

points in the food chain to judge whether imported or commercially available foods comply with current legislation. Intensive sampling by the same authorities would be carried out during an outbreak to identify its source. In commerce, sampling is performed to confirm compliance with specifications between suppliers and customers. Samples taken at intermediate steps of the processing line, from surfaces of equipment in contact with the product or from the processing environment, allow the food processor to verify adherence to Good Hygienic Practices (GHP) and the efficacy of preventive measures.

12.2.1 Steps in the Sampling Procedure

Initial sampling represents the step where physical samples are taken, according to a preestablished sampling plan, at specified sampling points. *Sample handling* encompasses all subsequent steps including the description, recording, and labeling of the samples, their intermediate storage before dispatch, their transport to the analytical laboratory, and their reception, registration, and storage up to the preparation of the analytical unit(s) before analysis. All steps up to the analysis must be controlled to ensure the quality of the sample units and to allow reliable traceability. The guidelines provided in this chapter are considered good practice and should help to optimize results.

12.2.2 Containers

Unless sample units comprise products in their original packaging, such as sachets, tins, boxes, bottles, or cups, the samples drawn must be placed in clean and sterile containers. The containers used should be of appropriate size and have a sufficiently wide opening to allow clean and easy transfer of the samples. Depending on the sample unit, different containers may be used, such as plastic bottles, jars, or bags, metallic cans, or boxes. Glass containers, however, should not be used in manufacturing facilities where breakage could lead to the presence of glass in the products.

Using sterile disposable containers is the most convenient, but for reasons of cost this may not be possible. If reusable containers are used, they should be thoroughly washed, rinsed, dried, and sterilized before further use. Depending on the material, containers are sterilized either at 121 °C for 15 min in an autoclave, or for at least 1 h at 160 °C in a hot air oven. Details of the different options for the sterilization of containers can be found in APHA (1992).

It is important to prevent samples from leaking during handling and, in particular, during transport. Screw caps are the most appropriate closing devices for jars and bottles, and plastic bags must be sealed securely. If rubber or cork is used for caps or closures, it must be confirmed that no adverse interactions with the samples can occur.

12.2.3 Sampling Utensils and Devices

Utensils used to collect the sample units should be sterile and adapted to permit the most appropriate and representative sampling according to the type of food. Sterile scissors, knives, saws, can openers, and other tools can be used to open boxes, packs, sacks, or cans. Samples of the actual food product can then be collected using sterile scoops, spoons, triers, probes, drill bits, forks, cork-borers, pipettes, or swabs, depending on the type of food sampled. Examples and further details can be found in FAO (1990).

For environmental sampling, utensils need to be adapted to the sampling site, with no limitations to their type. Sponges or swabs are most suitable to sample food contact sur-

faces, floors, or walls. Scrapers, scoops, spatulas, or brushes are suited to collecting residues in cracks, crevices, or underneath equipment. Pipettes (plastic) can be used to collect water in drains, and air sampling devices can be used to monitor the air.

Containers and utensils should be packaged individually and be presterilized, thereby avoiding the need for disinfection between samples. Disposable plastic gloves are recommended when collecting certain samples (e.g., sponge samples from equipment), a new glove being used for each sample. Sterilization of utensils by means of a torch should be avoided and may even be dangerous (e.g., risking explosions in dusty environments).

Sampling ports or taps directly installed on equipment, such as tanks, are usually sterilized in situ by means of steam. They require particularly careful attention (design, cleaning, and sterilization procedures) to avoid contamination of the product.

12.2.4 Sampling Procedures

The method of sampling should be adjusted to fit the purpose. For lot acceptance determinations, the intent is to collect sample units that are representative of the lot. To assess control of the processing environment, a routine procedure should have been developed including sample sites and method of sampling. When investigating the source of a problem, the sampling sites, foods, ingredients, times, frequencies, etc. are not predetermined and are left to the discretion of the sampler. While it is desirable to make every effort to collect samples that fulfill the intended purpose, the safety of the individual collecting the sample, and others who may be affected, takes precedence. Under dangerous circumstances, sample collection should not be attempted. Likewise, if the method of sampling can jeopardize the food and render it injurious to consumers, the sampling procedure should be changed or the sample should not be collected.

Samples should be collected by persons previously trained and instructed, using appropriate methods and, above all, aseptic techniques. The timing and sampling point in the food chain or a food process may be crucial for data interpretation. When regulatory authorities collect samples at a port-of-entry, timing is determined by the arrival of trucks or ships. Samples collected by authorities to verify compliance with legal requirements may be collected during storage, distribution, retail, or food service. Samples collected at different steps in the processing line, such as after production and packaging and before and after cleaning, allow food manufacturers to verify adherence to GHP and the efficacy of Hazard Analysis Critical Control Point (HACCP) plans.

For packaged products, sampling is straightforward and need only follow preestablished sampling plans. For ultra high temperature products, for example, routine samples are taken at defined intervals during processing, while *event samples* are taken after startup, after stops in production, or after changes of paper rolls and strips to monitor and assess the impact of such events (Cordier, 1990). In other circumstances, packed units may be taken from pallets according to a predetermined scheme.

If the packaging material needs to be opened to collect the sample unit, appropriate precautions must be taken to avoid contamination. External surfaces must be cleaned to remove dust and soil and, if multiple layers of packaging are used (e.g., bags of flour, sugar, or other dry products), the outer layer(s) can be removed. This allows access to cleaner surfaces, which may then be disinfected, if necessary, before opening or cutting.

Sufficient quantities should be collected to allow additional analyses to be performed as necessary, or for unforeseen inquiries.

If packages show signs of "blowing" (swelling), extra care should be exercised to avoid dissemination of contaminated material. In such circumstances, the whole package should be sent to the laboratory.

Ideally, the food should be mixed before collecting the sample units, but this may not be practical or possible. For liquid samples, such as milk, ice cream mixes, or beverages stored in vats or tanks equipped with stirrers, it is usually relatively easy to obtain representative samples. If there is no evidence of recent stirring and mixing, thorough mixing with a sterile ladle is recommended before collecting the sample.

In some foods, however, sampling specific components, sections, or layers of multi-component products is desirable. Particular care should be exercised to achieve a good separation. Examples of special considerations are provided in Table 12-1.

Water (potable water, processing water) should be sampled from taps or built-in sampling devices after flushing sufficient water through those taps or built-in devices to obtain a representative sample. A sterile ladle or other means can be used for water or brine solutions in open systems. Addition of a neutralizing solution is necessary to neutralize disinfectant residues, if present.

Air sampling in processing environments may be performed to assess the microbiological status of air. Many techniques have been described, from simple passive collection using sedimentation plates to active air samplers based on impaction or impingement. Those methods and available equipment are reviewed by Henningson & Ahlberg (1994).

Special consideration should be given to sampling during investigation of food-borne outbreaks since the sampling can differ considerably from routine inspections. Using professional judgment, samples of food or equipment surfaces should be collected at the most appropriate locations where contamination, survival, or growth might have occurred (Bryan, 1999).

12.2.5 Sample Labeling

Sample units must be clearly labeled and identified to permit good traceability. This can be achieved by writing descriptive terms or numbering each sample unit directly on the container or a firmly attached label, making sure that the ink cannot be washed off.

In addition, a sampling report should be prepared with relevant details stating, for example, the time of sampling, sample site, particular observations on packaging, etc. The comments may vary widely depending on the purpose of sampling and what is to be learned from the analysis. Thus, the report should indicate the reason for sampling and, if known by the person collecting the samples, the types of analyses to be performed. If necessary, for example, for legal reasons or in case of dispute, the report should be signed by the person responsible for the sample collection as well as by representatives of the parties concerned. In such instances, sample containers may be sealed with an official seal that makes tampering impossible.

12.3 INTERMEDIATE STORAGE AND TRANSPORTATION

Collected sample units should be transported to the laboratory as promptly as possible. However, in certain situations, intermediate storage cannot be avoided before samples are dispatched, e.g., when daily dispatch is impossible. For environmental sampling, samples may be collected at regular intervals from specific sites over a period of time and, when assembled, dispatched. When samples are stored, the storage conditions must

Table 12-1 Special Considerations When Sampling Food Commodities

Phase		State	Form	Food Ingredient	Recommendations for sampling	
Solid	Dry	Fine powder		Dry milk, cocoa powder	From center of container unless problems suspected at the surface	
			Large part	RTE* cereals, dry pasta	Select representative components and blend before drawing sample; may be rehydrated directly	
		Blocks		Dates, sugar, cheese	If hard, use sterile hammer and chisel (also mortar and pestle) to draw sample; try to draw samples from several locations	
			Multicomponent	Soup mix, spices	Entire unit or selected components drawn for sample	
			Particles	Ground meat	Multiple portions drawn from one or more packages or units	
Solid	Moist	Chunk		Tote of beef primal cuts	Selected pieces or liquid weep (drip) from large tote	
		Blocks		Whole poultry, cheese	Whole bird rinsed in diluent or media; try to draw samples	
		Carcass		Beef	Prescribed suspected area swabbed or sponged for testing	
		Sausage		Sliced, cooked	Rinse or blend	
				Fermented sausage	Selected areas for selected analyses, e.g., test for <i>S. aureus</i> near the surface, and enterohemorrhagic <i>E. coli</i> in the center	
		Multicomponent		Cream dessert, stew	Selected areas for selected analyses, e.g., test for <i>S. aureus</i> near or in cream filling; test for <i>C. perfringens</i> in liquid portion of the stew	
Solid	Frozen	Particles		IQF† peas/beans	Frozen sample either "stomached" or rinsed with diluent	
		Blocks		Frozen egg	Collected with (sterile) funnel and drill	
Liquid		Multicomponent		Pizza	Sector sampled while frozen unless specific components tested	
		Single phase		Water	Membrane filter kept in conditions that avoid death or multiplication	
		Multiple nonmiscible phases		Salad dressing	Well-mixed representative sample, or specific layer	
				High calorie supplement	Representative weighed sample	
		Homogeneous multiple phases				

* RTE—ready-to-eat

† IQF—instant quick frozen

prevent changes, i.e., microbial growth or death. Storing dry or shelf-stable products or samples poses relatively few problems, but wet and perishable samples are more delicate and need to be refrigerated or even frozen. The storage conditions considered most appropriate must be established to ensure that the target flora remains unchanged. Loss of viability during frozen storage and subsequent thawing can be a particular problem (e.g., *Campylobacter jejuni* vegetative cells of *Clostridium perfringens*).

For transportation, all samples must be packed to avoid breakage and spillage. Where necessary, containers should be protected by additional packaging material. Finished products, raw materials, and environmental samples should be packaged separately or shipped separately to avoid possible problems of cross-contamination.

Samples of perishable chilled or frozen products should be shipped in insulated containers to maintain the appropriate temperature with a refrigerant, such as ice, dry ice, or freezer packs. For particularly sensitive or important samples, temperature recorders or indicators should be included to record in-transit temperatures that could affect the analytical results.

12.4 RECEPTION OF SAMPLES

When received at the analytical laboratory, sample units should be inspected visually for damage or spillage, temperature checked as necessary, and the samples cross-checked against the sample report. Information that could influence the analytical results should be noted on the sample report. Today laboratory information management systems are widely used, allowing for easy and complete registration, thus ensuring good traceability.

12.5 SAMPLE ANALYSIS

12.5.1 Withdrawing Analytical Units

Samples should be analyzed as promptly as possible and, if not, stored under conditions that do not permit either death or multiplication of the target microflora.

Paramount in the process of withdrawing sample units is to use aseptic technique to prevent contamination of the analytical unit. The first step of the preparation for microbiological analysis is to withdraw a representative analytical unit from each sample unit. Samples must be mixed thoroughly to obtain a representative analytical unit. Liquid, semi-liquid, and, to some extent, powders in containers with a headspace, can be mixed by inverting or shaking the container. Analytical units should be removed as soon as possible after mixing. The external parts of packages or containers are first disinfected using chlorinated or iodinated solutions. Opening of packages is performed taking all necessary measures to prevent contamination. Sterile utensils are used to open packages, mix, and withdraw the necessary aliquots.

12.5.2 Dilution and Homogenization of the Analytical Unit

Weighing the analytical unit into the appropriate diluent (first dilution) must be performed aseptically. With dusty products, it is recommended to work under a hood or in a laminar flow cabinet to avoid dispersion of dust that might be contaminated with pathogens.

Most analytical methods recommend weighing the analytical unit with a precision of ± 0.1 g of the desired weight. The size of the analytical unit has a significant effect on the coefficient of variation of the sample weight. The larger the analytical unit, the lower

the coefficient of variation and the better the accuracy. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more.

For residue collected from the environment, only small quantities, commonly 1 g or less, may be available. Nevertheless, such samples can provide very valuable information.

Some products, such as liquids, semi-liquids, and paste-like samples, are normally mixed and dispersed rather easily. For products such as margarine, butter, chocolate, etc., gentle warming (to about 40 °C) improves dispersion and dissolution of the food matrix and facilitates the release of microorganisms. Other types of samples, in particular solids, require special treatments using homogenizers or blenders. Diluted homogenized sample units can then be further processed according to the protocols of specific analytical methods. Detailed information on sample dilution and homogenization is provided in various documents, such as ISO documents (1999).

Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. This has long been recognized and is discussed, for example, by Keller *et al.* (1974).

Use of stomachers or blenders usually has no impact on the viability of microorganisms. An exception is high-speed blenders that may damage certain cells, for example anaerobes, due to exposure to oxygen (Ray, 1979).

Dry foods must be rehydrated slowly to avoid die-off due to osmotic shock (e.g., freeze-dried cells) (Ray *et al.*, 1971). This was confirmed by van Schothorst *et al.* (1979), who showed that the conditions of rehydration of foods can have a major impact on the recovery of salmonellae. Slow rehydration, e.g., using a soaking procedure, resulted in higher recovery rates than shaking.

In foods, the ratio between dead, injured, and healthy cells varies, as does the extent of injury to individual cells. Numerous studies have been devoted to this subject and reviews are available (Ray *et al.*, 1971; Mackey, 2000).

12.6 RECOVERY OF INJURED CELLS

Injury of microbial cells can have a significant impact on the final results and must be taken into account during analysis. Injured or damaged microbial cells should be allowed time to repair that damage before growth is initiated. This "time to repair" is seen as an increased lag phase and affects the incubation time of both solid and liquid media. Time spans recommended in standard methods are generally those optimal to recover the maximum number of microorganisms. Reducing this incubation period can lead to a significant reduction in counts, or in the case of qualitative methods, to false negatives. This was shown for *Salmonella*, for example, when preenrichment for 6 h gave lower recoveries than preenrichment for 24 h. Extending preenrichment to 48 h did not increase the number of *Salmonella*, and numbers were sometimes lower than that at 24 h (D'Aoust *et al.*, 1992; Hammack *et al.*, 1993).

Food components can markedly affect recovery of both injured and healthy cells, as is shown by detection of *Salmonella* in different food matrices. Rapid rehydration of dehydrated products, such as milk powder or feeds, reduces the recovery rate (Van Schothorst

et al., 1979; D'Aoust & Sewell, 1986). Dehydrated culinary products, such as soups or concentrated bouillons, and raw materials, such as spices, onions, etc., require higher dilution rates (1:20 up to 1:100) or the addition of substances to neutralize the inhibitory effects of salt and certain food components (Andrews *et al.*, 1995). Bacteriostatic and/or bactericidal substances present in cocoa may inhibit growth of *Salmonella* during the preenrichment (Zapatka *et al.*, 1977). Inhibition is neutralized by adding casein or nonfat dry milk to the preenrichment broth (Poelma *et al.*, 1981; IOCCC, 1990). In fatty foods, surfactants such as Tween 80 improve recovery (D'Aoust *et al.*, 1982). Hydrocolloids may affect and inhibit recovery of salmonellae by thickening and changing the pH of the preenrichment broth. Using appropriate dilutions, adding enzymes to reduce viscosity, and adjusting pH have been shown to improve handling and recovery (Amaguaña *et al.*, 1996, 1998). Gel formation of gelatin during incubation will affect recovery, and dilution (1:20) or using papain to reduce the viscosity are recommended (Jay *et al.*, 1997; Amaguaña *et al.*, 1998).

Environmental samples can be heavily contaminated and addition of malachite green to the preenrichment broth to inhibit competitive microorganisms has been shown to enhance recovery of salmonellae (Van Schothorst & Renaud, 1985).

Optimization of preenrichment conditions is therefore essential for the detection of salmonellae and should be considered for other pathogens. Increasing the number and type of selective enrichment broths and selective plating media will not improve detection of injured and healthy cells if they are not permitted to recover and grow during preenrichment.

Selective agents in direct plating media can have an adverse effect on particular microorganisms (Ray, 1979). This is of particular concern if low levels need to be detected. Selective agents, such as sodium lauryl sulphate, Oxgall, brilliant green, bile salts, sodium desoxycholate, crystal violet, and others, are used in different media to detect Enterobacteriaceae, coliforms, or *Escherichia coli*. Comparative studies with basal agar supplemented with one of these selective components have shown inhibition rates between 0 and 99% for *E. coli* (Ray, 1979). This has been confirmed by others using different experimental approaches, underlining the need for careful choice of media in the microbiological analysis of foods.

Numerous attempts have been made to overcome this problem either through choice of selective agent, introducing a resuscitation step in the procedure, use of a nonselective medium, or addition of betaine or pyruvate to enhance recovery of injured or stressed cells (Ray, 1979; Johnson & Busta, 1984; Mackey *et al.*, 1994; Marthi & Lightfoot, 1990).

12.7 ERRORS ASSOCIATED WITH METHODS AND PERFORMANCE OF LABORATORIES

The errors associated with quantitative methods, such as colony count techniques, differ from those for qualitative methods, such as presence/absence tests. Errors affecting the quality of data obtained by analytical laboratories have been discussed in detail (Jarvis, 1989). Quality of results is characterized by the accuracy of the method, i.e., the ability to provide results equal to, or close to, the real value. The repeatability (r) of a method reflects the difference between two single results obtained when the same sample is analyzed by the same analyst under identical analytical conditions. The reproducibility (R), on the other hand, represents the difference obtained between two laboratories. Examples of r and R values for different analytes are provided in Table 12-2.

Over recent years, considerable efforts have been made in the accreditation of laboratories. This will have a positive outcome, provided accreditation is not just considered as a system or a commercial asset. If properly implemented, it should be used for continual

Table 12-2 Examples of Repeatability (*r*) and Reproducibility (*R*) for Different Analytes in Mashed Potatoes, Rehydrated with Cultures. (Data provided by the Silliker Laboratories).

Estimation of laboratory variance (*Repeatability*) using the average variation of homogeneity samples for proficiency test materials. Each set examined by the same technician (20 tests).

Method	s^2 [†]	<i>s</i>	CV [‡] (%)	Repeatability (<i>r</i>)
Aerobic mesophilic counts (<i>n</i> =80)	0.0126	0.1122	2.46	0.317
<i>C. perfringens</i> spores (<i>n</i> =40)	0.103	0.321	7.29	0.908
Coliforms (<i>n</i> =80)	0.057	0.239	6.42	0.677
Coliforms (MPN)* (<i>n</i> =80)	0.109	0.331	9.29	0.935
<i>E. coli</i> (MPN) (<i>n</i> =40)	0.150	0.387	9.73	1.096
Yeasts (<i>n</i> =40)	0.023	0.152	3.31	0.425
Molds (<i>n</i> =40)	0.022	0.150	4.16	0.424

Estimation of the variance among laboratories (*Reproducibility*) using the average variation from 13 proficiency test sets (1995–1998).

Method	s^2	<i>s</i>	CV (%)	Reproducibility (<i>R</i>)
Aerobic mesophilic counts (<i>n</i> =1669)	0.068	0.261	5.4	0.37
<i>C. perfringens</i> spores (<i>n</i> =660)	0.353	0.594	15.2	1.68
Coliforms (<i>n</i> =1439)	0.138	0.371	10.0	1.05
Coliforms (MPN) (<i>n</i> =1066)	0.191	0.437	11.4	1.24
<i>E. coli</i> (MPN) (<i>n</i> =706)	0.435	0.659	17.8	1.87
Yeasts (<i>n</i> =701)	0.129	0.359	8.7	1.02
Molds (<i>n</i> =735)	0.084	0.290	8.9	0.82

[†]*s*—standard deviation

[‡]CV—coefficient of variation

*MPN—most probable number

improvement of analytical performance and laboratory procedures, such as the quality of the media used and their preparation and control of incubator and water bath temperatures. Skills and training of personnel, standardization of laboratory practices, and so on, should also be addressed.

Participation of laboratories in proficiency tests organized and offered by national, professional, or commercial organizations and requested by accreditation bodies also represents an opportunity for improvements (Black & Craven, 1990; Peterz, 1992; Berg *et al.*, 1994). Such proficiency tests facilitate benchmarking performance of the laboratory and identification of weaknesses that need improvement. It must, however, be realized that the type of samples provided for proficiency testing has limitations related to the preparation and the viability of microbial population. Consequently, check samples for proficiency testing are not available for all food matrices. The concentration of pathogens is frequently relatively high and a competitive flora is not always included in check samples. Such samples do not, therefore, accurately assess the laboratory's ability to detect very low numbers of injured cells that may occur in actual food samples. The use of reference materials containing very low levels of injured cells, such as the *Salmonella* product developed in the Netherlands (Foundation, 2001), may be more useful in assessing the laboratory perfor-

mance or reliability of a method. Reference materials have been developed for various microorganisms (Peterz & Steneryd, 1993; In't Veld *et al.*, 1995).

Simplified or alternative methods are often used to cope with the large number of analyses and to obtain results more quickly. This is legitimate and can accommodate a sudden influx of samples, e.g., environmental, to detect a source of contamination. This is more effective than applying more cumbersome standard methods that limit the number of samples that can be analyzed.

It is extremely important to underline the need to use alternative methods that have been validated. This not only allows more results to be obtained sooner, but also guarantees reliability of results. A number of validation procedures exist, ranging from a simple peer review of alternative methods by an expert panel to extremely thorough procedures based on extensive comparative and collaborative studies (Andrews, 1996; Lombard *et al.*, 1996; Rentenaar, 1996; Scotter & Wood, 1996). The development of a technical protocol within Microval (Rentenaar, 1996) represents an example of how to standardize validation procedures and to develop an International Organization for Standardization/Comit European de Normalisation standard that will be issued during 2001.

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Salmonella in Dried Milk

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15.1 INTRODUCTION

Milk is widely used worldwide as an important part of the diet, especially for younger children. Raw milk is a very perishable product and efforts to preserve it have led to the development of a range of dairy products (Anon., 1995). One of the earliest means of preserving milk was natural fermentation (ICMSF, 1998), but modern technologies have improved the old preservation methods and added new ones (Robinson, 1986a, b; Varnam & Sutherland, 1994; ICMSF, 1998 at Chap. 16). In particular, drying has developed into one of the most used technologies. In addition to preserving the most nutritious elements, drying offers the advantage of weight reduction, making transportation more convenient. Dried milk has been shipped in large quantities around the world for decades. In many tropical countries, producing and/or importing dried milk provides children with this nutritious food.

Milk is pasteurized, or even sterilized, before drying to render the product safe because drying cannot be relied upon to kill pathogens in sufficient numbers (Daemen & van der Stege, 1982). Unfortunately, very occasionally recontamination has occurred and, especially when dried milk was used in infant formula, this led to cases or outbreaks of salmonellosis (Anon., 1997; Becker & Terplan, 1986; Gelosa, 1994; Louie, 1993; Usera *et al.*, 1996; Rowe *et al.*, 1987). Vast quantities of dried milk are consumed daily worldwide without evidence of causing salmonellosis. This may indicate that recontamination seldom occurs, or that low numbers of salmonellae do not cause an infection and that multiplication of *Salmonella* in the reconstituted milk must occur to cause illness. Data on the infective dose of salmonellae in children are not well documented (FAO/WHO, 2000) because infections due to dried milk are rare, and when they occur (Collins *et al.*, 1968; Furlong *et al.*, 1979), the number of salmonellae in the reconstituted product is rarely determined. Several aspects of the risk evaluation of *Salmonella* in dried milk will be discussed and serve as an example of setting a food safety objective (FSO).

15.2 RISK EVALUATION

15.2.1 Hazard Identification

All *Salmonella* serovars, with the exception of *Salmonella typhi* and *S. paratyphi*, which behave very differently, are considered in this risk evaluation. Salmonellae are

Gram-negative rods that can grow in milk with $a_w > 0.95$ and $\text{pH} > 4.9$ at temperatures between 8–45 °C (ICMSF, 1998). Salmonellae are not particularly heat resistant and when present in milk are readily killed by heat treatments such as the various time-temperature combinations used in commercial pasteurization (Thomas *et al.*, 1966; Read *et al.*, 1968). Salmonellae are widespread in most regions of the world and animals can be infected by a wide range of serovars (ICMSF, 1996; Ziprin, 1994). During infection, animals may excrete salmonellae in high numbers. Consequently, water and dust may become contaminated and, particularly when cows are shedding salmonellae, milk may be contaminated during milking. When contaminated water or dust enters a dairy plant, salmonellae may gain entry to production lines and the product may become contaminated (Van Schothorst & Kleiss, 1994).

15.2.2 Exposure Assessment

Pasteurization of milk was one of the first examples of a technology applied to render safe a potentially hazardous food. During pasteurization, at least a 10-log reduction of salmonellae is normally obtained by minimal processing conditions (72 °C for 15 s) (ICMSF, 1996). Often an even greater killing effect is achieved in the production of whole milk powder, because temperatures of 80–85 °C are applied for at least 5 s to inactivate most lipolytic enzymes.

Milk pasteurization is a process that can be closely controlled. Handling the dried milk is, however, more critical because many potential routes for recontamination exist (IDF, 1991, 1994). The frequency of recontamination is unpredictable, but when good hygienic practices are maintained, it is rare and the numbers of salmonellae involved are very low. Normal end-product analysis of dried milk will not reveal such low levels of recontamination; consequently, accurate estimates of the frequency of contamination and the true concentration are difficult to establish. In addition, milk powder must be reconstituted before consumption. Although the procedures recommended for preparing, storing, and using reconstituted milk are well documented, the potential opportunities for multiplication are not known. However, based on commercial experience and epidemiologic evidence, it is reasonable to assume that the presence of *Salmonella* in the product is infrequent and the concentration at the moment of consumption is low.

15.2.3 Hazard Characterization

This part of the risk evaluation describes the effect of the identified hazard on an individual or particular groups within a population. Salmonellosis is normally caused by the ingestion of salmonellae in food or water (Silliker & Gabis, 1986). The incubation period may vary from 5 h to 5 days (ICMSF, 1996). Symptoms include nausea, stomach cramps, vomiting, diarrhea, and fever (D'Aoust, 1991). Especially the very young, very old, and immunocompromised persons may become infected with low doses ($< 100 \text{ cfu g}^{-1}$) of salmonellae, and their symptoms may be more severe (Blaser & Newman, 1982; Glynn & Palmer, 1992). Salmonellae may cause death among the aged and other vulnerable persons. Moreover, salmonellosis may leave sequelae, such as rheumatoid arthritis (Archer, 1985; Keat, 1983; Smith, 1994). When estimating risk, it is thus very important to specify which type of illness is considered and the target population.

The type of food also may influence the dose-response relationship. Experience has shown that in foods with a high fat content (e.g., chocolate, cheese, or fermented meats),

low numbers of salmonellae (less than 10 cfu g^{-1}) may provoke illness (D'Aoust *et al.*, 1975; D'Aoust, 1985; Graven *et al.*, 1975; Greenwood & Hooper, 1983; Hedberg *et al.*, 1992; Lehmacher *et al.*, 1995). On the other hand, classical volunteer studies conducted with salmonellae in fluids indicated that up to 10^6 salmonellae were necessary (McCullough & Eisele, 1951a, b, c.). While data are not available for the number of cases of salmonellosis caused by reconstituted milk powder, it is reasonable to state that the number is very low in terms of number of servings per annum. Since reconstituted dry milk is often consumed by children, a vulnerable population group, a worst case scenario approach is used in this risk evaluation, i.e., it is assumed that one *Salmonella* per serving of milk powder may lead to gastroenteritis in children.

15.2.4 Risk Characterization

The world production of whole milk powder is estimated to be more than one million tons (10^{12} g) per annum. Assuming a serving size of 10 g , 10^{11} servings are consumed annually. Outbreaks and cases of salmonellosis due to the direct consumption of dried milk are rare (Collins *et al.*, 1968; Furlong *et al.*, 1979), but in this example it will be assumed that fewer than 1000 cases of salmonellosis result from contaminated milk powder annually. This gives a probability of illness of < 1 in 10^8 servings. In the absence of accurate data, it is not possible to validate this estimate, but this is most likely an over-estimation of the actual risk.

15.3 RISK MANAGEMENT

15.3.1 Acceptable Level of Consumer Protection

In most industrialized countries, the prevalence of salmonellosis from all sources averages about 50 cases per 100,000 population per annum (Gomez *et al.*, 1997). For this example, a public health goal could be stated as less than 1 case per 100,000 population per annum associated with consumption of dried milk. This means the product will not significantly add to the current burden of disease.

15.3.2 Establishing a Food Safety Objective

If the number of servings consumed annually is assumed to be one glass of milk per day (i.e., 10 g powder) by one-third of the world's population, this would mean that about 10 million servings per 100,000 persons would be consumed per annum or 10^8 g . Considering the estimated probability of infection to be < 1 in 10^8 servings, this would mean that the consumption of dried milk would be associated with < 1 case per 100,000 population per annum.

Using a worst-case scenario, i.e., that 1 *Salmonella* cell per serving could provoke illness, the following FSO could be proposed:

The concentration of *Salmonella* in dried milk powder must be $< 1 \text{ cfu } 100 \text{ kg}^{-1}$ at the time it is reconstituted for serving and consumed.

This FSO reflects a public health goal of < 1 case per 100,000 population per annum. The same FSO also could have been established by reviewing annual production records and the number of illnesses reported or estimated, as was calculated in the risk characterization section.

For this particular food-pathogen combination, no change in risk is expected until the milk powder is reconstituted, after which, the time and temperature of holding will determine whether salmonellae can grow and the rate of growth. The potential for growth and increase in risk, such as during subsequent storage, must be addressed through education, such as labeling and other means. In this example, the FSO is intended for milk powder that is consumed immediately following reconstitution.

The safety of reconstituted milk also can be affected by contamination introduced from other sources, such as the water used to reconstitute the milk, utensils, container, and the person involved in preparing the milk. Other pathogens may be of significance (e.g., viruses, shigellae) in addition to salmonellae. These concerns are not considered in this example.

15.3.3 Control Measures

In principle, several control measures are available to help prevent *Salmonella* contamination in powdered milk and thus to meet the FSO. First, elimination of salmonellae at the dairy farm would prevent contamination during milking and further handling. However, this is very difficult to achieve, because salmonellae are widely distributed in nature, including farm environments (Van Schothorst, 1986). A second option is to eliminate salmonellae from the whole factory environment, but this also is difficult to achieve. Consequently, efforts to eliminate salmonellae should be concentrated on the equipment and environment following pasteurization (Stadhouders, 1975; Jarl & Arnold, 1982; Terplan & Becker, 1989). In current industrial practice, a series of control measures are applied, from collection of the milk until the powdered milk is packaged, to avoid contamination to the extent possible. Collectively, the control measures ensure the FSO has been met. In this example, the control measures are described only briefly. More detailed information can be found in the literature (IDF, 1991, 1994). Control measures include:

- *Controlling initial levels in raw materials*—Effective hygienic measures are applied at the farm level and the collected milk is refrigerated to below 7 °C to prevent multiplication.
- *Reducing levels during pasteurization of raw milk*—Raw milk is pasteurized to eliminate any salmonellae present (initial concentration).
- *Preventing an increase in levels by avoiding recontamination*—Recontamination during drying, handling, storing, and packaging is prevented by adopting a number of good hygienic practices (GHPs) that minimize the chance of contamination, e.g., use of filtered air; good separation of the wet and dry processing steps.
- *Providing consumer information*—A risk management option is to provide consumers with information on the product's label. This information provides instructions on reconstituting the milk powder, including precautions for what should not be done during and after reconstitution. Governments may want to include this information in general food hygiene information programs.

In this example, testing the final product cannot be used as a control measure to achieve the required FSO. Even with the most stringent sampling plan recommended (i.e., two-class sampling plan, $n = 60$, analytical unit = 25 g), the mean concentration of *Salmonella* would have to be at 1 cfu in 526 g or greater before a positive lot could be

detected with a 95% probability, assuming a log-normal distribution and a standard deviation of 0.8 (Legan *et al.*, 2001).

15.3.4 Performance Criterion

A performance criterion is the required outcome of a step, or combination of steps, that contributes to ensuring that an FSO is met. Performance criteria are usually applied at steps where hazards can either be reduced or increased if appropriate control measures are not applied. Performance criteria may be more stringent than the FSO to ensure that the FSO is met. For milk powder that must be reconstituted before consumption, a more stringent performance criterion may be adopted to account for the slight abuse during preparation and use.

To arrive at a performance criterion for the control measures during manufacturing, the equation from Chapter 3 can be used:

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$H_0 - \Sigma R + \Sigma I \leq -8$$

where:

ΣR = total (cumulative) decrease of the hazard

ΣI = total (cumulative) increase of the hazard

H_0 = initial level of the hazard

Performance criterion, H_0 , R , and I are expressed in $\log_{10}$ units.

The basic control measures to achieve the FSO and its related performance criterion consist of killing the salmonellae that may occasionally be found in fresh milk and preventing recontamination of the heat-treated raw material.

15.3.4.1 Controlling Initial Levels in Raw Materials

Since the effectiveness of the heat treatment depends on the number of salmonellae, hygienic measures must be taken at the farm level to keep contamination of salmonellae as low as reasonably possible. The collected milk must then be cooled rapidly and maintained below 7 °C to prevent multiplication. Tankers must be refrigerated and/or insulated. Upon receipt at the dairy plant, the temperature should be checked as one of the several physico-chemical tests. Total viable counts and coliforms may be determined as a check on the effectiveness of the chilling and the general hygienic measures taken. In many countries, maximum total counts have been established as microbiological standards.

The first stages of milk powder production have little or no influence on the number of salmonellae in the milk. During clarification, filth, clumps of milk particles, etc., as well as an excess of animal cells (for instance leukocytes), are removed. A separator is used to remove a portion of the cream. When this equipment is adequately and frequently cleaned, separation has no influence on the number of salmonellae. Depending on the type of milk powder produced, some of the cream may be added back to the fluid milk to obtain the required fat content. Storing and handling the fat, holding and standardizing the milk, etc. should be performed in a manner that prevents contamination with salmonellae and does not permit their number to increase.

Given the above hygienic precautions, the initial number of salmonellae in raw milk is usually very low, and no more than 1 cfu ml⁻¹ (i.e., $H_0 = 0$).

15.3.4.2 Reducing Levels during Pasteurization of Raw Milk

Pasteurization is of great importance and is normally identified as a critical control point (CCP) in a Hazard Analysis Critical Control Point (HACCP) system. The temperature is monitored with a thermograph. Most equipment has a flow diversion valve and when the temperature drops below the target value, the milk is diverted to a holding vat for repasteurization; thereby, the flow rate is also monitored and maintained at the required rate and survival of salmonellae is prevented. During cooling, an overpressure should be kept on the pasteurized milk side of the heat exchanger to prevent recontamination. If the pasteurizer does not function correctly or if salmonellae are introduced during cooling, the required low level of salmonellae may not be achieved. Fouling of equipment and buildup of biofilms must be prevented. Inspections and/or tests to detect micro-leaks are used in good hygiene and maintenance practices.

If the initial concentration of salmonellae is assumed to be no more than 1 cfu g^{-1} , then to achieve a performance criterion of 1 cfu per 10^8 g , an $8 \log_{10}$ reduction needs to be achieved through pasteurization, i.e., $\Sigma R = 8$ (see Figure 15-1).

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$0 - \Sigma R + \Sigma I \leq -8$$

$$\Sigma R = -8$$

15.3.4.3 Preventing an Increase in Levels by Avoiding Recontamination in the Factory

Following pasteurization, the milk moves through an evaporator to concentrate it before spray drying. The temperatures used for condensing may further reduce the number of salmonellae, but evaporation is normally not important for the safety of the milk powder. Balance tanks are often used between condensing and spray drying to compensate for differences in flow rates. The balance tanks should be located in clean, *Salmonella*-free environments to prevent recontamination. The air entering the balance tank could be filtered as an additional preventive measure.

At this stage, it is critical to maintain a barrier between the wet operations of the factory and the dry operations where the spray dryer and the rest of the production line are

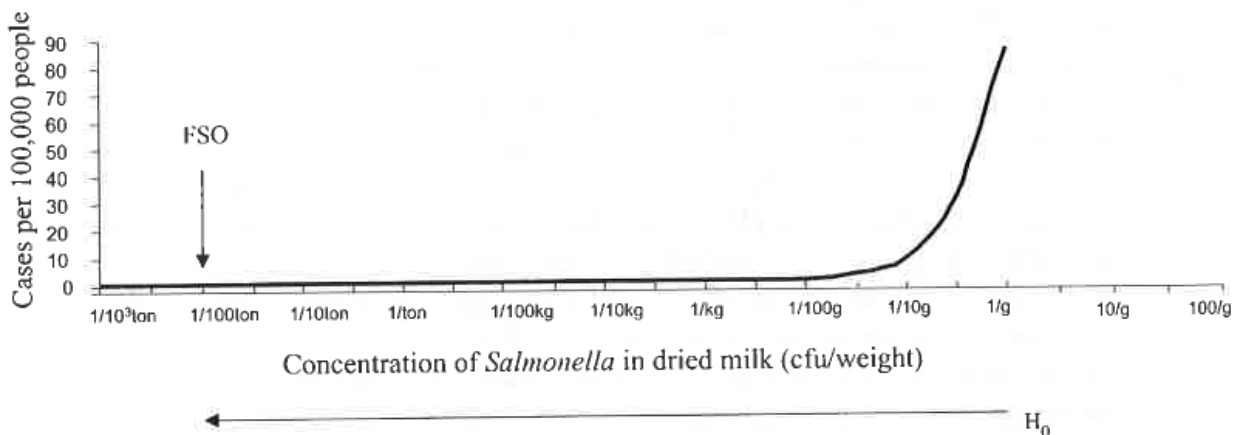


Figure 15-1 Pasteurization of raw milk and preventing recontamination in the factory.

located. People, forklift trucks, etc. should not enter the dry zone without taking precautions to prevent introducing contamination. Air entering this dry sensitive area should be of controlled hygienic quality, entry of pests should be prevented, humidity should be low, and dry cleaning should be the routine procedure used.

The condensed milk is transported via a high-pressure pump to a spray nozzle or another spraying device installed at the top of the spray dryer. Hot air is blown into the aerosol of fluid milk that has been generated by the spray nozzle. The water is evaporated and milk powder falls to the bottom of the dryer. The hot air, which may still contain very fine particles of milk powder, is passed through cyclones to remove the milk particles from the air. These particles are returned to the milk powder at the bottom of the spray dryer.

The powder needs further cooling, and additional air is blown over the film of milk powder leaving the spray dryer. This air must be of high hygienic quality, because if it were to contain salmonellae, the cells would contaminate the final product. After cooling, the powder is passed over a sieve to remove clumps. It is then transported, either mechanically or pneumatically, to a hopper, which feeds the packaging machine or intermediate storage bins. Maintaining a clean, dry environment that consistently tests negative for salmonellae is critical for all the steps that occur within the dry areas of the plant, including the packaging operation.

All the lines where the milk is still in a fluid phase can be cleaned with Cleaning In Place installations or manually using water, cleaning agents, brushes, etc. The environment in this area of the plant is also wet cleaned. In contrast, the spray tower and its environment, as well as the sections of the line and surrounding environment after the spray dryer, are all dry cleaned whenever possible. Occasionally wet cleaning is necessary, but in these instances the equipment or surrounding environment are dried as soon as possible afterward. Drains and pipes containing water should be properly sealed. Condensation on cold pipes should be prevented; when insulation is used, care should be taken to prevent buildup of moisture and microbial growth, a potentially significant source of contamination. The principle applied here is that salmonellae cannot multiply when water is not available.

If the initial concentration of salmonellae is assumed to be no more than 1 cfu g^{-1} and an $8 \log_{10}$ reduction is achieved through pasteurization (i.e., $\Sigma R = 8$), then, to achieve a performance criterion of 1 cfu per 10^8 g , there must be no contamination (i.e., $\Sigma I = 0$) (see Figure 15-1).

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$0 - 8 + \Sigma I \leq -8$$

$$\Sigma I = 0$$

15.4 PRODUCT AND PROCESS CRITERIA

The process criterion for the pasteurization would consist of heating the milk for at least 15 s at 72°C , or for 5 s at 80°C .

The most serious problem in controlling the hazard from salmonellae in dried milk is preventing recontamination. To achieve that, many GHP-related measures are necessary. In practice, this means eliminating salmonellae from the equipment and its immediate

environment and preventing salmonellae from entering this environment with air, dust, pests, packaging materials, personnel, etc. Although it is difficult to quantify such low contamination rates, a recontamination rate of < 1 *Salmonella* per 10^8 g would be required. To put this into perspective, 10^8 gram is 100 tons of product, and that implies preventing recontamination during 30 h of production using a medium-sized spray-drying tower.

15.5 GHP AND HACCP

The basis for the production of safe milk powder is the application of GHPs that have been developed through many decades of industrial production of this product. All milk powders receive a heat treatment during one or more stages of production, and consequently preventing salmonellae in the end product depends mainly on preventing recontamination after the final heat treatment.

During development of the HACCP plan, the sources of *Salmonella* contamination and possible multiplication have to be identified, control measures put into place, and monitoring procedures described and implemented. A well-known source of salmonellae is milk residues, which may be found on the roof of spray tower buildings. Birds may contaminate this powder, and when the secondary air intake is close to the roof, this air may transfer salmonellae to the powder if the air filtration is not controlled. Salmonellae may also occur in the dust or mud outside the factory and may even gain access in noncritical areas, such as warehouses for raw materials or finished goods. Operators, maintenance personnel, visitors, etc. should, therefore, change their shoes before entering the dry areas of the milk powder production line. Movement of air throughout the various rooms should be well controlled. A slight overpressure should be maintained in the spray tower building, as well as in the packaging room. Again, the quality of this air should be carefully controlled. The packaging material could be a source of salmonellae, so control measures to ensure its high hygienic quality should be in place. Many other good hygienic practices are put in place to prevent recontamination, as described elsewhere, for instance in the IDF bulletin, *Recommendations for the Hygienic Manufacture of Milk and Milk Based Products* (IDF, 1994).

15.5.1 Monitoring and Verification from the Raw Milk to the Consumer

The quality of the incoming milk, and thus the potential level of contamination with *Salmonella*, is not under direct control of the dairy plant. However, the plant can influence the hygienic conditions at the farm and during transportation of the milk. The hygienic condition of incoming milk can be monitored by a number of tests, such as freezing point determination, methylene blue tests, filth tests, tests for aerobic mesophilic microorganisms, cell counts, acidity, etc. If necessary, the incoming milk could be tested microbiologically to check, for example, that a level of 1 *Salmonella* ml⁻¹ is not normally exceeded, as a verification procedure.

The time and temperature specified as process criteria are monitored in most dairy plants with continuous temperature and flow rate recorders of different designs and levels of sophistication.

Elimination of *Salmonella* from the equipment and its environment and preventing their introduction requires a number of hygienic measures that need careful surveillance (see Chapter 10). Testing for indicator microorganisms, as well as for salmonellae, can

check the effectiveness of these hygienic measures. If no salmonellae are found in the most likely locations and if the numbers of indicators, such as Enterobacteriaceae, are in the normal order of magnitude, it can be assumed that the performance criteria are achieved.

Testing end products for salmonellae is, in principle, not necessary as a routine monitoring procedure. Greater reliance must be placed on monitoring the HACCP system and assessing control of the environment through visual inspections and microbiological testing. When the process and environment records indicate that the operation is under control, microbiological testing would not be sufficiently sensitive to determine whether the performance criterion has been met. Nevertheless, many in industry often test a few samples for the presence of salmonellae as part of their verification program. While a positive result would indicate that the process was not under control, a negative result cannot be relied upon to ensure that the performance criterion and FSO have been met (see sections 15.3.3 and 15.6.3).

15.6 ACCEPTANCE CRITERIA FOR FINAL PRODUCT

15.6.1 Organoleptic

Milk powder has certain organoleptic characteristics that can easily be checked at any stage in the food chain. Milk powder should be dry, free flowing, and easily dissolve in cold or lukewarm water. It has a distinct white or creamy white color, without brown (Maillard reaction) or black (burned) discoloration or particles. It should not have a rancid or other abnormal aroma. Unopened packages should not be damaged or show any sign of insect infestation. Although these organoleptic acceptance criteria are more related to quality than to safety, they indicate that GHP were adhered to during production, storage, and distribution. As mentioned above, deviations from GHP could lead to safety problems. Atypical product should, therefore, not be accepted without further inquiries or investigational sampling.

15.6.2 Chemical and Physical

A number of quality-related tests, such as moisture level, fat and protein content, solubility, etc., are used in commerce (CAC, 1999). Most of them are unrelated to the safety of the product. Sometimes tests for aflatoxin M, antibiotics, heavy metals, pesticides, etc. are performed to check compliance with legislation. Moisture or water activity tests are normally not necessary to determine whether growth of pathogens might have occurred. When moisture is raised to a_w levels allowing microbial growth, the powder becomes totally unacceptable and would be rejected through normal organoleptic examination.

15.6.3 Microbiological

Microbiological examination often includes a test for aerobic plate counts (APC), coliforms, or Enterobacteriaceae, and sometimes *Salmonella* (see also ICMSF Book 2, (1986)). For APC, case 2 is selected with a three-class plan consisting of $n = 5$, $c = 2$, $m = 3 \times 10^4$, and $M = 3 \times 10^5$. Case 2 is appropriate to this situation in which these counts indicate the normal level of microorganisms found in these products, without direct link to GHP or HACCP.

Table 15-1 Performance of Sampling Plans for *Salmonella* Expressed as the Mean Concentration Detected with 95% Probability Using a 25 g Sample Size and Assuming a Log-Normal Distribution and a Standard Deviation of 0.8

Case	10	11	12
No. of Samples	$n = 5$	$n = 10$	$n = 20$
Mean Concentration	32/1000g (1 cfu/32g)	12/1000g (1 cfu/83g)	5.4/1000g (1 cfu/185g)
Case	13	14	15
No. of Samples	$n = 15$	$n = 30$	$n = 60$
Mean Concentration	7.4/1000g (1 cfu/135g)	3.6/1000g (1 cfu/278g)	1.9/1000g (1 cfu/526g)

Case 5 can be used for coliforms with the following values: $n = 5$, $c = 1$, $m = 10$, and $M = 10^2$. Case 5 relates to indicators of adherence to GHP and HACCP. Lots of milk powder meeting this criterion should not be rejected, unless other findings raise concern.

The following guidance can be used for *Salmonella* testing. Cases 10, 11, and 12 normally apply to foods being tested for *Salmonella* and result in 5, 10, and 20 samples, respectively. The analytic unit from each sample is typically 25 g. For milk powder, case 11 is most appropriate because there may be no change in the level of *Salmonella* between when the powder is tested at the factory or port-of-entry and when the powder is reconstituted for serving. When the powder is intended for a high-risk population, the number of samples can be increased to 15, 30, and 60, with 30 samples being the most appropriate. The performance of these sampling plans can be related to a mean concentration of bacteria that could be detected with a probability of 95% using the approach of Foster (1971) and Legan *et al.* (2001) and assuming a log-normal distribution with a standard deviation of 0.8. The mean concentrations controlled appear in Table 15-1. Thus, for case 11, there would be 95% confidence of detecting *Salmonella* if present at a level of 1 cfu 83 g⁻¹ or greater and if the level of contamination is log normally distributed throughout the lot with a standard deviation of 0.8.

These criteria are applicable when no information is available on the history of the lot or the supplier's control system. For a full discussion on the need for testing and choice of sampling plans, the reader is referred to the previous chapters.

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Listeria monocytogenes in Cooked Sausage (Frankfurters)

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16.1 INTRODUCTION

This chapter considers *Listeria monocytogenes* in cooked sausage (i.e., frankfurters) as an example of a microbial hazard that is capable of growth in a wide variety of perishable ready-to-eat (RTE) foods. The use of performance criteria, process criteria, and validation in relation to the Hazard Analysis Critical Control Point (HACCP) plan is described. In addition, the importance of Good Hygienic Practices (GHP) and an example of a sampling program to assess control of a pathogen in the environment is discussed. In this example, the pathogen of concern is psychrotrophic and can establish itself as a resident and multiply in the refrigerated areas of the food operation, as well as in the refrigerated food. This chapter describes the application of principles introduced in previous chapters. Hypothetical values are used throughout the chapter wherever assumptions were necessary to illustrate a concept or procedure. No attempt has been made to validate their accuracy.

Sausage is one of the oldest forms of processed food and may actually have been the first processed meat product. Thousands of years ago, primitive civilizations began processing meat when they discovered that procedures such as salting, sun drying, and cooking could prolong the edible qualities of meat products, in addition to improving flavor. Almost all races and societies have used this type of food processing method at some point during their development. The term "sausage" is derived from the Latin word *salus*, meaning chopped or minced meat preserved by salting. In 1987, Frankfurt-am-Main, over the protests of Vienna (Wien), which considers itself the originator of the "frankfurter," celebrated the 500th birthday of its claim to being the birthplace of the frankfurter (hot dog). Hundreds of different sausage varieties are available, including cooked, uncooked, smoked, fermented, cured, etc., as well as varieties using combinations of these processes.

Cooked sausages, which include the ever-popular frankfurter as well as bologna and various luncheon meats, are prepared from mixtures of comminuted beef, pork, chicken,

and/or turkey to which salt, sugar, sodium nitrite, and spices are normally added. When making frankfurters, the meat/ingredient mixture, commonly referred to as the sausage emulsion, is stuffed into natural or artificial casings, which are then twisted to form sausage links. Frankfurters are typically cooked to an internal temperature of $\geq 70^{\circ}\text{C}$ to (a) coagulate protein, (b) fix the cured color, and (c) destroy certain pathogens and bacteria that would cause spoilage. Although not an absolute requirement, frankfurters and other similar sausages are commonly subjected to natural smoke. Alternatively, a commercially available liquid smoke solution can be added to the sausage emulsion or atomized onto the surface of frankfurters before or during heating. After cooking, frankfurters are cooled, packaged, and refrigerated for shipment to wholesale and retail markets (Figure 16-1). Skinless frankfurters, which are very popular, are produced in a similar manner, except that the artificial casing is mechanically peeled from the sausage after cooking.

Cooking renders the sausages free of *L. monocytogenes*. However, post-processing contamination can occur and in rare instances has led to illness, such as the 1998–1999 multistate outbreak with 101 confirmed cases, including 15 adult deaths and six miscarriages/stillbirths (CDC, 1999). It should be noted that multiplication of *L. monocytogenes* on frankfurters is probably necessary for disease to occur, but data on the dose-response relationship in humans are not available. This and other aspects of the risk analysis of *L. monocytogenes* in frankfurters are discussed below. Several reports dealing with risk assessment and *L. monocytogenes* have been published and contain additional background information (Farber *et al.*, 1996; Hitchins, 1996; Buchanan *et al.*, 1997; Bemrah *et al.*, 1998; FAO/WHO, 2000; Buchanan & Lindqvist, 2000; Ross *et al.*, 2000; FDA, 2001).

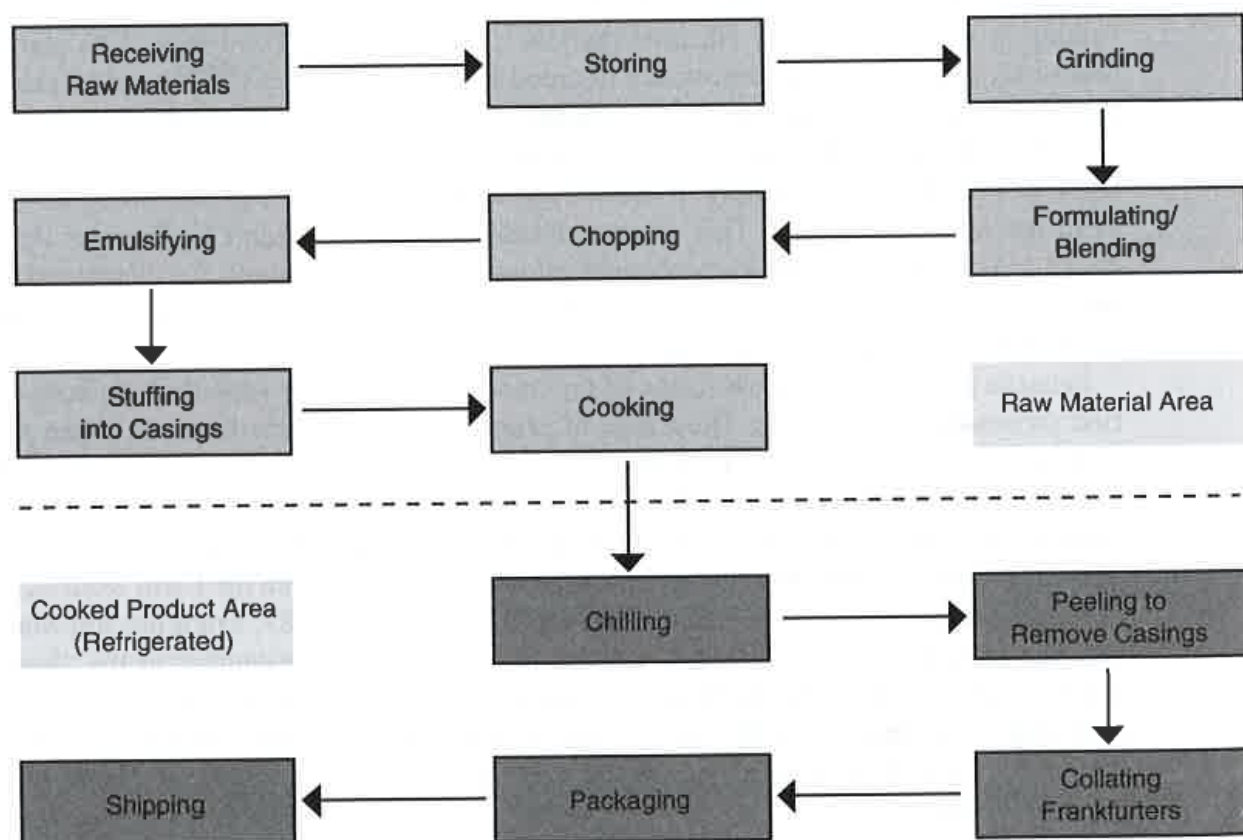


Figure 16-1 Flow diagram for typical frankfurter operation.

16.2 RISK EVALUATION

16.2.1 Hazard Identification

Epidemiologic data suggest that most exposures to *L. monocytogenes* are foodborne (Ciesielski *et al.*, 1988; Broome *et al.*, 1990; Farber & Peterkin, 1991; McLauchlin, 1993; Mead *et al.*, 1999). Although listeriosis occurs infrequently, at somewhere between 2–6 cases annually per million of population, between 20–30% of the cases are fatal (McLauchlin, 1993; Rocourt, 1996; Mead *et al.*, 1999). Thirteen serotypes of *L. monocytogenes* have been identified, but most illnesses have been associated with only three, namely serovars 4b, 1/2a, and 1/2b. Serovar 4b accounts for close to half of all the cases in Europe, while serovars 4b, 1/2a, and 1/2b are more equally represented in North America (Farber & Peterkin, 1991; McLauchlin, 1993; Rocourt, 1996; Rocourt & Bille, 1997). However, the potential for all serovars to cause human illness cannot be discounted.

All *L. monocytogenes* serotypes are considered in this risk evaluation. *L. monocytogenes* is a Gram-positive, non-spore-forming rod. It is facultatively anaerobic, growing at temperatures between -0.4 and 45°C , pH values 4.39–9.4, a water activity of 0.92 or higher, and a salt concentration of 10% or less (ICMSF, 1996). It is catalase positive, oxidase negative and produces β -hemolytic colonies on blood agar. The organism is widely distributed in nature and can be found on fruits and vegetables, in soil, water, sewage, silage, slaughterhouse waste, milk from healthy and mastitic cows, as well as human and animal feces (Farber & Peterkin, 1991, 1999). *L. monocytogenes* can be found as part of the normal flora of many animal species and humans, and it has been estimated that between 2 and 6% of humans and animals are transient carriers of the organism. Fecal excretion of the organism is not necessarily an indication of disease, and the role of healthy carriers in the epidemiology of listeriosis is still unclear (Rocourt, 1996).

L. monocytogenes is inactivated by heat treatments of 70°C or above, used in cooking or pasteurization. However, products, such as frankfurters, that are purchased as a pre-cooked sausage are sometimes eaten without further cooking or may be reheated by a variety of methods (frying pan, grilling, simmering water, microwave). The practice of reheating in a microwave oven was associated with one sporadic case of listeriosis in an elderly woman who had cancer (CDC, 1989). Meat products, such as cooked sausages, can be recontaminated between cooking and packaging in the processing facility. During extended refrigerated storage (e.g., ≥ 35 days), the organism could multiply to potentially hazardous levels on the surface of the frankfurters and in the exudate, especially if the product is temperature-abused. The free exudate in the package has a higher concentration of cells than the product itself and may be a source of cross-contamination in the kitchen.

16.2.2 Hazard Characterization

Human listeriosis is now recognized as a disease primarily caused by ingestion of *L. monocytogenes* in food. This also includes secondary transmission between a mother and her fetus or neonate. Despite its widespread presence in the environment, illness due to this organism occurs infrequently. The outcome of listeriosis can be severe, with an estimated case fatality rate of between 20–30% in the segments of the population most at risk, i.e., immunocompromised individuals, pregnant women, the very young (i.e., fetuses and neonates), and the elderly. This spectrum of individuals is estimated to com-

prise 15–20% of the population (Buchanan *et al.*, 1997) and is expected to increase, reflecting the trend in longer life expectancy and an overall aging population.

Unlike the symptoms caused by many foodborne pathogens, diarrhea and other gastrointestinal symptoms are not common symptoms of listeriosis, although the patient may experience malaise and a mild fever. In several foodborne listeriosis outbreaks, patients have exhibited only these mild symptoms (Salamina *et al.*, 1996; Dalton *et al.*, 1997). However, the majority of reported cases have been the invasive type of listeriosis, symptoms of which include meningitis, encephalitis, and septicemia. In pregnant mothers, unrecognized and untreated listeriosis can lead to abortion of the fetus, stillbirth, or premature delivery of a sick child. Serious sequelae, such as mental retardation and hydrocephalus, also have been reported following cases of foodborne listeriosis (Büla *et al.*, 1995).

There are no experimental dose-response data available for *L. monocytogenes* in humans (i.e., the minimum infectious dose (MID) is uncertain). As with many other foodborne pathogens, the MID will depend on factors such as the virulence of the strain, the amount of food consumed, the levels of the organism in the food, and the immune state of the host. Some animal data are available, mainly using the mouse model (Audurier *et al.*, 1980; Golnazarian *et al.*, 1989; FAO/WHO, 2000), but extrapolation of mouse data to the human situation is tenuous at best. While dose-response studies using human volunteers for certain pathogens have sometimes been conducted, this is not feasible with *L. monocytogenes*, since the risk of severe morbidity or mortality is too great. An alternate approach used with RTE foods is to combine epidemiologic data with estimates of the number of organisms present in the incriminated food. However, because outbreaks are an uncommon occurrence and it is difficult to obtain sufficient material in most cases, the amount of reliable data is scant and the information insufficient to draw reliable conclusions. The type of food consumed, perhaps, may influence the dose-response relationship.

When a risk estimate for *L. monocytogenes* is being made, it is important to determine which type of illness is being considered (i.e., mild versus invasive) and for which part of the population (i.e., “normal” versus “high-risk”). Since frankfurters are consumed by all segments of the population, different risk estimates ideally should be used for normal versus high-risk individuals.

Foodborne listeriosis appears generally to follow one of three scenarios. Scenario 1 consists of isolated cases for which information about the food is seldom available. Scenario 2 consists of an outbreak or cluster of cases involving a single lot of contaminated food. Both of these events typically involve errors in food handling that lead to a single lot of food becoming contaminated and an opportunity for multiplication before the food is consumed. Once the implicated food is eliminated, further cases cease to occur.

Scenario 3 consists of an outbreak involving anywhere from a few cases to several hundred cases scattered by time and location. This type of outbreak typically involves an unusually virulent strain that has become established in the food processing environment and contaminates multiple lots of food over days or months of production. Experience in cooked meat and poultry operations indicates that a niche or site within the cooked product environment wherein *L. monocytogenes* becomes established and multiplies is commonly involved. The sites may be impossible to reach and clean with normal cleaning and sanitizing procedures. In fact, the processing environment typically appears visually clean and acceptable. The sites serve as a reservoir from which the pathogen is dispersed during a food processing operation, contaminating food contact surfaces and, thereby, the

food. The niche usually affects only the product being handled (e.g., filled, sliced, conveyed, assembled, packaged) along one packaging line and not the product on an adjacent line. Microbiological testing is necessary to detect the niche. Examples of niches include hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close fitting layers of metal-to-metal or metal-to-plastic, worn or cracked rubber seals around doors, on-off valves and buttons for equipment, and saturated insulation.

In all three scenarios, there is an opportunity for *L. monocytogenes* to multiply in the food before it is consumed. Food processors should establish systems to prevent scenario 3 events and minimize the risk of scenarios 1 and 2.

16.2.3 Exposure Assessment

L. monocytogenes is found as a frequent contaminant of RTE foods (Farber & Peterkin, 1991, 1999). Many different foods have been involved in both sporadic cases and food-borne outbreaks, with the approximate levels detected ranging from 100 to 1×10^9 cfu g⁻¹ (Table 16-1). The meat industry has a long history of providing safe food, including frankfurters, notwithstanding the recent large listeriosis outbreak (CDC, 1999). Qvist & Liberski (1991) found *L. monocytogenes* to be present in four of 67 (6%) frankfurter samples, while Wang & Muriana (1994) detected the organism in seven of 93 (7.5%) packages of retail frankfurters examined. The US Department of Agriculture (USDA) monitoring program for RTE meats after packaging at the manufacturing facility found the prevalence of *L. monocytogenes* in small-diameter cooked sausages to be 5.3, 4.8, 4.1, 3.7, 3.3, 3.5, and 1.8% annually from 1993 to 1999, respectively.

Frankfurter processing involves a step-heating schedule to allow for the formation of the "skin," for setting of the emulsion, and to allow the curing reaction to occur. This step also reduces *L. monocytogenes* numbers. Zaika *et al.* (1990) prepared frankfurters from a sausage emulsion inoculated to contain around 10^8 cfu g⁻¹ of *L. monocytogenes*. After stuffing, all frankfurters were thermally processed (without smoke) according to a standard commercial heating schedule. Zaika and colleagues found that *L. monocytogenes* decreased approximately 1000-fold in frankfurters heated to an internal temperature of 71.1 °C. Hence, based on those data, cooking frankfurters to an internal temperature of 71.1 °C should eliminate higher levels of *L. monocytogenes* ($\sim 10^3$ cfu g⁻¹) than are likely to occur in raw frankfurter emulsions. In addition, post-process pasteurization can eliminate *L. monocytogenes* from the surface of frankfurters that have been contaminated between cooking and packaging. This procedure is currently practiced commercially by certain manufacturers throughout the world.

The cooking step for frankfurters can be controlled in facilities operating with effectively designed and implemented HACCP plans, but preventing contamination of the cooked product during cooling and packaging is much more difficult. Data gathered by the American Meat Institute in 1988 pointed to frankfurters as a likely carrier of *L. monocytogenes* and suggested that uncontrolled environmental conditions before packaging may play a major role in contaminating the finished product (Anon., 1989). Environmental sampling of a turkey frankfurter plant, whose product was linked to a case of listeriosis, found that contamination of the majority of frankfurters occurred at a single point during the peeling process, just prior to packaging (Wenger *et al.*, 1990). In retrospect, a properly applied environmental sampling program (see Chapter 10) might have detected the site and, if corrected, prevented contamination of the final product.

Table 16-1 Foodborne Outbreaks due to *L. monocytogenes*

Location (year)	No. of Cases (deaths)	Perinatal/nonperinatal	No. cfu g ⁻¹	Foods associated	Serotype	Reference
Boston, USA (1979)	20 (5)*	0/20	NK†	Raw celery, tomatoes, lettuce†	4b	Ho et al., 1986
New Zealand (1980)	29 (9)	22/7	NK	Shellfish, raw fish	most cases 1/2b	Lennon et al., 1984
Maritime Prov., Canada (1981)	41 (17)	34/7	NK	Coleslaw†	4b	Schlech et al., 1983
Massachusetts, USA (1983)	49 (14)	7/42	NK	Pasteurized milk†	4b	Fleming et al., 1985
California, USA (1985)	142 (48)	93/49	10 ³ – 10 ⁴	Queso blanco fresco cheese	4b	Linnan et al., 1988
Switzerland (1983–1987)	122 (31)	65/57	10 ⁴ – 10 ⁶	Raw milk cheese	4b	Bille, 1990
Connecticut, USA (1989)	9 (1)	2/7	NK	Shrimp†	4b	Riedo et al., 1994
United Kingdom (1987–1989)	355 (94) ^s	185/129	< 10 ² – 10 ⁶	Pâté†	4b; 4b(x)	McLauchlin et al., 1991
W. Australia (1990)	11** (6)	11/0	8.8 × 10 ³	Pâté†	1/2a	Kittson, 1992
Tasmania, Australia (1991)	4(0)	0/4	1.6 × 10 ⁷	Smoked mussels	1/2b	Mitchell et al., 1991
New Zealand (1992)	3 (2)	2/1	NK	Smoked mussels	1/2a	Baker & Wilson, 1993; Brett et al., 1998
France (1993)	39 (12)	31/8	< 100 – 1.0 × 10 ⁴	Pork rillettes (pâté)	4b	Goulet, 1995
France (1992)	279 (85)	92/187	NK	Pork tongue in jelly	4b	Goulet et al., 1993

Italy (1993)	23 (0)	0/23	NK	Rice salad*	1/2b	Salamina <i>et al.</i> , 1996
USA (1994)	45 (0)	1/44	2.9×10^{11}	Chocolate milk	1/2b	Dalton <i>et al.</i> , 1997; Proctor <i>et al.</i> , 1995
France (1995)	20 (4)	9/11	NK	Raw milk soft cheese	4b	Goulet <i>et al.</i> , 1995
Sweden (1994–1995)	6 (1) ^{††}	2/4	$< 100 - 2.5 \times 10^6$	Cold-smoked rainbow trout	4b	Ecklow <i>et al.</i> , 1996
Canada (1996)	2 (0)	0/2	2×10^9	Imitation crab meat	1/2b	Farber <i>et al.</i> , 2000
Sweden (NK)	5 (0)	0/5	1.9×10^5	Cold-smoked rainbow trout	1/2a	Miettinen <i>et al.</i> , 1999
Finland (1999)	18 (4)	0/18	$< 100 - 1.1 \times 10^4$	Butter	3a	Lyttikäinen <i>et al.</i> , 1999
USA (1998–1999)	101 (20)	NK	NK	Frankfurters, sliced deli meats	4b	CDC, 1999

* For 2 of these 5 deaths, an underlying disease, not listeriosis was apparently the cause of death.

† Not known.

‡ Foods only epidemiologically linked.

§ Outcome only known for 252 cases.

|| Only 314 patients categorized.

** Includes two sets of twins.

^{††} Not known if death due to listeriosis or leukemia.

Glass & Doyle (1989) reported that this pathogen can proliferate on vacuum-packaged, artificially contaminated (~ 0.01 *L. monocytogenes* cfu g⁻¹) retail frankfurters during storage at 4.4 °C; populations increased by 2–5 log₁₀ on samples that were judged organoleptically acceptable after 4 weeks. In another study, *L. monocytogenes* increased in number from 5×10^2 to 2.1×10^5 MPN g⁻¹ on vacuum-packed frankfurters stored at 4 °C for 20 days. Interestingly, non-inoculated control products contained 1.2×10^2 MPN g⁻¹ after the 20-day storage period, after initially testing negative for the organism (Bunčić, *et al.*, 1991). In a more detailed study of vacuum-packaged all-beef, poultry, or beef/pork frankfurters stored at 5 °C for up to 28 days, McKellar *et al.* (1994) found 40 of 61 (65.6%) frankfurters supported growth of *L. monocytogenes*, with an average increase of 1.26 log₁₀ within a 14-day period. Initial lactic acid bacteria concentrations, as well as phenol and nitrite levels, varied considerably during storage, unlike sodium chloride levels. In addition, average pH levels decreased significantly during storage by 0.19 pH units. Several statistical models were derived in an attempt to describe adequately the growth and death of the organism in all frankfurter samples. Although no one model was found to be sufficient, the best model implicated initial and final lactic acid bacteria counts and initial pH as factors that influence the growth of *L. monocytogenes*.

16.2.4 Risk Characterization

Cooked sausages, of which the frankfurter is but one example, are consumed in large quantities throughout the world. It is estimated that 20 billion frankfurters are consumed each year in the US, for an average of 60 frankfurters per person per year. A retrospective case-control study conducted in 1986–1987 involving 154 listeriosis patients found that sporadic cases were significantly more likely to have eaten either frankfurters that had not been fully reheated or chicken meat that was still pink (Schwartz *et al.*, 1988). It was estimated that 20% of sporadic cases were linked to the two food products. A subsequent case-control study identified as potential causes Mexican-style and feta cheeses, foods purchased from the deli section of grocery stores and, again, undercooked chicken, but not frankfurters (Schuchat *et al.*, 1992).

The population of the US in 1998 was 270 million. From 1996–1999, the estimated incidence of listeriosis from the FoodNet system was 5, 5, 6, and 5 cases per million per year, respectively (CDC, 2000) or about 1,350 cases per year. If it is assumed that 1% (13.5 cases) of the total listeriosis cases might be due to frankfurters and there are 20×10^9 servings of frankfurters per year, then that equals 13.5 cases for every 20×10^9 servings among the total population.

Approximately 20% of the US population (54 million) is immunocompromised. If it is assumed that this high-risk population consumes frankfurters at the same rate as the general population (i.e., about 60 frankfurters per year), then the total number of frankfurters consumed by this subpopulation would be $(54 \times 10^6) \times 60 = 3240 \times 10^6$ or 3.24×10^9 . If it is assumed that listeriosis is restricted to this immunocompromised population and 1% (13.5) of the total listeriosis cases might be due to frankfurters, then there would be 13.5 cases for every 3.24×10^9 servings among the high risk population.

The risk associated with frankfurters, however, is likely to be related to growth of *L. monocytogenes* and handling/preparation practices. Between 1993–1999, the prevalence of *L. monocytogenes* among the lots sampled by USDA-Food Safety and Inspection Service declined from 5.3 to 1.8%. Using a single dose approach (Buchanan *et al.*, 1997), one could assume that all cases of listeriosis are associated with the proportion of frank-

furters having a high level of *L. monocytogenes* (e.g., $\geq 1 \times 10^3$ cfu g⁻¹). Although there are no data for the proportion of frankfurters that may have this level, it will be assumed that 5% contain *L. monocytogenes* but only 10% of those have a high level (i.e., $\geq 1 \times 10^3$ cfu g⁻¹) when purchased. The total number of contaminated frankfurters having a high level of *L. monocytogenes* purchased by the high-risk population then would be $3.24 \times 10^9 \times 0.05 \times 0.10 = 16.2 \times 10^6$.

If it is further assumed that the method of handling and reheating is inadequate to eliminate the high level of *L. monocytogenes* in 10% of the servings, then the frequency of exposure to a high level of *L. monocytogenes* would be $16.2 \times 10^6 \times 0.10 = 1.62 \times 10^6$. If 1% of all listeriosis is associated with eating franks, then the 13.5 cases would more likely result from the estimated 1.62×10^6 franks having a high level of *L. monocytogenes* that are handled and prepared in such a manner that illness can result among susceptible individuals.

Thus, the probability (P) that the high-risk population would acquire listeriosis from frankfurters containing high levels of *L. monocytogenes* would be $P = 13.5$ cases per 1.62×10^6 servings (i.e., approximately one case per 120,000 servings) or $P = 8.3 \times 10^{-6}$.

Since numerous assumptions have been made and reliable data are lacking, it is impossible to validate this estimate or to calculate attendant uncertainties. For example, the estimate assumes that all the strains of *L. monocytogenes* detected on frankfurters are equally virulent, which is unlikely. The estimate does suggest, however, that the risk of acquiring listeriosis from frankfurters on a per serving basis is exceptionally low, even among the high-risk population. Experience indicates that when frankfurters are contaminated with a highly virulent strain, cases may occur among the high-risk population (CDC, 1999).

The draft risk assessment conducted jointly by the US Food and Drug Administration and the USDA estimated the median number of cases of listeriosis per serving of frankfurters to be 3.0×10^{-6} , 5.0×10^{-8} and 5.9×10^{-9} for the perinatal, elderly, and intermediate age-based populations, respectively (FDA, 2001). The perinatal population consisted of fetuses and neonates (16 weeks after fertilization to 30 days after birth) with exposure occurring in utero from contaminated food eaten by the pregnant mother. The elderly group included individuals who were 60 or more years of age. The intermediate age group included the remaining population, including susceptible individuals not included as perinatal or elderly, such as cancer, AIDS, and transplant patients, for whom there was insufficient data for them to be considered as a separate population.

On a per serving basis, *reheated* frankfurters were of low risk relative to 19 other categories of foods studied, ranking 15th out of 20 for the three population groups. Frankfurters consumed *without reheating* were of high risk, ranking first or second among the 20 categories for the three population groups, assuming that from 1–14% of frankfurters are consumed without reheating. Overall, frankfurters ranked number 4 or 5 among the 20 food categories for the three population groups when the median estimates were used to predict the relative risk for listeriosis on a per annual consumption basis, again assuming that 1–14% of frankfurters are consumed without reheating (FDA, 2001).

16.3 RISK MANAGEMENT

16.3.1 Acceptable Level of Consumer Protection

In most industrialized countries, the annual incidence of listeriosis is between 2–6 cases per million of the population (Rocourt & Bille, 1997). For the risk characterization,

an estimate of 1% of the total cases of listeriosis was used for the cases that might be associated with frankfurter consumption, i.e., 13.5 cases. An example of a public health goal could be to reduce the number of cases attributable to the consumption of frankfurters by some value (e.g., 50% reduction to no greater than 6.75 cases per million of the population).

16.3.2 Establishing a Food Safety Objective

Due to its widespread presence in the environment, eradication of *L. monocytogenes* from the food supply is impossible. There is general agreement that when the organism is ingested in low numbers, even susceptible individuals have little chance of developing listeriosis. A realistic food safety objective (FSO) must therefore be established that would, as far as possible, recognize that it is not possible with current technology to eliminate contamination of foods with *L. monocytogenes*. When total prevention is not possible, measures must be put into place to control recontamination to an acceptable level. To achieve these goals, it is essential that GHP and HACCP programs that are specific to the control of *L. monocytogenes* be applied at all stages of manufacture, storage, transport, and retail. In addition, research should continue to develop additional barriers to control the growth of *L. monocytogenes* in meat and poultry products. This might include the addition of food additives and/or the use of a competitive flora (McMullen & Stiles, 1996).

It is currently recognized that on a daily basis, humans are consuming *L. monocytogenes* in foods at levels of at least 100 cfu g⁻¹ and not becoming ill. For example, data from Denmark show an overall contamination rate for cold-smoked salmon products of around 25–32%, with 4–5% of those samples containing greater than 100 cfu g⁻¹ (Huss, 1997). Even with the large amounts of Danish cold-smoked salmon being consumed worldwide, there have been no recorded cases of listeriosis due to this product. In addition, countries such as Canada have established an action level for *L. monocytogenes* of 100 cfu g⁻¹ for low risk products and have not seen an increase in the baseline levels of human listeriosis. This is also consistent with the estimated dose-response curve for *L. monocytogenes* from Buchanan *et al.* (1997), which includes an estimate for immunocompromised individuals, is reasonably conservative, and allows for the possibility of a single cell causing serious illness (Figure 16–2). Finally, epidemiologic data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have contained levels well in excess of 100 cfu g⁻¹ (see Table 16–1). Thus, on the basis of epidemiologic and prevalence data, the following FSO is proposed:

The concentration of *L. monocytogenes* in frankfurters should not exceed 100 cfu g⁻¹ at the time of consumption.

This proposal is consistent with an earlier recommendation from the International Commission on Microbiological Specifications for Foods (ICMSF) (ICMSF, 1994). It also is compatible with a conclusion of the World Health Organization (WHO)/Food and Agriculture Organization (FAO) risk assessment for *L. monocytogenes* in RTE foods that a more strict tolerance of “not detected in 25 g” does not provide a higher level of protection (Ross *et al.*, 2000). Indeed, the incidence rate for listeriosis in the US, which has maintained the “negative in 25 g” tolerance, is not lower than other industrialized countries that have applied a tolerance of 100 cfu g⁻¹.

Probability of infection (%)

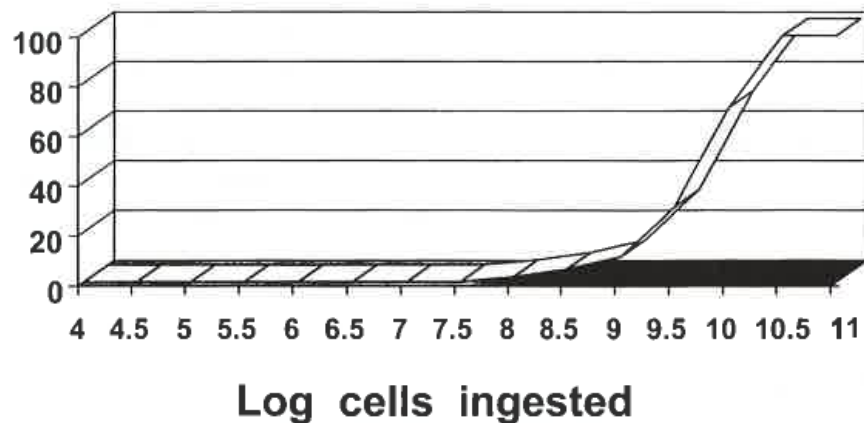


Figure 16-2 Estimated dose response curve for *Listeria monocytogenes*.

16.3.3 Control Measures

It is anticipated that industry can easily meet the proposed FSO for the product at the time of manufacture. However, if frankfurters are recontaminated after the commercial cooking, by the time they are consumed, there is potential for concentrations to exceed 100 g^{-1} , especially with a long refrigerated shelf-life, no additional barriers to growth, and/or temperature abuse. To reduce the incidence of listeriosis (e.g., to fewer than 5 cases per million population) by specifically reducing the number of cases due to frankfurters (e.g., from 13.5 to no greater than 6.75 cases), then other risk management options need be considered.

A flow diagram for a typical frankfurter operation is shown in Figure 16-1. HACCP systems and GHP procedures must be used to achieve the expected level of control and meet the FSO. It is important to consider available control measures in the context of the steps used in manufacture. In this example, controlling *L. monocytogenes* so the concentration will not exceed 100 cfu g^{-1} when frankfurters are consumed will involve combinations of the following control measures:

- *Controlling initial levels in raw materials*—Handling raw materials during storage and preparation to minimize an increase in numbers due to contamination or growth.
- *Reducing levels during cooking of frankfurters in the manufacturing process*—Cooking to eliminate *L. monocytogenes* in the raw meat emulsion. Establishing a performance criterion for cooking that results in process criteria (i.e., critical limits) that can be incorporated into the HACCP plan.
- *Preventing recontamination between cooking and packaging*—Minimizing recontamination between cooking and packaging by adopting GHP measures, e.g., separating raw from cooked product, and an environmental management and monitoring program.
- *Reducing levels in cooked product after packaging (in-pack pasteurization)*—Applying commercially feasible pasteurization processes for pasteurizing the product after packaging.
- *Preventing increase in levels between packaging and preparation for serving*—Controlling the increase of *L. monocytogenes* that subsequently may occur with

- recontamination and growth in packaged frankfurters during storage and distribution. Examples of control measures might include: adding safe, acceptable additives to control growth of *L. monocytogenes* on frankfurters, using code-dating practices and improved chill chain management to preclude an unacceptable increase in *L. monocytogenes*, or freezing the product.
- Reducing levels prior to consumption*—Improving storage and handling practices and inactivating *L. monocytogenes* through effective cooking and reheating prior to consumption. These outcomes could be achieved as control measures in food service operations or through education of consumers, particularly the more susceptible population and their health care providers.

16.3.4 Performance Criteria

A performance criterion is the required outcome of one or more control measures at a step or combination of steps applied to achieve an FSO (see Chapter 3). Performance criteria are usually applied at steps where hazards can either be reduced or where hazards may increase.

To arrive at a performance criterion for the control measures needed to meet an FSO for frankfurters, the equation from chapter 3 can be used:

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

$$H_0 - \Sigma R + \Sigma I \leq 2.0$$

where:

FSO = Food safety objective

H_0 = Initial level of the hazard

ΣR = Total (cumulative) reduction of the hazard from processing, etc.

ΣI = Total (cumulative) increase of the hazard

FSO, H_0 , R, and I are expressed in $\log_{10}$ units.

Manufacturers of frankfurters should assume that *L. monocytogenes* will be present in all species and types of raw meat and poultry. This is evident from reports in the literature and results from surveys such as the USDA baseline studies for ground beef, ground turkey, and ground chicken (Table 16-2) (USDA, 1995a; USDA, 1995b; USDA, 1996). Data of this nature can be used to establish the initial concentration (H_0). Another option would be to generate similar data from raw emulsions collected at the facility prior to cooking.

The USDA baseline studies reported the prevalence of *L. monocytogenes* in ground beef, chicken, and turkey to be in the range of 18–35% when analyzing 25 g samples. Further analysis of the positive samples yielded low numbers of *L. monocytogenes*. It can be concluded from the data that concentrations of 100 g^{-1} or fewer would be expected under normal conditions. Further reducing the likelihood of higher concentrations is the effect of blending and emulsification that occurs in the manufacturing process. These steps disperse the cells more uniformly throughout the meat emulsion and result in an overall lower number g^{-1} with little likelihood of localized pockets of higher concentrations.

16.3.4.1 Controlling Initial Levels in Raw Materials

Consideration should be given to multiplication of *L. monocytogenes* that might occur as raw meat and poultry is stored and prepared for cooking. It is important to note that

Table 16-2 USDA Baseline Survey Results for Ground Beef, Ground Turkey, and Ground Chicken

	Ground beef (563 samples)	Ground turkey (165 samples)	Ground chicken (162 samples)
Prevalence based on 25 g samples	18%	23%	35%
Geometric mean for positive samples only	3.9/g	3.8/g	2.24/g
95% confidence level	2.2–7.2/g	0.4–2.9/g	1.06–4.7/g

Samples found positive by the qualitative method were further analyzed to determine the number of *L. monocytogenes* g⁻¹ of ground meat. The results for the positive samples were as follows:

Number of <i>L. monocytogenes</i> /g	Ground beef (99 samples)	Ground turkey (52 samples)	Ground chicken (59 samples)
< 0.03	45.2	82.5	56.1
0.03–0.29	0.0	0.0	0.0
0.3–2.9	30.2	6.7	29.3
3.0–29.9	15.0	0.0	10.5
30–299.9	9.6	10.9	3.2
300 or higher	3 samples had > 110/g	0.0	0.9

Note: These data indicate, for example, that in the case of ground beef, 563 samples were analyzed using 25 g each. Only 99 samples were found to contain *L. monocytogenes*. Upon further analysis of the 99 samples, 90.4% had fewer than 30 cfu gram⁻¹. From an overall perspective, only 3 of the total 563 samples had greater than 110 *L. monocytogenes* gram⁻¹, the upper limit of detection used for testing.

In the case of ground turkey, 165 samples were analyzed using 25 g each. Only 52 were found to contain *L. monocytogenes*. Upon further analysis of the 52 samples, 89.2% had fewer than 30 cfu gram⁻¹. From an overall perspective, the highest number detected among the 165 samples was a sample containing 93 cells gram⁻¹.

the samples for the baseline studies were collected, packaged, and shipped in refrigerated containers to the laboratory. Samples were considered acceptable for analysis if they were received at the laboratory by the day after collection and with a temperature of 10 °C or less. Hence, the samples were at least one day older than when collected and some temperature abuse may have occurred.

To further place the possibility of multiplication during preparation into perspective, the data in Table 16-3, derived from the USDA Pathogen Modeling Program, indicate that from 4–9 days at 4 °C would be required for *L. monocytogenes* to increase 10-fold in meat or poultry with no added salt (0.5%) (Buchanan & Whiting, 1996). Food MicroModel (Ver 3.02) similarly predicts that it would take around 6 days for a 10-fold increase of *L. monocytogenes* (4 °C, 0.5% NaCl, pH 6.5). Longer times would be required in raw materials containing added salt. For example, the mechanically deboned poultry meat used for manufacturing frankfurters typically contains added salt and sodium nitrite and is chilled to well below 4 °C before shipping to the frankfurter manufacturing plant. The values in Table 16-3 include the lag phase estimated for each set of parameters. The length of the lag phase that actually occurs in raw meat under commer-

Table 16-3 Days for a 1 Log₁₀ (10-Fold) Increase in *L. monocytogenes* at 4 °C and pH 6.0

Sodium chloride (%)	Days for 10-fold increase			
	Aerobic conditions (e.g., meat at top of container)		Anaerobic conditions (e.g., meat below surface of container)	
	No NaNO ₂ Added	150 µg g ⁻¹ NaNO ₂ added	No NaNO ₂ Added	150 µg g ⁻¹ NaNO ₂ added
0.5	5.3	8.9	4.1	8.8
1.0	5.6	9.4	4.5	9.6
1.5	6.0	10.0	4.9	10.5
2.0	6.4	10.6	5.3	11.5
2.5	6.8	11.4	5.8	12.4
3.0	7.3	12.3	6.2	13.4

cial conditions has not been determined, but the raw meat and/or poultry used for the manufacture of frankfurters is normally less than 7 days from the date of slaughter.

From the data in Table 16-2 it is evident that the numbers of *L. monocytogenes* are very low in raw meat and poultry. Furthermore, the data in Table 16-3 indicate that significant multiplication during preparation is unlikely. For this example, it will be assumed that the initial concentration may under certain circumstances increase 10-fold prior to commercial cooking. Thus, in this example, the initial number in the raw materials prior to cooking is assumed to be no more than 1000 g⁻¹ (i.e., H₀ = 3).

16.3.4.2 Reducing Levels during Cooking of Frankfurters in the Manufacturing Process

If it is assumed that initial numbers of *L. monocytogenes* in the raw materials could be as high as 1000 g⁻¹ (i.e., H₀ = 3), then a performance criterion for the reduction step (i.e., cooking) to meet the FSO would, theoretically, only need to be a 1 log₁₀ reduction as given below and illustrated in Figure 16-3a.

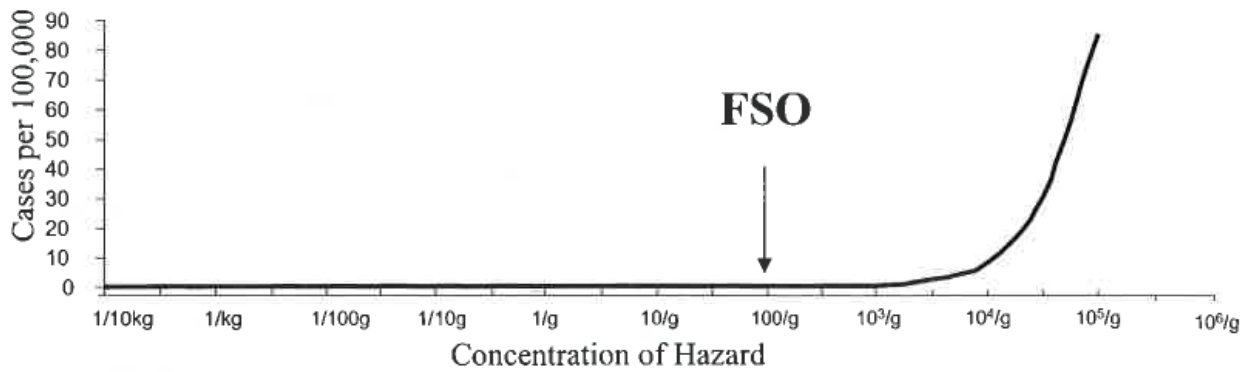
$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

$$3 - \Sigma R + 0 \leq 2.0$$

$$\Sigma R = 1$$

However, if any viable cells remain after cooking, by the time the frankfurters are consumed, there is the potential for concentrations to exceed 100 g⁻¹, especially with a long refrigerated shelf-life, no additional barriers to growth, and/or if temperature abuse occurs. Therefore, to ensure that survivors do not remain and multiply during distribution and storage, it is necessary to apply a more stringent cooking process. For this example, it will be assumed that a 6 log₁₀ reduction (R = 6) during cooking will achieve the desired result, given an initial concentration of no greater than 1000 g⁻¹ (H₀ = 3). Thus, a 6 log₁₀ reduction would result in a final concentration of ≤ 1 cfu kg⁻¹ after cooking. This could be expressed as a performance criterion:

The concentration of *L. monocytogenes* after cooking shall be ≤ 1 cfu kg⁻¹.



1) FSO could be met with only 1 $\log_{10}$ reduction:

$$\begin{array}{c} \leftarrow H_0 \\ \Sigma R = 1 \end{array}$$

2) Cooking during manufacture (6 $\log_{10}$ reduction, $\Sigma R = 6$) is applied to reduce levels to meet a performance criterion of $\leq 1 \text{ kg}^{-1}$ ($\leq 10^{-3}$ per g)

$$\begin{array}{c} \leftarrow H_0 \\ \Sigma R = 6 \end{array}$$

Figure 16-3a Control measure: cooking.

This is demonstrated in the following equation and illustrated in Figure 16-3a.

$$H_0 - \Sigma R + \Sigma I \leq 1/\text{kg} (\leq 10^{-3}/\text{g})$$

$$3 - \Sigma R + 0 \leq -3$$

$$\Sigma R = 6$$

If the initial number is less than 1000 g^{-1} through raw material selection and/or preventing any increase during handling and storage (e.g., $\leq 100 \text{ g}^{-1}$), then a 5 $\log_{10}$ reduction would be adequate to meet the performance criterion of $\leq 1 \text{ kg}^{-1}$.

16.3.4.3 Prevent Recontamination Following Cooking

L. monocytogenes is a very hardy organism and can be recovered from different locations in the plant environment, depending on the level of control. In the absence of an effective control program, the organism can persist for prolonged periods in frankfurter production environments. During a 1989 investigation, the same strain of *L. monocytogenes* was recovered from a patient, from leftover frankfurters in the patient's refrigerator, and 4 months later from the frankfurter peeler in the manufacturing facility (Wenger *et al.*, 1990). In another outbreak in the US associated with frankfurters, it was reported that removing a refrigeration unit resulted in new or increased contamination of production equipment (CDC, 1999). This outbreak involved cases from August 2, 1998 through January 16, 1999, suggesting that multiple lots of product were implicated. Attempts to verify that the strain had become established in the environment, however, were unsuccessful. Thus, even if process criteria are met for cooking, recontamination of processed

product is a possibility unless proper attention has been paid to the GHPs that are targeted toward control of *L. monocytogenes*.

Certain measures that fall within the realm of GHP have been found to be important for control of *L. monocytogenes* in cooked meat and poultry operations. The measures include plant layout, equipment design, maintaining equipment, cleaning and sanitizing procedures that are specific to listeriae control, maintaining a clean and dry environment, use of enclosed steam to sanitize equipment as a scheduled routine procedure, and low temperature storage (Tompkin *et al.*, 1999).

Two factors determine the effectiveness of a listeriae control program: routine environmental testing and the response to a positive finding. Without an environmental testing program, it is not possible to assess control. Furthermore, in the event a positive product contact sample is detected, corrective actions should be initiated to eliminate the source of contamination, thereby minimizing the risk of product contamination. To verify control, plants should implement an environmental monitoring program for *L. monocytogenes* or an indicator, such as *L. innocua* or *Listeria spp.* The program must be specific to each plant and should detail the areas to be sampled, the analytic method to be used, the frequency of sampling, and the action to be taken when *Listeria spp.* are detected (Tompkin *et al.*, 1992, 1999).

An effective monitoring program to assess control of the cooked product environment should consider the following strategies:

- preventing the establishment of *L. monocytogenes* in niches or other sites that can lead to contamination of RTE foods,
- implementing a sampling program that can assess in a timely manner whether the environment in which RTE foods are produced is under control,
- responding to each positive product contact surface sample as rapidly and as effectively as possible, and
- verifying that the problem has been corrected and providing data (e.g., tabulated, trends in graphical form) to facilitate short- and long-term assessment of control.

Experience in frankfurter operations indicates that sites within the cooked product environment wherein *L. monocytogenes* becomes established and multiplies (i.e., niches) are important contributing factors to product contamination. The sites serve as a reservoir from which the pathogen is dispersed and contaminates product contact surfaces. Furthermore, an environmental sampling program, such as described in Chapter 11, is necessary to assess whether the environment is under control and to detect the presence of a niche.

Response to a positive product contact sample (e.g., a sponge sample collected from a conveyor belt used in a frankfurter operation) is best when the following items are considered. Assuming an effective control program is in place, the primary source of *L. monocytogenes* in frankfurter operations is contamination from one or more niches. In general, contamination flows downstream with the process flow, much like a river. To regain control, it is necessary to locate the source where growth and contamination are occurring. This can best be accomplished by creating a map of the cooked product rooms and the equipment layout (see Chapter 11). The results of samples collected from each piece of equipment should be recorded on the map, including both positive and negative results. The layout map should then be reviewed for patterns. Which sites are more frequently positive? Where in the process flow do the first positives occur? It is important when seeking the source to analyze all the sponge samples separately and not as com-

posites. In addition, sampling should be aggressive both in terms of location and frequency throughout the period of operation. Unfortunately, niches can rarely be detected unless the equipment is operating and product is being produced. While pursuing the source of contamination, consideration must be given to the possibility that a niche may not be involved (contamination could result, e.g., from someone touching the floor or other unclean surface and returning to handle exposed product).

When equipment has been identified as the source, the following steps can be effective. First, dismantle the equipment, collecting samples of suspicious material as this is being done. Second, replace obvious defective parts (e.g., hollow rollers) and clean and sanitize the equipment as it is being reassembled. If this procedure fails to eliminate the cause (or source) of contamination, it may be necessary to remove sensitive electronics, oil, and grease, and heat the equipment. This can be accomplished by placing the equipment in an oven and heating to an internal temperature of 71 °C with high humidity. Alternatively, the equipment can be shrouded with a tarp and steam can be injected into the space until the equipment reaches an internal temperature of 71 °C. Internal temperatures can be monitored with strategically placed thermocouples.

In frankfurter operations the following are examples of identified sources of contamination:

- rubber gaskets around the doors and other openings to the brine chill system
- saturated insulation on pipes carrying refrigerant to the brine chill system
- peelers that remove artificial casings before packaging
- casing removal systems
- hollow rollers on conveyors
- on/off valves and buttons for various equipment
- within complex collating equipment
- hollow support rods on the frames of equipment.

It is difficult to assign a performance criterion to recontamination, as any recontamination has the potential to reach high numbers through growth during the subsequent distribution and storage.

To ensure that the performance criterion of $\leq 1 \text{ kg}^{-1}$ (i.e., $\leq 10^{-3}$ per g) is not exceeded due to recontamination, there must be no recontamination and "I" in the following equation must be zero. This is illustrated in Figure 16-3b.

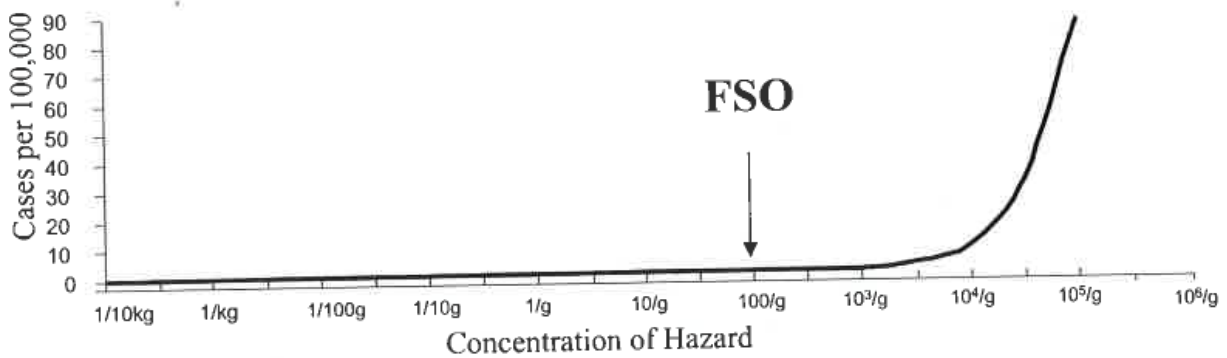
$$H_0 - \Sigma R + \Sigma I \leq 1 \text{ kg}^{-1} (\leq 10^{-3} \text{ per g})$$

$$3 - 6 + \Sigma I \leq -3$$

$$\Sigma I = 0$$

16.3.4.4 Reducing Levels in Cooked Product after Packaging (In-Package Pasteurization)

Experience indicates that recontamination after cooking is the most common reason for the presence of *L. monocytogenes* in packaged, cooked sausages, such as frankfurters. If the increase in concentration due to recontamination is assumed to be as high as 10 g^{-1} , then an in-package pasteurization treatment could be applied as a means to achieve a 4 log reduction and still meet the performance criterion of $\leq 1 \text{ kg}^{-1}$ (i.e., 10^{-3} per g). As demonstrated in the following equation, if cooking provides an initial 6 log reduction and recontamination results in 10 cfu g^{-1} , then an in-package pasteurization treatment could be used to provide a final 4 log reduction and meet the performance criterion of $\leq 1/\text{kg}$.



Preventing recontamination between cooking and packaging through effective GHPs and an environmental sampling program

$$H_0 - \sum R + \sum I \leq \text{Performance criterion (1 kg}^{-1} \text{ or } \leq 10^{-3} \text{ per g)}$$

$$3 - 6 + \sum I \leq -3$$

$$\sum I \leq 0$$

Figure 16-3b Control measure: preventing recontamination.

Hence, the combined reduction steps for cooking and in-package pasteurization result in a 10 log reduction (i.e., $\sum R = 10$). This is illustrated in Figure 16-3c.

$$H_0 - \sum R + \sum I \leq 1 \text{ kg}^{-1} (\leq 10^{-3} \text{ per g})$$

$$3 - \sum R + 4 \leq -3$$

$$\sum R = 10 \text{ (Cooking 6D, In-pack pasteurization 4D)}$$

16.3.4.5 Preventing Increase in Levels between Packaging and Preparation for Serving

In this and the next example, an in-package pasteurization treatment is not applied. If the increase in concentration due to recontamination is again assumed to be as high as 10 g⁻¹ (i.e., a 4 log₁₀ increase from 1 kg⁻¹) and if the FSO of 100 g⁻¹ at time of consumption is to be met, there can be no greater than a 10-fold increase during storage and distribution before the food is consumed (see also Figure 16-3d).

$$H_0 - \sum R + \sum I \leq \text{FSO}$$

$$3 - 6 + 4 \leq 2$$

$$\sum I \leq 1$$

If recontamination occurs, then an increase of 10-fold or more must be controlled by adding inhibitory ingredients, freezing the product for distribution, and/or establishing use by date coding that are within the time for a 10-fold increase.