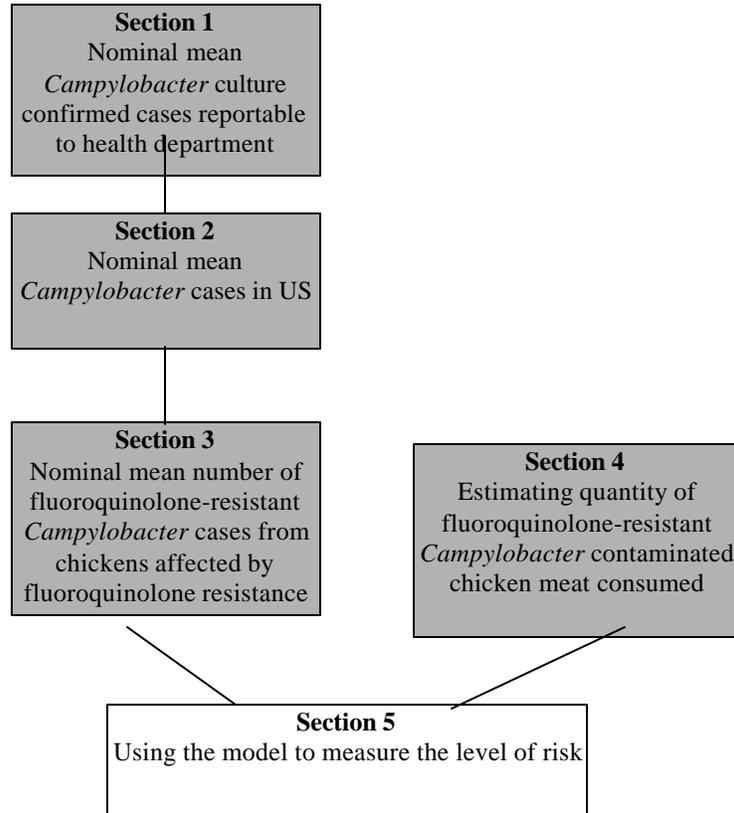
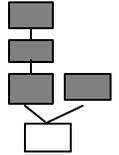

Section 5

Using the model to measure the level of risk.





Discussion of results

This risk assessment model has provided a quantitative estimate of the human health impact resulting from fluoroquinolone-resistant *Campylobacter* on poultry. 1998 and 1999 were modeled side-by-side in an @RISK/Excel spreadsheet simulation model. Any parameter that was common to both years was modeled in one cell and referred to wherever necessary, which ensured consistency between model iterations.

The model was run for 10,000 iterations to produce the relative frequency plots and statistics. It was run for 300 iterations to produce points on the spider plots, a number sufficient to stabilize the reported means. All models used Latin Hypercube sampling.

The model produced a number of outputs for both 1998 and 1999:

- Estimates of the probability a person would be affected by the risk in question for various U.S. sub-populations. Probabilities were provided as fractions and 1 in x estimates;
- Estimates of nominal mean number of *Campylobacter* cases in U.S. population (**12_T**);
- Estimates of nominal mean number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken (**13_T**);
- Estimates of nominal mean number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken, seeking care, treated with fluoroquinolone and therefore affected by the fluoroquinolone resistance (**14_T**); and
- Estimates of total consumption of boneless, domestically reared chicken contaminated at slaughter plant with fluoroquinolone-resistant *Campylobacter* in U.S. in pounds (**V_i**).

Figures 5.1a and 5.1b, displayed on the next two pages, show cumulative uncertainty distributions. The estimates are all 'nominal mean' estimates assessing the human health illness rates rather than the actual number of cases there may be in a year as a result of random chance.

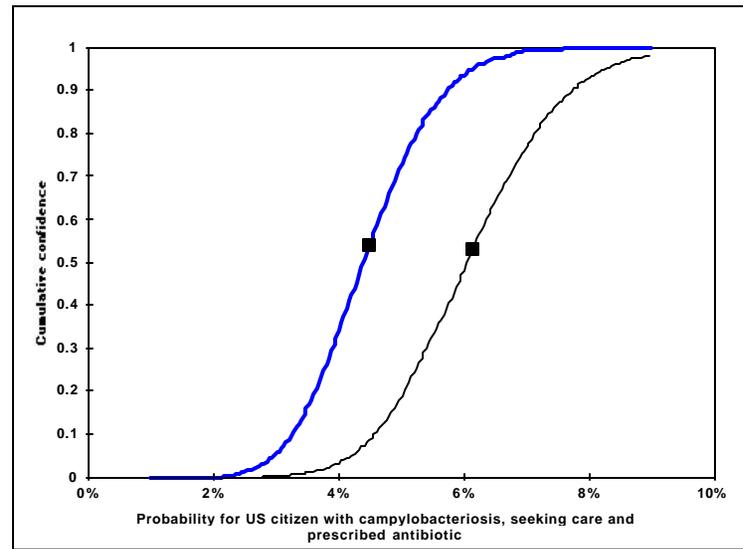
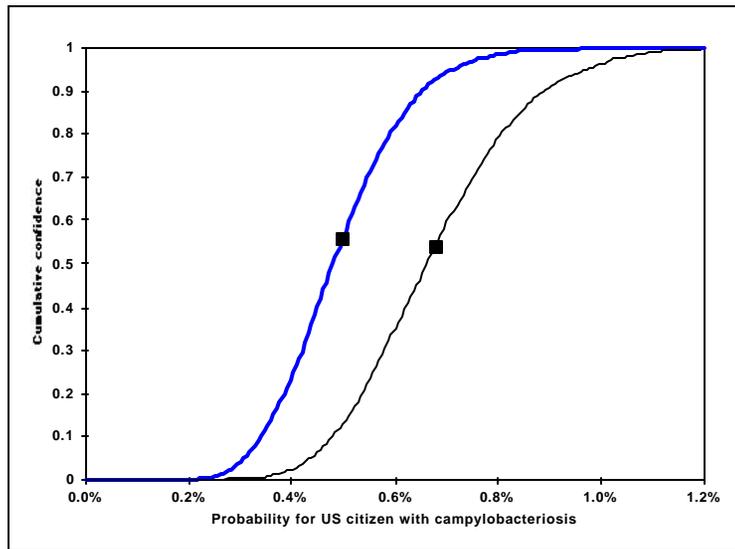
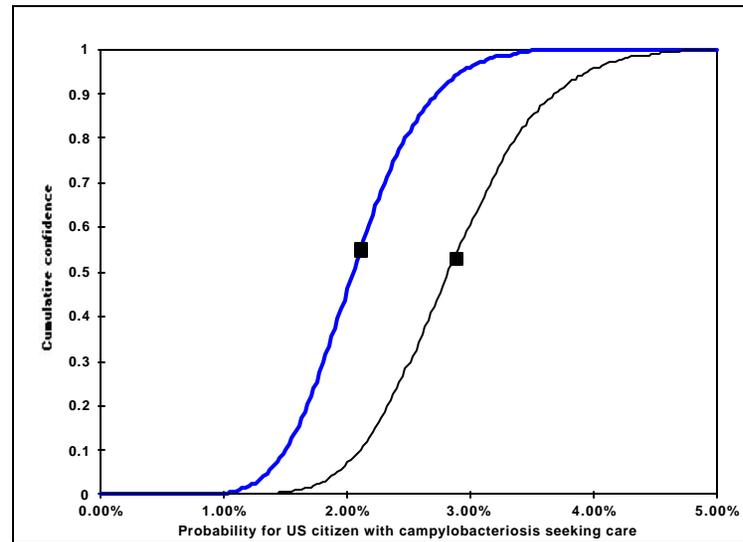
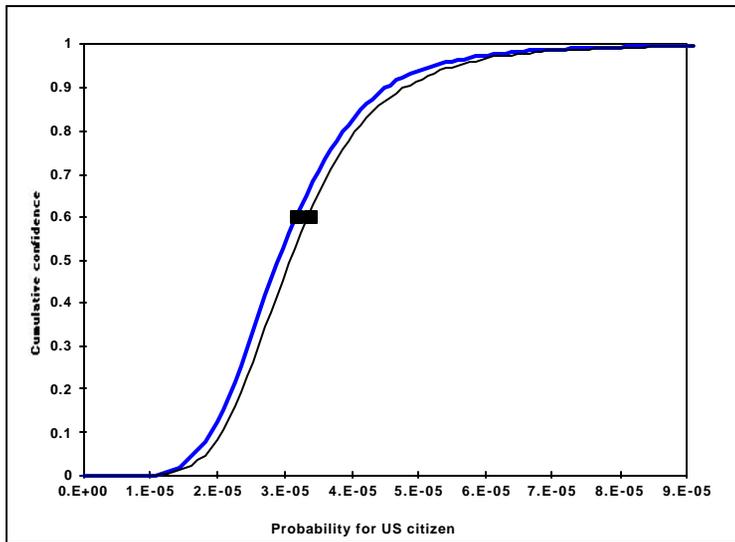


Figure 5.1a Confidence distributions for 1998 (heavier lines) and 1999 (lighter lines) values for the **probabilities** described in this section for the four different denominators representing different populations at risk – black squares denote expected values

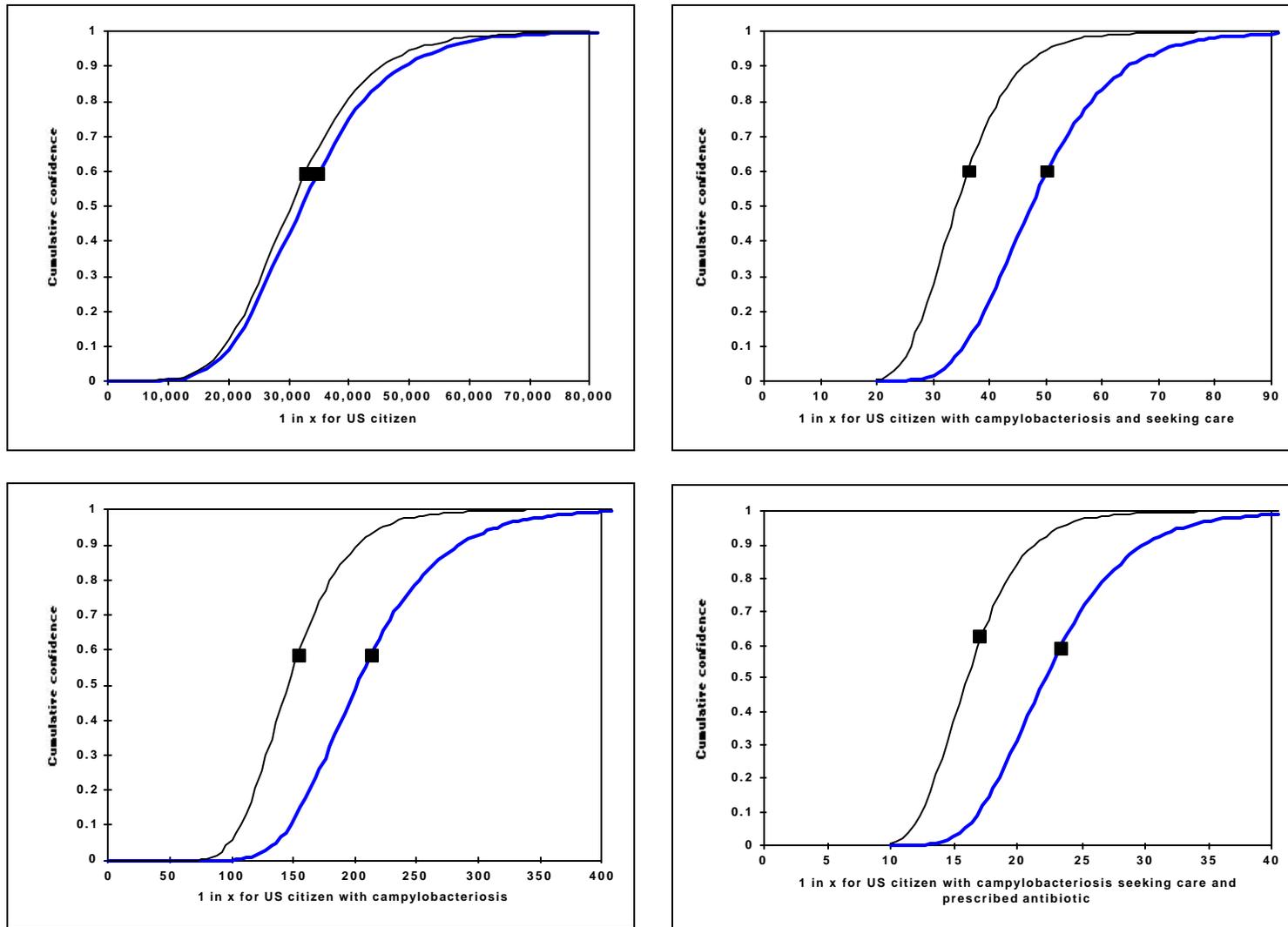
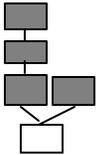


Figure 5.1b Confidence distributions for 1998 (heavier lines) and 1999 (lighter lines) values for the probabilities described in this section (in **1 in x** format) for the four different denominators representing different populations at risk – black squares denote expected value



K_{all} and K_{res}

Aside from the probabilities, two ‘K’ values were calculated, K_{all} and K_{res}, which represent the potential of poultry meat contaminated with *Campylobacter* and fluoroquinolone-resistant *Campylobacter* respectively to result in human illness. These parameters are calculated as follows:

$$K_{all} = \frac{\text{Nominal mean number of } \textit{Campylobacter} \text{ cases attributable to chicken}}{\text{Estimated amount of } \textit{Campylobacter}\text{-contaminated chicken meat consumed}}$$

$$K_{res} = \frac{\text{Nominal mean number of fluoroquinolone-resistant } \textit{Campylobacter} \text{ cases from chicken}}{\text{Estimated amount of fluoroquinolone-resistant } \textit{Campylobacter}\text{-contaminated chicken meat consumed}}$$

The K values can be thought of as the probability that a pound of *Campylobacter* contaminated chicken meat (in general, and resistant) will result in a case of campylobacteriosis (in general and resistant). If the distributions of the total number of *Campylobacter* that reside on resistant and susceptible *Campylobacter*-contaminated carcasses are the same, and if resistant and susceptible *Campylobacter* have similar survivability and virulence, it is reasonable to assume that these values will be roughly equivalent. The importance of these K-values as a predictive tool was discussed in the Introduction. The theory behind them is discussed later in this section. Figures 5.2 to 5.4 plot these K estimates. There is strong agreement between years: i.e., the differences between the 1998 and 1999 distributions for both parameters are very small compared to the total uncertainty being described by the distributions’ ranges. The difference in the spread of the 1998 and 1999 K_{all} distributions noted in Figure 5.2 is due to the increase in the catchment population and the concomitant decrease in uncertainty. There is also reasonable overlap between K_{res} and K_{all}, though K_{res} is consistently estimated as larger than K_{all}. Two of the most logical reasons for this difference are that the prevalence estimate of fluoroquinolone resistant *Campylobacter* on carcasses is too small (about half of what it should be) because:

1. The estimate used in this analysis came from an unweighted analysis of NARMS chicken isolate test results. An analysis that weighted the state prevalence by the production in pounds of chicken gives a significantly higher result (12.0% for the weighted modeled result vs. 10.3% for the unweighted modeled result in 1999).
2. NARMS testing procedures take one chicken isolate from a cultured dish, and test that isolate for resistance. This would provide a good estimate of resistance prevalence if all *Campylobacter* on a fluoroquinolone resistant-contaminated carcass were resistant. However, if there are also susceptible *Campylobacter* present, the isolate selected from a cultured dish may be a susceptible *Campylobacter* mixed in a population of resistant *Campylobacter*. So, for example, if a carcass contaminated with resistant-*Campylobacter* had, on average, a 50% mix of resistant and susceptible *Campylobacter*, the observed resistance prevalence from NARMS isolates would be about half the true prevalence. Data are not currently available on the distribution of ratio between susceptible and resistant *Campylobacter* on a carcass, but would be extremely useful to get a clearer picture of the risk issue.

In addition to the two reasons for underestimation of K_{res} above, it may also be that the assumptions, i.e., same distribution of number of *Campylobacter* reside on resistant and susceptible *Campylobacter*-contaminated carcasses, and resistant and susceptible *Campylobacter* have similar survivability and virulence, in comparing the two K values may need to be reevaluated.

If differences are observed in K_{res} or K_{all}, when making comparisons between years, these differences may be explained by changes in the: 1) prevalence of resistance in travelers, 2) prevalence of resistance on imported food, or 3) use of the drug in other food animal species and many other factors.

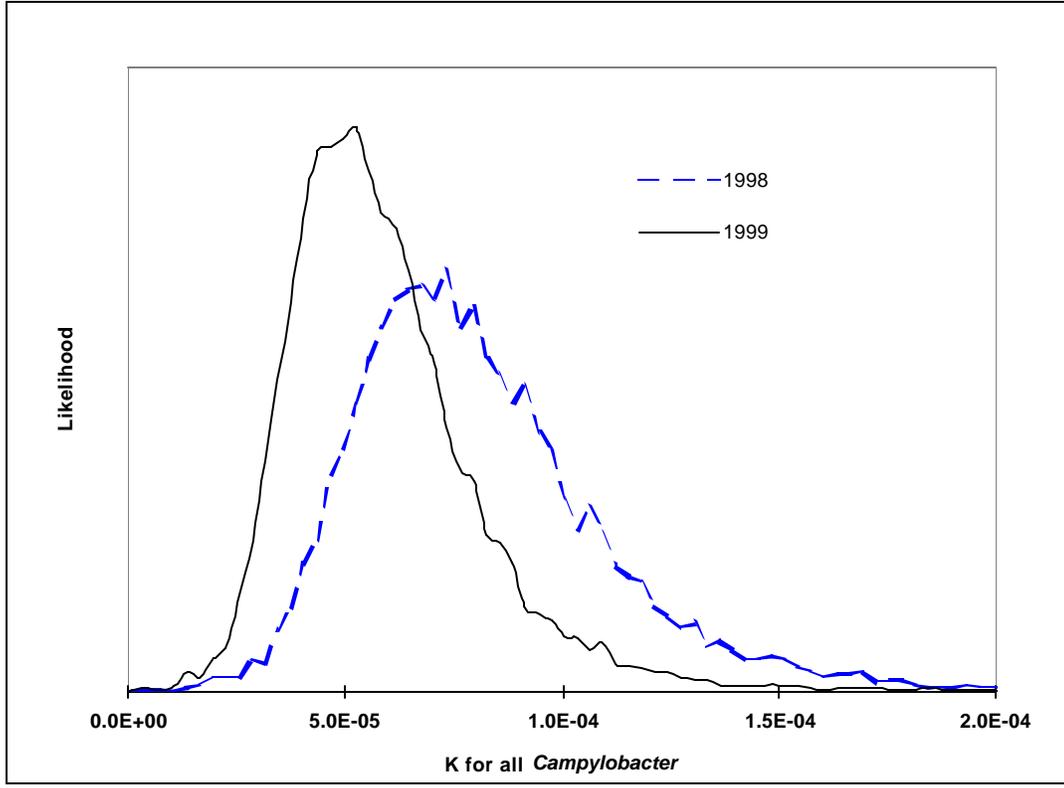
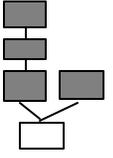


Figure 5.2. Estimates of K_{all} for 1998 and 1999

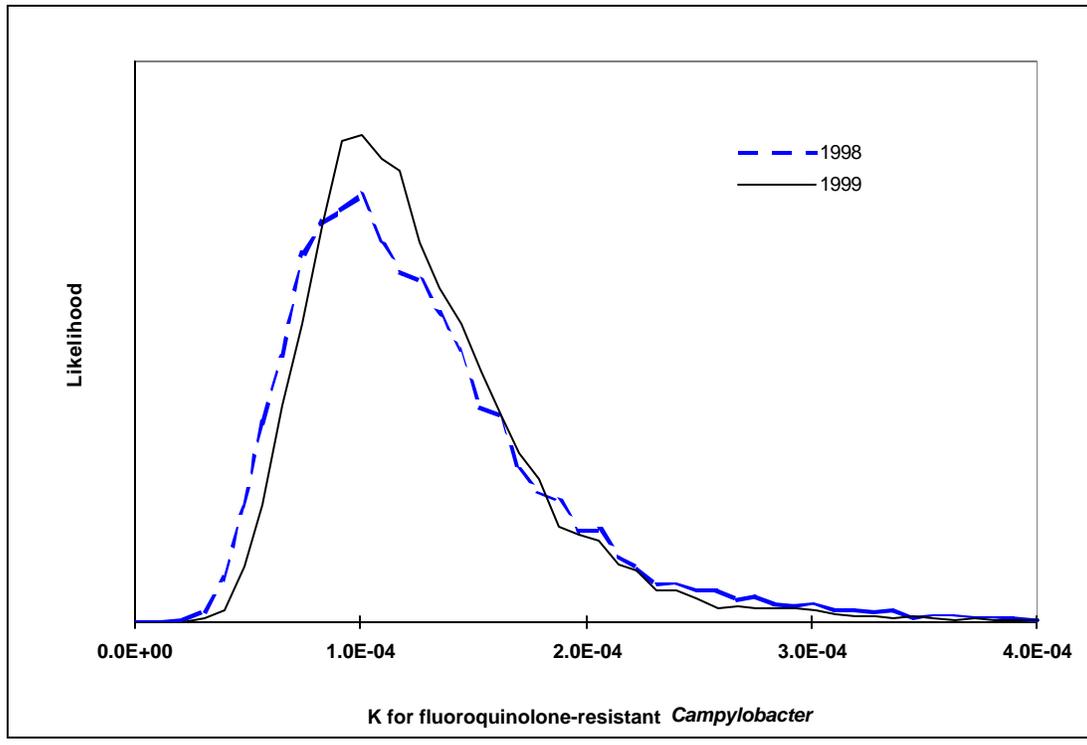


Figure 5.3. Estimates of K_{res} for 1998 and 1999

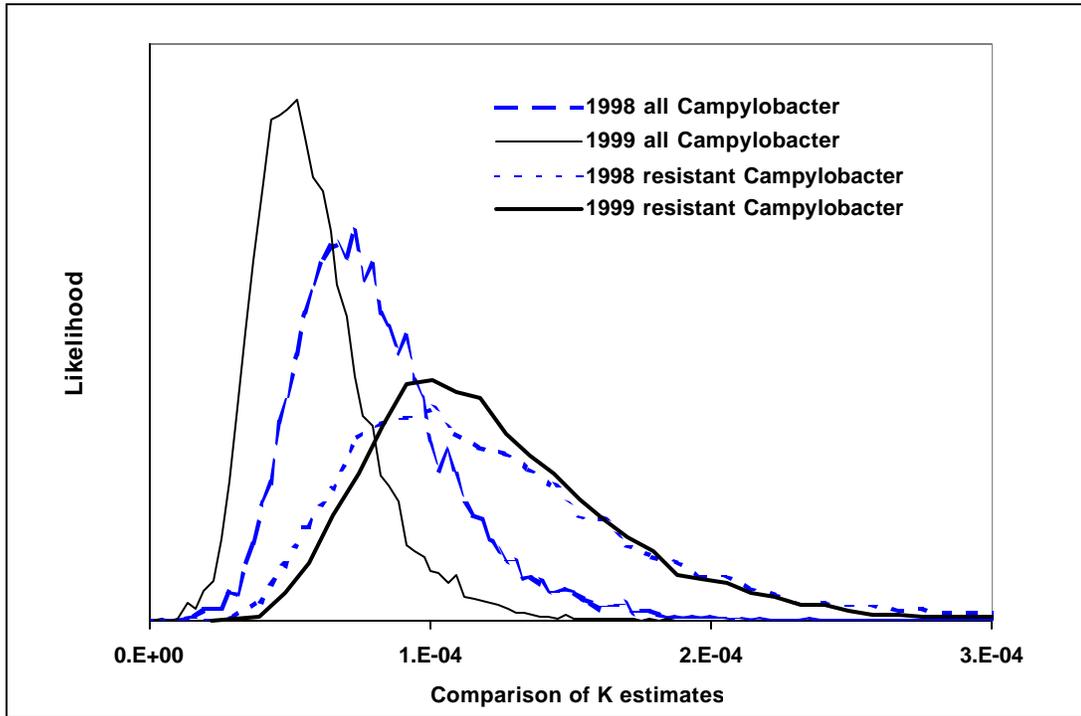
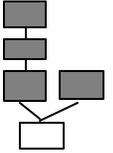
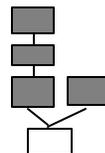


Figure 5.4. Comparison of K_{all} and K_{res} for 1998 and 1999



Measuring the level of risk

The results and principles of Sections 1 to 4 of this model can be used to measure and monitor the level of risk to the U.S. population posed by fluoroquinolone resistant *Campylobacter* from domestically reared broilers.

Measuring the level of human health impact

1. Probability

The level of risk was assessed by calculating the ratio of the nominal mean number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken, seeking care, treated with fluoroquinolone and therefore affected by the fluoroquinolone resistance each year (λ_{4T}) to the size of the population at risk. There are various options one may select as the population at risk, shown in the table below:

Table 5.1. Confidence intervals for estimates of **probability** of being affected by fluoroquinolone resistant *Campylobacter* for various groups

Exposed group	1998			1999		
	5 th percentile	Mean	95 th percentile	5 th percentile	Mean	95 th percentile
U.S. citizens	0.0018%	0.0032%	0.0053%	0.0019%	0.0034%	0.0056%
U.S. citizens with campylobacteriosis	0.31%	0.50%	0.72%	0.44%	0.68%	0.98%
U.S. citizens with campylobacteriosis seeking care	1.38%	2.11%	2.95%	1.94%	2.89%	3.99%
U.S. citizens with campylobacteriosis seeking care and prescribed antibiotic	3.01%	4.49%	6.17%	4.24%	6.15%	8.28%

Table 5.2. Confidence intervals for estimates of **1 in x** of being affected by fluoroquinolone resistant *Campylobacter* for various groups

Exposed group	1998			1999		
	5 th percentile	Mean	95 th percentile	5 th percentile	Mean	95 th percentile
U.S. citizens	56,795	34,945	18,808	52,166	32,912	17,792
U.S. citizens with campylobacteriosis	319	214	139	227	156	102
U.S. citizens with campylobacteriosis seeking care	72	50	34	51	36	25
U.S. citizens with campylobacteriosis seeking care and prescribed antibiotic	33	23	16	24	17	12

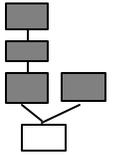


Table 5.1 gives estimates of the **probability**, with confidence intervals, that an individual randomly chosen from the selected denominator population at risk in 1998 and 1999 would have numbered among those for whom fluoroquinolone resistant *Campylobacter* in broilers resulted in a health impact (λ_{4T}). Table 5.2 offers an alternative expression of the probability as **1 in x** that many people find easier to interpret. The tables show mean estimates and the uncertainty around these values.

The size of the risk may be viewed differently depending on an individual's personal circumstances. For the average U.S. citizen, the risk may well be perceived presently as being small: we have estimated that 1 in 34,945 people were affected in 1998 and 1 in 32,912 in 1999, for example. On the other extreme, people with reduced immunity who may be more likely to seek medical help, may perceive the risk as quite significant. The results are presented with four different denominators.

The first denominator distributes the risk among the entire U.S. population. The great majority of the U.S. population consumes chicken, and the consumption of a fluoroquinolone resistant *Campylobacter* contaminated chicken product, or consumption of another food item contaminated by chicken (e.g. salad) is a random process. Thus, the great majority of people are exposed to the risk and the randomness of the process means that most people are not in full control of that risk. They may consume the food at a restaurant, other type of food outlet or the home of someone else. Considering only those people in the U.S. population who consume chicken could refine this denominator.

The second denominator distributes the risk among people who contract campylobacteriosis from any source. These people will potentially seek medical care and may be prescribed a fluoroquinolone. This denominator puts the risk from fluoroquinolone resistant *Campylobacter* from broilers into context with the total sources of *Campylobacter* infections. Thus, one can make statements like "0.68% of people contracting campylobacteriosis in 1999 were affected by the risk".

The third denominator distributes the risk among those people who contract campylobacteriosis from any source and then seek some medical care. These people are sufficiently ill that they decide they need help. This denominator includes consideration of those people who may be more susceptible to *Campylobacter* than most.

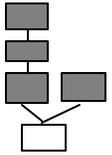
The fourth denominator distributes the risk among those people who contract campylobacteriosis from any source, seek some medical care and are prescribed an antibiotic. Both they themselves and their medical practitioner consider these people sick. This represents the group that is most seriously at risk from the failure of fluoroquinolone therapy.

2. Number of cases

The level of human health burden may alternatively be measured simply as the number of people who contract fluoroquinolone resistant campylobacteriosis in a year where *Campylobacter* is associated with domestically reared broilers (λ_{4T}).

3. Incremental days of illness

A third option is to measure the human health impact as the number of extra people-days of illness that occur as a result of fluoroquinolone resistant *Campylobacter* associated with domestically reared broilers. This would potentially recognize that those people with invasive infection would have a much larger incremental duration of illness than those with enteric infection. However, problems arise in the definition of duration. In addition, there is no substantial evidence to suggest that people with enteric infection and bloody diarrhea will be ill longer than those with enteric infection and non-bloody diarrhea. Since some 99.6% of estimated cases of Campylobacteriosis are enteric infections, calculating the number of incremental days of illness would amount to multiplying the number of enteric infections by some constant factor which was a difference of two medians, equivalent to a 3 day difference (92) or a mean difference of 2 days in the CDC Campylobacter Case Control Study (28).



If fluoroquinolone-resistant *Campylobacter* were demonstrated to induce more severe or longer illness than susceptible strains, then the increased incremental days of illness would be a good measure of the human health impact. The current data describing duration of diarrhea for resistant and susceptible illnesses are not sufficiently robust to use in this model.

Theory behind, and use of, the parameter K

If one selects an infected item of food at some point in the production of a food product (e.g. an infected carcass at the spin chiller of a production plant which will contain some random number of servings), there are any number of potential probabilistic pathways for which the consumption of this item will result in the infection of one or more people. The paths are probabilistic because of the inherent randomness of the system, so there must be some (unknown) probability distributions of the number of people that could become infected, ill, etc. from an individual serving. The shape of this distribution cannot be known because of the myriad ways that a person can become affected as a result of the consumption of an infected serving. The persons affected need not even be direct consumers of the serving: for example, they can become affected from other food that has come into contact with the serving in question, through contact with others who have consumed the serving, or from pets who have consumed the product. The shape of the distribution is a result of any remaining processing of the item, the history of its handling during distribution, the current consumption and food handling behavior of the consuming population, as well as the distribution of the pathogen load among infected product and the dose-response relationships for the various segments of the consuming population.

In the case of chickens, the number of people infected by a food pathogen is orders of magnitude lower than the numbers of servings infected with that pathogen, so this distribution must have a mean k that is much smaller than 1 (Figure 5.5)¹. Moreover, the probability of infecting two people from a serving will intuitively be considerably less than the probability of infecting just one person.

Applying the conditional probability identity principle, we can write:

$$\lambda = K_{\text{res}} * V_i$$

where:

I is the mean number of people per year who will experience an adverse human health effect as a result of consuming a pound of fluoroquinolone resistant *Campylobacter* contaminated broiler meat;
 V_i is the quantity (lbs.) of fluoroquinolone resistant *Campylobacter* contaminated broiler meat consumed in a year in the U.S.

¹ When K_{res} is much less than 1, the unknown parameter K_{res} can be interpreted as approximately equal to the probability that a random consumer will experience the human health impact by consuming 1lb of contaminated broiler meat. The relationship described by K essentially takes the role of the more traditional dose-response model, excepting that one has implicitly included some cross-contamination among people who have also consumed chicken, variations in pathogen load among infected servings and variation in organism-host interaction.

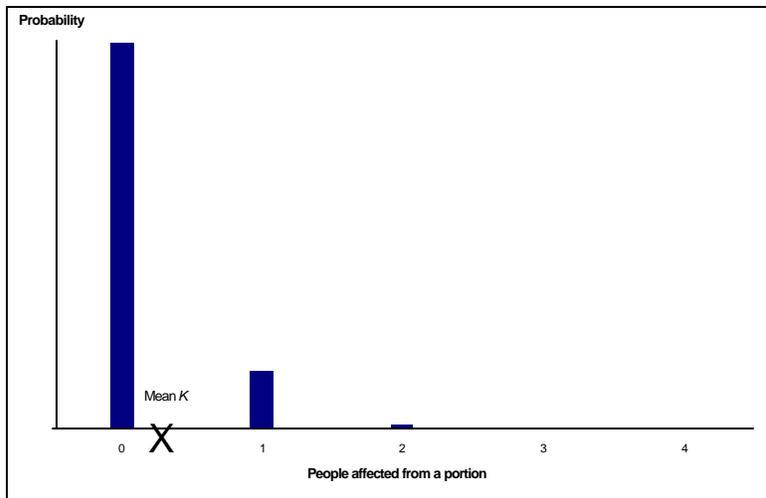
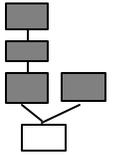


Figure 5.5. Probability distribution of number of affected people as a result of consuming one infected portion

Just as with the microbial pathogenicity approach to dose-response modeling using dose-response equations, the model parameter needs to be determined from data. In essence, this requires estimating the quantity of infected broiler meat consumed by the public in some recent time interval and estimating what **I** must have been, given the number of people experiencing the human health impacts of interest as a result of consuming those contaminated servings.

To use the model to predict the effects of various input parameters, K_{res} and V_i must be decomposed into products of the component inputs required in deriving them. For example, $V_i = V_c * p_c * p_{rc}$. Then λ can be modeled as a function of p_{rc} values chosen to be of interest while other inputs may or may not be held constant to reflect conditions of interest. The following graph displays the prediction of λ_3 as a function of p_{rc} ranging from 0 up to 25%. Further refinements of the predictive properties of the model are shown in Equation sets 1 and 2 in the Introduction.

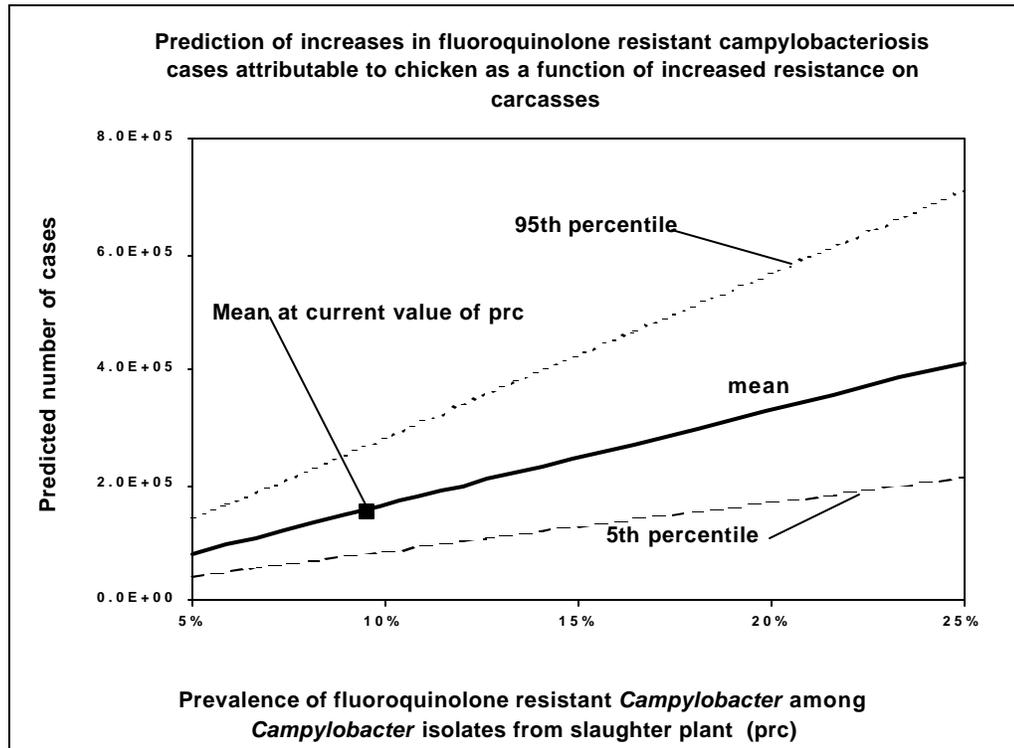
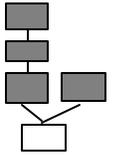
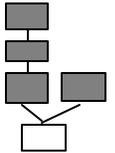


Figure 5.6. Using the risk assessment model to predict changes in I_{3T} , on the vertical axis, due to increases in p_{rc} , on the horizontal axis.

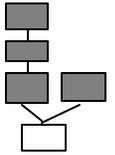


Sensitivity analysis

A sensitivity analysis was performed on the risk assessment model to determine which parameters are contributing to the model outputs' total uncertainty. The purpose of this exercise is to determine a) the model parameters to which the model outputs are most sensitive, and b) where extra information would be most useful in reducing the uncertainty about a model parameter and thus in the model outputs.

Five model outputs were used for the uncertainty analysis: λ_{3T} – the nominal mean number of fluoroquinolone resistant *Campylobacter* cases attributable to chicken; λ_{4T} - the nominal mean number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken, seeking care, treated with fluoroquinolone and therefore affected by the fluoroquinolone resistance; V_i – the total consumption of boneless, domestically reared chicken contaminated at slaughter plant with fluoroquinolone-resistant *Campylobacter* in U.S.(lbs); and the ratios K_{res} and K_{all} described above.

The sensitivity analysis was carried out by fixing each model parameter to the 5th, 25th, 50th, 75th and 95th percentiles of its uncertainty distribution in turn, whilst leaving all other model parameters with their uncertainty distributions. For each percentile, the model is simulated (with 300 iterations, sufficient to stabilize the output mean) to determine the mean output value. The result is a spider plot (118,119). The x-axis shows the percentile used for each model parameter and the y-axis shows the magnitude of the mean of the output in question. The degree of influence of an input parameter equates to the range of output mean values corresponding to the input percentiles. For example, Figure 5.8 shows that for 1999 eliminating the uncertainty about p_{th} would be the most effective mean for reducing the uncertainty in the estimate of λ_{4T} .



Sensitivity analysis for λ_{3T}

Figure 5.7 illustrates the parameters that contribute the most to the uncertainty in the value for λ_{3T} . The parameter p_{rh} produces the greatest vertical range for both 1998 and 1999 and therefore is the most influential input parameter. The next most important parameters are p_{ca} and p_+ . The parameters p_{rh} and p_{ca} plot with positive gradients so λ_{3T} would be larger the larger the true value of p_{rh} and p_{ca} . The parameters p_{cn} and p_+ plot with a negative gradient, so the lower their true values, the higher the true value of λ_{3T} .

From Figure 5.7 we can conclude that, to reduce the uncertainty in the human health impact of fluoroquinolone-resistant *Campylobacter* in broilers, the collection of the following data would be useful (in order of importance):

- Proportion of *Campylobacter* infections from chicken that are fluoroquinolone resistant (p_{rh});
- Probability a case of campylobacteriosis is attributable to chicken (p_{ca});
- Probability that a stool will be requested and submitted from a patient with non-bloody diarrhea (p_{cn}); and
- Probability that the culture will confirm *Campylobacter* given it was tested (p_+).

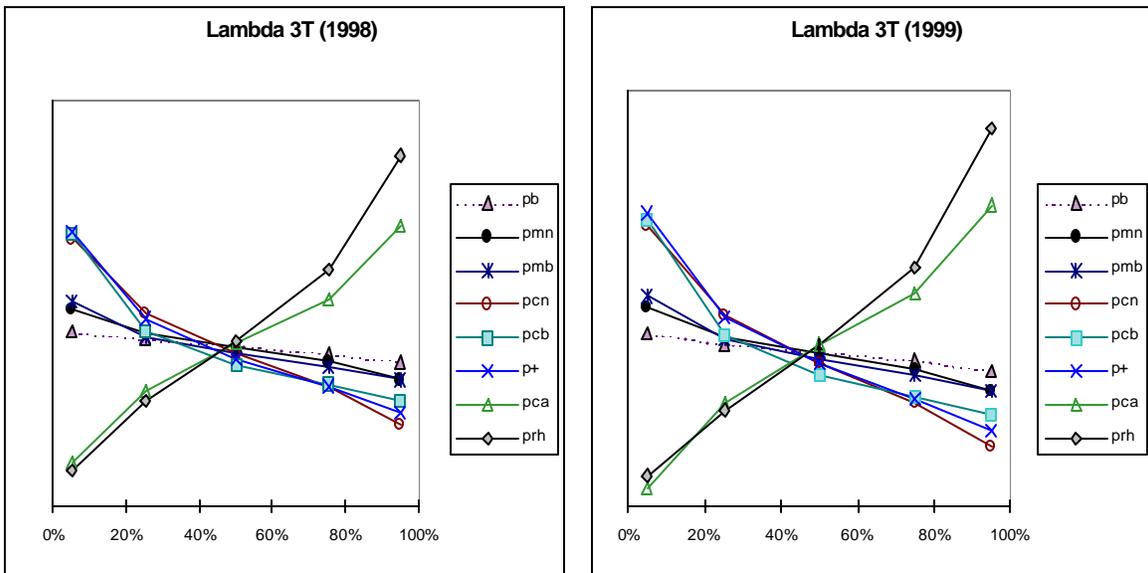
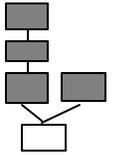


Figure 5.7. The parameters that contribute the most to the uncertainty in the value for λ_{3T} .



Sensitivity analysis for λ_{4T}

Figure 5.8 illustrates the parameters that contribute the most to the uncertainty in the value for λ_{4T} . The parameter p_{rh} produces the greatest vertical range for both 1998 and 1999 and therefore is the most influential input parameter. The next most important parameters are p_{cn} and p_{+} .

From Figure 5.8 we can conclude that, to reduce uncertainty in the human health impact of fluoroquinolone resistant *Campylobacter* in broilers, collection of the following data would be useful (in order of importance):

- Proportion of *Campylobacter* infections from chicken that are fluoroquinolone resistant (p_{rh});
- Probability that a stool will be requested and submitted from a patient with non-bloody diarrhea (p_{cn}); and
- Probability that the culture will confirm *Campylobacter* given it was tested (p_{+}).

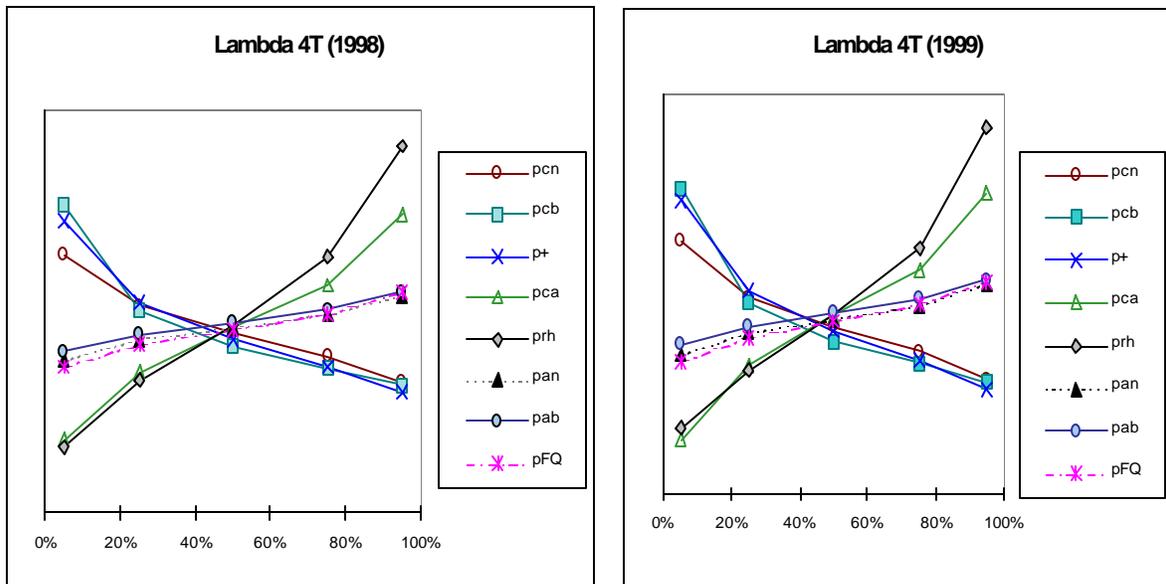
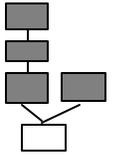


Figure 5.8. The parameters that contribute the most to the uncertainty in the value for λ_{4T} .



Sensitivity analysis for V_i

Figure 5.9 illustrates the parameters that contribute the most to the uncertainty in the value for V_i . There are only two uncertainty parameters in determining this output, p_c and p_{rc} , and p_{rc} (the prevalence of fluoroquinolone-resistant *Campylobacter* among *Campylobacter* contaminated broiler carcasses) is clearly contributing the greatest uncertainty to the determination of V_i .

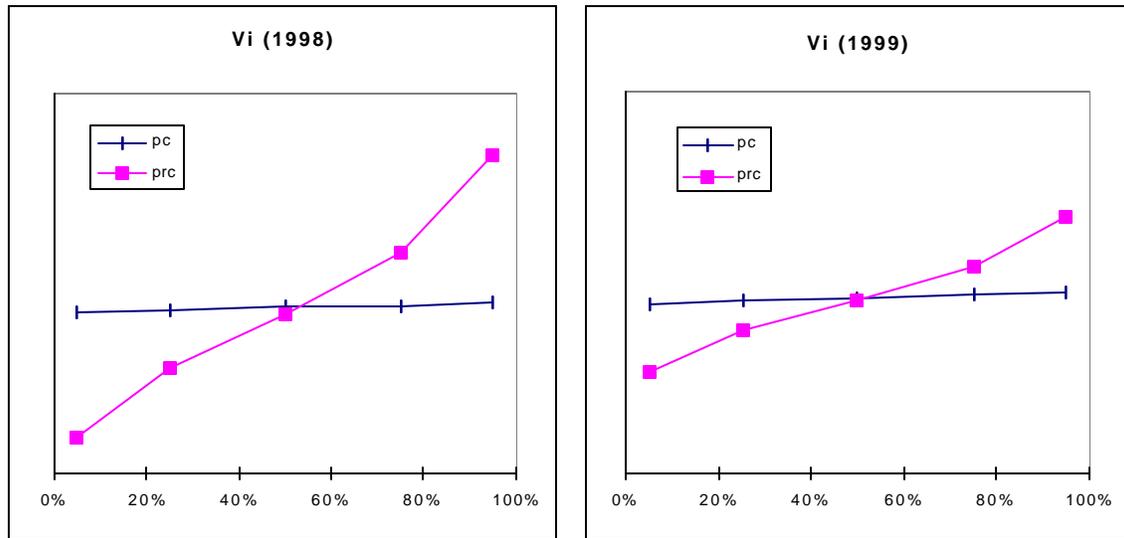
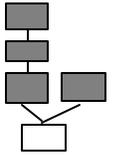


Figure 5.9. The parameters that contribute the most to the uncertainty in the value for V_i .



Sensitivity analysis for (K_{all})

Figure 5.10 illustrates the parameters that contribute the most to the ratio K_{all} . The parameters p_{ca} and p_{cn} produce the greatest vertical range and therefore are the most influential input parameters. While the parameter p_c is shown on the graphs for both 1998 and 1999, it does not add to the uncertainty in K_{all} , as indicated by the relative flatness of the line for p_c . The parameter p_{ca} is the only significant parameter plotted that contributes to the uncertainty from modeling contamination of chicken meat, i.e. all the other parameters correspond to determining the human health impact which means that we have more uncertainty about the human health side than the broiler side.

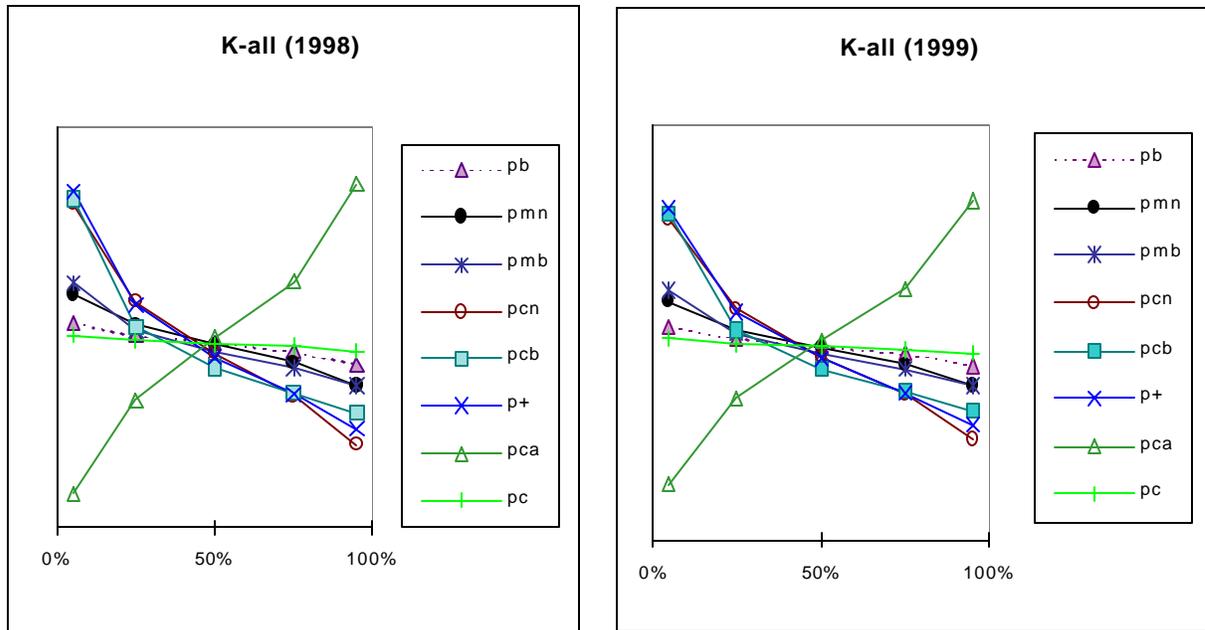
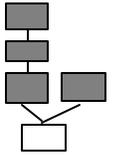


Figure 5.10. The parameters that contribute the most to the uncertainty in the value for K_{all} .



Sensitivity analysis for (K_{res})

Figure 5.11 illustrates the parameters that contribute the most to the ratio K_{res} . The parameters p_{rh} and p_{cn} produce the greatest vertical range and therefore are the most influential input parameters. The parameters p_{rc} and p_{ca} are the only significant parameters plotted that contribute to the uncertainty from the poultry side, i.e. all the other parameters correspond to determining the human health impact which means that we have more combined uncertainty on the human health side than the broiler side.

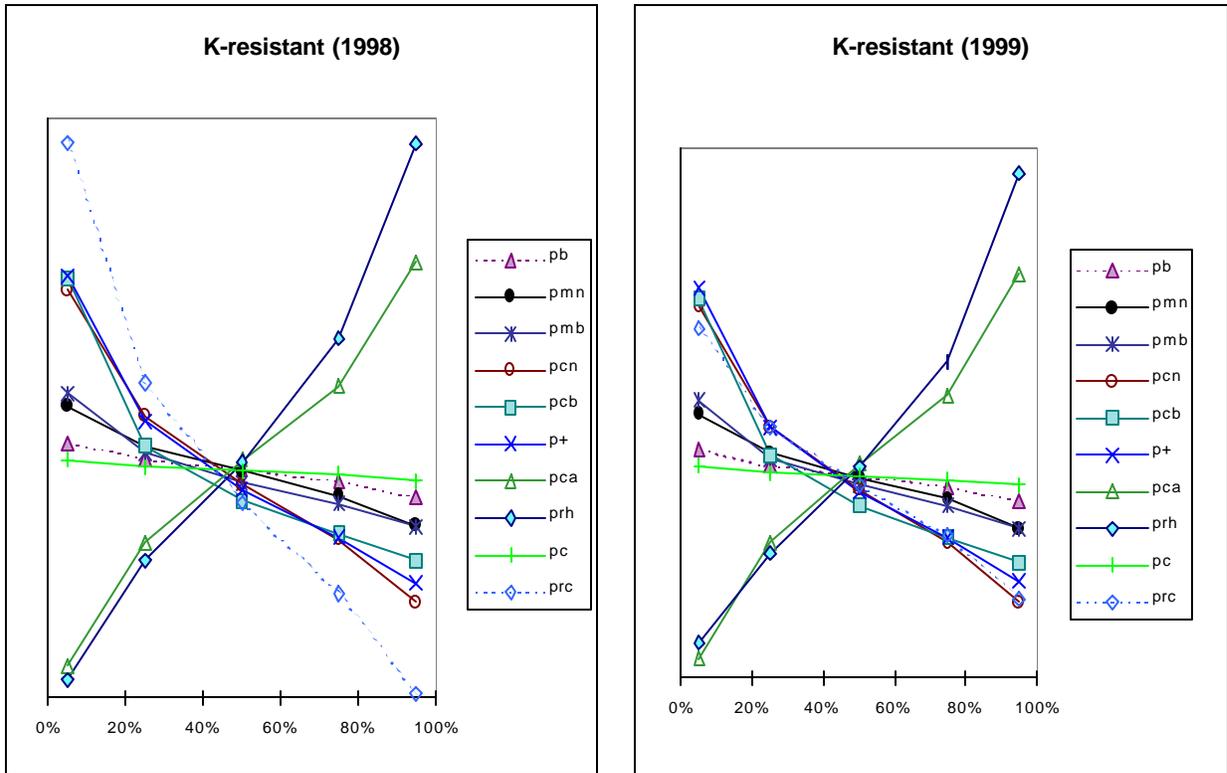
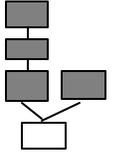


Figure 5.11. The parameters that contribute the most to the uncertainty in the value for K_{res} .

Sensitivity Analysis Summary

Quantitatively assessing the uncertainty for the parameters modeled indicates that there is more combined uncertainty on the human health side than the broiler side. Qualitative issues in assessing the data used in the risk assessment were raised in the Sections and given as limitations, assumptions and data gaps and are collectively listed in Appendix B. Other qualitative and methodological issues raised are described in the respective sections of the document. Consideration of both quantitative uncertainty and qualitative aspects of data are important in the collection of data useful for risk assessment.



Effect of considering clustering of isolates by state in the estimation of resistance among *Campylobacter* isolates in poultry

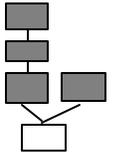
Chicken NARMS isolate susceptibility test results for 1999 were obtained for states with federally inspected poultry plants representing over 95% of chicken production. We obtained these data to allow us to assess whether an estimate of the resistance among *Campylobacter* isolates in poultry would be significantly different if we were able to weight isolate test results by the production in pounds of chicken for each state.

The data are shown in the spreadsheet model of Figure 5.12, which also illustrates the crude estimate (based on aggregating all isolate test results) and the estimate weighted by state production volume.

The results of this analysis are shown in Figure 5.13. The state-weighted estimate has almost the same degree of uncertainty (spread) but estimates the prevalence to be approximately 1.7% higher than the crude aggregate estimate. It would therefore be more accurate in the risk assessment model to use state-weighted estimates. However, since the state-by-state data were only obtainable for 1999, it was decided to use the crude method to estimate both years' prevalence to maintain consistency. If 1998 data became available, broken down by state, we would be able to update both years' estimates of the model parameter p_{rc} and therefore update estimates of K_{res} .

*Effect on model of underestimating total prevalence of *Campylobacter* among broiler carcasses p_{rc}*

The estimate of human health impact for the years 1998 and 1999 produced by the risk assessment model are unaffected by the consistent underestimation of p_{rc} . However, the model output K_{res} is inflated by a factor that is approximately $12\%/10.4\% = 1.15$. This makes it difficult to validate the estimate for K_{res} by comparison with the estimate for K_{all} . Predicting any future human health impact is essentially unaffected since the inflation factor is a constant through the model and cancels out.



	A	B	C	D	E	F	G
1							
2		STATE	Nr sampled	Nr Resistant Isolates	Relative Pounds Produced	Prevalence contribution by state	
3		C	29	6	0	0.00%	
4		BB	5	0	7	0.10%	
5		A	3	0	3	0.07%	
6		K	3	0	1	0.02%	
7		O	6	1	18	0.47%	
8		Q	4	0	5	0.09%	
9		T	17	2	40	0.68%	
10		U	8	1	6	0.13%	
11		V	1	1	4	0.26%	
12		E	63	1	139	0.45%	
13		J	9	0	ND	0	
14		P	44	2	90	0.62%	
15		S	10	0	16	0.14%	
16		AA	30	3	66	0.87%	
17		F	54	3	119	0.90%	
18		G	9	0	16	0.16%	
19		L	52	4	142	1.40%	
20		W	32	3	93	1.17%	
21		X	9	1	27	0.52%	
22		CC	10	0	24	0.22%	
23		B	22	4	38	0.84%	
24		D	15	4	23	0.73%	
25		I	4	0	1	0.01%	
26		M	6	0	1	0.01%	
27		N	12	1	14	0.20%	
28		R	20	6	41	1.38%	
29		Y	5	3	9	0.53%	
30					941		
31							
32						National prevalence estimate	
33						Estimate weighted by each state's production	11.96%
34						Raw estimate determined by modeling aggregated isolates	10.32%
35						Difference between estimates (weighted-raw)	1.65%
36							
37		Formulae table					
38		B3:E29	Data				
39		E30	=SUM(F3:F29)				
40		F3:F12, F14:F29	=RiskBeta(D3+1,C3-D3+1)*E3/\$E\$30				
41		F13	0				
42		F33 (output)	=SUM(F3:F29)				
43		F34 (output)	=(RiskBeta(SUM(D3:D29)+1,SUM(C3:C29)-SUM(D3:D29)+1)*1000)/E30				
44		F35 (output)	=F33-F34				
45							

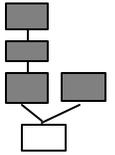


Figure 5.12. Spreadsheet model containing isolate test data and methods of estimating prevalence (spreadsheet values in column F show a single random realization of the model) (Ref. 112)

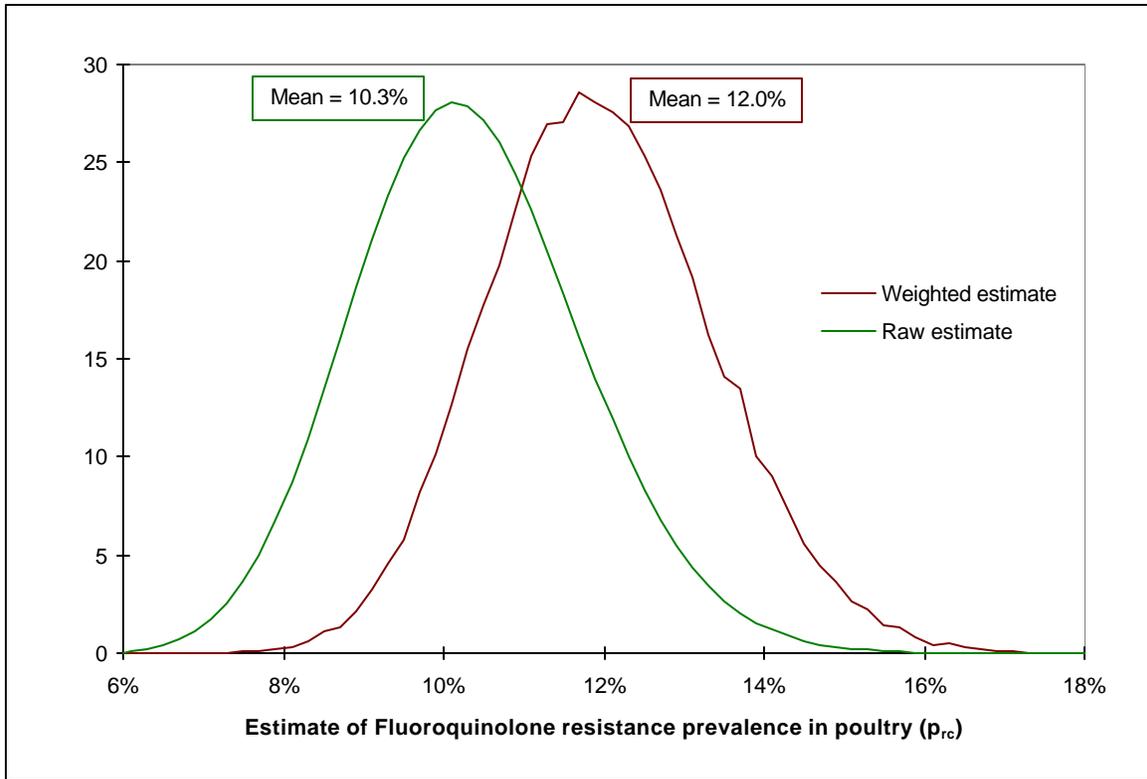


Figure 5.13. Estimates of fluoroquinolone resistance prevalence amongst *Campylobacter* contaminated poultry for 1999: Line labeled 10.3% – crude estimate aggregating all isolate test results; Line labeled 12.0% - estimate weighted by production volume from state of origin of each isolate.