Annex I

FAO/WHO Hazard Characterization of *Salmonella* spp.

Extracted from the FAO/WHO Risk Assessment of *Salmonella* spp. in broilers and eggs and reprinted with permission.

Risk Assessment Drafting Group

Anna Lammerding
Microbial Food Safety, Risk Assessment, Health Protection Branch, Health Canada, Canada

Aamir M. Fazil
Health Protection Branch, Health Canada, Ontario, Canada.

Eric Ebel
United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, United States of America

Fumiko Kasuga
National Institute of Infectious Diseases, Department of Biomedical Food Research, Japan.

Louise Kelly
Department of Risk Research, Veterinary Laboratories Agency, United Kingdom

Wayne Anderson
Food Safety Authority of Ireland, Ireland

Emma Snary
Department of Risk Research, Veterinary Laboratories Agency, United Kingdom

Roberta Morales
Research Triangle Institute, Senior Scientist, Health and Human Resource Economics, Centre for Economic Research, United States of America

Andrea Vicari
FAHRM, College of Veterinary Medicine, North Carolina State University, United States of America

Wayne Schlosser
United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, United States of America

Shigeki Yamamoto
National Institute of Infectious Diseases, Department of Biomedical Food Research, Japan.

Reviewers

The risk assessment was reviewed both during and after its elaboration including expert consultations, selected peer-reviewers and members of the public in response to a call for public comments.

Participants of expert consultations

Amma Anandavally, Export Quality Control Laboratory, Cochin, India.

Robert Buchanan, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, United States of America.

Olivier Cerf, Ecole Nationale Vétérinaire d'Alfort (ENVA), Maisons-Alfort Cedex, France.

Jean-Yves D'Aoust, Health Protection Branch, Health Canada, Canada.

Paw Dalgaard, Danish Institute for Fisheries Research, Ministry of Food Agriculture and Fisheries, Denmark.

Michael Doyle, Center for Food Safety, University of Georgia, United States of America.

Emilio Esteban, Food Safety Initiative Activity, Centres for Disease Control and Prevention, United States of America.

Lone Gram, Danish Institute of Fisheries Research, Department of Seafood Research, Technical University of Denmark, Denmark.

Inocencio Higuera Ciapara, Research Centre for food and Development (CIAD), Mexico

John Andrew Hudson, The Institute of Environmental Science and Research Ltd, New Zealand

David Jordan, New South Wales Agriculture, Wollongbar Agricultural Institute, Australia

Jean-Louis Jouve, Principle Administrator - DG XXIV, Brussels, Belgium.

Julia A. Kiehlbauch, Microbiology Consultant, United States of America.

Susumu Kumagai, The University of Tokyo, Graduate School of Agriculture and Life Sciences, Japan.

Roland Lindqvist, National Food Administration, Sweden.

Xiumei Liu, Department of Microbiology and Natural Toxins, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventative Medicine, Ministry of Health, China

Carol Maczka, United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, United States of America.

Patience Mensah, Noguchi Memorial Institute for Medical Research, University of Ghana, Ghana.

George Nasinyama, Department of Epidemiology and Food Safety, Faculty of Veterinary Medicine, Makerere University, Uganda.

Gregory Paoli, Decisionalysis Risk Consultants Inc., Canada.

Irma N.G. Rivera, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil.

Son Radu, Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia, Malaysia.

Tom Ross, University of Tasmania, School of Agricultural Science, Australia

Dulce Maria Tocchetto Schuch, Agriculture Ministry, Laboratorio Regional de Apoio Animal - Lara / RS, Brazil

Eystein Skjerve, The Norwegian School of Veterinary Science, Department of Pharmacology, Microbiology and Food Hygiene, Norway.

Ewen C. D. Todd, The National Food Safety & Toxicology Center, Michigan State University, United States of America.

Robert Bruce Tompkin, ConAgra Inc., United States of America.

Suzanne Van Gerwen, Microbiology and Preservation Unit, Unilever Research, The Netherlands.

Kaye Wachsmuth, Office of Public Health and Science, United States Department of Agriculture, Food Safety and Inspection Service, United States of America.

Helene Wahlström, National Veterinary Institute, Sweden.

Richard C. Whiting, Food and Drug Administration, Center for Food Safety and Applied Nutrition, United States of America.

Charles Yoe, Department of Economics, College of Notre Dame of Maryland, United States of America.

Reviewers in response to call for public comment

The United States Food and Drug Administration
The Industry Council for Development
Prof. O. O. Komolafe, Department of Microbiology, College of Medicine, Malawi

Aclnowledgement
Allan Hogue (Office of Public Health and Science, United States Department of Agriculture, Food Safety and Inspection Service, United States of America)

Hazard Characterization for *Salmonella* spp.

SUMMARY

This section reviews the basic characteristics of the organism, human host factors, and composition factors of the food matrix that influence the outcome of exposure to nontyphoidal *Salmonella enterica*. Human volunteer feeding trial data for various *Salmonella* serotypes and dose-response models that have been developed based on those studies are reviewed. Limitations in the results from human feeding trials are discussed. Additional data were collected from salmonellosis outbreak reports that provided detailed information on parameters such as the numbers of the pathogen in the contaminated food, approximate amount of food eaten, numbers of people who consumed the food, numbers of people exposed who developed the clinical symptoms of acute gastroenteritis, age information, and, in some cases, prior health information. The existing dose-response models were compared to the outbreak data as a validation step. These models failed to adequately represent the observed outbreak data. Consequently, a new dose-response model was developed, based on the outbreak data, and was used with exposure assessment information for eggs and broiler chickens to derive the risk estimates. In addition, an analysis of the outbreak data was done to attempt to derive quantitative estimates for the effect of host age and *Salmonella* serotype on the probability of acute gastroenteritis. No differentiation could be made on the basis of the dose-response outbreak data available at this time. The dose-response relationship derived from the outbreak data measured the host response in terms of acute gastroenteritis. Follow-up patient information on progression of the primary illness to more severe consequences was not detailed in the outbreak reports; in addition, the severity of illness, i.e. severity characterized by hospitalization, bacteremia, reactive arthritis, other symptoms or death, is often complicated by factors that are difficult to quantify, and hence the corresponding risk estimates were not calculated.

ORGANSISM, HOST AND MATRIX CHARACTERISITCS

Characteristics of the Organism

In order for infection with nontyphoid *Salmonella* to occur, the organism must survive a rather hostile environment. It must adapt to differences in growth conditions between the outside environment and the host and within highly variable microenvironments within the host. The invasive journey towards illness in the host must negotiate distinct temperature differences, osmolarity, oxidation-reduction potentials, environmental iron concentrations, pH and organic and inorganic nutrient environments (Slauch *et al*., 1997). An infective *Salmonella* must then survive peristalsis, the epithelial surface and the host immune response.

Non-typhoid *Salmonellae* possessing certain adaptive characteristics are more likely to produce foodborne disease. First, they must be acid tolerant to survive the pH of the stomach. They must also be able to attach themselves to and invade the intestinal epithelia and Peyer's patches (D'Aoust, 1997). Bacterial virulence factors include those that promote adhesion to host cells in the intestines: specific fimbriae, chromosome-coded bacterial surface adhesins, hemagglutinins,

and epithelial cell induction of bacterial polypeptides which can promote colonization and adhesion.

Resistance of *Salmonellae* to lytic action of complement varies with the length of the O side chains of lipopolysaccharide (LPS) molecules (D'Aoust, 1991). Smooth varieties are more resistant than rough types. O side chains of the LPS also have been shown to affect invasiveness and enterotoxin production (Murray, 1986).

Siderophores, which chelate iron, are necessary for the accumulation of sufficient environmental iron to allow growth of *Salmonellae*. Siderophores include hydroxamate, phenolate, and catechol types. Porins are hydrophobic bacterial cell proteins which enhance the virulence of *Salmonella* by repression of macrophage and polymorphonuclear-dependent phagocytosis. *Salmonella* porins may however have a limited importance in pathogenicity. Chromosomal determinants include specific virulence genes whose potential for action is tightly controlled by regulatory genes. Expression of the genes is determined by the environment and invasion occurs by the two-component regulatory system PhoPQ which enables survival of *Salmonellae* within the hostile environment of phagocytes (Slauch *et al*., 1997).

Virulence plasmids in the range of 50-100 kb have been associated with the ability to spread after colonization, invasion of the intestine, ability to grow in the spleen, and a general suppression of the host immune response (Slauch *et al*., 1997). The presence of virulence plasmids in *Salmonellae* is limited. Chiu *et al* (1999) studied virulence plasmids in 436 clinical human samples in Taiwan: 287 isolates were from faeces, 122 from blood and the remaining were isolated from other sites. Sixty-six percent of the non-faecal isolates compared with 40% of the faecal isolates contained a virulence plasmid. All the isolates (n = 50) of the three highly invasive serotypes - S. Enteritidis, *S. dublin* and *S. choleraesuis* contained virulence plasmids. Virulence plasmids have also been confirmed in *S. typhimurium, S. gallinarum-pullorum* and *S. abortusovis*, but are notably absent in *S. typhi*, which is host-adapted and highly infectious.

Other factors that affect the ability of the organism to cause disease include the presence of cytotoxins and diarrhoeagenic enterotoxins. The enterotoxin is released into the lumen of the intestine and results in the loss of intestinal fluids (D'Aoust, 1991).

Antimicrobial resistance can have two effects on the outcome of exposure: there can be an accompanying change in the virulence of the organism, and/or there can be a poorer response to treatment because of the empiric choice of an antimicrobial to which the organism is resistant (Travers and Barza, 2002). An increase in virulence could result from linkage of resistance factors to other virulence genes, such as those for adherence, invasion and toxin production. A study by the U.S. Centers for Disease Control and Prevention (Lee *et al*., 1994) revealed that subjects with infections caused by antimicrobial-resistant *Salmonella* were significantly more likely to be hospitalized than those with antimicrobial-susceptible infections (35% vs. 27%, P = .006) and this difference persisted even after correction for underlying illness. Patients infected with resistant strains also tended to be ill longer (median, 10 days vs. 8 days) and hospitalized longer (median, 5 vs. 4 days). Most subjects were treated with an agent to which the organism was susceptible, and therefore the difference in hospitalization rates probably reflected increased virulence of the infecting organism rather than inappropriate choice of treatment. Thus, the data

suggest that antimicrobial-resistant strains are somewhat more virulent than susceptible strains, in that they cause more prolonged or more severe illness than do antimicrobial-susceptible strains (Travers and Barza, 2002).

Two potentially confounding factors in the study were the host susceptibility in terms of age, and potential differences in virulence between serotypes; neither of which were controlled for in the study (Travers and Barza, 2002). Black race and less than one year of age appeared to be host characteristics associated to a resistant infection; however differences in the distribution of infecting serovars among ethnic and age groups contributed to the occurrence of such effects. Varying food preferences or methods of food preparation might have been at the basis of different serovar distribution. The same consideration may explain the results of an earlier study which associated infection with *S. heidelberg*, penicillin intake, Hispanic origin, more than 60 years of age and antacid use to infection with a multi-resistant *Salmonella* (Riley *et al*., 1984). The conclusion of this study, that multi-resistant organisms are more dependent on host characteristics than sensitive organisms to cause disease, should be qualified accordingly.

Host Characteristics

Literature tends to be biased towards reporting statistically-significant and positive results. This review can only reflect such a bias, and the focus is evidently on host factors for which a statistically significant association to *Salmonella* gastroenteritis and related complications has been reported. Where clear indication on a non-significant finding is made in the original study, such a finding is also reported. Also, since not all studies considered the same factors, the significance of a factor in a given study may merely depend on the presence or absence of other ones. For instance, while a Swiss study considered travel abroad an important source of resistant *Salmonellae* (Schmid *et al*., 1996), such an association was not seen in a United States study (Lee *et al*., 1994). Such apparent inconsistencies may have various explanations but the discussion of which is beyond the scope of this review.

Host factors that can affect the outcome of exposure to the pathogen by ingestion, and which are considered in this review are the following:

➢ Demographic and socioeconomic factors	Age Gender Race & ethnicity Nutritional status Social/economic/environmental factors Travel abroad
➢ Genetic factors	HLA-B27 gene
➢ Health factors	Immune status Previous exposure Concurrent infections Underlying diseases Concurrent medications Pregnancy

Demographic and socioeconomic factors

The following factors are considered in this section: age, gender, race and ethnicity, nutritional status, socioeconomic and environmental factors, and travel abroad.

Age.

A common observation is that the age of patients with *Salmonella* infections is distributed according to a bimodal distribution with peaks in children and elderly. In a Belgian hospital-based study covering isolates for a 20-year period (1973-1992), *S. typhimurium* and *S.* Enteritidis were mainly isolated in children of less than 5 years of age (Le Bacq *et al*., 1994). The age distribution was, however, less accentuated for *S.* Enteritidis than for *S. typhimurium*. Both serovars were more likely to lead to bacteremia in middle and older age groups than in those younger than 5 years of age (Le Bacq *et al*., 1994), confirming a previous observation made in the United States (Blaser and Feldman, 1981). Another study reports on *Salmonella* isolates from a Hong Kong hospital for the period 1982-1993 (Wong *et al*., 1994). Among both intestinal and extraintestinal isolates, *S. typhimurium*, *S. derby* and *S. saintpaul* predominated in infants. In patients older than 1 year of age, *S. derby* and *S. typhimurium* were the most common intestinal isolates, while *S. typhi*, *S. typhimurium* and *S.* Enteritidis were the most common extraintestinal isolates. In a British population-based study, highest age-specific isolation rates for *S.* Enteritidis were observed in children aged under 2 years, and *S. typhimurium* in those under 1 year (Banatvala *et al*., 1999).

In children younger than one year of age, the peak incidence is generally observed in the second and third months (Ryder *et al*., 1976; Davis, 1981). The study from Hong Kong showed, however, a peak at 12 months of age (Wong *et al*., 1994). In a study of Peruvian children, the IgG and IgM titres against *Salmonellae* serogroups AO, BO and DO were higher at 12 months of age than at 2 or 3 months of age, which was interpreted as an indication of acquired immunity (Nguyen *et al*., 1998). In the US, infants under the age of 1 year have the highest reported incidence rate of salmonellosis, with the highest rate in infants 2 months of age, and an abrupt decrease after infancy (Olsen, 2001). Most cases are relatively mild; however, as with the immunocompromised and the elderly, children also face a relatively higher rate of severe outcomes, including death, than other demographic categories. Olsen *et al*. (2001) note a 4-13-fold higher rate of invasive disease in young children than other age groups. Buzby (2001) noted that most children who contract salmonellosis are believed to have been infected from contaminated food, as outbreaks in child-care facilities are rare. However, a matched case-control study among children in France found that cases were more likely to report a case of diarrhoea in the household 3 – 10 days before onset of illness, particularly in the age group less than 1 year old, indicating a role of person-to-person transmission of salmonellosis in infants (Delarocque-Astagneau *et al*., 1998).

It is noted that age associations may be influenced by other factors. In the very young, this includes increased susceptibility upon first exposure, but also that medical care is quickly sought for infants and incidents reported, and they are also more likely to be tested than adults with foodborne illness. The exception is the very elderly with diarrhoea whom may also be expected to be more frequently cultured than other age groups (Banatvala *et al*., 1999). As mentioned earlier, differences in the distribution of infecting serovars among age groups was considered the reason for an apparent increased risk of resistant *Salmonella* infection in infants (Lee *et al*.,

1994). When exposed to the same contaminated food in an outbreak, with the assumption that the individuals involved were exposed to a similar dose, no significant age-related difference was observed between those who became ill, and those who remained healthy (range 1 – 61 years old, median, 30; 12 children under the age of 15-years-old, four of whom became ill) in an outbreak investigated by Rejnmark and colleagues (1997). Similarly, no age-related association with hospitalization was noted in that investigation. Cowden and Noah (1989) postulated that the popularity of eggs and egg dishes in the diets of weaned and older children poses a serious problem. This suggests an increased rate of exposure to S. Enteritidis. Moreover, age association may reflect behavioral characteristics. For instance, eating snow, sand, or soil - a behavior more likely in children - was found to be associated with infection by *S. typhimurium* O:4-12 (Kapperud *et al*., 1998b). Handling pets, including reptiles, and farm animals, followed by hand-to-contact without washing increases exposure opportunities.

Gender.

In terms of number of isolates, several studies indicate that men seem to be generally more affected than women in a several studies. A male-to-female ratio of 1.1 has been reported on various occasions (Blaser and Feldman, 1981; Le Bacq *et al*., 1994; Wong *et al*., 1994). However, in other studies, the isolation rate for women exceeded that for men between the ages 20 and 74 year old, although boys 15 years or under had a slightly higher age-specific isolation rate than girls (Olsen *et al*., 2001). The significance of such a finding does not appear to have been addressed. Several factors, such as proportion of the two genders as well as different age distributions for males and females within a country or hospital catchment area, may play an important role. In the evaluation of a single study, it should be pointed out that the occurrence of other factors, e.g., use of antacids or pregnancy, relates to one gender more often or exclusively and gender may thus have the effect of a confounder. Furthermore, differences in food handling practices and hygiene during food preparation and/or amount of food consumed may also be contributors to any apparent gender differences.

Race and ethnicity.

The potential role of race and ethnicity has seldom been considered. As mentioned above, an association with black race and Hispanic origin was reported for resistant *Salmonella* infections (Lee *et al*., 1994; Riley *et al*., 1984). In the former case, the association was explained by differences in the distribution of infecting serovars among ethnic groups, which in turn depended on varying food preferences or methods of food preparation.

Nutritional status.

An association between altered nutritional status and acute gastroenteritis has been shown in AIDS patients (Tacconelli *et al*., 1998). Apart from this report, no direct reference to the role of nutritional status was found in the literature.

Social/economic/environmental factors.

Isolation rates of several *Salmonella* serovars among groups of different socioeconomic extraction have been compared on the basis of the Townsend score, an index for deprivation (Banatvala *et al*., 1999). While isolation rates for *S. typhimurium* were not related to the Townsend score, highest isolation rates of *S.* Enteritidis were observed in more prosperous areas.

It was advanced that populations living in such areas more frequently ingested vehicles harboring *S.* Enteritidis.

Sanitation deficiencies have been associated with high rates of enteric disease but direct reference to the potential role of *Salmonella* spp. is scarce. In the 1950s, lack of sanitation, poor housing, limited water supply and poor personal hygiene were associated with high *Shigella* rates in Guatemala (Beck *et al*., 1957). A similar observation was made in the United States where, in areas of inadequate sanitary facilities, poor housing and low income, *Shigella* infections were the major causes of diarrhoeal disease. In particular, there were nearly twice as many cases of diarrhoea among persons living in dwellings having outhouses than among those whose houses had indoor restrooms (Schliessmann *et al*., 1958). In certain Guatemalan villages, the habits of the people and the density of the population were found to be more important determinants than type of housing (Bruch *et al*., 1963). In a study conducted in Panama, six representative types of dwellings were considered as an index of social and economic influences on the prevalence of enteric pathogens among infants with diarrhoeal disease (Kourany and Vasquez, 1969). Each dwelling type differed characteristically from one another but five of the six types were considered substandard and their occupants were of low socioeconomic status. Infection rates for enteropathogenic *Escherichia coli*, *Shigella* and *Salmonella* among infants from the various groups of substandard dwellings ranged from 6.0 to 10.2%, in contrast to the zero infection rate observed in infants from the better-type housing. It is worth noting that the literature on sanitation and housing was mainly published in the 1950's and 1960's. It is possible that safety improvement in the water supply consequent to economic development has sensibly diminished the importance of those factors in several countries.

A French study on sporadic *S.* Enteritidis infections in children investigated the influence of diarrhoea in another household member in the 3 to 10 days before a child shows clinical symptoms. The strength of the association with such a factor appeared stronger for cases in infants (1 year of age or less) as compared to cases in children between 1 and 5 years of age (Delarocque-Astagneau *et al*., 1998). On the basis of this observation, as well as other results of the study, it was postulated that *S.* Enteritidis infection in children of less than 1 year of age may arise from person-to-person contact, while children between 1 and 5 years of age contract the infection by consuming raw or undercooked egg products or chicken.

A seasonal pattern in isolations, which generally shows increased rates during hotter months, has been documented. For instance, increased isolation rates for *S.* Enteritidis, *S. typhimurium*, *S. virchow* and *S. newport* were observed in summer in a British study (Banatvala *et al*., 1999). The French study mentioned in the previous paragraph noted that the association between *S.* Enteritidis infection and prolonged storage of eggs was stronger during the summer period.

Travel abroad.

Travel abroad is a risk factor for *Salmonella* gastroenteritis that has been consistently demonstrated in both North America and Europe. For California residents, Kass *et al*. (1992) demonstrated an association between sporadic salmonellosis and travel outside the United States within 3 weeks prior to the onset of illness. Possible variations related to serovar in sporadic salmonellosis were indicated by a study concerning residents of Switzerland (Schmid *et al*., 1996). Having been abroad within three days prior to clinical onset of the illness was found to be

associated with both *S.* Enteritidis and serovars other than Enteritidis, although to a greater extent for the latter case. Little difference was seen between the results of all *S.* Enteritidis phage types (PT) and of *S.* Enteritidis PT4. While most patients with *S.* Enteritidis were more likely to have traveled within Europe, the majority of non-Enteritidis infections might have been imported from outside Europe. Individuals of a British region with *Salmonella* infection were more likely to have reported travel abroad in the week before the onset of illness (Banatvala *et al*., 1999). Frequency of overseas travel between patients with *S.* Enteritidis or *S. typhimurium* was not different, but it was among patients with other serovars. Indication of how travel abroad may lead to salmonellosis can be found in a study referring to residents of Norway (Kapperud *et al*., 1998a). This study suggested that about 90% of the cases from whom a travel history was available had acquired their infection abroad but failed to show an association to either foreign travel among household members or consumption of poultry. However, consumption of poultry purchased abroad during holiday visits to neighboring countries was the only risk factor considered by the study that remained independently associated with the disease. Only cases of *S. typhimurium* allowed for a separate analysis that showed an association with both poultry purchased abroad and foreign travel among household members.

Genetic factors

As far as acute gastroenteritis caused by *Salmonella*, no host genetic factors have been reported. Reports concerning race and ethnicity should be considered in light of eating habits.

The putative association of the gene Human Leukocyte Antigen B27 (HLA-B27) for patients with spondyloarthropathies, in particular reactive arthritis and Reiter's syndrome, has been described. The HLA-B27 gene has a very high prevalence among the native peoples of the circumpolar arctic and sub-arctic regions of Eurasia and North America, and in some regions of Melanesia. In contrast, it is virtually absent among the genetically unmixed native populations of South America, Australia, and among equatorial and southern African Bantus and Sans (Bushmen) (Khan, 1996). Fifty percent of Haida Indians living on the Queen Charlotte Islands of the Canadian province of British Columbia have the HLA-B27 gene, which is the highest prevalence ever observed in a population. The prevalence among Americans of African descent varies between 2 to 3%, while 8% of the Americans of European descent posses the gene (Khan, 1995).

Health factors

Immune status.

The host immune status is, as in any other infectious disease, a very important factor in determining both infection and clinical illness. In general terms, its importance does not seem to have been the direct goal of any formal work and has thus to be indirectly assessed though other factors, e.g. age, or acquired immunodeficiency. Evidence for the development of immunity against nontyphoidal *S. enterica* was recognized in human volunteer experiments (McCullough and Eisele, 1951b). When subjects who became ill on the first challenge were later re-challenged, if they became ill again the severity of the illness was usually less than that of the initial illness, despite higher challenge doses being used. This is in contrast to experiments with typhoid, where vaccines gave protection against low but not high challenges doses, and once clinical disease occurred, the severity was not altered by previous vaccination. Evidence that immunity is partially serotype specific is suggested by the increased incidence of salmonellosis

amongst people who have traveled, and are presumably exposed to different serotypes and strains of *Salmonella* in food and water in other countries. There is a need to examine country- or region-specific population immunities in general to better understand the applicability of dose-response models to populations/countries/regions other than those where dose-response data were acquired.

Concurrent infections.

Persons infected with Human Immunodeficiency Virus (HIV) tend to have recurrent enteric bacterial infections. Such infections are often virulent and associated with extraintestinal disease (Smith *et al*., 1988; Angulo and Swerdlow, 1995). The following six risk factors for enteric salmonellosis have been identified in HIV-infected patients: increasing value on the prognostic scoring system APACHE II (Acute Physiology and Chronic Health Evaluation); altered nutritional status; previous antibiotic therapy; ingestion of undercooked poultry/eggs or contaminated cooked food; previous opportunistic infections; stage C of HIV infection (Tacconelli *et al*., 1998).

Underlying diseases.

The significance of Acquired Immunodeficiency Syndrome (AIDS) has been discussed in the previous paragraph. The risk represented by other underlying conditions was evaluated in a large nosocomial foodborne outbreak of *S.* Enteritidis that occurred in 1987 in New York (Telzak *et al*., 1991). Gastrointestinal and cardiovascular diseases, cancer, diabetes mellitus and alcoholism as well as use of antacids and antibiotics were the factors considered. However, diabetes was the only condition that was independently associated with infection after exposure to the contaminated meal. Although diabetic cases were more likely to develop symptomatic illness compared to non-diabetic, the difference was not statistically significant. Decreased gastric acidity and autonomic neuropathy of the small bowel (which leads to reduced intestinal motility and prolonged gastrointestinal transit time) are the two biologically plausible mechanisms for the increased risk of *S.* Enteritidis infection among diabetics. Among patients with sporadic salmonellosis in Northern California, diabetes mellitus and cardiac disease were both associated to clinical illness (Kass *et al*., 1992). This study contemplated 14 health conditions. Non-gastrointestinal medical conditions and, to a larger extent, a recent history of gastrointestinal disorder was associated with sporadic *S. typhimurium* O:4-12 infection in Norway (Kapperud *et al*., 1998b). It was however noted that physicians are more likely to require a stool culture from patients with preceding illness. In a British epidemiologic study, cases of *Salmonella* infection were more likely to report a long-term illness (including gastroduodenal conditions) than controls (Banatvala *et al*., 1999). All individuals with diabetes mellitus, malignancy, or immunodeficiency were cases.

Concurrent medications.

A number of investigations have examined the effects of antacids and prior or concurrent antimicrobial usage as factors influencing likelihood of contracting salmonellosis or affecting the severity of the outcome. The evidence found in the literature concerning their association with human salmonellosis is contrasting. While some studies have shown an association with antacid use (Banatvala *et al*., 1999), others have failed to do so (Telzak *et al*., 1991; Kapperud *et al*., 1998b). A similar situation is found for the use of antibiotics in the weeks/days preceding the infection or disease onset: some studies have demonstrated an association (Pavia *et al*., 1990;

Kass *et al*., 1992; Bellido Blasco *et al*., 1998) but others have not (Telzak *et al*., 1991; Kapperud *et al*., 1998b; Banatvala *et al*., 1999). Having a resistant *Salmonella* infection has been associated with previous antibiotic use (Lee *et al*., 1994). A delay between antimicrobial use and onset of symptoms suggests that the effect may be due to prolonged alteration of the colonic bacterial flora, resulting in decreased resistance to colonization (Pavia *et al*., 1990).

Among the 11 different medical therapies considered by a US North California study on sporadic clinical salmonellosis, which included antacids and antibiotics, only hormonal replacement therapy (principally conjugated estrogen) in older women was found to be associated to clinical salmonellosis (Kass *et al*., 1992). An association between serovars other than *S.* Enteritidis and intake of medications other than antacids was shown in Switzerland (Schmid *et al*., 1996). Regular use of medications was a risk factor for *S. typhimurium* O:4-12 infection in Norway (Kapperud *et al*., 1998b). In the same study, use of antacids and antibiotics were not risk factors.

(v) Pregnancy

There is a little information concerning the effect of salmonellosis on specifically on pregnant women and fetuses or neonates. No studies were found to indicate that pregnant women are at an increased risk for *Salmonella*-induced enteritis. However, when a pregnant woman suffers from foodborne infection the fetus or neonate may also be affected. A recent review by Smith (2002) on *Campylobacter jejuni* infection during pregnancy, summarizes the small amount of available data on the consequences of maternal *C. jejuni* enteritis and/.or bacteremia. Outcomes may include abortion, stillbirth, premature labor, bacteriemic newborn infants, and newborns with diarrhea or bloody diarrhea. Similar outcomes might be expected for some cases of salmonellosis in pregnant women.

Factors Related to the Matrix/Conditions of Ingestion

Empirical observation, mainly from outbreak investigations, shows that foodborne salmonellosis can be related to a variety of food items. Table 1.1 lists major foodborne outbreaks of human salmonellosis and shows the wide range of foods implicated in these outbreaks (D'Aoust, 1997).

TABLE 1.1: MAJOR FOODBORNE OUTBREAKS OF HUMAN SALMONELLOSIS AND IMPLICATED FOOD ITEMS (ADAPTED FROM D'AOUST, 1997)

Year	Country(ies)	Vehicle	Serovar
1973	Canada, United States	Chocolate	*S. eastbourne*
1973	Trinidad	Milk powder	*S. derby*
1974	United States	Potato salad	*S. newport*
1976	Spain	Egg salad	*S. typhimurium*
1976	Australia	Raw milk	*S. typhimurium PT9*
1977	Sweden	Mustard dressing	*S. Enteritidis PT4*
1981	The Netherlands	Salad base	*S. indiana*
1981	Scotland	Raw milk	*S. typhimurium PT204*
1984	Canada	Cheddar cheese	*S. typhimurium PT10*
1984	Canada		*S. typhimurium PT22*
1984	France, England	Liver pate	*S. goldcoast*
1985	United States	Pasteurized milk	*S. typhimurium*
1985	Scotland	Turkey	*S. thompson, S. infantis*
1987	Republic of China	Egg drink	*S. typhimurium*
1987	Norway	Chocolate	*S. typhimurium*
1988	Japan	Cuttlefish	*S. champaign*
1988	Japan	Cooked eggs	*Salmonella spp.*
1988	England	Mayonnaise	*S. typhimurium DT49*
1990	Sweden		*S. Enteritidis*
1991	Germany	Fruit soup	*S. Enteritidis*
1993	France	Mayonnaise	*S. Enteritidis*
1993	Germany	Paprika chips	*S. saintpaul, S. javiana, S. rubislaw*
1994	United States	Ice cream	*S. Enteritidis*
1994	Finland, Sweden	Alfalfa sprouts	*S. bovismorbificans*
1998	United States	Breakfast cereal	*S. agona*
1998	England	Chopped liver	*S. Enteritidis PT4*
1999	United States	Orange juice	*S. muenchen*

Gastric acidity is recognized as an important defense against foodborne pathogens. Pathogen, host and food factors interact in determining whether a sufficient number of bacteria are able to withstand stomach acidity and go on to colonize the gut. Such an interaction appears extremely dynamic. Although *Salmonellae* prefer to grow in neutral pH environments, they have evolved complex, inducible acid survival strategies that allow them to face the dramatic pH fluctuations encountered in nature and during pathogenesis (Bearson *et al*., 1997). While the human stomach normally has a pH of 2, several host factors may cause decreased gastric acidity. Examples reported in the previous section are older age, diabetes mellitus, and use of antacid drugs. As for factors specifically related to food, it appears that a systematic treatment of this topic has not yet been carried out. Circumstantial evidence suggests that the following elements are of particular relevance: amount of ingested food, nutrient composition including fat content of the food, buffering capacity of the food time of the meal, and nature of contamination. The reference to food rather than to food item emphasizes the importance of considering the whole meal.

In a *S. typhimurium* outbreak, it was observed that persons who had eaten two or more pieces of chicken tended to have shorter incubation periods. However, both attack rate and illness severity did not appear to be a function of the amount of chicken consumed. It was concluded that the amount of food consumed provides only a crude estimate of dose because a homogenous distribution of the pathogen among the chicken pieces is unlikely (Glynn and Palmer, 1992). This also means that since infectivity is not uniformly distributed within a food, a larger meal may increase the chances of ingesting an infected portion. D'Aoust (1985) noted that in foodborne outbreaks involving fatty vehicles, relatively low doses can lead to substantial numbers of illness (chocolate: <100 cells of *S. eastbourne*, 50 cells of *S. napoli*; cheddar cheese: 100-500 cells of *S. heidelberg*, 1-6 cells of *S. typhimurium*). Microorganisms trapped in hydrophobic lipid moieties may survive the acidic conditions of the stomach and thus the fat content of contaminated foods may play a significant role in human salmonellosis. In contrast, experimental evidence in rats shows that *Salmonella* infection is not affected by milk fat (Sprong *et al*., 1999). *Salmonellae* were actually protected from acid killing when inoculated onto boiled egg white, a food source high in protein and low in fat (Waterman and Small, 1998). The same study shows that the pH of the microenvironment occupied by the bacteria on the surface of a food source is critical for their survival.

The effect of substrate was studied in volunteers challenged with *Vibrio cholerae* fed in a medium with buffering capacity (Cash *et al*., 1974). The group of subjects that overcame the effect of a bicarbonate vehicle in less than 30 minutes (approximately half of the challenged individuals) experienced a lower attack rate than the group experiencing a prolonged buffering effect. Ingestion of low numbers of *Salmonella* between meals, i.e., on an empty stomach, was associated with an increased attack rate (Mossel and Oei, 1975). It was postulated that at such moments the pyloric barrier would initially fail. The authors also speculated that some food items, such as chocolate and ice cream, are more likely to be ingested between meals and thus lead to illness even with only a few organisms. A protective effect of alcoholic beverages was observed in a *S.* Enteritidis outbreak (Bellido Blasco *et al*., 1996). Besides the direct effect of ethanol on bacteria, alcohol may stimulate secretion of gastric acid. Last but not least, an important factor in determining the survival of bacteria in the stomach may be how uniformly a food is contaminated. Although a uniform distribution is usually assumed, the very nature of bacterial growth in colonies would suggest that agglomerations of bacteria occur within the food. It can be speculated that the outer layers of bacteria would protect the inner ones, allowing some pathogen to survive the gastric passage.

HUMAN FEEDING TRIALS

There have been a total of nine published studies of experimentally induced salmonellosis conducted between 1936 and 1970 using a variety of serotypes and strains. Serotypes and strains used in these series of feeding trials are listed in Table 1.2.

TABLE 1.2: LIST OF HUMAN FEEDING TRIALS THAT HAVE BEEN PERFORMED.

	Serotype	Strains	Reference
1	*S. typhimurium*		(Hormaeche *et al*., 1936)
2	*S. anatum*		(Varela and Olarte, 1942)
3	*S. meleagridis*	I, II & III	(McCullough and Eisele, 1951a)
	S. anatum	I, II & III	(McCullough and Eisele, 1951a)
4	*S. newport*		(McCullough and Eisele, 1951c)
	S. derby		(McCullough and Eisele, 1951c)
	S. bareilly		(McCullough and Eisele, 1951c)
5	*S. pullorum*	I, II, III & IV	(McCullough and Eisele, 1951d)
6	*S. typhi*		(Sprinz *et al*., 1966)
7	*S. sofia & S. bovis-morbificans*		(Mackenzie and Livingstone, 1968)
8	*S. typhi*	Quailes, Zermatt, Ty2V, 0-901	(Hornick *et al*., 1970)
9	*S. typhi*	Quailes	(Woodward, 1980)

Although the list of human feeding trials for *Salmonella* in humans is more extensive than may exist for other bacterial pathogens, some of these studies were deemed to be unsuitable and were not used in further analysis to derive conclusions about the pathogenicity of *Salmonella* in general in humans. The earliest study used 5 subjects who were all fed a dose of approximately 9-$\log_{10}$ in water and all exposed individuals were subsequently infected (Hormaeche *et al*., 1936). In a later study, (Varela and Olarte, 1942) apparently only one volunteer was used and became ill after ingesting a dose of 10-$\log_{10}$ in water. The study conducted by MacKenzie and Livingstone (Mackenzie and Livingstone, 1968) involved a nasal inoculation of approximately 25 cells in one volunteer who subsequently became ill. These three studies were not informative due to the use of only large doses with 100% attack rates, the testing of only one dose with one subject, or the method of inoculation. Studies conducted using *S. typhi* (Sprinz *et al*., 1966; Hornick *et al*., 1970; Woodward, 1980), were considered to be inappropriate in the current analysis, primarily because of the difference between the illnesses caused by typhoid and non-typhoid *Salmonella*e. *S. typhi* is highly invasive and causes typhoid fever, a systemic bacteremic illness as opposed to non-typhoid salmonellosis characterized by gastroenteritis and marked by diarrhoea, fever, and abdominal pain with rare systemic invasion.

The most extensive human feeding trials of non-typhoid *Salmonella* were conducted in the late 1940s to early 1950s (McCullough and Eisele, 1951a; McCullough and Eisele, 1951c; McCullough and Eisele, 1951d). A total of six different *Salmonella* serotypes were used with up to 3-4 different strains of some of the serotypes. The subjects used in the feeding trials were healthy males from a penal institution. Feeding trials using *S. pullorum* I, II, III & IV were considered to be inappropriate for deriving estimates about the infectivity of non-typhoid *Salmonella* for humans, because, as noted by other researchers (Blaser and Newman, 1982; Coleman and Marks, 1998) this is primarily a fowl-adapted strain. It was noted that a dramatically higher dose was required to produce illness using *S. pullorum* and the clinical picture of illness, when it did occur, was characterized by an explosive onset and fast recovery (McCullough and Eisele, 1951d). At dosages producing illness, the organism could only be isolated from the stools for the first day or two and not thereafter. In addition, Fazil (1996) conducted an evaluation of the feeding trial data and found that the dose-response relationship for *S. pullorum* was significantly different from the other strains used in the feeding trials.

In order to evaluate the data derived from the human feeding trials the experimental design used by the researchers is briefly described (McCullough and Eisele, 1951a; McCullough and Eisele, 1951c):

Human Volunteers

- The subjects selected for the experimental feeding trials were healthy males from a penal institution;
- According to the authors, chronic complainers and those who had frequent gastrointestinal disturbances in the past were eliminated from the trials;
- After an initial selection of volunteers, at least three weekly stool cultures were done;
- Only those individuals with no *Salmonella* or other easily confused organisms in the stools were carried further in the experiment;
- An initial serum agglutination test was done against the organism to be administered;
- Subjects that showed a moderate or high agglutination titre against a particular organism were in general not used in the experiments with that species.

Source of *Salmonella* Strains

- Strains of *Salmonella* used in the feeding trials were obtained from market samples of high moisture spray-dried whole egg powder.

Method of feeding

- Cultures for feeding trials were sub-cultured on trypticase soy agar;
- After 24 hours of incubation, the resulting growth was suspended in saline and standardized turbidimetrically;
- The dose was administered in a glass of eggnog shortly following the noon meal;
- A group of men usually consisting of 6, received the same experimental feeding dose;
- Control feedings were provided by eggnog alone or by prior feeding of the test organisms at what the authors observed to be non-infective levels.

Observations after feeding (Figure 1.1)

- Following the feeding, men were interviewed and observed three times a week for a period of two weeks and once a week thereafter;
- Additional visits were made when required by the condition of the volunteer;
- Men were questioned with regard to symptoms;
- Temperatures were recorded;
- Faecal cultures were obtained;
- When indicated, blood counts and cultures were also done;
- Blood samples for agglutination were drawn at weekly intervals for 4 weeks longer;
- Faecal samples were collected and cultures were done on all men 3 times a week for the first 2 weeks, after that once a week until at least three consecutive negative samples had been obtained.

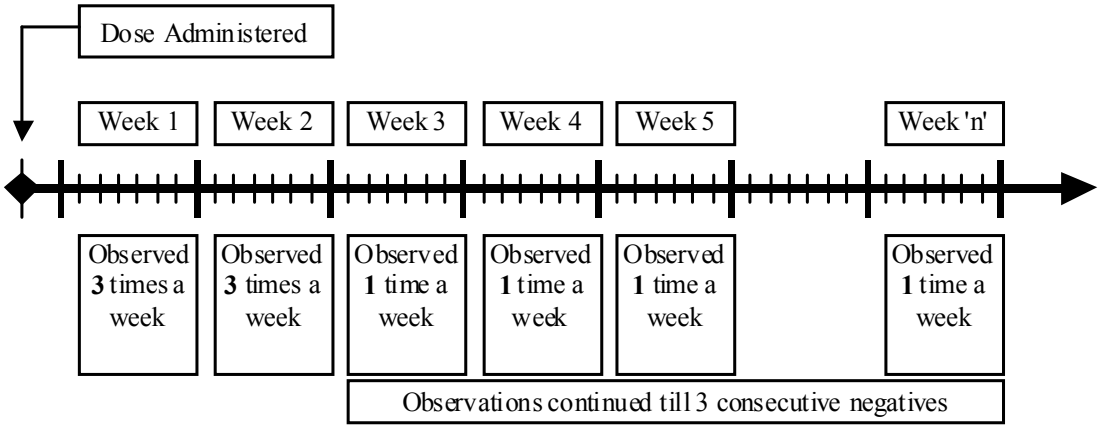

FIGURE 1.1: SCHEMATIC FOR OBSERVATIONS DURING HUMAN FEEDING TRIAL EXPERIMENTS OF MCCULLOUGH AND EISELE (1951A AND 1951C).

Infection definition (faecal shedding)

- Infection was defined as the recovery of the administered strain from faecal samples.

Illness definition criteria

- Illness was characterized by the existence of the following two conditions:
  - Documentation of symptoms
  - Recovery of the organism from stool (infection)
  - And one or more of the following:
    - diarrhoea or vomiting,
    - fever
    - rise in specific agglutination titre
    - or, other unspecified signs.

The feeding trial data have been reviewed and critiqued by various researchers. Blaser and Newman (1982) reviewed the infective dose data for *Salmonella* and identified several deficiencies:

1. The feeding of the pathogen to the volunteers was conducted after their noon meal when gastric acid was probably high;
2. It was observed that over half the volunteers who became ill had earlier been fed lower doses of the same serotype. These earlier feedings may have confounded the results by introducing a degree of immunity thus making infection less likely, or, alternatively the earlier feedings may have had a cumulative effect that made infection more likely;
3. A failure to assess the minimal infective dose;
4. The use of too few volunteers at low doses.

In the United States *Salmonella* Enteritidis in eggs risk assessment report (USDA-FSIS, 1998) additional deficiencies in the feeding trial data were identified:

1. The use of healthy male volunteers could likely underestimate the true pathogenicity to the overall population;
2. The size of the groups used at each of the doses was relatively small, with 18 of the 22 test-doses using less than 6 people;
3. There were no low doses tested. The smallest dose that was tested was greater than 10^4 cfu *Salmonella*;
4. The lowest dose that caused an infection was also the lowest dose tested.

Additional points related to some of the critiques should also be noted. While it is true that the feeding of the dose after the noon meal when gastric acid was high could potentially reduce the estimated infectivity of the pathogen (Blaser and Newman, 1982), the dose was administered using eggnog, a high fat content medium. The eggnog could have conferred a level of protection against the effects of gastric acid thus potentially negating the acid effects. It seems reasonable however to assume that given the fact that the subjects used in the feeding trials were healthy males, the infectivity estimated for this population will be some factor less than for the general population and more so for the more susceptible members of the general population. Overall, the criticisms of the feeding trial data are for the most part fair in their assessment of the potential biases in the results that may be expected.

The human feeding trial, as described earlier, measured both infection and illness. Most dose-response relationships are developed using infection (faecal shedding) as the dependent variable, primarily out of necessity due to the nature of the data. It should be noted that the use of the infection endpoint in deriving a dose-response relationship could introduce a level of conservatism into the dose-response relationship depending on how the conditional dependence of illness, which is essentially the output of ultimate interest, following infection is treated. In the human feeding trial it was also pointed out that approximately 40% of the volunteers that were shedding were reported to be last positive on or before the second day following administration, apparently clearing the infection two days post administration (Coleman and Marks, 1998). These authors noted that there is some ambiguity in estimating infection based on faecal shedding for less than two days. The available data measuring illness as the endpoint is sparse, without any response being observed until a dose of approximately 6-$\log_{10}$. It has been noted (Blaser and Newman, 1982) that the strict criteria used by the researchers to define illness may have resulted in volunteers with mild complaints being classified as asymptomatic excretors rather than ill subjects. Although concerns have been raised as to the experimental design of the human feeding trials, it is appropriate to consider it at this juncture as still holding value in providing a basis upon which to at least start exploring the dose-response relationship.

Tables 1.3 to 1.7 present the original data from the McCullough and Eisele studies. These data are also summarized in Figure 1.2.

TABLE 1.3: FEEDING TRIAL DATA FOR *S. ANATUM I, II* AND *III* (MCCULLOUGH AND EISELE, 1951A)

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. anatum I	1.20E+04	4.08	2	5	0.40
S. anatum I	2.40E+04	4.38	3	6	0.50
S. anatum I	6.60E+04	4.82	4	6	0.67
S. anatum I	9.30E+04	4.97	1	6	0.17
S. anatum I	1.41E+05	5.15	3	6	0.50
S. anatum I	2.56E+05	5.41	5	6	0.83
S. anatum I	5.87E+05	5.77	4	6	0.67
S. anatum I	8.60E+05	5.93	6	6	1.00
S. anatum II	8.90E+04	4.95	5	6	0.83
S. anatum II	4.48E+05	5.65	4	6	0.67
S. anatum II	1.04E+06	6.02	6	6	1.00
S. anatum II	3.90E+06	6.59	4	6	0.67
S. anatum II	1.00E+07	7.00	6	6	1.00
S. anatum II	2.39E+07	7.38	5	6	0.83
S. anatum II	4.45E+07	7.65	6	6	1.00
S. anatum II	6.73E+07	7.83	8	8	1.00
S. anatum III	1.59E+05	5.20	2	6	0.33
S. anatum III	1.26E+06	6.10	6	6	1.00
S. anatum III	4.68E+06	6.67	6	6	1.00

TABLE 1.4: FEEDING TRIAL DATA FOR *S. MELEAGRIDIS I, II* AND *III* (MCCULLOUGH AND EISELE, 1951A)

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. meleagridis I	1.20E+04	4.08	3	6	0.50
S. meleagridis I	2.40E+04	4.38	4	6	0.67
S. meleagridis I	5.20E+04	4.72	3	6	0.50
S. meleagridis I	9.60E+04	4.98	3	6	0.50
S. meleagridis I	1.55E+05	5.19	5	6	0.83
S. meleagridis I	3.00E+05	5.48	6	6	1.00
S. meleagridis I	7.20E+05	5.86	4	5	0.80
S. meleagridis I	1.15E+06	6.06	6	6	1.00
S. meleagridis I	5.50E+06	6.74	5	6	0.83
S. meleagridis I	2.40E+07	7.38	5	5	1.00
S. meleagridis I	5.00E+07	7.70	6	6	1.00
S. meleagridis II	1.00E+06	6.00	6	6	1.00
S. meleagridis II	5.50E+06	6.74	6	6	1.00
S. meleagridis II	1.00E+07	7.00	5	6	0.83
S. meleagridis II	2.00E+07	7.30	6	6	1.00
S. meleagridis II	4.10E+07	7.61	6	6	1.00
S. meleagridis III	1.58E+05	5.20	1	6	0.17
S. meleagridis III	1.50E+06	6.18	5	6	0.83
S. meleagridis III	7.68E+06	6.89	6	6	1.00
S. meleagridis III	1.00E+07	7.00	5	6	0.83

TABLE 1.5: FEEDING TRIAL DATA FOR *S. NEWPORT* (MCCULLOUGH AND EISELE, 1951C)

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. newport	1.52E+05	5.18	3	6	0.50
S. newport	3.85E+05	5.59	6	8	0.75
S. newport	1.35E+06	6.13	6	6	1.00

TABLE 1.6: FEEDING TRIAL DATA FOR *S. BAREILLY* (MCCULLOUGH AND EISELE, 1951C)

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. bareilly	1.25E+05	5.10	5	6	0.83
S. bareilly	6.95E+05	5.84	6	6	1.00
S. bareilly	1.70E+06	6.23	5	6	0.83

TABLE 1.7: FEEDING TRIAL DATA FOR *S. DERBY* (MCCULLOUGH AND EISELE, 1951C)

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. derby	1.39E+05	5.14	3	6	0.50
S. derby	7.05E+05	5.85	4	6	0.67
S. derby	1.66E+06	6.22	4	6	0.67
S. derby	6.40E+06	6.81	3	6	0.50
S. derby	1.50E+07	7.18	4	6	0.67

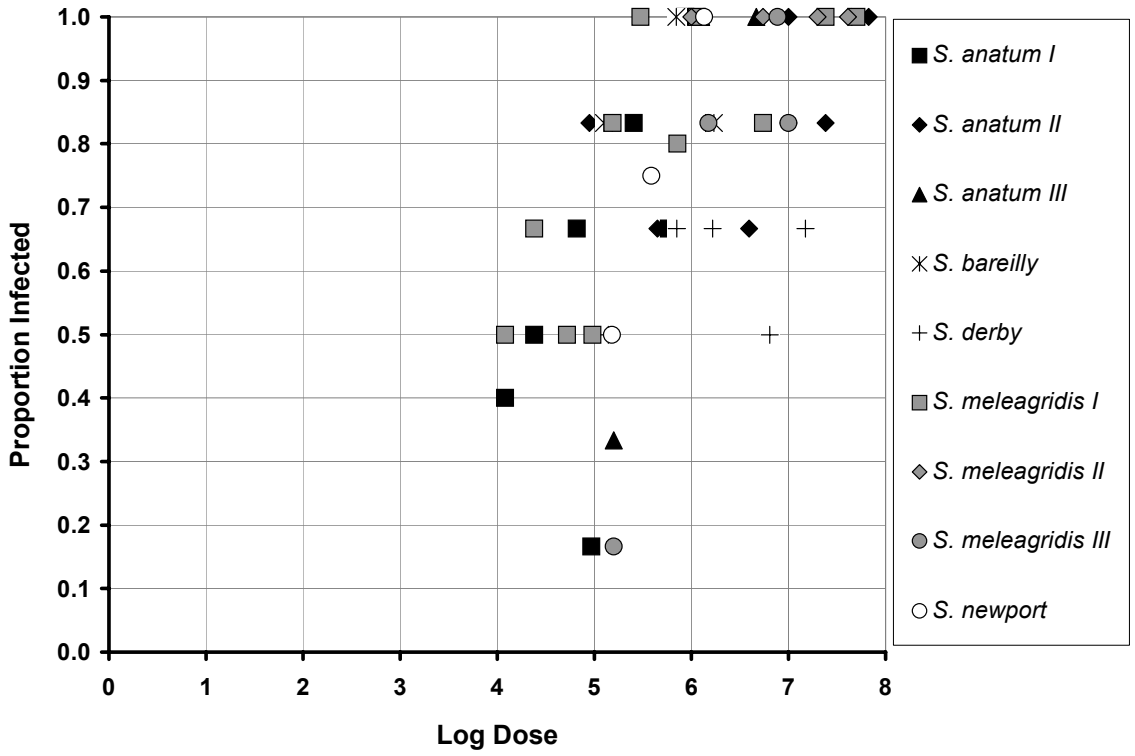

FIGURE 1.2: SUMMARY OF FEEDING TRIAL DATA (MCCULLOUGH AND EISELE, 1951A; 1951C)

It has also been noted that in the feeding trials, some of the volunteers were administered doses more than once. The earlier doses, which were lower and at which no response was observed, may have resulted in either a cumulative or immunity effect. In order to attempt to remove this bias, the doses and subjects at which repeat feedings were conducted were edited out and the data re-evaluated. The edited data for naïve subjects only are presented in Tables 1.8 to 1.12, and summarized in Figure 1.3.

TABLE 1.8: FEEDING TRIAL DATA FOR *S. ANATUM I, II* AND *III* FOR NAÏVE SUBJECTS

Serotype	Dose	$\log_{10}$ Dose	Positive (Inf)	Total	Proportion
S. anatum I	1.20E+04	4.08	2	5	0.40
S. anatum I	6.60E+04	4.82	4	6	0.67
S. anatum I	5.87E+05	5.77	4	6	0.67
S. anatum I	8.60E+05	5.93	4	4	1.00
S. anatum II	8.90E+04	4.95	3	4	0.75
S. anatum II	4.48E+05	5.65	4	6	0.67
S. anatum II	2.39E+07	7.38	3	3	1.00
S. anatum II	4.45E+07	7.65	3	3	1.00
S. anatum III	1.59E+05	5.20	1	3	0.33
S. anatum III	1.26E+06	6.10	6	6	1.00
S. anatum III	4.68E+06	6.67	3	3	1.00

TABLE 1.9. FEEDING TRIAL DATA FOR *S. MELEAGRIDIS I, II* AND *III* FOR NAÏVE SUBJECTS

Serotype	Dose	log_{10} Dose	Positive (Inf)	Total	Proportion
S. meleagridis I	1.20E+04	4.08	3	6	0.50
S. meleagridis I	2.40E+04	4.38	4	6	0.67
S. meleagridis I	5.20E+04	4.72	3	6	0.50
S. meleagridis I	1.15E+06	6.06	6	6	1.00
S. meleagridis I	5.50E+06	6.74	5	6	0.83
S. meleagridis I	2.40E+07	7.38	4	4	1.00
S. meleagridis II	1.00E+06	6.00	6	6	1.00
S. meleagridis II	5.50E+06	6.74	6	6	1.00
S. meleagridis II	2.00E+07	7.30	3	3	1.00
S. meleagridis III *	1.58E+05	5.20	1	3	0.33
S. meleagridis III	1.50E+06	6.18	5	6	0.83
S. meleagridis III	7.68E+06	6.89	4	4	1.00

TABLE 1.10. FEEDING TRIAL DATA FOR *S. NEWPORT* FOR NAÏVE SUBJECTS

Serotype	Dose	log_{10} Dose	Positive (Inf)	Total	Proportion
S. newport	1.52E+05	5.18	3	6	0.50
S. newport	3.85E+05	5.59	4	4	1.00
S. newport	1.35E+06	6.13	3	3	1.00

TABLE 1.11. FEEDING TRIAL DATA FOR *S. BAREILLY* FOR NAÏVE SUBJECTS

Serotype	Dose	log_{10} Dose	Positive (Inf)	Total	Proportion
S. bareilly	1.25E+05	5.10	5	6	0.83
S. bareilly	6.95E+05	5.84	3	3	1.00
S. bareilly	1.70E+06	6.23	3	3	1.00

TABLE 1.12. FEEDING TRIAL DATA FOR *S. DERBY* FOR NAÏVE SUBJECTS

Serotype	Dose	log_{10} Dose	Positive (Inf)	Total	Proportion
S. derby	1.39E+05	5.14	3	6	0.50
S. derby	7.05E+05	5.85	2	3	0.67
S. derby	1.66E+06	6.22	3	4	0.75
*S. derby**	6.40E+06	6.81	1	3	0.33

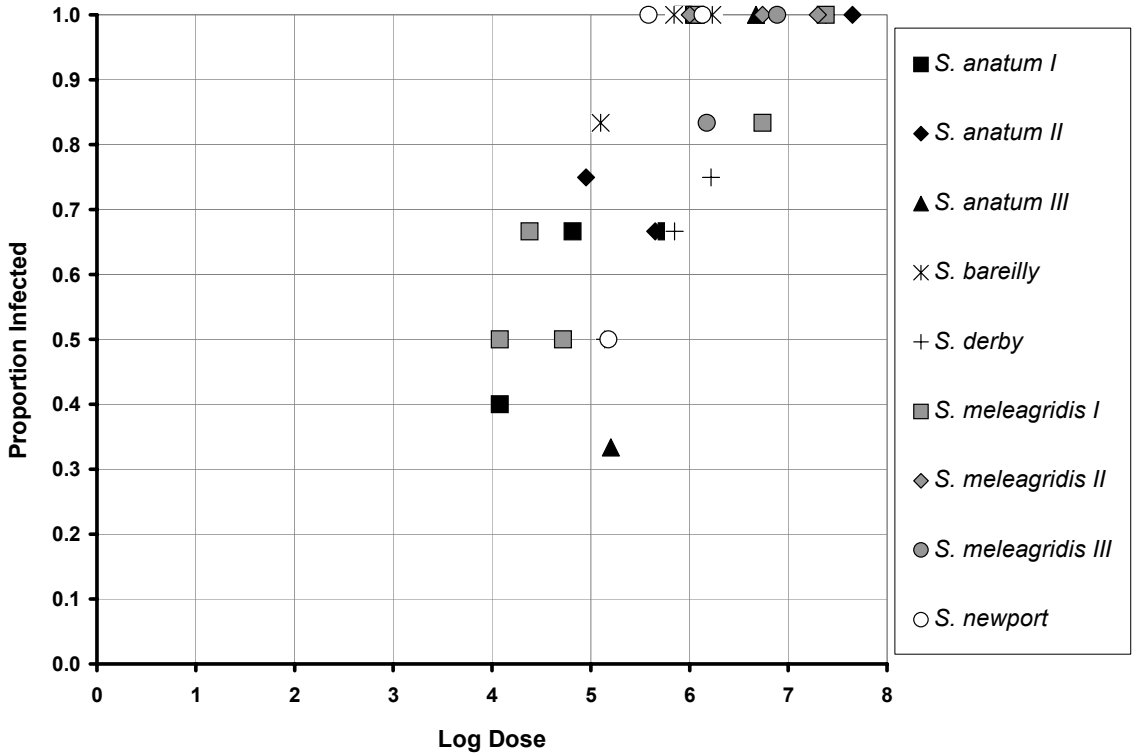

FIGURE 1.3. SUMMARY OF FEEDING TRIAL DATA FOR NAÏVE SUBJECTS.

DOSE RESPONSE ASSESSMENT

This section presents the quantitative information that is available for *Salmonella* spp. infectivity or illness, from which dose-response relationships can be estimated. It is not possible to provide all the details necessary to give a complete coverage of the theory behind the dose-response relationships in this document. However, a comprehensive treatment of dose-response models and assumptions related to the mathematical derivation of the various equations is given in the FAO/WHO Hazard Characterization guidelines document.

Dose-Response Models for *Salmonella*

Several approaches and models to characterize the dose-response relationship for *Salmonella* have been presented in the literature or in official reports and documents. This draft document discusses three different approaches for modeling *Salmonella.* The first model is the beta-Poisson model fit to the human feeding trial data for *Salmonella* (Fazil, 1996). The second model was proposed in the United States *Salmonella* Enteritidis risk assessment (USDA-FSIS, 1998) and was based on the use of a surrogate pathogen to describe the dose-response relationship. The third model, introduced in the Health Canada *Salmonella* Enteritidis risk assessment, used a Weibull dose-response relationship updated to reflect outbreak information using Bayesian techniques. In addition to these models, the current analysis also explores the effect of fitting the beta-Poisson model on the human feeding trial data for naïve subjects only.

Dose-response model fit to non-typhi Salmonella human feeding trial data

The human feeding trial data have been analyzed using the beta-Poisson, lognormal (log-probit) and exponential dose-response functional forms (Fazil, 1996). Three doses in the data set were identified as "outliers" (i.e. *S. anatum* I: 9.3E+5; *S. meleagridis* III: 1.58E+5; *S. derby* - 6.4E+6) and were subsequently removed from the analysis. The analysis concluded that both the lognormal and beta-Poisson functional forms fit the majority of the data. However, based upon theoretical considerations (threshold vs. non-threshold[1]) the beta-Poisson model was proposed as the model to describe the dose-response relationship for *Salmonella*. In addition it was reported that all the serotypes could be adequately described using a single beta-Poisson dose-response curve. The parameters of the beta-Poisson dose-response model for non-typhi *Salmonella* in general were reported as alpha = 0.3126, and beta = 2885. The uncertainty in the parameters was estimated using a bootstrap approach, which generated sets of parameters that satisfied the model fitting conditions. The potential for a greater probability of illness for susceptible and normal populations was not addressed in the analysis.

$$Pill = 1 - \left(1 + \frac{Dose}{\beta}\right)^{-\alpha}$$

Model Used:	**Beta-Poisson**
Parameters:	*Alpha = 0.3126* *Beta = 2885*
Comment:	Uncertainty in the parameters estimated using a bootstrap approach which generated a set of alpha and beta parameters that could be randomly sampled in order to incorporate uncertainty.

Dose-response model fit to non-typhi Salmonella naïve human feeding trial data

The model parameters reported by Fazil (1996) did not consider the effect that multiple feedings may have on the dose-response relationship. As a result, for this present review, the data using only naïve subjects (Tables 1.8 to 1.12 and Figure 1.3) were re-fit to the beta-Poisson model and the parameters for this model were estimated. The data were fit using maximum likelihood techniques, as described by various authors (Haas, 1983; Haas *et al*., 1993; Regli *et al*., 1991; Teunis *et al*., 1996). The parameters of the beta-Poisson dose response model fit to the data for naïve subjects was estimated to be alpha = 0.4047, and beta = 5587. The uncertainty in the parameters was estimated using the bootstrap approach.

[1] Threshold models assume that there is some finite minimum dose below which no response can occur, non-threshold models assume that the minimum possible dose that can cause a response is one cell, even though the probablility may be very low for one cell to successfully survive all the host defenses.

$$Pill = 1 - \left(1 + \frac{Dose}{\beta}\right)^{-\alpha}$$

Model Used:	**Beta-Poisson**
Parameters:	*Alpha = 0.4047* *Beta = 5587*
Comment:	Uncertainty in the parameters estimated using a bootstrap approach which generated a set of alpha and beta parameters that could be randomly sampled in order to incorporate uncertainty.

The beta-Poisson dose-response curves generated using the original dose-response data and the data edited to reflect only naïve subjects are shown in Figure 1.4. Also shown in the figure are the feeding trial data to illustrate the fit to the data.

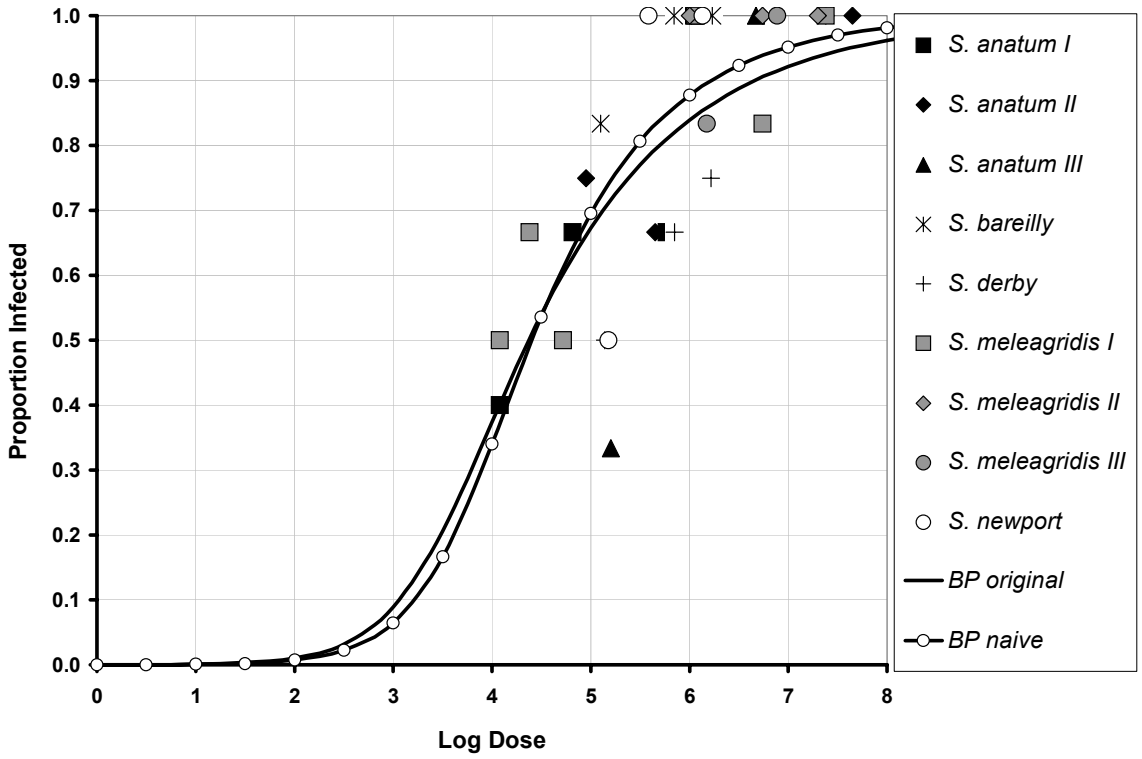

FIGURE 1.4: COMPARISON BETWEEN DOSE-RESPONSE MODEL FIT TO ORIGINAL FEEDING TRIAL DATA AND FEEDING TRIAL DATA FOR NAÏVE SUBJECTS.

As shown in Figure 1.4, both models fit the feeding trial data well and the difference between the curves using the original data and the data that reflects only naïve subjects is small. Interestingly, the curve fit to the naive data tends to estimate a greater probability of infection at doses above approximately 10^4, than the curve fit to the original data, perhaps reflecting a tendency in the data for a slightly greater susceptibility for naive subjects. Within the lower dose regions, the two curves are very similar, and the dose translating to a probability of infection for 50% of the population is virtually identical for the two curves (2.36e4 vs. 2.54e4 for the original and naïve models). The low dose extrapolation for the two dose-response curves was also very similar. As a result of the similarities between the models and the concerns that have been raised about potential immunity or cumulative effects, the beta-Poisson model fit to the data of naïve subjects is used in the remainder of this analysis, as the representation of the human feeding trial data fit to the beta-Poisson model.

USDA/FDA Salmonella Enteritidis Risk Assessment

The hazard characterization in the United States *Salmonella* Enteritidis Risk Assessment (USDA-FSIS, 1998) evaluates the public health impacts of exposure to SE through shell eggs and egg products in terms of numbers of illnesses and specific public health outcomes on an annual basis. Considerations in quantifying the dose-response relationship included the selection of an appropriate functional form, extrapolation of fitted curves to low-dose ranges, and the use of surrogate organisms in the absence of feeding trial data specific for SE.

In the initial quantification of a dose-response relationship for *Salmonella* Enteritidis, a beta-Poisson dose-response curve was fit to the pooled data from all *Salmonella* feeding trials (McCullough and Eisele, 1951a; McCullough and Eisele, 1951c) and the fitted model compared to epidemiological information from available *Salmonella* Enteritidis outbreak data. The model validation on the epidemiological data showed that outbreaks associated with *Salmonella* Enteritidis exhibited a higher attack rate than would be estimated using the pooled human feeding trial data for *Salmonella*. Furthermore, an analysis of variance (ANOVA) on the *Salmonella* human feeding trial data for dose and serotype effects revealed two distinct, statistically significant dose-response patterns (representative of doses $>10^3$ organisms) among the *Salmonella* serotypes in the human feeding studies data (Morales *et al*., 1996; Jaykus *et al*., 1997).

The inability of several dose-response models, fitted to the *Salmonella* data, to predict the high attack rates associated with low doses such as the 1994 *S.* Enteritidis outbreak from ice cream (Hennessy *et al*., 1996) was likewise previously noted by Morales *et al*. (1996). In order to capture the region of concern (i.e., the low-dose range with corresponding high attack rates evident in the outbreak investigation data), human feeding study data utilizing a low-dose organism was selected for subsequent dose-response modeling as a surrogate for SE. The absence of human feeding study data for SE prompted the selection of *Shigella dysenteriae* (Levine and DuPont, 1973) as a proxy for modeling "low-dose" *Salmonella* serotypes (attack rates >0 with doses = 10^3 organisms).

Epidemiological evidence from outbreak investigations was once again used to conduct a model validation check on the two dose-response models generated (beta-Poisson curves fit to human feeding trial data for pooled *Salmonella* species and to the low-dose proxy *Shigella dysenteriae*). A review of the epidemiological outbreak investigations showed that many of the reported doses resulting in illnesses were several orders of magnitude lower than the doses reported in the

Salmonella feeding trials. Further, the doses which caused outbreaks were likewise several orders of magnitude lower than the doses which were predicted by the dose-response models constructed from the *Salmonella* feeding trial data. Model validation to the available outbreak investigation data subsequently served as the basis for selection of a dose-response relationship (Figure 1.5). The outbreak investigation data used for dose-response model validation are detailed in Table 1.13.

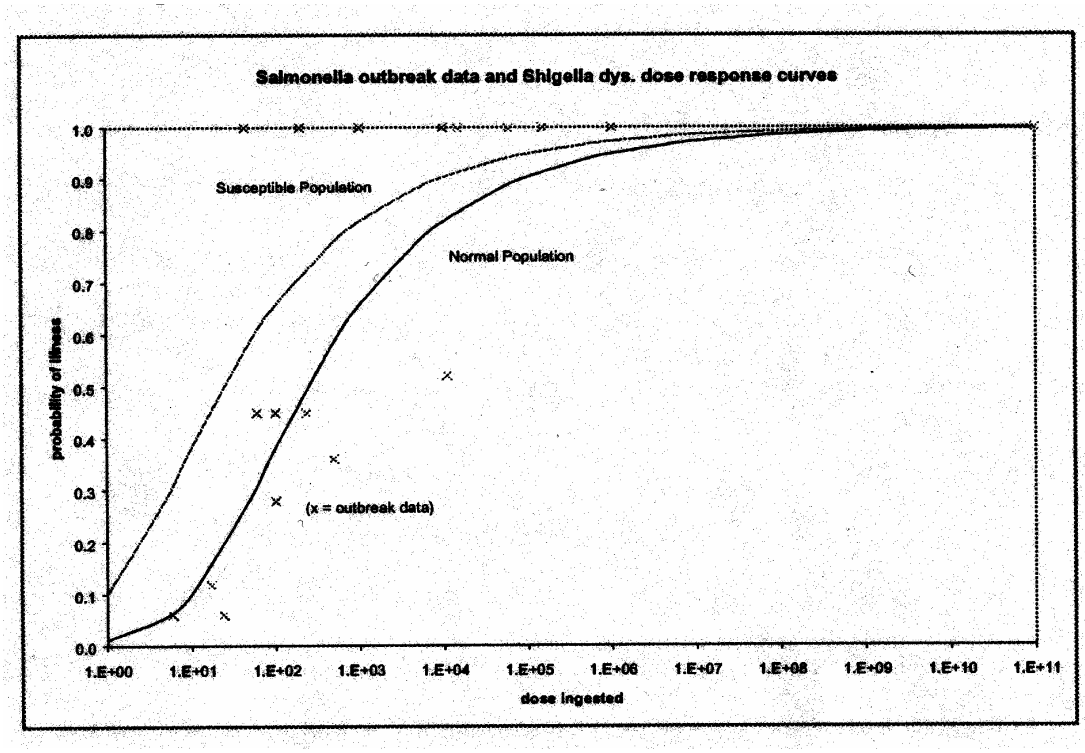

FIGURE 1.5. USDA COMPARISON OF AVAILABLE *SALMONELLA* OUTBREAK INVESTIGATION DATA AND BETA-POISSON DOSE-RESPONSE CURVES FOR SHIGELLA DYSENTERIAE ESTIMATED FOR NORMAL AND SUSCEPTIBLE SUBPOPULATIONS (USDA-FSIS, 1998).

TABLE 1.13. *SALMONELLA* OUTBREAK INVESTIGATION DATA USED IN THE US *SALMONELLA* ENTERITIDIS RISK ASSESSMENT TO COMPARE WITH *SHIGELLA* DOSE-RESPONSE CURVES (USDA-FSIS, 1998).

Reference	Serovar	Dose	$\log_{10}$ Dose	Number Ill	Attack Rate
Boring *et al*., 1971	Typhimurium	1.7E+01	1.23	16,000	12%
Lipson, 1976	Schwarzengrund	4.4E+01	1.64	1	100%
Fontaine *et al*., 1978	Newport	6.0E+01	1.78	48	45%
D'Aoust *et al*., 1975	Eastbourne	1.0E+02	2.00	95	45%
Fontaine *et al*., 1980	Heidelberg	1.0E+02	2.00	339	28%
George, 1976	Heidelberg	2.0E+02	2.30	1	100%
Fontaine *et al*., 1978	Newport	2.34E+02	2.36	46	45%
Fontaine *et al*., 1980	Heidelberg	5.0E+02	2.70	339	36%
Armstrong *et al*., 1970	Typhimurium	1.1E+04	4.04	1,790	52%
Lang *et al*.. 1967	Cubana	1.5E+04	4.18	28	100%
Lang *et al*., 1967	Cubana	6.0E+04	4.78	28	100%
Reitler *et al*., 1960	Zanzibar	1.5E+05	5.18	6	100%
Angelotti *et al*., 1961	Infantis	1.0E+06	6.00	5	100%
Reitler *et al*., 1960	Zanzibar	1.0E+11	11.00	8	100%
Hennessy *et al*., 1996	Enteritidis	6.0E+00	0.77	>1,000	6%
Vought and Tatini, 1998	Enteritidis	2.4E+01	1.38	>1,000	6%
Levy *et al*., 1996	Enteritidis	1.0E+03	3.00	39	100%
Levy *et al*., 1996	Enteritidis	1.0E+04	4.00	39	100%

The dose-response relationship subsequently used was a beta-Poisson model fit to the human feeding trial data for *Shigella dysenteriae* M131 with parameters alpha = 0.2767, and beta = 21.159 (http://www.fsis.usda.gov/OPHS/risk/semodel.htm, July 2000). Uncertainty was introduced into the beta parameter by characterizing it as a normal distribution truncated at zero, with a maximum of 60 and a mean and standard deviation of 21.159 and 20 respectively for the proportion of the population assumed to be in good health (normal subpopulation). In addition, the beta parameter of the *S. dysenteriae* beta-Poisson model was reduced by a factor of 10, thus shifting the curve to the left to estimate a higher probability of illness for susceptible individuals (susceptible subpopulation). Uncertainty in the beta parameter for the susceptible subpopulation was therefore introduced using a normal distribution with a mean and variance of 2.116 and 2.0 respectively and a minimum of 0 and maximum of 6.

$$Pill = 1 - \left(1 + \frac{Dose}{\beta}\right)^{-\alpha}$$

Model Used:	**Beta-Poisson**	
Parameters:	Normal	*Alpha = 0.2767* *Beta = Normal (μ:21.159, σ:20, min:0, max:60)*
	Susceptible	*Alpha = 0.2767* *Beta = Normal (μ:2.116, σ:2, min:0, max:6)*
Comment:	Human feeding trial data for *Shigella dysenteriae* used as a surrogate. Susceptible population characterized by reducing beta parameter by a factor of 10. Simulation of public health outcomes for normal and susceptible subpopulations incorporates the uncertainty represented in the beta parameters.	

Health Canada <u>Salmonella</u> Enteritidis dose-response relationship

The Health Canada *Salmonella* Enteritidis risk assessment used a re-parameterized Weibull dose response model. Bayesian methods were employed as a means to provide a consistent framework for combining information from various sources including feeding and epidemiological studies (Paoli, 2000; Ross, 2000). The Canadian *Salmonella* Enteritidis risk assessment has not currently been published, however a brief description of the procedure used is provided, and the model generated is compared to the other alternatives.

The Canadian model begins with the Weibull dose-response model.

$$P = 1 - \exp\left(-\theta \times d^{b}\right)$$

Where "d" is the dose.

The model was re-parameterized as summarized below (Paoli, 2000; Ross, 2000), and this is the equation that is referred to in the remainder of this section.

$$P = 1 - \exp\left(-\exp\left\{b\left[\ln\left(d\right) - \kappa\right]\right\}\right)$$

$$\beta = \ln\left(b\right)$$

$$\kappa = \frac{-\ln\left(\theta\right)}{b}$$

The parameter "b" in the model was characterized by performing a meta-analysis of all the bacterial feeding trial data. This analysis determined that the log transformed value of "b",

termed β (β = ln[b]) could be well described using a normal distribution with mean of –1.22 and a standard deviation of 0.025. This characterization of β, for all bacterial pathogens represents between-study variability, which is used as a reference prior (Paoli, 2000; Ross, 2000). Epidemiological data, specifically information generated from the Schwann's ice-cream outbreak (Hennessy *et al*., 1996; Vought and Tatini, 1998), was incorporated into the model by adjusting the parameter "θ".

In order to adjust the parameter "θ", the following equation in terms of epidemiological information was used (Paoli, 2000; Ross, 2000).

$$\theta = \frac{-\ln(1-P)}{X^b}$$

Where "P" represents the attack rate reported in an epidemiological outbreak and "X" represents the dose estimated to have caused the outbreak.

Within the model, the dose ingested was defined stochastically so as to reflect the uncertainty associated with the data. A single value for the attack rate "P" was used, and this was estimated to be 6% (Hennessy *et al*., 1996). The dose was estimated based on the concentration reported and the amount of ice cream consumed. The concentration (cfu/g) was characterized using a lognormal distribution with a mean of 0.15 and a standard deviation of 0.1. The amount of ice cream consumed was estimated using a pert distribution with a minimum of 60, a mode of 130, and a maximum of 260.

A separate dose-response relationship was generated for the susceptible population, which was based on epidemiological information. Specifically, information from a waterborne outbreak of *S. typhimurium* in Riverside, California (Boring III *et al*., 1971), which reported on age specific attack rates, was used to shift the value of "θ"according to the following equation (Paoli, 2000; Ross, 2000; Baumler *et al*., 2000).

$$\theta_{susceptible} = \theta_{normal}\left(\frac{\ln\left[1-beta_{sus}\{a_s,b_s\}\right]}{\ln\left[1-beta_{norm}\{a_n,b_n\}\right]}\right)$$

Where the parameters ("a" and "b") for the beta distributions are estimated from the reported epidemiological data on the total number of individuals exposed and the number that became ill. The sub-scripts "s" and "n" refer to the data for susceptible and normal populations respectively.

$$\theta_{susceptible} = \theta_{normal}\left(\frac{\ln\left[1-beta_{sus}\{a_s,b_s\}\right]}{\ln\left[1-beta_{norm}\{a_n,b_n\}\right]}\right)$$

Model Used:	**Re-parameterized Weibull**
Parameters:	*Beta = Normal (μ:-1.22, σ:0.025)* *Concentration = Lognormal (μ:0.15, σ:0.1)* *Amount consumed = Pert (min:60, mode:130, max:260)* *Attack Rate = 6.6%* a_s = 231 b_s = 987 a_n = 749 b_n = 5966

Several parameters in the dose-response models described, incorporated uncertainty into their characterization. In order to display the dose-response curves in the following sections, the uncertainty in those parameters has been simulated and the specified moments displayed.

The following abbreviations are introduced and will be used when referring to the dose-response curves: Canadian normal population dose-response ("Can-norm"), Canadian susceptible population dose-response ("Can-susc"), the United States normal population dose-response ("US-norm"), the United States susceptible population dose-response ("US-susc"), and beta-poisson dose-response curve fit to naïve subject human feeding trial data ("Naïve-BP"). These are shown in Figures 1.6 to 1.8

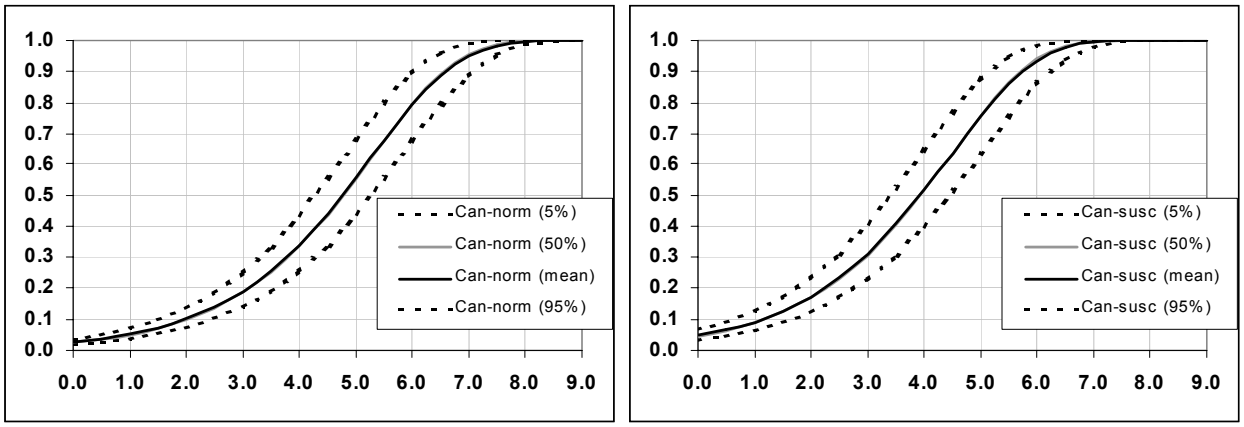

Figure 1.6. Dose-response curves for normal (Can-norm), upper panel, and susceptible (Can-susc) populations, lower panel, as estimated in Canadian *Salmonella* Enteritidis risk assessment.

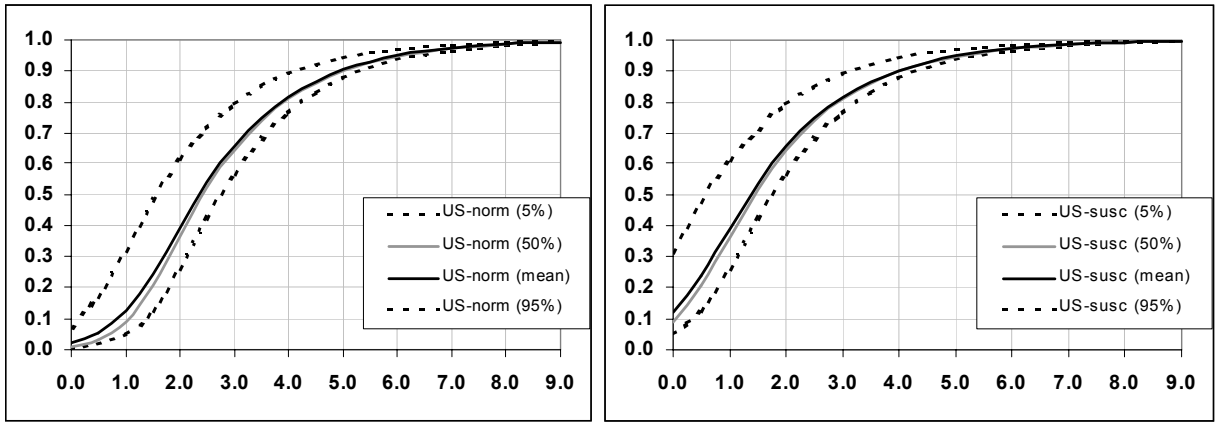

FIGURE 1.7. DOSE-RESPONSE CURVES FOR NORMAL (US-NORM), UPPER PANEL, AND SUSCEPTIBLE (US-SUSC) POPULATIONS, LOWER PANEL, AS ESTIMATED IN THE UNITED STATES *SALMONELLA* ENTERITIDIS RISK ASSESSMENT.

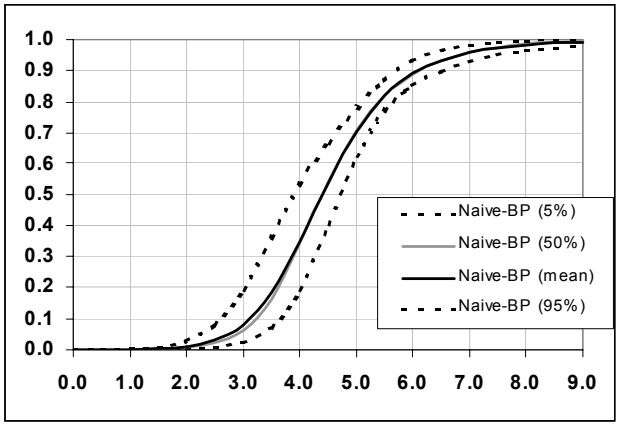

FIGURE 1.8 BETA-POISSON DOSE-RESPONSE CURVE FIT TO NAÏVE SUBJECT NON-TYPHI *SALMONELLA* HUMAN FEEDING TRIAL DATA (NAÏVE-BP).

The five dose-response curves are plotted together in Figure 1.9 to assist in the comparison of the curves.

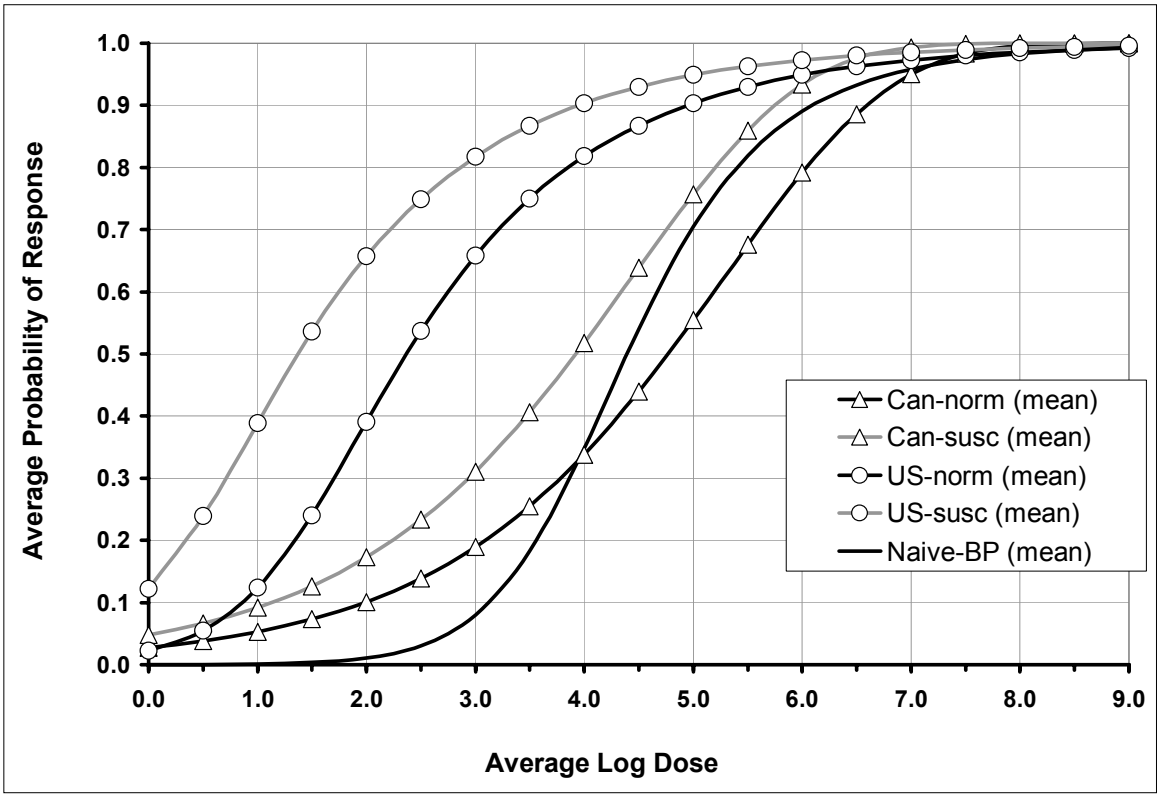

FIGURE 1.9. COMPARISON BETWEEN FIVE DOSE-RESPONSE CURVES: CANADIAN NORMAL POPULATION MODEL, CANADIAN SUSCEPTIBLE POPULATION MODEL, UNITED STATES NORMAL POPULATION MODEL, UNITED STATES SUSCEPTIBLE POPULATION MODEL, AND BETA-POISSON MODEL FIT TO NAÏVE SUBJECT DATA SET.

Since the 50th percentile and the mean are very similar in all 5 dose-response curves (Figures 1.6 to 1.8), only the mean values for the curves are plotted. The 95th percentile and 5th percentile boundaries for the curves are omitted from this figure for visual reasons.

There is some overlap between the "Can-norm" dose-response curve and the "Naïve-BP" dose-response curves. However, the "Naïve-BP" curve estimates a higher probability of response than the "Can-norm" for individuals exposed to a dose greater than approximately 10^4 cells. At an average dose of less than approximately 10^4 cells the "Can-norm" dose response curve estimates a greater probability of response than the "Naïve-BP". In fact, at an average dose of 2 $\log_{10}$ (100 cells) the "Can-norm" dose-response curve estimates a probability of response of approximately 10% compared to approximately 1% for the "Naïve-BP" dose-response curve. The adjustment of the Canadian dose-response curve to reflect epidemiological information, specifically the 6% response rate at a dose of approximately 1 $\log_{10}$ (Hennessy *et al*., 1996; Vought and Tatini, 1998) is evident in the behavior of the curve at that lower dose region.

The "US-norm" and "US-susc" dose-response curves, which are based on using *Shigella* as a surrogate pathogen, estimate a higher probability of illness at a given dose than the other dose-response curves across almost the entire dose range except the lowest (10 or less organisms). At

the 2 $\log_{10}$ (100 cells) average dose level, the normal population using the United States dose response curve would be estimated to have approximately a 40% average probability of response and the susceptible population would be estimated to have approximately a 65% probability of response. This can be compared to 10% and 18% for normal and susceptible populations using the Canadian dose-response curves.

The dose-response curves thus have a significant degree of deviation from each other. Selecting a dose-response curve from this information would have to be based on several considerations that include: the level of conservatism that one wishes to employ; the theoretical acceptability of using a surrogate pathogen; the biological plausibility of various functional forms for modeling dose-response relationships; the biological endpoint or public health outcome of interest; or the acceptability of the human feeding trial data in capturing the overall response for a population.

In order to gain additional insight into the pathogenesis of *Salmonella*, the available data from epidemiological information were explored.

EPIDEMIOLOGICAL INFORMATION

Epidemiological data can provide valuable insight into the pathogenicity of microorganisms as it applies to the general population. In a sense, outbreaks represent realistic feeding trials with the exposed population often representing a broad segment of society. The doses are essentially real-world levels, and the medium carrying the pathogen represents a range of characteristics (protective, fatty, long residence time, etc). Ideally, an epidemiological investigation should attempt to collect as much quantitative information as possible in order to lend itself to better characterizing the dose-response relationship for microbial pathogens. In order to refine the dose-response relationship so that it has greater applicability to the general population, two pieces of information are required in an epidemiological investigation: the dose, the population exposed and the number of people exhibiting a response (illness, fever, etc.).

The dose that is suspected to have caused illness in a specific outbreak is often the most difficult measure in an investigation. The lack of dose estimates can be attributed to either the inability to obtain samples of contaminated food or the lack of emphasis being placed on the value of such information. Oftentimes, contaminated food is tested and only the presence or absence of the suspected pathogen is reported. This information is often viewed as sufficient to incriminate the food; however it does little in furthering our knowledge of the dose-response relationship.

The attack rate represents the response in a dose-response relationship. In order to estimate the attack rate, an accurate estimate of the population that was exposed to the contaminated food as well as the number of individuals that got sick is required. In addition, it is valuable to know the characteristics of the exposed and affected population, in order to account for potential susceptibility issues.

Summary of epidemiological and outbreak information

The following sections present and summarize outbreaks found in the literature that included quantitative information from which the dose and attack rate could be estimated. It is important to note that although these outbreaks include quantitative data, some assumptions had to be made depending on the nature of the information. In the interest of transparency, the following sections

present the information from the original epidemiological reports in as much detail as possible and where appropriate, the assumptions that are used are clearly indicated.

In addition, reports that are currently unpublished were received from Ministry of Health and Welfare, Japan (1999). Although these reports have not been published, and the details of the methods used in the investigations have not been stated (other than through personal communication), they represent a valuable source of information on the real world dose-response relationship and expand our database of *Salmonella* pathogenicity considerably. The data in these reports are generated as part of the epidemiological investigations that take place in Japan following an outbreak of foodborne illness. In accordance with a Japanese notification released on March 1997, large scale cooking facilities which prepare more than 750 meals per day or more than 300 dishes of a single menu at a time are advised to save food for future possible analysis in the event of an outbreak. Fifty-gram portions of each raw food ingredient and each cooked dish are saved for more than 2 weeks at temperature lower than minus (-) 20ºC. Although this notification is not mandatory, it is also applicable to smaller scale kitchens with social responsibility such as those in schools, daycare centers and other child-welfare and social-welfare facilities. (Some of the local governments in Japan also have local regulations that require food saving, but the duration and the storage temperature requirements can vary among them).

In the evaluation of the outbreak data, whenever sufficient information was available, susceptible and normal populations were separated out of the database to aid in further analysis. Children of age 5 years or younger were considered to be susceptible populations. The criteria or assumptions used to identify potentially susceptible populations are noted in the individual outbreak summaries.

In addition, the uncertainty associated with each of the outbreak parameters are also summarized and defined at the end of each outbreak description. The published reports were used as a basis upon which to derive a reasonable characterization of the uncertainty. However, it should be recognized that since only rarely is sufficient information given upon which to derive a range of uncertainty for the parameters, the uncertainty ranges used are only a crude estimate. In addition, in several reports there is no information whatsoever to use as a basis for uncertainty estimates, in these cases a consistent default assumption was used. To capture the dose uncertainty, a 25% over- and under-estimate for the reported concentration and amount consumed was used.

Number: 1
Reference: (Boring III *et al*., 1971)
Serovar: S. *typhimurium*
Setting: Citywide municipal water
Medium: Water

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
17	#/Liter	0.75	Liter	1.28E+01	Concentration found in tap water using composite sample
1000	#/Liter	0.75	Liter	7.50E+02	Order of magnitude for concentration found in tap water based on single sample collected independently

Exposed	Response	Attack Rate	Comments
8788	1035	11.78%	Reported average attack rate for all individuals
7572	805	10.63%	Attack rate reported for individuals > 5 years old (assumed "normal" population).
1216	230	18.91%	Attack rate reported for individuals < 5 years old (assumed "susceptible" population).

Comments
Composite water samples were collected late in the epidemic (nine days after initial case) and water in the composite samples had been stored for one to four days at room temperature prior to culturing. Since varying amounts of water, from a few ml to as much as 500ml, were pooled from several sample bottles, it is possible that numbers in some samples were greatly diluted by negative samples. The pooled sample consisted of water from 74 different samples, and only 5 of the 74 samples were actually positive. The concentration of 1000/L was an order of magnitude estimate following a single isolation made independently from a 1ml sample (suggesting an order of magnitude of 1000 organisms/liter). The concentration in the water was therefore assumed to range between the two reported concentration estimates (50/Liter to 500/Liter concentration range used), with water consumption of 0.75 liters which results in a dose range of between 37 and 375 cells.

A house-to-house survey was conducted that comprised 8788 people, with 1035 reporting gastroenteric illness. The report also identified attack rates according to age, which was used in the current analysis as an estimate of the potential attack rate for susceptible and normal populations. Children less than 5 years old (assumed potentially susceptible) were reported to have an 18.9% attack rate, compared to approximately 11% for the rest of the population. The uncertainty in the average attack rate was calculated by allowing for 5% under or over-reporting. Given 1035 people reporting gastroenteric illness, only 983 may have actually been sick with the other 5% claiming to be sick; alternatively 1087 people may have actually been sick with the additional people not reporting to be sick. It was assumed that the contamination in the water supply was randomly distributed throughout such that all 8788 people that reported drinking water were exposed. It should also be noted that the attack rates listed in this table assume exposure to the pathogen only once during the outbreak.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution					Pert Distribution		
Min	Max		Value		Min	ML	Max
37	375		7572		765	805	845
37	375		1216		219	230	242
37	375		8788		983	1035	1087

Number: 2
Reference: (Fontaine *et al*., 1980)
Serovar: *S. heidelberg*
Setting: Restaurant
Medium: Cheddar Cheese

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
0.36	#/100g	28	g	0.10	Concentration reported by Food Research Institute, Wisconsin, United States
1.8	#/100g	28	g	0.50	Concentration reported by CDC in Atlanta, United States
1.08	#/100g	28	g	0.30	Average of two reported concentrations
108	#/100g	28	g	30.24	Average concentration adjusted for a 99% die-off prior to culturing
1080	#/100g	28	g	302.40	Average concentration adjusted for a 99.9% die-off prior to culturing

Exposed	Response	Attack Rate	Comments
205	68	33.17%	Attack rate based on exposed employees in incriminated restaurants, consumers at incriminated restaurants, and employees at restaurants which received contaminated cheese lot shipments and at which employee cases existed

Comments
Samples analyzed by the CDC in Atlanta were reported to have an MPN of 1.8 organisms/100 grams while the Food Research Institute in Wisconsin reported an MPN of 0.36 organisms /100 grams. According to the restaurant, the serving size was approximately 28 grams of cheese per meal. The potentially very low infectious dose for this outbreak was noted by the researchers and the potential for up to a 99.9% die-off occurring prior to culturing was acknowledged. The concentration in the food at consumption was assumed to range between 108 to 1080 cells per 100 grams (99% to 99.9% die-off prior to culture). The dose ingested, accounting for the amount consumed was estimated to range between 30 and 300 cells.

The attack rate in this outbreak was reported to range from 28% to 36%. The exposed population was estimated to be 205, consisting of employees in incriminated restaurants, consumers at incriminated restaurants, and employees at restaurants which received contaminated cheese lot shipments and at which employee cases existed. The number of positives (57 to 74) was back calculated from the reported attack rate range and the exposed population.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution					Pert Distribution		
Min	Max		Value		Min	ML	Max
30	300		205		57	68	74

Number: 3
Reference: (Lang *et al*., 1967)
Serovar: *S. cubana*
Setting: Hospital
Medium: Carmine dye capsules

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
30,000	#/capsule	0.5	capsule	15,000	Lower dose estimate based on some patients being given ½ a capsule
30,000	#/capsule	2.0	capsule	60,000	Upper dose estimate based on some patients being given up to 2 capsules

Exposed	Response	Attack Rate	Comments
?	21	?	Recognized cases during outbreak
?	12	?	Confirmed cases as a result of dye capsule ingestion.

Comments
This outbreak involved a susceptible population that consisted of debilitated and aged people, infants and persons with altered GI function. Carmine dye capsules are used as a fecal dye marker for such things as the collection of timed stool specimens, GI transit time and the demonstration of GI fistulas. The number of capsules given to patients ranged from 1/2 to 2, as a result the dose ingested was assumed to range from 15,000 to 30,000.

There were a total of 21 recognized cases during this outbreak, however 4 were reported to have been infected prior to admission and 5 cases were suspected to have been secondary transmission. Therefore, there were 12 confirmed cases directly as a result of carmine dye capsule ingestion. Unfortunately, for attack rate estimation the total number of exposed individuals was not determined, however the authors of the report note that there were some people who received carmine but were not infected. It was thus inferred that the attack rate was some value less than 100%. As an upper and lower bound it was assumed that 14 to 20 individuals received dye capsules.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution					
Min	Max	Min	ML	Max		Value	
15,000	30,000	14	17	20		12	

Number: 4
Reference: (Angelotti *et al*., 1961)
Serovar: *S. infantis*
Setting: Home
Medium: Ham

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
23,000	#/g	50	g	1,150,000	Lower dose estimate based on lower weight of slice and only one slice consumed
23,000	#/g	200	g	4,600,000	Upper dose estimate based on higher weight of slice and up to 2 slices consumed.

Exposed	Response	Attack Rate	Comments
8	8	100%	

Comments
This outbreak occurred in a family consisting of adults and at least two grade school age kids. Smoked ham purchased from a supermarket was taken home and refrigerated for approximately 5 hours. Eight people in the family ate either raw or fried slices of ham, and all 8 experienced acute diarrhea with gastroenteritis symptoms within 8 to 24 hours. An uneaten portion of ham was obtained and examined in laboratory 2 days after the outbreak occurred. Various bacteria were isolated from the raw ham: total aerobic plate count (268,000,000/gram), coliform bacteria (15,000/gram), *Streptococcus faecalis* (31,000,000/gram), staphylococci (200,000,000/gram) and *S. infantis* (23,000/gram). Staphylococci were negative for coagulase production and negative for enterotoxin production. Stools from 4 of the 8 persons affected were examined 10 days after the outbreak: mother, father and two grade school age sons. *S. faecalis* was isolated from both parents and one son. *S. faecalis var. liquefaciens* was isolated from another son, and *S. infantis* was isolated from both parents but not the sons. The researchers noted that *S. infantis* in the ham, stools and the long incubation period implies infection of *Salmonella* etiology. However, a mixed infection is a possibility.

The weight of a slice of ham was estimated to range from 50 to 100 grams, with 1 to 2 slices consumed. The dose was thus estimated to range from 1,150,000 to 4,600,000 cells of *S. infantis.* The exposed and positive populations in this case were quite well established; therefore accounting for uncertainty in these parameters was unnecessary.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
1,150,000	4,600,000		8			8	

Number: 5
Reference: (Armstrong *et al*., 1970)
Serovar: *S. typhimurium*
Setting: Various parties and banquets
Medium: Imitation ice-cream

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
113	#/75gram	75	grams	113	Reported concentration and amount consumed at limited menu venue

Exposed	Response	Attack Rate	Comments
1400	770	55%	Reported attack rate at limited menu venue

Comments
This episode involved 14 outbreaks with a total of 3450 people attending the various events at which imitation ice-cream (chiffonade) was identified as the vehicle of infection. The authors estimated a 52% attack rate based on a survey of persons attending seven of the events. The menus at the various events were relatively extensive; however one of the outbreaks involved a large affair with a limited menu in which the authors cite that nearly all the people had eaten all of the foods offered. Using this outbreak the attack rate was estimated to be 55% (1400 people attending and 770 people sick).

The chiffonades were stored at -20C for 1 month before quantitative cultures were done, and the MPN was reported to be 113 or less *Salmonella* per 75-gram serving. The reduction in numbers that could be expected due to freezing was experimentally determined by artificial inoculation of *S. typhimurium* into chiffonade and storing these samples at -20C. Artificial inoculation experiments indicated that $\log_{10}$ reductions would have occurred during the storage period however no more than a 2-$\log_{10}$ reduction was likely to have occurred during the one-month storage. As a result, the concentration was estimated to range between 1,130 (1-$\log_{10}$ reduction) to 11,300 (2-$\log_{10}$ reduction) per serving. In this outbreak the exposed population was reasonably well established, however the positive population was assumed to have a 5% under and over-reporting (732 to 809).

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution					Pert Distribution		
Min	Max		Value		Min	ML	Max
1,130	11,300		1400		732	770	809

Number: 6
Reference: (Fontaine *et al*., 1978)
Serovar: *S. newport*
Setting: Interstate: (Maryland: households, Colorado: households, Florida: naval base)
Medium: Hamburger

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
6	#/100gram	100	gram	6	Lowest reported concentration
23	#/100gram	100	gram	23	Highest reported concentration

Exposed	Response	Attack Rate	Comments
?	48	?	Total number of people affected over entire geographic area of outbreak.

Comments
The concentration of *S. newport* in ground beef was determined from MPN to be between 6 to 23 per 100 grams. Accounting for freezing, the authors cite that experimental evidence would indicate a 1- to 2-$\log_{10}$ reduction due to freezing which would place the concentration at 60-2300 per 100 gram. However, cooking, even undercooking is likely to produce a reduction prior to consumption. If the effects of cooking are conservatively assumed to be 1-2 $\log_{10}$, then the concentration prior to consumption is again estimated to be 6 to 23 per 100grams. Assuming consumption of 100 grams the dose that was capable of causing an infection in some people can be estimated to be approximately 6 to 23 organisms. Unfortunately, this outbreak was geographically widespread and the authors did not report the total number of individuals that were exposed. The attack rate is therefore undetermined in this outbreak.

Number: 7
Reference: (Fazil, 1996)
Serovar: *S. newport*
Setting: Naval Base
Medium: Hamburger

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
4	#/100gram	100	gram	4	Low reported concentration (6 cfu/100 g) with 25% allowance for uncertainty (approx. 4 cfu/100 g)
30	#/100gram	100	gram	30	High reported concentration (23 cfu/100 g) with 25% allowance for uncertainty (approx. 30 cfu/100 g)

Exposed	Response	Attack Rate	Comments
7254	19	0.3%	Attack rate estimated with all recruits at the base exposed
3627	19	0.5%	Attack rate assuming 50% were actually exposed
1813	19	1.0%	Attack rate assuming 25% were actually exposed
725	19	2.6%	Attack rate assuming 10% were actually exposed

Comment

The data in this outbreak is derived from the prior episode described (Fontaine *et al*., 1978). However, Fazil (1996) examined the naval outbreak in greater detail through a series of personal communications with the United States Navy to attempt to determine an attack rate. A total of 21 cases occurred at the naval training center, 2 were asymptomatic food handlers and 19 were trainees.

The entire complex had a population of 12483 with the military population listed as 9904 (full time military personnel and trainees). Meals were served at several locations, and included: the galley, the staff galley and the exchange cafeteria. The outbreak was reported to have occurred at the "Training Station", which is a separate area within the center where training is conducted. There were 7254 recruits who were fed at the galley that serviced the trainees. Therefore, depending on the assumed number of people that ate a contaminated hamburger, an attack rate can be estimated/approximated. Assuming 7254 individuals exposed (all present which is unlikely), the attack rate is estimated to be 0.3% (19/7254). It was assumed that a more likely exposure population was 25% (1813) with an uncertainty range of between 10% (725) to 50% (3627). It was assumed that the positive population was well characterized given the nature of the location for the outbreak. At the naval base the trainees would have had access to convenient medical attention. It should be noted that if there was reporting bias it is more likely to be under-reporting as opposed to over-reporting

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution					
Min	Max	Min	ML	Max		Value	
4	30	725	1813	3627		19	

Number: 8
Reference: (Narain and Lofgren, 1989)
Serovar: *S. newport*
Setting: Restaurant
Medium: Pork and Ham sandwiches

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
4.40E+07	#/gram				Concentration found in pork sandwich stored by one of the patients. No indication of how much pork or ham sandwiches individuals consumed.

Exposed	Response	Attack Rate	Comments
200 ?	105	52.5%	Total number of people who got ill and were exposed at the restaurant. The 200 people who are listed as exposed were actually the number of people that ate at the restaurant during the period according to the owner's recollection.

Comments
A total of 105 people were reported to have become ill during this episode that was attributed to ham and pork sandwiches. The sandwiches were suspected to have been contaminated at the restaurant and a refrigerated portion of a pork sandwich from a patient yielded $44x10^6$ *S. newport* per gram.

The attack rate that might be inferred from information provided in this report is unknown. The restaurant reported serving approximately 200 people during the period of which 105 got ill. However the information required is an estimate of the number of people that actually ate ham and pork sandwiches and were thus exposed to contaminated food. It can be assumed that not everyone ate the ham and pork sandwiches. If it is assumed that 60% of the people visiting the restaurant ate the contaminated food, then 120 people may have been exposed. At the other extreme it could also be assumed that only 105 people were actually exposed and 105 got sick, the attack rate at 100%.

Number: 9
Reference: (Craven *et al*., 1975)
Serovar: *S. eastbourne*
Setting: Interstate-Homes
Medium: Chocolate balls

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
2.5	#/gram	450	gram	1,130	Reported concentration with dose estimated based on the consumption of an entire bag of chocolates (approximately 50 chocolate balls)
2.5	#/gram	225	gram	563	Reported concentration with dose estimated based on the consumption of half a bag of chocolates
2.5	#/gram	45	gram	113	Reported concentration with dose estimated based on the consumption of approximately 5 chocolate balls

Exposed	Response	Attack Rate	Comments
?	80	?	Total number of cases in geographically widespread outbreak. No information on exposed population

Comments
This outbreak involved a potentially susceptible population and involved chocolate balls. The median age of the cases in this outbreak was 3 years old. The attack rate cannot be determined in

this case because no information was provided in the report and the geographically widespread nature of the outbreak makes inferences difficult. The outbreak occurred simultaneously in the United States and in Canada. The description of the Canadian portion of the outbreak is described in the next section (D'Aoust *et al*., 1975).

The New Jersey health department reported a mean concentration of 2.5 *Salmonella*e per gram of chocolate from samples obtained from homes where cases occurred. A bag of the chocolate was reported to be 1 lb or approximately 450 grams; therefore the maximum dose causing infection in some people was estimated to be no more than approximately 1000 cells (2.5/gram x 450grams). Alternatively, the dose could be as low as 100 cells if only 40 grams was consumed (2.5 x 40).

Number: 10
Reference: (D'Aoust *et al*., 1975)
Serovar: *S. eastbourne*
Setting: National - homes
Medium: Chocolate balls

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
2	#/ball	50	balls	100	Lower reported concentration and dose estimate based on consumption of entire bag
9	#/ball	50	balls	450	Upper reported concentration and dose estimate based on consumption of entire bag
2	#/ball	5	balls	10	Lower reported concentration and dose estimate based on consumption of entire bag
9	#/ball	5	balls	45	Upper reported concentration and dose estimate based on consumption of entire bag

Exposed	Response	Attack Rate	Comments
?	95	?	Total number of cases in geographically widespread outbreak. No information on exposed population

Comments
This outbreak again involved a potentially susceptible population, as 46% of the cases were children aged 1-4 years old. There were a total of 95 reported cases. The outbreak was attributed to chocolate balls. Each ball was reported to weigh approximately 10 grams, with a bag of chocolate containing approximately 50 balls. The contamination of the chocolate balls was estimated to be 2 to 9 *Salmonella*e per chocolate ball. This outbreak was the Canadian part of the outbreak that also occurred simultaneously in the United States and described previously (Craven *et al*., 1975).

The dose causing illness in some of the exposed population was estimated by the authors based on the consumption of a bag of chocolate. This estimate, which may be high considering the

consumption of 50 chocolate balls, would place the dose at approximately 100 to 450 cells. Depending on the assumption of the amount of chocolate that was consumed the dose causing illness could be as low as 2 cells if only one ball was consumed at the lowest concentration. However, it is difficult to determine with the given information exactly how much chocolate sick individuals consumed, and what the concentration was in the chocolate that was consumed. The overall attack rate for this outbreak is also difficult to estimate, similar to Craven *et al*. (1975), due to the geographically widespread nature of the outbreak.

Number: 11
Reference: (Levy *et al*., 1996; USDA-FSIS, 1998)
Serovar: *Salmonella* Enteritidis
Setting: Hotel
Medium: Raw shell eggs (hollandaise sauce)

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
1,000	#/gram	10	gram	10,000	Concentration reported from informal quantification, dose estimated from consumption of 2 tablespoons of sauce
10,000	#/gram	10	gram	100,000	1-$\log_{10}$ higher concentration from informal quantification results, dose estimated from consumption of 2 tablespoons of sauce

Exposed	Response	Attack Rate	Comments
39	39	100.00%	Attack rate estimated from all individuals consuming hollandaise sauce becoming ill

Comment
In this outbreak a total of 56 persons who ate at a Washington, D.C. hotel had onset of diarrhea. The D.C. public health department conducted an investigation on the outbreak and incriminated hollandaise sauce as the likely vehicle. According to the USDA (USDA-FSIS, 1998) only 39 persons ate the hollandaise sauce and all 39 became ill, which would imply a 100% attack rate. The attack rate in this case was assumed to be 100%, with a good characterization of the exposed and positive populations.

The actual concentration of *Salmonella* Enteritidis causing illness in this outbreak was not reported in the publication describing the outbreak (Levy *et al*., 1996); however the USDA-FSIS (1998) reported the results of some testing. This informal quantification, which was not performed to extinction, tested a sample of sauce recovered from a patron who had taken it home in a "doggie bag" and refrigerated it for 72 hours. The concentration in this sample was reported to be 10^3 per gram. It was assumed that 2 tablespoons (approximately 10 grams) were consumed by the patrons of the restaurant, placing the dose at approximately 10^4 (USDA-FSIS, 1998). To allow for the uncertainty associated with the concentration estimates and the potential underestimate, an additional 1 $\log_{10}$ was allowed for in the concentration range.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
10,000	100,000		39			39	

Number: 12
Reference: (Vought and Tatini, 1998; Hennessy *et al*., 1996; USDA-FSIS, 1998)
Serovar: *Salmonella* Enteritidis
Setting: USA, Interstate
Medium: Ice-cream

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
0.152	#/gram	65	grams	9.88	Low expected concentration using Bayesian analysis with dose calculated using smallest reported consumption amount.
0.152	#/gram	260	grams	39.52	Low expected concentration using Bayesian analysis with dose calculated using highest reported consumption amount.
0.894	#/gram	65	grams	58.11	High expected concentration using Bayesian analysis with dose calculated using smallest reported consumption amount.
0.894	#/gram	260	grams	232.44	High expected concentration using Bayesian analysis with dose calculated using highest reported consumption amount.

Exposed	Response	Attack Rate	Comments
452	30	6.6%	Attack rate calculated based on cross sectional study for which exposure and response details were available.

Comment

This was an interstate outbreak attributed to ice-cream. Hennessy *et al*. (1996) provide details on the epidemiological characteristics of the outbreak and the concentration of *S*. Enteritidis found in samples of ice-cream using traditional MPN techniques. The effect of frozen storage was also experimentally investigated. The authors found no evidence of a decrease in numbers during storage at -20C for 16 weeks, unlike the work of Armstrong *et al*. (1970), described previously. A re-analysis of the quantitative MPN results was performed at a later date using alternative statistical tools to better estimate the concentration in the ice cream (Vought and Tatini, 1998). The expected concentration was reported as 0.152 MPN/gram at the lower range and 0.894 MPN/gram at the upper range. In addition, a small group of people that were investigated in more detail were reported to have consumed from 65 to 260 grams. The uncertainty in the dose was therefore assumed to range from 10 cells to 235 cells.

The outbreak was reported to have affected a large number of people, however from the report by Hennessy *et al*. (1996) we have details on a smaller cross-sectional group for which interviews were conducted. A total of 541 people were interviewed that had purchased incriminated ice cream, of which 452 were reported to have consumed the product. To allow for some uncertainty in the exposed population, it was assumed that this could be 10% less than the number that reported eating the ice cream. A total of 30 individuals in the population in the cross-sectional study became ill. The number of positives was assumed to have a 5% under and over-reporting.

Outbreak Parameter Uncertainty

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution			Pert Distribution		
Min	Max	Min	ML	Max	Min	ML	Max
10	235	407	451	452	29	30	32

Number: 13
Reference: (Taylor *et al*., 1984)
Serovar: *S. typhimurium*
Setting: Home
Medium: Ice cream

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
1.00E+06	#/ml	1000	ml	1.00E+09	Dose estimated for fatality in 13 year old boy
1.00E+06	#/ml	750	ml	7.50E+08	Dose for individuals consuming 750ml
1.00E+06	#/ml	250	ml	2.50E+08	Dose for individuals consuming 250ml
1.00E+06	#/ml	100	ml	1.00E+08	Dose for 2 year old girl consuming 100ml

Exposed	Response	Attack Rate	Comments
7	7	100%	

Comments
This outbreak involved a family and one neighbor, and was attributed to home-made ice cream. The ages of the exposed population were as follows: father 35, mother 30, sons ages 13, 9 and 8, daughters 6 and 2, and a male neighbor 22-years old. Ice cream was obtained from the freezer at the farm and found to have 10^6 *Salmonella*e/ml. One of the sons, aged 13 years, who ate the most ice cream (1000 ml) died from his illness. Various amounts of ice cream ranging from 100ml to 1000ml were reported to have been consumed by the family members. Since the actual sample of ice cream was obtained from the freezer only a few days after the event, the concentration reported was assumed to be reflective of that at the point of consumption. The uncertainty in the dose was modeled using the given concentration, and accounting for the different amounts consumed. In the current analysis the child of 2 years of age was assumed to be potentially more susceptible while the rest of the individuals were assumed to represent a normal population. In this particular case, there was no uncertainty in the exposed and positive populations.

OUTBREAK PARAMETER UNCERTAINTY

DOSE			EXPOSED POPULATION		POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
1.0e+8	7.5e+8		7			7	
2.5e+8	7.5e+8		6			6	

Number: 14
Reference: (D'Aoust *et al*., 1985), (D'Aoust, 1985)
Serovar: *S. typhimurium*
Setting: Nationwide
Medium: Cheddar Cheese

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
0.36	#/100g	100	g	0.36	Min. concentration in samples from plant
9.3	#/100g	100	g	9.3	Max. concentration in samples from plant.
3.5	#/100g	100	g	3.5	Avg. concentration in samples from plant.
1.5	#/100g	100	g	1.5	Min. concentration in food samples from patients.
9.1	#/100g	100	g	9.1	Max. concentration in food samples from patients.
4.2	#/100g	100	g	4.2	Avg. concentration in food samples from patients.

Exposed	Response	Attack Rate	Comments
?	1500	?	

Comments
This outbreak involved more than 1500 people with cheddar cheese implicated as the vehicle of infection. Cheese samples were obtained from the plant as well as from homes of some of the individuals that were ill. The level of contamination on the cheese from the plant was found to be between 0.36 to 9.3 *Salmonella*e per 100 grams (D'Aoust *et al*., 1985), while the level of contamination on cheese from individual homes was found to be between 1.5 to 9.1 *Salmonella*e per 100grams (D'Aoust, 1985). The average concentration from cheese plant samples was estimated to be 3.5 per 100 grams while those from homes were estimated to be 4.2 per 100 grams. The authors noted that the number of *Salmonella*e probably did not change substantially during storage and the levels estimated reflect the levels at the time of consumption. It was estimated that approximately 100 grams of cheese was consumed, based on the level of consumption reported for six individuals that ranged from 20 grams to 170 grams.

The attack rate in this case is again difficult to estimate due to a lack of information on the exposed population and the inability to make reasonable assumptions given the information and the widespread distribution of the outbreak.

Number: 15
Reference: (George, 1976)
Serovar: *S. schwarzengrund*
Setting: Hospital
Medium: Pancreatin

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
1000	#/g	0.2	g	200	Reported concentration and dose estimated from consumption of 200mg by single susceptible individual

Exposed	Response	Attack Rate	Comments
1	1	100%	

Comments
This case involved a susceptible individual (1-year-old child) who developed diarrhea when treated with pancreatic extract (pancreatin is an extract from the pancreas of mammals used to assist in the digestion of food) that was contaminated with *S. schwarzengrund*. The pancreatic extract was found to be contaminated with 1000 *Salmonella*e per gram and the child got sick following ingestion of 200 mg. It should be noted that this case involves only one individual and the 100% attack rate quoted for this dose could skew the true attack rate which could be less for a group of individuals receiving this dose. For example it could be possible that this one individual might be the only one that got sick if 20 similarly susceptible individuals were given the same dose. In that hypothetical situation the attack rate would be estimated to be only 5%.

Number: 16
Reference: (Lipson, 1976)
Serovar: *S. schwarzengrund*
Setting: Hospital
Medium: Pancreatin

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
8	#/g	5.6	g	44.8	Reported concentration and dose estimated based on last 24 hours of feedings consisting of four 1.4 gram amounts.

Exposed	Response	Attack Rate	Comments
1	1	100%	

Comments
This case involved a susceptible individual (9-month-old child with cystic fibrosis) who was fed pancreatin contaminated with *S. schwarzengrund*. The pancreatin was found to be contaminated at a level of 8 *Salmonella*e per gram. The child was given approximately 700 mg with each 6-hourly feed for the first 10 days, increasing to approximately 1.4 g in the 36 hours before the

symptoms began. The authors note that he had therefore ingested less than 22 organisms per day initially and less than 44 organisms per day in the last 36 hours. If the dose is not cumulative over 24 hours then the infective dose would be approximately 44 organisms (24 hours, fed every 6 hours which translates to 4 feedings; each feeding is 1.4 grams which translates to 5.6 grams. 5.6 grams x 8 per gram = approx. 44 cells). The points raised about one individual exposed and the attack rate estimates in the previous case (George, 1976) also apply in this case.

Number: 17
Reference: (Greenwood and Hooper, 1983)
Serovar: *S. napoli*
Setting: Nationwide
Medium: Chocolate bars

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
16	#/10gram	64	g	102	Average reported concentration and consumption amount by one individual that got sick
58.5	#/10gram	64	g	374	Highest average concentration reported in a packet of 6 bars
240	#/10gram	64	g	1,540	Highest concentration reported in an individual bar

Exposed	Response	Attack Rate	Comments
1	1	100%	Widely geographically spread outbreak, with a large potentially exposed and sick population, however details only available on one individual.

Comments
This was a nationwide outbreak attributed to chocolate bars (16 grams each) contaminated with *S. napoli*. Although the overall attack rate in the population exposed cannot be determined, details were given on three individuals: a mother and two sons. All three ate two bars on the first day, and one son ate two more bars on the second day. The son that ate chocolate bars on both days became ill. He may have received a larger dose, or, alternatively, not all the bars were contaminated and the ill child ingested a single contaminated bar. We can only state that the attack rate for the one child that ate four chocolate bars was 100%.

A box of chocolates, which consisted of 8 packets with 6 bars in each packet, was obtained from a retailer from whom two patients had purchased chocolate. This box of chocolates was analyzed and 42 of the 48 bars examined were positive with the average concentration on the positive bars reported to be 16 organisms per 10 grams. The highest concentration on a bar was 240 organisms per 10 grams and the lowest was 3 organisms per 10 grams. It was also observed that the level of contamination per packet was not consistent. Packets consisting of 6 bars that were all positive also tended to have a higher contamination level. Of the 8 packets examined, the packet with the highest average concentration was 58.5 per 10 grams.

Since information is only known about one case, these data were not considered for further analysis.

Number: 18
Reference: Ministry of Health and Welfare, Japan (1999)
Serovar: *S.* Enteritidis (PT4)
Setting: Restaurant
Medium: Roasted Beef

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
2,000	#/gram	120	g	240,000	Reported concentration and consumption

Exposed	Response	Attack Rate	Comments
5	3	60%	Reported exposed and positive numbers

Comments
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 135,000 (1500 cfu/gram x 90gram) to 375,000 (2500 cfu/gram x 150gram). Since the size of the exposed population was reasonably small, it can be assumed that the uncertainty associated with the exposed and positive populations is minimal.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
135,000	375,000		5			3	

Number: 19
Reference: Ministry of Health and Welfare, Japan (1999)
Serovar: *Salmonella* Enteritidis
Setting: Caterer
Medium: Grated yam diluted with soup

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
32,000	#/gram	60	g	1,920,000	Reported concentration and consumption

Exposed	Response	Attack Rate	Comments
123	113	91.87%	Reported exposed and positive numbers

Comments
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for

the dose were estimated to be 1,080,000 (24,000 cfu/gram x 45gram) to 3,000,000 (40,000 cfu/gram x 15gram). The exposed and positive populations in this case were potentially uncertain. Since the degree of uncertainty is unknown, it was assumed that the reported exposed population could not have been exceeded; however, there could have been 10% fewer people actually exposed. The number of positives reported was assumed to represent the most likely number, however a 5% under- and over-reporting were allowed for.

Outbreak Parameter Uncertainty

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution			Pert Distribution		
Min	Max	Min	ML	Max	Min	ML	Max
1,080,000	3,000,000	111	122	123	107	113	119

Number: 20
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT22)
Setting: School lunch
Medium: Beef and bean sprouts

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
40	#/gram	22	g	880	Reported concentration and consumption

Exposed	Response	Attack Rate	Comments
10552	967	9.16%	Reported number of potentially exposed population
5276	967	18.33%	Attack rate with 1/2 of the population exposed
3517	967	27.50%	Attack rate with 1/3 of the population exposed
2638	967	36.66%	Attack rate with 1/4 of the population exposed

Comments
The number of potentially exposed elementary school students (6- to12-years old) was very large since a central cooking facility serves 15 schools. Patients were found from almost all the schools, however, there was an indication that most of the exposures occurred at 5 schools. It is highly unlikely that all 10,775 people were exposed to contaminated food. As a result, it was assumed that a proportion, ranging from 1/2 to 1/4 of the total potentially exposed population was actually exposed. There could also be uncertainty in the number of positives, however given the size of the denominator (exposed population) and the size of the numerator (positives), incorporating a 5% allowance for under and over-reporting has very minimal effect on the attack rate uncertainty range.

In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 495 (30 cfu/gram x 16.5gram) to 1,375 (50 cfu/gram x 27.5gram).

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution					
Min	Max	Min	ML	Max		Value	
495	1375	2638	3517	5276		967	

Number: 21
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Home
Medium: Egg

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
<0.03	#/gram	60	g	<1.8	

Exposed	Response	Attack Rate	Comments
5	3	[60.00%]	

Number: 22
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Hotel
Medium: Scallop roasted with egg yolk (product 1), Shrimp roll in bread (product 2), Hamburg steak (product 3)

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
47,000	#/gram	40	g	1,880,000	Concentration and consumption amount reported for product 1

Exposed	Response	Attack Rate	Comments
115	63	54.78%	

Comments
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the values reported. The lower and upper bounds for the dose were estimated to be 1,057,500 (35,250 cfu/gram x 30gram) to 2,937,500 (58,750 cfu/gram x 50gram). The exposed and positive populations in this case were also potentially uncertain. Since the degree of uncertainty is unknown, it was assumed that the reported exposed population could not have been exceeded; however, it was assumed that there could have been 10% fewer people actually exposed. The number of positives reported was assumed to represent the most likely number, however a 5% under- and over-reporting was allowed for.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution			Pert Distribution		
Min	Max	Min	ML	Max	Min	ML	Max
1,057,500	2,937,500	104	114	115	60	63	66

Number: 23
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Confectionery
Medium: Cake

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
6,000	#/gram	100	g	600,000	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
13	11	84.62%	Reported attack rate

Comments
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 337,500 (4500 cfu/gram x 75gram) to 937,500 (7500 cfu/gram x 125gram). Since the size of the exposed population was reasonably small, it can be assumed that the uncertainty associated with the exposed and positive populations is minimal.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
337,500	937,500		13			11	

Number: 24
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT1)
Setting: School lunch
Medium: Peanut sauce

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
1.4	#/gram	35	g	49	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
5320	644	12.11%	Reported attack rate

Comments
The attack rate that was reported for this outbreak was based on the entire school population that receives lunch from the central kitchen being exposed. With a large exposed population like this one, which can be highly uncertain, the estimated attack rate can vary widely. It is highly unlikely that the entire reportedly exposed population was actually exposed to the contaminated food. Unlike the prior school outbreak, there was no indication in this case of some schools being more likely to have been exposed than others. As a result, it was assumed that a proportion, ranging down to 1/2 of the total potentially exposed population was actually exposed. There could also be uncertainty in the number of positives, however given the size of the denominator (exposed population) and the size of the numerator (positives), incorporating a 5% allowance for under and over-reporting has very minimal effect on the attack rate uncertainty range.

In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 28 (1.05 cfu/gram x 26.25gram) to 77 (1.75 cfu/gram x 43.75gram).

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution					
Min	Max	Min	ML	Max		Value	
28	77	2660	3990	5320		644	

Number: 25
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Daycare
Medium: Cooked chicken and egg

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
27	#/gram	150	g	4,050	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
16	3	18.75%	Exposed and positive adults at day care
117	50	42.74%	Exposed and positive children at day care
133	53	39.85%	Exposed and positive population at day care

Comments
The food was a rice dish covered with cooked chicken and eggs. Of 133 exposed people, 16 were adults (3 became ill) and 117 were children (50 became ill). Daycare-aged children were assumed to be of increased potential susceptibility to foodborne pathogens. The exposed and positive populations were assumed to be well characterized in this case because of the outbreak setting (daycare).

In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 2,278 (20.25 cfu/gram x 112.5gram) to 6,328 (33.75 cfu/gram x 187.5gram).

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
2,278	6,328		16			3	
2,278	6,328		117			50	
2,278	6,328		133			53	

Number: 26
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT1)
Setting: School lunch
Medium: Peanut sauce

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
<100	#/gram	80	gram	8000	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
2267	418	18.44%	Reported exposed and positive population

Comments
The attack rate that was reported for this outbreak was based on the entire school population that receives lunch from the central kitchen being exposed. With a large exposed population like this one, which can be highly uncertain, the estimated attack rate can vary widely. It is highly unlikely that the entire reportedly exposed population was actually exposed to the contaminated food. In addition, the reported concentration per gram of food was less than 100 cfu, which introduces a second significant uncertain parameter.

Number: 27
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Hospital
Medium: Raw egg in natto

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
1.20E+06	#/gram	50	g	6.00E+07	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
191 ?	45	23.56%	Reported exposed and positive population

Comments
Eggs were pooled in the preparation of this food. The number of exposed was the number of people who were served with this dish. One hundred and twenty-eight people from 191 served answered the food-intake questionnaire. (Some of the hospital patients could not talk.) Among 128 responses, 36 did not actually consume this dish. Among the 45 cases, 2 were tuberculosis (TB) patients and apparently had taken antibiotics. The number of TB patients in the actual exposed population is unknown. This outbreak is highly unusual because the dose is very high but the attack rate is very low. In addition, the outbreak is reported to have occurred in a hospital, an environment in which we might expect, depending on the condition, the exposed population to be more susceptible than the overall population. Because of the uncertainties in these data and the potential confounding factors, this outbreak was not included for further analysis.

Number: 28
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT4)
Setting: Hospital
Medium: Grated yam diluted with soup

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
2,400	#/gram	60	g	144,000	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
343 ?	75	21.87%	

Comments
This outbreak is unusual, like the previous hospital-associated outbreak. Eggs were pooled and mixed well in preparing this dish. The actual number of individuals exposed is suspected to be lower than originally reported. The reported attack rate is lower than would be expected at this high dose level. It should be noted that some of the patients had antibiotic treatment, which may be a confounding factor in interpretation of these data.

Number: 29
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT1)
Setting: Hospital
Medium: Tartar sauce

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
100	#/gram	36	g	3,600	

Exposed	Response	Attack Rate	Comments
126	36	28.57%	

Comment
This outbreak is also unusual, similar to the previous two hospital outbreaks, although in this case the dose is not as high as reported in Nos. 27 and 28. Information about confounding factors in these hospital outbreaks, such as diagnoses and treatments that patients were undergoing, was not available. Therefore, the three Japanese hospital outbreaks were not included in further analysis.

Number: 30
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT1)
Setting: Restaurant
Medium: Cooked egg

Concentration		Amount Ingested			
Value	Units	Value	Units	Dose	Comments
200	#/gram	30	g	6,000	Reported concentration and attack rate and average amount consumed

Exposed	Response	Attack Rate	Comments
885	558	63.05%	

Comment
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the values reported. The lower and upper bounds for the dose were estimated to be 3.375 (150 cfu/gram x 22.5gram) to 9,375 (250 cfu/gram x 37.5gram). The exposed and positive populations in this case were also potentially uncertain. Since the degree of uncertainty is unknown, it was assumed that the reported exposed population could not have been exceeded; however, it was assumed that there could have been 10% fewer people actually exposed. The number of positives reported was assumed to represent the most likely number, however a 5% under- and over-reporting was allowed for.

Outbreak Parameter Uncertainty

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		Pert Distribution			Pert Distribution		
Min	Max	Min	ML	Max	Min	ML	Max
3,375	9,375	797	884	885	530	558	586

Number: 31
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis (PT4)
Setting: Confectionery
Medium: Cake

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
14	#/gram	30	g	420	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
5103	1371	26.87%	

Comment
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the values reported. The lower and upper bounds for the dose were estimated to be 236 (11 cfu/gram x 22.5gram) to 656 (18 cfu/gram x 37.5gram). The exposed and positive populations in this case were also potentially uncertain. Since the degree of uncertainty is unknown, it was assumed that the reported exposed population could not have been exceeded; however, it was assumed that there could have been 10% fewer people actually exposed. The number of positives reported was assumed to represent the most likely number, however a 5% under- and over-reporting was allowed for.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution		**Pert Distribution**			**Pert Distribution**		
Min	Max	Min	ML	Max	Min	ML	Max
236	656	4593	5102	5103	1302	1371	1440

Number: 32
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *Salmonella* Enteritidis
Setting: Daycare
Medium: Egg salad

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
0.78	#/gram	30	g	23.4	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
156	42	26.92%	

Comment
This outbreak was assumed to represent a susceptible population since the outbreak occurred in a daycare facility. In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the values reported. The lower and

upper bounds for the dose were estimated to be 13 (0.59 cfu/gram x 22.5gram) to 37 (0.98 cfu/gram x 37.5gram). The exposed and positive populations were assumed to be well characterized in this case because of the outbreak setting (daycare).

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
13	37		156			42	

Number: 33
Reference: Ministry of Health and Welfare, Japan, 1999
Serovar: *S. oranienburg*
Setting: Hotel
Medium: Grated yam diluted with soup

Concentration		Amount Ingested			
Value	**Units**	**Value**	**Units**	**Dose**	**Comments**
5.00E+07	#/gram	150	g	7.50E+09	Reported concentration and amount consumed

Exposed	Response	Attack Rate	Comments
11	11	100.00%	

Comment
In order to incorporate uncertainty in the dose, the concentration and amount consumed were assumed to have a potential range of 25% of the one reported. The lower and upper bounds for the dose were estimated to be 4.22e+9 (3.75e+7 cfu/gram x 112.5gram) to 1.17e+10 (6.25e+7 cfu/gram x 187.5gram). Since the size of the exposed population was reasonably small, it can be assumed that the uncertainty associated with the exposed and positive populations is minimal.

OUTBREAK PARAMETER UNCERTAINTY

DOSE		EXPOSED POPULATION			POSITIVE		
Uniform Distribution							
Min	Max		Value			Value	
4.22e+9	1.17e+10		11			11	

Epidemiological Data Summary and Analysis

Twenty-three of the thirty-three outbreak reports collected from the published literature and from unpublished data received by FAO and WHO following the call for data, contained sufficient information on the number of people exposed, the number of people that became ill, and the number of organisms in the implicated food in order to calculate a dose response relationship. Three of the twenty-three outbreaks were excluded because the immune status of the persons exposed could not be determined. The remaining twenty outbreaks comprise the database used to calculate a dose response relationship.

Eleven of the twenty outbreaks in the database occurred in Japan and nine occurred in the United States. Several serotypes were associated with the outbreaks including Enteritidis (12 outbreaks), Typhimurium (3), and in single outbreaks, Heidelberg, Cubana, Infantis, Newport, and Oranienburg. Several vehicles were implicated including food (meat, eggs, dairy products, and others), water, and a medical dye capsule (carmine dye).

Reports provided by the Ministry of Health and Welfare of Japan (1999) represent a valuable source of information on the real world dose-response relationship and expand our database of *Salmonella* pathogenicity considerably. The data in these reports are generated as part of the epidemiological investigations that take place in Japan following an outbreak of foodborne illness. In accordance with a Japanese notification released on March 1997, large scale cooking facilities which prepare more than 750 meals per day or more than 300 dishes of a single menu at a time are advised to save food for future possible analysis in the event of an outbreak. The notification is also applicable to smaller scale kitchens with social responsibility such as those in schools, daycare centers and other child-welfare and social-welfare facilities. Fifty-gram portions of each raw food ingredient and each cooked dish are saved for more than 2 weeks at temperature lower than minus (-) 20ºC. Although this notification is not mandatory, the level of compliance is high. (Some of the local governments in Japan also have local regulations that require food saving, but the duration and the storage temperature requirements can vary among them).

A summary of the doses, attack rates, serovars and characteristics of the exposed populations derived from the outbreak reports described in the preceding section is given in Table 1.14 and Figure 1.10. The analysis of the epidemiological data was intended to serve three purposes:

1. To determine if there is any epidemiological evidence for greater attack rates in susceptible vs. normal populations;
2. To determine if there is any epidemiological evidence for different attack rates for *Salmonella* Enteritidis vs. other *Salmonella* serotypes;
3. To compare the epidemiological data for dose and attack rate with the estimates generated by the dose-response models.

The data in Table 1.14 and Figure 1.10 are coded according to the outbreak number assigned in this document. If additional information related to a specific data point is required, for example the assignment of two data points, the details of the outbreak can be referred to in the previous section. The related assumptions for inclusion, exclusion, or multiple data points are certainly issues for discussion and debate, and therefore included in the summary of reported outbreaks.

Table 1.14. Summary of outbreak data.

Outbreak Ref #	Serovar	Food	Pop[1]	Dose[2] log_{10} cfu	Attack Rate[3] %	Reference
1	*S. typhimurium*	Water	N	2.31	10.63%	Boring *et al*., 1971
1	*S. typhimurium*	Water	S	2.31	18.91%	Boring *et al*., 1971
2	*S. heidelberg*	Cheddar Cheese	N	2.22	32.76%	Fontaine *et al*., 1980
3	*S. cubana*	Carmine dye	S	4.57	70.93%	Lang *et al*., 1967
4	*S. infantis*	Ham	N	6.46	100.00%	Angelotti *et al*., 1961
5	*S. typhimurium*	Imitation ice-cream	N	3.79	55.00%	Armstrong *et al*.,1970
7	*S. newport*	Hamburger	N	1.23	1.07%	Fazil., 1996 Fontaine *et al*., 1978
11	*S.Enteritidis*	hollandaise sauce	N	4.74	100.00%	Levy *et al*., 1996 USDA-FSIS., 1998
12	*S.Enteritidis*	Ice-cream	N	2.09	6.80%	Vought & Tatini, 1998 Hennessy *et al*., 1996
13	*S. typhimurium*	Ice-cream	N	8.70	100%	Taylor *et al*., 1984
13	*S. typhimurium*	Ice-cream	S	8.00	100%	Taylor *et al*., 1984
18	*S. Enteritidis*	Roasted Beef	N	5.41	60.00%	Ministry Health & Welfare, Japan, 1999
19	*S. Enteritidis*	Grated yam with soup	N	6.31	93.93%	Ministry Health & Welfare, Japan, 1999
20	*S.Enteritidis*	Beef and bean sprouts	N	2.97	26.86%	Ministry Health & Welfare, Japan, 1999
22	*S. Enteritidis*	Scallop with egg yolk	N	6.30	56.01%	Ministry Health & Welfare, Japan, 1999
23	*S. Enteritidis*	Cake	N	5.80	84.62%	Ministry Health & Welfare, Japan, 1999
24	*S. Enteritidis*	Peanut sauce	N	1.72	16.41%	Ministry Health & Welfare, Japan, 1999
25	*S. Enteritidis*	Chicken and egg	N	3.63	18.75%	Ministry Health & Welfare, Japan, 1999
25	*S. Enteritidis*	Chicken and egg	S	3.63	42.74%	Ministry Health & Welfare, Japan, 1999
30	*S. Enteritidis*	Cooked egg	N	3.80	64.18%	Ministry Health & Welfare, Japan, 1999
31	*S. Enteritidis*	Cake	N	2.65	27.33%	Ministry Health & Welfare, Japan, 1999
32	*S. Enteritidis*	Egg salad	S	1.40	26.92%	Ministry Health & Welfare, Japan, 1999
33	*S oranienburg*	Grated yam with soup	N	9.90	100.00%	Ministry Health & Welfare, Japan, 1999

[1] N: Normal population, S: Susceptible population, B: Both populations exposed
[2 & 3] Expected value based on defined uncertainty ranges and distributions.

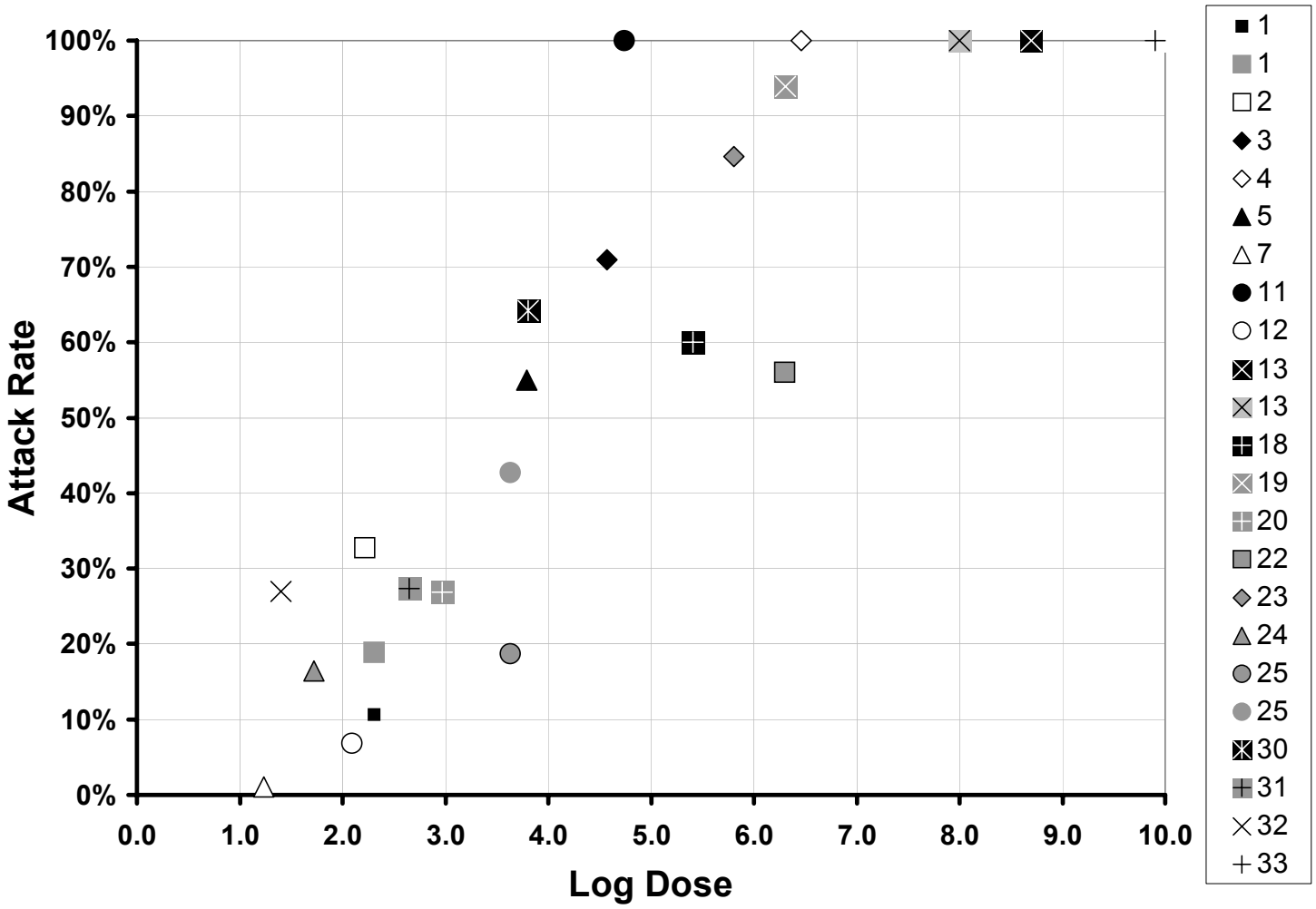

FIGURE 1.10. SUMMARY OF EPIDEMIOLOGIC DATA. LEGEND NUMBERS INDICATE OUTBREAK NUMBER GIVEN IN TEXT.

The data shown in Figure 1.10 appear to reflect our theoretical assumptions regarding the increasing trend in attack rates as dose increases. In addition, although there is a degree of clustering in some of the data points, a dose-response relationship is visually evident.

As noted earlier, some data were excluded from this summary and further analysis. For example, outbreaks # 27, 28 and 29 were attributed to *Salmonella* Enteritidis in a hospital setting, where the exposed population would be expected to be more susceptible. The characteristics of the individuals that were exposed to the food are highly uncertain, so it may in fact be the case that the condition for which they were hospitalized is such that their immunity was not compromised. However even if they are assumed to have normal susceptibility, these outbreaks were still distinctly different from outbreaks with a similar dose level, if the reported exposures were accurate. Alternative explanations for these data sets are that the individuals served the meal did not actually consume the implicated food, or that concurrent antibiotic therapy prevented the ingested *Salmonella* from colonization and illness production.

Susceptible vs. Normal Populations

The observed outbreak data were used to gain some insight into the potential differences that may exist between "susceptible" and "normal" populations. The database of quantitative outbreak information collected during the course of this work includes several outbreaks that were determined to be associated with "susceptible" and "normal" populations. Unfortunately, limited data only allowed a comparison to be made based on an age basis. Susceptibility in this analysis was therefore limited to outbreak data for individuals less than 5 years old being classified as "susceptible" and other outbreak data as representing a "normal" population. This was the case for all but one of the "susceptible" data points (estimated 85% attack rate, approx. 4.5 $\log_{10}$ dose), that occurred in a hospital and was attributed to carmine dye capsules. The "susceptible" and "normal" outbreak data were compared on the basis of reported attack rate corresponding with reported dose. Given the potential range in the observed data (dose and attack rate could vary based on the nature of the epidemiological investigation) the comparison was intended to look for overall trends first, and then if necessary additional analysis could be done. A plot of dose against attack rate for the "susceptible" and "normal" populations is shown in Figure 1.11.

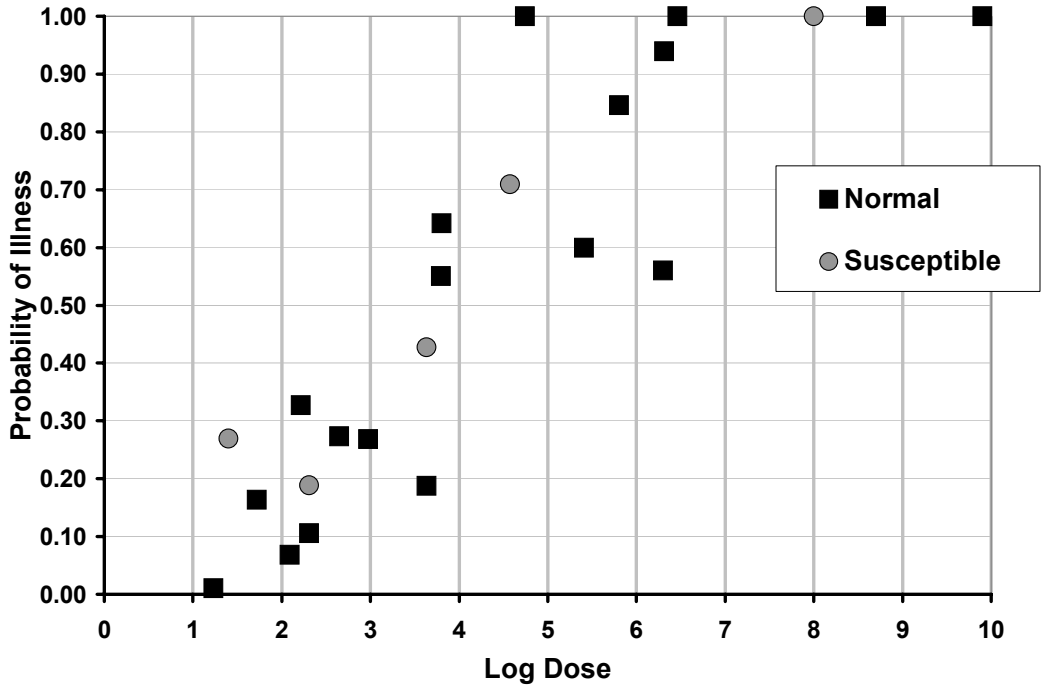

FIGURE 1.11. ATTACK RATES CORRESPONDING TO DOSE FOR "NORMAL" AND "SUSCEPTIBLE" POPULATIONS IN REPORTED OUTBREAKS.

Comparing the attack rates for the "susceptible" and "normal" populations shown in Figure 1.11, there does not seem to be any clear evidence to state that the probability of illness at any one dose is higher for "susceptible" populations compared to "normal" populations. The highest attack rate at a dose of between 1 and 2 $\log_{10}$ (26.9%) was attributed to a "susceptible" population, however an outbreak in the same dose interval, attributed to a "normal" population had a reported attack rate of 24.2%. Similarly, at other dose intervals there are outbreaks attributed to "normal" populations with either very similar or higher attack rates than outbreaks involving "susceptible" populations. Given the data that currently exists from outbreaks, there is insufficient evidence to conclude that "susceptible" individuals, as defined in this database, have a higher probability of illness compared to the "normal" population.

It should be noted that within the database of outbreaks, there are two outbreaks in which a "susceptible" and "normal" population were identified in the same outbreak with differing attack rates. The "susceptible" definition in these cases was again based on an age criteria (<5 years old and >5 years old). In these two outbreaks, shown in Figure 1.12, the attack rate was clearly reported to be higher for the susceptible population compared to the normal population. Taken in isolation, it could be concluded from this information that there is clearly a higher probability of illness for the susceptible population compared to the normal population. However, if we look at the whole picture, we can see other outbreaks involving a "normal" population with higher attack rates at similar doses.

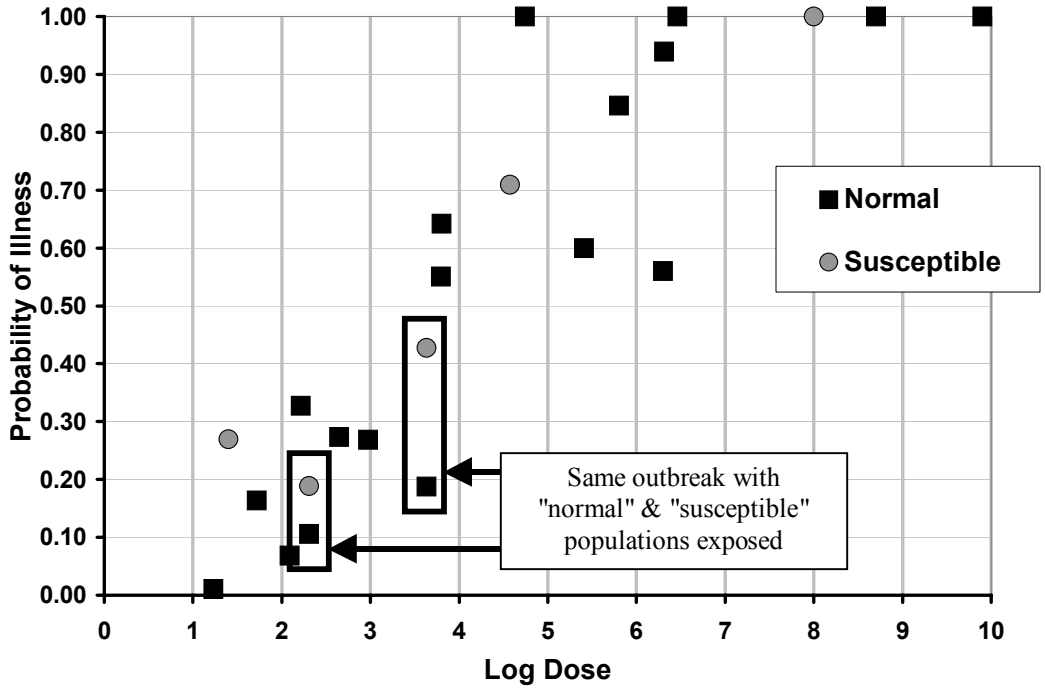

FIGURE 1.12. ATTACK RATES FOR TWO OUTBREAKS IN WHICH DIFFERENT POPULATIONS IN THE SAME OUTBREAK WERE IDENTIFIED.

Given the outbreak data that are currently available, it is not possible to conclude that some segments of the population are more susceptible to getting ill upon exposure to *Salmonella* spp. than others. Furthermore, it is impossible to derive a quantitative estimate of the increased probability of illness for some segments of the population compared to others. The dose-response relationship for the probability of illness for different segments of the population was therefore assumed to be the same.

The key distinction that needs to be made in this conclusion is that the probability of illness is assumed to be indistinguishable given the current data and the susceptible populations defined in the database. It is important to recognize that even if the probability of becoming ill, defined in the dose-response assessment as any degree of gastroenteritis, the *severity* of the illness may be markedly different for certain segments of the population. To quantify the probabilities of different outcomes, quantitative patient follow-up information and data on physicians' visit, hospitalizations, death, or other chronic outcomes is needed.

***Salmonella* Enteritidis vs. other *Salmonella* serovars**

In a similar manner to the comparisons made for susceptible and normal populations, the attack rates in outbreaks associated with *S.* Enteritidis were compared to outbreaks associated with other *Salmonella* serovars. This information is summarized in Figure 1.13.

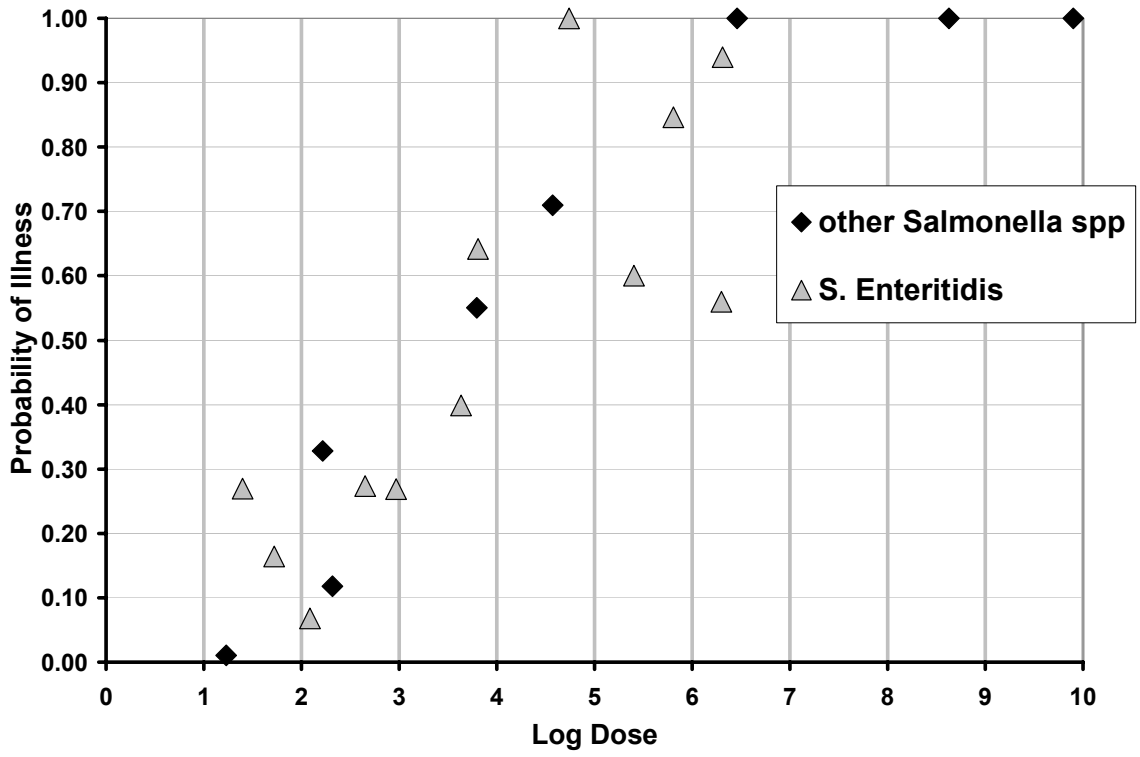

FIGURE 1.13. ATTACK RATES CORRESPONDING TO DOSE FOR S. ENTERITIDIS AND OTHER SALMONELLA SPP. IN REPORTED OUTBREAKS

The attack rates observed in outbreaks associated with other *Salmonella* serovars are indistinguishable from outbreaks associated with *Salmonella* Enteritidis. At some dose ranges, the highest attack rate reported is for *Salmonella* Enteritidis while at others the highest attack rate is for other serovars. Based on this information, *Salmonella* Enteritidis and other serovars were treated as equivalent for the purposes of the dose-response relationship. It is acknowledged however that less virulent strains may be infrequently the cause of foodborne outbreaks and hence would not be captured in this database.

In summary, it was concluded that for the purposes of the current assessment and based upon the existing observed evidence: 1) a single dose-response relationship for the probability of illness would be used for all members of the population; and 2) *Salmonella* Enteritidis and other *Salmonella* serovars are assumed to have a similar probability of initiating illness at the same dose.

Comparison of Outbreak Data with Existing Salmonella spp. Dose-Response Models

Three dose-response models for *Salmonella* exist in the literature. The first, (Fazil, 1996) is the beta-Poisson model (Haas, 1983) fit to the human feeding trial data for *Salmonella* infection (McCullough & Eisele, 1951a; 1951c; 1951d). The second model was proposed in the United States *Salmonella* Enteritidis risk assessment (USDA-FSIS, 1998) and was based on the use of a surrogate pathogen to describe the dose-response relationship. This model assumed a shift in the dose-response model for "susceptible" and "normal" populations. The third model was introduced in a *Salmonella* Enteritidis risk assessment done by Health Canada (Paoli, 2000), which was based on a Weibull dose-response relationship that was updated to reflect selected outbreak information using Bayesian techniques. Similar to the United States model, this one also assumed a higher probability of illness for susceptible populations. The models and their comparison with the outbreak data are shown in Figures 3.14 to 3.16.

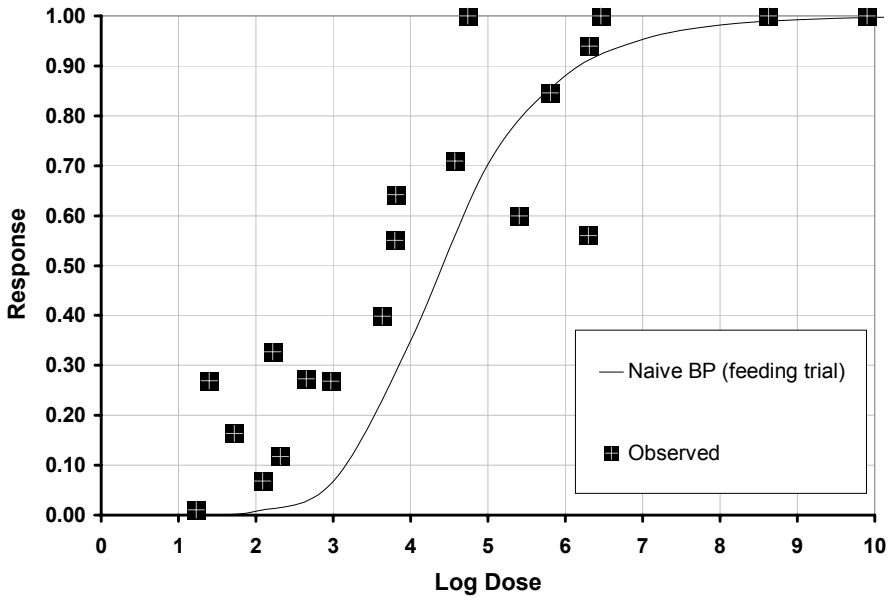

FIGURE 1.14. BETA-POISSON DOSE-RESPONSE MODEL FIT TO NAÏVE HUMAN FEEDING TRIAL DATA COMPARED WITH REPORTED OUTBREAK DATA.

Naïve human feeding trial data (beta-Poisson model)

The model suffers from the nature of the feeding trial data (i.e. the subjects used were healthy male volunteers) and may not reflect the population at large. The model also tends to greatly underestimate the probability of illness as observed in the outbreak data (Figure 1.14), even under the extremely conservative assumption that infection, as measured in the dose-response curve equates to illness.

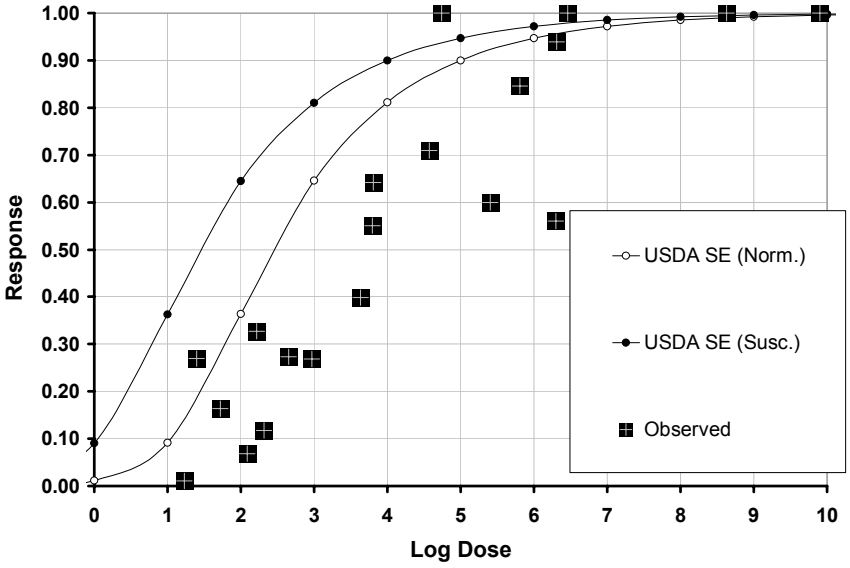

FIGURE 1.15. USDA-FSIS SALMONELLA ENTERITIDIS DOSE-RESPONSE MODEL COMPARED WITH REPORTED OUTBREAK DATA.

USDA-FSIS *Salmonella* Enteritidis (beta-Poisson model)

The model (uses human feeding trial data for *Shigella dysenteriae* as a surrogate pathogen with illness as the measured endpoint in the data. The appropriateness of using *Shigella* as a surrogate for *Salmonella* is questionable given the nature of the organisms in relation to infectivity and disease. Compared to the outbreak data (Figure 1.15), and on a purely empirical basis, this curve tends to capture the upper range of the data, but overestimates the probability of illness that is observed in the outbreak data.

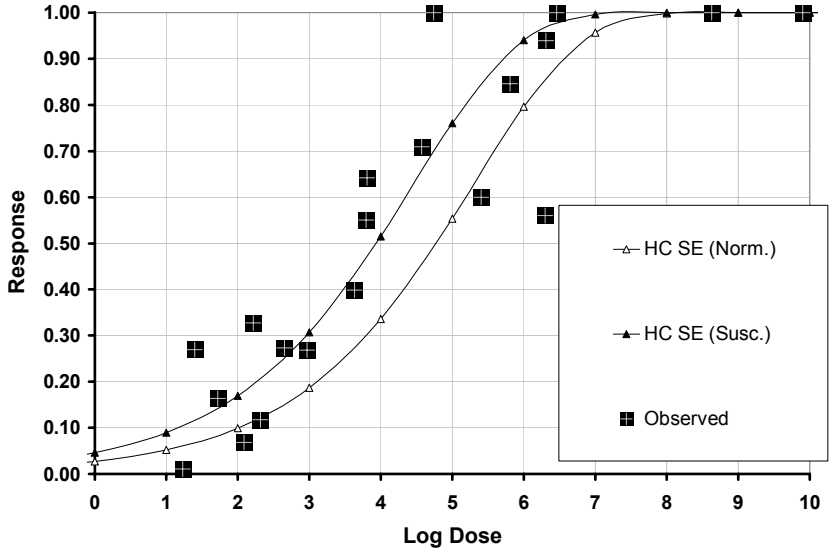

FIGURE 1.16. HEALTH CANADA SALMONELLA ENTERITIDIS DOSE-RESPONSE MODEL COMPARED WITH REPORTED OUTBREAK DATA.

Health Canada *Salmonella* Enteritidis (Weibull-Gamma model)

To date this model has not been fully documented and lacks transparency. The model uses data from many different bacterial pathogen-feeding trials and combines this information with key *Salmonella* outbreak data using Bayesian techniques. Using data from many bacterial feeding trials and the current lack of transparency regarding their influence is a point of caution. Empirically, the curve describes the outbreak data (Figure 1.16) at the low dose well but tends towards the lower range of response at higher doses.

Dose-Response Model Based on Outbreak Data

The availability of a reasonably large data set representing real world observations for the probability of illness upon exposure to *Salmonella* spp. (outbreak data), allowed a unique opportunity to attempt to develop a dose-response relationship based upon this data. The beta-Poisson model (Equation 1) was used as the mathematical form for the relationship and this was fit to the outbreak data.

$$Pill = 1 - \left(1 + \frac{Dose}{\beta}\right)^{-\alpha} \qquad \textbf{Equation 1}$$

The maximum likelihood technique was used as the basis to generate the best fitting curve to the data. The fit was optimized using an iterative technique that minimized the deviance statistic based upon a binomial assumption (Haas, 1983).

The outbreak data have merits as real world observations of the probability of illness upon exposure to a dose, however there are some drawbacks in the data as well. Specifically, it should be recognized that there is a degree of uncertainty in the outbreak data, primarily due to the uncontrolled settings under which the information and data are collected. In some cases, the actual dose ingested can be uncertain, while in other cases the true number of people exposed or ill during the outbreak can be under or overestimated.

The uncertainty in the outbreak data set was incorporated into the fitting routine by reviewing the outbreak information and assigning an uncertainty distribution on observed variables that were potentially uncertain. A detailed summary of the assumptions associated with each outbreak and the estimation for the range of uncertainty for each of the variables were described in Section 1.2.2. A summary of the data set, with uncertainty for the variables, is given in Table 1.15.

TABLE 1.15. UNCERTAINTY RANGES ASSIGNED TO VARIABLES IN REPORTED OUTBREAK DATA.

Otbrk Ref #	Serovar	log_{10} Dose (Uncertainty)		Response [Attack Rate] (Uncertainty)	
		Min	Max	Min	Max
1	*S. typhimurum*	1.57	2.57	11.20%	12.36%
2	*S. heidelberg*	1.48	2.48	28.29%	36.10%
3	*S. cubana*	4.18	4.78	60.00%	85.71%
4	*S. infantis*	6.06	6.66	100.00%	100.00%
5	*S. typhimurium*	3.05	4.05	52.36%	57.64%
7	*S. newport*	0.60	1.48	0.54%	2.59%
11	*S. Enteritidis*	4.00	5.00	100.00%	100.00%
12	*S. Enteritidis*	1.00	2.37	6.42%	7.64%
13	*S. typhimurum*	8.00	8.88	100.00%	100.00%
18	*S. Enteritidis*	5.13	5.57	60.00%	60.00%
19	*S. enteritidis*	6.03	6.48	87.70%	103.51%
20	*S. enteritidis*	2.69	3.14	18.61%	36.41%
22	*S. enteritidis*	6.02	6.47	52.17%	61.32%
23	*S. enteritidis*	5.53	5.97	84.62%	84.62%
24	*S. enteritidis*	1.45	1.89	12.19%	23.96%
25	*S. enteritidis*	3.36	3.80	39.85%	39.85%
30	*S. enteritidis*	3.53	3.97	60.14%	70.90%
31	*S. enteritidis*	2.37	2.82	25.62%	30.04%
32	*S. enteritidis*	1.11	1.57	26.92%	26.92%
34	*S oranienburg*	9.63	10.07	100.00%	100.00%

In order to fit the dose-response model to the uncertain outbreak data, the data were resampled based on the uncertainty distributions, generating a new data set at each sample. The dose-response model was then fit to each of the resampled data sets. This procedure was repeated approximately 5000 times, generating 5000 dose-response data sets, to which 5000 dose-response curves were fit. The fitting procedure used (Haas, 1983) places a greater emphasis on fitting the curve through the larger scale outbreaks compared to the smaller outbreaks. This is primarily as a result of the binomial assumption and the greater variance associated with data from a small observation compared to a large one. Figure 1.17 shows an example of the dose-response curves that are generated by fitting to the uncertain data.

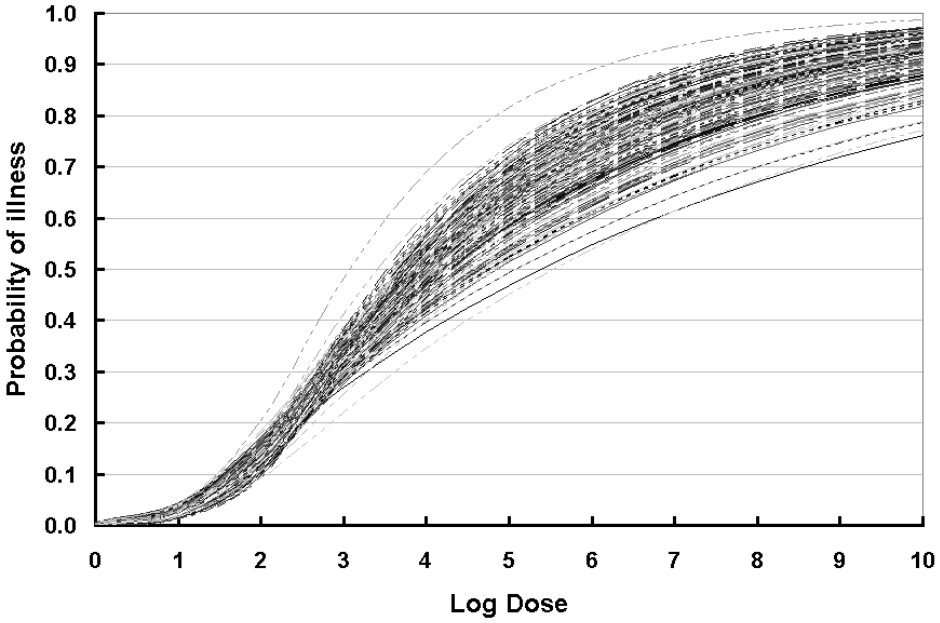

FIGURE 1.17. DOSE-RESPONSE CURVES GENERATED BY FITTING TO SAMPLES FROM UNCERTAIN OUTBREAK OBSERVATIONS

The observed outbreak data were found to be over-dispersed compared to what would be expected from the binomial assumption inherent in the deviance statistic that is minimized during fitting. As a result it was not possible to get a statistically significant single "best fitting" curve to the expected value of all the outbreak data points. However, the characterization of the observed outbreak data by the fitted dose-response model was better than that of the other dose-response models described previously. It is important to note that the range of possible responses at any one given dose shown in Figure 1.17 do not represent the statistical confidence bounds of the dose-response fit, but rather the best fit of the beta-Poisson model to different realizations of the observed data, given its uncertainties.

Figure 1.18 shows the comparison between the fitted curves and the expected value for the observed data. The upper bound, lower bound, expected value, 97.5^{th} percentile and 2.5^{th} percentile for the dose-response curves fit to the 5000 data sets are also shown. The fitted dose-response range captures the observed outbreak data quite well, especially at the lower and mid dose range. The greater range at the high doses is due to the existence of several large scale outbreaks at the lower- and mid-doses through which the curves attempt to pass, while the two high-dose data points are for relatively small scale outbreaks that allow greater "elasticity" in the fit.

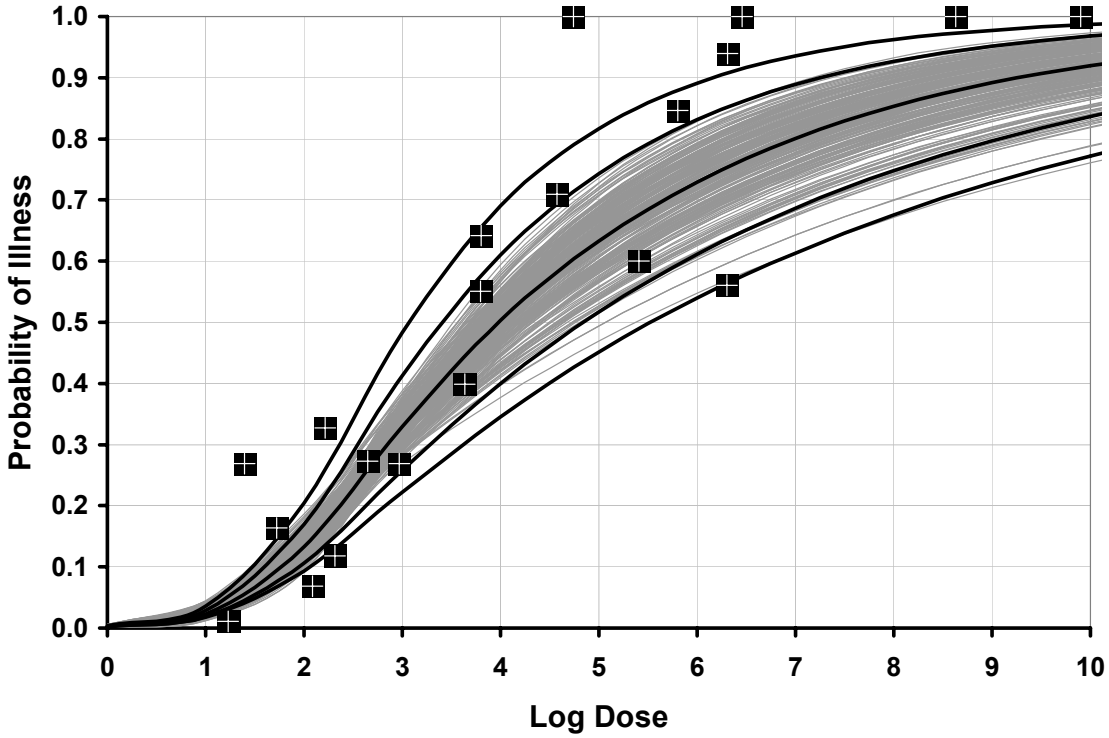

FIGURE 1.18. UNCERTAINTY BOUNDS FOR DOSE-RESPONSE CURVES COMPARED WITH EXPECTED VALUE FOR THE OUTBREAK DATA.

Since the fitting procedure generated a dose-response curve for each of the 5000 data sets, there are also 5000 sets of beta-Poisson dose-response parameters (alpha & beta). In order to apply the dose-response relationship in a risk assessment, the ideal approach would be to randomly sample from the set of parameters that are generated, thereby recreating the dose-response curves shown in Figures 1.17 and 1.18. As an alternative, it is also possible to use the upper, lower, expected value, 2.5th percentile or 97.5th percentile to represent the uncertainty ranges in the dose-response relationship as opposed to a full characterization resulting from the sampling of the parameter sets. The parameters that generate dose-response curves that approximate the bounds shown in Figure 1.18 of the dose-response relationship are summarized in Table 1.16.

TABLE 1.16. BETA-POISSON DOSE RESPONSE PARAMETERS THAT GENERATE THE APPROXIMATE BOUNDS SHOWN IN FIGURE 1.18.

	Alpha	**Beta**
Expected Value	0.1324	51.45
Lower Bound	0.0763	38.49
2.5th Percentile	0.0940	43.75
97.5th Percentile	0.1817	56.39
Upper Bound	0.2274	57.96

Figure 1.19 summarizes all the dose-response models described so far as well as the outbreak data. It also highlights the expected result of a better characterization of the outbreak data using the current model compared to the alternatives.

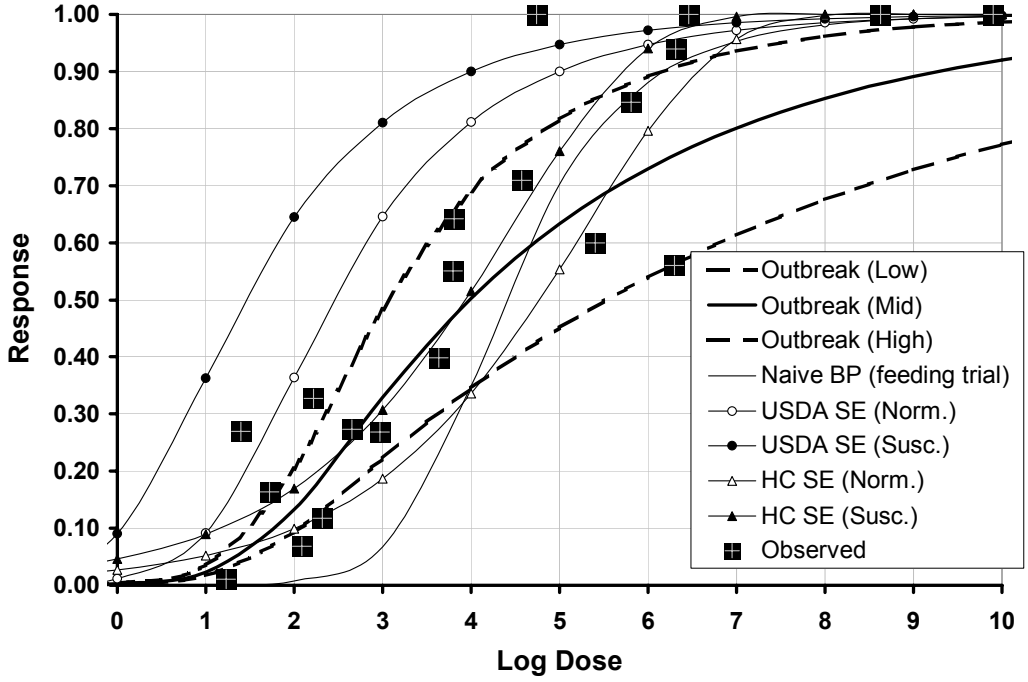

FIGURE 1.19. COMPARISON OF ALL DOSE-RESPONSE MODELS WITH REPORTED OUTBREAK DATA

In dose-response analysis, the critical region is the lower dose region. These are the doses that are most likely to exist in the real world and this is also the region for which most experimental data are non-existent. The outbreak data extend to a much lower dose than is common in experimental feeding trials, and as such may offer a greater degree of confidence in the lower dose approximations generated by the outbreak dose-response model. Table 1.17 and Figures 1.20 and 1.21 summarize the low dose estimates for the various dose-response models.

TABLE 1.17. PROBABILITY OF ILLNESS ESTIMATED BY ALTERNATIVE DOSE-RESPONSE MODELS AT SELECTED LOW MEAN DOSE VALUES.

	Mean $\log_{10}$ Dose {Mean Dose}			
	0 {1 cell}	**1 {10 cells}**	**2 {100 cells}**	**3 (1000 cells}**
Outbreak (Mid)	0.25%	2.32%	13.32%	32.93%
Naive BP (feeding trial)	0.01%	0.08%	0.75%	6.77%
USDA SE (Susc.)	9.06%	36.27%	64.44%	81.08%
USDA SE (Norm.)	1.12%	9.14%	36.43%	64.54%
HC SE (Susc.)	4.65%	8.99%	16.97%	30.72%
HC SE (Norm.)	2.65%	5.16%	9.95%	18.72%

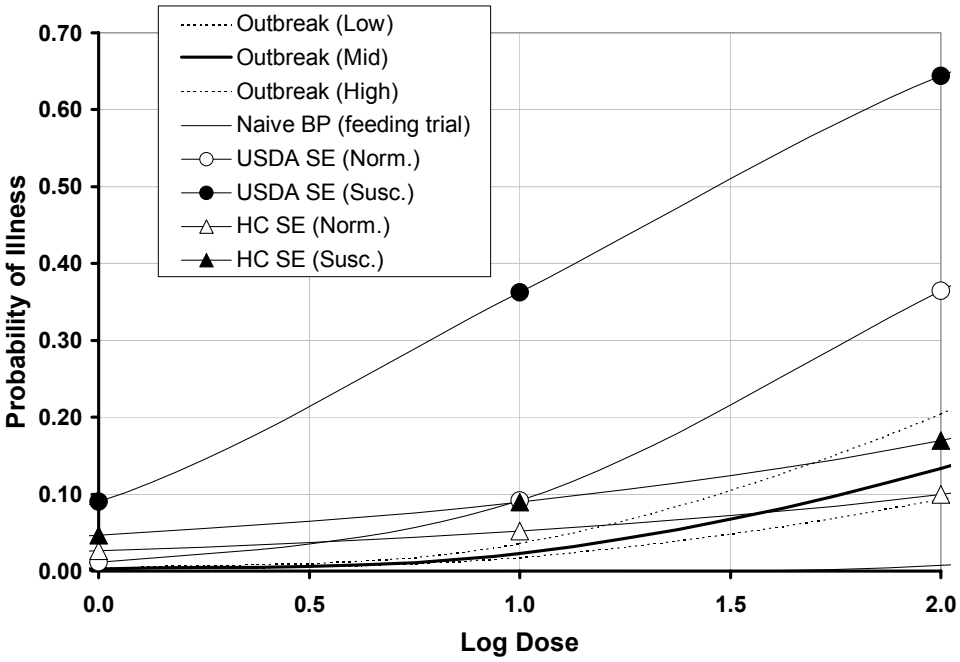

FIGURE 1.20. COMPARISON OF ALTERNATIVE DOSE RESPONSE MODELS IN THE 0 TO 2.0 MEAN LOG_{10} DOSE INTERVAL.

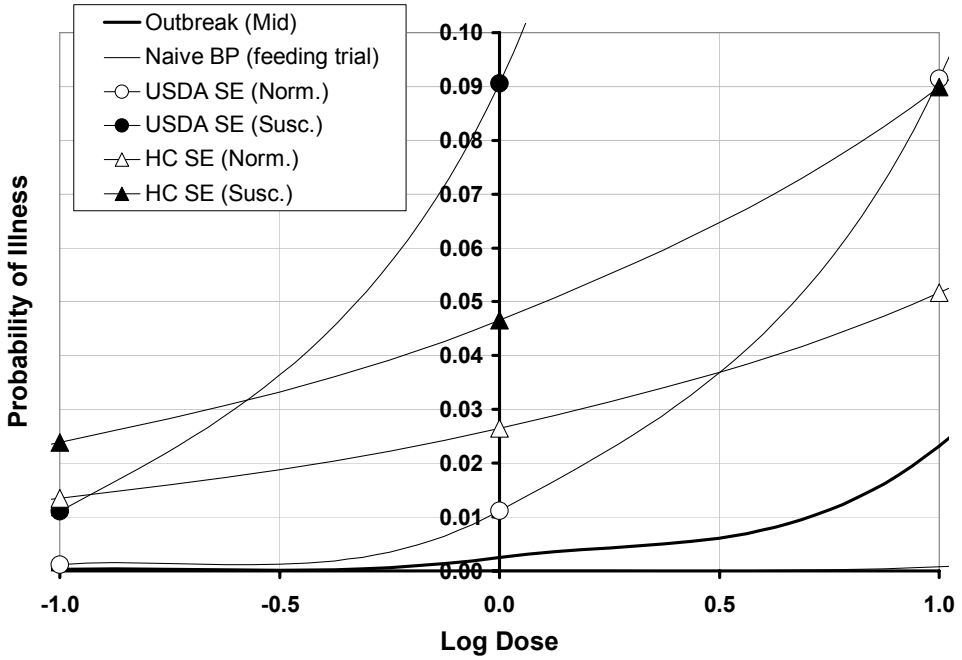

FIGURE 1.21. COMPARISON OF ALTERNATIVE DOSE RESPONSE MODELS IN THE –1.0 TO 1.0 MEAN LOG_{10} DOSE INTERVAL.

There is a wide range of estimates generated by the dose-response models. At a dose of 1000 cells, the USDA SE model for the normal population estimates a 65% probability of illness, and an 81% probability for the susceptible population. The Health Canada SE model estimates a 31% probability for susceptible populations and 19% for normal populations while the outbreak model estimates a probability of 33%. At a dose of 100 cells, the USDA SE model continues to be the most conservative with estimates ranging from 37% to 64%, while the outbreak model estimates a probability of 13%, that lies between the range (10% to 17%) estimated by the Health Canada SE model. Perhaps the most telling feature of low dose estimates is the probability of illness estimated by the models upon ingestion of 1 cell. The USDA and Health Canada SE models for susceptible populations estimate 9% and 5% probabilities respectively. In the case of the normal population, the Health Canada SE model estimates a higher probability (2.7%) than the USDA model (1.1%). The outbreak model estimates the probability at 0.24%, approximately an order of magnitude lower than the Health Canada model for normal populations.

In conclusion, the dose-response model based upon the observed outbreak data provides an estimate for the probability of illness that is based on real world data. Given the assumptions associated with some of the other models (surrogate pathogens, infection response with healthy male volunteers and lack of transparency with non-linear low dose extrapolation), the outbreak model offers the best current alternative for estimating the probability of illness upon ingestion of a dose of *Salmonella*.

DISCUSSION AND CONCLUSIONS

It has been postulated that some strains of *S.* Enteritidis, particularly the phage-types isolated from the increased number of egg-related outbreaks seen in recent years, may be more virulent than other serovars of *Salmonella*. From the outbreak data used to examine the dose-response relationship, there was no evidence that *S.* Enteritidis had a different likelihood of producing illness than other serovars. In total, 12 sets of data were evaluated for *S.* Enteritidis, against 8 sets of data for other serovars. However, increased severity of illness once infected was not evaluated.

It was concluded that there is insufficient evidence, in the current outbreak database to conclude that some segments of the population have a higher probability of illness compared to others. There was some indication in two instances, in which two populations potentially exposed to *Salmonella* in the same outbreak exhibited different attack rates. There is therefore a possibility that the probability of illness upon exposure may be different for some members of the population compared to others. However, in the absence of additional information the probability of illness could be assumed to be the same for all members of the population, although the severity of the illness could be potentially different.

This document did not consider a quantitative evaluation of secondary transmission (person-to-person) or chronic outcomes. In addition, the impact of the food matrix was not incorporated into the assessment. These may be considerations for future document development.

The dose-response model fit to the outbreak data offers a reasonable estimate for the probability of illness upon ingestion of a dose of *Salmonella*. The model is based on observed real world data, and as such is not subject to some of the flaws inherent in using purely experimental data. Nevertheless, the current outbreak data also have uncertainties associated with them and some of the outbreak data points required assumptions to be made. Overall, the dose-response model generated in the current exercise can be used for risk assessment purposes and generates estimates that are consistent with those that have been observed in outbreaks.

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