

1 Chapter 5 Public Health Module

3 A. Overview

5 The Public Health Module links exposure in cooked servings of ground beef to
6 alternative surrogate dose-response models to predict cases of illness associated with *E.*
7 *coli* O157:H7. Variables in this module include annual estimates for serving sizes and
8 frequencies by age group for various preparation methods of ground beef inferred from
9 consumption data from surveys of short duration. Limited data are available to predict the
10 number of severe cases as additional public health endpoints and differential
11 susceptibility of more sensitive human sub-populations.

13 Two published risk assessments are available for consideration of data sources and
14 approaches for modeling adverse public health effects due to foodborne illness from *E.*
15 *coli* O157:H7 in ground beef (Cassin 1998; Marks 1998). The Marks manuscript (1998)
16 was the major source for some sections of the text for this chapter.

18 Data are available from human clinical trials for many pathogens (Teunis 1996) to predict
19 the probability of illness given the dose of pathogen administered to healthy adult
20 volunteers. However, such data for modeling the probability of illness associated with a
21 given dose of *E. coli* O157:H7 in human volunteers are not available. Two published risk
22 assessments (Cassin 1998; Marks 1998) fit data for *Shigella* human clinical trials (Levine
23 1973; Dupont 1969, 72) to a single model form, the Beta-Poisson (Haas 1983) for
24 shigellosis as a surrogate model to predict the probability of illness (pathogenicity) for *E.*
25 *coli* O157:H7. One risk assessment team chose to calculate risk with attendant
26 uncertainty only for the healthy adult population consuming hamburgers away from
27 home using a shigellosis model as a surrogate for *E. coli* O157:H7 (Marks 1998). The
28 other team chose to model probability of *E. coli* O157:H7 illness equally for children and
29 adults (Cassin 1998). Disease severity was not modeled in either published risk
30 assessment for adults (Cassin 1998; Marks 1998), but Cassin (1998) used point estimates
31 of severity from an outbreak to predict disease severity for children, probability of the
32 serious complication hemolytic-uremic syndrome (HUS) given hospitalization = 10% and
33 probability of death given HUS = 5%.

35 Inputs and Outputs for the public health module:

37 The input to the public health module from the preparation module will consist of the
38 number of contaminated servings and the density of the pathogen *E. coli* O157:H7 within
39 contaminated servings.

41 The output of the module will be the annual number of cases of illnesses of different
42 severity that might occur based on the data, assumptions, and judgement. The Public
43 Health endpoints considered for healthy adults may include diarrhea and bloody diarrhea
44 or hemorrhagic colitis (HC) which will be estimated from surrogate dose-response
45 models described in a later section as number of cases per year associated with
46 consumption of beef. No data are available that directly relate dose of surviving *E. coli*

1 O157:H7 in cooked ground beef servings to adverse effects in humans or animals. The
2 potential surrogates for this data gap are described in detail later in this chapter.

3
4 Other endpoints for adults are uncertain. Progression of bloody or non-bloody diarrhea in
5 otherwise healthy adults to more severe endpoints, such as HUS and thrombotic
6 thrombocytopenic purpura or TTP, appears to occur infrequently and may be modeled
7 using attack rates from outbreak and surveillance data (appended Table 1). Some
8 attention will be needed to estimate uncertainties associated with the assumptions and the
9 limitations of these data. For example, use of attack rates to model disease progression
10 and severity assumes that dose is a constant for the entire population. This appears to be a
11 very tenuous and unrealistic assumption that merits extensive deliberation and perhaps
12 consideration of potential alternative data sources or approaches. Dose-dependence
13 strongly influences both probability and severity of illness as described in this chapter.

14
15 Endpoints for more susceptible sub-populations are even more uncertain. Specific data
16 are needed to define susceptible sub-populations, such as children under a certain age, the
17 immunocompromised or institutionalized, or the elderly over a given age, that might be
18 more susceptible than healthy adults to *E. coli* O157:H7 illness. The available data are
19 described later in this chapter. Our Canadian colleagues considered the same surrogate
20 dose-response model for the entire human population. If more susceptible sub-
21 populations could be defined, data appear to be lacking for prediction of the probability
22 or severity of illness for children or other potentially susceptible sub-populations as a
23 function of ingested dose. Data are available from animal and human clinical studies that
24 depict families of dose-response curves that account for differential susceptibility for
25 another enteric pathogen (Coleman, in preparation). To our knowledge, such data are not
26 available for *E. coli* O157:H7. It is unclear how the probability of illness would be
27 modeled for more susceptible sub-populations. Some options to consider are use of more
28 conservative model forms or shifting the dose-response model for adults to the left and/or
29 imposing greater slopes for the dose-response models of susceptible sub-populations.

30
31 As described for adults, if alternative surrogate dose-response models can be generated
32 for more susceptible sub-populations, additional endpoints may include hospitalization,
33 severe complication (eg, HUS, TTP), and death. Neither published risk assessment
34 (Cassin 1998; Marks 1998) modeled the elderly as a susceptible sub-population or
35 modeled more severe effects in the elderly. This issue merits further deliberation,
36 especially considering outbreak data from institutional settings (Su 1995). Some deaths
37 among children and immunocompromised adults and the elderly have been noted in *E.*
38 *coli* O157:H7 outbreaks (Su 1995). A simple model may be developed based on rates of
39 hospitalization, HUS and/or TTP, and death reported in epidemiological studies
40 summarized in Table 1 of this chapter.

1 Table 1: Public Health Statistics

Statistic	Reference
Diarrhea	
0.6% to 2.4% of all diarrheal cases associated with O157	Su (1995)
O157 isolated from 13 (2.9%) of 445 children's stools submitted during 1 yr in Seattle, WA	Bokete (1993)
Bloody Diarrhea, Hemorrhagic Colitis (HC)	
38% - 61% O157 illnesses result in HC	Su (1995)
95% of the 93 sporadic cases of O157 in Washington State in 1987 had bloody diarrhea	Ostroff (1989)
451 (90%) of 501 cases of diarrhea in a multi-state outbreak caused by <i>E. coli</i> O157:H7 were bloody	Bell (1994)
15%-36% all bloody diarrhea or HC caused by O157	Su (1995)
13% -73% HC cases result in hospitalization	Su (1995)
1% HC cases result in mortality	Roberts (1998), citing Boyce 1995 and Ryan (1986)
Hemolytic Uremic Syndrome (HUS)	
140 (89%) of 157 HUS cases post-diarrheal	Siegler (1994)
102 reported post-diarrheal HUS cases 1996	CDC (1996)
2%-7% O157 illnesses progress to HUS	Griffin (1991)
5%-10% of O157 w/ bloody diarrhea progress to HUS	Griffin (1995)
10% infected children under 10 receive medical attention for HUS	Tarr (1995).
HUS caused chronic renal sequelae, usually mild, in 51% of survivors, 48% of 157 HUS cases over 20-yr period in Utah	Siegler (1994)
Neurological complications, commonly mild, may occur in 30% to 50% of HUS patients, but serious complications may arise.	Su (1995)
9 (7.7%) of 117 HUS children had renal failure and survived; one (<1%) required kidney transplant	Martin (1990)
Severe kidney or neurological impairments (end stage renal disease or stroke) occurred in 9 (6%) of 157 HUS cases	Siegler (1994)
60% of pediatric HUS patients that develop chronic kidney failure die prematurely	Buzby (1996)
3-5% acute mortality for HUS	Mahon (1997), citing Martin (1990), Tarr (1987), and Rowe (1991)
8 (5%) of 157 HUS cases resulted in mortality	Siegler (1994)
5%-10% mortality for HUS	Su (1995), citing Karmali (1989) before long-term studies reported
HUS incidence in children <18 in MN inc from 0.5 to 2.0 per 10 ⁵ 1979-1988	Martin (1990)
HUS incidence in children <15 in King Co, WA inc 2.5 x from 1971-76 to 1976-80	Tarr (1987)
HUS incidence in children in UT 1971-90 ranged from 0.2-3.4 per 10 ⁵ w/ no evidence of increase	Siegler (1994)
1994-97, annual no. outbreaks declined 30% and no. ill per year declined 45%	Tables 6-9 of Chapter 1
Incidence HUS approx 1-3 per 10 ⁵ children-yr for children under 5; 1-2 per 10 ⁵ children-yr for older children	Martin 1990, Rowe 1991, Kinney 1988, Tarr 1987, Siegler 1994
Thrombotic Thrombocytopenic Purpura (TTP)	
3/37 (8%) HC cases progressed to TTP in one outbreak	Su (1995) citing Ostroff (1990)
Case-mortality rate for TTP varies among outbreaks and is uncertain	none

1 Table 1 (cont'd)

Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP)	
94 (5%) of 1855 of outbreak cases of O157 progressed to HUS or TTP	Table 10 of Chapter 1
NUMBER OF CASES	
The number of cases reported to NDSS increased from 1420 to 2741 from 1994-96 (0.82-1.18 per 10 ⁵)	CDC (1997a)
8 per 10 ⁵ per-yrs O157 cases 1985-86 in WA (pop study)	MacDonald (1988)
2.1 per 10 ⁵ per-yrs O157 illnesses 1987 in WA (1 st yr surveillance)	Ostroff (1989)
2.9 per 10 ⁵ per-yrs O157 cases 1996 FoodNet sites avg.	CDC (1997b)
2.1 per 10 ⁵ per-yrs O157 cases 1996 FoodNet sites avg.	CDC (1998)
10-20 * 10 ³ cases (seeking medical care) nationally per yr (4-8 per 10 ⁵ per-yrs)	CDC (1993)
45% of O157 ill persons did not seek medical care	Cieslak (1997)
20-40 * 10 ³ total infections nationally per yr	Roberts (1998)
19 (1%) of 1855 O157:H7 outbreak cases resulted in mortality	Table 10 of Chapter 1
Ground beef identified as likely vehicle in 248 of 1764 (14%) outbreak illnesses 94-97	Table 3 of Chapter 1
Unknown vehicle in 515 of 1764 (29%) outbreak illnesses 94-97	Table 3 of Chapter 1
Ground beef id as likely vehicle in 1267 of 3587 (35%) outbreak illnesses 82-97	Table 1 of Chapter 1
Unknown vehicle in 561 of 3587 (16%) outbreak illnesses 82-97	Table 1 of Chapter 1

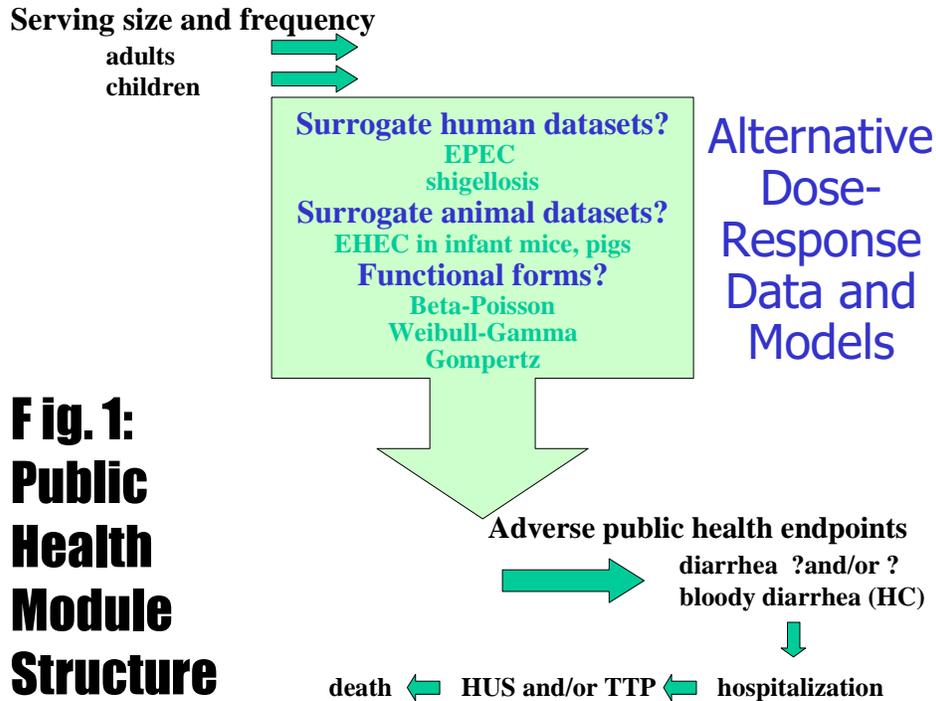
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B. Module structure

4

5 The outputs of the Preparation Module, the level and occurrence of *E. coli* O157:H7 in
6 cooked ground beef meals, become the inputs to the Public Health Module (Figure 1).
7 The meals will be characterized from consumption data by age of consumers such as the
8 CSFII for 1994-96. The size and frequency of ground beef meals will be derived for
9 preparation at home and away from home. The doses per serving of ground beef will then
10 become inputs into alternative surrogate dose-response models which output the
11 probability of illness. It is unclear how the dose-response models might be adjusted for
12 more susceptible sub-populations. The probabilities of illness for adults and perhaps other
13 more susceptible sub-populations derived from dose-response models must then be
14 adjusted to predict the numbers of cases for different endpoints of interest. One method to
15 achieve this adjustment is to apply attack rates estimated in outbreaks and surveillance
16 studies as a surrogate to model progression of illness to more severe endpoints. It is
17 unclear whether or not the endpoints HUS and TTP should be modeled separately. For
18 children, the predominant concern will be HUS (Su 1995).



**Fig. 1:
Public
Health
Module
Structure**

C. Variable description and evidence

1. Data sources for consumption of ground beef, including hamburger

Although results are not currently available, the following are potential sources of information on consumption.

1a. Continuing Survey of Food Intake by Individuals 1994-96

The 1994-96 CSFII was conducted by the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA). Each year of this 3-year data set comprises a nationally representative sample of noninstitutionalized persons residing in the United States. Regional estimates, but not state-level estimates, are available from the CSFII/DHKS 1994-96 data. The sample is a stratified, multistage area probability sample. The stratification plan took into account geographic location, degree of urbanization, and socioeconomic characteristics. Low-income individuals were oversampled, but age groups were not oversampled.

In the CSFII 1994-96, 2 nonconsecutive days of dietary data for individuals of all ages were collected between January 1994 and January 1997 through in-person interviews using 24-hour recalls. The 3-year CSFII data set includes information on food and nutrient intakes by 16,103 individuals who provided at least 1 day of dietary data, for a total number of person-days of over 30,000. The response rate for completing at least one day of food intake was 80%.

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1 Detailed food codes enable the user to obtain statistics on consumption of hamburgers,
2 other ground beef, other beef, and other processed foods or recipes containing beef, such
3 as mixed beef/pork hot dogs and beef enchiladas. For each food type of interest, the data
4 can be used to estimate the frequency of consumption (percent of person days on which
5 hamburger consumption occurs), as well as the average level of consumption. A detailed
6 recipe data base can be used to convert amounts of beef-containing recipes (such as
7 enchiladas) to amounts of beef consumed.

8
9 Preliminary data from CSFII were provided (Ralston 1998). Some of the findings include
10 the observation that of the 359 children under the age of 1 included in the survey,
11 approximately 3% consumed hamburger. One could argue from these results that
12 hamburger is an unlikely vehicle for *E. coli* O157:H7 illness for this age group.

13 14 1b. The 1996 Hamburger Preparation Quiz and Consumption Diary

15
16 The HPQCD was collected by the Market Research Corporation of America as a
17 supplement to their on-going Menu Census Survey during March 1996 - February 1997.
18 The Menu Census Survey is a nationally representative mail survey in which respondents
19 complete a 2-week diary on food consumption followed by a questionnaire on attitudes
20 related to food purchases. Respondents who consumed hamburger, chicken or eggs were
21 asked to fill out a supplement to Menu Census Diary, which included data on whether
22 hamburgers were frozen, how they were thawed, how they were cooked, and the color of
23 the cooked patty.

24
25 The Menu Census Survey covers about 2000 households who are selected from a 12,000
26 household purchase diary survey. Both the larger sample and the Menu Census Survey
27 are selected as stratified samples to match U.S. Census data for geographic and
28 demographic cells. However, the respondents to the survey supplement were not
29 perfectly representative of the U.S. population. ERS has estimated weights to correct for
30 these imbalances.

31
32 The consumption diary supplement was completed for 1580 households, recording 6400
33 hamburger eating occasions, of which 3600 were at home.

34 35 1c. National Health and Nutrition Examination Survey

36
37 The Third National Health and Nutrition Examination Survey (NHANES III), 1988-94,
38 was conducted on a nationwide probability sample of approximately 33,994 persons 2
39 months and over. The survey was designed to obtain nationally representative
40 information on the health and nutritional status of the population of the United States
41 through interviews and direct physical examinations. The survey includes a dietary recall
42 covering one day per individual.

43
44 The survey is a stratified multistage design. The sample over-samples children under 5,
45 and adults over 60. These groups have been clearly identified as more vulnerable to
46 foodborne illness, and identifying high-risk consumers within these groups is facilitated

1 by this over-sampling. (See website for further information:
2 <http://www.cdc.gov/nchswww/products/catalogs/subject/Nhanes3/nhanes3.htm#description1>)

3 4 1d. Making inferences from the available consumption data

5
6 An estimate of the risk per meal, or the probability of illness per eating occasion can be
7 calculated. Specifically, the distribution of the amounts of consumed meals can be
8 estimated, and then, for each eating occasion, an associated probability of illness can be
9 determined. As an example, information is provided from an older source for serving size
10 and frequencies (USDA Continuing Survey of Food Intake by Individuals (CSFII), 1989-
11 91).

12
13 A simple distribution is desired for which probabilities can be easily and directly
14 calculated for consumption. Distributions from the class of distributions defined by Burr
15 (Johnson 1970) were considered (Marks 1998). Among these, the Burr type XII function
16 provided a good fit to the data for hamburger prepared away from home (CSFII), 1989-
17 91). The minimal value for consumption was 6.93 grams (approximately 0.25 ounces).
18 The other parameters of this distribution were estimated by computing $y = \ln(1/(1-F(x)))$
19 for values of x , treating y as an dependent variable, and solving for the unknown
20 parameters. The model chosen had minimal residual standard error for predicting y . The
21 modal value derived for this distribution was 50.40 grams; the median value derived was
22 65.56; and the mean value derived was 74.67 grams. Because of the large number of
23 observations used in deriving these estimates, the parameter values can be considered
24 constant in subsequent Monte Carlo simulations (Marks 1998).

25 26 **2. Clinical data from human and animal experiments**

27
28 Most of the clinical data surveyed in this section are dose:frequency data which report the
29 percentage of a group of volunteers with an adverse effect at each dose administered.
30 Some data are also available that report a measurement of the adverse effect, such as
31 volume of liquid stool (Bieber 1998). The latter type of study may be more useful for risk
32 assessment purposes than dose-frequency studies. Data for bacterial strains administered
33 to healthy animals at high doses are difficult to interpret. Further research may be needed
34 to resolve difficulties in relating the conditions used in the human clinical trials to
35 naturally occurring foodborne disease. For example, effects of food matrices, stomach
36 acid neutralization, and lack of expression of virulence gene products in microbiological
37 media appear to be strong and potentially competing influences on prediction of illness.
38 In addition, uncertainties in low-dose extrapolation will be considerable. Data and
39 comments on approaches to account for these factors are of great interest to FSIS for this
40 risk assessment.

41
42 Strains of the pathogen *Escherichia coli* O157: are classified along with strains of O26
43 and O111 serotypes in the enterohemorrhagic *E. coli* (EHEC; Levine 1987) which can
44 cause non-bloody diarrhea, bloody diarrhea (hemorrhagic colitis, HC), and more severe
45 complications, including hemolytic uremic syndrome (HUS) and thrombotic
46 thrombocytopenic purpura (TTP; Su 1995). The EHEC are classified in a larger group
47 termed VTEC or verocytotoxic *E. coli*, which all produce toxins but may not share other

1 specific virulence factors that contribute to pathogenesis in humans. Additional
2 pathogenic *E. coli* strains causing infant diarrhea are classified as enteropathogenic *E.*
3 *coli* or EPEC (Levine 1987). *Escherichia. coli* O157:H7 is closely related to certain
4 EPEC strains and may have evolved from a common ancestor (Whittam 1993). The
5 similarity in these two types of pathogenic *E. coli* is the basis for considering the EPECs
6 as a potential surrogate in this chapter.

7 8 2a. Evidence for Selection of Surrogate Pathogen-Host Systems

9
10 No quantitative data are available for EHEC or VTEC strains of *E. coli* for dose-response
11 modeling in humans. Development of surrogate information for dose-response modeling
12 has not been widely explored. The following criteria are proposed for consideration in
13 selection of a surrogate for a pathogen: similarities in: 1) genetics of the pathogens,
14 especially for their virulence factors which may include pathogenicity islands and toxin
15 genes; 2) mechanisms of pathogenesis, including site of attachment and pathology and
16 extent of invasion of the pathogen into host cells, tissues, and body fluids; and 3) mode of
17 disease transmission. The similarity in the virulence genes of the pathogens may be an
18 important criterion for selection of surrogates. The behaviors of a pathogen, including the
19 last two criteria, may arise from bacterial genes or the interaction of bacterial genes or
20 gene products with the host. Obviously, quantitative data for dose and response should
21 exist for the surrogate, especially for low doses typical of foodborne exposures.

22
23 Potential surrogates for *E. coli* O157:H7 were identified which partially satisfy the three
24 criteria, including infant diarrheal *E. coli* strains (types 55, B₅ and 111, B₄, H locus
25 unspecified), June, 1953; type B171-8 (O111:NM), Bieber 1998) and two species of the
26 genus *Shigella* (*S. dysenteriae* type I, strains M 131 and A-1; *S. flexneri*, strain 2457T;
27 Levine 1973; DuPont 1969; Dupont 1972). The three potential surrogates share some
28 genetic similarities (Whittam 1993; Doyle 1989), but differ from *E. coli* O157:H7 in
29 mechanism of pathogenesis (Falkow 1996; Roth 1995; Salyers 1994). The potential
30 surrogates can be transmitted person-to-person as observed for *E. coli* O157:H7, though
31 not all surrogate species are regarded as foodborne pathogens (Doyle 1989). The
32 available evidence for use of EPEC and *Shigella* as surrogates is evaluated separately
33 below.

34
35 The infant diarrheal *E. coli* strains are classified in the “virotype” termed
36 enteropathogenic *E. coli* or EPEC (Salyers 1994; Levine 1987). *Escherichia. coli*
37 O157:H7 appear closely related to certain EPEC, including serotypes 55 and 111
38 administered in the human feeding studies, and *E. coli* O157:H7 strains share a common
39 pathogenicity island (LEE) for their chromosomal virulence genes, including the *eae*
40 associated with attaching and effacing lesions and the attachment peptide intimin (Jarvis
41 1996; Kaper 1998a,b). The site of attachment and pathology for EPEC strains appears to
42 be the distal small intestine in humans, whereas the EHEC strains appear to attach and
43 cause damage in the proximal colon (ascending and transverse; cecum and ascending)
44 after several days of intestinal colonization (Kaper 1998b; Su 1995). Conventional and
45 gnotobiotic piglets mirror some aspects of the pathology of humans, EPEC strains
46 causing pathology in small and large intestines, whereas EHEC strains cause lesions only

1 in the large intestine (Kaper 1998a). In fact, the histopathology of attaching and effacing
2 lesions has been observed in tissue culture cells, animal models (gnotobiotic piglets,
3 infant rabbits, young cattle, chickens, and macaque monkeys), and human EPEC victims
4 (Kaper 1998b) and EHEC victims (Tarr, pers comm). Other details of pathogenicity that
5 are shared by the two pathogens include: 1) localized adherence to host epithelial cells in
6 the lower GI tract ; 2) signal transduction, host actin rearrangement, and effacement of
7 villi on attached host cell; and 3) intimate, membrane-membrane adherence involving the
8 gene encoding the protein intimin and pedestal formation (Finlay 1995; Jarvis 1996). No
9 invasion of host cells is observed for either of these *E. coli* strains. The striking genetic
10 homology of some strains and the common histopathology of attaching and effacing
11 lesions for *E. coli* O157:H7 and EPEC infections suggest that the data for the infant
12 diarrheal *E. coli* strains may be relevant for adults exposed in the high dose
13 (approximately 10^{7-10}) region.

14
15 The EPECs appear to cause endemic illness in infants and children in developing
16 countries, occasional outbreaks of neonatal diarrhea in hospitals around the world, and
17 perhaps sporadic cases of infant diarrhea (Doyle 1989). However, the EPEC strains rarely
18 appear to be the cause of disease (Ferguson 1952) or foodborne disease (Doyle 1989) in
19 adults. Adults appear to be asymptomatic carriers of EPEC strains, which may be of low
20 pathogenicity in adults because immunity is acquired with age (Doyle 1989).
21 Asymptomatic carriers of *E. coli* O157:H7 have been identified in outbreaks (Su 1995).
22 In human feeding studies, low doses of EPEC were not administered (June 1953),
23 presumably because low virulence was expected, and observed, in adults. Some adult
24 volunteers did develop generally mild symptoms of gastroenteritis after ingesting high
25 doses of EPECs (Levine 1987). Other studies (Bieber 1998) suggest more variability
26 might be associated with pathogenicity of strains. However, full immunity to infection is
27 not generally observed even among adults. Although the clinical data for the EPECs in
28 adult volunteers are not conclusive for risk assessment purposes, we considered the
29 EPEC as a potential surrogate in our exploration of dose-response modeling for *E. coli*
30 O157:H7.

31
32 Regarding *Shigella* as a potential surrogate for *E. coli* O157:H7, other researchers used a
33 *Shigella* dose-response model for *E. coli* O157:H7 (Cassin 1996; Cassin 1998). Modern
34 taxonomists consider the distinction between the genera *Escherichia* and *Shigella*
35 unwarranted (Salyers 1994). Indeed, genetic similarity of bacterial species in general
36 should be interpreted with caution. No conclusive evidence of similarity in virulence
37 genes or mechanisms of pathogenesis exists to support the appropriateness of the invasive
38 *Shigella* species as surrogates for the non-invasive *E. coli* O157:H7. The largest body of
39 evidence for both pathogenesis mechanism and dose-response modeling from human
40 feeding studies exists for *S. flexneri*.

41
42 Pathogenesis in shigellosis involves bacterial adherence to a host epithelial cell in the
43 colon; invasion of host M cells in the colon; escape from the M cell into adjacent
44 epithelial cells; replication in the host cell cytoplasm; movement between adjacent cells;
45 inflammation; and undermining of the integrity of patches of host cells, resulting in local
46 areas of tissue necrosis (Neidhardt 1996; Finlay 1995; Salyers 1994). The pathogenesis of

1 shigellosis generally does not include bacteremia or spreading from the colonic epithelial
2 cells into the bloodstream, but typically includes ulceration of the colon and exudation of
3 blood and pus in diarrheal stools, generally termed dysentery (Salyers 1994). All four
4 species of *Shigella* (*S. flexneri*, *S. dysenteriae*, *S. sonnei*, and *S. boydii*) have similar
5 virulence genes essential for the invasive pattern of infection (Salyers 1994).

6
7 Key mechanisms of the pathogenesis of *Shigella spp.* and *E. coli* O157:H7 differ. *E. coli*
8 O157:H7 does not utilize the invasive virulence mechanism or cause the level of
9 inflammation typical of shigellosis (Salyers 1994). However, both *E. coli* O157:H7 and
10 the *Shigella spp.* can cause similar pathological symptoms of dysentery in human hosts
11 (Salyers 1994). An unrelated parasite (*Entamoeba histolytica*) also causes symptoms of
12 dysentery. Whether the criterion of induction of similar pathological symptoms in the
13 host is adequate evidence to justify use of these microbes as surrogates for a common
14 dose-response relationship is unclear. The symptoms of dysentery are usually self-
15 limiting in healthy adults, but can be fatal in infants and young children, presumably due
16 to partial immunity and better hygienic practices among adults (Salyers 1994). Similar
17 age-dependency of attack rates for *E. coli* O157:H7 have also been observed (Su 1995).

18
19 To this point in the discussion of the primary pathogenesis mechanism for shigellosis,
20 Shiga toxin has not been mentioned. *Shigella flexneri* does not produce Shiga toxin; *S.*
21 *dysenteriae* type I has chromosomal genes for Shiga toxins, which have activity in model
22 systems as enterotoxins, neurotoxins, and cytotoxins (Salyers 1994). *Escherichia coli*
23 O157:H7 strains lack the chromosomal gene for Shiga toxin, but appear to have acquired
24 the phage-associated virulence factor for the Shiga toxins (Whittam 1993). The role of
25 Shiga toxin as a primary virulence factor in pathogenesis involving either *S. dysenteriae*
26 or *E. coli* O157:H7 is equivocal (Su 1995; Salyers 1994; Levine 1973). Shiga toxin is not
27 required for *Shigella spp.* to adhere, invade, multiply in the host cytoplasm, invade
28 adjacent cells, and cause cell necrosis (63) or for *E. coli* O157:H7 (or the EPECs) to
29 adhere and cause attaching and effacing lesions (Su 1995). Rather, the Shiga toxin may
30 function later in pathogenesis, and may impact severity of disease and systemic
31 complications of infection with *E. coli* O157:H7 such as HUS (O'Brien 1996; Salyers
32 1994) and *S. dysenteriae* (Salyers 1994).

33
34 Such a role for Shiga toxin enhancing the severity of disease appears likely from work
35 with a murine EPEC strain genetically engineered to express Shiga toxin I (Bloom 1998).
36 The engineered EPEC strain expressing Shiga toxin (RDEC-H19A) caused hemorrhagic
37 colitis in rabbits, whereas the parent EPEC strain caused less severe symptoms of illness
38 (Boedeker 1998). The biological effects of the Shiga toxin may differ for the invasive *S.*
39 *dysenteriae* and non-invasive *E. coli* strains which might release toxin intra- and extra-
40 cellularly, respectively (O'Brien 1996).

41
42 The proposed criterion of mechanism of transmission lends some support for selection of
43 shigellosis as a surrogate for *E. coli* O157:H7. Both diseases can be transmitted person-
44 to-person (Su 1995; Salyers 1994). Transmission by this route suggests that small doses
45 may be sufficient to cause illness, but the existence of quantitative experimental data to
46 support this hypothesis is uncertain.

1 The body of evidence for shigellosis as a surrogate is summarized below. Quantitative
2 data supporting dose-response assessment include one strain of *S. flexneri* (2457T) which
3 was administered in milk to 43 and 193 fasting healthy adult male volunteers in two
4 experiments, respectively (DuPont 1969; Dupont 1972). Additional observations exist for
5 *S. dysenteriae* strains from single experiments similarly conducted, using strain M 131
6 with 30 volunteers and strain A-1 with 10 healthy adult male volunteers (Levine 1973).
7 The A-1 strain which has been omitted from several analyses (Crockett 1996; Cassin
8 1998; Marks 1998) was much less pathogenic than the included strain M 131. Both
9 strains will be considered herein. Some low doses of *S. dysenteriae* (10 cells of strain M
10 131) were administered, which reduces the uncertainty associated with high to low dose
11 extrapolation that is problematic for the EPEC data.

12
13 Despite the lack of conclusive evidence that the *Shigella* dose-response relationships
14 provide a suitable surrogate for *E. coli* O157:H7, the *Shigella* datasets were selected for
15 surrogate dose-response modeling for *E. coli* O157:H7 in the past (Cassin 1998; Marks
16 1998) and will be considered for this risk assessment. The invasive pathogenesis of the
17 *Shigella* may result in greater pathogenicity than a similar dose of the non-invasive *E.*
18 *coli* O157:H7 (O'Brien 1996). The shigellosis surrogate dose-response model may thus
19 provide an unknown level of conservatism for modeling illness associated with the non-
20 invasive pathogen *E. coli* O157:H7.

21 22 2b. Human clinical trials: EPECs

23
24 In addition to shigellosis data, Marks (1998) also considered human clinical data for
25 enteropathogenic *E. coli* (EPEC; June 1953) as a potential surrogate for *E. coli* O157:H7.
26 Recently, additional studies with the EPECs and *Shigella* have been identified (Bieber
27 1998; Levine 1989) that are described below along with other data from human and
28 animal clinical trials.

29
30 Bieber 1998: Healthy adult volunteers (ages 18-48) were administered 150 mL of sodium
31 bicarbonate solution (1.3%) one minute prior to ingestion of challenge bacteria in
32 phosphate buffered saline with bicarbonate at doses of 5×10^8 , 2.5×10^9 , or 2×10^{10}
33 colony forming units of enteropathogenic *E. coli* (EPEC) wild strain B171-8 and mutants
34 of this strain. {The bicarbonate treatments are expected to maximize sensitivity of the
35 volunteers by minimizing exposure of the challenge bacterial dose to the acidic
36 environment of the stomach. The researchers expect that by thus maximizing the
37 delivered dose to the site of bacterial attachment in the colon, pathogenicity can be
38 observed at lower doses than in normally resistant healthy adults.}

39
40 EPEC and *E. coli* O157:H7 strains share the same pathogenicity island (LEE) that results
41 in expression of genes that are essential to key initial events in pathogenesis, pedestal
42 formation and development of attaching and effacing lesions. {Verify that O157:H7 also
43 produce type IV pili, encoded by the BFP operon in EPECs.}

44
45 Mutant EPEC strains were generated within the BFP plasmid to test the hypotheses that
46 BFP mutants with disruption of adherence capacity would be avirulent and that the

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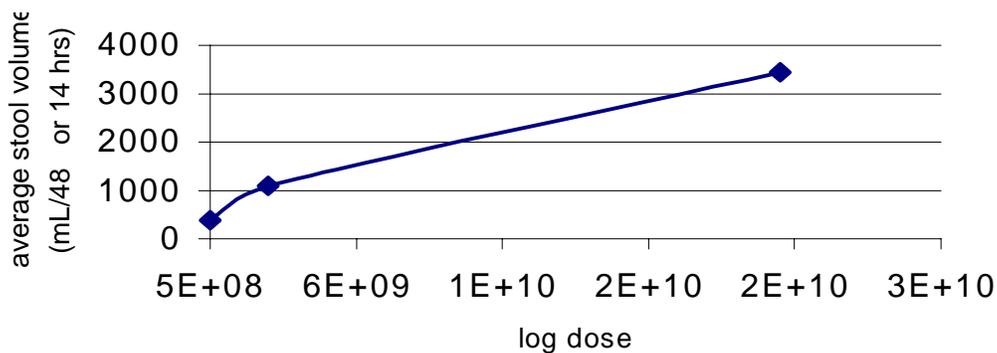
October 28, 1998

1 adherence-competent wild strain that causes actin condensation and pedestal formation in
2 tissue culture cells would cause diarrhea in human volunteers. Results reported include
3 not only dose-frequency information, but also an estimate of severity of illness: volume
4 of liquid diarrhea. Volunteers who received the wild type EPEC strain exhibited dose-
5 dependent diarrheal response. In fact, the response was so severe for the two volunteers at
6 the highest dose that the study was ended before the 48-hour observation period, at 14-
7 hours post-administration. For the wild type EPEC strain, 11/13 volunteers developed
8 clinical diarrhea, whereas 2/16 volunteers administered the delta A mutant strain, 3/14
9 volunteers administered the Gm mutant, and 4/13 volunteers administered the Fm mutant
10 developed diarrhea. Dose-dependency was not well demonstrated for the mutants. None
11 of the volunteers administered the highest dose for deltaA mutants or the two highest
12 doses for the Fm mutant became ill. For the delta T strain, none of the volunteers for the
13 two lowest dose groups became ill, whereas $\frac{3}{4}$ at the highest dose group became ill.

14

15 The data illustrated in Figure 2 reflect dose dependency for illness and severity of illness.
16 These data may be useful to consider as a surrogate for *E. coli* O157:H7, given some
17 additional investigation. The raw data supporting the graph of the reported data was
18 requested from the study authors. The raw data would be helpful because of differences
19 in the time interval for collection of feces. The highest dose group had such severe
20 symptoms that their portion of the study was halted after only 14 hours post challenge,
21 whereas the first two points reflect cumulative fecal volumes up to 48 hours post-
22 challenge. Also, data for individual volunteers would be more useful than averages for
23 risk assessors to model variability and uncertainty.

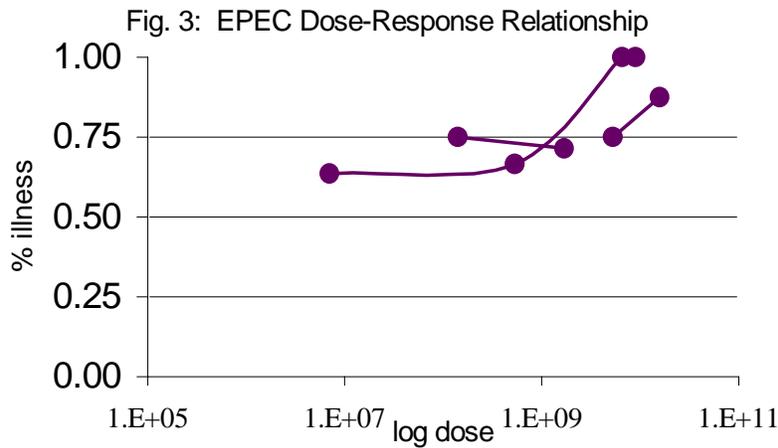
Fig. 2: EPEC Wild Strain Dose-Response Relationship



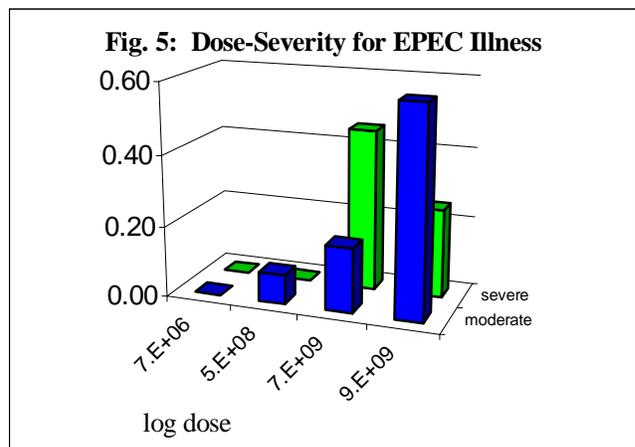
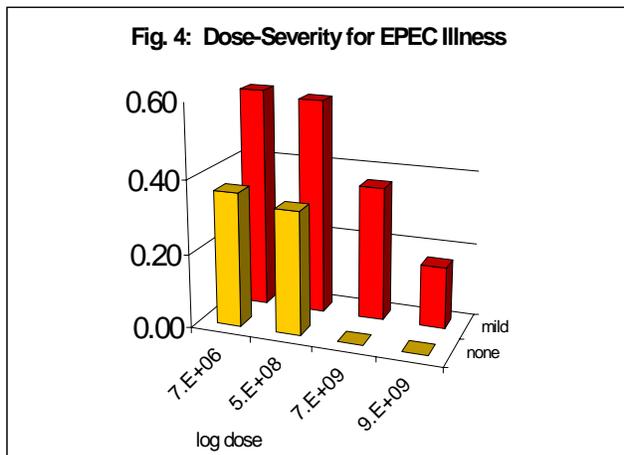
24 Note that *E. coli* O157:H7 does not produce Type IV pili. Both similarities and
25 differences thus exist between EPEC and *E. coli* O157:H7, such as localized adherence
26 (LA phenotype) as micro-colonies on cultured cell lines and autoaggregation into
27 spherical bacterial aggregates in tissue culture media (autoaggregation phenotype) for
28 EPECs only. Therefore, EPECs are not perfect surrogates, but are considered as part of
29 the body of evidence available.

30

1 Figure 3 depicts the human dose-response relationship for administered infant diarrheal
 2 *E. coli* types 55, B₅ and 111, B₄, H locus unspecified; June, 1953). The strains were
 3 administered at high doses (10^7 to 10^{10}) in milk to 77 volunteers in three experiments
 4 approximately 2.5 hours after the noon meal.



5
 6 Figures 4 and 5 depict dose-dependence of disease severity after administration of high
 7 doses of strains, presumably EPEC (Ferguson 1952). A mixture of three strains (*E. coli*
 8 111 B₄, strains 69, 72, and 95 isolated from diarrheal infants) were administered to 114



9 healthy adult male volunteers (ages 15-48 years) at doses of 7×10^7 , 5×10^8 , 7×10^9 , 9
 10 $\times 10^9$ in milk approximately 2.5 hours after their noon meal. The administered EPEC
 11 strains were the predominant fecal bacterium for nine of 11 volunteers in the highest dose
 12 group by 24 hours post-administration, while the remaining volunteers became fecal
 13 positive within 72 hours. At 11 days post-administration, the test strains continued to be
 14 excreted in feces along with other fecal colonizers. Each volunteer developed antibodies
 15 directed against the O antigen of the organism, with five also developing antibody against
 16 other aspects of the bacterial surface. The onset of symptoms for the high dose group was
 17 abrupt, beginning approximately 10 hours after administration, except for a single
 18 observation of symptoms after only 5 hours. Lower dose groups exhibited longer
 19 incubation times (48 hours), lower levels of serological response, and shorter duration of

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October 28, 1998

1 illness and fecal shedding. An asymptomatic volunteer administered 5×10^8 was fecal
2 negative for the administered strains throughout the study. The severity of illness ranged
3 from nausea and cramps to violent diarrhea, cramps, nausea, and vomiting and was
4 judged by an undescribed grading system into 4 illness categories, severe, moderate,
5 mild, or none. Samples of blood, urine, and throat swabs were negative for the
6 administered strains throughout the study.

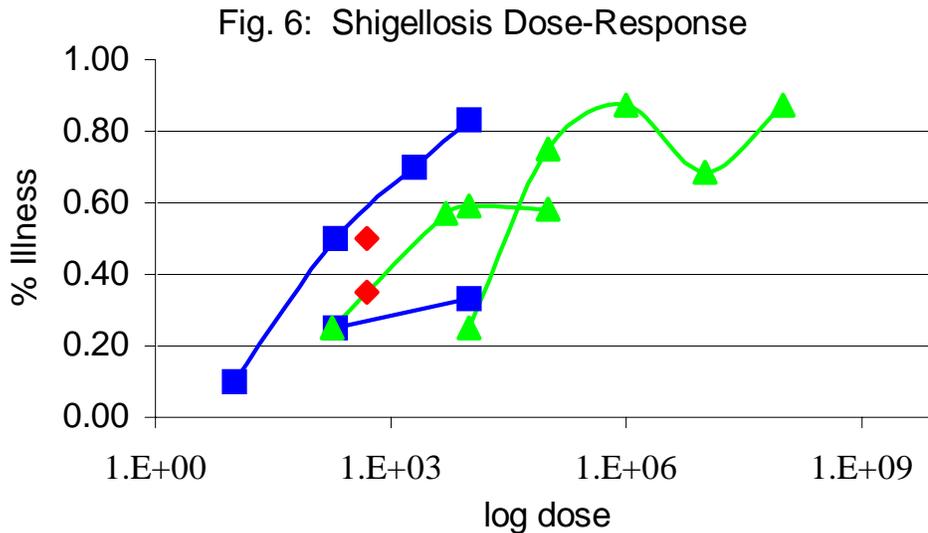
7
8 An interesting observation from this study is that an *E. coli* strain from the normal human
9 flora administered at the highest dose rate (9×10^9) as for the EPEC strains produced no
10 symptoms of illness (diarrhea, vomiting, cramps, lassitude, fever, or immune responses).
11 The study authors conclude that some normal adults appear resistant to the EPEC strains
12 tested, but immunocompromised adults have reportedly developed EPEC illness.

13
14 (Levine 1985 abstract): A class I EPEC strain O127:H6 and a derivative (deletion mutant
15 without the adhesion plasmid) were administered to nine and ten healthy adult volunteers
16 at a dose of 10^{10} organisms in an unspecified substrate. Nine of ten volunteers
17 administered the parent EPEC strain developed diarrhea (mean liquid stool volume 1,178
18 mL), whereas two of nine administered the plasmid-minus strain developed milder
19 diarrhea (mean 433 mL, $p < 0.006$). The parent strain induced both IgA and IgG directed
20 against an outer membrane protein present in EPEC (and EHEC?) strains, but not in
21 ETEC or meningitic strains.

22
23 A class II EPEC strain O114:H2 was administered to 11 volunteers at doses of 10^8 or 10^{10}
24 in an unspecified substrate. Diarrhea developed in six of 11 volunteers (mean 1,156 mL).
25 The conclusion of the study authors was that class II EPECs utilize a different mechanism
26 of pathogenesis which does not involve adhesion typical of the Hep-2 response essential
27 for pathogenicity of class I EPECs.

28 29 2c. Human clinical trials Shigella

30
31 One strain of *S. flexneri* (2457T; triangular (green) symbols) was administered in milk to
32 43 and 193 fasting healthy adult male volunteers in two experiments, respectively
33 (DuPont 1969; Dupont 1972). Additional data appear to exist for 55 volunteers
34 administered the same *S. flexneri* strain (dose 100, 180, 10^4 , and 10^{5-8} , Dupont 1989), but
35 are not yet included herein. Two *S. dysenteriae* strains (M 131) were similarly
36 administered in one experiment to 30 volunteers and another strain (A-1) to 10 volunteers
37 (square (blue) symbols; Levine 1973). One *S. sonnei* strain (diamond (red) symbols, 53G)
38 was administered to 20 and 38 volunteers at a single dose, 500 cells in milk. The A-1
39 strain was isolated from a Guatemalan patient with mild dysentery, and a more
40 pathogenic M 131 strain was a pandemic isolate from a severe dysentery case. The 53G
41 strain was isolated from a Japanese child. The lowest doses administered were 10 cells
42 for strain M 131 (*S. dysenteriae*), 200 cells for *S. flexneri*, and 500 cells for *S. sonnei*. The
43 percentage of 152 volunteers administered 100-500 bacteria ranged from 25% to 50% for
44 the four strains. Figure 6 below depicts dose-response relationships for shigellosis
45 (symbol legend: square *S. dysenteriae*; diamond *S. sonnei*; and triangle *S. flexneri*).



1

2 2d. Clinical data from animal experiments:

3

4 Thayyar-Madabusi (1998) summarized data from Pai (1986) depicted in Figure 7 and 8
5 below. Infant rabbits were observed for one day to ensure absence of diarrhea. An *E. coli*
6 O157:H7 strain was administered by gavage to three-day-old rabbits. Endpoints observed
7 were morbidity (diarrhea; Figure 7) and mortality (Figure 8). Counts per gram of
8 intestinal tissue were also reported.

Fig. 7: Dose-Dependent *E. coli* O157:H7 Morbidity

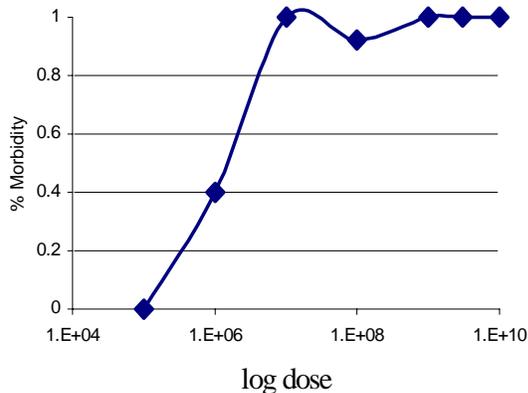
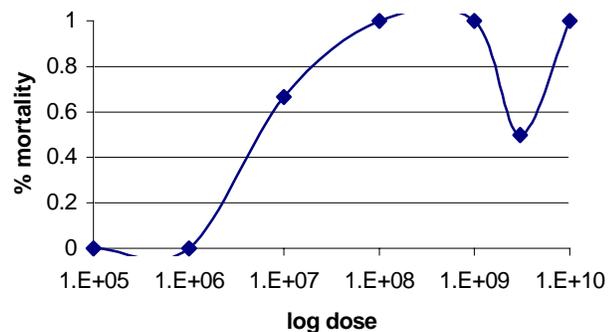


Fig. 8: Dose-Dependent *E. coli* O157:H7 Mortality



9 Data for additional animal models will also be explored for this assessment. The data
10 from animal models include: edema disease of pigs (Griffen 1995); a rabbit model using
11 genetically engineered EPEC rabbit strain expressing the Shiga toxin genes (Bloom
12 1998); neonatal calves (Dean-Nystrom 1998); and many other animals (baboons,
13 macaque monkeys, chickens, mice, greyhounds, gnotobiotic piglets, and rabbits (Moxley
14 1998). Data for normal and susceptible animals, such as infant or gnotobiotic animals,

1 may be helpful in extrapolation of dose-response curves for normal adults to more
2 sensitive sub-populations of humans.

3 4 **3. Some Alternative Dose-Response Model Forms**

5
6 The observed data from human or animal clinical studies can be fit to a number of
7 empirical models. For salmonellosis human clinical data, the model form alone can
8 account for 75 order of magnitude difference in predictions for illness at doses of a single
9 bacterial cell (Coleman 1998). The dramatic influence of model form upon the magnitude
10 of the risk estimate is caused by differences in the behavior of alternative models when
11 extrapolating to low doses (Coleman, in preparation). The Gompertz model form is an
12 extreme value distribution which has sub-linear behavior in the low dose region, whereas
13 the Beta-Poisson model form has linear extrapolation. An additional model form which
14 has been applied in microbial dose-response modeling is the Weibull-Gamma. We
15 propose fitting the available data to each model form for the risk assessment in order to
16 account for model uncertainty in our analysis.

17
18 Most of the administered dosage levels for shigellosis and EPEC illness may be much
19 higher than what might be expected to be ingested based on the estimated number of
20 organisms that will be simulated in previous modules. Extrapolation from high to low
21 doses is necessary for the derived dose-response models. Some of the data from the
22 *Shigella* studies have been analyzed by other researchers (Cassin 1998; Marks 1998;
23 Crockett 1996; Holcomb, in preparation). These researchers have used the Beta-Poisson
24 model to describe the relationship between the probability of illness, p , and the ingested
25 number of organisms, D . Haas (1983) and Vose (1998) give an heuristic derivation of the
26 function using simple microbiological models and attach biological interpretations to the
27 parameters of the model. The equations for three model forms proposed for use in this risk
28 assessment are listed below.

29
30 Beta-Poisson (Haas 1983; Crockett 1996)

$$31 \quad 1 - (1 + \text{dose}^x/\beta)^{-\alpha}$$

32
33
34 Weibull-Gamma (Holcomb, in preparation)

$$35 \quad 1 - [(1 + \text{dose}^x/\beta)]^{-\alpha}$$

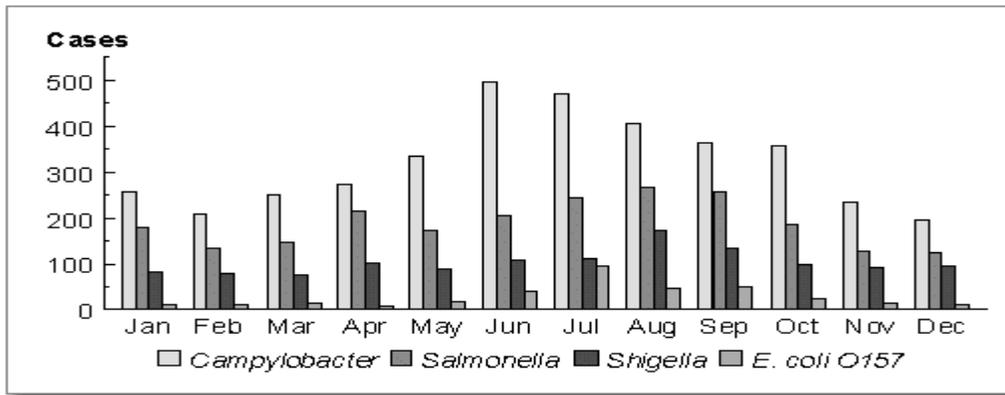
36
37 Extreme value (Gompertz, Coleman 1998)

$$38 \quad 1 - \exp(-\exp(\alpha_i + \beta * \text{dose}^x))$$

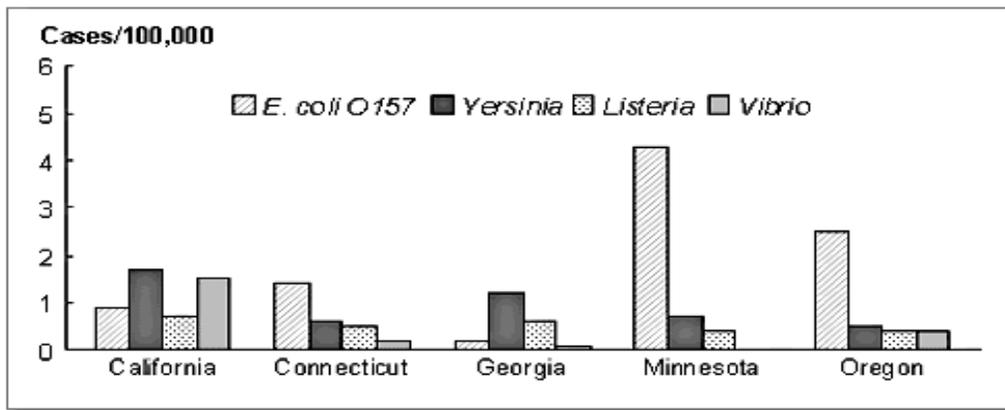
39 40 **4. Defining More Susceptible Sub-Populations and Progression of Severe Endpoints**

41
42 The FoodNet an active surveillance program, undertaken collaboratively by the Centers
43 for Disease Control and Prevention (CDC), the USDA Food Safety Inspection Service,
44 and the Food and Drug Administration, provides data for rates of diarrheal diseases by
45 region, and season, age, and gender. Regional and seasonal differences by site are
46 depicted in Figures 9 and 10 (source: www.cdc.gov/ncidod/dbmd/foodnet).

1 Fig. 9: Seasonal variation in cases.



2
3 Fig 10: Geographic variation in cases.



4
5 Data are available from two full years of surveillance (1996, 1997). The numbers of
6 culture-confirmed cases of illness reported for the pathogen *E. coli* O157:H7 were 388
7 for the population of approximately 14 million persons represented in the survey for 1996
8 and 340 for the population of approximately 16 million persons represented in the survey
9 for 1997 (USDA 1997, 1998).

10
11 The CDC (1998) recently provided additional unpublished data to the FSIS team that
12 further describe age-dependent rates of illness (Figures 11, 12). The rate for adults >50
13 years of age was an average of ≤ 2 cases per 100,000 for each 5-year interval. These data
14 do not appear to indicate a higher probability of illness from this pathogen for the elderly
15 compared to younger adults, but children are clearly more likely to incur illnesses than
16 adults. The most susceptible population appears to be children between 1 and 2 years of
17 age with a maximum rate of 20 cases per 100,000 for this age interval. The rate for the
18 least susceptible population between 40 and 45 years of age was 20-fold less,
19 approximately 1 case per 100,000. The age-dependent pattern of illness may be affected
20 to some extent by age differences in the proportion of ill persons who provide a stool
21 specimen for testing for the presence of *E. coli* O157:H7.
22

Fig. 11: Culture-Confirmed *E. coli* O157:H7 Illnesses by Age
 (source: FoodNet 1996 - 1997)

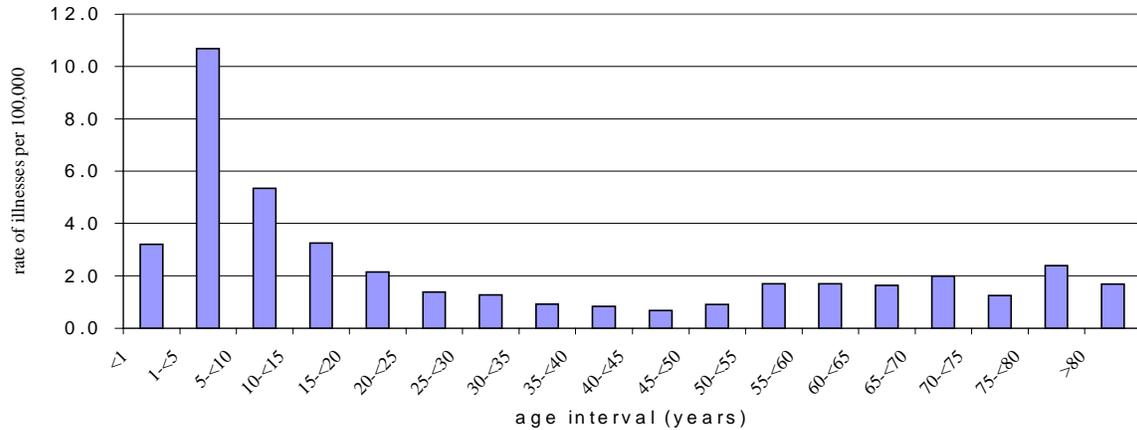
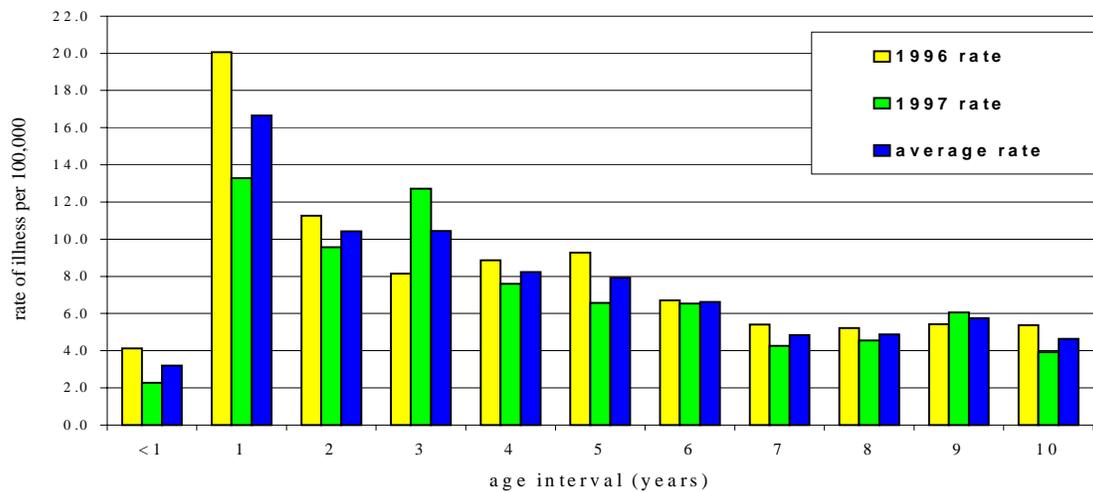


Fig. 12: Culture-Confirmed *E. coli* O157:H7 Illnesses by Age



1
 2 Additional data on rates of *E. coli* O157:H7 illness exist from active surveillance studies
 3 in Washington state (Ostroff 1989) and King County (McDonald 1988). The age-
 4 intervals differ among the studies, so direct comparison of the studies with the FoodNet
 5 data is problematic. The number of cases identified was much smaller in the older studies
 6 (25 in McDonald 1988; 93 in Ostroff 1989; 388 in FoodNet 1996; 340 in FoodNet 1997).
 7 In each study, children <5 or <10 appear to be more likely to become ill than other age
 8 groups based on population adjusted rates of illness. Data depicting rates of illness for the
 9 elderly are difficult to interpret. The age-interval ≥ 50 (McDonald 1988) actually appeared
 10 less susceptible than younger adult groups. This finding might be an artifact of the small
 11 number of observations over all age groups (25 cases) or the width of this interval to
 12 include “late middle-aged” persons along with the elderly. Ostroff (1989) and FoodNet
 13 studies (USDA 1997, 1998) suggest that the elderly may be at slightly higher risk of
 14 illness than other adults, while statistical analysis of the FoodNet data may not support
 15 significant differences in the rates of *E. coli* O157:H7 illness for adults >20.

1 The FoodNet web site reports data for some enteric pathogens indicating gender and age-
 2 gender interactions in rates of illness. However, active surveillance data (USDA 1997,
 3 1998) from both the 1996 (54% female) and 1997 (52% female) do not appear to support
 4 gender differences for *E. coli* O157:H7.

5
 6 **5. Selected Interpretive Summaries of Key Epidemiology Studies**

7
 8 **Ostroff 1989:** The first year of statewide disease surveillance for *E. coli* O157:H7
 9 included 93 cases of illness within the state of Washington and an estimated rate of *E.*
 10 *coli* O157:H7 illness of 2.1 cases per 100K. The median age of cases was 14 and the
 11 range was 11 months to 78 years. Figure 1 in the paper reported age-specific rates of
 12 cases per 100,000 person-years; estimates of histogram values from the figure are
 13 summarized in Table 2 below.

14

Table 2: Population-adjusted rates of illness per 100,000 (Ostroff 1989) for ages						
≤5 years	5-9 years	10-14 years	15-19 years	20-59 years	60-69 years	≥70 years
~6	~5	~4	~3	~1	~1.5	~1.5

15
 16 The gender-specific incidence was 2.1 per 100K for males and 2.2 for females. Of
 17 reported cases, 12% advanced to HUS (9/93 cases) or TTP (2/93 cases). HUS occurred in
 18 25% of cases under 10 years of age and in 4% of cases over 10 years of age (relative risk
 19 7.1, 95% CI 1.8-34.1). Both adults who developed TTP were immunocompromised, one
 20 of whom died. (No deaths were attributable to children or to HUS in the year of study.)
 21 Four secondary cases developed between children or from a child to an adult in 5% of
 22 households with cases, and a mean incubation interval of 4.8 days is consistent with the
 23 3-8 day incubation period noted by the study authors from the literature. Duration of
 24 diarrhea was an average of 6 days, range 1-18 days. No more than 11% of the 93
 25 identified cases could be attributed to consumption of raw milk or raw ground beef.

26
 27 **MacDonald 1988:** This one-year prospective, population based study conducted in
 28 HMOs in Puget Sound area of Washington state May 1985 to April 1986 reported 25
 29 isolations of *E. coli* O157:H7 among 6485 stool specimens included in the study (0.4%
 30 positive overall). The gender distribution for cases was 14 male and 11 female. The age
 31 distribution of cases spanned 1-70 years (mean 29). The mean duration of illness was 8.6
 32 days and the range spanned 3-21 days. A figure in the paper reported age-specific rates of
 33 cases per 100,000 person-years; estimates of histogram values from the figure are
 34 summarized in Table 3 below.

35

Table 3: Population-adjusted rates of illness per 100,000 (MacDonald 1989) for ages					
≤9 years	10-19 years	20-29 years	30-39 years	40-49 years	≥50 years
~15	~7	~10	~5	~10	~4

36
 37 An overall rate of 8 cases per 100K person years was reported, although no confidence
 38 intervals were provided. Of the 25 patients, 14 were hospitalized for a reported mean
 39 duration of 8.4 days, range 2-8 days. None of the 25 cases progressed to HUS to TTP.

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October 28, 1998

The only death mentioned in the study was also associated with chronic diarrhea over a one year period during which *Giardia lamblia* and *Salmonella* were detected in stool cultures in addition to *E. coli* O157:H7 after one year of sampling. Less than 30% of the 25 identified cases could be attributed to consumption of rare ground beef and raw milk.

Summary: The body of evidence for national estimates of cases of illness associated with *E. coli* O157H7 will be used to “ground truth” the risk assessment model simulated results as described in chapter 1. The range of rates of reported culture-confirmed *E. coli* O157H7 illness per 100,000 estimated from the five FoodNet sites (1996-1997) by site were 0.2 to 5.4 cases per 100,000. The estimate from Ostroff (1989) for Washington state active surveillance program was 2.1 cases per 100,000, and the estimate from the MacDonald (1988) HMO study in Seattle was 7.6 cases per 100,000. The data summarized in Table 4 depict the range of possible extrapolations by site to a US national estimate, assuming the U.S. population (265 million for FoodNet studies; 256 million others) is representative of each site.

Source	Rate per 100K	Estimated number of US cases/yr
FoodNet, 1997 (GA)	0.2	530
FoodNet, 1996 (MN)	5.4	14,310
Ostroff 1989 (WA)	2.1	5,368
MacDonald 1988 (Seattle)	7.6	19,426

Bell 1994: The *E. coli* O157:H7 outbreak in Washington state in 1993 included 501 culture confirmed cases or HUS cases identified in the state between December 1, 1992 and February 28, 1993. The median incubation period was 3 days, The cases included primary (79%) and secondary (10%) illnesses or unclassified (11%). Of 501 illnesses, 151 (31%) were hospitalized for a median of 4 days (1-118 days). Of 151 hospitalized, 45 (9%) developed HUS. Of 45 HUS cases, three died. The median age of cases was 8 years (4 months to 88 years), and the median age of HUS cases was 5 years. Mean age of HUS cases was 8 years (1-68). Additional cases of bloody diarrhea (130) identified during the outbreak period were not culture-confirmed as O157:H7 positives, and were excluded from case estimates.

MMWR 1993: The CDC summarized the following results from all four states involved in the 1993 *E. coli* O157:H7 outbreak in the Pacific northwest associated with hamburgers.

State	# Ill	# Hospitalized	# HUS cases	# Deaths
WA	477	144	30	3
ID	14	4	1	0
CA	34	14	7	1
NV	58	9	3	0
Total	583	171	41	4

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October 28, 1998

1 **Fukushima 1996; Michino 1996; Michino 1998:** Radish sprouts were identified as the
 2 most likely food vehicle for the world's largest *E. coli* O157:H7 outbreak in Sakai City,
 3 Japan (Fushima 1996), which was associated with school lunches that did not include any
 4 beef foods. Approximately 7,000 people, mostly school children, were affected in the
 5 1996 outbreak. The pathogen was not isolated initially from seeds, seed culture solution,
 6 current crop of radish sprouts, cattle (200) from surrounding farms, farm workers, and
 7 water (well, drainage, river, waterway) (Michino 1996). Subsequently, the pathogen was
 8 reportedly isolated from radish seeds produced in Oregon.

Sakai City (Michino 1996)	Sakai City (Michino 1998)	All 1996 outbreaks in Japan
6309 cases (children aged 6-10)	7966 cases	10,275 cases
84 adults (school workers)		
72 asymp. Carriers		
56 secondary cases		
997 hospitalized (15% total)	606 (7.6% total)	827 (8% total)
102 HUS cases (10% hospitalized)	106 HUS cases (17.5%)	150 HUS (18%)
2 deaths (2% HUS cases)	3 deaths (2.8% HUS cases)	6 deaths (4% HUS)

11 6. Dose-Reconstruction

13 The term "infective dose" has its origin in toxicology studies, controlled animal dosing
 14 experiments with multiple administered doses (Davis 1973). Use of the term "infective
 15 dose" in the risk assessment arena (Coleman 1998) appears to incorrectly imply that a
 16 single "true" infective dose for a population or a sub-population of potential hosts exists.
 17 The actual ingested doses which causes illness would vary according to the factors of the
 18 disease triangle (host, pathogen, and environment, and interactions). Some scientists
 19 seem to use the term "infective dose" for describing pathogenicity or the likelihood of
 20 illness. However, the mathematics for modeling dose-response relationships from
 21 observations of a defined population of animals in a controlled dosing experiment in the
 22 toxicology field have not been extended to the less structured observations of human
 23 populations that incur natural infections of foodborne disease for which both the ingested
 24 doses and the population responses are incompletely characterized. An attempt to
 25 consider the data and account for variability and uncertainty is needed for risk assessment
 26 purposes. Therefore, the following example is offered as an initial attempt to introduce
 27 concepts necessary to conduct more formal dose-reconstruction. The output of a dose-
 28 reconstruction is not a point estimate, but a distribution that reflects uncertainty due to
 29 many factors, including sampling and measurement errors (Marks 1998).

31 Actual ingested doses that caused disease in retrospective epidemiologic investigations of
 32 outbreaks of foodborne disease must be inferred from companion samples of remaining
 33 lots of suspect food. Suspect foods are unlikely to be representative of foods processed
 34 and handled properly. Using levels of pathogens quantified in a small number of samples
 35 of suspect foods as "normal" exposure might bias the results of a risk assessment and
 36 predict more cases than would actually be expected to occur due to inflated estimates of
 37 exposure.

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1 Some researchers believe that low numbers of *E. coli* O157:H7 cells per eating
2 occurrence (<1000) are sufficient to cause foodborne disease (AGA 1995) based on
3 bacterial levels in companion samples of foods implicated in the 1993 U.S. outbreak.
4 Only six samples of frozen and thawed raw ground beef were enumerated for this
5 outbreak (FSIS 1993; Johnson 1995). The maximum detected density was a Most
6 Probable Number (MPN) of 15/g. Freeze/thaw cycles may have reduced recovery from
7 such samples by <1 log, so the actual density could have been 150/g prior to freezing
8 (FSIS, unpublished results), not accounting for sampling and measurement errors which
9 could be significant (Vought 1998; Tatini, pers. comm.). The patties in the outbreak were
10 reportedly 45 grams (regular patties, included in children's meals) and jumbo patties (114
11 grams; Bell 1994). The levels of *E. coli* O157:H7 might be as high as 6750 counts per
12 serving in regular burgers and 17,100 in jumbo burgers. The reported internal cooking
13 temperature for the process which was used during the outbreak ranged from 42 - 81 °C
14 for 16 patties tested, and 10 of the 16 patties reached internal cooking temperatures less
15 than 60°C (135 °F; Bell 1994). A log lethality for these conditions might be expected to
16 be less than 2 logs (Appendix figure from Juneja 1997). Due to undercooking, it seems
17 possible that approximately one hundred or more *E. coli* O157:H7 may have survived per
18 serving. This evidence is insufficient to determine whether or not a single *E. coli*
19 O157:H7 cell surviving cooking can cause illness. It does appear that low doses (~10²)
20 can cause illness. However, the existence of thresholds below which illness does not
21 occur in individuals in the general population or more susceptible sub-populations are
22 uncertain.

23
24 The findings of asymptomatic carriers in outbreaks (Su 1995) and in a healthy 6-month-
25 old infant from a farm family (Wilson 1996) and immunity against Shiga toxins and *E.*
26 *coli* O157:H7 lipopolysaccharide (Wilson 1996) all support the hypothesis of a threshold
27 exposure below which illness is not observed. A threshold greater than 1 *E. coli* O157:H7
28 cell was also suggested by Griffin (1991) before the outbreak in the Northwestern US. It
29 appears that colonization of the human GI tract by *E. coli* O157:H7 is necessary, but not
30 sufficient to cause illness. To our knowledge, mechanistic data to model pathogenesis,
31 the progression from asymptomatic colonization to illness and to more severe
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