6 Research Needs

INTRODUCTION

The strength of risk assessment modeling is its iterative nature. Models can be built with incomplete data and assumptions, and updated as new scientific studies are completed. The revised SERA model is based on the best available data, but also includes as inputs many assumptions and some ambiguous data and principles from scientific theory. Key uncertainties were identified for the current body of evidence and addressed as described in the various Annexes to this risk assessment and other chapters. Good risk assessment differentiates what is known from what is not known, so that future research initiatives can be directed toward filling the data gaps that would most enhance the scientific basis for food safety regulations. Thus, the goal of this chapter is to describe ongoing studies and new research initiatives that could target the most important research needs for risk assessment modeling. Filling these research needs would improve our understanding of the farm-to-table system modeled in this assessment by identifying the variability of the variables that affect risk reducing the uncertainty in the model. An overview of the presentation of the major research needs for the SERA revision is presented in Table 6-1 below. Some research needs are likely to require long-term commitments from multi-disciplinary teams and may require expert consultations before more explicit applications in risk assessment modeling are possible. The potential usefulness of new research initiatives to risk assessment and public health protection is addressed.

Table 6-1 Overview of research needs.

Origin	Annex or Chapter	Research needs
	B. Prevalence in shell eggs	
	C. Initial levels in shell eggs	Prevalence and levels by site of contamination
Farm	D. Cooling of shell eggs	Probability of hens infected given young infected flocks
Farm		Growth kinetic parameters by site of contamination, and
Establishment		previous history of storage
Retail and	E. Growth	Time and temperature assumptions for storage
Home	Exposure Assessment	
		Paired pre- and post-pasteurization data
		Storage practices of liquid
Establishment	F. Levels in egg products	Growth of *Salmonella* in liquid product pre and post pasteurization
		Data at additional temperatures by site of contamination
Establishment		for shell egg pasteurization
and HRI or		Data of liquid products with and without additives
Home Kitchen		Time and temperature assumptions for cooking
	G. Lethality	Extent of undercooking
		Classifications of high risk foods
		Fractions of eggs consumed undercooked
		Variability in host and *Salmonella* populations
		Data depicting relationships between dose and severity
		Progression of infection to adverse effects and severity
		Methodology for expanded epidemiologic investigations,
	H. Consumption	particularly for reconstruction of doses causing and not
Home or HRI	Hazard Characterization	causing illnesses in outbreaks

STUDIES IN PROGRESS

The data analysis for the draft revision of the SERA risk assessment in shell eggs and egg products was completed in the fall of 2002. The following studies already proposed or in progress appear relevant to FSIS data needs for the SERA revision. As raw data from these studies become available in the coming years, FSIS could conduct data analysis and update the SERA model to determine the impact of the new data on model predictions. The need for additional studies addressing other aspects of these topics may be considered upon completion of the work in progress.

- A preliminary dataset from the FSIS baseline survey for liquid egg products was used to determine the distribution of the initial levels of *Salmonella* spp. in liquid egg products before pasteurization. This study is important, as the usefulness of the pasteurization process is dependent on the starting level of bacteria present within the raw liquid eggs. In this risk assessment, preliminary data from a portion of this survey was used to determine this distribution from the partial dataset available in December 2002. Upon completion of the FSIS baseline survey for liquid egg products expected in March 2003, the full dataset will be analyzed and compared to the derived distribution. In addition, data for serotypes identified from egg product samples from this study will also be summarized and considered in the final risk assessment upon completion of the FSIS dataset. These data are important for consideration of the fraction of isolations attributable to SE versus other *Salmonella* spp. relevant to the mechanisms of contamination and potentials interventions to reduce risk.

- FSIS/RTI web-based Consumer Behavior survey questionnaire is currently under review by the Office of Management and Budget (OMB). If this survey is approved and funded, it could provide information permitting reliable quantification of consumer handling and preparation of eggs for future risk assessment models. The times and temperatures of egg storage and the methods and efficiencies of cooking eggs prepared in households are important predictors of the levels of SE surviving within a contaminated egg serving. The fractions of egg handling and preparation attributable to given scenarios might also determined from this questionnaire. The sensitivity analysis in Section 4 identified the following as priorities for data needs relevant to this item: times and temperatures of eggs and egg products under home storage; and fractions of undercooked eggs and egg ingredients. Future models could be updated to determine the impact of these data relative to the assumptions used in the current model.

- The American Egg Board has funded research to measure times and temperatures that eggs might experience from the farm to the processing plant and ultimately to the consumer's table. This time and temperature information is important, as these variables will strongly influence the potential and the extent of SE growth. Future models could be updated to determine the impact of these data relative to the assumptions or expert opinions from academia and industry for times and temperatures of egg storage used in the current model.

    - Researchers at The Pennsylvania State University are conducting a study entitled "Temperature sequence of an egg from oviposition through retail distribution." This study will encompass seven states representing different regions of the country, winter and summer seasons, in-line and off-line processing, and open sided and forced ventilation houses. This study will: determine ambient and internal temperatures of eggs from farm to retail; identify variables associated with US production and processing that influence times and temperatures of egg storage; and model the various time and temperature sequences of processing and handling to predict resulting egg temperature under many scenarios. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, times and temperatures on farm and temperatures in retail are likely to be influential data gaps for updating the assumptions used in the risk assessment model with scientific data.

    - Researchers from the University of Pennsylvania and the University of Bristol are conducting a study entitled "SE concentration in shell eggs in the U.S. as determined by time and temperature." This study will: develop an experimental inoculation model for the reliable production of eggs from laying hens containing SE; confirm the intra egg location of SE from experimentally inoculated laying hens; determine the impact of time and temperature abuse on the intra-egg concentration of SE; define the impact of aging on the intra-egg growth of SE under ideal and abusive storage conditions; and address five targeted sampling locations: hen house, processing plant, cartooned storage, distribution, and retail supermarket. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the fraction of eggs that are contaminated on the vitelline membrane of the yolk is likely to be an influential data gap for updating the risk assessment model with additional scientific data.

- The Agricultural Research Service has funded researchers at the SE Poultry Research Laboratory to conduct a study entitled: Detection and control of SE in Poultry (planed for 2001-2006). This study will focus on the following research needs.

  - Improved methods for detecting SE infections in laying flocks and SE contamination in eggs. This will help to reduce false negative rates associated with current methodology. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the likelihood of hen positives given flock positives is likely to be an influential data gap for updating the risk assessment model with additional scientific data.

  - Characterizing how, where, when, and in what numbers egg contamination by SE occurs. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the fraction of eggs that are contaminated on the vitelline membrane of the yolk is likely to be an influential data gaps for updating the risk assessment model with additional scientific data.

  - Reducing airborne dust and SE in poultry hatching cabinets and breeder houses using an electrostatic space charge system (ESCS), and determine the mechanism by which ESCS kills airborne and surface SE. This aspect of the study could identify effective interventions to reduce transmission of SE within a flock, thereby reducing the risk to the consumer.

NEW RESEARCH NEEDS

Numerous research needs were identified during the data analysis for the exposure assessment described in Annexes B through H. The research needs for exposure assessment are presented below by annex. An additional entry for this section relates to time and temperature assumptions for storage, including post-processing behaviors of consumers and preparers of egg servings, discussed in the Exposure Assessment chapter rather than in an Annex.

Distribution of *Salmonella* Prevalence within Shell Eggs (Annex B)

The site of *Salmonella* contamination within the egg is important because the potential and the extent of SE growth differ by site. To determine this, a nationally representative survey conducted over all seasons to estimate the fraction of annual domestically produced shell egg-positives for *Salmonella* in yolk, on vitelline membrane, in albumen close to and far from the yolk, and on inner shell is needed. This study should include variables for molting status, age, and breed of hen and *Salmonella* strain as these factors may affect *Salmonella* survival and recovery from different compartments of intact shell eggs. In this risk assessment, these estimates are either assumed or identified from several different studies. A survey analyzing these issues would decrease the uncertainty associated with many of the above estimates. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the site and level of contamination is likely to be an influential data gap for updating the risk assessment model with additional scientific data.

Salmonella present on the eggshell surface prior to washing can penetrate the outer shell, persist in the pores of the shell, and gain internal access to the egg contents. Studies using experimentally infected hens and artificially contaminated eggshell surfaces were used to estimate the prevalence of eggshell contamination and the likelihood of penetration, respectively. However, it is unclear to what extent this takes place in hens naturally infected with *Salmonella* and processed under commercial conditions. To estimate the prevalence of eggshell contamination and the likelihood of shell penetration, eggs produced by hens naturally infected with *Salmonella* should be investigated for *Salmonella* on the eggshell surface and evidence of shell penetration. This should include SE and other *Salmonella* spp., as shell penetration is not exclusive for SE. These data are needed to verify assumptions in the model for shell eggs and to better elaborate the mechanisms of contamination of egg products.

This draft risk assessment used data from surveys conducted with hens at the time of slaughter (i.e., spent hens) to identify the proportion of individually infected hens within a SE-infected flock. Because the relationship between egg-producing hens and spent hens is unclear, a nationally representative baseline survey is needed for SE and other *Salmonella* spp. within-flock prevalence over all seasons utilizing rigorous *Salmonella* isolation techniques to minimize potential bias in methods associated with high false negative rates. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the fractions of flocks positive and of hens positive within positive flocks are likely to be influential data gaps for updating the risk assessment model with additional scientific data.

A priority research need beyond the scope for the current model regards data depicting how farm management practices may influence flock or egg positive fractions and levels. Data demonstrating the impact of manure management, flock size, feeding practices, rodent control, and biosecurity would likely be informative. These issues could be important for future modeling efforts targeting potential interventions for egg contamination on the farm.

Initial Levels of Contamination in Shell Eggs (Annex C)

The level of *Salmonella* spp. initially deposited within the egg is important for determining the potential and extent of SE growth. A nationally representative survey conducted over all seasons to estimate the counts of *Salmonella* in yolk, on the vitelline membrane, in albumen close to and far from the yolk, and on the inner shell is needed. Variables for molting status, age, and breed of hen and *Salmonella* strain should be considered. In this risk assessment, SE-infected eggs produced by experimentally infected hen studies were used to estimate the levels of SE within an egg. However, due to the low samples numbers and potential of SE to have grown before analysis, there is a large amount of uncertainty associated with some of the above estimates. A survey analyzing these issues would decrease the uncertainty associated with many of the above estimates, in particular the level of SE within the yolk. The studies proposed by the American Egg Board and Agricultural Research Service may partially fulfill this research need. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the initial levels are likely to be influential data gaps for updating the risk assessment model with additional scientific data.

Exponential Cooling Rates for Storage of Shell Eggs (Annex D)

During the processing of shell eggs, eggs are cooled to prevent microbial growth and preserve egg quality. In this risk assessment, cooling rates were applied to various levels of egg processing to predict the internal egg temperature. However, it is unclear what fraction of eggs in US production is applicable to each cooling rate. To determine this, a study of the fractions of U.S. egg production applicable to each cooling rate model from available studies of eggs within pallets of selected materials used in commercial egg packing is needed.

A validation study for the derived adjustment for the effect of location of shell eggs within pallets is also needed. Data were available to describe the worst-case for cooling of an egg in the center of the pallet. Although adjustments were developed for eggs in other locations of pallets based on theoretical equations of heat transfer, experimental studies are needed to describe the actual cooling behavior of eggs in pallets to determine the appropriateness of the theoretical adjustments.

Modeling Growth of *Salmonella* in Shell Eggs (Annex E)

The potential for, the rate of, and the extent of SE growth within a contaminated egg will largely determine risk of illness to the consumer. A large body of experimental evidence was considered in the data analysis phase of this risk assessment to model the growth of SE in shell eggs. However, data were sparse for estimating growth parameters for SE, and there were no data that measured growth curves typically generated in predictive microbiology studies. In particular, there are no data for the lag phase duration times. Other *Salmonella* spp. contaminations in some specific compartments of intact shell eggs. These sites included on inner shell (*Es*), in albumen close to (*Eac*) and far from the yolk (*Eaf*), and on the vitelline membrane (*Ev*). In contrast, data were more extensive for estimation of growth parameters for yolk contaminations (*Ey*) from experimentally inoculated eggs. While albumen is recognized as a sub-optimal environment for SE growth compared to yolk, little data is available from controlled studies describing the likelihood of growth/no growth events for any compartments of intact shell eggs. However, multiple researchers have developed theoretical approaches to model growth as a stochastic or random process. This scientific theory was applied in the absence of data for modeling SE growth in shell eggs in stages, first as likelihood of growth and approximations for the extent of growth. Therefore, studies validating these theoretical stochastic growth models for SE are needed. An additional line of research could involve development of alternative methods for estimating and modeling the key events of pathogen growth more mechanistically. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, growth parameters in yolk and yolk membrane breakdown are likely to be influential data gaps for updating the risk assessment model with additional scientific data.

The current model predicts risk for given scenarios of pasteurization efficacy. Further, the current model assumes that growth after pasteurization is consistent with kinetics of growth before pasteurization. One study reported that the extent of growth in reconstituted dried albumen was substantially higher than that of untreated albumen. In addition, in-shell pasteurization could have profound effects on the time to yolk membrane breakdown, and subsequent enhancement of growth. Quantitative data are needed to measure the impact of these factors on growth after pasteurization. Specifically, data are needed to determine the likelihood and extent of growth for low numbers of SE or other *Salmonella* spp. surviving shell egg pasteurization.

Levels of *Salmonella* spp. in Liquid Egg Products (Annex F)

Upon completion, the FSIS baseline study will provide data for prevalence and levels of *Salmonella* spp. in egg product samples collected immediately before pasteurization. However, additional studies may be needed to resolve questions about valve effects and other factors that might confound the survey results. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the initial levels, particularly in yolk products, may be an influential data gap.

Lethality Models for Liquid Egg Products and Shell Egg Contents (Annex G)

While the ongoing FSIS baseline study will provide data for prevalence and levels of *Salmonella* spp. in egg product samples collected immediately before pasteurization, data are needed after pasteurization as well. Paired data from pre- and post-processing at commercial plants (and perhaps at end-user establishments?) are needed to quantify the magnitude of the lethality achieved in commercial pasteurization processes. The available data on decline after pasteurization may not be representative of the commercial processes used currently. The current model predicts risk for given scenarios of pasteurization efficacy. Data for modeling more explicitly the efficacy of various commercial pasteurization processes are needed for the full range of egg products produced in the US. These data could be incorporated in future risk assessment models and also could serve as validation for the efficacy of commercial processes to achieve lethality performance standards in future regulatory initiatives for *Salmonella* spp. in egg products.

Data are also needed determining the growth potential for *Salmonella* spp. that survive pasteurization processes for egg products. These data are needed to replace assumptions in the current model predicting growth after pasteurization from the data for growth in raw egg matrices.

Some experimental data are available to estimate lethality curves for both in-shell and egg product pasteurization. However, the representativeness of the experimental methods used or the fraction of US egg production applicable to each experimental method is unknown. In the case of lethality for shell eggs, the experimental data used in this risk assessment provided the $\log_{10}$ reduction only at two different temperatures. Thus, it was not possible to model lethality accurately over a range of temperatures as was possible for modeling growth in yolk. In addition, the experiments for shell eggs were conducted by inoculating SE in the center of yolk in an egg. Because high levels of SE contamination can occur within the albumen, information concerning the lethality of *Salmonella* within the albumen is needed. These data could be incorporated in future risk assessment models and also could serve as validation for the efficacy of commercial processes to achieve lethality performance standards in future regulatory initiatives SE and other *Salmonella* spp. in eggs.

Egg Consumption (Annex H)

The data available from the Continuing Survey for Food Intake by Individuals (CSFII) for servings of eggs as ingredients and main dishes are well represented. The survey includes few observations of consumption of eggs as beverages. However, from the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, serving sizes of beverages do not seem to be an influential data gap in the draft simulation model.

The classification of high-risk foods and the fraction of egg servings consumed undercooked and the extent of undercooking are not well characterized. Research needs for experimental data on lethality for cooking procedures are discussed below. Data from nationally representative surveys are desirable to replace the assumptions used in the model. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the fraction of egg ingredient servings is likely to be an influential data gap.

The CSFII data do not address questions regarding batch scrambling of eggs. For example, the number of individuals consuming an egg and the number of eggs contributing to a serving are unknown. The impact of the assumptions in the model may warrant further data collection.

Post-processing Behavior of Consumers and Preparers (Exposure Assessment)

Although studies are proposed and funded that might address some research needs for this topic, the time and temperature assumptions about storage and preparation of eggs will be strong drivers of growth and decline in the model. The high importance and uncertainty associated with post-processing behavior might warrant additional research initiatives. Primary research into these practices is needed to ensure that risk managers have sufficient information about actual levels of protection achieved by various consumer practices. From the sensitivity analysis described in the Risk Characterization chapter and summarized in Section 4, the times and temperatures of storage, the fraction of mixed ingredients, and the cooking and undercooking of eggs and egg ingredients are likely to be an influential data gaps for updating the risk assessment model with additional scientific data.

Other Research Needs (Annex I and Hazard Characterization)

The FAO/WHO salmonellosis dose-response model used in this risk assessment is far from a deterministic and certain model. Extrapolation is needed to predict from epidemiologic studies to the likelihood and severity of illness in the US population. The predictions of the model based on outbreak strains may overestimate risk from the population of strains in present in US eggs and egg products. Variability is incompletely characterized, and uncertainty might be reduced with additional research. A more mechanistic understanding of the host, pathogen, and food matrix factors influencing the likelihood and severity of illness is needed, particularly for low doses of SE and other *Salmonella* spp. in eggs and egg products. The research needs are extensive and may require long-term systematic and collaborative studies, as well as development of new methodology and models. The need for analytical-deliberative process for mechanistic modeling is commonly acknowledged. The topic is on the agenda for future WHO/FAO expert consultations and for other groups in professional societies and the European Union (http://www.cost920.com/00012.html).

Data from case-control studies to determine the fraction of US salmonellosis cases attributable to egg and egg products consumption are needed. Such studies would be helpful to anchor the model predictions of magnitude of the public health impact and the effectiveness of future risk management strategies in reducing egg-associated salmonellosis cases in the US.

When enhanced epidemiologic investigations are possible (see Foodborne Disease Outbreak Questionnaire at http://www.foodriskclearinghouse.umd.edu/dose_resp.htm), data on possible doses consumed and responses resulting could be estimated and used to re-construct a dose-response relationship. However, additional methodology is needed for formal dose-reconstruction that accounts for measurement and sampling errors for food microbiology methods. Various intrinsic and extrinsic factors of foods, eggs in particular, are associated with methodological limitations of recovery, detection, and enumeration of pathogens typically expected to be clustered or non-homogeneously distributed in foods. For example, study designs to address this research need could target repeated sampling to describe distributions of pathogens in lots of suspect foods. Such data would increase the confidence in estimates of ingested doses that resulted in illness or no adverse effects.

Additional strategies might be considered to generate data relating ingested doses of foodborne pathogens and likelihood of illness, such as the following:

- Food microbiology studies could be established through collaborations between government, industry, and academia. When positive lots or positive flocks are identified, the distribution of levels of *Salmonella* in naturally contaminated foods could be determined to verify or refute the assumption of homogeneous distribution in foods. These data would enhance the understanding of the food system and provide risk managers a more direct measure of the potential effectiveness of sampling plans in detecting pathogens that may not be distributed homogeneously in foods.

Mechanistic data for salmonellosis dose response modeling is needed to characterize the variability in host, pathogen strain, and environment, as well as interactions that influence predictions of likelihood and severity of illness. A clearer understanding of the nature and magnitude of adverse effects predicted would better inform risk assessors and risk managers about the relative impact of exposures to the strains in servings of eggs and egg products. This research is needed for:

- Normal and susceptible subpopulations
- Serotype and strain differences in pathogenesis and virulence
- Progression to more severe or systemic complications of salmonellosis, including factors associated with more severe illness such as high doses

These data are important for cost-benefit analysis of interventions to reduce the number of severity of illnesses and for future development of food safety objectives linked to public health endpoints.

RESEARCH NEEDS IDENTIFIED FROM SENSITIVITY ANALYSIS OF SIMULATION MODEL

The results of the sensitivity analysis for the revised simulation model described in the Risk Characterization chapter are summarized below. Small changes in the values of the most influential input variables resulted in 10-100-fold changes in the prediction of illness, with other variables constant. Variables that result in 10-fold or lower changes in the prediction of illness may be important, but appear of lesser influence given the current model structure and assumptions. Variables are also identified that are not influential, given the current model structure.

For the shell egg model, the time and temperature assumptions were most influential in predictions of illness. The most important variables were storage times and temperatures on farm and in homes, times for eggs produced off-line, and temperatures at retail. The following variables were also influential in predicting illness: probability of infected flocks; the probability of infected hens within infected flocks; the fraction of eggs SE-positive on the vitelline membrane at low levels; the initial density in albumen; the growth parameters for yolk and yolk membrane breakdown; the fraction of servings with egg ingredients; and the lethality for boiled eggs. Variables that are not influential for this model include assumptions for cooking methods, cooling rates, and serving sizes.

For the egg products model, the assumptions for storage times and temperatures and undercooking were most influential in predictions of illness from all the egg products modeled. For yolk and whole egg products, the growth parameters in yolk were also important. Initial levels may be important for egg products. Variables that are not influential for this model include serving type fractions and serving sizes.

SUMMARY

The revised SERA model is based on the best available information. There were many knowledge and data gaps identified in the data analysis phase of the project. In general, these gaps were filled using assumptions and principles from scientific theory. The new research initiatives identified in this chapter targeting significant risk assessment data gaps, and other areas of research, will improve the scientific basis of future iterations of the risk assessment model.