Foreword

These *Salmonella* Control Guidelines were prepared as an educational document for American Feed Industry Association (AFIA) member companies to provide suggested practices to minimize contamination of animal feed, ingredients and pet food products with the subject organism. It is the responsibility of all manufacturers of animal feed, ingredients and pet food to take the necessary steps to provide *Salmonella*-negative product. Thus, these guidelines were written to promote *Salmonella* control at all animal feed, ingredient and pet food facilities. It is expected that these practices will also help reduce other bacteria populations of concern.

The guidelines are intended to update and replace a comparable set of recommendations prepared by AFIA in 1990, entitled “Recommended *Salmonella* Control Guidelines for Processors of Livestock and Poultry Feeds” which replaced the 1970 recommendations by the U.S. Department of Agriculture (USDA) in cooperation with a group of three associations, which included the AFIA.

The original USDA recommendation, “Recommended Sanitation Guidelines for Processors of Livestock and Poultry Feeds,” was the third in a series of publications. “Recommended Sanitation Guidelines for Processors of Poultry and Animal Byproducts,” and in “The Processing of Fish Products,” were similarly developed by USDA in 1964 and 1965, respectively.

Control of *Salmonella* in feeds, ingredients and pet foods has been a long-standing objective of conscientious producers of ingredients and finished feeds and foods. That effort was materially assisted in both direction and effect with publication of the original guidelines. It is hoped that this third generation of guidelines for feed, ingredient and pet food processors will provide additional assistance in a continuing effort directed at controlling *Salmonella* utilizing the latest scientific principles and data.

Personnel at each facility should determine if and how any or all of these suggested practices may apply to their operation. Some suggestions may not be practical or applicable for a particular facility. These suggestions should be considered in the redesign of current facilities or the design of new facilities.

This document is intended for industry guidance only, and it provides recommendations on how best to effectively control *Salmonella* in feed, feed ingredients and pet food.

Comments and suggestions are welcome. Correspondence should be sent to the American Feed Industry Association, 2101 Wilson Blvd., Suite 916, Arlington, VA 22201, afia@afia.org.
Introduction

The old adage that understanding the problem is half the solution is applicable to control of *Salmonella* in animal food. Equally applicable is the general principle that a subject should first be defined before understanding can commence.

*Salmonella* is the general name of a form of bacteria originally isolated in 1885 by Dr. A.E. Salmon, after whom it is named. *Salmonella* are rod-shaped, gram-negative one-celled organisms that cannot be seen, smelled or tasted. In the *Salmonella enterica* group, there are more than 2,400 different serotypes or strains of the bacteria. *Salmonella* are ubiquitous in nature, being found just about anywhere one may look. They are common to the general environment. Of specific interest is the fact that they may be found on many raw foods, including those of oilseed and animal origin, on animals and humans, and in their gastro-intestinal tracts. Since ingestion of *Salmonella* may trigger illness generally referred to as salmonellosis, control procedures are called for to minimize the possibility of animal feed, ingredient and/or pet food contamination. Fortunately, the application of common sense to ingredient procurement, feed, ingredient and pet food processing and distribution, plus good housekeeping, can be most helpful in precluding problems associated with contamination.

In recent times, there has been an evolution in the use of nomenclature regarding the *Salmonella* genus. The common nomenclature is to use the genus *Salmonella* (in italics) and the serotype (ex. Enteritidis), which is understood to be *Salmonella enterica* (species) enterica (subspecies) Enteritidis (serotype). Thus, the serotypes are commonly stated as *Salmonella* Enteritidis or *S. Enteritidis.*

The term “control” is used because of the nature of *Salmonella* and its widespread presence simply precludes elimination. This fact was recognized at a 1984 International Symposium on *Salmonella,* where a group of world-renowned scientists stated that …“the eradication of *Salmonella* in domestic animals is not attainable at this time ….” Humphrey (2004) observed, “Given its ubiquity, it is unlikely that *Salmonella* will be eradicated from the food chain.”

While eradication may not be possible, control is possible and should be exercised as a means of reducing or precluding contamination. These guidelines may be used to aid the efforts directed at achieving control of *Salmonella.* The goal of feed, ingredient and pet food processors should be to significantly reduce the incidence of *Salmonella* in all aspects of production.
Salmonella contamination will not normally be uniform within a lot of ingredients or mixed feed or pet food. Hence, an otherwise representative sample of the feed material may not actually be representative regarding the absence of Salmonella. This being the case, analytical results showing no Salmonella present are described as “Salmonella negative, as tested” rather than “Salmonella free, as tested.” By contrast, positive analytical results of properly secured and analyzed samples do indicate contamination. This important distinction should be kept in mind when interpreting analytical results.

Domestic and foreign studies have reported the contamination of feed ingredients and manufactured feeds with Salmonella organisms. Ingredients may be contaminated with Salmonella prior to being received at the processing facility. Such an ingredient may contaminate a relatively large quantity of mixed feed or pet food ingredient mix into which it is incorporated. Additionally, a single ingredient can contaminate processing equipment and the general facility environment. Contaminated equipment, organic material build-up, and dust may, in turn, cause contamination of subsequent batches of feed. These guidelines are designed not only for feed and pet food facilities, but for ingredient suppliers as well.

Heat and chemical processing, including pelleting, extruding, rendering, baking and use of some chemical control compounds are currently known to be effective processing procedures normally performed by feed, ingredient or pet food processors that can significantly reduce Salmonella. Heat, length of heating time, moisture level of the product and pressure applied may be sufficient to kill the organisms. However if there is an extremely high level of Salmonella contamination in the feed, ingredient or pet food, or the products are pelleted or extruded at a relatively low temperature, complete destruction would be unlikely. Technology advances will likely provide additional equivalent Salmonella reduction procedures that may be used in the future. This will be especially relevant for non-heat treated, meal-form animal feeds and ingredients.

This publication presents recommended guidelines for all manufacturers of feeds, ingredients or pet foods and outlines sound management and operating procedures designed to aid in significantly reducing the incidence of Salmonella in finished products. Emphasis is placed on selective purchasing with a documented supplier management program, Good Manufacturing Practices (GMPs), Hazard Analysis and Critical Control Points (HACCP), prerequisite programs, and robust continuous improvement activities.

In general, these control principles are applicable to the wide variety of facilities which range from relatively simple farm-type to large, sophisticated commercial operations. The specific application will vary with the physical facilities and business of the individual firm. Salmonella mitigation programs based only on testing finished products cannot provide adequate assurance of safe manufacturing and distribution. Therefore, these guidelines represent a comprehensive approach to Salmonella control, encompassing a number of opportunities to reduce the likelihood
of *Salmonella* contamination. Sero-typing of *Salmonella* and other *Enterobacteriaceae* by Pulsed Field Gel Electrophoresis (PFGE) will assist in trace-back and control steps.

To secure maximum benefit from these guidelines, employees must be trained in appropriate plant sanitation practices and GMPs, and alerted to the need for strict adherence to prescribed operating procedures. Key personnel should be trained as security or sanitation officers to ensure all aspects of the guidelines are consistently and effectively utilized.

Representative laboratory samples should be used to monitor the effectiveness of the program. If laboratory testing of incoming ingredients or finished products discloses *Salmonella* contamination, appropriate measures must be taken.

In addition to the specific best practices discussed, it is important to have appropriate documentation practices in place such that plant procedures, corrective actions, monitoring and verifying documentation, testing records, etc., are readily available to plant personnel and for review.

This guide is not a substitution for daily due diligence and adherence to plant sanitation practices. Instead, it offers some options for reducing the likelihood of *Salmonella* contamination in plants and subsequent products. The guide does not propose to encompass all plant type of operations. It only offers principles and guidelines for all plants to use in accomplishing the goal of *Salmonella* reduction.
Table of Contents

Content                                                                 Page
Foreword.................................................................................................................................1
Introduction...............................................................................................................................2
Raw Materials Purchasing Practices.....................................................................................6
Ingredient Shipping/Receiving ..............................................................................................6
Physical Facilities ..................................................................................................................7
Plant Employees and Visitors ...............................................................................................9
Plant Procedures and Policies ...............................................................................................10
  Sanitation and Cleaning ........................................................................................................10
  Pest Control ..........................................................................................................................10
  Materials Handling ...............................................................................................................11
  Dust Control and Air Flow ....................................................................................................11
  Moisture Control .................................................................................................................12
  Miscellaneous Controls .......................................................................................................12
Equipment Maintenance and Operation .................................................................................13
Packaging, Storage and Transportation ................................................................................13
Control Procedures ................................................................................................................14
  Process Control – Pelleting and Extrusion .........................................................................14
  Optional Treatments .............................................................................................................15
  Decontamination ................................................................................................................16
Sampling and Analysis ............................................................................................................18
  Procedures for Sampling Aseptically .................................................................................18
  Criteria for Laboratory Selection .......................................................................................21
  Laboratory Methods ............................................................................................................22
  Environmental Monitoring ..................................................................................................22
Notes .........................................................................................................................................23
Summary and Conclusions .......................................................................................................24
Literature Cited and References ............................................................................................24
Acknowledgements .................................................................................................................25
Guidelines for *Salmonella* Control

**Raw Materials Purchasing Practices**

It is difficult to produce *Salmonella*-negative feeds without attention to raw materials. While the process is arduous, establishing a formal supplier evaluation and approval process is the most effective approach toward reducing or minimizing the risk of *Salmonella* from ingredients.

Establishing a formal supplier-approval process should include third-party audits of the manufacturing facilities involved and/or vendor audits. In addition, first-hand facility visits and conferences with supplier-management personnel should be accomplished prior to establishing written ingredient specifications. Companies should ascertain whether or not the suppliers of these ingredients are following appropriate guidelines and practices for their industry.

In addition to conveying expected results (*Salmonella*-negative), written supplier management programs, including signed and approved ingredient specifications, should be specific to be effective and should address the following issues:

- Raw materials procurement (avoiding risky, dangerous or off-quality materials)
- Manufacturing procedures, GMPs, HACCP and/or other food safety programs
- Documentation of ingredient production process control

**Transportation expectations including the following:**

- Vehicle maintenance
- Vehicle sanitation
- Vehicle use (i.e. what materials might be allowed or are specifically prohibited on back-hauls)

**Ingredient Shipping/Receiving**

Every ingredient load arriving at the facility should be subjected to arrival inspections and include the following:

- Inspection of documentation, invoices and seals
- Assessment of transport vehicles with respect to maintenance, sanitation and cleanliness
- Verification of ingredient identity
- Verification that ingredient quality specifications are met (including Certificate of Analysis)

Transport ingredients in vehicles that are in good condition and provide good protection. Keep unloading areas clean.

Visually inspect all incoming ingredients for evidence of water damage, contamination and normal aroma.
• Inspection of product for quality indicators, including the following:
  o Visible evidence of water damage
  o Visible fecal or pest contamination
  o Temperature check, if applicable
  o Normal aroma for that ingredient
  o Specific raw material analytical acceptance criteria - protein, moisture, etc.
• Criteria for rejecting loads
• Procedures for dealing with Salmonella-positive results
• Criteria for termination of the relationship
• Certificate of Analysis requirements

Ingredient loads that do not meet company ingredient specifications should be rejected. For receiving, loads deemed to be within company ingredient specifications should be sampled using the procedures described in the sampling section below. Samples of every ingredient load should be retained until finished products have been in commerce for a period of at least 3 months past the expiration date of the finished product. Any analytical results from samples should be compared with ingredient suppliers’ data on a regular basis and supplier/purchasing profiles should be maintained. Supplier non-conformances should be tracked and communicated for continuous improvement.

Once established, manufacturers should actively verify compliance to specification and document compliance verification activities. In addition, high-risk supplier manufacturing facilities should be assessed on an annual basis to ensure Salmonella control measures are being maintained.

**Physical Facilities**

Many aspects of the physical facilities are already determined upon completion of facility, and reconstruction activities or construction of new facilities may be cost prohibitive. However, it is important to understand and minimize the facilities’ impact on contamination and feed quality. Therefore the following guidelines should be utilized:

• Facilities should be located on a site that is suitably drained, i.e. no water pooling.
• Access roadways should be properly graded, compacted and well drained.
• Facilities should be designed to a capacity adequate to produce the volume of product required.
• Facilities should have adequate ingredient storage capacity, so the facility can reject an ingredient load if necessary.
The lay-out, design, construction and size of the facilities and equipment should permit adequate cleaning and/or disinfection.

Facilities and equipment should be designed as much as possible without ledges, rafters, or protrusions that can collect dust and debris.

Adequate facilities should be provided to hold raw materials in a manner which prevents mixing or cross-contamination except as required by product formulations.

Adequate site security should be provided to minimize the possibility of product contamination by either accidental or deliberate means.

Facilities should be provided and maintained for the temporary storage of waste materials prior to removal from the premises in a sanitary fashion. Waste containers should be clearly identified, leak-proof and, where appropriate, covered. Waste containers should be cleaned and sanitized at an appropriate frequency to minimize contamination potentials.

Premises should be designed for wet weather operation, so that the facility is able to load and/or unload feeds and ingredients without significant water damage to products or ingredients.

Ventilation within the facility should provide sufficient air exchange to prevent accumulation of steam, condensation or dust and to remove contaminated air, but ventilation should always move from clean (finished areas) to dirty (raw areas) through a means of automatic control.

Facilities should have adequate natural and/or artificial lighting. Where necessary, ceilings and overhead fixtures should be designed, constructed and finished to prevent the accumulation of dust, dirt and condensation.

Water used in feed manufacture should be ample, clean and potable with conduits used for water distribution throughout the facility being inert in nature. Adequate water pressure and temperature (>82°C) should also be provided.

Water should be routinely tested for suitability for its intended purpose.

Windows, doors and other openings should prevent the entrance of pests.

The human traffic flow patterns should be laid out to avoid traffic from raw to finished areas.

Facilities for employees should include the following:

- Separate facilities for workers on the pre-processing side and workers on the post-processing side of the plant.
- Adequate facilities for showering and dressing so as to minimize contamination carried on clothing, shoes or the person.
- Adequate lavatory and toilet accommodations that include:
  - Hot and cold running water,
  - Soap, or other acceptable agents (in sanitary dispensers),
  - Toilet tissue
  - Automatic “no-touch” towel dispensers or blow dryers
• The drainage of sewage and plant wastes should be properly installed, have adequate slope and capacity to remove readily all waste to minimize or prevent stoppage/backflow of the system. The drains shall be properly designed for ease of cleaning and frequent sanitation.
• Practical efforts should be taken to exclude insects, rodents, birds and other pests from the processing facilities.
• Plants should be laid out to prevent dead spots or dead ends where feed or ingredients can accumulate. Process flow should be designed in a linear fashion, so that once product disinfection is achieved, the product will not pass back through unsanitized areas.
• Periodic cleaning of ingredient bins and finished product storage areas should be performed.
• Maintain good ventilation of ingredients and products in storage to minimize moisture condensation or migration. Avoid intake of potentially contaminated air into storage and processing areas.
• Ingredient receiving pits attract pests and birds – keep pits covered when not in use, use doors to limit access, maintain good dust-control measures, and keep areas dry.
• Ingredients should not be stored in finished product areas, and finished products should not be stored in ingredient areas or bins.

**Plant Employees and Visitors**

Appropriately qualified and/or experienced and dedicated persons should be utilized to direct and supervise operations. Personnel should be trained to understand the importance of the processes for which they are responsible. Technical or organizational measures should be taken to avoid or minimize any cross-contamination and errors. It is highly recommended that a dedicated quality management member be employed for purposes of designing, training and executing a robust and comprehensive food safety program.

Employees should be instructed and otherwise encouraged to maintain a high degree of cleanliness of self and work clothing. Each employee should be required to report to work in clean clothes and maintain adequate, daily personal hygiene. It is highly recommended that employers provide clean uniforms for use by the employees. The uniforms, footwear and other garments used in the facility should stay on site and not be allowed to be taken home by the employees. If reusable garments are used, the facility should provide for proper sanitizing.

Persons suffering from communicable illness should not be allowed to work in the facility. Every person must wash their hands with soap and warm water after each use of the restroom and break facilities.
Procedures and training should be put in place to control access of contractors, transport operators, customers and other visitors to the site and their movements around the facility. Procedures should also be in place to ensure that all visitors to the site, including staff, contractors, transport operators and customers, are aware of the potential impact of their actions on all aspects of product safety. Avoid unnecessary foot traffic from outside sources in all facility areas because *Salmonella* contamination may be carried on the feet and shoes. Pay particular attention to foot traffic from such areas as barns, stock pens and stock trucks to all facility areas. This includes employees who care for or raise livestock or poultry. Provide for sanitizing of footgear if deemed necessary and practical.

**Plant Procedures and Policies**

**Sanitation and Cleaning**

The buildings and equipment should be cleaned regularly to prevent accumulation of dirt, dust, spilt feed, organic material or raw materials on the floor or surrounding grounds. Cleaning should include the following: the interior and exterior of production machinery as well as ceilings, roof structure, wall cavities, ledges or rafters. Cleaning and sanitizing procedures should be appropriate for each specific piece of equipment or area. Dry cleaning of spillages by sweeping and/or vacuuming is preferable to wet, as water contributes to *Salmonella* growth. If the use of water is necessary, appropriate water temperatures and sanitizing steps should be specified (and appropriate to the area being cleaned).

Premises and work areas should be cleaned after each shift or more frequently to eliminate build-up of organic material using good housekeeping and sanitation practices. Specifically, “Transition Areas” where the product and personnel change from pre- to post-process areas should be sanitized on a shift interval at minimum. Settled dust should be removed using a vacuum cleaner rather than sweeping. Vacuum cleaners should be dedicated to either pre- or post-process and should not be used in other locations. Vacuum collection of dust is preferable to using compressed air, which would increase air-borne dust. Employees should avoid using compressed air whenever possible to remove dust from equipment or from clothing of personnel.

**Pest Control**

Keep all areas within and around the plant free from refuse and trash. Unused equipment should be stored in a manner that eliminates pest infestation. Keep the grounds surrounding the facility well drained and free of unnecessary vegetation, such as weeds and high grass. Clean storage bins and flat storage areas on a regularly, scheduled basis. Minimize ingredient and product spillage and clean up spills promptly.
Take all practical measures to prevent insects, birds, rodents and other animals from gaining access to or remaining around the facility. An effective, documented pest-control program should be in place to minimize the potential impact of rodents, wild birds and insect infestations on product quality. It is recommended that a third-party, licensed pest control company be contracted to assist in continuous pest control design, monitoring and treatment.

**Materials Handling**

High-moisture, raw protein ingredients, such as those used to manufacture digests or pet foods, should be stored under refrigeration/freezing conditions (with a defined temperature maximum not to exceed 4.4°C) immediately after entering the receiving area of the plant. These ingredients should be handled and processed in areas separate from the finished product processing and storage areas. Dedicated utensils and material conveyance equipment should be used in the raw area and not transferred to finished product areas. Documented programs should be developed to provide clear indication of pre- and post-process cleaning utensils and storage containers. Cleaning programs should address the cleaning and sanitizing procedures for equipment and utensils used to handle and process raw materials.

Low-moisture, dry ingredients should not be handled with receiving equipment or utensils that come into contact with high-moisture raw ingredients. In the processing and storage areas, avoid accumulation of dust, organic material accumulation, spillage and broken bags. Any such material suspected of having become contaminated with *Salmonella* should be disposed of as waste, further processed or reprocessed. Such further processing must include methodology to kill the organism. Any wet or under-processed material should also be disposed of as waste or recycled through the heat-processing step(s). Under-processed material can be defined as any material not meeting minimum processing conditions to kill the organism.

**Dust Control and Air Flow**

Dust is inherent in feed manufacturing. However, dust is a primary vehicle by which *Salmonella* can be transmitted within the facility. Therefore, measures taken to control and minimize dust are crucial to the success of any *Salmonella* control program. Air drawn into the facility should not be taken from areas likely to be contaminated. The control of air flow (and the dust contained in that air) should be designed so that contamination does not spread from the raw material areas into the finished product areas of the plant.

Continuous attention should be paid to controlling and removing accumulated dust that settles in processing and storage areas. Dust collection systems should be adequate to control dust and to aid in keeping the facility clean. Remove settled dust with a vacuum cleaner equipped with a
highly efficient filter rather than sweeping. Avoid using compressed air to remove dust from equipment or from clothing of personnel.

Since dust from the collector system and “sweepings” are often contaminated with Salmonella, they should be disposed of and employees who handle the materials should shower and change clothes before assuming other duties within the facility, specifically post-process duties.

The air used for cooling pelleted or extruded products is of particular concern, since these products will normally be Salmonella-negative as a result of the temperatures and pressures involved in these processes. While use of internal air is not preferable in cooling, but commonly used in pelleting facilities, air filtration and dust control should be in place to reduce possible recontamination of pelleted feeds.

**Moisture Control**

Keep feed materials dry at all times. Moisture is critical for Salmonella growth. Roofs, ceilings and walls should be leak-proof. Construct storage-area walls and floors in such a manner as to keep out moisture. Avoid or correct conditions conducive to the formation of condensation in buildings and equipment. Keeping ingredients, finished products, containers, storage areas and transporting vehicles as dry as possible will prevent growth of Salmonella. Containers used for wet ingredients, such as fresh meats, should be thoroughly cleaned and sanitized, including bases if integrated, immediately after use.

The low water activity level found in most dry ingredients and finished products usually results in severely dehydrated bacteria. It is only when there is adequate moisture, temperature and growing conditions that these stressed bacteria recover and multiply.

Pellet coolers should be operated in a manner to prevent condensation on interior surfaces that encourage Salmonella growth and should be regularly monitored for contamination. All equipment should be thoroughly cleaned and sanitized if Salmonella-positive results are obtained during monitoring.

**Miscellaneous Controls**

Monitor, control and log processing temperatures to ensure adequate conditions are achieved to reduce possible contamination levels of Salmonella per the documented plans. After heat processing, cooling and drying should occur as rapidly as possible to reduce condensation in processing and conveying equipment, storage containers, packaging equipment, and in the distribution of finished products.
Keep portable equipment used for handling ingredients separate from those used for finished feeds. When wet cleaning is necessary, follow documented procedures.

**Equipment Maintenance and Operation**

Construct all equipment including bins, blenders, mixers, conveyors and baggers in such a way as to minimize the buildup or collecting of material within the equipment and to provide adequate access for inspection and cleaning. Construct and install all processing and handling equipment in a manner to minimize leakage, spillage and dust accumulation. Inspect equipment at regular intervals. Clean equipment as necessary to remove accumulations of organic material. Clean magnets at regular intervals to prevent feed materials from building up at these points.

Equipment should be designed, constructed, installed and maintained to ensure the following:

- It is capable of delivering the requirements of the process.
- It has appropriate dust extraction capability.
- It allows for routine cleaning, maintenance and inspection.
- It is maintained in good and safe working order.
- It is capable of delivering feed to appropriate quality standards.

When possible, sanitary designs should be used for equipment construction, improvements or changes.

**Packaging, Storage and Transportation**

Since new bags are usually free of *Salmonella*, previously used bags or containers are not recommended for finished product packaging unless sanitized. Make a continuing effort to minimize storage time of ingredients and finished feeds, thus decreasing the opportunity for contamination with *Salmonella* and other microbes. Equipment used to transport ingredients and finished feeds should be clean, dry and free from conditions conducive to *Salmonella* contamination.

Bulk feeds should be covered during transportation to prevent moisture accumulation and prevent birds from perching in the feed ingredient and feed product. Feed ingredient and feed products should be stored in containers in which condensation is minimized. Vehicles handling the product in transport should be of good physical integrity, free of debris, clean and dry with appropriate seals for transportation to secure the ingredient and/or finished product.
Control Procedures

Process Control – Pelleting and Extrusion

The use of high temperatures to accomplish pasteurization during processing is based on the destructive effects of time and temperature on microorganisms. However, equal numbers of bacteria placed in a process are not always destroyed with the same ease by heat.

Microbiologists have identified at least 11 factors or parameters of microorganisms and their environment that can affect heat destruction. These factors include moisture or water activity, fat levels, presence of salts, presence of carbohydrates, pH, protein content, number of organisms, age of organisms, inhibitory compounds, and time and temperature history. Despite the influence these factors can have on the resistance of microorganisms to heat, thermal destruction during the processing steps of pelleting or extrusion is the most critical control step for destruction or reduction of Salmonella and other pathogenic microorganisms.

Thermal death time (TDT) is the time necessary to kill a given number of organisms at a specified temperature. Death is defined as the inability of the organisms to form a visible colony. By this method, the temperature is kept constant, and the time necessary to kill all cells is determined. TDT curves have been established for Salmonella and other microorganisms. As indicated in Figure 1, temperatures reached in pelleting and extrusion are critical in Salmonella control. The graph in Figure 1 is based on a high moisture matrix – the actual time/temperature requirements must be determined based on the specific matrix and processing methods.

**Figure 1: Thermal Death Time (TDT) Curve for Common Microorganisms**

Data Derived from IFT Report to FDA, 2000
Microbial populations are not killed instantly upon exposure to heat, moisture and pressure. In Figure 1, the bacterial population is approximately 5,000 organisms at 55°C. This population is reduced approximately 90% (500 organisms) at 58°C. This population is further reduced by approximately 99% (50 organisms) at 64°C. To ensure *Salmonella*-negative tests, the bacteria population should be reduced by approximately 99.9999%, thus the extension of Figure 1 data out to zero bacterial population at approximately 75°C. These data points in Figure 1 will vary somewhat based on initial bacterial populations, ingredient matrix and processing methods. So again, *the actual time/temperature requirements must be determined based on the specific matrix and processing methods.*

One second of moist heat (at 22% moisture and $10^6$ log initial population) at 77°C can kill *Salmonella* organisms as long as all processed material actually reaches these recommended temperatures internally or throughout. While this should be the target or intended minimum processing temperature, it may not be reached during the startup phase of the pelleting or extrusion process. For this reason, it may be necessary to recycle the first material coming through the pelleting or extrusion process to allow for system warm-up and adequate processing temperature. If pet food is intended for export, certain countries require that raw material of animal origin be processed at a core temperature of at least 133°C for at least 20 minutes at a pressure of 3 bars (43.5 pounds per square inch) or processed by an officially approved alternative system of heat treatment offering equivalent guarantees with regard to microbiological safety.

Pellet mills and extrusion equipment should be carefully monitored to insure proper operation with respect to temperatures as set forth by the facilities’ written plan. Thermocouples or temperature-monitoring devices should be properly calibrated and strategically placed in the process flow to monitor actual product temperatures. The effectiveness of the process parameters employed should be validated as described in the section entitled “Sampling and Analysis.”

**Optional Treatments**

Ultrasonics and/or other electronic technologies may also be applicable to specific matrices to accomplish destruction of *Salmonella* and other micro-organisms.
While heat treatment is the most effective control method, sometimes it is not appropriate and other options are available. However, the use of chemical treatments (organic acids and formaldehyde) should be approached with caution as recent research has suggested that such treatments interfere with Salmonella detection methods rather than killing the organism (Carrique-Mas et al., 2007).

FDA-approved formaldehyde products utilized at approved use rates and applications with ingredients and processed feeds can be effective in reducing Salmonella and other microbial contamination. These chemical treatments may also have a positive residual effect in finished feed in the reduction of Salmonella contamination, and they may help reduce contamination of processing and feeding equipment.

**Decontamination**

The cleaning and sanitizing procedures to use if Salmonella is detected in a facility are varied. Areas that can be readily accessed should be thoroughly cleaned as described in the “Sanitation and Cleaning” section. In some instances, equipment may need to be partially disassembled to allow access for cleaning. When partial disassembly is not a solution, dry flushing with salt, calcium carbonate or other relatively abrasive low-cost ingredient may be options. This flush should be isolated, discarded and not re-used within the facility. In theory, inclusion of approved formaldehyde compounds and/or organic acids in the flush may provide additional reduction in Salmonella.

Physical cleaning should be followed by chemical or equivalent disinfection procedures. Suppliers of cleaning or disinfection products should be consulted to help establish appropriate chemical/disinfectant usage. If wet cleaning is needed, a chlorine-based solution may be used following the manufacturers’ directions. Any residual water after wet cleaning should be removed and all contact surfaces should be dry. If disinfection measures are inadequate, Salmonella organisms may adapt to the stress conditions and become more resistant to control efforts.

Currently, U.S. Food and Drug Administration (FDA) regulations allow the irradiation of lab animal chow to 50 kGy to control microorganisms, and poultry feed to 25 kGy for the control of Salmonella. Irradiation is the process of applying gamma rays, or electrons for the purpose of sterilizing or preserving materials.

The energy for the commercial irradiation of food or feed in the United States comes from exposure to either gamma rays via radioactive materials (usually cobalt [\(^{60}\text{Co}\)]) or accelerated electrons from electron beam (e-beam) technology. While neither radiation source is capable of making materials radioactive and no evidence suggests that toxic products are present in legally irradiated food products, differences exist between gamma rays and e-beams.
Although e-beam technology uses no radioactive materials and is much faster than gamma rays, gamma rays are capable of penetrating ten times further in depth as compared to e-beams. Although irradiation has been approved by the U.S. and more than 40 countries for microbial disinfection of more than 100 food products, it can produce about a 15% loss in the potency of fat soluble vitamins (A, D, E and K), thiamin and pyridoxine as well as peroxidation (rancidity) of fats. Yet irradiation is used to sterilize or sanitize common household products such as baby-bottle nipples, personal hygiene products, cosmetics, bandages, polymerized flooring materials, Teflon-coated skillets, insulation on electrical wire and spices.

The use of ultraviolet light (UV) light is a well-established disinfection method for air and surfaces, including packaging materials. Both FDA and USDA have concluded that the use of UV treatment is safe and effective, if applied correctly. Furthermore, UV light treatment is a simple, effective and economical treatment method as compared to other technologies. It is also a cold, dry process that does not generate chemical residues.

However, as with other technologies, UV light treatment must be understood and correctly applied for best results. UV light occupies the spectrum of light between X-rays and visible light. For practical purposes the UV spectrum is divided into UVC (short-wave length); UVB (medium-wave length) or UVA (long-wave length). While all wave lengths of UV light have some germicidal activity, UVC (short-wave length) is by far the most effective, with activity against bacteria, viruses, protozoa, molds, yeasts and algae.

However, UV light does not penetrate the target deeply and meat or milk that has been exposed directly to UV light may develop off-flavors. These off-flavors may arise from the absorption of ozone, nitrogen compounds or as a result of oxidation (rancidity) of fats. These effects might be avoided by covering the product with a layer of inert gas prior to treatment, but, while unpleasant, these off-flavors do not appear to be harmful.

Newer technology used in food and medical applications include high-alcohol products applied in a spray of heated carbon dioxide gas (to cleaned surfaces only), and blasting cleaned surfaces with dry ice. These newer technologies require specific training in the use of specialized application equipment. In addition, ultra-high pressure is being used on food items with high water activity.

All cleaning procedures should be documented in Standard Operating Procedures, so they can be executed in a consistent manner.
Sampling and Analysis

An ingredient sampling and testing program should be considered to monitor compliance with microbiological specifications. Testing should be by an official or validated method. This program should be documented and include an action plan on responding to a positive result, notifying the vendor and even rejection of the specific lot of ingredients or finished products tested.

Of concern in establishing such a testing program, is the likelihood of finding positive tests that may result in recalls. The alternative is to hold products until test results are received and evaluated. This may be economically costly.

To develop a sampling and testing program, secure samples of ingredients upon arrival at the mill and of finished product at the time of bagging. Routinely subject these samples to appropriate physical examination for overall quality and condition, being alert for any signs or evidence that could indicate contamination. Employ the aseptic techniques described below in securing and handling samples of ingredients and finished feeds.

The results of the laboratory examination will indicate the adequacy of the facility’s Salmonella control plans, purchasing, handling, processing and storage practices in procuring and producing Salmonella-negative products. Routine sampling for non-microbiological testing may be accomplished with sanitary equipment, but sterile equipment is recommended for any microbiological testing to minimize false positive results as a result of poor sampling techniques and non-sterile sampling equipment and containers.

Procedures for Sampling Aseptically

Purpose: Salmonella can be found nearly anywhere and since it is invisible to the naked eye, collecting and analyzing samples is the only way to know if it is present. However, if samples are collected incorrectly, the Salmonella from potential contaminants (like dust, hair, clothing or hands) can produce Salmonella-positive results, when in fact the product is not positive. Collecting samples aseptically (i.e. aseptic sampling) allows us to be almost certain that Salmonella-positive samples reflect what is actually in the feed or ingredient and not what is on or near the person collecting the sample.

The Following Sampling Supplies Are Needed

1. Sterile or sanitary disposable sample containers. Containers that may have been opened prior to sample collection should be discarded.
   a. Sterile bags designed for sample collection may be obtained from scientific or food service supply companies and are the best choice.
   b. Sanitary food storage bags may be used with the following cautions:
Sample bags are used once and discarded. Bags used previously for any purpose must not be used.

- Avoid thin bags, and
- Choose bags that have not been opened until you are ready for sample collection.

2. Choose bags that close securely once the sample is collected such as zip-lock or roll-top closures.

3. Sterile or sanitary sample collection devices.
   - Sterile spatulas or spoons may be obtained from scientific or food service supply companies and are the best choice.
   - Sanitary food service items such as plastic spoons or cups (paper or plastic) may be used but with the following cautions:
     - Items are used once and then discarded. Items that have been previously used for any purpose may not be re-used.
     - Items must be stored in a secure air-tight container and removed only when ready for use.

4. Sample transport container such as a plastic cooler or a plastic storage bin. Avoid Styrofoam or cardboard containers because they cannot be adequately sanitized and are not durable. Also avoid clear or see-through containers since they can trap solar energy. The container must have no sharp edges or corners on the inside and should allow the person sampling to keep control of all necessary supplies. This transport container allows for the convenient transport of all needed supplies to the sampling site as well as protection of the samples following collection. The sample transport container CANNOT be the same container used for supply storage.

5. Sterile or sanitary gloves will provide protection from hand contamination. Bare hands may be used, but the hands of the person collecting the sample must be washed and sanitized prior to sample collection then sanitized via germicidal wipes between samples.

6. Label the samples with permanent, water-proof marker that will not wash off bags or sample containers.

7. Use a sample log book for recording information about samples. Avoid use of spiral notebooks in favor of books with permanent pages such as composition books.

8. Air-tight storage container such as a plastic storage bin with a lid. Container should be large enough to easily contain all necessary supplies and stored in a location that is relatively free of dust.

9. Germicidal wipes.

10. Sanitizing liquid for disinfecting containers following sampling.

Plan and Prepare for Sampling

1. Determine how many samples are needed and the most appropriate place to collect those samples.

2. Locate the storage container and take the following actions in a location that is relatively free of dust:
   - Remove the sample transport container and sanitize the interior with a germicidal wipe or sanitizing liquid and set it aside to dry.
b. Remove the sample log book, the permanent marker and appropriate amounts of supplies (i.e. sample containers, sampling devices and gloves). DO NOT carry the entire box, bag or container of supplies to the sampling location, but it is important to have extra supplies to allow for errors.

c. Label the bags or sample containers and record the information in the log book and include the date of sampling as well as the name of the person collecting samples.

d. Pack the sample transport container. If loose items such as gloves or sampling devices are included, pack those items in separate, sterile or sanitary bags. Also include a disposable plastic shopping bag for collection of trash.

3. Think through the sampling process and verify the following:
   a. Nothing has been overlooked or disregarded,
   b. An adequate number of samples will be collected,
   c. Appropriate supplies and amounts are included, and
   d. Containers are properly labeled.

Sample Collection

1. It is important to work efficiently, so that samples are collected correctly, and exposure to potential contamination via dust or dirt is diminished.

2. In particularly dusty or dirty locations, it is important to limit the chances that supplies will be contaminated through the following actions:
   a. Where possible, place the sample transport container in a relatively secure, sanitary, draft-free location before opening. This will be used as “home base” for the collection of samples.
   b. Open the sample transport container and supply containers for as brief a period as possible for removal of appropriate amounts, then close quickly.
   c. Carry only the necessary supplies to the location, collect the sample(s).
   d. Carry used supplies and the sample back to the “home base.”

3. Every attempt should be made to collect samples that reflect or represent the entire load or batch of feed or ingredient. When sampling a previously loaded vehicle or material from storage, take samples from each of 10 different locations. For bulk shipments, take a sample at varying intervals of time as material is unloaded or loaded so the sample represents the contents of the load.

4. When collecting samples avoid touching the inside of sample containers or sampling devices.

5. Open sample containers for only the amount of time necessary to insert the sample, then securely close the container.

6. If mistakes are made or there is any doubt, discard the supplies (and sample if in doubt) and begin the sampling process over using the extra supplies.

7. Once the sample is collected, place the sample in the sample transport container and discard used supplies in the trash bag.

8. When all samples are collected and materials have been transported back to the supply storage location, complete the following:
   a. Remove samples, supplies and trash from the sample transport container.
   b. Wash or wipe the entire transport container inside and out, then sanitize and set aside to dry.
   c. Discard trash, used materials and unused supplies.
d. Record sample collection information or observations in the log book.

e. Wipe the outside of each sample container with a germicidal wipe, using a separate wipe for each sample.

f. Prepare samples for immediate transport to the laboratory for analysis, being sure that samples are protected from heat and light.

g. Repack supply storage container, close tightly and return to storage site.

For the purpose of these guidelines, *Salmonella*-negative ingredients and finished feeds are those in which *Salmonella* is not detected when sampled and analyzed by procedures outlined in these guidelines. Because of the many variables involved, no one sampling plan will fit every product’s requirements. Consideration should be given to the potential incidence and level of contamination, composition of the material, form of material, and the various treatments to which it has been subjected. The product should also be categorized as to whether it is homogenous or heterogeneous in nature.

Similar sampling methods of dust from unloading pits, ingredient bins, coolers, dryers and finished product bins can provide an indication of the effectiveness of *Salmonella*-control measures.

If water is not supplied from a municipal water treatment facility, it would be prudent to test water sources on a scheduled basis as well. It is important that the back-flow prevention devices are inspected on a scheduled basis and water testing be done at the point of use in the process as well as incoming to the facility. Point-of-use testing is conducted to assure that piping through the facility was done appropriately and not connected to cleaning systems. Also, piping should be dedicated for cleaning and back-flow prevention devices on those systems as well.

The above sampling and analysis plan can be used as a guide or starting point for the establishment of a monitoring program for *Salmonella*. The important thing to keep in mind is to know what your plan can tell you and to be able to interpret the analytical results. Once a facility is contaminated, it may take an extended period of time to eliminate the sources of contamination.

**Criteria for Laboratory Selection**

Analysis for *Salmonella* is a procedure normally requiring the use of a commercial laboratory. However, if facilities manage their own laboratory examinations, it is highly recommended the laboratory be disconnected from the facility or managed on the pre-process (pre-sterilization) side of the facility. Selection of a competent and reliable laboratory is essential for confidence in the analytical results. The following preferred criteria should aid in the selection process.
The ideal laboratory for *Salmonella* testing should do the following:

- Use FDA or AOAC International recognized procedures.
- Allow no variations to the recognized procedure.
- Utilize positive and negative controls.
- Have confirmation ability using alternative tests (rapid biochemical, serological).
- Specify sample size assayed.
- Use a check sample program for validation.
- Retain samples for a specified period of time.
- Obtain ISO accreditation.

**Laboratory Methods**

The method currently preferred by many parties is that recommended by the Food and Drug Administration’s (FDA) Center for Veterinary Medicine for isolating and identifying *Salmonella* in rendered products. It is published in the Bacteriological Analytical Manual (BAM) of FDA which is distributed by the AOAC International. It is often referred to as the BAM method.

The method can be obtained from FDA or AOAC. The respective web addresses are as follows:
- Food and Drug Administration: [www.fda.gov/cvm](http://www.fda.gov/cvm)
- AOAC International: [www.aoac.org](http://www.aoac.org)

**Environmental Monitoring**

It is highly recommended that a program of routine sampling of the production environment be utilized for evaluating microbial cleanliness and the microbial load present in the facility and equipment. Sampling results before and after cleaning can provide a good measure of the effectiveness of the sanitation program and highlight the potential need for further cleaning, revision of cleaning practices, or special focus on specific areas. Trend analyses of the routinely sampled areas will provide data that supports the programmed sanitation frequency, or will indicate the need for a program adjustment.

When performing environmental swabs it is recommended to analyze for *Enterobacteriaceae*, which is a large “family” of bacteria that includes *Salmonella* and *E.coli*. Analyzing for *Enterobacteriaceae* will provide more comprehensive and quantitative feedback on the sanitation efforts and microbial condition versus looking for just a single pathogen such as *Salmonella*, which may be present at very low levels and difficult to find even when present. *Salmonella* cells
that survive in the processing environment may be damaged and use of pre-enrichment steps is generally required for their growth.

One cautionary note is that while Enterobacteriaceae counts are good indicators of “microbial clean,” a low count does not guarantee the absence of Salmonella, nor does a high count indicate Salmonella is present – only that the likelihood of its presence is greater. Also, a sample with very high Enterobacteriaceae count can actually over-grow a low level presence of Salmonella organisms during the sample enrichment phase, and should not be interpreted as “negative” for Salmonella.

Environmental swabbing and analysis should be performed using a system of zones that are designated based on the proximity to the product flow itself. An example would be Zones 1 through 4, with Zone 1 being product contact surfaces, Zone 2 being surfaces immediately over or next to product, then moving to Zones 3 and 4, with Zone 4 being furthest from the product. Expectation of microbial counts and reaction to microbial levels will be somewhat dependent on the surface sampled.

As an example, Zone 1 sampling sites would include the interior of storage containers and storage bins and conveying equipment. Zone 3 could include floors, drains and HVAC vents in the areas immediate to the product zones, transition rooms from pre- to post- process areas, personnel, and cleaning utensils. Zone 4 could include employee break rooms and traffic aisles. Each facility should design its own sampling plan and decision tree based on its risk analysis.

Notes

Analytical results from competent, qualified laboratories using accepted analytical methods will be no better than the quality of the sample submitted. Samples for analysis should be as representative as possible of the material sampled, taken in an aseptic manner, and the integrity should be protected for the analytical results to have meaning.

To sample material in an aseptic manner, the sampling instruments and containers should not be contaminated before use. If spoons (or other comparable instruments) are used, they can be sanitized as a group and a separate spoon should be used for each subsample. Before re-use, the spoons should be cleaned and re-sanitized. They can be placed in boiling water or heated in an oven to over 180-200°F Fahrenheit for at least 10 minutes. Boiling water for 10 minutes will kill vegetative cells, but may not kill bacterial spores, so it cannot be considered sterilization.

Dry sterilization of spoons and other heat-treatable instruments can be accomplished by wrapping the items in aluminum foil, then exposing these items to 191°C for at least 15 minutes. Care must be taken not to re-contaminate the spoons when opening the packaging.
For sanitary sampling for non-microbial testing, new paper cups may also be used for sampling. Paper cups are purchased in a plastic bag. Facility employees are instructed not to touch samples and to keep cups tightly closed within the plastic bag when not in use. Samples are collected only in new paper cups. Cups are used only once and then discarded. New sample bags (zip-lock or similar bags) must be single use and clean, but they are not considered to be sterile.

For sterile sampling for microbial testing, it is recommended that sterile spoons or scoops in individually wrapped packaging be used for each sample. Samples are placed in sterile plastic bags following collection for transport to the laboratory. Although simple, this method is quite effective at preventing cross contamination. Sterile plastic bags or jars can be purchased from laboratory supply stores for sterile sampling.

**Summary and Conclusions**

The North American feed industry has a strong interest in providing safe feed and food for livestock and companion animals. Under current U.S. regulations, feeds contaminated with certain serotypes of *Salmonella* may be considered adulterated and subject to recall by regulatory authorities. Due to scientists being able to detect ever-smaller amounts of potential food contaminants, food safety is at the forefront of new government regulations for animal feed, pet food and human foods. The purpose of these guidelines is to provide educational material for personnel training and practical recommendations for *Salmonella* control and reduction to the many facets of the livestock feed and pet food industries.

Since *Salmonella* organisms are ubiquitous, that is they may be found in most environments, no ingredient of biological origin is completely risk-free. Complete elimination of *Salmonella* is not feasible or realistic. The science-based recommendations outlined in this guide, in conjunction with SOPs, GMPs and HACCP programs, can assist feed manufacturers, pet food manufacturers, ingredient suppliers and renderers in reducing the incidence of *Salmonella* contamination of feeds and pet foods. Some recommendations can be easily applied, while others may require significant financial resources to implement.

The recommendations stated here are intended as general guidelines. It is fully recognized that not all of these recommendations will apply to any specific facility. The recommendations that can be practically applied will likely contribute to *Salmonella* risk reduction. These recommendations will likely be useful in reducing risk from other pathogens as well.

**Literature Cited**


**References**


Acknowledgements

AFIA is deeply indebted to Dr. Frank Jones, professor emeritus, University of Arkansas, for his authorship and guidance in the development of this document. Additionally, AFIA thanks the following individuals for their contributions:

- Dr. Bruce Arentson, Kent Feeds, now at The Nutro Company
- Mr. Louis Carasquillo, American Dehydrated Foods Inc.
- Dr. George Collings, Collings Nutrition Solutions
- Mr. Tom Dobbs, Griffin Industries, Inc.
- Mr. Chuck Hayden, Trouw Nutrition USA LLC
- Dr. Dale Hill, ADM Alliance Nutrition, Inc.
- Ms. Carole Koch, C.J. Foods, Inc.
- Mr. Galen Rokey, Wenger Manufacturing, Inc.
- Mr. Steve Wawrzyniak, G.R.A. Consultants LLC
- Mr. Brian Streit, Natura Pet Products Inc.