ANNEX A

General Introduction to the Annexes

The purpose of this introductory annex is twofold: First, it provides an overview of the content of subsequent annexes (Annexes B through I) that give the rationale for the model used in the risk assessments. Included is a description of the data and the analysis procedures used for determining the distributions and values of parameters for the risk assessment models. The data analyses in Annexes B through H are inputs to the draft exposure assessment model for eggs from farm to table. The modeling applications of the results of these data analyses are described in the Exposure Assessment (chapter 3). The model predicts, as final outputs, the frequency and extent of *Salmonella* Enteritidis (SE) or *Salmonella* spp. contamination of servings of eggs or egg products. Annex I describes the data from epidemiologic investigations of foodborne salmonellosis and the procedures used in developing the FAO/WHO dose-response model.

The second purpose of this introductory annex is to provide background information on two subjects, knowledge of which is required to understand better the information presented in subsequent annexes. The first of these subjects is a comprehensive "picture" of the biology relevant in developing a risk assessment model for SE in eggs. The second of these subjects is a description of variability and uncertainty in risk assessment inputs.

Transparency in a risk assessment does not end at presenting a clear description of the procedures of calculations used to compute risk. Equally important is communicating the motivation for choosing an approach and deciding to make the calculation in the first place. We have made every effort to provide interested parties a clear understanding of the limitations of

the risk assessment models so to enhance the likelihood that such parties will provide us with useful ideas or information that might lead to improvements in future risk assessments. Thus, the annexes present an in-depth explanation of our reasoning and procedures for constructing the model.

Before proceeding, however, we are presenting in this annex a general discussion of the biological picture that guided us in constructing the model. Though we present in Annex E data that questions our picture in one important aspect, we are using the picture described below and elaborated in the annexes despite conclusive evidence of its validity. This is done because the model represents a plausible approach, given the evidence, from which risk calculations can be made.

In the Introduction to these risk assessments we stated that one of our goals was to separate variability and uncertainty. There are many terms researchers use in describing uncertainty. For our purposes, it is sufficient to classify the types of uncertainties into two broad categories: uncertainty calculated from the data; and "state of knowledge" uncertainty in the absence of data.

OVERVIEW OF ANNEXES B THROUGH I

Annex B provides information about the prevalence of SE-contaminated flocks and eggs in the United States. Molting of flocks and penetration of *Salmonella* through the outer shell of the egg are specifically considered as factors contributing to SE prevalence. The factors that affect prevalence of *Salmonella* in eggs also might affect the levels of the initial contamination; however, we are unaware of data to estimate whether such a correlation exists.

Annex C provides information about the initial contamination level of SE in shell eggs, distinguishing levels occurring between yolk and albumen. The amount of growth of SE cells depends upon their growth kinetics, which in turn depends upon the internal temperature of the egg. Thus, to model the effects of time and temperature storage scenarios on the levels of SE contamination, it is necessary to model how rapidly the egg cools and the growth kinetics of SE within the egg as a function of temperature.

Hence Annex D describes an exponential cooling rate model that was developed to estimate the internal temperatures of eggs as they cool. And Annex E describes the models used to estimate the growth kinetics of SE in shell eggs as temperatures change.

The models described in Annexes D and E were used to model growth of *Salmonella* spp. in eggs for given time/temperature storage scenarios. If contaminated eggs are broken and the contents are used in producing liquid egg products, then *Salmonella* within the eggs will contaminate the liquid product. *Salmonella* spp. on the exterior of the shell during the breaking process may also contaminate the liquid product.

Annex F presents an estimate of the distribution of *Salmonella* spp. levels in liquid egg products immediately before pasteurization, based on an analysis of data collected from the FSIS Egg Baseline Survey of *Salmonella* spp. levels in liquid egg product.

The results from Annex F together with predictions based on the models described in Annexes B to E allowed us to model the distributions of *Salmonella* levels in liquid egg products for any given time/temperature scenario, provided some assumptions. In particular, the effect on the distribution of *Salmonella* levels in liquid product if eggs are from SE-free flocks versus

those for flocks assumed not to be SE-free can be evaluated for given scenarios of handling eggs before pasteurization. This is important because performance standards, which essentially specify a required probability of assuring no viable *Salmonella* cells after pasteurization for given conditions, are dependent upon the estimated distribution of *Salmonella* levels in the pre-pasteurized product. Thus, for example, performance standards for liquid product from eggs handled in certain ways would depend on whether SE-free flocks are used, provided the exposure assessment indicated the distribution depends significantly on whether the eggs are from SE-free flocks.

In addition to the above modeling, we also modeled risk that exists today under present regulatory requirements. Annex G presents data and development of inactivation models for different types of egg products and shelled eggs.

Annex H describes how data from the USDA Continuing Survey of Food Intake by Individuals (CSFII) were used to identify the amount and frequency of consumption of eggs and egg products. These data combined with estimates of the level of *Salmonella* in a serving of eggs or egg products completes the exposure profile.

Annex I presents a report prepared by a Joint Expert Meetings on Microbiological Risk Assessment on the Joint FAO/WHO Risk Assessment of *Salmonella* spp. in Eggs and Broiler Chickens. The dose-response model for non-typhoid salmonellosis presented in the report was used in these risk assessments. The technical details of the methodology cited in the FAO/WHO report are not fully transparent; thus, while the derived dose-response model was used here to compute the probabilities of illness, the procedures used for deriving this model cannot be endorsed by FSIS unless further documentation is provided.

BIOLOGICAL CONCEPTS RELEVANT IN DEVELOPING A RISK ASSESSMENT MODEL FOR SE

Current data demonstrate differences in the incidence of SE egg contamination, SE levels, and growth kinetics by site of contamination within the egg. Development of models for predicting such values were based largely on data from studies with experimentally inoculated hens or eggs. Several biological concepts were significant in development of the data analysis approaches used for the risk assessment of SE in eggs, the most important of which are briefly described below.

Describing contamination of eggs with SE

The growth potential, frequencies of occurrences, and SE levels in eggs depend strongly upon the site of contamination during egg formation within the hen (vertical transmission) or after lay (horizontal transmission) for SE and other *Salmonella* spp. A hen can become infected with SE by oral, aerosol and other modes of horizontal transmission. Subsequent infection of the ovary or oviduct and subsections within these organs can lead to contamination of different egg sites. Growth potential as supported by the availability of nutrients may be dependent on the site that SE contaminates the egg. We identified six types of SE contamination events (*Ex*, where *x* is a letter or a set of letters identifying the site of the egg that is contaminated).

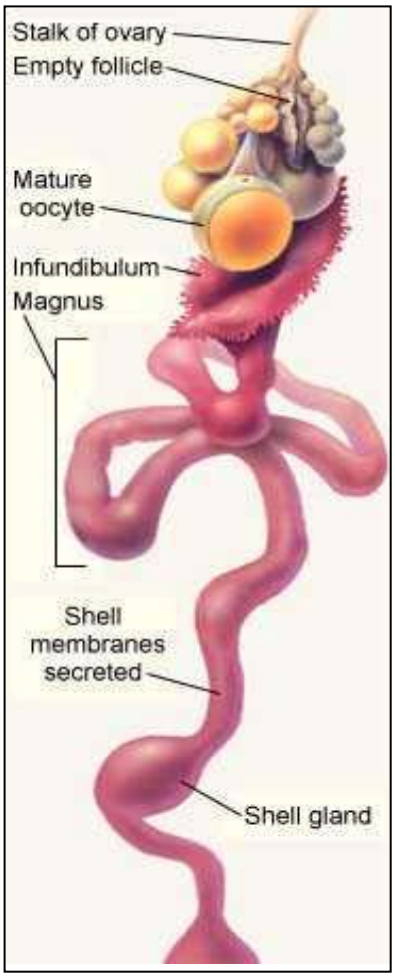

Figure A1 Anatomy of the reproductive tract of hen (source:http://chickscope.beckman.uiuc.edu/explore/embryology/day05/ovary.html).

1) SE can be vertically transmitted within the hen (Figure A1), migrating to and colonizing the ovary and oviduct tissues. SE can contaminate the ovule or yolk contents before release from the ovary (*Ey*), as described in Figure A2 and highlighted in the text box below.

2) While within the ovary or during release of a yolk from the ovary follicle into the opening of the oviduct (infundibulum), SE can contaminate the vitelline membrane of the yolk (*Ev*).

3) As the yolk descends along the oviduct where the first layers of albumen are laid down around the yolk, SE can contaminate the albumen close to the yolk (*Eac*).

4) As the forming eggs further descends along the magnum of the oviduct where the outer layers of albumen are laid down, SE can contaminate the albumen far from the yolk (*Eaf*).

5) As the inner shell membranes then the outer shell are laid down, SE can contaminate the inner shell membranes by vertical transmission (*Es*).

6) SE can contaminate the exterior surface of the shell by horizontal transmission after lay from the environment of the hen (*Ep*).

Contamination events (*E*) for SE within shell eggs are either vertical or horizontal transmissions (see text box below).

Type of Event	Contamination Site	Transmission
Ey	In the interior yolk (*y*) contents	Vertical
Ev	On the vitelline membrane surface, (*v*) but not yolk interior	Vertical
Eac	Within the inner layer of albumen close to the yolk	Vertical
Eaf	In the outer albumen far from the yolk	Vertical
Es	In or on the inner shell membranes	Vertical
Ep	Penetrating egg from outside environment	Horizontal

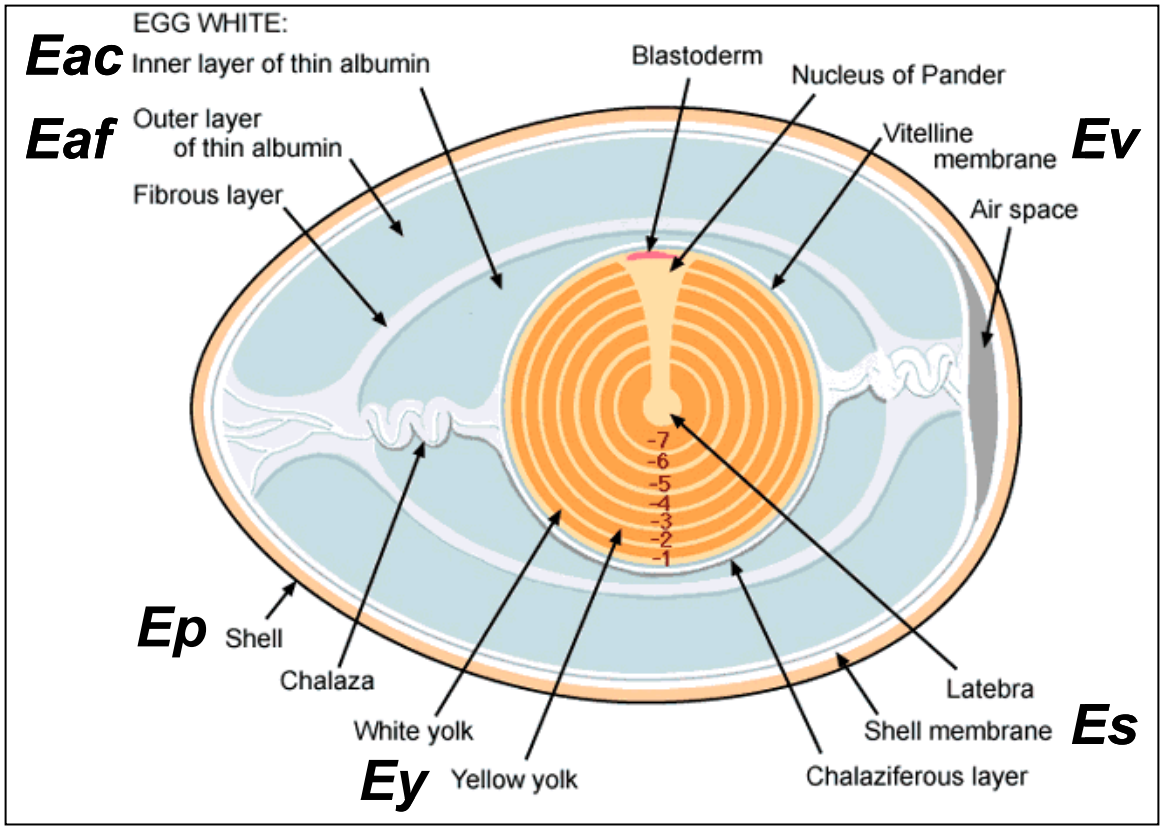

Figure A2 Anatomical picture of egg compartments that correspond to potential SE contamination sites *Ey*, *Ev*, *Eac*, *Eaf*, *Es*, and *Ep* as explained in Text Box below. (Source:http://chickscope.beckman.uiuc.edu/explore/embryology/dayo1/the_shell.html).

Initial growth and physiological state of SE in eggs.

Growth in first 24 hours after lay

The amount of initial growth of SE within the first 24 hours after lay was addressed. Humphrey[1] presented a graph depicting a 10-fold increase in the number of SE cells within the first 24 hours after lay. His claim of a 10-fold increase of SE within the 24 hours after lay was explained by hypothesizing that SE is able to utilize internal reserves of iron and grow while the pH is neutral. However, no data were adduced that directly demonstrated this amount of growth within the 24 hours after lay. This assumption is discussed more in depth in Annex E; however, as a consequence of its considerations, we do not assume a particular phase of growth associated with the first 24 hours after lay. It is possible that growth rates within the first 24 hours are different than those after the first 24 hours; however, no data are available to provide information on the "true" growth curve in albumen. The belief that SE growth is possible in albumen beyond 24 hours played a crucial role in developing the exposure assessment model. Attachment 1 in Annex E is included to set forth some explanations for this potential growth.

Lag phase - physiology

A difficulty of interpreting data from experimental infection of hens or contamination of eggs is that SE cells prepared for experimental inoculation are often in stationary phase and subject to a lag phase of unknown magnitude. We believe naturally contaminating SE would behave differently from experimentally inoculated cells. Part of the difficulty of determining growth of *Salmonella* in the egg is the fuzzy picture of the status of the growth phase of the SE within the egg, in particular the physiological state of the SE in the hen before invading the forming egg and immediately after invasion.

Virtually no data exist to characterize the lag phase durations or the physiological states of SE (or changes of them within the hen or within naturally contaminated eggs) before and after the egg is laid. However, information regarding these features is important as the lag phase, dependent on the bacterial physiological state and change of environment, will determine the time before SE growth within the egg commences. This information could explain the variability regarding the quantity of SE within young eggs laid by naturally-infected hens.[2,3] Explanations of these results would depend upon knowledge of the possible growth that could occur before the egg is laid, which in turn depends upon knowledge of the physiological states of SE cells as the cell's environment changes from hen to egg. The picture we present in this area is based on a literature-derived speculative understanding and on our understanding of the biological events that could take place. However, this picture cannot be justified independently by available data.

The physiological states of SE before deposition into the egg will, in part, determine the lag as transition into a new environment requires time for SE cells to adjust before growth is possible. We assumed SE cells within the ovary or oviduct are not in the exponential phase of growth. This assumption is based on the following:

1) SE-infected hens do not typically demonstrate clinical signs of illness or slowing of egg production rate. This suggests SE growth is controlled by either the hen, the bacteria, or a combination of both; therefore SE are unlikely to be growing exponentially. For instance, because high levels of SE growth within hen reproductive tissue would be more likely detected by innate and adaptive immune surveillance systems than would low numbers of SE, an equilibrium between the hen and the bacteria might be forged that allows lower levels of SE to persist in the hen.[4] This strategy of pathogenesis would maximize colonization or establishment over time in the reproductive tract and minimize the likelihood of inactivation due to the immune defenses of the hen.

2) The internal host environment is limited by sequestration of free iron, likely prohibiting rapid growth of SE colonizing the surface of reproductive tissues.

3) The majority (>90%) of *Salmonella* might be located on the surface of infected tissue as demonstrated by colonization of the mammalian gastrointestinal tract.[5] This suggests most infecting SE could colonize the hen's reproductive tissues as biofilms or microcolonies in which few cells are capable of leaving lag phase and doubling multiple times before lay.

Taken together, the above points suggest SE would not typically be in exponential phase during colonization of the ovary or oviduct. On the other hand, SE cells are capable of invading into ovarian cells[6] and are likely to do the same in the oviduct. This process results in rapid SE growth within the host cell and the release of immune activating and chemotactic chemicals. SE cells emerging from invaded host tissue could be in an exponential phase of growth and could, if deposited within an egg, adapt quickly for rapid growth. However, as mentioned above (point 3), the majority of SE seems to remain attached to the exterior of host cells and would not be vigorously engaged in this rapid growth process. This latter claim is further evidenced by the observation that lesions, due to tissue destruction, are not typically observed in reproductive tissue of SE infected hens. Therefore, SE cells colonizing the reproductive tissue are unlikely to be growing exponentially.

We further visualize that, because of the greater amount of time before lay, cells deposited earlier in egg formation (*Ey* or *Ev* or *Eac* contamination) would experience more growth than SE cells deposited later in egg formation (*Eaf* or *Es* or *Ep* contamination.) Moreover, there might be features of the albumen surrounding the yolk that would enhance SE growth. This latter possibility is supported by data from Humphrey and Whitehead[7] indicating relative SE growth in albumen near the yolk is greater than that in albumen further from the yolk. However, others have reported "no general correlation" of growth of SE and other *Salmonella* strains in albumen incubated in the presence or absence of yolk.[8] The reasons for these results are not clear.

We visualize that after the egg is laid, the pH rises and the environment changes. Any limited SE growth that might have occurred while the egg was forming in the hen will likely be inhibited due to the altered environment. Through a mechanism that is explained in Attachment 1 to Annex E, the bacterial cells, even in the iron-depleted environment of the albumen and the alkaline pH, are able to grow to significant amounts.[9,10] But the details of this growth are unknown. Based on the above discussion, we expect SE cells would need to go through a lag phase before growing exponentially. But the length of this lag phase is not known. Would the amount of SE growth (or the possibility of growth) depend upon the nature of the contamination – *Eac* or *Eaf* – and the numbers in the initial contamination, so that perhaps certain phenomena that are needed for growth only happen under certain circumstances? The above picture allows us to rationalize or explain observed bimodal distributions of results from studies of inoculated and naturally contaminated eggs. The two subpopulations identified represent two different sets of events that would lead to stark differences among the results. For example, the observed high levels of SE may reflect *Eac* contaminations that could grow within the albumen near the yolk before lay while the albumen pH is neutral; the low levels of SE may reflect *Eaf* contaminations for which negligible growth is expected in albumen further away from the yolk, over the shorter interval before lay.

It is important to recognize that the contaminated eggs in experiments for which data are used extensively for determining values of parameters of exposure assessment models were inoculated with stationary phase SE culture preparations.[10,11] Predictive microbiology research supports the premise that the duration of the lag phase is influenced strongly by the condition of the inoculum. As discussed above, in the natural setting, before the egg is laid, SE within the egg could have experienced some limited growth due to the internal reserve of nutrients within the SE cell. However, in time, as explained in Attachment 1, SE is able to utilize the iron that is available in the environment, and begin to grow again. We believe the lag phase of these cells before this latter growth would be shorter than that of the inoculated cells that were in stationary

phase. The biological reason for longer expected lags may be the need for physiological adjustment by SE from the nutrient-rich conditions of culture broth to the more stressful environment of egg albumen. Both dynamic pH and competition for free iron could be associated with longer lags in experimentally inoculated eggs than those for naturally infected hens. In either case, though, the lag phase durations are not known. "State of knowledge" assumptions were thus made concerning the duration of lag phase, as explained in later sections. More research is needed in this area. In the risk assessment, the assumed lag times for cells naturally contaminating eggs was assumed to be shorter than those used in laboratory experiments.

High levels of SE in contaminated egg at lay

Two developing theories are now relevant to our considerations of SE levels in eggs at lay: 1) quorum sensing, and 2) aggregation of cells into colonies or clusters that exhibit swarming or coordinated behavior. These theories explain why high levels of SE can occur in contaminated eggs.

1) Quorum sensing is a mechanism by which cells, within a densely populated area, are able to share resources that enable them to benefit mutually from each other, in this case, are able to enhance their growth. This phenomenon explains why, for example, lag phase duration could be considerably shorter when there is a high density of cells compared to the duration when there is a low density of cells. Quorum sensing is thought possible because bacterial populations activate density-dependent transcription of target genes only upon accumulation of small molecules, termed autoinducers, which are secreted into the microenvironment. Evidence for the theory of quorum sensing supports this mechanism as a broadly conserved or common characteristic of bacterial pathogens of plants and animals. Pathogenic bacteria, including SE, express small diffusible compounds that are secreted into the environment, such as acyl homoserine lactones (AHLs) that function as signals for sensing bacterial density. Alterations in gene expression by density cues are crucial to appropriate expression of specific sequences of virulence genes for invasion by SE of tissues of various hosts, including rodents, hens, and humans. The SE strains expressing AHLs also grow to higher densities than those that do not express AHLs, and thus can be more virulent in chicks and more efficient in causing egg contamination.[12] Even greater efficiency in contaminating eggs was demonstrated in strains that express both AHLs and the glycosylated high molecular mass (HMM, synonymous with previously defined HMW) lipopolysaccharide associated with extracellular matrices or biofilms.[12]

2) Swarming, or aggregation, refers to cells moving together, much as a pack of wolves during the hunt. Cells expressing HMM LPS produce biofilms enriched in flagella and fimbriae that enable swarming behavior. Guard-Petter[12] cited studies with diverse enteropathogens that correlated swarming phenotypes expressing HMM LPS with enhanced virulence and migration of multicellular aggregates across surfaces. The combination of these

characteristics of SE variants producing AHLs and HMM LPS was only recently reported for SE, and greater potential for egg contamination was verified experimentally in mixed phenotype SE infections.[13]

Yolk membrane breakdown (YMB)

After the egg is laid, through the process of osmosis, water seeps into and enlarges the yolk. This allows yolk material, particularly iron or other nutrients, to seep outside the yolk and become available to SE cells in the albumen or those that are lodged on the vitelline membrane. There could be some growth enhancement of SE due to even a small seepage of yolk material. In time, the membrane weakens until a point is reached where there is more free exchange of material between the albumen and the yolk, upon which SE can grow and divide rapidly. A primary question is how quickly this latter event, "yolk membrane breakdown" or YMB, happens in an egg. The model developed for this risk assessment assumes that the duration of YMB is short, and thus models the event of YMB for a given egg as occurring at a specific time. At that time, the kinetics of *Salmonella* growth in yolk material begin to operate. States of knowledge assumptions were made concerning the lag phase duration before SE begins to grow rapidly. It is evident that the likelihood of this event is temperature dependent, as well as dependent on the levels of *Salmonella* in the egg and their location within the egg. Unfortunately, direct information, particularly of the effects of the latter two factors, is not available.

UNCERTAINTY IN THE RISK ASSESSMENTS

A risk assessment can be thought of as a collection of probability distributions with links between them, representing the pathways that the hazardous agent takes in reaching the consumer (in this case). The distributions describe the possible values of variables that are directly or indirectly related to the hazardous agent or its pathway. For example, variables in these risk assessments included the number of *Salmonella* spp. cells in eggs at some specific time and the amount of egg product consumed at a meal. To define a probability distribution for a variable, a well-defined population (people, eggs, and so forth) to which the values of the variables apply, must be identified.

In all cases in these risk assessments, "variable" refers to a random variable that can take on different values for units or objects of a well-defined population, where the frequency of the possible values of that variable within the population are determined by a probability distribution. This definition is meant to include the degenerate case when there is only one possible value for the variable, usually determined by an assumption. For example, the lethality for a given process may be assumed constant for a given scenario of a risk assessment. The word "variability" for a variable then refers to the distribution of that variable over a well-defined population; to determine the variability of a variable is essentially the same as determining the distribution of that variable.

Before proceeding with this discussion, the concept of a parameter, as it is used in the following discussion needs to be explained. In common usage, parameter often refers to a numeric constant. However, parameters refer to any type of object whose values or specific identities determine the characteristic or actions or results of something – in this case, the

calculations of these risk assessments. Clearly then, functions and "populations" are parameters of a risk assessment, since the estimated risk depends upon the functions and populations considered; change the functions and populations, and the risk changes. When the true population is not known and data from other populations are available, then selecting the "population" to use introduces potential biases and thus introduces uncertainty. Examples of this occur when, for example, animal data are used to "represent" dose-response for humans, or, as in these risk assessments, spent hen data are used as a proxy for commercial hens.

Parameters in these risk assessments always refer to entities (usually constant numbers) that affect the calculation of risk. For example, the parameter a could be the characterization that the variable x has a normal distribution with mean a; if the value of a changes, the distribution of x changes. In a risk assessment, values of parameters are assumed by some means, and the uncertainty of the assumed value reflects, in some sense, the degree of knowledge of the assumed value. A confusion of terminology arises when one wants to consider the variable x as a parameter; that is to say, treat it as a constant, and associate an uncertainty to it based on the distribution associated with x. In part, we are presenting this discussion to help avoid such confusion.

Typically, perfect knowledge of the "true" distribution of a variable is unachievable. Rather, the distributions are estimated by a variety of methods, depending upon the information that is available. Two methods are germane to this discussion: Method 1) probability distributions are estimated through a statistical analysis of data that are, in some well defined way, "representative" of the population being studied; Method 2) an assessment of anecdotal evidence based on perceptions of what is or might be, ideally from individuals who have had experience with the variable of concern.

In the following, the nomenclature that is used to distinguish values of parameters that are estimated by the two ways discussed above is given. The assumption for Method 1 is that data are collected and represent, in a probabilistic fashion, a well defined population so that the values of the data can be said to be "stochastic" realizations of some random variable. Statistical procedures can be applied to the data to derive estimates of the values of the relevant parameters that determine or characterize the distribution. For the purposes of these risk assessments, parameters that are used to characterize the distribution that are estimated from these data are termed stochastic parameters.

Procedures have been devised to assess the accuracy of an estimated parameter from collected data. This assessment of the accuracy reflects the "uncertainty" of the estimated values of the parameters. The uncertainty so derived is referred to as "stochastic uncertainty."[14] Thus, when distributions for some variable are determined from data assumed to be probabilistically representative of some well-defined population, there is a clear distinction between what is termed variability and uncertainty; the predicate "stochastic" is attached to the parameters and the uncertainties associated with the estimation of possible values of the parameters. For example, if $\therefore$ is a parameter whose value is statistically estimated from data, then $\therefore$ is referred to as a stochastic parameter, and the uncertainty of its values is referred to as stochastic uncertainty.

On the other hand, not all parameter values can be estimated by Method 1. The determination or assumption for the values may be based on the opinions of experts, with the possible aid of only anecdotal data. In this sense, the values determined for parameters depend strictly on one's "state of knowledge;"[14] thus this phrase is the predicate that is attached to such parameters so determined. That is, a parameter is a "state of knowledge" parameter when its values are not

determined from probabilistic representative data using statistical procedures of estimation. In such a situation, it is not possible to make an assessment of the accuracy of the assumed values in the same way that such an assessment is made from data that are representative of a well-defined population. Rather, the assessment of accuracy is based on the same type of judgment that is used to derive the parameter's estimate. Consequently, there is no clear distinction between the assumed values and the assessment of the accuracy of the assumed values. In this situation, the uncertainty is termed "state of knowledge" uncertainty; a "likelihood" of the possible values of the parameters determined in this fashion does not exist, at least in the same way that such likelihood exists for the assessment of stochastic parameters. Rather the assessment and the assigned likelihoods are subjectively determined, dependent upon the beliefs of the people who made the evaluation. Consequently, in this situation, if possible, we have specified a set of values or a distribution we believe corresponds to the distribution of the variable. If distinct values are identified, reflecting the "uncertainty" of possible values for a parameter, then the risk assessment is computed separately for each of the distinct values, at least theoretically.

The following points of clarification that relate to these risk assessments are needed.

1) There exist some parameters for a distribution of a variable or set of variables that are stochastic and some that are state of knowledge. In this case, the risk assessments assumed values for the state of knowledge parameter, and then estimated, conditional on these values, the values of the parameters, with their attendant uncertainty.

2) Related to the above, the functions that were used to represent a distribution for a variable or relationships between variables were parameters, for these risk assessments in all cases, except for the consumption model, were considered states of knowledge parameters. For some variables, several functional forms were compared; based on some measure of goodness of fit or other considerations, one of the functions was chosen to represent the distribution for that variable, or to describe a relationship between variables. However, in some cases, information was not available to make such comparisons; thus, one function was chosen, based on a common practice (e. g., a normal distribution) or as an accepted default (e.g., beta-Poisson for dose response). In one case, a clear selection could not be made, thus two functions were used in these risk assessments; the risk assessments were performed using one function and then repeated using the other so to account for uncertainty of this parameter. However, with this one exception, the uncertainty of these risk assessments does not include uncertainty due to selection of the functional forms of distributions.

3) The population that is represented by data can also be thought of as a state of knowledge parameter, as discussed above, while the parameters that define the distribution of the variables associated with the population are stochastic. In other words, there exists data representing a population that is not the same as the population for which a distribution is desired. In all such cases for

these risk assessments, an uncertainty associated with this parameter (the proxy population) regarding its relationship to the desired population was not accounted for. For example, the data from the USDA spent hen survey,[15] which represents the population of spent hens, does not represent commercial egg laying hens, so that the validity of using derived distributions from that data to estimate distributions for commercial hens is based on judgment. In the risk assessments no procedure was used that accounts for the uncertainty of these types of judgment.

4) Uncertainty calculations, of either type, were made with almost all parameters that characterize probability functions of functions that describe relationships between variables identified in the risk assessments. A primary exception is the distribution of the amount of egg consumed, for which standard errors of the computed percentiles are not included in these risk assessments.

5) Stochastic uncertainties for estimated values of stochastic parameters are characterized by assigning "probability" distributions to possible values for the parameters. For the risk assessments the distributions were determined by using asymptotic normal distributions used for approximating confidence regions for estimates of parameter values or by using a bootstrap procedure.

State of knowledge uncertainty implies a set of possible values for parameters that are determined by judgment. To determine the magnitude of this type of uncertainty for the outputs of the risk assessments, we defined subsets of assumed values from the set of possible values. For each subset, risk calculations were made, which included the estimated probabilities of adverse events and other desired outputs of the risk assessments, together with attending stochastic uncertainty evaluations, expressed as confidence intervals. This can lead to an enormous number of calculations. To limit the number of calculations, one procedure is to choose values that represent the extremes of risk and the midpoint within the range of the possible values for the identified parameters (if possible), and compute the risks for these combinations. More involved calculations could be made with the purpose of finding a functional relationship between the possible values of the parameters and the risks. In effect, the output of these types of calculations can be thought of as multivariate, with fixed independent variables (representing the possible values of the state of knowledge parameters).

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