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Review of Microbiological Risk Assessment and Drinking Water Supplies

Final Report to the Department of the Environment

DWI 3940/1
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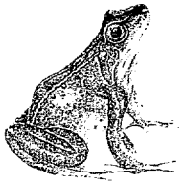

REVIEW OF MICROBIOLOGICAL RISK ASSESSMENT AND DRINKING WATER SUPPLIES

Final Report to the Department of Environment

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EXECUTIVE SUMMARY

In the United States, models have been developed to predict the risks of microbiological infection from drinking water supplies. These are used by the U.S. Environmental Protection Agency not only for the development of microbial standards but also for determining the level of drinking water treatment required to ensure an acceptable risk of infection. One model has predicted that there may be a lifetime risk of death as high as 1 in 20 from exposure to waterborne virus.

This report was commissioned by the Department of the Environment with the objectives of both reviewing such models world-wide and considering the development of models for application in the UK to specific pathogens.

The first part of this report reviews and critically assesses the risk assessment models developed world-wide for pathogens in drinking water. No information was found for countries other than the US. The major criticism of US models is that no account is taken of what proportion of consumers are exposed to what numbers of pathogens. Indeed, one manifestation of the assumptions made is that consumers are effectively either exposed to just one pathogen or to zero pathogens. This may not be appropriate to drinking water supplies where micro-organisms appear to be clustered. Clustering would render a smaller proportion of consumers exposed to much higher numbers of pathogens. It is concluded that by ignoring pathogen clustering, US models could overestimate the risk from more infectious pathogens (e.g., viruses and protozoa) but underestimate the risk from less infectious bacterial pathogens.

The remainder of this review considers the development of risk assessment models in the UK. It is suggested that any pathogen which presents a hazard through drinking water should be accommodated. Epidemiological information on individual pathogens highlights the necessity to customise UK risk assessment models for each pathogen. The most cost-effective approach for risk modelling in the UK is to further our understanding of pathogen numbers in the drinking water supply. In view of pathogen clustering and the highly infectious nature of certain pathogens, it is proposed that dose-response data may be redundant in drinking water models. In effect, infection could be modelled on the proportion of consumers exposed to a dose of one or more pathogens. Such an approach would provide a 'worst case scenario'.

Little information is available on pathogen numbers in the UK drinking water supply, because very large volumes need to be sampled to detect them. Here, a method is demonstrated for modelling pathogen numbers across the supply using raw water data and treatment removal rates. That would enable the cost-effectiveness of various risk reduction options to be modelled. In addition to effects of environmental inputs and treatment processes on health could be assessed. The overall conclusion of this review is that data and technology may be available to develop a UK risk assessment model for *Cryptosporidium*.

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1. INTRODUCTION

Drinking water supplies in the UK are regularly monitored for faecal indicator organisms to warn of the potential presence of faecal contamination and hazard from pathogenic micro-organisms (The Water Supply (Water Quality) Regulations (1989)). Organisms belonging to the group called total coliforms and the individual species *Escherichia coli* should be absent from 100-ml volume samples taken from random and fixed point consumer premises within each supply zone. However water treatment systems do not provide complete removal of micro-organisms and it seems likely that some pathogens will remain, albeit at extremely low concentrations. Furthermore the absence of total coliforms or *E. coli* in 100 ml volume samples does not guarantee the absence of pathogens such as *Cryptosporidium* in much larger volumes, e.g. 100 litre samples. As a consequence, despite the general improvement in microbial quality as measured by the absence of faecal indicator organisms a residual risk to public health may exist. In the United States mathematical models are being developed to assess the risks from *Giardia* and viruses in drinking water (Regli *et al.* 1991, Haas *et al.* 1993).

1.1 The benefits and uses of practical application of quantitative assessments of risk from pathogens in drinking water

The benefits of applying a suitable risk assessment model for a particular pathogen in drinking water supplies are:-

- microbiological health risks from low levels of pathogens remaining after treatment in the supply may be estimated (Haas *et al.* 1993).
- microbiological standards for drinking water supplies may be developed to meet a particular health criterion (Rose and Gerba, 1991).
- options to minimise risks from defined microbiological substances may be identified, enabling costs and benefits to health and environment to be quantified
- microbiological health risks from the ineffective operation of a particular treatment process or variation in raw water quality may be evaluated

In their publication discussing the application of microbiological risk assessment models in drinking water, Regli *et al.* (1991) list a number of questions which such models would help to answer. These are:-

1. Does a system really need a filter?
2. What is an adequate watershed control programme?
3. How much disinfection, depending on the level of contamination in the source water, should a system that filters apply?

4. When is the minimum 3- and 4-log removal-in-activation requirement for *Giardia* and viruses, required under the Surface Water Treatment Rule (SWTR), not enough?

5. When is disinfection warranted in a groundwater system?

6. What level of disinfection should be provided?

Modelling risks from drinking water consumption would be useful for infections which may be spread through other sources, e.g. milk, food; perhaps providing answers to the question 'What proportion of cases arise form drinking finished water?" Even though many incidents of water-borne transmission of viruses (Anon, 1983) and other pathogens are not recognised or reported, the available epidemiological data indicate that the role of water in the overall incidence of viral diseases may be limited. Other means of transmission, particularly personal contact, probably are responsible. Risk assessment models could prove a useful complimentary approach for outbreak control teams.

1.2 Objectives of the contract

There are doubts about the validity of the US risk assessment models, the strength of the data used to develop the models and the applicability of these models for use in the UK. The aim of this contract is to critically review and assess risk assessment models developed in other countries, including the US, and assess the applications and data inputs needed for UK models.

This review is divided into five sections. These are

1. Review and assessment of world-wide application of risk assessment models for drinking water supplies.

2. Application of risk modelling methodology to pathogens in the UK.

3. Report on the necessary data inputs for a UK model and assess the state of appropriate data knowledge in the UK.

4. Advice on cost effective methods to improve the quality of data inputs for a UK model.

5. Identify risk reduction options for UK and assess with respect to health, environmental and cost benefits.

The contract will go further than US models by considering the possibility of predicting levels of pathogens in the drinking water supply from treatment and loading in source water.

1.3 The nature of microbial disease

The main features of microbial diseases are:-

- they are acute,
- epidemics feature, followed by secondary transmission,
- recovery leads to immunity and carriers
- certain groups (e.g. immunocompromised, elderly, young) are at greater risk
- higher risk in low socio-economic status individuals with poor living conditions
- depending on the vehicle of transmission, large numbers of people may be infected.

These have implications in development of microbiological risk assessment models.

2. REVIEW AND ASSESSMENT OF WORLD-WIDE APPLICATION OF RISK ASSESSMENT MODELS FOR DRINKING WATER SUPPLIES

The objective of this section is to review the microbiological risk assessment models currently developed worldwide. To gain information on models which are being developed in countries other than the US, letters were written to researchers involved in water reuse in Spain (Prof. Mujeriego), South Africa (Prof. Grabow), Israel (Prof. Shelef) and Greece (Prof. Ganoulis). Names of further contacts were provided in their responses, but there was not time in the contract to follow them up. Therefore only published models used in the USA are discussed here.

The microbiological risk assessment models used by the United States Environmental Protection Agency have been developed by Charles N Haas, Joan B Rose, Charles P Gerba and Stig Regli. They model the quantification of microbial risk through the following stages:-

1. Select pathogen
2. Measure numbers in finished water
3. Estimate volume of finished water consumed per person/day.
4. Determine dose/infectivity response curve.
5. Estimate daily and yearly infection rates from risk model using 2,3,4 above
6. Estimate illness rates
7. Estimate mortality rates
8. Estimate uncertainty in risk assessment

2.1 Principles of US risk assessment models

The underlying principle of the US risk assessment models is to assess exposure of drinking water consumers to pathogen from the pathogen density in the supply and the volume of water each consumer drinks. Exposure is the number of pathogens to which each consumer is exposed. It is calculated as the product of the volume of water consumed and the concentration of pathogens in that water volume.

$$\text{Exposure} = \text{pathogen density} \times \text{volume consumed.}$$

Haas (1993) and Haas *et al.* (1993) use a single point estimate both for pathogen density in the drinking water supply and for volume of water consumed. They use a point estimate of 0.0012 virus per litre for virus density and a point estimate of 2 litres of tap

water per person per day for volume of water consumed. Their point estimate used for virus reflects an approximation for the most exposed individual.

Both the concentration of pathogens in water taken from a consumer tap at a particular time and the volume of unboiled tap water consumed by each individual vary considerably. Thus the range of pathogen exposures which an individual may be exposed to is considerable. This is discussed below.

Dose/response assessment is of central importance in US risk modelling. This defines the relationship between the exposure to pathogen and the likelihood of infection, illness or mortality.

Exposure is translated into estimated risk through the dose-response equation.

Risk ~ g(Exposure. Dose-response)

Thus risk may be expressed as a function:-

Risk ~ g(pathogen density. volume consumed. dose-response)

Haas *et al.* (1993) considers two types of risk assessment model for viruses in drinking water. They differ in how the risk quantified is presented:-

- the point estimate of risk quantification
- the interval estimate of risk quantification

The point estimate of risk quantification

From the dose-response curve for rotavirus infectivity (Ward *et al.* 1986), Haas calculates a point estimate of the daily risk of illness to be 0.000717, which is equivalent to an annual risk of disease of 0.23. This risk reflects the most exposed individual.

The interval estimate of risk quantification

The interval of risk merely reflects the uncertainty interval for the point estimate of risk. Haas *et al.* (1993) calculate the 95% confidence interval for the daily risk to be 0.000317 - 0.00188. They characterise the uncertainty only from the point of view of the dose-response data. No account is taken of uncertainty in exposure calculation or variation in pathogen density or volume of water consumed.

2.2 Criticism of using single point estimates to calculate exposure.

In the US models for viruses (Haas *et al.* 1993) and other pathogens (Haas, 1993), exposure to pathogen for the most exposed individual is calculated from single point estimates for pathogen density and volume of water consumed.

2.2.1 Point estimate for consumption of water

Haas (1993) writes, "For the purpose of performing the point estimate, it is necessary to determine the amount of water ingested per exposure..... US. EPA generally uses 2 litres per person per day for risk estimation from drinking water; however recent reanalysis calls this figure into question (Roseberry and Burmaster, 1992)".

In fact, this figure is actually quite a good point estimate for the most exposed individual, since analysis of the lognormal data presented by Roseberry and Burmaster, (1992) shows that 2.5% of the population drinks more than 2.7 litres of tap water per person per day.

The fact that consumptions of tap waters in the US are lognormally distributed means there is considerable variation between individuals in the volume consumed. Thus while the median volume consumed is 1.198 litres of tap water per person per day, some 2.5% of the population drink less than 0.341 litre per person per day (Roseberry and Burmaster, 1992). Thus the top 2.5% of 'tap water drinkers' consume over 8-times more tap water than the bottom 2.5%.

2.2.2 Criticisms of point estimate for pathogen density

No account is taken of how many consumers consume what proportion of pathogens

The problem with using a single point estimate for a pathogen density is that the variation in pathogen density within a water supply may be very large, posing the question as to what pathogen density should be used in the model. Different consumers will drink water containing different numbers of pathogens depending on their property's location in the supply zone. By analogy with densities of total heterotrophic bacteria which are lognormally distributed, densities may vary more than 10^5-fold within a given supply during the year (Maul *et al.* 1985, see also Section 4.1.6). Thus, some consumers may be exposed to 100,000 or more total heterotrophic bacteria per 100 ml while others may be exposed to less than 1 per 100 ml. Using a single point estimate for pathogen densities measured from a particular location in a supply on a particular day may be analogous to thinking up a random number. The single point estimate for pathogen density does not take into account heterogeneity of pathogen distribution within the supply and does not allow for regional and seasonal variation.

Probably aware of this problem, Haas (1993) effectively looks at the "worst case scenario" by considering the maximum-exposed individual (MEI). He considers it "appropriate to look at the risk from waterborne pathogens at the households in closest proximity to a water treatment plant (i.e., having received the least contact time for disinfection)". This may not be the place to look for the highest pathogen levels because we currently do not understand why pathogen densities are clustered and, in particular, where they are most highly clustered. Haas assumes a decrease in pathogen density with distance from the treatment works. Perhaps, hydraulic and sedimentation properties are more important, leading to hot spots within the supply. Post-treatment contamination could occur anywhere in the supply, particularly at parts further away from the treatment

works where uncontrolled de-pressurisations and mains repairs occur. Furthermore, total heterotrophic bacteria increase on average three-fold between the summer and winter months.

Calculating the risk for the most exposed individual provides limited information. First, if the risk of infection for the MEI was high we would not be too surprised because we know from epidemiological evidence that infections have been contracted through polluted municipal water supplies. More important, however, no account is taken of what proportion of the total drinking water consumers are exposed to the same high dose. If 0.001% of consumers fall into the category of MEI then the health effects across the whole population would be insignificant compared to say 10% of consumers. Only the statistical distribution of pathogen densities within the drinking water supply contains the information to answer the fundamental question in risk assessment, "What proportion of consumers are exposed to what densities of pathogens?". Very little is known about the pathogen density statistical distribution in the drinking water supply and furthermore, it will vary from zone to zone depending on season, quality of the raw water, efficiency of treatment processes on pathogen removal and post-treatment contamination. For this reason US models do not consider pathogen density statistical distributions but use point estimates.

In this review, a working assumption is made that pathogen densities are log-normally distributed within the drinking water supply. The evidence for this is presented in Section 4.1.6, together with the implications. For reference, a log-normal distribution for total coliform concentrations measured in the drinking water supply from a UK water company (collected under The Water Supply (Water Quality) Regulations (1989)) is presented in Figure 2.1. This figure is discussed in Section 2.2.2.

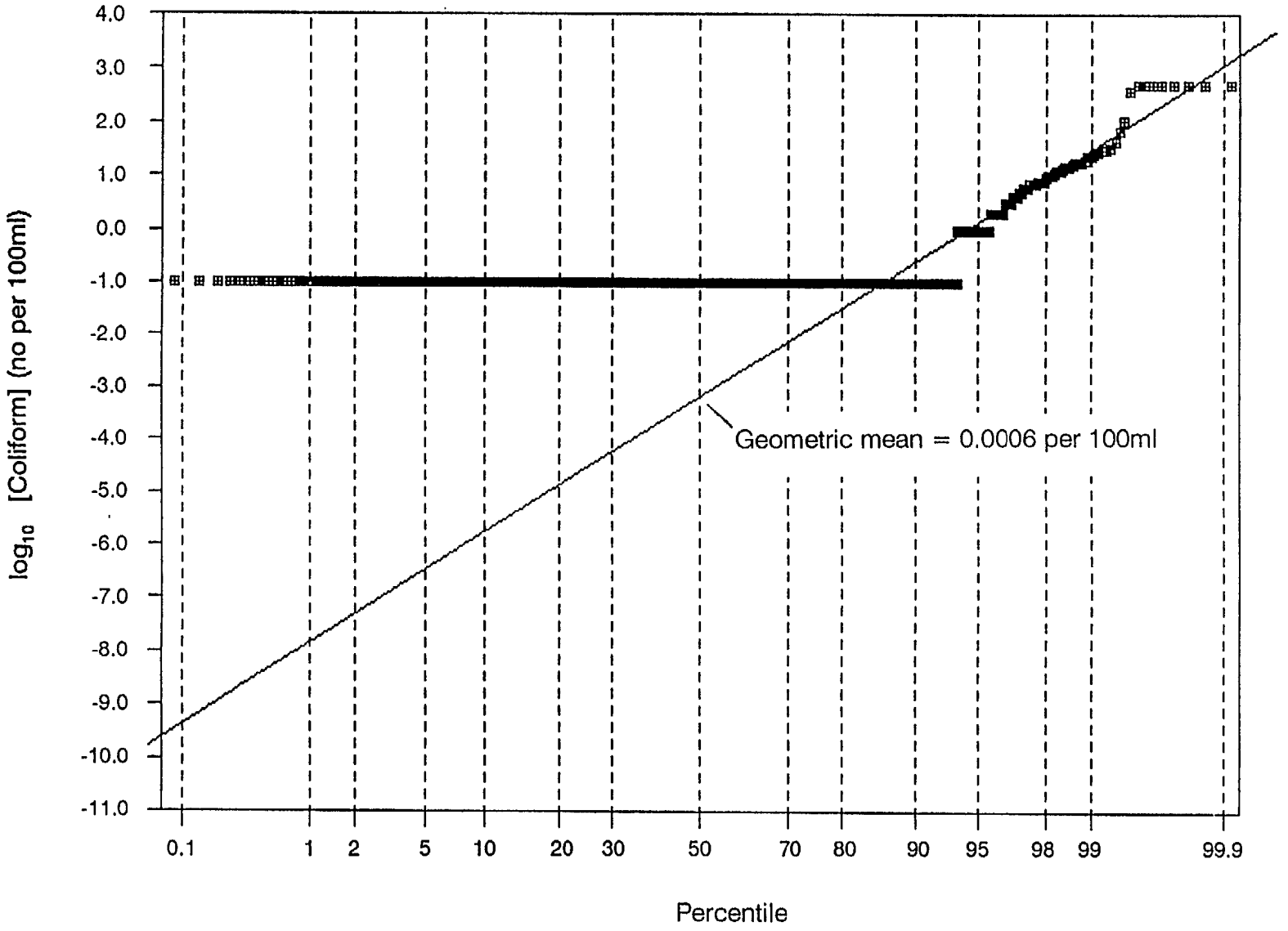

Figure 2.1 Concentrations for total coliform bacteria recorded from the drinking water supply of a UK water company and plotted on a lognormal probability plot.

Point estimates used by Haas are not appropriate statistically

Table 2.1 presents the pathogen concentrations for finished drinking waters used by Haas (1993). Haas admits that particular concerns exist about the direct utility of these numbers in his risk assessment models. He notes the following criticisms:-

- the efficiency of the pathogen enumeration method is less than 100%
- not all numerable organisms are viable.

but assumes that these two effects compensate for each other.

Table 2.1 Pathogen densities in finished drinking waters summarised by Haas (1993) for use in risk assessment models.

Pathogen	Average density (per litre)	Quoted Reference
Enteric viruses	0.0006	Payment *et al.* (1985)
Giardia cysts	<0.0025[b]	Rose *et al.* (1991a)
	0.045[g]	LeChevallier *et al.* (1991b)
Cryptosporidium oocysts	0.001[a]	Rose *et al.* (1991a)
	0.015[g]	LeChevallier *et al.* (1991b)

[a]Geometric mean calculated from log(y + 1)
[b]None detected in drinking water samples
[g]Geometric mean of positive samples (less than 30%)

Haas does not consider that the average pathogen densities quoted in Table 2.1 are appropriate for risk assessment models because some estimate of the upper likely concentration of pathogens is required to simulate the maximum-exposed individual. He therefore makes the assumption that a reasonable upper tail estimate is equal to twice the average microbial density (arithmetic or geometric mean). This assumption, however, is invalid for several reasons:-

1. Haas does not explain why he multiplies the average by a factor of two. It does not appear to be based on any assumption about statistical distribution of pathogen densities within the supply.

2. The arithmetic mean as used by Payment *et al.* (1985) is not an appropriate estimate of central tendency for a lognormal distribution, particularly when 0 concentrations

mean below the limits of detection. Taking an arithmetic mean of lognormally distributed data leads to a gross overestimation of central tendency (as demonstrated below).

3. Taking the geometric mean of positive data (LeChevallier *et al.* 1991b) and ignoring the 70% or more of 0 concentrations, is not appropriate. Similarly calculating the geometric mean by adding 1.0 to each value (thus converting zeros to 1) is not acceptable as performed by Rose *et al.* (1991a). Both these approaches could lead to a gross overestimation of the true geometric mean (as demonstrated below).

For a normally distributed parameter, the median value equals the arithmetic mean, or in the case of a lognormal distribution, the geometric mean. Thus, in the study of LeChevallier *et al.* (1991b), where 73% of samples registered 0 *Cryptosporidium* oocysts per 100 litres, the median oocysts concentration is <1 per 100 litres. However, the geometric mean calculated from the 27% of samples which were positive was 1.52 oocysts per 100 litres (written as 0.015 per litre in Table 2.1). Thus the median and geometric mean (as calculated from positive samples) do not agree, as expected. Similarly for *Giardia* cysts, 83% of samples recorded 0 per 100 litres, the median concentrations again being <1 per 100 litres. The geometric mean calculated from the 14 samples which were positive was 4.45 cysts per 100 litres (written as 0.045 per litre by Haas (1993) in Table 2.1).

Payment *et al.* (1985) detected viruses in 7% (11 of 155) of the finished water samples (1,000 litres) in Montreal. He calculated an average density of 0.0006 per litre. This value of is of little use because the nature of the statistical distribution of viral densities is unknown. All that can be said is that the median viral density is <1 per 1,000 litres. The value of 0.0006 per litre reflects a viral density which could occur at some region of the supply. Haas *et al.* (1993) and Haas (1993) multiply this value by two (almost for good measure, it would seem) to approximate the case for the most exposed individual for risk assessment analysis.

Haas could have estimated the pathogen density for the most exposed individual by simply using the highest pathogen density recorded in a treated drinking water sample. These are summarised in.Table 2.2

The problem with zeros.

In Table 2.3 the proportion of drinking water samples registering 0 pathogens are presented from the US and Canadian published data used by Haas for risk assessment purposes. Between 73 and 100% of the samples registered zero pathogens. This is analogous to coliform data in 100 ml volume samples collected under The Water Supply (Water Quality) Regulations, where 95% at least of samples are required to register 0 coliforms per 100 ml. These zero samples present a problem for statistical analysis, in particular calculation of means and standard deviations. They are valid results and therefore cannot be ignored as LeChevallier *et al.* (1991b) did. At the same time, they cannot be used because they represent samples below the limits of detection with the

volume sampled. They should therefore be recorded as <1 per 100 ml (in the case of coliforms) and their true concentration is unknown but may range from just less than 1 per 100 ml down to 0.00000001 per 100 ml in the case of total coliforms (Figure 2.1).

Table 2.2 Maximum pathogen densities in finished drinking waters in studies performed in US and Canada.

Pathogen	Maximum density	Reference
Enteric viruses	20 per 1,000 l	Payment *et al.* (1985)
Giardia cysts	0 per 378 l	Rose *et al.* (1991a)
	64 per 100 l	LeChevallier *et al.* (1991b)
Cryptosporidium oocysts	1.7 per 100 l	Rose *et al.* (1991a)
	48 per 100 l	LeChevallier *et al.* (1991b)

Table 2.3 Proportion of drinking water samples registering zero pathogens per volume sampled

Pathogen	Number of negative Samples (Volume of sample)	% Negative	Reference
Enteric viruses	144 of 155 (1,000 l)	93%	Payment *et al.* (1985)
Giardia cysts	All (378 l)	100%	Rose *et al.* (1991a)
	68 of 82 (100 l)	83%	LeChevallier *et al.* (1991b)
Cryptosporidium oocysts	30 of 36 (378 l)	83%	Rose *et al.* (1991a)
	60 of 82 (100 l)	73%	LeChevallier *et al.* (1991b)

There are two ways round this problem. The first would be to take larger volume samples such that over 50% of samples were positive for *Cryptosporidium*. This would give a median value of >0 per measured volume. The median could then be used as an approximation of the geometric mean. Indeed, one of the recommendations from work funded by Foundation for Water Research to investigate coliforms statistics (Gale, 1994a) was to undertake a large volume sampling programme for coliforms from consumer taps. The second would be to plot all the data including zeros on a normal probability plot (see

Figure 2.1). This would not only provide information on the nature of the statistical distribution (i.e. Poisson, normal or lognormal) but also perhaps provide some rough approximation of parameters such as the geometric mean and logarithmic standard deviation.

The lognormal distribution for densities of microorganisms in the drinking water supply

In Figure 2.1, coliform density data (per 100 ml) collected from random consumer premises under The Water Supply (Water Quality) Regulations (1989) are plotted on a normal probability plot after logarithmic transformation. The best fit line through the data registering one or more coliforms per 100 ml represents a lognormal distribution. Where the coliform density is <1 per 100 ml, the sampling registers 0 coliforms (represented as -1.0). The range of coliform densities described by the lognormal distribution appears to be very large, ranging between 10^{-10} per 100 ml (1 per 1 000 000 000 litres) to 550 per 100 ml.

The median (or geometric mean) may be estimated as where the best fit line intercepts the 50 percentile. Its value is 0.0006 per 100 ml, which is 6 per 10,000 litres. Using this data set, 'averages' were calculated as in the publications on which Haas based his risk assessment models. These are presented in Table 2.4. It appears that in all cases the 'average' coliform concentration is hugely overestimated (by 10,000 fold) compared to the true geometric mean density.

Table 2.4 'Average' coliform densities as calculated by different methods and used by Haas in risk assessment modelling.

Average	Value	Reference
True geometric mean	0.00062 per 100 ml	
Arithmetic mean	3.25 per 100 ml	Payment *et al.* (1985)
Geometric mean, Log (1 + y)	1.14 per 100 ml	Rose *et al.* (1991a)
Geometric mean coliform positive samples	5.55 per 100 ml	LeChevallier *et al.* (1991b)

No account is taken of clustering of pathogens

Consider the most contaminated *Cryptosporidium* sample collected from finished waters (LeChevallier *et al.* 1991b). Some 48 oocysts were detected in 100 litres. The US EPA assume everybody drinks 2 litres per day. So does everybody get more or less one of each of the 48 oocysts or does one person get all 48 in his 2 litres? This is the crucial question of microbiological risk assessment modelling and the question which the US models do not consider. Again the answer depends on the statistical distribution of pathogens.

The US models assume a homogeneous (random) distribution of pathogens as defined by the Poisson model. Thus, the 48 oocysts are homogeneously distributed across the 100 litres and, on average, each 2 litre sample drunk per day will contain 0.96 oocysts. The average consumption of oocysts would therefore be 0.96 per day per person. The Poisson distribution (with mean 0.95 oocysts per person per day) predicts (from statistical tables) that 38.7% of consumers will ingest 0 oocysts per day, 36.7% will get 1 oocyst, 17.5% will ingest 2 oocysts, 5.5% will get 3 oocysts and 1.6% will get 4 or more oocysts. A lognormal distribution, however, assumes those 48 oocysts are more clustered. Thus, it is possible that 98% of consumers could ingest no oocysts per day but one or two consumers may ingest 48 oocysts in one day.

The important difference is that a homogeneous dispersion means that a large proportion of consumers gets a small but similar dose. For a heterogeneous dispersion most consumers are not exposed but a few get much higher doses than expected from the average.

2.2.3 Summary of pathogen density estimates

1. The major problem in modelling microbiological risks in drinking water supplies is the lack of information on pathogen densities; and in particular their statistical distribution. This reflects their low densities, with many drinking water samples registering 0 pathogens per volume analysed. These data pose a problem to statistical analysis.

2. US risk assessment models do not consider statistical distributions of pathogen densities across drinking water supplies and hence ignore the fundamental question, "What proportion of consumers are exposed to what densities of pathogens?".

3. Estimates of geometric/arithmetic means for pathogen densities in finished water used by Haas for risk assessment purposes are unrealistically high, and furthermore do not reflect any statistical property of the statistical distribution (Poisson or lognormal) for pathogen densities.

4. The point estimates used by Haas for pathogen densities do not reflect those experienced by the most exposed individual, but merely reflect a pathogen density which could occur somewhere in the supply on the basis of published data.

5. The point estimate for pathogen density in the most exposed individual may be an underestimation because the US model assumes a Poission model which does not account for clustering.

6. Calculating the risk for the most exposed individual is of limited use anyway, because:-

   a) the proportion of the consumers within the total population that are exposed to this density is not known. It could be an insignificant proportion or a considerable proportion. The exact proportion would depend on the degree of clustering of pathogens within the entire supply zone as defined by the statistical distribution of densities.

   b) the effect of exposure to lower densities than that experienced by the most exposed individual is not quantified. Since the major proportion of consumers fall into this category it is of more importance in public health.

   c) If the risk of infection to the most exposed individual was high one would not be too surprised because it is well known that infection through the drinking water supply is possible.

2.3 Review and assessment of dose-response data

Dose-response models used for different pathogens in the US risk assessment methodology are now reviewed and assessed. In addition, a literature review has been performed to identify further information on volunteer studies for pathogens such as *Cryptosporidium.*

The dose-response model relates the number of pathogens consumed to the incidence of illness. The dose-response model is typically a sigmoidal curve. A generalised example is shown in Figure 2.2. The curve is defined mathematically and is fitted to data obtained from experiments with human volunteers (not shown in Figure 2.2). It can be seen that as the dose of pathogen increases so does the probability of infection. From the curve, the dose at which half of the volunteers are infected may be determined (Arrow A). This dose is referred to as the HID_{50}. Some workers (Rose and Gerba, 1991) report the dose at which 1% of the volunteers are infected. This dose is termed the HID_{01} (not shown on model). The curve also enables the probability of infection from just one organism to be estimated (Arrow B). Another concept is that of the minimal infectious dose.

There are two areas which need addressing in the application of dose-response models in the assessment of microbiological risk from drinking waters. These are:-

i. type of mathematical equation (*model*) relating concentration of pathogen consumed (*dose*) to number of people ill (*response*), and

ii. waterborne pathogens for which data are available for developing such models.

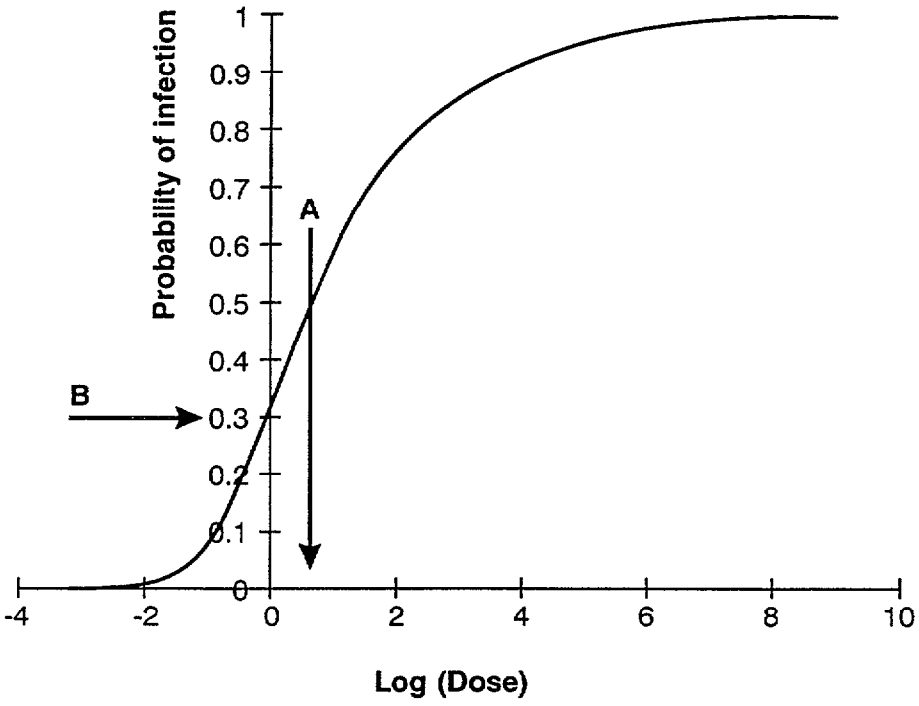

Figure 2.2 Typical dose response curve.

Mathematical models.

Two approaches are used in modelling infection. These are deterministic and stochastic. In the deterministic model, pathogens in the dose are considered to act cooperatively. Illness results from their joint action. In the stochastic model, pathogens are assumed to act independently.

Haas (1983) considered three probability models for their appropriateness to relate infection by low level exposure to enteric pathogens in drinking water. The lognormal model is deterministic, while the single-hit exponential and β-distributed models are stochastic.

Pathogens for which dose-response data appropriate to the drinking water supply are available.

Experiments with human volunteers have been conducted for a number of bacteria, protozoans and viruses. In such experiments, sets of volunteers were exposed to different (known) dosages of microorganisms. Rose and Gerba (1991) list parameters characterising dose-response models for 15 microorganisms. The type of model used by Rose and Gerba (1991) and the values for parameters defining the model are presented in Table 2.5. Rose and Gerba also calculated the dose required for infection of 1% of volunteers (HID_{01}) and the probability of infection from exposure to one organism (Table 2.5). At WRc dose-response models have been simulated on computer. For a number of organisms listed in Table 2.5, the values quoted for HID_{01} and the probability of infection from exposure to one organism in Rose and Gerba (1991) do not agree with computer simulations using the parameters listed.

Dose-response curves using parameters provided by Rose and Gerba (1991) in Table 2.5 are plotted for four pathogens in Figure 2.3. For rotavirus, *Shigella flexneri 2A* and *Salmonella typhi* dose-response curves are β-distributed. The curve for *Giardia lamblia* is single hit exponential. Comparison of the dose-response curves shows that the risk of infection is 1,000 lower for the bacteria (*Shigella flexneri 2A* and *Salmonella typhi*) than for rotavirus and *Giardia lamblia* at a similar level of exposure. Thus, the probability of infection from a single cell of *Salmonella typhi* is 0.0038%, while that for a single rotavirus particle is 31% (according to data from Rose and Gerba, 1991). However, as shown in this section there is considerable controversy about the infectivity of salmonellae, the current opinion suggesting that infectious doses are somewhat lower. Thus, a risk assessment model using the dose-response data (Rose and Gerba, 1991) for *Salmonella typhi* may considerably underestimate the risk.

Table 2.5 **Parameters for dose-response models for waterborne pathogens. Data from Rose and Gerba (1991). Infection was the end result measured for development of each of these models with the exception of *Shigella dysenteriae*.**

Microorganism	Model	Parameters	Probability of infection from one organism
Campylobacter	Beta-Poisson	0.039 and 55	0.007
Salmonella	Beta-Poisson	0.33 and 139.9	0.0023
Salmonella typhi	Beta-Poisson	0.21 and 5531	0.000038
Shigella	Beta-Poisson	0.16 and 155	0.001
Shigella dysenteriae	Beta-Poisson	0.5 and 100	0.000497
Shigella flexneri	Beta-Poisson	0.2 and 2000	0.0004
Vibrio cholera classical	Beta-Poisson	0.097 and 13020	0.000007
Vibrio cholera El Tor	Beta-Poisson	0.000027 and 1.33	0.000015
Poliovirus 1	Beta-Poisson	15 and 1000	0.0149
Poliovirus 3	Beta-Poisson	0.5 and 1.14	0.031
Echovirus 12	Beta-Poisson	1.3 and 75	0.017
Rotavirus	Beta-Poisson	0.232 and 0.247	0.31
Entamoeba coli	Beta-Poisson	0.17 and 1.32	0.091
Entamoeba histolytica	Beta-Poisson	13.3 and 39.7	0.28
Giardia lamblia	Exponential	-0.0199	0.0198

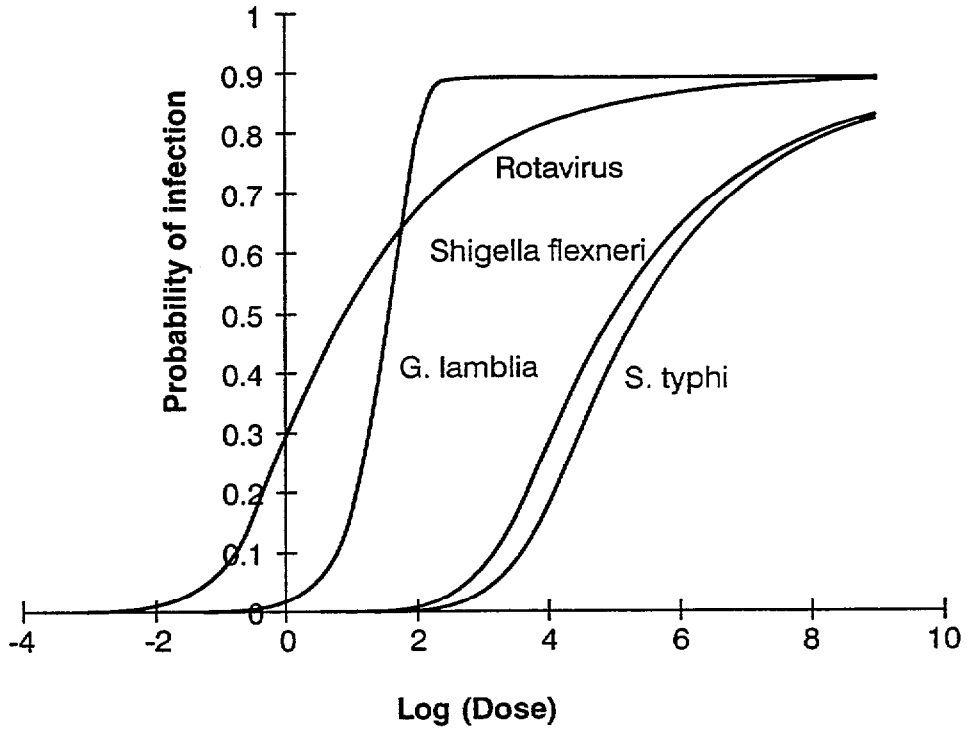

Figure 2.3 Dose response curves generated for viral, protozoan and bacterial waterborne pathogens using data from Rose and Gerba (1991).

Haas (1993) also presents parameters characterising dose-response models for certain pathogens. These are presented in Table 2.6. It is interesting to note that these parameters differ to those reported by Rose and Gerba (1991).

Table 2.6 Best-fit dose response parameters for potential waterborne pathogens in humans (taken from Haas, 1993).

Organism	α	N50
Polio III	0.500	3.4
Rotavirus	0.141	5.6
Giardia	--	35.0
Polio I	15.0	47.3
Echovirus 12	1.30	52.8
Entamoeba coli	0.170	76.5
Shigella dysenteriae	0.50	300.0
Polio I	0.119	67516.4

Dose-response data for certain pathogens of waterborne health significance are now considered in more detail.

Salmonella spp.

There is considerable disagreement in the minimum number of ingested salmonellae reported as necessary to produce clinical symptoms in humans. Blaser and Newman (1982) review the infectious dose data for salmonellosis in humans. They conclude that results of studies involving volunteers show that large inocula of salmonellae are necessary. Experiments reported in 1951 involving volunteers showed that more than 10^5 cells of *Salmonella melagridis* or *Salmonella anatum* obtained from spray-dried whole egg were required to produce illness. However, retrospective investigations of outbreaks of salmonellosis suggest that the implicated infectious dose was often low. In six of 11 outbreaks, the actual doses ingested were calculated to be $<10^3$ organisms. Indeed for a waterborne outbreak of *Salmonella typhimurium* involving 16,000 people, as few as 17 organisms were estimated retrospectively to cause infection. Doses of 100 - 250 organisms of *Salmonella eastborne* were calculated for an outbreak caused through contaminated chocolate balls. D'Aoust (1985) calculated retrospectively from individual cases that between one and six cells of *Salmonella typhimurium* are required (in cheddar cheese) to support infection of humans. D'Aoust (1985) suggested that the fat content of contaminated foods such as cheddar cheese may play a significant role in human salmonellosis. Organisms trapped in hydrophobic lipid moieties may readily survive the acidic conditions of the stomach and pass into the intestine where the proliferate to toxic levels.

The general consensus therefore is that just a few cells of salmonellae can be infectious.

Mintz *et al.* (1994) studied the effects of ingested dose of *Salmonella enteritidis* on incubation period, symptoms and severity of acute salmonellosis in a food poisoning outbreak at a wedding reception in 1990. In the study 169 persons, who developed gastroenteritis after eating a sauce made from eggs, were divided into three groups based on self-reported dose of sauce ingested. As dose increased, median incubation period decreased and greater proportions reported body aches and vomiting. Increased dose was associated with increased weight loss, rating of illness severity, and the number of days of confinement to bed. Although the study did not provide information on dose-response relationships, the findings present important considerations for drinking water risk assessment models, since pathogens may be clustered. Glynn and Bradley (1992) also studied the relationship between infecting dose and severity of disease in reported outbreaks of salmonella infections. They found no evidence for a dose-severity relationship for *Salmonella typhi*. However, such relationships were found for other *Salmonella spp.* including *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella infantis*.

Giardia lamblia

In their risk assessment model for waterborne giardiasis, Rose *et al.* (1991b) used data from Rendtorff's experiments in which *Giardia* cyst doses ranging from 1 to 10^6 were fed to prison volunteers. The cysts were collected from the faeces of infected humans. A positive response was measured by cyst excretion in the faeces. They modelled the probability of infection (response) from cyst dose by the exponential equation. They report that 'the exponential model was statistically consistent with the Rendtorff data. A value for r was calculated from the best fit model to be -0.0198. The data which Rendtorff (1954) obtained are presented in Table 2.7.

Table 2.7 Data for experimental infection for *Giardia lamblia* cyst exposure (Rendtorff, 1954).

Cyst Dose	Volunteer response Number infected	Proportion infected
1	0 of 5	0
10	2 of 2	1.00
25	6 of 20	0.30
100	2 of 2	1.00
10,000	3 of 3	1.00
100,000	3 of 3	1.00
300,000	3 of 3	1.00
1,000,000	2 of 2	1.00

In Figure 2.4, the proportion of volunteers infected is plotted as a function of cyst dose. The best fit exponential model is plotted for each of the doses administered. This is the

dose-response curve. One criticism is that for most of the doses only two or three volunteers were used, and for those doses all volunteers were infected. For two doses where five or more volunteers were used, not all volunteers were infected.

In Rendtorff's experiments infections were easily induced in the volunteers. However, no disease or diarrhoea could be attributed to the infection. Nash *et al.* (1987) state a number of possible reasons for this, "including differences in virulence among isolates of *Giardia* or the prior development of resistance to those as yet unidentified factors responsible for the development of diarrhoea in giardiasis. Although Rendtorff's studies were well done and informative, the choice of the inoculating isolate of *Giardia* was necessarily a random selection (from human faeces)". *Giardia* isolates from humans differ biochemically and biologically. These differences may explain some of the variability in the clinical features noted in human infections. Gene probe analysis of DNA from 15 human isolates of *Giardia* revealed marked differences. Isolates also differed in surface antigens and excretory-secretory products. Nash *et al.* (1987) demonstrated strain variation in the pathogenicity of *Giardia* infections in humans. Human volunteers were inoculated with one of two distinct human isolates of *Giardia lamblia*, GS/M and Isr. Each volunteer was given about 50,000 trophozoites. All five volunteers inoculated with GS/M became infected and three became ill as well. Two had diarrhoea and typical symptoms of giardiasis. None of the volunteers inoculated with Isr became infected.

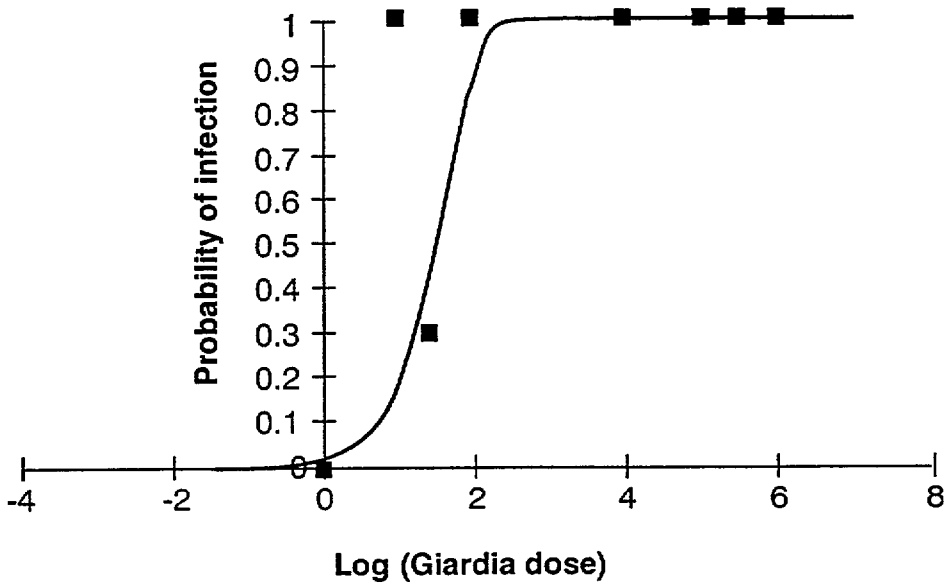

Figure 2.4 Exponential dose-response curve for *Giardia lamblia* (data from Rendtorff (1954)) used in the waterborne giardiasis model (Rose *et al* 1991).

Cryptosporidium parvum

DuPont *et al.* (1995) have performed a study on informed volunteers to determine the infective dose of *Cryptosporidium parvum* for humans. Volunteers were healthy and antibody negative. Oocysts were obtained after passage in newborn calves. Doses used ranged between 30 and 1,000,000 viable oocysts and were administered in capsules. Infection occurred in 16 of 26 of the volunteers (Table 2.8). The ID_{50} was estimated to be 214 oocysts. Six of the 16 infected volunteers developed clinical illness consisting of diarrhoea, nausea and abdominal pain. Secondary spread to household contacts was not observed. It should be noted, however, that volunteers were advised about principles of hygiene and therefore have may taken extra precautions to reduce person-to-person secondary transmission within their household. Rose *et al.* (1995) have modelled the data using an exponential model with r = 0.00467. The probability of infection from a single organism is 4.7×10^{-3} and a dose of 30 oocysts would initiate infection of 20% of those exposed.

Table 2.8 Data for experimental infection for *Cryptosporidium parvum* oocyst exposure (Dupont *et al.*, 1995).

Oocyst Dose	Volunteer response Number infected	Proportion infected
30	1 or 5	0.20
100	3 of 8	0.38
300	2 of 3	0.67
500	5 of 6	0.83
>1,000	7 of 7	1.00

Echovirus-12

Shiff *et al.* (1984) performed a study on healthy adult volunteers to determine the dose of echovirus-12 required to establish infection in humans. Volunteers were given 0 - 330,000 pfu of echovirus-12 in chilled nonchlorinated drinking water (presumably free of other pathogens!). The proportions of individuals infected at each dose are presented in Table 2.9. Infection was determined by seroconversion or intestinal shedding of virus. The data obtained with four doses were used to produce a dose-response curve by probit analysis. From that curve, the HID_{50} (dose required to infect 50% of volunteers) of echovirus-12 was determined to be 919 pfu (95% fiducial limits from 573 pfu to 1,503 pfu). The dose required to infect 1% of volunteers (HID_{01}) was 17 pfu with 95% fiducial limits of (1.0, 56 pfu). The results of Schiff *et al.* (1984) indicated that previous infection with echovirus-12 dose not provide lasting protection against reinfection.

Table 2.9 Data for experimental infection for Echovirus 12 exposure (Schiff *et al.* 1984).

Dose of virus (pfu)	Volunteer response Number infected	Proportion infected
0	0 of 34	0.00
330	15 of 50	0.30
1000	9 of 20	0.45
3300	19 of 26	0.73
10000	12 of 12	1.00

Rotavirus

Ward *et al.* (1986) determined the infectious dose for human rotavirus. Rotavirus was obtained from a stool specimen of a hospitalised child. Subjects used for this study were first screened for serum neutralising antibody to the rotavirus. The antibody titres varied considerably ranging from <2 to 1,600. Thus, many subjects had relatively high titres of serum neutralising antibody to the strain used. Ward *et al.* (1986) reported, however, high antibody titres had no significant effect on the probability of either infection or illness in subjects given an infectious dose of rotavirus. Eight of the nine subjects with the highest preinoculation titres (geometric mean titre 708) became infected, and five experienced illness.

Ward *et al.* (1986) administered doses ranging from 0.009 to 90,000 focus-forming units orally to 62 volunteers (healthy 18 - 45 year old men) after consumption of 50 ml of sodium bicarbonate (4%). The proportions of volunteers developing infection (shedding of virus or rise in antibody titre) after a given dose are plotted in Figure 2.5. The best fit dose-response curve based on the β-Poisson model with the parameters presented in Rose and Gerba (1991) is also plotted in Figure 2.5. That model suggests that doses of just 5 focus forming units would infect 50% of adults and that a dose of just one rotavirus would infect some 31% of adults. In Ward's experiments, 17 of the 30 infected individuals became ill.

Norwalk Virus

Norwalk virus infection is a common cause of gastroenteritis in humans. Dose-response trials have been conducted by Graham *et al.*. (1994). Norwalk virus was administered to 50 volunteers. Clinical features and virological and immunological responses were studied. It should be noted that sodium bicarbonate solution was taken by each person during administration of the virus inoculum. The study by Graham *et al.* (1994) does not provide dose-response data which are useful for risk assessment models since a standard dose was given to each volunteer.

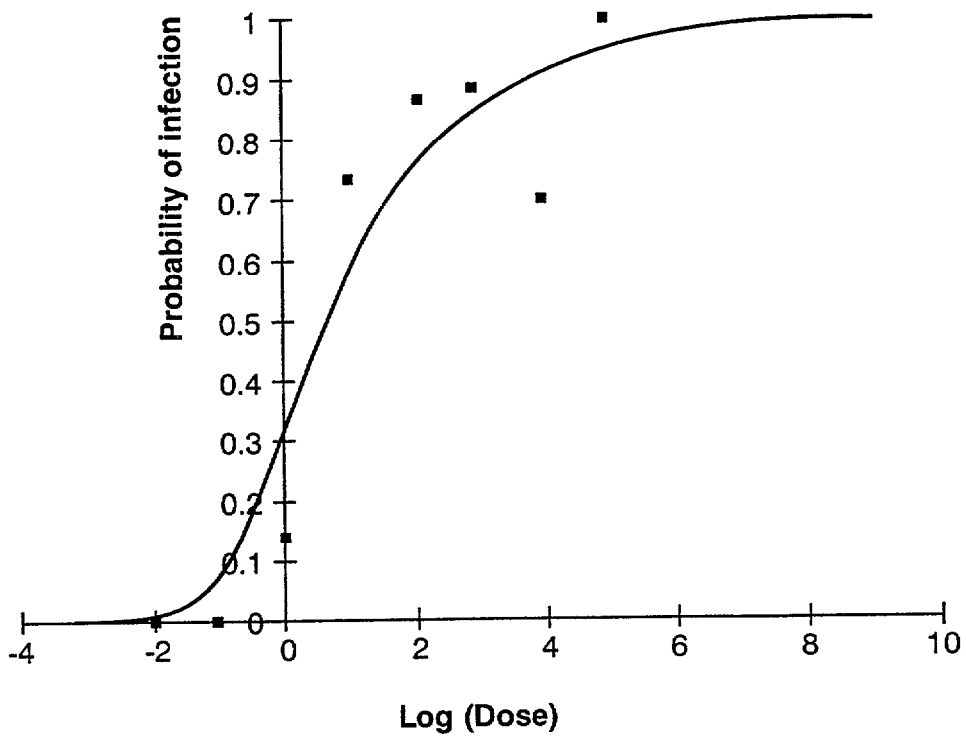

Figure 2.5 Dose-response data for human rotavirus from trials on volunteers (Ward *et al* 1986). ß-Poisson dose-response curve constructed using parameters presented in Rose & Gerba (1991).

Human Caliciviruses

Cubitt (1989) reports a small volunteer study in which three of four adults developed mild or moderate symptoms of gastrointestinal illness (diarrhoea and nausea) after they were given between 100 and 1,000 human calicivirus particles. This information is not sufficient for dose-response modelling.

Enteric adenoviruses

No volunteer studies involving fastidious adenovirus strains have been published to date.

Astrovirus

Kurtz *et al.* (1979) showed that adult volunteers could be infected. They excreted virus and some developed mild diarrhoea symptoms. In addition, the majority seroconverted but no conclusions could be drawn about potential pathogenicity in babies and young children, the ones most likely to be ill. This study did, however, confirm that the virus is capable of infecting man.

Poliovirus

In early experiments (cited in Katz and Plotkin (1967)) human volunteers were administered doses of the SM strain of poliovirus type 1 in gelatin capsules. The results are shown in Table 2.10.

Table 2.10 Infection with attenuated poliovirus Type 1 (SM strain of adult volunteers (taken from Katz and Plotkin (1967)).

Dose (pfu)	Result	% infected
0.2	0 of 2	0
2	2 of 3	67
20	4 of 4	100
200	4 of 4	100

Katz and Plotkin (1967) studied the minimal infective dose of attenuated poliovirus Type III in 22 premature infants in the USA. Infection was judged as detection of poliovirus in stools. Three concentrations of the vaccine virus were used and quantified in units of TCD_{50}, which reflects the concentration which infects 50% of tissue culture. The results are presented in Table 2.11. Their results showed that 30% of subjects given enough virus to infect 50% of tissue cultures (i.e. 1 TCD_{50}) were infected. The calculated 50% infective dose for the infants was 4 TCD_{50}.

Table 2.11 Infection with attenuated poliovirus Type III (Fox strain) of premature infants (Katz and Plotkin (1967)).

Dose (TCD_{50})	Result	% infected
1	3 of 10	30
2.5	3 of 9	33
10	2 of 3	67

E. coli O157:H7

The infectious dose of *E. coli O157:H7* that leads to symptomatic infection is speculated to be low on the base of a retrospective-case control study from a swimming-associated outbreak (Keene *et al.*. 1994). The evidence for this conclusion was weak, however, and did not take into account the fact that bacterial concentrations tend to be clustered into pollution hotspots in lakes (Gale *et al.* 1990).

Enteroadherent *Escherichia coli*

Two strains, *O?:H33/35* and *O78:H33/35*, of enteroadherent *E. coli* (EAEC) were isolated from patients who developed diarrhoea after travelling to Mexico and administered to healthy adult volunteers (Mathewson *et al.* 1986). Volunteers were given 2 g of sodium bicarbonate as an Antacid immediately before challenge. Very high doses of 7×10^8 or 10×10^9 were given to each volunteer. Not surprisingly every volunteer shed the EAEC strain in their stools. Varying proportion developed diarrhoea or enteric symptoms. The challenge studies of Mathewson *et al.* (1986) really only provide epidemiological evidence that EAEC may be an agent in diarrhoea and are of little use for developing dose-response relationships for risk assessment modelling.

Vibrio cholerae.

The infective dose of *Vibrio cholerae* is 10^{11} organisms; although after neutralisation of gastric acid this drops to between 1,000 and 10,000. Asymptomatic or mild infections may outnumber severe cases by as many as 100 to one in endemic areas, and may be important in sustaining epidemics. Some people are more vulnerable to cholera than others. Breast feeding confers protection not received by babies who are feed formula milks. Gastric hypoacidity increases susceptibility, and this may be the mechanism by which *Helicobacter pylori* infection increases vulnerability. Cholera gravis (resulting in death within a few hours) occurs more often in those with blood group O. Dose response relationships in risk assessment models should accommodate such features if predicted risks are to be realistic.

Shigella spp.

From their retrospective study of a swimming-associated outbreak, Keene *et al.* (1994) also speculated that the infectious dose for *Shigella sonnei* is low. Dupont *et al.* (1989) summarised the published data of dose-response of volunteer studies with *Shigella spp.* The proportion of volunteers developing infection after an administrated dose are presented in Table 2.12

Table 2.12 Dose-response data for *Shigella spp.* from trials on healthy adult volunteers (Dupont *et al.* 1989).

Test Strain	Dose	No. of Ill volunteers	Proportion Ill
S. flexneri 2a			
2457/T	100	14 of 36	0.39
	180	9 of 36	0.25
	5,000	28 of 49	0.57
	10,000	58 of 103	0.56
	>100,000	38 of 59	0.64
S. dysenteriae 1			
A-1	200	3 of 8	0.38
	10,000	2 of 6	0.33
M-131	10	1 of 10	0.10
	200	2 of 4	0.50
	2,000	7 of 10	0.70
	10,000	5 of 6	0.83
S. sonnei			
53G	500	7 of 20	0.35
53 G	500	19 of 38	0.50

2.3.1 Critical assessment of dose-response data used for microbiological risk assessment

The dose-response curve is the 'heart' of the US risk assessment models for drinking water. Dose-response models are available for several pathogens of waterborne importance. Some of the problems with using dose-response data in risk assessment models for drinking water supplies are now discussed.

1. Point estimates for pathogen exposure used in US risk assessment models for drinking water are typically less than one. For example, Haas *et al.* (1993) use an

exposure estimate of 0.0024 viruses per person per day. The meaning of doses of fractions of a pathogen should be questioned. For the dose-response curve for rotavirus (Figure 2.5) and poliovirus (Table 2.10), doses of less than one were administered. In principle, it is inconceivable that a fraction of a pathogen could even be administered, let alone cause an infection. Indeed, an individual either ingests a certain whole number of pathogens or zero pathogens. It would seem therefore that dose-response curves should not go down below 1 pathogen, except in the case of zero pathogens when the probability of infection is zero. In theory, however, it is quite acceptable to model the beta-Poisson dose-response curves down to doses below 1 pathogen. Thus, a dose of 0.009 rotavirus (Figure 2.5) means that on average there is nine rotavirus particles per 1,000 dose volumes administered to volunteers. The beta-Poisson dose-response curve will account for most volunteers not receiving any virus but a small (about 1%) proportion of volunteers receiving one, and an even smaller proportion receiving two viruses, and so on. This does however question whether the beta-Poisson curve is really the best model for a dose-response curve, since risks for doses between zero and one pathogen are effectively based on information contained in the curve for doses of one or more pathogens. Indeed, the ideal dose-response curve would not consider fractions of pathogen. It would contain risks from discrete doses, 0, 1, 2, 3, 4, 5..... of pathogen. The statistical distribution of pathogens in the drinking water supply would then relate the proportions of consumers exposed to each dose.

2. Dose-response curves obtained to date used small numbers of volunteers. The confidence intervals (uncertainty) in data are therefore large. This is apparent in the dose response data for *Giardia lamblia* (Rendtorff, 1954). Haas *et al.* (1993) show the error bars (95% confidence intervals) for each of the doses on the rotavirus data of Ward *et al.* (1986) to be large. Indeed, for a dose of one rotavirus particle the 95% confidence interval for proportion of individuals infected is 0.01 to 0.50.

3. Only a proportion (which is unknown) of the total numbers of pathogens may be infectious.

4. Dose-response models are reported for infection, rather than illness or mortality. Ratios relating mortality and illness rates to infected individuals are needed for each pathogen.

5. Dose-response models do not take into account the findings for salmonella, at least, (Mintz *et al.* 1994) that severity and duration of illness are related to dose ingested. However, in the dose-response study with rotavirus on human volunteers, Ward *et al.* (1986) report that the percentage of infected subjects who experienced illness was unrelated to dose.

6. There is considerable disagreement in the minimum number of salmonellae necessary to produce clinical symptoms. Infectious doses may be somewhat lower than suggested by dose-response data quoted for US risk assessment models (Rose and Gerba, 1991).

7. In the rotavirus study of Ward *et al.* (1986) and also Norwalk virus studies (Graham *et al.* 1994, Johnson *et al.* 1990), volunteers were given sodium bicarbonate as an "Antacid" first. This may promote survival of the virus. Thus, dose-response curves may predict higher risks from these viruses than under normal conditions.

8. Most dose-response studies performed on salmonellae have involved food as the vehicle for ingestion of the organisms. Those data may therefore not be applicable to waterborne risk assessment models. Indeed, D'Aoust (1985) suggests that the fat content of contaminated foods may play a significant role in human salmonellosis by determining the number of organisms reaching the intestine.

2.4 Dose-response data are not used optimally in US drinking water risk assessment models.

The point exposure per person per day to pathogen is translated into a risk of infection from the dose-response curve. Haas (1993) gives a worked example for poliovirus III (shown in Table 2.13), showing how US models achieve this.

Table 2.13 Calculation of daily risk of infection from poliovirus (Haas, 1993).

Point estimate for daily water consumption per person = 2 litres
Point estimate for virus concentration = 0.0012 per litre (see Section 2.1)

Point estimate for daily exposure to virus = 0.0024 viruses per day per person

Using the Poisson-beta model for poliovirus ($\alpha = 0.50$, $N_{50} = 3.4$, see Table 2.6), a daily risk of poliovirus infection through drinking water is calculated to be 0.00106.

It is apparent, however, that there is a problem. The point estimate exposures (e.g. 0.0024 viruses per person per day) used in the US risk assessment model are much smaller than the doses administered to volunteers in producing dose-response curves (Section 2.3). To overcome this problem, the US models extrapolate their dose-response curve down to doses of fractions of a pathogen, well below those for which dose-response data are available. The weakness is that those parts of the curve play a 'passive' role in the best fit procedure used to produce the curve. Only, the parts of the curve where data are available will contribute to the best fit procedure.

In the author's opinion, dose-response curves are not used optimally in US risk assessment models. Thus, there really is no need to extrapolate dose-response curves into realms for which data were not available. Indeed there is no need to model even into

fractions of a pathogen. The information is there in the part of the curve describing risks of infection from 1, 2, 3, 4, 5.... pathogens.

2.4.1 Recalculating risks considering actual exposures gives a discrepancy compared to extrapolation.

Using the data from Table 2.13 and the same Poisson-beta model, the risk may be calculated a different way, by considering the risk from exposure to 0, 1, 2, 3, 4, 5.... organisms, reflecting a real life situation. This avoids using the region of the model where extrapolation into fractions of a pathogen is needed. It enables risk calculations from parts of the curve where dose-response data were obtained. The calculation shown in Table 2.14. was performed on a spread sheet. The approach again assumes an average virus exposure of 0.0024 viruses per person per day. Using the equation for the Poisson distribution (mean = 0.0024), the probabilities of any person consuming 0, 1, 2, 3, 4, 5....viruses per day are calculated in the second column. In the third column, the probabilities of infection from 0, 1, 2, 3, 4, 5...viruses are calculated using Haas's dose response equation. In the forth column the two probabilities are multiplied together to give the probability of risk of infection from each pathogen exposure in the population. Summing the probabilities in the forth column gives the daily risk of infection from all pathogen doses, 0.00065, in the population. This is about half the risk predicted by Haas's extrapolation approach.

Table 2.14 Calculating daily risk of infection from poliovirus by considering risks from consumption of 0, 1, 2, 3, 4, 5....viruses - avoiding Haas' extrapolation of dose response curves.

Number of pathogens in water (x)	Probability of ingesting x number of pathogens (Px)	Probability of infection from x number of pathogens (Pi)	(Px x Pi)
0	0.9976	0	0
1	0.002394	0.271	0.000649
2	0.00000287	0.398	0.0000011
3	2.3×10^{-9}	0.476	2.3×10^{-9}
.			
.			
100	0	0.894	0
Total	1		0.00065

Px, calculated from Poisson distribution using mean of 0.0024 viruses per person per day
Pi, calculated from beta dose-response model ($\alpha = 0.50$, $N_{50} = 3.4$) of Haas (1993)

2.4.2 Using the Poisson model with such low point estimates effectively means US dose-response models only consider exposure to one pathogen.

By assuming a Poisson model, no account is taken of clustering of pathogens within the drinking water supply (discussed in Section 2.2.2). Furthermore, the very low pathogen exposure estimates used in conjunction with the Poisson model render the probability of exposure to more than one pathogen very remote. Consider Table 2.14. With an average exposure of 0.0024 per person per day, the Poisson distribution calculates that over 99.76% of people will not be exposed to viruses. About 0.24% of people will be exposed to just one virus. However, the probability of being exposed to two or more viruses is 0.00029%, i.e. negligible.

Thus, in effect US risk assessment models either consider persons being exposed to no viruses or persons being exposed to just one virus.

It should be noted that if viruses are clustered then an even higher proportion of people will not be exposed to any viruses. However, a very small proportion of consumers may be exposed to very large numbers of pathogens, e.g. 1000 per day. In the case of viruses and perhaps protozoa, where single organisms may cause illness, US models may overestimate the risk. In the case of certain bacterial pathogens, e.g. *Vibrio cholerae*, for which thousands of organisms are needed to infect an individual, US models underestimate the risk.

2.5 Characterisation of risk in US risk assessment models

Risk characterisation is the determination of which risk should be expressed and how it should be expressed.

2.5.1 Which risk?

For pathogens there are three different risks. These are risk of infection, risk of illness, and risk of mortality. Which risk is most appropriate depends on the nature of the pathogen, and the susceptibility of different groups within the population to morbidity or mortality. For AIDS patients, mortality would be the appropriate risk category for *Cryptosporidium*, while for the healthy adult population infection would be sufficient. For hepatitis E virus, the appropriate risk category for pregnant women would be mortality. Most US risk assessment models somewhat avoid the problem of characterising risk for different population groups by aiming for finished waters of microbial quality such that risks of infection from *Giardia* and enteric viruses are less than one infection per 10,000 people per year (Regli *et al.*, 1991, Rose *et al.* 1991b, Rose and Gerba, 1991). The pathogens and the risk characterisations in US risk assessment models are summarised in Table 2.15.

Table 2.15 Pathogens and risk characterisations in US risk assessment models for drinking water.

Pathogen	Risk	Reference
Giardia	Infection	Rose *et al.* (1991b)
Viruses	Infection, Mortality	Haas *et al.* (1993)

The *Giardia* (Rendtorff, 1954) and rotavirus (Ward *et al.* 1986) dose-response models measure infection as the detection of cysts or virus particles (respectively) in faeces from the volunteers. Thus US risk assessment models using those dose-response data effectively predict the risk of infection as determined by excretion of pathogen in faeces. Haas *et al.* (1993) develop the virus model a stage further by considering the probabilities of an infected person developing illness, and an ill person dying. They note the paucity of data on mortality rates from different pathogens but cite information suggesting mortality rates from coxsackie and echoviruses of 0.12 - 0.94%. The risk of mortality from hepatitis A virus in the US is 0.6%. Infectivity/morbidity and infectivity/fatality ratios have been reported for a variety of enteric viruses contaminating drinking water (Gerba and Haas, 1988). These are reported in Table 2.16.

Table 2.16 Mortality rates for enteroviruses

Enterovirus	Mortality rate, %
Hepatitis A	0.6
Coxsackie A2	0.5
A4	0.5
A16	0.12
Echo 6	0.29
9	0.27
Polio 1	0.9

2.5.2 How is risk expressed in US models

The SWTR was drawn up by the US EPA to ensure that the population consuming water would not be exposed to a risk of greater than one infection of giardiasis or enteric virus per 10,000 people per year. US risk assessment models therefore report annual risks.

US models consider each day as a discrete exposure. The daily risk of infection is calculated from the dose-response curve using a daily point estimate for pathogen

exposure (as shown in Section 2.4). The daily exposure is then translated into an annual risk, P_{year}, by the equation

$$P_{year} = 1 - (1 - P_{day})^{365}$$

where P_{day} is the risk of infection from the 2 litres of tap water consumed each day.

2.6 Application of US risk assessment models to the general community.

US risk assessment models do not consider spread through secondary infection, e.g. person-to-person contact, after primary infection of persons through a point source such as the municipal water supply. Rates of secondary spread may be considerable for certain pathogens (see Section 3) and should be considered in drinking water risk analysis models to assess the full impact of a waterborne outbreak on the general community. Modelling secondary spread could be achieved through mathematical models for infectious diseases (Wickwire, 1977) and is not discussed further in this review.

This section considers whether the dose-response curves used in risk assessment models are applicable to the general community.

2.6.1 Broad cross sections of the community

The US risk assessment model for viruses in drinking water (Haas *et al.* 1993) may underestimate the risks to the general community by an unknown factor which varies from community to community. This is because the dose response data on which the model was based were obtained from healthy adult volunteers (Ward *et al.* 1986) and not children, AIDS patients, pregnant women or other susceptible persons. Even within groups of healthy adult volunteers, there is considerable variation in the effect of pathogen dose. Thus, from the dose-response curve for rotavirus (Figure 2.5), it is apparent, that while a single rotavirus particle may cause infection in 31% of the healthy adults, some 8% of the healthy adults are not infected by as many as 10,000 rotavirus particles. This variation is accommodated by the dose-response curve and presents no problem for risk assessment models. Where there is a problem, however, is in application of those risk assessment models to broad cross-sections of the community with persons other than healthy adults. Each risk assessment model is only applicable to persons representative of the group from which the dose-response data were collected (healthy adults in the virus model of Haas *et al.* (1993)).

Healthy adults represent the group most resistant to micro-organism infection (except in the case of mortality from hepatitis A virus). Rotavirus and astroviruses cause illness in infants. *Cryptosporidium* may be fatal in AIDS patients. The risk of mortality from hepatitis E virus in pregnant women is high (10 - 20%). Another problem is that the proportions of children, AIDS cases, pregnant women and adults may vary from community to community. Furthermore, certain viruses, e.g., have more impact amongst communities of lower socioeconomic status. US risk assessment models do not cater for

these more susceptible groups within the population. To apply risk assessment models to the general community dose-response data from persons typical of the general community must be obtained.

2.6.2 Degree of immunity and vaccination

The susceptibility of humans to infection is determined by many factors, the most important of which may be the degree of immunity developed as a result of previous infection by the same or a related virus or from vaccination. Rotavirus disease in adults is usually the result of reinfection rather than primary infection because almost all adults have serological evidence of a previous infection. Reinfection may be possible because of loss of immunity or exposure to a different serotype of rotavirus. However, the presence of serum neutralising antibodies (i.e. previous exposure) did not fully protect volunteers against rotavirus illness in the study of Kapikian *et al.* (1983). In the experiments to determine the dose-response relationship for rotavirus, Ward *et al.* 1986 selected adults after prior screening for a low titre of serum neutralising antibody. Such volunteers are unlikely to represent the general community.

The immune response and protection following natural infection or vaccination with an enteric virus have been characterised in studies with poliovirus (Ghendon *et al.* (1961)). Immunoglobulin A (IgA) secreted into the intestine after poliovirus infection has been implicated as the primary factor responsible for alimentary resistance. IgA may also protect against rotavirus. However, this antibody defence mechanism may not operate against Norwalk virus (Cukor *et al.* (1982)) or echovirus-12 (Schiff *et al.* (1984)). Thus the state of alimentary resistance associated with enteric viral infections and predicted on the basis of studies with poliovirus may not be universally applicable. This means that microbiological risk assessment models will need to treat multiple exposures in different ways depending on the virus.

Factors responsible for susceptibility to Norwalk virus infection are poorly understood. There are disparate results regarding the nature of host immunity to Norwalk virus between seroepidemiological studies in developing nations and volunteer studies in US adults. Black *et al.* (1982) and Ryder *et al.* (1985), in separate studies in Bangladesh and Panama, found that serum antibody titres of >1:100 were associated with protection of children against subsequent episodes of Norwalk gastroenteritis. This pattern was not found in volunteer studies among adults in the USA. Blacklow *et al.* (1979) demonstrated that ill volunteers challenged with Norwalk virus were more likely to have high than low prechallenge serum antibody titres whereas Parrino *et al.* (1977) showed that serum antibody titres to Norwalk virus were not protective against illness in a study of 12 volunteers multiply challenged with Norwalk virus. The 6 who became ill initially also experienced gastroenteritis when rechallenged 27 - 42 months later. The 6 who remained well did not experience gastroenteritis on rechallenge. Four volunteers who became ill were challenged a third time 4 - 8 weeks after the second challenge. Only one experienced gastroenteritis: that volunteer had a high antibody titre before the third challenge.

Johnson *et al.* (1990) found that all volunteers (12 of 12) with high (>1:200) prechallenge titres of serum antibody to Norwalk virus experienced illness while only 19 of 30 with low

(1:100) prechallenge titres experienced illness after the first challenge. They concluded that preexisting serum antibody to Norwalk virus does not seem to be associated with protective immunity. However, after repetitive exposure antibody levels were associated with protection. There appears to be a group in the USA who maintain low serum Norwalk antibody levels yet are resistant to Norwalk infection. Whether specific immunologic, genetic, or other host factors cause this resistance is unclear.

2.6.3 Age of consumers

Dose-response data have been obtained from experiments involving healthy adults (with the exception of premature infants for poliovirus Type III (Katz and Plotkin, 1967)). In the case of rotavirus, for example, the most severe illnesses involve infants and young children. Adults are generally protected from infection by enteric adenoviruses (Chiba *et al.* 1983) which account for 5 - 10% of childhood diarrhoea (Kim *et al.* 1990). Dose-response models used in risk assessment do not take into account the fact that immunity in the case of hepatitis A virus, for example, is directly related to age. In a study by the PHLS (Thornton *et al.* 1995) of a drinking water supply contaminated with sewage, it was found that those who were immune to hepatitis A virus were significantly older than those who were susceptible (mean ages: 43.5 years and 19.0 years, $p < 0.0001$). In a recent unmatched case-control study performed in response to a *Cryptosporidium* outbreak it was found that the median age of the control group was 10 years compared to 3 years for the infected cases. Furthermore, in that particular study it was found that consumption of vegetables appeared to have a protective effect. Whether this reflected different eating habits in the two age groups is not clear, but risk assessment models should take such facts into account.

The stomach acts as a barrier to ingested bacteria mainly through a pH-dependent mechanism. Patients who have had gastrectomies, are achlorhydric, or are taking antacids appear to be more susceptible than others to infection with salmonellae. Observations of susceptibility to other ingested pathogens such as *Vibrio cholerae* and *Giardia lamblia* indicate a similar pattern (Giannella *et al.* 1972). Children less than two months of age produce little hydrochloric acid, and the incidence of achlorhydria is greater in persons over 60 years old. These phenomena could to some extent account for the increased susceptibility of the very young or the elderly to infection with salmonella. Risk assessment models need to take these considerations in account.

2.6.4 Conclusions

There are many poorly understood and complex issues which render current dose-response models inapplicable to the general community.

2.7 Outputs of US risk assessment models

Microbiological risk assessment models have been applied in the US in three ways. The first method uses values measured for pathogen densities in the finished water supply to

calculate risks of infection. As discussed above in Section 2.2.2, the problem is deciding what value to use for a point estimate of pathogen density. The second approach effectively does the opposite by calculating from dose-response models a critical pathogen density below which the annual risk of infection is considered as acceptable by the US EPA. The latter approach provides data for the setting of microbial standards in drinking water supplies. The third approach considers pathogen levels in source waters and estimates how effective water treatment needs to be to ensure risks of less than 1 per 10,000 per person per year. Outputs from these approaches are now reviewed.

2.7.1 Risks predicted by US risk assessment models

Haas *et al.* (1993) estimate of the daily probability of virus infection from drinking water is 0.000717 (95% confidence interval, 0.0000317 to 0.00188). This is for the most exposed individual using a point estimate virus density based on data of Payment for finished drinking water in the Montreal area. It is interesting to note, however, that the daily risk of illness (0.000717) was in very good agreement with the value of 0.00082 per day obtained in the prospective epidemiological study of Payment for the Montreal area. Haas *et al.* (1993) also consider the potential for mortality. They assume that 1 incident in a thousand of viral illness derived from drinking water result in death (Gerba and Haas, 1988). Using the risk interval for illness and assuming exposure over a lifetime of 70 years, the interval for the risk of death from a lifetime's exposure to viruses in drinking water is 0.0008 to 0.047.

The upper limit (1 in 20) for mortality from viruses in drinking water is not negligible. However, this value is calculated using a point estimate for a most exposed individual. Put in context, this predicted risk is of little use because no information is provided on what proportion of the consumers are exposed. Judging from Payment's data, at least 93% of consumers (see Table 2.3) would have been exposed to lower doses than that used in Haas's model. Thus, the calculated risk of death only applies to less than 7% of the population. Furthermore, it is shown in Section 2.4.1 that extrapolating the dose response curve to very low values may overestimate the risk by a factor of two. In addition by using a Poisson model and not considering pathogen clustering (Section 2.4.2), the US risk assessment models may considerably overestimate the proportion of consumers exposed to virus and hence the risk.

2.7.2 Development of microbial standards

The US EPA set an acceptable risk limit of 1 infection per 10,000 persons per year as acceptable. Rose and Gerba (1991) calculated that *Giardia* levels should be below 0.2 cysts/100 l and poliovirus and rotavirus levels should be less than 0.1 and 0.3 pfu/100 l to achieve an annual risk of not more than 1 infection in 10,000.

Regli *et al.* (1991) present maximum mean densities of pathogens computed from dose-response curves to give annual risks of infection of 10^{-4}. These are presented in Table 2.17.

Table 2.17 Maximum mean concentrations of various pathogens computed from dose-response curves to give an annual risk of infection of 10^{-4} (taken from Regli *et al.* 1991).

Organism	Density (number/litre)
Rotavirus	0.000000222
Polio III	0.000000265
Entamoeba coli	0.000000625
Giardia	0.00000675
Polio I[a]	0.0000151
Polio I[b]	0.00191
Echovirus 12	0.0000685

[a]using dose-response data of Minor *et al.* (1981)
[b]using dose-response data of Lepow *et al.* (1962)

2.7.3 Determining the effectiveness of drinking water treatment for different source waters (Rose *et al.* 1991b; Rose and Gerba, 1991)

Rose *et al.* (1991b) and Rose and Gerba (1991) avoid the problem of lack of information on pathogen densities in the drinking water supply by considering the quality of raw waters and predicting the health effects from the resulting treated water after varying degrees of pathogen removal by water treatment. This effectively tests the effectiveness of various levels of water treatment on health risk.

Rose and Gerba (1991) used *Giardia* cysts concentrations measured in 40 source water samples collected over the period of a year. Samples were grouped according to *Giardia* concentration allowing the frequencies to be calculated (Table 2.18). Densities were then divided by 1,000 to simulate densities in the drinking water supply after a 3 log removal by water treatment. Densities were then translated into risk through the dose-response curve for *Giardia* (Rendtorff, 1954).

Table 2.18 Calculation of risk of Giardia infection from cyst densities in source water after 3 log removal (Rose and Gerba, 1991).

Source Water Cyst density per 100 litres	Frequency	Risk
<1	60	0
0 - 0.9	5	0.000,000,28
1 - 5	20	0.000,008,9
6 - 10	2.5	0.000,002,7
11 - 100	2.5	0.000,032
100 - 1000	10	0.000,13

This is a much more useful approach than using single point estimates (Haas *et al.* 1993) because:-

1. The frequencies of *Giardia* cyst density within the drinking water supply are considered. Thus, to some extent the all important question of what proportion of consumers are exposed to what levels of pathogen density is approached. For example, it is apparent from Table 2.18 that the *Giardia* risk to the 10% most exposed consumers is greater than or equal to 0.00013 per day. Similarly, it may be stated that the daily risk to 85% of the consumers is less than or equal to 0.89×10^{-6}.

2. Real values (for source waters, at least) are used instead of the meaningless averages (see Section 2.2.2 for criticisms of average point estimates).

Criticisms of the Rose and Gerba (1991) study are:-

1. Although they approach the question of what proportion of consumers are exposed to what densities of cyst they do not use that frequency information in calculating an overall risk across the population.

2. 60% of source water samples recorded <1 cyst per 100 litres. Thus, a 3 log reduction on treatment will give <1 per 100,000 litres. Rose and Gerba incorrectly calculate the risk to be 0 (Table 2.18). It will be small but finite (Haas *et al.* (1993)) calculated a risk from viruses based on a density of 0.0012 viruses per litre.

3. For each pathogen density estimated in drinking water, a Poisson distribution is assumed.

4. They base conclusions about water treatment on incorrectly calculated geometric averages, stating, "Geometric averages of 1 - 100 organisms/100 litres (source

water) require 3 - 5 logs of treatment reduction to achieve the 1:10,000 risk". From the data in Table 2.18 they calculate a geometric average of 1.64 cysts per 100 litres for source water. Since 60% of samples register <1 cyst per 100 litres the median, and hence the geometric mean, is also <1 cyst per 100 litres (see Section 2.2.2).

2.8 Uncertainty Analysis

Uncertainty in risk assessment models arises for two reasons:

1. lack of knowledge

2. statistical variability, i.e. chance.

While the second can be quantified by statistical theory, the first requires assumptions which may later prove to be incorrect.

Uncertainties in risk assessment models are usually evaluated by Monte Carlo analysis. Essentially Monte Carlo analysis combines the statistical distributions for several input variables to estimate the statistical distribution for an output; in this case, the risk of infection from drinking water. The US authors (Haas *et al.* 1993; Regli *et al.* 1991) refer to bootstrapping, which is an approach based on Monte Carlo simulations for assessing the uncertainty in parameter estimations due to variability of small samples.

A Monte Carlo risk assessment model for microbiological infection from drinking water supplies should, in theory, combine the following inputs:-

- statistical distribution for pathogen densities across drinking water supply
- statistical distribution for water consumption across population
- dose-response model

to produce a probability distribution for risk of infection. However, the US models of Regli *et al.* (1991) and Haas *et al.* (1993) consider only the uncertainty in the dose response data. In particular, they report the confidence contour for the parameters α and N_{50} which define the Poisson-beta model for rotavirus. The US models do not consider the statistical distributions of pathogen densities or water consumption and hence uncertainties in pathogen exposure. Uncertainties for pathogen exposure would be quantifiable if the statistical distribution is known for a particular factor. For example if the concentrations of *Cryptosporidium* oocysts were known to be lognormally distributed (with parameters μ and σ) then we could calculate with certainty the confidence limits for the proportion of the consumer population exposed to drinking water with more than one oocyst per litre.

There are some disadvantages with the Monte Carlo analysis; namely the time required and the potential complexity. Slob (1994) has presented a 'desk calculator' approach which allows a simple and quick analytical uncertainty analysis. Burmaster and Anderson

(1994) propose 14 principles of good practice to assist people in performing Monte Carlo risk assessments.

2.9 Conclusions

1. US risk assessment models for drinking water supplies are no more than dose-response curves. Single point estimates used for pathogen exposures in US models are of little use.

2. US risk assessment models do not consider the fundamental question, "What proportion of consumers are exposed to what numbers of pathogens?" Variation in pathogen densities within the supply is not considered. Both would be defined by the statistical distribution of pathogen densities.

3. Uncertainties are based on uncertainty of the dose-response data and do not consider uncertainty in pathogen exposure estimates.

4. The mathematical models (beta-Poisson for most pathogens) for dose-response curves may not be appropriate to micro-organisms because fractions of a pathogen are considered.

5. US models do not model secondary spread. Dose-response curves are not appropriate to the general community, including infants, the elderly, the immunocompromised, and persons of varying acquired immunities. Some dose-response data were obtained after antacid consumption and may overestimate risk.

6. Extrapolation of risks from fractions of a pathogen dose is unnecessary and produces inaccurate results.

7. Applying the Poisson model to very low point estimates for pathogen exposure effectively limits US models to considering exposure to zero pathogens or exposure to just one pathogen. The US model for viruses effectively considers the probability of exposure to more than one virus as negligible.

8. Applying the Poisson model when in fact pathogens are clustered in the supply would overestimate risk from more infectious agents such as viruses and protozoa, but underestimate risk from less infectious organisms such as certain bacteria.

9. Bacteria, in particular salmonellae may be more infectious than suggested by dose-response data.

3. APPLICATION OF RISK MODELLING METHODOLOGY TO PATHOGENS IN THE UK

In the contract proposal, it was stated that the objective of this stage is to assess the validity of each risk modelling methodology for application in the UK and recommend an approach that is appropriate to the UK with particular reference to specific pathogens. This is probably the wrong way to approach the problem because a basic risk assessment model for a specific pathogen is complete when the dose-response curve and the statistical distribution of densities in the drinking water supply are defined for that pathogen. Only the correct dose-response curve and statistical distribution of densities are appropriate. Where this information is available for a specific pathogen, risk assessment modelling may be performed. Further refinement, however, will be needed based on epidemiologic data for each specific pathogen. Thus, factors such as:-

- increased mortality in different age groups (e.g. hepatitis A virus)
- increased mortality in different subgroups of the population (e.g., pregnant women in the case of hepatitis E virus)
- different rates of secondary spread (hepatitis E vs. hepatitis A viruses)

need to be considered.

Before developing and applying microbiological risk assessment models to drinking water supplies, two questions should be answered:-

1. For which pathogens should risk assessment methodology for UK drinking water supplies be developed?
2. What refinements based on epidemiologic information need to be made to risk assessment models for specific pathogens?

The answer to the first question is that the risk from any pathogen which presents a potential or proven waterborne hazard should be modelled. This is because one objective of risk assessment models is to identify the most cost effective options to reduce risk. In this respect, specific waterborne pathogens cannot be considered alone. Implementing one option at the expense of another to reduce the risk from a specific pathogen may increase the risk from other pathogens. The answer to the second question requires detailed epidemiologic information on each pathogen.

In this section, therefore, pathogens which are known to present or could present a waterborne hazard in the UK are reviewed. Many of the pathogens discussed are more prominent in parts of the world other than the UK. However, they are included because a microbiological risk assessment model that works should be able to predict low or negligible risks from such pathogens. Infected carriers entering the UK from parts of the world where such pathogens present a serious health problem will inevitably release

pathogens into the UK sewage system. The risk assessment model as well as the drinking water treatment processes must be able to cope with such pathogens.

Emphasis is placed on the newly-emerging pathogens (e.g., *E. coli O157*, *Helicobacter pylori*, hepatitis E virus, astroviruses and caliciviruses). It would be of particular benefit to develop risk assessment models for such pathogens because their contributions through waterborne infections to public morbidity and mortality are not as yet quantified.

The contract lists the following considerations:-

- abundance in UK
- major sources in UK
- other sources
- likelihood of waterborne transmission
- risk characterisation
- unique features to UK

These factors cover epidemiologic information which would require customisation of risk assessment models for specific pathogens. Where possible these features are addressed for each pathogen in this section. The abundance of pathogen in the UK is considered in Section 3.4.

3.1 Protozoa

Giardia lamblia

Giardia lamblia is a flagellated protozoan parasite that infects the upper intestinal tract of humans and many animal species. It is the most common gastrointestinal parasitic infection of humans in the US and may be found in water contaminated with faeces. Transmission of infection is by the faecal-oral route. Many cases of giardiasis reported in the UK result from travel to endemic areas. Many outbreaks in the developed world have been waterborne. An outbreak associated with mains water in the UK has been documented (Jephcott *et al.* 1986). Epidemics have occurred after contamination of municipal water supplies (Juranek, 1979). Person-to-person contact is also considered important. In developed countries, infections occur most frequently in children, homosexuals, institutionalised individuals, travellers, and backpackers. Outbreaks in the UK have also been associated with contact with farm animals and pets. Although many people are asymptomatic cyst excreters, other infected individuals may complain of diarrhoea, nausea, vomiting, flatus, and abdominal cramp.

Cryptosporidium

The enteric protozoan *Cryptosporidium* has been recognised as an increasingly important cause of both outbreak-related and sporadic disease in humans (Casemore, 1990). *Cryptosporidium* causes a self-limited infection in immunocompetent hosts. Person-to-person transmission of *Cryptosporidium* is the major route of transmission (Smith, 1992) especially among children in day care centres, hospitals and nurseries. Waterborne cryptosporidiosis in immunocompetent populations is well-established. *Cryptosporidium* oocysts are commonly recovered from a variety of surface water sources, including supplies intended for drinking water abstraction (LeChevallier *et al.* 1991a). Humans can acquire cryptosporidiosis from a variety of animal hosts. Thus, oocysts from infected livestock and agricultural runoff contribute to the numbers of waterborne oocysts which are potentially infectious to man (Smith, 1992). Wastewater may contain varying numbers of oocysts - up to 13,700 oocysts per litre have been reported in crude sewage (Smith, 1992). It has been suggested that agricultural sources are of a major concern - levels of 149,000 oocysts per litre being detected in effluent from a slaughter house. Oocysts, excreted in faeces, are thick-walled and survive for long periods of time in water. They are not inactivated by the chlorine concentrations typically applied by water treatment works. Several outbreaks have been associated with contaminated municipal water sources. In the 1987 Carrolton (USA) outbreak resulting from sub-optimal flocculation and filtration at the treatment works, 13,000 persons were affected (Hayes *et al.* 1989). In 1993, the largest documented waterborne disease outbreak in USA history occurred in Milwaukee with 403,000 people estimated as developing watery diarrhoea after drinking municipal water contaminated with *Cryptosporidium parvum* (MacKenzie *et al.* 1994). For some reason, not attributable to mechanical breakdown of the flocculators or filters, the water treatment works failed to maintain treated water at low turbidity enabling oocysts to pass through. Contact with surface water has been demonstrated to be a risk factor for sporadic cases.

In immunocompromised patients, the disease is severe, persisting indefinitely, and can be life-threatening. Infection in such patients typically causes unremitting diarrhoea that does not respond to therapy. In the US, cryptosporidiosis is reported in 10% of AIDS patients and is associated with considerable mortality. Probable waterborne transmission of cryptosporidiosis to persons with AIDS during a community outbreak has been documented (Clifford *et al.* 1990). Sorvillo *et al.* (1994), however, conclude that the municipal drinking water supply is not an important risk factor for cryptosporidiosis in AIDS patients residing in Los Angeles (USA). Their evidence was based on the finding that water filtration could not be demonstrated to exert a protective effect. Thus, the prevalence of cryptosporidiosis among persons with AIDS was lower (4.2%) in an area receiving unfiltered water (Area A), than in an area (6.2% incidence rate) receiving filtered water (Area B). Furthermore, addition of filtration in Area A did not result in an increased reduction in cases compared to that observed in Area B. Sorvillo *et al.* (1994), however, acknowledge that their findings may not be generalisable to other areas. It also was not established that heavy *Cryptosporidium* contamination of the drinking water supply did actually occur in their study.

3.2 Bacteria

Helicobacter pylori

This organism is associated with duodenal and gastric ulcers and also gastric carcinoma (Anon, 1994). It was classified as a member of the *Campylobacter* genus before 1989 when a new genus, *Helicobacter*, was proposed. *Helicobacter pylori* exhibits a narrow host range naturally infecting only humans and nonhuman primates, in which it specifically colonises the gastric mucosa. It is uniquely adapted to survive in the acidic environment. Interest on possible routes of transmission has focused research on the presence of *H. pylori* in the mouth and faeces of infected individuals.

Reservoirs of *H. pylori* are the digestive tracts of humans and some primates. Transmission from reservoirs is considered to be person-to-person. This assumption is supported by the finding of clustering of similar strains within families and by consistent demonstration of close interpersonal contact as a risk factor from infection. Transmission can exist between couples: 68% of spouses of *H. pylori*-infected people were affected, whereas 9% of spouses of uninfected people were infected. Secondary transmission of *H. pylori* through person-to-person contact after primary outbreak from a waterborne incident should therefore be considered in risk assessment models.

Person-to-person contact may occur through faecal-oral transmission and oral-oral transmission. *H. pylori* has been detected in the oral cavity, in dental plaque, and in the saliva of one person. *H. pylori* is eliminated in faeces after turnover of the gastric mucosa. Consumption of raw vegetables fertilised with human faeces was found to be a risk factor for infection in Santiago, Chile (Hopkins *et al.* 1993), and consumption of municipal water was found to be a risk factor in children in Lima, Peru (Klein *et al.* 1991). *H. pylori* has been detected by PCR in sewage water in Peru (Westblom *et al.* 1993).

Diarrheagenic *Escherichia coli*

These organisms are major components of the normal intestinal microflora in humans and animals. Although most strains are relatively harmless in the bowel, others possess virulence factors that are related to diarrhoea disease. At least five types of *E. coli* intestinal pathogens have been recognised. The terminology used to describe diarrheagenic *E. coli* is confusing. Tarr (1995) lists the following terms to describe diarrheagenic *E. coli*:-

- Enteroaggregative *E. coli* (EaggEC) or enteroadherent *E. coli* (EAEC)
- Enteroinvasive *E. coli* (EIEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)

- SLT-producing *E. coli* (SLTEC) or verocytotoxigenic *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC),

depending on their principal pathogenic properties. However, the classification oversimplifies the situation because the traits are not mutually exclusive. It is apparent that diarrheagenic bacteria cause disease through a medley of traits and strict classification may not be possible. This makes the task of developing risk assessment models for such organisms all the more difficult.

The SLT-producing *E. coli* includes *E. coli O157:H7*. These contain the SLT-genes which code for production of the Shiga-like toxin (SLT), so-called because it is immunologically related to Shiga toxin, the principal extracellular cytotoxin of *Shigella dysenteriae* serotype 1. The toxin from *E. coli O157:H7* (initially isolated from the filtrate of stools from infected children) is lethal to cultured Vero (African green monkey kidney) cells and is hence called a verotoxin. *E. coli* isolates of varied serotypes producing this verocytotoxic activity are also termed verocytotoxigenic *E. coli* (VTEC). Shiga toxin is nearly identical in structure to SLT I (or verotoxin I) of *E. coli O157:H7*. Both are A-B bacterial toxins in which the A subunit contains an enzymatically active molecule and the B subunit binds the toxin to the target cell in the gut. SLT-production among *E. coli* is transmissible and may involve a bacteriophage. It has been speculated that bacteriophages are responsible for the dissemination of the Shiga toxin/SLT genes within the *E. coli* gene pool but not among shigellae (O'Brien *et al.* 1992).

Auxiliary virulence mechanisms of *E. coli O157:H7* have been partially elucidated. A transmembrane protein called intimin (also found in enteropathogenic *E. coli*) is produced which mediates actin aggregation in the target cells.

Escherichia coli O157:H7; and enterohaemorrhagic *E. coli* (EHEC)

Escherichia coli O157:H7 is an important and common pathogen of the human gastrointestinal tract. Vehicles of infection incriminated in outbreaks of *E. coli O157:H7* infection are listed in a recent review by Tarr (1995) and include food of bovine origin (hamburgers), unpasteurised cow's milk, mayonnaise, swimming pool water (Keene *et al.* 1994) and drinking water (Dev *et al.* 1991). Person-to-person transmission is also well-documented. The case of four patients with *E. coli O157* infection was reported in a Scottish village (Dev *et al.* 1991). Two of the patients were also infected with *Campylobacter jejuni*. Analysis of the drinking water supply to patient's homes revealed heavy contamination with faecal *E. coli*, but *E. coli O157* was not isolated. It is believed that a subsidiary water supply opened up in the dry weather may have been contaminated with cattle slurry. The isolation of *E. coli* 0157:H7 from the faeces of healthy cattle demonstrates that cattle are potential reservoirs for this organism (Borvzyk *et al.* 1987).

E. coli O157:H7 causes a spectrum of illnesses ranging from asymptomatic carriage to haemorrhagic colitis. Nonbloody diarrhoea progresses to bloody diarrhoea within 1 or 2 days in most cases of *E. coli O157:H7* infection. The appearance of blood is accompanied by abdominal pain. Bloody diarrhoea in *E. coli O157:H7* infection lasts between 4 and 10 days. Many patients require hospitalisation because of dehydration. Haemolytic-uremic

syndrome (HUS) develops one week after the onset of diarrhoea and represents damage to the vascular endothelial cells following absorption of bacterial toxin (SLT) from the gut. HUS is characterised by anaemia and acute renal failure. It can affect people of all ages but is most frequently diagnosed in children under 10 yrs of age. Approximately 10% of *E. coli O157:H7* infections in children under 10 yrs old progress to HUS requiring hospitalisation for dialysis or transfusion. Death results in some 15% of HUS cases.

Enterotoxigenic *E. coli* (ETEC)

These strains of *E. coli* elaborate on an enterotoxin similar to that of *Vibrio cholerae*. Two types of enterotoxin are produced by ETEC. The heat-labile toxin (LT) is a protein which is destroyed by heat and acid. It acts like cholera toxin by activating the enzyme adenylate cyclase, causing secretion of fluid and electrolytes into the intestinal lumen. LT is similar immunologically to cholera toxin. The second toxin is heat stable (ST), activates guanylate cyclase, and has no biochemical similarity to cholera toxin.

Pathogenic *Citrobacter freundii*

Pathogenic *Citrobacter freundii* produce a homologue of the Shiga-like toxin (SLT) II (cited in Tarr, 1995). Shiga toxin (and SLTs I and II) have multiple properties that contribute to their role in diseases caused by toxin-producing bacteria.

Salmonella spp.

There are four subgenera of Salmonella and over 2,000 serotypes. *S. typhi* is a specific human pathogen. *S.typhi, S. paratyphi* A and *S. paratyphi* B may invade tissues causing septicaemia with high temperature. Other serotypes in man cause transient intestinal infection with diarrhoea. Many *Salmonella* infections are symptomless.

Human carriers are the source of infection from *S. typhi* and *S. paratyphi* A. Most salmonellae are primarily pathogens of animals which also provide important reservoirs of infection. *Salmonella* bacteria may be present in food products grown in faecally-polluted environments, commonly being isolated form poultry and livestock. Waterborne outbreaks have predominantly been associated with *S. typhi* and much less frequently with *S. paratyphi* B.

Campylobacter spp.

Worldwide, campylobacters are much more important than salmonellae as causes of acute gastroenteritis, but not as important as shigellae. Several major outbreaks of *Campylobacter enteritis* have been linked to the ingestion of contaminated food, milk or water. For water hygiene, the thermophilic campylobacters are of greatest significance. Isolation of *Campylobacter jejuni* from the water supply has been achieved in one waterborne outbreak (Melby *et al.* 1990).

Vibrio cholerae

Vibrio cholerae is a Gram-negative bacteria, which infects the gastrointestinal tract and through a protein toxin, causes the disease called cholera. John Snow demonstrated the contagious nature of the disease in England in 1854. The epidemiology of cholera has been characterised by pandemics, in which the disease spreads across continents (Crowcroft, 1994). Until recently, *Vibrio cholerae O1* was thought to be the only serotype that caused cholera. The emergence of a new serotype, *Vibrio cholerae O139*, in south Asia in 1991, has marked the beginning of the eighth pandemic, while the seventh (*Vibrio cholerae O1*), which started in Indonesia in 1961 continues. The spread of cholera may be rapid and unpredictable because of international travel.

Untreated faeces and inadequate sanitation in underdeveloped countries promote spread of the disease. The rapid spread of cholera across Peru and South America was facilitated by poor maintenance of municipal water systems and absent or ineffective chlorination. Cholera is not a problem in the developed world because of appropriate sewage and drinking water treatment. Furthermore, the infective dose of *Vibrio cholerae* is 10^{11} organisms, so the drinking water supply would have to be grossly contaminated.

Contaminated drinking water can transmit cholera. However, it is sometimes blamed because it is a plausible source rather than because it has been proved responsible. Indeed in at least 11 epidemics drinking water has played no part in the transmission (Crowcroft, 1994). However, a matched case-control study in Ecuador (1991) showed that drinking unboiled water was the single most important risk (Weber *et al.* 1994). Drinking unboiled water (odds ratio = 4.0), drinking beverages from a street vendor, eating raw seafood and eating cooked crab were associated with illness. Always boiling drinking water at home was protective against illness, as was the presence of soap in the kitchen.

During epidemics, *Vibrio cholerae* may be isolated from rivers and water supplies, but rarely in high concentrations. Poor sanitation maintains transmission during epidemics. It is estimated that a quarter of the population of South America and the Caribbean has no access to safe water, and a third has no hygienic means to dispose of faeces. Cases imported into countries with good sanitation do not usually lead to secondary transmission. The Pan American Health Organisation has proposed a $200 billion plan to construct and maintain facilities for treatment and supply of drinking water and proper disposal of human waste for all of South America. This, according to Weber *et al.* (1994) would provide the best prevention of illness and death from cholera in that continent.

3.3 Viruses

Many of the more than 100 enteric viruses that may be present in human faeces or urine have been associated with water-transmitted disease. Metcalf *et al.* (1988) list the pathogenic viruses that, through human faeces, may pollute wastewater and hence have a potential for causing illness after transmission via the water cycle (Table 3.1).

Table 3.1 Enteric virus groups containing pathogens that may pollute water sources (from Metcalf *et al.* 1988).

Virus Group	Nucleic acid type	Number of serotypes in group	Number of serotypes transmitted via water
Enteroviruses	ss RNA		
Polioviruses		3	?
Coxsackie A		23	?
Coxsackie B		6	?
Echovirus		31	?
Enteroviruses 68-71		4	?
Hepatitis A (type 72)		1	1[b]
Adenoviruses	ds DNA	41	16[c]
Gastroenteritis viruses			
Rotavirus	dsRNA	5	probably all
Norwalk virus	ssRNA	several	probably all
Astrovirus	ssRNA	at least 5	not known
Miscellaneous			
NANB hepatitis virus	ssRNA	at least 2	at least 1

[b]transmitted by shellfish as well as water route

[c]14 serotypes are known swimming pool-transmitted pathogens. Two serotypes causally associated with gastroenteritis may be water-transmitted pathogens.

3.3.1 Viruses causing gastroenteritis

The major causes of gastroenteritis in the United States, and in the rest of the world as well, are viruses, accounting for 30 to 40% of acute episodes (Kotloff *et al.* 1989). The leading human pathogens can be grouped into five categories: rotavirus, enteric adenovirus, Norwalk virus, calicivirus, and astrovirus. All may be transmitted through drinking water.

Enteric Adenovirus

Most adenoviruses cause upper respiratory infections, but a new group, known as serotypes 40 and 41, are responsible for gastroenteritis in children less than two years of age (Kotloff *et al.* 1989, Chiba *et al.* 1983). Approximately 5 to 10% of childhood

diarrhoea (in Korea and Guatemala) is associated with enteric adenovirus, without any seasonal occurrence (Kim *et al.* 1990). Unlike rotavirus or Norwalk virus, infection with enteric adenovirus has a long incubation period lasting 8 - 10 days, and illness can be prolonged for as much as 14 days. Adults are generally protected from enteric adenovirus infection (Chiba *et al.* 1983).

Rotaviruses

Rotaviruses are recognised as the most important cause of severely dehydrating diarrhoea in infants and young children. Early childhood infection is essentially universal, with symptomatic infections most prevalent between 6 and 24 months. In the USA, the estimated 3.5 million cases of rotavirus-caused paediatric gastroenteritis result in 110 000 hospitalisations and about 150 deaths each year.

Rotaviruses frequently cause illness in adults, although these are more mild than in infants. More severe adult infections occur during outbreaks in geriatric in-patient settings. Infected individuals often excrete $>10^{12}$ rotavirus particles/g of faecal matter (Flewett, 1983). Rotavirus particles can survive for days under environmental conditions (Moe and Shirley, 1982). Rotavirus differs from other faecal-oral pathogens in that level of hygiene and socioeconomic conditions have relatively little influence on the overall incidence of infection. Infected siblings and asymptomatic infected parents are likely sources of infection for the infant, while adult infections often follow contact with infected children. Reports of infection from contact with animals are rare. Waterborne outbreaks of rotavirus disease have been reported, of which the most notable occurred in China in 1982 (Hung *et al.* 1984).

Human Enteric Caliciviruses (Norwalk Virus)

The virus family *Caliciviridae* includes the human caliciviruses, small round structured viruses (SRSV) and hepatitis E virus (Carter *et al.* 1991). Norwalk virus is the prototype SRSV.

Norwalk virus and the Norwalk-like viruses are important human pathogens that cause epidemic acute viral gastroenteritis. Immune electron microscopy studies have defined four distinct serotypes of SRSV represented by prototype strains of Norwalk virus, Hawaii virus, Snow Mountain virus and Taunton virus. Viruses in this group are spread by the faecal-oral route, and outbreaks of water- and foodborne gastroenteritis are well documented. At least 42% of outbreaks of nonbacterial gastroenteritis in the US are caused by Norwalk or Norwalk-like viruses. Norwalk virus is recognised as an important cause of waterborne illness being responsible for about 23% of waterborne outbreaks. Norwalk virus has been reported to be very resistant to chlorine (Keswick *et al.* 1985), which may explain its importance in outbreaks of waterborne disease.

Norwalk virus was first described in an outbreak of acute gastroenteritis in Norwalk, Ohio, USA in 1968. In a two-day period during that outbreak, acute GI developed in 50% of 232 students and teachers at school. Furthermore, secondary infection was observed in

32% of family contacts of primary cases. Virus particles were detected in faecal material and a stool filtrate from an infected adult could reproduce the disease when administered orally to healthy adult volunteers (Dolin *et al.* 1971). Some 50% of adult volunteers developed illness, with diarrhoea, vomiting, nausea and fever. The incubation period ranged from 10 to 51 h and the illness lasted 24 - 48 h.

The Hawaii virus was first identified in 1977 by immune electron microscopy as a Norwalk-like (27 nm) virus in the stool of a volunteer who was challenged with a stool suspension derived from a 1971 family outbreak of gastroenteritis (Thornhill *et al.* 1977).

Astroviruses

Astroviruses are small (28 nm) non-enveloped RNA viruses first observed in the faeces of infants with diarrhoea. The virus particles possess a smooth margin with five- or six-pointed star motif on their surfaces. There are up to seven serotypes of human astroviruses. The complete genomic sequence of human astrovirus serotype 1 isolated in Newcastle upon Tyne has been published (Willcocks *et al.* (1994). Astroviruses display similarities in particle composition to the picornaviruses, and to caliciviruses with respect to the synthesis of a subgenomic RNA.

Human astroviruses most frequently cause disease in young children, and by 5 years of age more than 80% of children show serological evidence of previous infection. The incidence of illness increases in the elderly.

Work performed by Dr Myint at Leicester University has demonstrated the risk of infection by human astrovirus-4 from bathing in sea waters polluted by sewage discharges. Myint's research showed high levels of astrovirus in all beaches - from 10 per litre in some beaches up to 100,000 per litre in poorer quality beaches. Myint went on to show that bacteria indicators in seawater do not correlate with the virus levels - viruses surviving much longer than bacteria.

The relative contribution of astroviruses to the total incidence of virus-associated diarrhoea is not precisely known. Since the illness is mild, many cases will go unreported. Furthermore, difficulties in positively identifying astrovirus particles may compound this. Astroviruses were discovered by electron microscopy. EM remains the only diagnostic method with which all the viruses associated with diarrhoea can be detected. Polyacrylamide gel electrophoresis or antibody-based tests are only applicable to some viruses. It is generally believed from results of a six-year retrospective surveillance (using EM) that astroviruses are a relatively minor cause of viral gastroenteritis, with rotaviruses and adenoviruses being the main viruses involved (Lew *et al.* 1990). However, the importance of astroviruses may have been underestimated because they are more easily missed than the larger rotavirus and adenovirus particles, even when present in large numbers (Willcocks *et al.* 1992). Furthermore, the use of EM alone can result in the mis-classification of astroviruses, particularly as some particles do not contain the 'star-like' morphology. Unclear astroviruses may be misidentified as the small round viruses (SRV) which have smooth margins or even small round structured viruses (SRSV), characterised by fuzzy surfaces.

Surveillance studies on stool samples form children in Thailand using a monoclonal antibody-based method instead of EM showed astroviruses to be the second most prevalent agent associated with diarrhoea (Herrmann *et al.* (1991)). Enteric adenoviruses were found in 2.6%, astrovirus in 8.6% and rotavirus in 19% of stools from all children with gastroenteritis. A gene-probe based method (Willcocks *et al.* 1992) for the detection of astrovirus in stool samples showed, in a study for the Newcastle area of the UK, that astrovirus was more common than enteric adenoviruses 40 and 41. Willcocks *et al.* (1992) cite other reports showing underestimation of astrovirus.

3.3.2 Hepatotrophic Viruses

At least five viruses cause acute hepatitis. The viruses of hepatitis A (HAV) and hepatitis E (HEV) are spread predominantly by the faecal-oral route, whereas hepatitis B, C and D viruses (HBV, HCV and HDV) are spread by blood and other body fluids.

Hepatitis A Virus

Hepatitis A virus is an enterovirus that is transmitted by the faecal-oral route. Infection is worldwide and is spread predominantly by person-to-person contact. Common source outbreaks may occur as a result of faecal contamination of drinking water supplies and food. The largest recorded outbreak of HAV infection, affecting over 290,00 people occurred in Shanghai in 1988 and was attributed to consumption of raw shellfish. In the US, more than 20,000 cases of hepatitis A virus are reported annually, with approximately 2,500 cases in the UK in 1994. The reported cases, however, probably reflect less than 20% of the total. Serological evidence showed that in London (1985), 32% of the population (30 - 40 year age group) to have been exposed to HAV (Forbes and Williams, 1990). In 1977 the figure was 47%. Forbes and Williams (1990) suggest that improvements in the British standard of living explain the falling prevalence of exposure amongst London blood donors between 1977 and 1985.

HAV usually causes a minor or unnoticed illness in children and young adults, and on a worldwide scale fewer than 5% of cases are recognised clinically. Clinically apparent hepatitis A becomes more likely with increasing age at exposure in all populations studied. This is a factor which risk assessment models should take into account. Although the incidence of infection decreases with age in the UK, the ratio of deaths to incidence increases almost log-linearly with increasing age above 20 years.

Hepatitis E virus

A considerable percentage of cases of acute viral hepatitis in young to middle age adults in Asia (India, Nepal, Pakistan, Burma, USSR) and Africa are caused by an agent that is not HAV or HBV. The disease is primarily associated with ingestion of faeces contaminated drinking water. The term ET-NANBH (enterically transmitted nonA, nonB hepatitis) has been coined and is now referred to as hepatitis E. This type of hepatitis was first documented in India in 1955, when 29,000 cases were identified following

widespread faecal contamination of the city's drinking water. NANBH has developed in individuals returning home to USA from Karachi.

The clinical and epidemiological features of hepatitis E virus are:-

- faecal-oral transmission through contaminated water
- outbreaks involving up to tens of thousands
- highest attack rates found among individuals between 15 and 40 years old
- associated with high mortality rate (20%) in infected pregnant women.

Hepatitis E virus is 32-34 nm in diameter and contains a single-stranded polyadenylated RNA. Hepatitis E virus is substantially different from picornaviruses (including hepatitis A) with respect to its genomic organisation. Bradley (1992) speculates that hepatitis E virus may belong to a larger family of single-stranded RNA viruses (the Caliciviruses) including Norwalk virus.

3.3.3 Other viruses of current concern

Human Immunodeficiency Virus

Since discovery of HIV, the causative agent of AIDS, health officials have maintained that AIDS is almost exclusively transmitted through sexual contact, by infected blood and blood by-products. Even so, reports that HIV can be transmitted not only from blood and semen but also from saliva, tears, urine and vaginal excretions continue to raise concerns about the possibility of alternate transmission routes. Johnson *et al.* (1994) present information regarding the concentration of HIV in human waste and survivability of HIV outside the human body. They conclude that the probability for occupational transmission of HIV from wastewater approaches zero, and less than for other bloodborne pathogens. Although HIV is spread rapidly and widely among intravenous drug users, transmission by accidental needle stick is relatively rare (0.01%). It has been argued that the quantity of blood transferred is critical and that disease transmission depends on relatively large transfer of fluid. Levels of HIV in blood may be much lower than for other bloodborne viruses such as hepatitis B virus for which the probability of transmission from needle stick injury is 30%. Furthermore HIV may only be transmitted through infected cells (i.e. human T cells). Disinfection studies with HIV have shown that NaOCl readily inactivates the virus. Riggs (1989) concludes that there is no risk of HIV infection through drinking water.

3.4 The incidence of gastrointestinal and hepatic infections in the UK.

The numbers of cases of gastrointestinal infections in England and Wales during 1994 (taken from Communicable Disease Report, 5, 2) are presented in Table 3.2. These represent cases reported to GPs.

Table 3.2 Numbers of reported cases of gastrointestinal infections and hepatitis A virus infections in England and Wales 1994.

Adenovirus (EM faeces)	1,511
Adenovirus type 40/41	287
Astrovirus	539
Calicivirus	184
Rotavirus	15,422
SRSV	1,744
Hepatitis A virus	2,543
Campylobacter	44,315
Shigella	6,312
Enteropathogenic *E. coli* (children <3 years)	387
Cryptosporidium	4,424
Entamoeba histolytica	715
Giardia	6,009

Includes cases caught abroad

There is evidence that each of the above pathogens could be transmitted through water. However, there is little information on the exact number of cases infected through consumption of contaminated water supplied by water companies. It is interesting to note in an incident in Kildare, Ireland (October, 1991) when the water supply to about half of the population of a town of 11,000 people became contaminated with sewage that there was no evidence of an outbreak of hepatitis A virus despite its being present in the community. However, an outbreak of gastroenteritis affecting about 6,000 residents was reported almost simultaneously with the occurrence of 'dirty water'.

3.4.1 Surveillance of waterborne disease in England and Wales.

Epidemiological data may fail to reflect a complete picture of the role of water in the transmission of viral diseases. There are several reasons for this:-

- In the case of hepatitis A virus the long incubation period renders recognition and investigation of outbreaks difficult

- Diagnosis of a single aetiological agent in viral infections as well as the detection of a common source in outbreaks are difficult.

- Many virus infections are sub-clinical

- Detection of viruses in environmental and drinking water samples presents problems that are not usually encountered with clinical samples. The most serious problem relates to virus quantities, which are usually very low.

Recent publicised events such as contamination of drinking water supplies with cryptosporidial oocysts (Richardson *et al.* 1991) and an impression that many waterborne incidents are not reported completely to the PHLS have shown a need for a national surveillance system to collect information about illnesses attributable to water. The PHLS Communicable Disease Surveillance Centre (CDSC) has devised a pilot scheme to actively seek information on suspected and confirmed cases of disease attributed either to water consumption or exposure to recreational water at home or abroad. Reporting of contamination incidents, both microbiological and chemical, is encouraged.

Infectious disease events were reported in two categories:-

- water related infectious disease

- water related infection hazard, i.e. contamination of potable water with pathogens or *Escherichia coli.*

Each category was classified as definite, probable or possible, according to the strength of the evidence. In the six months between October 1991 and March 1992, eight cases of water related infectious disease were reported and seven incidents suggesting water related infection hazard (Nazareth *et al.* 1994). In two incidents, classified as definite hazards, cryptosporidial oocysts were detected in water samples leaving a large treatment works. The low number of events suggested that waterborne incidents are rare in the UK, although it was apparent that not all incidents were reported to CCDCs.

Examples of waterborne microbiological outbreaks in the UK

1. Gutteridge and Haworth (1994) report an outbreak in 1992 of gastrointestinal illness associated with contamination of the mains supply by river water. The outbreak arose because the mains water supply to a farm was connected to an irrigation system using river water. Back syphonage into the mains occurred because a valve was left open. *Campylobacter spp* were found in faeces of two children. In total 42 cases of acute gastrointestinal illness were recorded.

2. The outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire (Richardson *et al.* (1991). Over 500 cases were confirmed by laboratory analysis and up to 5,000 people overall may have been affected. Treated water leaving the

works contained oocyst concentrations of 0.002 - 5/l. Inefficiency of the settlement process and recycling of backwash water with high oocysts loadings may have contributed to the outbreak.

3.5 Conclusions

The following conclusions are made concerning application of risk assessment models for specific pathogens in the UK.

1. The risk from any pathogen which presents a potential or proven waterborne hazard should be modelled. This is because one objective of risk assessment models is to identify the most cost effective options to reduce risk. In this respect, specific waterborne pathogens cannot be considered alone. Implementing one option at the expense of another to reduce the risk from a specific pathogen may increase the risk from other pathogens.

   To avoid complacency, pathogens which cause epidemics in the developing world (e.g. cholera) are included. The reasons for this are:-

   - infected individuals may enter the UK
   - one aim of risk assessment modelling would be to assess the health risks of reducing chlorination to eliminate disinfection by-products.

   The pathogens which should be modelled for drinking water supplies include:-

   - Hepatitis A virus
   - Hepatitis E virus
   - Rotavirus
   - *Cryptosporidium*
   - *Giardia lamblia*
   - *Campylobacter*
   - *Vibrio cholerae*
   - Salmonellae
   - Shigellae
   - *E. coli O157*
   - Caliciviruses
   - Astroviruses

2. Epidemiologic information on individual pathogens highlights the necessity to customise risk assessment models for each pathogen. Thus,

   - rotavirus and enteric adenovirus do not affect adults

- mortality from HAV is higher in older persons exposed for the first time,

- mortality from HEV is high (20%) in pregnant women,

- secondary transmission among exposed household members is low for HEV but considerably more significant for HAV.

3. Secondary transmission from a point source (e.g. drinking water supply) through person-to-person contact is important for several pathogens including *E. coli O157*, hepatitis A virus, *Helicobacter pylori*, rotavirus, Norwalk virus, *Cryptosporidium* and *Giardia lamblia*. The impact of secondary transmission from a primary waterborne source should be considered in risk assessment models for such pathogens.

4. Surveillance of waterborne incidents of gastrointestinal illness is difficult. Many incidents of gastrointestinal illness are not reported and reported incidences should not be used as bench marks to test risk assessment models. Furthermore the relative significance of certain pathogens, for example, astrovirus may be underestimated through current detection methods (e.g., electron microscopy).

5. Risk characterisations for UK models should vary according to pathogen type and the proportion of high risk consumers (e.g. pregnant women, AIDS patients).

4. REPORT ON THE NECESSARY DATA INPUTS FOR A UK MODEL AND ASSESS THE STATE OF APPROPRIATE DATA KNOWLEDGE IN THE UK.

Risk assessment models developed in the USA use two steps. These are exposure assessment and dose-response assessment. One limitation of the US models is the lack of assumptions and information on the statistical distribution of pathogen densities in the drinking water supply. This reflects the low density of pathogens in most supplies and the potential for variation. A second limitation is the relative inflexibility of US models through not considering the whole of the multiple barrier approach to microbiological hazards in drinking water supplies.

The stages and barriers for transmission of a human pathogen through the faecal-oral route in the water cycle are shown in Figure 4.1. Development of UK risk assessment models which consider the impact of the stages and barriers in the water cycle on microbiological risk would offer several advantages over US models. These include:-

- assessment of cost effectiveness of risk reduction options
- assessment of impact of changes in environmental inputs
- assessment of impact of changes in treatment strategy

To develop such risk assessment models information is effectively needed on each of the stages and barriers (shown in Figure 4.1) for each pathogen type. Integrating such models with effects on the statistical distribution of pathogen densities in the drinking water supply, would predict an answer of the all important question -

What proportion of consumers are exposed to what numbers of pathogens?

The proportion of consumers exposed to particular densities of pathogens depends not only on the statistical distribution of the density of pathogens within the drinking water supply but also on the volume of water consumed. The latter is more easy to quantify and there have been several studies on consumption of tap water in the UK and the US. Consumption data are reviewed in Section 4.2. There are, however, several problems with quantifying the concentration of pathogens across the drinking water supply. This is considered in Section 4.1.

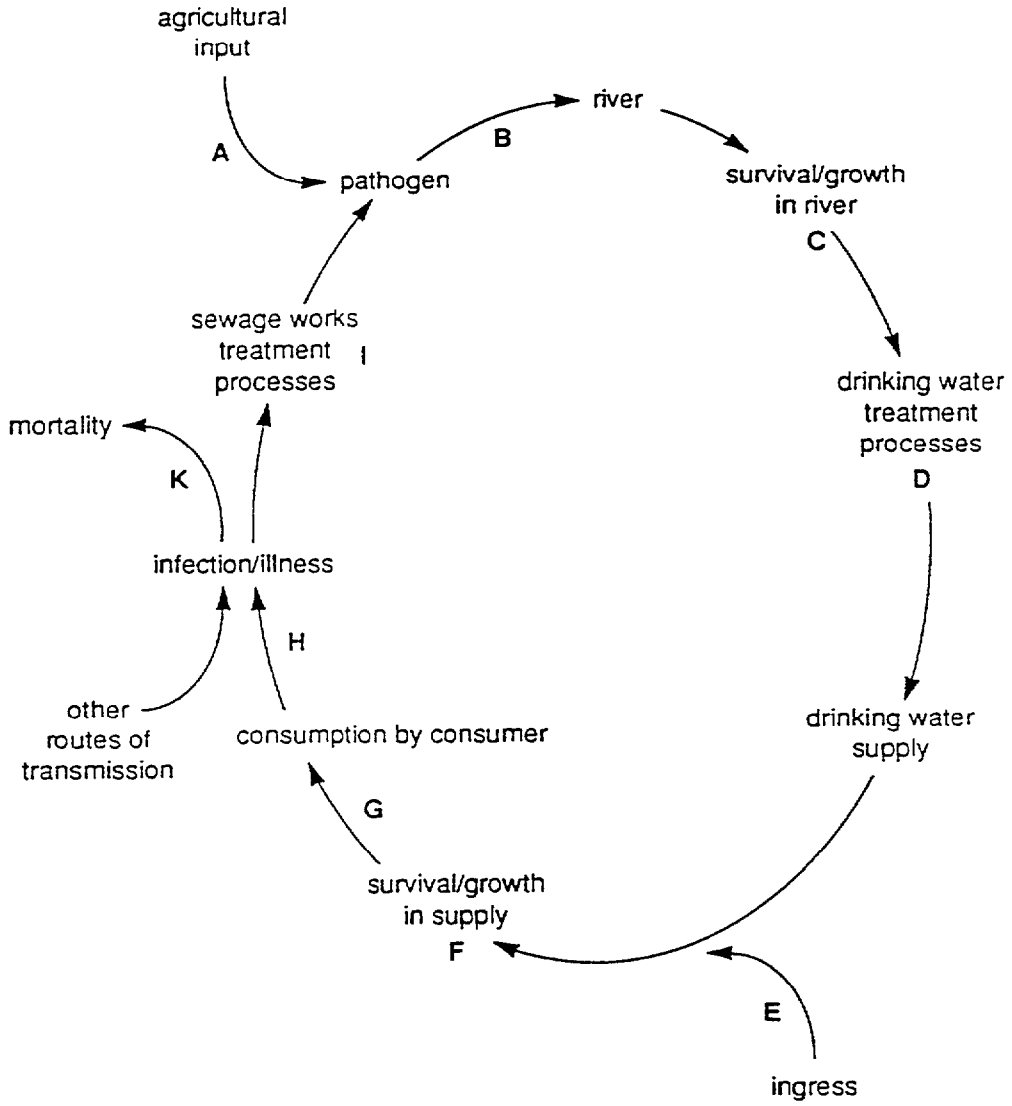

Figure 4.1 Stages in risk assessment model for pathogens in the drinking water supply.

4.1 Data needed to estimate what proportion of the consumers are exposed to what concentrations of pathogens in the treated drinking water supply in the UK

Problems in quantifying the distribution of pathogens across drinking water supplies relate to imponderables and also limits in our current status of knowledge. Unknowns include the following:-

- pathogen concentrations in the drinking water supplies where sampling recorded <1 per volume (i.e., below limits of detection).
- variation of pathogen concentrations not only spatially (e.g. from clumping) across different regions of the supply zone, but also temporally, e.g. total heterotrophic bacteria and total coliform densities increase seasonally with temperature (Gale, 1994a).
- pathogen concentrations in the environment vary according to the area and the incidence of disease in the human and animal population.
- numbers of pathogens entering the supply through ingress and post treatment contamination.

The concentrations of pathogens in the drinking water supply will vary depending on:-

- net input into UK rivers from agricultural sources prior to abstraction
- input into sewage in UK and removal by sewage treatment
- survival in the river/environment
- removal by drinking water treatment
- growth/survival in the drinking water supply
- post-treatment contamination

Furthermore exact exposures by individual consumers will be influenced by the statistical distribution of microorganisms in the drinking water distribution.

4.1.1 Overview of occurrence and detection of waterborne pathogens

Black and Finch (1993) have reviewed the literature worldwide for the detection of waterborne pathogens in various waters. Their findings are summarised in Table 4.1.

Table 4.1 Detection of pathogens in various waters worldwide (taken from Black and Finch, 1993)

Organism	Drinking Water	Surface Water	Waste Water
Bacteria			
Campylobacter	no	yes	no
Coliform bacteria	yes	yes	yes
Escherichia coli	yes	yes	yes
Helicobacter spp	no	yes	no
Salmonella	no	yes	yes
Vibrio spp	yes	yes	no
Viruses			
Adenovirus	no	yes	no
Coxsackievirus	no	yes	no
Echovirus	no	yes	no
Enterovirus	no	yes	yes
Hepatitis	no	yes	yes
HIV	no	no	yes
Norwalk virus	no	yes	no
Poliovirus	no	yes	yes
Rotavirus	no	yes	no
Protozoa			
Cryptosporidium spp	no	yes	no
Giardia lamblia	no	yes	no

Their review is not fully comprehensive in that campylobacters and *Cryptosporidium* have been detected in drinking water supplies (See Section 3) as have enteroviruses (Payment *et al.* 1985). Furthermore, Gerba *et al.* (1984) has reported the isolation of both hepatitis A virus and rotavirus from drinking water samples in Mexico. *Giardia* and *Cryptosporidium* have also been detected in wastewater samples. However, the findings of Black and Finch (1993) present the overall picture that information on viral densities, in particular, for drinking water supplies is limited but more available for surface waters (which are used for abstraction).

The determination of pathogen concentrations in the drinking water supply is effectively dependent on the ability to detect and quantify pathogens. The major problem of detecting pathogens in environmental or drinking water samples is their extremely low densities, requiring analyses of large volume samples for detection, or some selective concentration/filtration method.

Low virus concentration precludes direct antigen detection in water samples because of low sensitivity. Gene probe methods (without amplification by PCR) are able only to

detect down to 10,000 virus particles. Viruses must be therefore be concentrated for detection. Furthermore methods of detection vary in difficulty and are far from routine for detection of hepatitis A virus and Norwalk virus. Water-transmitted enteric viral pathogens are divided into three groups (Metcalf *et al.* 1988) based on their cultivability in cell culture. These are:-

- routinely cultivable (enteroviruses and adenoviruses)
- not routinely cultivable (hepatitis A virus and rotavirus)
- not cultivable (Norwalk virus)

The detection strategy adopted for their detection is based on these differences. Cell culture methods are the most sensitive, detecting down to one infectious physical particle. Such methods are routinely used for enteroviruses and adenoviruses. The nonroutinely cultivable (e.g., hepatitis A virus and rotavirus) and the noncultivable (e.g., Norwalk virus) offer the greatest detection challenge. Although rotavirus is not an ordinarily cultivable virus, it can often be detected by enumeration of foci of infected cells that result from its limited replication in cell cultures. The foci are detected using immunochemical procedures. The method is only semiquantitative at best and cannot be applied to hepatitis A virus and Norwalk virus. Gene probe technology potentially offers possibilities for the detection of these viruses (Metcalf *et al.* 1988).

Another problem for risk assessment models is the possibility that culture-based methods do not detect the presence in water of bacteria which, though not culturable, may nevertheless still be intact and viable, and potentially able to cause disease.

4.1.2 The occurrence of pathogens in surface waters

Giardia and *Cryptosporidium*

USA data

Rose *et al.* (1991b) considered two categories of surface water: polluted waters contaminated by sewage and agricultural discharges; and pristine waters originating from protected watersheds without point source pollution or input from human activities. Ongerth (1989) showed concentrations of *Giardia* cysts from three pristine rivers in the Pacific Northwest to be lognormally distributed. For each river, no cysts were found in half or more of the samples. This is analogous to statutory water quality monitoring for coliforms where 95 - 99% of samples register 0 coliforms per 100 ml. Concentrations for these samples are not really 0 per 100 ml but <1 per 100 ml (Section 2.2.2). True concentrations may be 0.01 per 100 ml, for example, and require larger volumes for accurate measurement. By extrapolation of cyst concentration data on lognormal probability plots (c.f. Figure 2.1), Ongerth (1989) calculated median concentrations for *Giardia* cysts to be between 0.003/L to 0.06/L for the three pristine rivers. From extrapolation of the lognormal probability plots (Ongerth, 1989) it would appear that

concentrations of *Giardia* cysts in 99% of samples from one of the rivers (Green River) vary between ~0.001/litre and ~6/litre. This is some 6,000-fold variation in the same river during a 9-month period.

Rose *et al.* (1991a) compared occurrences of *Cryptosporidium* and *Giardia* in potable water supplies in the US. *Cryptosporidium* oocysts were detected in 55% of the surface water samples, while *Giardia* cysts were found in 16% of the same samples.

UK *Cryptosporidium* data

An infected calf may excrete as many as 10^{10} oocysts per day for as long as 14 days. Oocysts from infected animals and man enter surface waters through sewage effluent, farm drainage and runoff. Furthermore, oocysts may survive for long periods in the environment. This has prompted comprehensive surveys on the occurrences of oocysts in waters used for abstraction to be carried out in the UK.

During a survey of lowland surface waters, 10 sites on three rivers in England were monitored (Carrington and Miller, 1993). The rivers in England were chosen because i) they were used for abstraction, ii) they received sewage effluents, and iii) they were subject to agricultural inputs. For two of the rivers, oocysts were present in less than 5% of samples. In the third river, however, oocysts were present in around 50% of the samples. Unfortunately, the data presented by Carrington and Miller (1993) are little use because the sample volumes are not reported and not consistent from sample to sample or river to river. Means and ranges for oocyst concentrations from the positive samples were presented, and again are of little use because the zero concentrations were ignored (Section 2.2.2). Similar criticism applies to a study of UK upland water sources (Loch Lomond) in which Parker *et al.* (1993) reported 32 of 279 samples being positive for *Cryptosporidium* oocysts. Parker *et al.* (1993) sampled between 100 and 1,000 litres but do not report exact volumes for samples registering zero oocysts.

In the UK Water Industry's *Cryptosporidium* monitoring programme 12% of lowland river water/reservoir samples were positive for oocysts. 8.5% of upland reservoir/lake samples were oocysts-positive. In contrast, only 1% of borehole raw waters were oocysts-positive. Analysis of sewage effluent showed 75% of samples were positive. Again no information was provided on sample volumes, so comparisons may not be valid.

4.1.3 Removal of pathogens by drinking water treatment

There is considerable information on the removal efficiencies for groups of pathogens by different drinking water treatment processes. Table 4.2. (provided by Dr T. Hall of WRc) shows estimates of the performance of a range of water treatment unit processes for log removal or inactivation of micro-organisms of public health significance.

Table 4.2 Estimates for removal or inactivation of micro-organisms of public health significance.

Unit process	*Giardia*	*Crypto-sporidium*	Viruses	Total Coliform
Rapid gravity filtration	1 - 2	1	<1	<1
Chemical coagulation/RGF	2 - 3	2 - 3	1 - 2	1 - 3
Chemical coagulation/ clarification/RGF	2 - 3	2 - 3	1 - 2	>4
Slow sand filtration	2 - 3	2 - 3	1 - 3	1 - 2
Microfiltration	>4	>4	0.5 - 2	>8
Chlorine Ct = 15 min	<0.5	no effect	>4	>4
Ozone Ct = 1.6 min	2 - 3	no effect	>4	>4
Ultra violet 25 mW/cm2	<1	no effect	2 - 3	>4
Chlorine dioxide Ct = 10 min	1 - 2	no effect	2 - 3	>4

Giardia and *Cryptosporidium*

A pilot scale study on the removal of *Cryptosporidium* oocysts showed that chemical coagulant treatment streams removed 99.8%% of oocysts. Alum coagulation removed 99% of *Giardia* cysts at pH 5.6 and 6.2. Several reports have highlighted the importance of optimised coagulation conditions for effective removal of *Giardia* cysts by coagulation and also rapid sand filtration. Slow sand filters have been shown to remove 99.98% of *Cryptosporidium* oocysts and up to 99.99% of *Giardia* cysts in raw . It has been shown that the removal rate of *Giardia* cysts by slow sand filtration decreased with temperature; only 93.7% being removed at 0.5°C. Chlorine concentrations needed for inactivation of *Cryptosporidium* oocysts are far in excess of those that could be practically achieved during water treatment; it has been demonstrated that a small proportion of oocysts survive 8,000 mg/l chlorine for 24 h. *Giardia* cysts can be destroyed by a 2 mg/l residual after 10 minutes contact time at pH 6 to 7, or a 3 mg/l residual at pH 8 (Ainsworth, 1990).

Viruses

Disinfection processes used in drinking water treatment appear to be relatively effective against viruses, with chlorination removing more than 99.99% (Table 4.2). However, there is evidence that certain viruses of waterborne health significance are exceptions.

Hepatitis A virus

Peterson *et al.* (1983) measured the effects of chlorine on HAV infectivity in marmosets, which were inoculated intramuscularly. Without any chlorination, the inoculum induced hepatitis in 100% of marmosets. Treating the virus suspension for various periods (15, 30 or 60 min) with 0.5, 1.0 or 1.5 mg/l of free residual chlorine induced hepatitis in 8 to 14% of marmosets. Inoculum treated with 2.0 or 2.5 mg/l of free chlorine was not infectious in the 13 animals tested. Peterson *et al.* (1983) concluded that treatment levels of 0.5 to 1.5 mg/l free residual chlorine inactivated most but not all HAV in the preparation, whereas concentrations of 2.0 and 2.5 mg/l free chlorine destroyed the infectivity completely. They concluded that HAV is somewhat more resistant to chlorine than are other enteroviruses. Average chlorine concentrations in UK drinking water supplies range between 0.1 and 0.3 mg/l suggesting that the UK chlorination process alone is not sufficient to remove HAV.

Grabow *et al.* (1983) however, presented evidence that chlorine disinfection of drinking water supplies will successfully inactivate HAV particularly under the right pH conditions. They found the inactivation of hepatitis A virus, rotavirus SA-11 and poliovirus type II by chlorine was pH dependent being more effective at pH 6 than pH 10. At all three pH levels, HAV was more sensitive to chlorination than poliovirus type II, but it was always more resistant than rotavirus SA-11.

Pilot plant studies showed that coagulation, settling and filtrations in a pilot plant reduced the densities of hepatitis A virus, rotavirus and poliovirus by 2 to 3 log orders (Rao *et al.* 1988).

Hepatitis E virus

During the New Delhi, India, epidemic of hepatitis in 1955/56, highly polluted river water was being treated with five to six times the normal dosage of alum and four times the normal dosage of chlorine. That prevented the occurrence of all enteric disease of bacterial or viral origin, except for hepatitis (cited from Rao *et al.* 1988). Since the Delhi outbreak was probably caused by hepatitis E virus, the implications are that this virus can survive drinking water treatment.

Norwalk Virus

Keswick *et al.* (1985) investigated the inactivation of Norwalk virus in drinking water by chlorine. They tested the inactivation of the virus using infectivity studies in human volunteers. They found Norwalk virus in water was more resistant to chlorine inactivation than poliovirus type 1, human rotavirus (Wa), simian rotavirus (SA-11), or f2 bacteriophage. A 3.75 mg/l dose of chlorine was found to be effective against other viruses but failed to inactivate Norwalk virus. This is much higher than doses applied in UK drinking water supplies. Routine chlorination alone cannot be relied upon to inactivate Norwalk virus. In a camp in Maryland, water pumped from a well to a storage tank was found to contain 0.7 to 1.0/L mg of iodine before and during an outbreak of Norwalk virus which affected 133 persons.

4.1.4 The occurrence of pathogens in the drinking water supply in the UK

Giardia and *Cryptosporidium*

Parker *et al.* (1993) reported that out of 299 samples of Loch Lomond origin taken from the drinking water supply, oocysts were detected in 14 (4.7%). Viability was as high as 50%. Unfortunately, no information on sample volumes is presented. Water utilities use the SCA "Blue Book" method to analyse for *Cryptosporidium*. From the First National *Cryptosporidium* questionnaire, it appeared that most companies were collecting 100 to 500 litres of raw water and generally 500 to 1,000 litres of treated water, although up to 5,000 litres was used. In a UK water industry *Cryptosporidium* monitoring programme 4.6% of customer's taps registered positive for *Cryptosporidium*. 5.6% of treated water samples from service reservoirs were positive. In the 1988 Ayrshire outbreak, Smith *et al.* (1989) report that two final water samples of 300 litres and 500 litres registered oocyst concentrations of 4.8/litre and 0.04/litre, respectively.

4.1.5 The statistical distribution of pathogens in the supply

The statistical distribution of pathogens within the drinking water supply is the single most important factor in microbiological compliance, risk assessment modelling, and impact on public health. With respect to public health, it defines how many of the population are exposed to what density of pathogens. With respect to compliance monitoring, it defines what proportion of samples of a specified volume register positive for a given microorganism, (e.g. coliform, total heterotrophic bacteria, or *Cryptosporidium*). It is also the area in microbiological drinking water supply research where least information is available. This reflects the low concentrations of coliforms and in particular pathogens in the drinking water supply, necessitating large volume sampling programmes to yield fruitful results.

Furthermore, there is a tendency for incorrect assumptions and rash statements to be made. It has been written at an International Conference on Risk Assessment (1992) that, "Those applying microbiological risk assessment to drinking water have certain advantages. For example,.....water is a *relatively homogeneous* medium. Consequently, reasonably valid assumptions about consumption and distribution can be made quite easily". **This statement, while more appropriate to water samples taken from a well-shaken bottle, is incorrect when applied to water samples taken from consumer premises in a water supply zone.** Indeed, evidence will be presented in this section that microorganisms are heterogeneously distributed within drinking water supply systems. Instead of being homogeneously dispersed throughout the drinking water supply, pathogens and other microorganisms, including indicator bacteria, appear to be clustered into certain regions of the supply, leaving other regions virtually void of pathogens.

The implications of this are considerable for public health and risk assessment, and compliance. In effect small proportions of the population will be exposed to relatively high densities of pathogens in the drinking water supply, while the majority will not be exposed to any.

Evidence for a lognormal distribution of bacterial densities in the drinking water supply

Studies in the city of Metz (France) have shown that densities of total heterotrophic bacteria are spatially heterogeneous within a water supply zone at a given time (Maul *et al.* 1985). Indeed, it was shown that water supply zones are comprised of several subsystems which differ in bacterial density. Work performed at WRc (Gale 1994b) furthers the work of Maul *et al.* (1985) demonstrating that the different sub-regions of spatial heterogeneity may be linked through a lognormal statistical distribution.

Total heterotrophic bacteria densities

Total heterotrophic bacteria (2 day, 37°C) densities (obtained from a water supply zone in the UK during the period of one year) are plotted on a Normal probability plot after log-transformation (Figure 4.2). The highest concentration recorded was 300 per ml and the lowest <1 per ml. The data for samples with concentrations of 1 or more per ml appear to be approximately log-normally distributed. The best fit line was constructed through all data except the 15% of samples registering <1 per ml. From that line it would appear that 99.9% of concentrations ranged between 0.018 per ml (0.05 percentile) and 2,000 per ml (99.95 percentile), i.e. concentrations varied over 10^5-fold within one zone during the year. A cumulative frequency distribution for the Metz system showed spatial variations of 10^5-fold on the same day (Maul *et al.* 1985). The log-normal model accommodates the high degree of heterogeneity observed in total heterotrophic bacterial densities in the water supply zone during the year. Through the geometric mean (GM) and logarithmic standard deviation (LSD), which define the log-normal distribution, all the heterogeneous densities occurring at different spatial locations across the supply zone are accommodated.

To investigate seasonal effects, the total heterotrophic bacteria density data presented in Figure 4.2 were divided according to season (summer months, April to September 1990 and winter months, January to March 1990 and October to December 1990). Bacterial densities were approximately log-normal in distribution during both seasons, but differed considerably (Figure 4.3), being shifted to higher densities in summer months. Comparing the median values, densities were four-fold higher in the summer months than in the winter months. From cumulative frequency plots presented in Maul *et al.* (1985), median total heterotrophic bacterial densities increased five-fold from January to May, increasing a further five-fold one day in June.

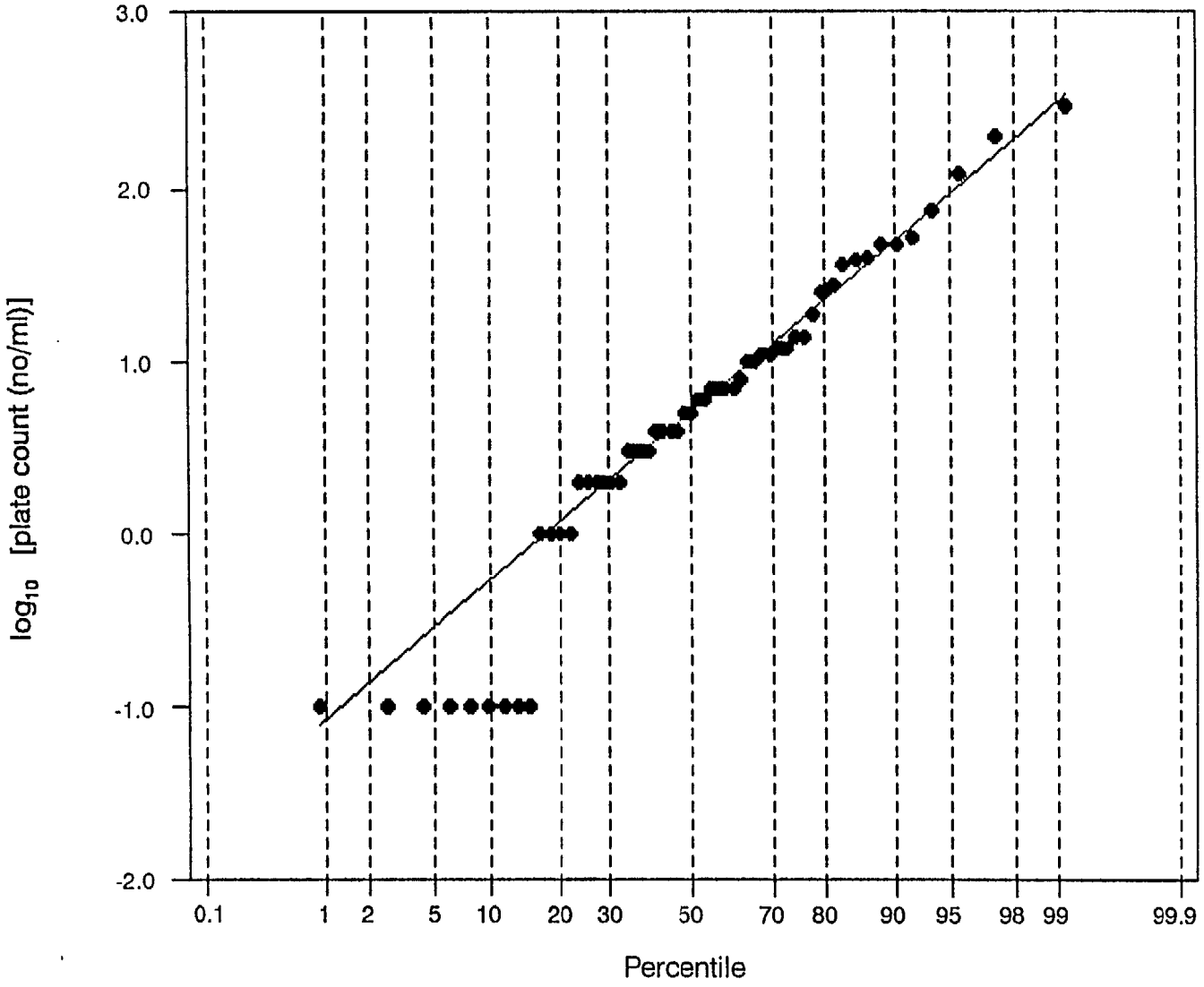

Figure 4.2 Concentrations for total heterotrophic bacteria (2 day; 37°C) recorded during a year from a water supply zone in a UK water company and plotted on a normal probability plot.

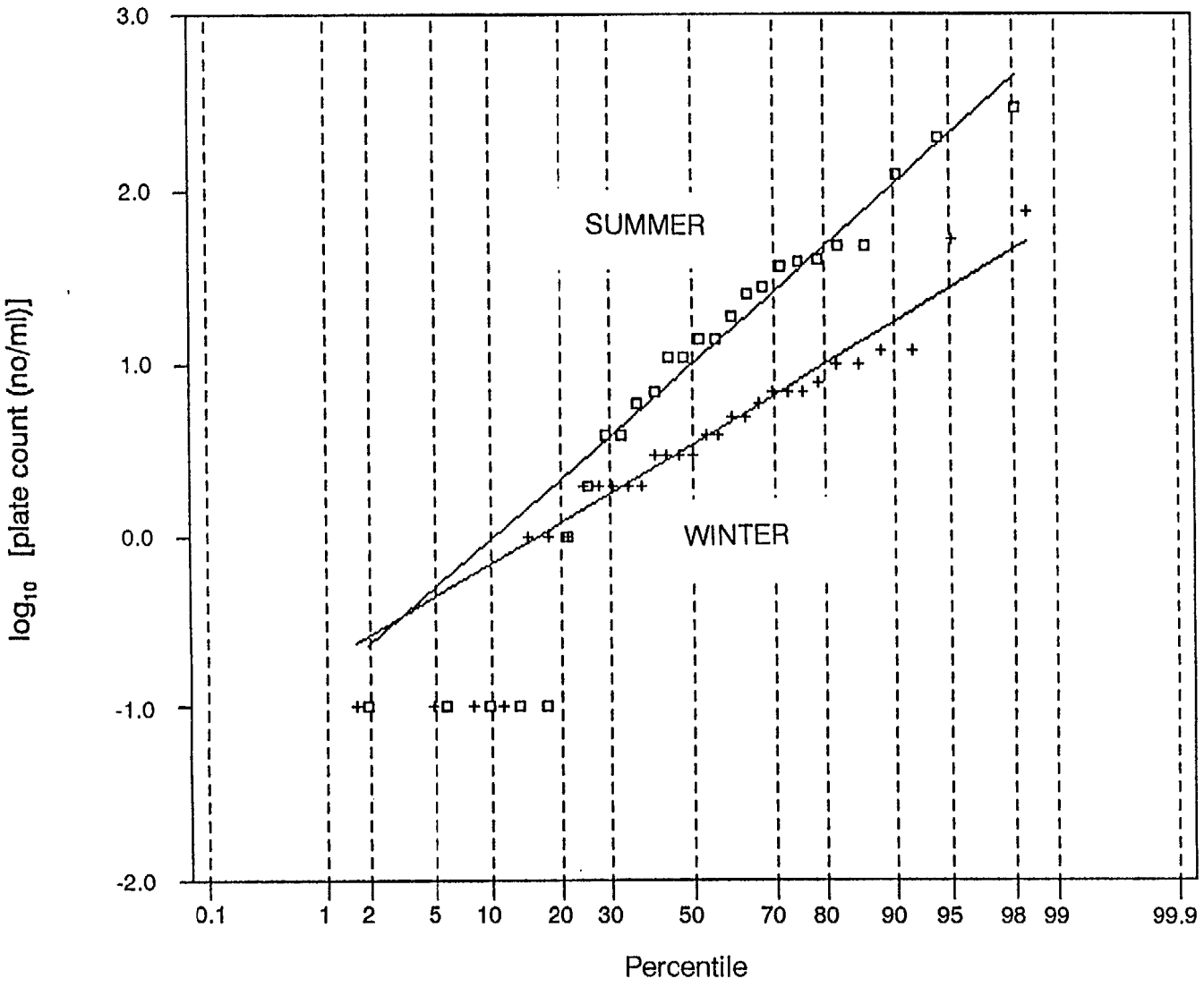

Figure 4.3 Concentrations of total heterotrophic bacteria (2 day; 37°C) from a water supply zone in a UK water company are higher in the summer months (April to September) than the winter months (Jan to March; October to December).

Total coliform bacteria densities

Many 100 ml water samples are collected from consumer premises across the UK throughout the year and analysed for total coliforms. This high frequency, regular monitoring programme is vital for rapid response to acute deterioration in microbiological water quality. For the majority of water supply zones (99% in 1993), over 95% of the samples register 0 coliforms per 100 ml. Such data provide little information for operational or statistical analysis. This is because the 'zero concentrations' do not mean zero coliforms but <1 coliform per 100 ml.

In Figure 2.1, the logarithms of the coliform densities recorded from zones in a water company were plotted on a Normal probability plot. Data from samples with concentrations of 1 or more coliforms per 100 ml (appearing at the right hand end of the plot) resemble the corresponding parts of log normal distributions for total heterotrophic bacteria (Figure 4.2). By analogy, therefore, coliform concentrations could also be log-normally distributed. The difference between the coliform concentrations and the total heterotrophic bacteria concentrations is that in the former around 95% of samples registered <1 per 100 ml, while for the latter only 15% of samples recorded <1 per ml. To produce a log normal model for coliform concentrations, a best fit line was constructed through the portion of the plot representing samples with 1 or more coliforms per 100 ml. These data account for 6.4% of samples in Figure 2.1. The remaining samples (93.6%) registered 0 per 100 ml and appear in Figure 2.1 as the 'flat portion' with values of -1.0 (0 concentrations were set a value of 0.1 prior to log-transformation, since there is no log of zero). The best fit line suggests that densities for regions where sampling registered 0 coliforms per 100 ml could range between 0.1 per 100 ml (i.e. 1 per litre) and 10^{-9} coliforms per 100 ml (i.e. 1 coliform in 10^8 litres). It is interesting to note that 10^8 litres is a cube with dimensions of 50 metres, and is similar to a large service reservoir. Thus, volumes as large as a service reservoir could exist with just a single coliform present. At the other end of the density scale, some samples contain as many as 500 coliforms per 100 ml. This huge range in density of microorganisms within the drinking water supply is an important consideration in risk assessment modelling.

From the intercept of the best fit line with the 50 percentile (Figure 2.1) a value of 6.2×10^{-4} coliforms per 100 ml (6.2 per 1,000 litres) was estimated for the geometric mean (Table 2.4). The logarithmic standard deviation defined by the slope of the best fit line is 2.01 $\log_{10}$ per 100 ml.

Implications of lognormal distribution for pathogen densities in risk assessment modelling.

1. The lognormal distribution defines what proportion of samples contain more than a certain density of pathogens

Lognormal probability plots show the huge variations in bacterial densities in a drinking water supply. Through the lognormal distribution defined by its geometric mean and logarithmic standard deviation this heterogeneity is accommodated. Furthermore, through

the lognormal distribution the proportion of samples registering more (or less) than a certain number of organisms is defined. This is of fundamental importance in microbial risk assessment where an estimate of how many consumers are exposed to what density of pathogen is needed. Thus, for example for Figure 2.1, we can read the following exposures from the plot:-

- 50% of consumers are exposed to coliform densities of less than 6.2 per 1000 litres.
- 10% of consumers are exposed to coliform densities of greater than 2.4 per litre
- 5% of consumers are exposed to coliform densities of greater than 1.2 per 100 ml
- 1% of consumers are exposed to coliform densities of greater than 30 per 100 ml
- 0.1% of consumers are exposed to coliform densities of greater than 1,016 per 100 ml

This information is exactly what is needed for microbiological risk assessment modelling.

Coliform compliance monitoring under The Water Supply (Water Quality) Regulations (1989) is analogous. It is shown in Figure 2.1 that 6.4% of 100 ml volumes contain one or more coliforms.

There is no reason in risk assessment modelling why pathogen densities in the drinking water supply should not be treated in a similar way to coliform densities. The problem is determining the values for the geometric mean and logarithmic standard deviation which define their statistical distribution.

2. The lognormal distribution allows the effects of water treatment on microbiological compliance and risk to be modelled

Vertical shifts in the line on a probability plots represent changes in the water quality across the whole zone and are reflected in a change in the geometric mean. Figure 4.4 shows three log-normal distributions for coliform densities with the same LSD (slope) but differing in GM. Effectively each and every density in line Y is 100-fold smaller than the corresponding density on line X. Similarly each and every density on line Z is 10,000-fold smaller than the corresponding point on line X. This is reflected in the geometric means (10^{-2} per 100 ml for line X, 10^{-4} per ml for line Y and 10^{-6} per 100 ml for line Z). The three lines could reflect coliform densities at different stages of water treatment. Thus line X, with 20% of 100 ml samples registering coliforms could be the raw water. The first stage of treatment gives a 2 log-removal across the board; the distribution of coliform densities being represented by line Y, in which 5% of 100 ml samples are positive. The second stage of treatment gives a further 2 log-removal, shifting vertically from Y to Z, in which only 1% of 100 ml samples are coliform positive. Line Z is thus the finished water, fit for consumption.

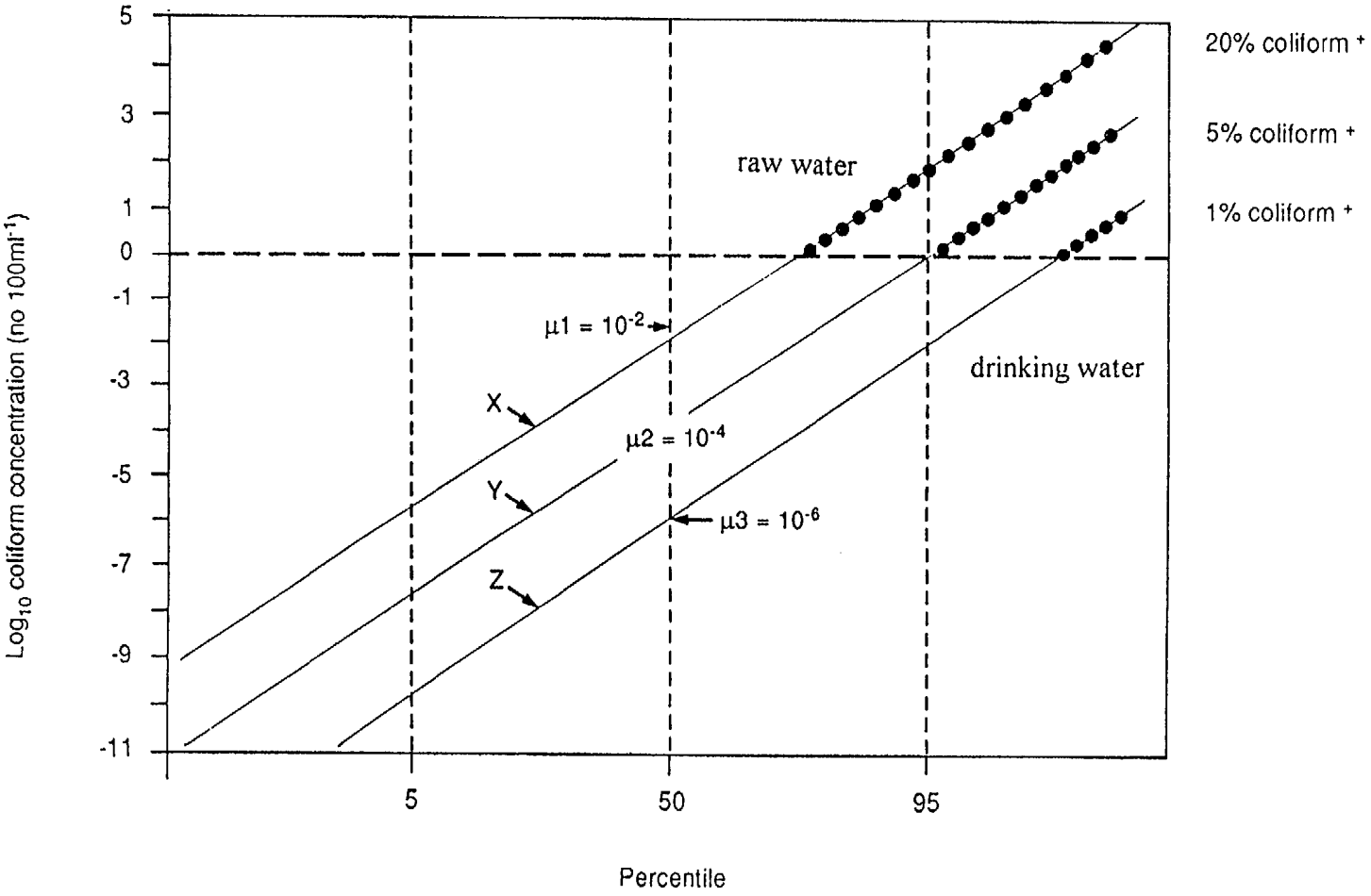

Figure 4.4 Modelling the statistical distribution of coliforms (or pathogens) in the drinking water supply after treatment of raw waters.

Evidence for a lognormal distribution of pathogens in the drinking water supply

In Section 4.1.2 studies by Ongerth (1989) are reported showing that *Giardia* cysts in pristine rivers are lognormally distributed. In fact the statistical distribution for *Giardia* cysts in a river could resemble line X in Figure 4.4, if units were cysts per 100 litre rather than per 100 ml. Removal rates for *Giardia* cysts by water treatment are logarithmic (Table 4.2). Thus, the effect of water treatment would be to shift line X in Figure 4.4. vertically downwards maintaining the lognormal distribution but simply decreasing the geometric mean. This would suggest that *Giardia* cysts are lognormally distributed in the drinking water supply, perhaps resembling line Z in Figure 4.4 (assuming units are cysts per 100 litres).

For example, applying a 4 log removal by treatment (c.f. Figure 4.4) to the lognormal distribution for *Giardia* cyst densities in Green River water samples (Ongerth, 1989) would predict that 99% of samples from the treated water supply would contain between 0.0000001 cysts/litre and 0.0006 cysts/litre.

4.2 Drinking Water Consumption

Hopkin and Ellis (1980) have reported the findings of a study on drinking water consumption in Great Britain. For adults the mean total liquid intake was 1.79 l per person day. Hot drinks and non-tap-water based drinks accounted for 1.67 l per person day, leaving only 0.12 l per person day as cold tap-water-based drinks.

A comprehensive statistical analysis of tap water intake by children and adults has been performed in the USA (Roseberry and Burmaster, 1992) showed consumption within each age group to be lognormally distributed. Statistical distributions are defined by the log geometric mean and logarithmic standard deviation for each age group. These statistics as reported in Roseberry and Burmaster, 1992) are presented in Table 4.3. In addition the central tendencies are reported as geometric means in the third column to give a feel for average volumes consumed by each age group.

It appears that on average persons in the over 65 yr age group drink the most tap water (1,197 cm^3 per day), some 4.5-fold more than children under 1 yr of age (267 cm^3 per day). From the values of μ and σ in Table 4.3, the volumes consumed by the upper 5% of the population may be calculated. Thus, 5% of the population consume more than $e^{(\mu + 1.64\sigma)}$ cm^3 per day in each age group. It is calculated that 5% of the over 65 yr age group consume more than 2,614 cm^3 per day (tap water). This is similar to that used in the US risk assessment models which assume 2 l /person/day and predict lifetime risks of death from waterborne virus (for the most exposed individual) to be as high as 1 in 20.

Table 4.3 **Summary statistics (calculated from natural logarithms of intakes in cm^3 per day) for best fit lognormal distributions for tap water intake rates. Data taken from Roseberry and Burmaster (1992). Geometric mean intakes calculated as e^{μ} are also presented.**

Age Group	Ln Geometric mean (μ)	Logarithmic standard Deviation (base e) (σ)	Geometric mean (cm^3 per day) e^{μ}
0 < age < 1	5.587	0.615	267
1 <= age < 11	6.429	0.498	619
11 <= age < 20	6.667	0.535	786
20 <= age < 65	7.023	0.489	1122
65 <= 65	7.088	0.476	1197
All	6.87	0.530	963

4.3 Conclusions

1. Some information is available for pathogen concentrations in surface waters both in the UK and USA. UK data are not presented in a useful format for risk assessment modelling. USA *Giardia* data, however, are although only for relatively pristine rivers. UK *Cryptosporidium* data should be reanalysed statistically on probability plots.

2. Considerable information is available on rates of pathogen removal both by drinking water and sewage treatment processes (data not presented). Log removal efficiencies by the individual treatment processes are available for broad groups of pathogens. However, certain viruses appear to be more resistant to chlorination than others. Indeed, Norwalk virus and perhaps hepatitis viruses appear to be more resistant to chlorination levels typically used in drinking water supplies.

3. Although pathogens such as *Cryptosporidium* have been detected in UK drinking water supplies, little information is available regarding their exact densities. This is because densities are so low that most samples register zero per volume analysed.

4. No information is available about the statistical distribution of pathogen densities in the drinking water supply in the UK or the USA. Analogies, however, may be made to densities of total heterotrophic bacteria which are approximately lognormally distributed. Furthermore the lognormal distribution of *Giardia* cyst densities in US rivers may be preserved in drinking water treatment.

5. Information on tap water consumption in the USA is suitable for risk assessment modelling.

5. ADVICE ON COST EFFECTIVE METHODS TO IMPROVE THE QUALITY OF DATA INPUTS FOR A UK MODEL.

The cost of building better models and collecting more accurate data must be balanced by the improved value of the information obtained. A risk assessment model is only as good as the weakest link. Thus, improving data in one area, for example, dose-response information, will not be cost effective unless complimentary and equal improvements are made in other areas, for example, statistical distributions of pathogen densities in the drinking water supply.

There are, however, areas where a fundamental change in assumption could make a dramatic improvement in drinking water models. In particular, UK models should avoid using a single point estimate for pathogen density and not adopt the US assumption of a Poisson distribution. Drinking water microbiological risk assessment models should now be developed through consideration of the proportions of consumers exposed to different pathogen densities (as shown for coliforms in Section 4.1.6). This would avoid the meaningless concept of the most exposed individual and provide an assessment of risk across the whole population. It would appear that US researchers and risk modellers do not appreciate the implications of a lognormal statistical distribution of pathogen densities within the supply. In particular they do not know how to accommodate all the zero data points. Calculating means based only on the positive data (LeChevallier *et al.* 1991b), or considering zeros as zeros (Payment *et al.* 1985) may give misleading and invalid statistical parameters. Adopting a lognormal distribution is the most cost effective way to solve these problems.

The weakest link of US microbiological risk assessment models for drinking water supplies appears to be our understanding of pathogen densities within the drinking water supply. Indirect evidence is presented in Section 4.1.6 that *Giardia* cysts may be lognormally distributed in drinking water supplies in the US. This is not to say that dose-response modelling could not be improved. Sections 2.3 and 2.4 illustrate some problems with applying dose-response curves to drinking water samples and across the general community.

Cost effective methods to improve risk assessment models are now considered.

5.1 Dose-response data for UK models

There is unlikely to be one single dose-response curve for each specific pathogen that will make risk-assessment models applicable to the general community. It is apparent for each pathogen that different dose-response curve data would be needed for healthy adults, infants, pregnant women, AIDS patients, the elderly, people with different vaccination backgrounds and natural immunities. Furthermore, currently available dose-response data are complicated by selection of volunteers according to low antibody titre or provision of an antacid before the pathogen dose. Further volunteer studies are not the most cost

effective way forward and may complicate matters further. Mathematical models relating risk of infection to actual pathogen doses (0, 1, 2, 3, 4, 5....pathogens) should be developed for available dose-response data.

One method to obtain dose-response data applicable to a general community would be a prospective epidemiology study. This would not only be very expensive but also fundamentally flawed because of the difficulty in determining the statistical distribution of pathogen density within the supply during any study.

Before considering expenses of developing dose-response data, the following question should be considered:-

Are dose-response data really needed in UK drinking water risk assessment models?

Answer: Perhaps Not.

The 'heart' of the US risk assessment models is the dose-response curve. Indeed, Haas (1983) has considered these in great detail, with relatively little consideration of pathogen density in the models later developed (Haas *et al.*, 1993). But are they really necessary in drinking water models? All a dose-response curve does is relate proportion of infected people to dose. Indeed it is shown in Section 2.4.2 that the US model for viruses (Haas *et al.* 1993) in reality only considers exposure to doses of 0 or 1 pathogen. So really the only information needed in US models is what proportion of people register infection after ingestion of a dose of one pathogen, e.g. for rotavirus this is 31% (Table 2.5).

UK risk assessment models could avoid a specific dose-response curve for each pathogen by making the assumption that persons exposed to one or more pathogens through the drinking water supply will be infected. The advantage of this approach is that the worst case scenario is predicted. Three facts add credibility to this approach.

1. Exposure to less than one pathogenic organism will not cause infection. US risk assessment models, through extrapolation of dose-response data, consider effects of fractions of a pathogen in the case of rotavirus. This is not appropriate for drinking water supply microbiology.

2. A single pathogen is capable of causing infection. From the dose-response curve for rotavirus, some 31% of healthy adults are expected to be infected by a single rotavirus particle. For protozoa, relatively small doses affect large proportions of volunteers. The dose-response curve for *Giardia lamblia* shows that 100 cysts will infect some 86% of volunteers. Indeed in Rendtorff's experiment, 2 out of 2 volunteers were infected by a dose of just 10 cysts. A dose of 214 *Cryptosporidium parvum* oocysts will infect 50% of volunteers. There is also evidence from retrospective studies of foodborne salmonella outbreaks that single organisms are capable of causing disease.

3. Micro-organisms, including perhaps pathogens, are clustered in drinking water supplies. Indeed, one water company operator once remarked, "One does not normally get just one coliform in a sample, it's usually hundreds" referring to

coliform-positive 100-ml volume samples. This could suggest that persons ingesting a dose of pathogens from a drinking water supply will get more than just one pathogen per glass. Consideration of the coliform density data (Figure 2.1) collected from a UK water company under the Water Supply (Water Quality) Regulations (1989) shows:-

- 93.7% of samples recorded 0 coliforms per 100 ml
- 2% of samples recorded 1 coliform per 100 ml.
- 4.3% of samples recorded more than 1 coliform per 100 ml.
- 2% of samples recorded 10 or more coliforms per 100 ml.

In statutory monitoring data pooled from 11 supply zones of much poorer microbiological quality from another UK company:-

- 80.2% of samples recorded 0 coliforms per 100 ml
- only 3.8% of samples recorded 1 coliform per 100 ml
- 7.4% of samples recorded >10 coliforms per 100 ml
- 2.1% of samples recorded >100 coliforms per 100 ml

It is apparent that although the majority of samples register 0 coliforms per 100 ml, the chance of ingesting 10 or more coliforms per 100 ml is almost double the chance of just a single coliform. This reflects the lognormal nature of the statistical distribution for coliform densities. Thus, the lognormal distribution for pathogens in a drinking water supply may diminish the need for dose-response curves in UK risk assessment models for the more infectious agents such as rotavirus.

To further this approach information on the proportions of the general community resistant to infection, through acquired immunity, is needed.

This approach would not be appropriate to the less infectious pathogens such *as Vibrio cholerae* (Crowcroft, 1994) and *Cryptosporidium parvum* (DuPont *et al.* 1995). Dose-response information for each pathogen type is needed in MRA modelling to account for differences in infectivity between highly infectious agents like rotavirus and less infectious agents such as *V. cholerae.*

5.2 Pathogen Exposure in UK models

The major problem in microbiological drinking water supply research is that pathogens are so dilute (usually) that even the majority of large volume samples (1,000 litres) are negative (i.e. zero, Table 2.3). This makes determination of the statistical distribution for pathogens in the supply difficult. Even for coliforms in 100 ml volumes, 95% or more samples typically register zero (Figure 2.1).

More powerful methods of pathogen detection are dependent on selective and efficient methods of pathogen concentration from large volume samples. Development of such methods is expensive. Furthermore large volume sampling programmes, which would provide more information on pathogen and coliform statistical distributions, appear to be unpopular with water supply companies.

One solution, which is cost-effective, therefore is to estimate the statistical distribution of pathogens in the drinking water supply from:-

a) the statistical distribution of pathogens in the raw water. This could be measured by sampling using reasonable volumes since pathogens are present in raw water in higher numbers.

b) the log-reduction by a particular drinking water treatment process on the distribution for each pathogen

Section 4.1.6 describes how this approach would be used to predict a lognormal distribution for *Giardia* cyst densities within the US drinking water supply. It is suggested that *Cryptosporidium* oocyst density data for UK surface waters are analysed as lognormal distributions. By applying the removal efficiencies (Table 4.2) for different treatment processes the statistical distribution for *Cryptosporidium* oocysts in drinking water networks supplied by different source waters could be predicted. This would provide an estimation of what proportions of consumers are exposed to what densities of oocysts, enabling quantitative risk assessment to be performed.

Two advantages of this approach relate to its flexibility. These are:-

1. The effects of adding new treatment processes or failure of a treatment process on health across the community could be predicted.

2. Effects on health of fluctuations in quality (i.e. oocyst numbers) in the raw water could be predicted. It may be possible to consider effects of agricultural inputs of *Cryptosporidium.*

5.3 Tap water consumption data for UK models

This is probably the best defined information available for drinking water risk assessment models. All that needs to be done is to apply the log-normal analysis of Roseberry and Burmaster (1992) to the UK data (Hopkins and Ellis, 1980). Market research to get

improved information on consumption (seasonal and age group variation) of unboiled tap water is not needed for risk assessment models until dose-response relationships and pathogen density data have been improved.

5.4 Other methods to improve the quality of data for UK models

Better reporting of waterborne illnesses

Validation of any risk assessment model is as important as developing it and requires accurate information regarding UK waterborne outbreaks including subsequent secondary transmissions of infection. To this end the PHLS Communicable Disease Surveillance Centre has devised a scheme to encourage reporting of such cases. However, this scheme is only at the pilot stage. Furthermore, it relies on cases being reported to GPs. As far as the PHLS is concerned there is no outbreak if nobody registers illness at the GP. Information on the number of unreported cases relative to reported cases would be useful for calibration of risk assessment models, which will predict the total number of cases.

Furthermore, the PHLs' task of diagnosing illnesses in patients could be facilitated by better detection methods for viruses. Electron microscopy (EM) is the main diagnostic method for viruses in diarrhoea. EM, however, may bias results by missing astroviruses in particular. There is a requirement for a more widely applicable diagnostic method for viruses including astrovirus, small round viruses, small round structured viruses, adenovirus and rotavirus. Furthermore the method should rely less on the good morphological preservation of the sample and the skill of the operator than EM. It should also be applicable to those viruses which remain uncultivable. Serological methods, especially those based on monoclonal antibodies have been successful to some extent.

Quantitative information on potential for secondary spread

Microbiological risk assessment models developed to date for drinking water supplies use water as the primary source of infection. However, for many pathogens transmitted by the faecal-oral route there is potential for secondary infection through person-to-person contact or the infected person-to-food route. Indeed person-to-person, rather than common source, transmission probably accounts for most cases of hepatitis A virus infection. Algorithms to predict the effect of secondary spread from a point source waterborne infection are needed.

5.5 Conclusions

US models risk assessment models for drinking water concentrate on the dose-response curve. It is proposed that a UK risk assessment model should concentrate on exposure to pathogens through drinking water.

Advice on cost effective methods to improve inputs for a UK model is:-

1. The most cost effective method to improve quality of inputs for a UK model is to consider modelling the statistical distribution of pathogen densities in the drinking water supply. This could be achieved by considering the effect of different drinking water treatment processes on available data for pathogen densities in the raw waters through lognormal probability plots. This approach would allow both environmental fluctuations in pathogen input and effect of treatment process on health risk to be modelled.

2. The data and technology are available to develop a UK risk assessment model for *Cryptosporidium*.

3. Conducting further volunteer studies (with more subjects than in previous experiments) to obtain more detailed and specific dose-response data would be a hugely expensive task, difficult to direct, and would probably complicate interpretation of risk assessment models further.

4. Dose-response curves may not be necessary for drinking water models. In the case of viruses, protozoa and perhaps salmonellae, small doses (<100 organisms) infect considerable proportions of healthy volunteers. A feature of the lognormal nature of the pathogen density distribution is that pathogens are likely to occur in clumps, i.e. consumers either are exposed to high enough doses to induce infection or are exposed to zero pathogens. **Thus, infection could be modelled on the proportion of consumers exposed through drinking water to doses of one or more pathogens.** This would give a worst case scenario across the community.

5. Information on proportions of the general population which are resistant to high doses (through immunity from previous exposure) is needed to develop the approach further.

6. In the course of time, volunteer studies will undoubtedly be carried out for different pathogens and their dose-response curves will be of interest to risk assessment models for drinking water supplies.

7. Expense on further tap water consumption data is not justified until problems with pathogen density have been overcome.

6. IDENTIFY RISK REDUCTION OPTIONS FOR UK AND ASSESS WITH RESPECT TO HEALTH, ENVIRONMENTAL AND COST BENEFITS.

While US models indicate to some extent to what levels pathogen concentrations should be reduced they provide no information on the most cost effective way to achieve the reduction. Models such as Figure 4.1 would allow all the risk barriers and inputs for a particular pathogen to be considered as risk reduction options. These would include sewage treatment, input of pathogens to the environment and drinking water treatment itself. Integrating data for the various barriers and inputs into a model to predict the statistical distribution of pathogen densities in the drinking water supply (Figure 4.4) would enable the effectiveness of the risk reduction options on health and environment to be assessed. By adding in the capital costs and operating costs for the different barriers in relation to removal efficiency for each pathogen, the cost effectiveness of the risk reduction options could be assessed.

In Tables 6.1 and 6.2 capital and operating costs, respectively, are provided for the different processes of drinking water treatment. Table 4.2 provides the removal efficiencies of the different processes for *Cryptosporidium* and groups of pathogens. Starting with the statistical distribution for *Cryptosporidium* densities in a UK raw water (e.g., Line X, Figure 4.4) and using data from Table 4.2, the statistical distribution for oocysts in the drinking water supply could be predicted after combinations of different treatment processes (Lines Y or Z, Figure 4.4). The proportions of consumers exposed to oocysts could be estimated from these lines. By considering the costings (Tables 6.1, and 6.2) for each process the most cost effective combination of drinking water treatment processes for *Cryptosporidium* could be determined. Further data on sewage treatment removal and input of *Cryptosporidium* into the raw waters could be used to consider changes in Line X (the raw water quality). This would further the approach to considering the cost effectiveness of controlling agricultural inputs or sewage treatment processes.

6.1 Conclusions

1. Through lognormal probability plots, costs for changing treatment strategies or reducing environmental inputs may be related to pathogen exposure through drinking water supplies. This would identify the most cost effective risk reduction options for each pathogen.

2. Operating and captial costs are available for drinking water treatment processes.

Table 6.1 **Capital cost estimates (in £'000) for unit processes for a range of plant sizes - taken from Water Industry construction cost estimating manual (TR61) WRc UC1946, May 1993.**

Unit process	1 Ml/d	10 Ml/d	100 Ml/d
Rapid gravity filtration (RGF)	110	430	1,630
Chemical coag/RGF	120	450	1,720
Chemical coag/FBC/RGF	230	1,000	5,270
Chemical coag/DAF/FBC	544	1,600	9,100
Microfiltration	200	990	8,500
Chlorine M&E	29	61	130
Chlorine M&E + contact tank	38	106	339
Ozone	100	400	2,500
Ultra violet	15	50	300

Table 6.2 **Operating Cost Estimates in p/m^3 treated. Estimates based on National Comparability Study (1986/87) from data given in, "A guide to water treatment processes and practices", WRc 854-S, 1989.**

Treatment type	1 Ml/d	10 Ml/day	100 Ml/d
RGF only	5 - 10	2 - 6	1 - 3
Chemical coag/RGF	7 - 12	4 - 8	3 - 5
Coagulation/Clarification/RGF	8 - 13	5 - 9	4 - 6
Microfiltration	5	3	2
Chlorine	1	1	0.5
Ozone	2	1.5	1
Ultra violet	1	1	0.5

7. CONCLUSIONS

This report comprises five sections, reflecting the objectives of the contract. The conclusions are treated separately for each objective.

7.1 Review of risk assessment models world-wide.

Information of microbiological risk assessment models for drinking water supplies developed and used in the United States by their Environmental Protection Agency was obtained from the literature. To seek information on risk assessment models being developed world-wide, researchers in Israel, South Africa, Greece and Spain were approached. However, no information was obtained. Therefore this report only considers US models. The conclusions from a critical review and assessment are:-

1. US risk assessment models for drinking water supplies are no more than dose-response curves.

2. Single point estimates used for pathogen exposures from drinking water in US models are of little use and inappropriate statistically.

3. The fundamental question in drinking water risk assessment, "What proportion of consumers are exposed to what numbers of pathogens?" is not considered in US models.

4. Uncertainties are based on uncertainty of the dose-response data and do not consider uncertainty in pathogen exposure estimates.

5. A manifestation of the assumptions made about pathogen densities in the drinking water supply is that in US models consumers are effectively restricted to doses of either zero pathogens or one pathogen. Evidence is presented that pathogens are clustered within the drinking water supply and it is conceivable, by analogy to coliforms, that some consumers could be exposed to much higher doses than just one pathogen. By ignoring clustering, US models may overestimate risks across the community from more infectious agents such as viruses and protozoa, but underestimate risk from less infectious organisms such as certain bacteria.

6. The mathematical models used in the US risk models for dose-response curves may not be appropriate to micro-organisms in drinking water supplies. Indeed, US models rely on extrapolation to doses of fractions of a pathogen. This is unsound and would be unnecessary if mathematical models relating risk from integer doses of 0, 1, 2, 3, 4, 5.... pathogens were developed.

7. Dose-response curves are not appropriate to the general community, including infants, the elderly, the immunocompromised, and persons of varying acquired immunities. Some dose-response data were obtained after antacid consumption and

may overestimate risk. Bacteria, in particular salmonellae, may be more infectious than suggested by dose-response data.

8. US models do not model secondary spread.

7.2 Developing UK risk assessment models for specific pathogens

Epidemiological data and UK occurrences were reviewed for waterborne pathogens including those of emerging interest. The conclusions for development of UK models for specific pathogens are:-

1. Risk assessment models should be developed in the UK for any pathogen which presents a potential or proven waterborne hazard. This includes pathogens which are more prevalent in the developing world because of the possibility of infected individuals entering the UK.

2. Specific waterborne pathogens should not be considered alone, particularly if models are to be used for identifying the most cost effective risk reduction options or with the aim of reducing chlorination to eliminate disinfection by-products.

3. Epidemiological information on individual pathogens highlights the necessity to customise UK risk assessment models for each pathogen.

4. The impact of secondary transmission from a primary waterborne source should be considered in risk assessment models for pathogens such as *E. coli O157*, hepatitis A virus, *Helicobacter pylori*, rotavirus, Norwalk virus, *Cryptosporidium* and *Giardia lamblia.*

5. Risk characterisations for UK models should vary according to pathogen type and the proportion of high risk consumers (e.g. pregnant women, AIDS patients) should be considered.

7.3 Review of necessary data inputs for a UK model and assessment of the state of appropriate data knowledge in the UK

1. No information is available in the UK to determine what proportion of consumers are exposed to what numbers of pathogens. Indirect evidence is presented that pathogen densities are lognormally distributed in the drinking water supply.

2. Some information is available for pathogen concentrations in surface water in the UK.

3. Considerable information is available on rates of pathogen removal by drinking water and sewage treatment processes.

4. UK *Cryptosporidium* data for source waters should be analysed on lognormal probability plots.

5. Information on tap water consumption in the UK is available.

7.4 Cost effective methods to improve data inputs for a UK model.

1. Further volunteer studies to obtain more dose-response data are not the most cost effective way forward. Indeed, it is suggested that because of pathogen clustering dose-response curves may not be necessary for drinking water risk assessment models. It is possible that most drinking water consumers are either exposed to high enough doses of pathogen to induce infection or are exposed to zero pathogens.

2. The most cost effective method to improve data inputs for a UK model is to consider modelling the statistical distribution of pathogen densities in the drinking water supply. This would indicate what proportion of consumers are exposed to one or more pathogens. A method which accounts for source water loading and efficiency of treatment processes is proposed. This could be applied to UK *Cryptosporidium* data.

3. The data and technology are available to develop a UK risk assessment model for *Cryptosporidium.*

7.5 Modelling the cost effectiveness of risk reduction options

1. Through lognormal probability plots, costs for changing treatment strategies or reducing environmental inputs may be related to pathogen exposure through drinking water supplies. This would identify the most cost effective risk reduction options for each pathogen.

2. Operating and capital costs are available for drinking water treatment processes.

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