WHO Guidelines for the Safe Use of Wastewater and Excreta in Agriculture

Microbial Risk Assessment Section

S. A. Petterson & N. J. Ashbolt

Summary

Quantitative Microbial Risk Assessment (QMRA) provides an alternative or supplementary framework to epidemiology for identifying potential excess risk for defined pathways of particular pathogens from source to recipient. Both QMRA and epidemiology are environmental health assessments that fit between the public health outcome and setting of health target components of the WHO harmonised framework (Bartram *et al*., 2001). QMRA translates the environmental occurrence of pathogens to the probability of infection (microbial risk) following the paradigm used for chemical risk assessment, and has the potential to provide much greater sensitivity in identifying risk. The usefulness of QMRA, however, is dependent upon the quality and appropriate use of available data for describing the occurrence, persistence and human dose-response of pathogens in the environment.

Given the vagaries in data on immune status and variability, QMRA can only predict potential excess risks, and for specific pathogens and pathways. Further, limited dose-response data is available, and in some cases varies by more than a 1000-fold for different strains of the one pathogen. For the time being, therefore, QMRA should not be seen as directly comparable to epidemiological data, but rather, a tool to assess the sensitivity of changes in performance of (treatment) elements and to identify major risk groups and pathways.

The review provides a summary of available data, its use in QMRA and some example outcomes that may aid in setting health targets. For example, Echovirus infection risks via aerosols during wastewater irrigation can be modelled using Gaussian plume dispersion methods and indicat little health risk from aerosols even at distances as close as 20m to the irrigation source when the effluent concentration was $\leq$ 1 plaque forming unit (pfu).L^{-1}. However as the virus concentration increased, acceptable separation distance (irrigation plume to receptor) increased to 400m for 100 pfu.L^{-1} and $\geq$ 600m for $\geq$1,000 pfu.L^{-1}. The acceptable separation distance was defined as the distance at which the calculated infection risk was $\leq 10^{-4}$ per year. For highly infectious Rotavirus and *Giardia*, the acceptable separation distances increases substantially.

An example of the application of sensitivity testing of various model components is illustrated for virus risk associated with wastewater irrigated salad crops. For the lettuce model, virus inactivation was the most important component for reducing risk. On the surface of the lettuce leaf, conditions greatly promote virus inactivation due to exposure to sunlight, high temperatures and desiccation. Inactivation rates were therefore very high (best estimates of 2.5 d^{-1} fast phase, and 0.5 d^{-1} slow phase) and therefore, if sufficient time is allowed between final irrigation and consumption (for example 14 days) then exposure of the consumer to infectious viruses would be very low.

Application of the methods and approaches suggested in this review should enable the quantitative assessment of microbial risks in many wastewater/excreta reuse applications in agriculture. Current concepts of limits for thermotolerant coliforms or *Ascaris* eggs may still be applicable, but the strength of QMRA is in its provision to assess treatment step performance needs and identify zones for critical control. Many controls may pragmatic rather than relying on microbiological analyses, such as withholding times after application of the reuse material or simply the provision of sufficient numbers of treatment ponds and estimation of their residence times.

Introduction

Use of recycled excreta, sewage effluents and nitrified river waters may provide for sustainable agriculture, yet transmission of enteric pathogens is a fundamental public health consideration for such practices. Evaluation of hazards from any particular agricultural practise to the consumer, worker or neighbouring community has traditionally been undertaken from sound experience and an epidemiological perspective. As discussed elsewhere in these guidelines, epidemiology is the study of exposure factors and the occurrence of disease in human populations (Blumenthal *et al.*, 2001).

Within an epidemiological framework the excess risk of a particular disease (such as diarrhoea) attributable to the reuse pathway is measured using scientific evidence. The primary limitation of this approach is the uncertainty associated with the collection of information and the influence of confounding factors; both of which reduce the sensitivity of epidemiological methods for identifying excess risk. Most outbreaks of waterborne disease are therefore not identified by epidemiological methods unless at least one percent of the population in a community becomes ill within a few months (Regli *et al.*, 1991). Not surprisingly therefore, direct epidemiological evidence for excess risk resulting from wastewater use in agriculture is extremely limited (Shuval *et al.*, 1985, 1986; Blumenthal *et al*., 2000; Cifuentes *et al.*, 2000; Devaux *et al*., 2001).

Quantitative Microbial Risk Assessment (QMRA) provides an alternative framework that may be used in conjunction with epidemiological methods for identifying potential excess risk. Both QMRA and epidemiology are environmental health assessments that fit between the public health outcome and setting of health target components of the WHO harmonised framework (Bartram *et al*., 2001). QMRA translates the environmental occurrence of pathogens to the probability of infection (microbial risk) following the paradigm used for chemical risk assessment (Haas, *et al*., 1999), and has the potential to provide much greater sensitivity in identifying risk. The usefulness of QMRA, however, is dependent upon the quality and appropriate use of available data for describing the occurrence, persistence and human dose-response to pathogens in the environment.

Quantitative risk assessment techniques were originally developed for evaluating the risk associated with exposure to chemical hazards (National Academy of Sciences, 1983). When QMRA was first undertaken, the conceptual framework for undertaking chemical risk assessment was applied directly for evaluating microbial risk, and consisted of the following steps:

> **Hazard identification:** the range of pathogens (classes and species of disease-causing organisms) that are to be considered in the risk investigation are identified;
>
> **Exposure assessment:** the magnitude of exposure (the number of organisms consumed) for each identified hazard is characterised;
>
> **Dose-response assessment:** the expected physical response (infection/disease) to the hazard in the population is evaluated (dose-response relationship); and

Risk characterisation: the likelihood of infection and illness in the exposed population is calculated and assessed.

The chemical risk assessment framework allowed for the numerical tracking of pathogens through the environment, however some important differences between microbial and chemical agents were identified, leading to limitations with the chemical risk framework Craun *et al.,* (1996):

1. The concentration of pathogens in environmental samples can grow or decline due to reproduction and inactivation.

2. Microorganisms are not uniformly distributed, but rather may exhibit a heterogeneous distribution due to clumping or aggregation.

3. Infectious diseases differ from chemical agents as a person who is infected may proceed to infect additional people. These secondary cases may be people who have had no direct contact with the initial vehicle of exposure (secondary spread).

4. Variation in susceptibility: There are a complex set of immune responses including short and long term immunity that may alter the dose-response relationship and the severity of outcomes. Some sections of the community including children, the elderly, pregnant women and severely immunocompromised individuals are particularly sensitive (Gerba *et al*., 1996). Furthermore, immunosuppressed hosts are not only more likely to become infected but also develop chronic disease with more severe health outcomes.

Further developments of the QMRA framework have attempted to incorporate the unique characteristics of microorganisms into risk models. The International Life Sciences Institute (ILSI) Risk Science Institute (RSI) in co-operation with the USEPA Office of Water convened a working group to develop a conceptual framework to assess the risks of human disease associated with pathogenic micro-organisms (Craun *et al*., 1996) (Box 1). Based on this ILSI framework, two case studies were presented: firstly, a static model for *Cryptosporidium* in drinking water (Teunis and Havelaar, 1999) with an emphasis on correctly analysing microbial datasets and describing uncertainty and variability within the risk model; and secondly, a dynamic model for rotavirus (Soller *et al*., 1999), based on the epidemiological approach previously presented for recreational water exposure to *Giardia* (Eisenberg *et al*., 1996). The epidemiological framework for evaluating pathogen risk presented by Eisenberg *et al.* (1996) took a population perspective in the development of a mathematical model. The model made explicit the mechanistic aspects of the infectious disease process and incorporated such data as incubation period, immune status, duration of disease, and the rate of symptomatic development. Chick *et al.* (2001) have also described models with different levels of person-to-person spread and immunity which suggests that epidemiological models are required to complement previous approaches in MRA to fully assess environmental pathogen pathways. The ILSI framework was later enhanced through some minor refinements and elaboration based on the outcomes of these two case studies (ILSI, 2000) and a second joint workshop with US-EPA (2002).

BOX 1: The International Life Sciences Institute (ILSI) Framework for assessing the risk of human disease following exposure to pathogens (Summarised from Craun et al., 1996)

The working group convened by the International Life Sciences Institute (ILSI) Risk Science Institute (RSI) in co-operation with the U.S. EPA Office of Water, was asked to consider a number of issues including:

1. the dynamic and iterative nature of the risk assessment process
2. the role of risk managers, risk assessors and stakeholders; and
3. the wide variety of potential scenarios such as the risk of human disease associated with pathogens in drinking water, recreational water, or sludge, foods, devices and other media.

Discussion of these issues led to the development of a conceptual framework that is illustrated in Figure 1 and summarised in the following sections.

Figure 1. Generalised framework for assessing pathogen exposures from wastewater/excreta reuse in agriculture

Problem Formulation

Problem formulation is the systematic planning step that identifies the goals, breadth and focus of the risk assessment, the regulatory and policy context of the assessment, and the major factors that will need to be addressed for the assessment. A critical component of the problem formulation phase is to determine the purpose of the risk assessment, and the unique questions that the assessment is to address.

The problem formulation phase then proceeds to an initial characterisation of exposure and health effects. A conceptual model is developed that describes the interactions of a particular pathogen or medium and defined population and exposure scenario. The model also describes the specific questions to be addressed, the relevant information needed, the methods that will be used to analyze the data, and the assumptions inherent in the analysis. The conceptual model provides direction for the analysis phase of the assessment.

Analysis Phase

The analysis phase consists of two elements, characterisation of exposure and the characterisation of human health effects. While these two elements are considered to be separate the analysis should be interactive to ensure that they are compatible.

Characterisation of Exposure

Characterisation of exposure involves an evaluation of the interaction between the pathogen, the environment and the human population. Three elements of analysis may be involved:

Pathogen Characterisation: Determining the properties of the pathogen that affects its ability to be transmitted to and cause disease in the host.

Pathogen Occurrence: Characterising the occurrence, distribution and physical state of the pathogenic microorganism including information on the ability of the pathogen to survive, persist and multiply.

Exposure Analysis: Characterising the source and temporal nature of human exposure.

Exposure Profile: The exposure profile provides a qualitative and/or quantitative description of the magnitude, frequency and patterns of exposure for the scenarios developed during problem formulation. A critical component of the exposure profile is an assessment of the assumptions and uncertainties that are made during the analysis.

Characterisation of Human Health Effects

Characterisation of human health effects involves the interactive analysis of three critical components: host characterisation, evaluation of human health effects, and quantification of the dose-response relationship.

Host Characterisation: Evaluation of the characteristics of the potentially exposed human population that may influence susceptibility to a particular pathogen including age, immune status, use of medications, genetic predisposition, pregnancy and nutritional status. The analysis may also consider whether and how social and/or behavioural traits influence susceptibility or severity.

Health Effects: The clinical illness associated with the pathogen or medium is characterised. The whole spectrum of clinical manifestations should be considered including symptomatic and symptomatic infection, duration of clinical illness, mortality and sequelae. Data from epidemiological investigations are the primary input for this phase.

Dose-Response Analysis: This analysis evaluates the relationship between dose, infectivity and the manifestation of clinical illness.

Host-Pathogen Profile: The host-pathogen profile provides a qualitative and/or quantitative description of the nature and potential magnitude of adverse human health effects for the scenarios developed during problem formulation. A critical component of the host-pathogen profile is an assessment of the assumptions and uncertainties that are made during the analysis.

Risk Characterisation

The likelihood of adverse human health effects occurring as a result of a defined exposure scenario to a microbial contaminant or medium is estimated. Risk characterisation consists of two major steps:

Risk Estimation: Risk estimation describes the types and magnitude of effects anticipated from exposure to the microbe or medium.

Risk Description: All assumptions that were made throughout the risk assessment should be clearly identified and their impact on the assessment described. The uncertainties associated with problem formulation, analysis, and risk characterisation should be identified and quantified where possible. The confidence in the risk estimates should be expressed and include consideration of the sufficiency and quality of the data, and evidence of causality.

The risk characterisation should include a discussion of whether the assessment adequately addresses the questions delineated during problem formulation.

The QMRA framework has been applied to case studies in the United States (Asano *et al.*, 1992; Tanaka *et al.*, 1998; Dowd *et al.*, 2000), Israel (Shuval *et al.*, 1997) and Australia (Gardner *et al.*, 1998; Petterson *et al.*, 2001a,b; Storey and Ashbolt, 2002) for evaluating microbial risk from wastewater reuse in agriculture. The underlying data sources and assumptions of each model are summarised in Table 1. Each component of these models will be discussed more fully in the following sections.

1 Problem formulation and Pathogen Hazards (associated with wastewater/excreta reuse)

1.1 Pathogen Hazards

The first step in any microbial risk assessment is to identify the pathogen hazards that are to be investigated. There are literally hundreds of different pathogenic microorganisms that may be present in human faeces collected from communities. These organisms are grouped according to common characteristics and classified as viruses, bacteria, protozoa and helminths (see Box 3).

It is simply not possible for a risk assessment to take into detailed consideration all potential pathogenic microorganisms. Rather, the most relevant pathogens affecting the study population must be identified and targeted in the risk investigation.

Individual pathogens may also be selected to represent an entire group; for example, Rotavirus may be modelled to represent all enteric viruses, as it is both common and one of the most infectious enteric viruses. Furthermore, a single synthetic pathogen may be considered, with the most hazardous attributes, such as considerable environmental persistence and highly infectious representing features of hepatitis A and rotaviruses respectively. The results from the representative are expected to conservatively reflect the behaviour of all pathogens in that particular group, and may be referred to as the Reference Pathogen. The primary limitation of this approach is that all pathogens within each group do not behave identically. It is therefore important to select the reference pathogen carefully and conservatively. When selecting appropriate representative organisms the following factors should be taken into consideration:

1. The occurrence and distribution of enteric disease in the exposed population. The prevalence of enteric disease varies throughout the world depending on geographic location, socioeconomic status, sanitary conditions and season.

Diseases of particular concern for the study area along with the rate of endemic disease may be identified from epidemiological studies (local or from similar types of environments).

2. The severity and infectivity of different diseases. An individual pathogen may be targeted due to the severity of consequences (e.g. hepatitis E) or if it is prevalent and rapidly spread in the community (e.g. Rotavirus).

3. The persistence and behaviour of a pathogen in the environment are critical aspects relating to its environmental significance as a wastewater/excreta hazard. An enteric pathogen may be prevalent in a population and result in severe health consequences, however if that organism has been shown to be rapidly inactivated during wastewater treatment and under environmental conditions, then its significance for risk assessment is drastically reduced.

Table 2 includes a summary of pathogen types that have been modelled for risk assessment including the primary advantages and limitations of each as an index of their pathogen group.

Box 2 Enteric Pathogen Hazards: Viruses, Bacteria, Protozoa and Helminths found in excreta; including example reference pathogens & their characteristics, and typical environmental form for the group

Hazard group	Example Reference Pathogens	Characteristics of reference pathogens	Environmental stage, size (µm) and shape for group
Viruses	rotavirus	Highly infectious, not as persistent as HAV, Norwalk-like viruses and some other enteric viruses	Virion (0.02-0.08) generally spherical, protein coat protecting nucleic acid (DNA or RNA)
Bacteria	*Salmonella* sp. or *E. coli*	Always present in sewage, readily inactivated by disinfection	cell or dormant cell (0.1-2) cocci-rod
Parasitic protozoa	*Cryptosporidium parvum*	Not as prevalent as *Giardia*, but highly persistent and halide resistant.	Cyst or oocyst (4-40) oval-spherical
Helminths	*Ascaris lumbricoides*	Most persistent in soil/faeces, embryo must develop prior to human exposure	Ova (egg, 30-80) variable

It is also important to note that acute diarrhoea may be followed by subsequence diseases (sequelae) of greater severity in a limited sub-population, although the one pathogen is involved. Examples of these are:

- Diabetes, which has been linked to Coxsackie B4 virus;
- Myocarditis, which has been linked to Echovirus;
- Reactive arthritis and Guillian-Barré syndrome associated to *Campylobacter jejuni* (reviewed by Nachamkin, 2002); and

- Gastric cancer which has been linked to *Helicobacter pylori*.

With the exception of *Helicobacter pylori*, the association of pathogens with acute waterborne disease and sequelae has been well established. Due to the long time between infection and subsequent sequelae, it is very likely that additional pathogens will be linked to chronic diseases in the future. A QMRA for waterborne *Campylobacter* infection and modelling of health burden (DALY) from diarrhoea, reactive arthritis and Guillian-Barré diseases has been presented by Havelaar *et al.* (2000).

1.2 Hazard Pathways and Scenarios

Potential routes of exposure to pathogens are identified as hazard pathways. Examples of four possible points of exposure are illustrated in the conceptual model provided in Figure 1. These are 1) direct contact with raw wastewater/excreta, 2) direct contact with the treated wastewater/excreta, 3) consumption of crops (with or without a withholding period since last application of waste), and 4) inhalation of pathogens from application aerosols. A fifth exposure pathway is also identified in Figure 1, that being from the waste to animals to humans, where the animal host may also amplify the pathogen in the 'environment'.

It is important to draw out these hazard pathways, not only to identify the data needs, but also for subsequent identification of potential control points to manage the risks. Furthermore, in addition to the hazard pathways, there are likely to be numerous scenarios that increase pathogen risk (Figure 2). Examples here are storm events where wastewater/excreta is moved to areas normally protect from contact, and breakdown in treatment leading to higher pathogen numbers in product(s). Such scenarios may also be manageable, and hence here we interface QMRA with the hazard analysis critical control point (HACCP) approach (Deere *et al.*, 2001) by identifying the higher risk pathways and events that management should focus on and potential target levels to control particular pathogens below.

2 Exposure Analysis

The second stage in the risk process requires the flow of pathogens through the hazard pathway(s) to the point(s) of exposure to be modelled. A generic framework for undertaking exposure assessment for the use of wastewater in agriculture is shown in Figure 2. The contribution of each component in the model should at least be ranked if not quantified, so that the assessment can focus on the higher risk pathways/scenarios first. Latter, sensitivity analysis can be used to examine the range of performance for each system component, which may also be used to suggest management options to control risks. The accuracy and reliability of each model is dependent upon the quality and appropriate use of available data. A summary of the primary data sources and their limitations is provided in section 2.1, and issues of variability and sensitivity analysis are provided in section 2.3.

Figure 2 Generic flow diagram illustrating factors influencing microbiological risks associated with wastewater/excreta reuse for agriculture

2.1 Data Sources

2.1.1 Pathogen Numbers in Human Excreta

The number of pathogens present in excreta varies as a function of the health of the host and the local environment. Communities with poor hygiene and a high proportion of children will produce excreta especially rich in enteric pathogens. Healthy individuals do not normally excrete pathogens for prolonged periods and therefore their contribution to pathogens in excreta is subject to wide fluctuations.

Due to the very limited quantitative data on individual pathogens in human excreta, it may be better to estimated numbers from epidemiologic data. Estimates should consider the incidence of gastrointestinal (and other appropriate) infections, along with typical excretion times and densities to generate mean or probability density functions representing the range of pathogens expected in a population's faeces, as described in Box 3.

2.1.2 Pathogen Numbers in Sewage Effluent

As with excreta, the number of pathogens present in wastewater varies as a function of numerous factors including geographic location, socioeconomic status, sanitary conditions and season. Nevertheless, nearly all communities' sewage contain pathogens, and the larger the contributing population, the less variable the concentration.

A summary of the pathogenic bacteria, viruses and parasites found in sewage along with reported concentrations is given in Table 3. While a large number of studies have been undertaken to characterise pathogen occurrence, the numbers of analysed samples is often small or not even reported. This is largely due to the complexity and expense associated with laboratory methods. These datasets are useful for providing approximate ranges and limits of pathogen numbers, however they do not necessarily represent the real variability that may be expected in pathogen concentration in sewage effluents.

Addition data on pathogen occurrence in sewage will not only provide better descriptions for QMRA, but may also provide a more sensitive assessment of community infection (Ranta *et al*., 2001).

Box 3 Estimating pathogen numbers in excreta from epidemiological data

In the absence of appropriate pathogen data for a population's excreta, either point estimates (means) or a probability density function (PDF), can be synthesised from the incidence of infection along with typical excretion times and densities for key pathogens. Point estimates can be used (with arithmetic means [Haas, 1996]) or a PDF described, such as the lognormal by the mean and its standard deviation and the PDF calculated by Monte Carlo simulations in one of many spreadsheet available

(Haas, 1997). An example with means and within parentheses, $\log_{10}$ means and $\log_{10}$ standard deviations are provided for four pathogens in the table below:

Pathogen	Infection rate (% of pop in region type)		Excretion time (d)		Excretion density (#.g^{-1})	
	Developed	developing	Adult	Child	Adult	
Campylobacter jejuni	15.6[a]		(1.18, 0.325)[d]		(8, 1)[d]	
enterohaemorrhagic *E. coli* (EHEC)	0.013[b] 3-9 in meat workers[j]	?	Less in adults, but continuous in some[j]	(1.23, 0.8)[k]	?	
Cryptosporidium parvum	0.31[a]		(1.48, 0.173)[e]		(7, 1)[g]	
Giardia lamblia	0.84[a]	27.4[i]	(1.18, 0.325)[d]	(year long)[h]	(7, 1)[h]	
rotavirus	0.95[c]		(1.0, 0.30)[f]		(10, 1)[f]	
Ascaris			(2.48, 2.22)[d]		(4, 1)[d]	
Mean,SD per gram faeces for population*	(,)	(,)	(,)	(,)	(,)	

[a] Mead *et al*. (1999), [b] Baljer and Wieler (1999), [c] Wheeler *et al*. (1999), [d] Faechem *et al*. (1983), [e] Stehr-Green *et al*. (1987), [f] Gerba *et al*. (1996), [g] Girdwood and Smith (1999), [h] Jakubowski *et al*. (1991), [i] Newman *et al*. (2001) values for Brazilian children, [j] Stephan *et al*. (2000), [k] Belongia *et al*. (1993). Values in parentheses are $\log_{10}$ means and $\log_{10}$ standard deviations respectively. * Log_{10} mean and SD calculated with @Risk V4.5 (Palisade Corp., USA) within Excel (Microsoft Corp.).

2.1.3 Removal of Pathogens during Wastewater Treatment

Wastewater treatment processes remove a high proportion of pathogens from the water column, but very large numbers of pathogens can be present in raw wastewater at times. The actual rate of removal can be variable during 'normal' operation, and may decrease significantly during short or long-term periods of poorer plant performance. Depending on the data available for the study area regarding the occurrence of pathogens in wastewater, it may be necessary to assume a treatment performance (point estimate or PDF) for pathogen removal from the treatment plant. A summary of reported removal rates for a range of treatment processes is included in Table 4a&b.

2.1.4 Pathogen Transport

Crop contamination

Quantifying the level of pathogen contamination on food crops following irrigation with wastewater or biosolids application is necessary for the risk model, however it has not been well characterised. There are three main pathways for crop contamination: the spray irrigation of surface crops, surface splash following

application of excreta/biosolids to soil during rainfall, and sub-surface-drip irrigation or rain leachate through biosolids to subterranean crops (e.g carrots).

The interaction of microbes to environmental surfaces is complex involving adsorption, desorption and inactivation or growth. Hence, the behaviour of a microbe in the vicinity of a plant surface is controlled by the relative surface characteristics of the microbe and the plant. Factors of principal importance are ionic and hydrophobic properties of the microbe and surface, its state of aggregation and the presence of other materials adsorbed to the pathogen's surface. The composition of the plant cell surface along with the pH, ionic composition and concentration influence the likelihood of microbial attachment (Gerba, 1984).

Previous risk assessments that examined spray irrigated surface crops relied on an important simplifying assumption: that any microorganism contained in the residual wastewater remaining on the irrigated crop would cling to the surface of the crop after the wastewater itself evaporated (Asano *et al.*, 1992; Shuval *et al.*, 1997). This assumption allowed for the level of contamination to be estimated using only the pathogen concentration in the irrigation water, and an estimated quantity of water retained on the crop (see Table 1). It is important to consider, however that this assumption did not allow for variations in microbial adsorption behaviour or rapid inactivation of pathogens during irrigation (which is likely in warm, light environments). An alternative model for describing microbial attachment to crops is therefore desirable, including a model for describing microbial attachment to sub-surface crops, such as described by Petterson *et al*. (2001).

Aerosols

Bacteria and viruses have been demonstrated in aerosols emitted by treatment facilities and sprinkler irrigation systems (Camman *et al.*, 1988; Fanin *et al.*, 1985, Applebaum *et al.*, 1984; Teltsch *et al.*, 1980). While the potential for exposure to pathogenic bacteria and viruses has been well documented, quantification of this process for risk assessment is still in its infancy.

Camann (1980) presented a simple Gaussian dispersion model to describe the transport of bacterial pathogens in irrigation aerosols as a function of effluent source concentration, irrigator characteristics and atmospheric conditions. The model predicts atmospheric pathogen concentration (organisms/m^3 air) as a function of downwind distance from the irrigation source, and incorporates pathogen inactivation into the calculation. The model was adapted by Gardner *et al.* (1998) to assess the risk from virus and *Giardia* levels measured in a range of sewage effluents in Queensland, Australia. This approach appears to provide enormous potential for quantifying aerosol dispersion (Box 4), however has not as yet been widely tested and applied.

Bioaerosol concentrations downwind of areas undergoing land placement of biosolids were investigated by Dowd *et al.* (2000). Actual data on airborne *Salmonella* and indicator viruses (Male-specific [F+] coliphages enumerated on *E. coli* (Famp)) obtained at a biosolid placement site (Dowd *et al.*, 1997) were used with mathematical models to obtain probable numbers of organisms located downwind from the application site. Dose-response models were subsequently used to determine the risk of infecting populations (such as land application workers or residents of nearby

population centres) that inhale pathogenic organisms originating from locations were land placement of biosolids is occurring.

Box 4: Gaussian Dispersion model for quantifying the health risk of spray irrigating treated sewage effluent

Gardner *et al.* (1998) developed a quantitative risk assessment model for spray irrigating sewage effluent by combing an existing Gaussian pathogen dispersion model (Camann, 1980) with quantitative microbial risk assessment methodology. The procedure for applying the Gaussian dispersion model as described by Gardner *et al.* (1998) is outlined below.

Model Description

The model describes the downwind dispersion and survival of pathogens in aerosols generated during the spray irrigation of effluent. The model assumes that aerosols diffuse in a random manner, resulting in a Gaussian (normal) distribution of pollutants at any specific downwind distance (x). Aerosols released from a point source will achieve a medium plume height (H) and diffuse in both the horizontal (y) and vertical (z) directions during travel along the downwind plume centerline distance (x). Gaussian dispersion underpins most odour and atmospheric pollutant models and its characteristics are well understood and described (e.g. Turner, 1994). The major advance made by Camann (1980) was to define the aerosol/pathogen emission rate from the irrigation source and describe the microbial survival upon aerosolation, and with aerosol age (i.e. downwind distance). The general expression for the microbial dispersion model is: $C_{(x)} = D_{(x)} \times Q_a \times M_{(x)} + B$

where $C_{(x)}$ is the pathogen density in the atmosphere (cfu.m^{-3} or pfu.m^{-3}) at any downwind distance x. $D_{(x)}$ is the atmospheric dispersion factor described by the Gaussian model (s.m^{-3}). Q_a is the aerosol source strength adjusted for loss of microbial viability during the spray process (pfu.s^{-1} or cfu.s^{-1}). M_x is the fraction of micro-organisms which remain viable at distance (x) from the source (dimensionless). B is the background microbial density in the atmosphere (cfu.m^{-3} or pfu.m^{-3}).

The pathogen source strength of the aerosols, Q_a, is a function of: effluent flow rate F (L.s^{-1}); pathogen concentration in effluent q (pfu.L^{-1}); aerosolation efficiency A i.e. fraction of sprayed effluent that becomes an aerosol; micro-organism impaction factor I i.e. the aggregate of factors affecting microorganism survival during the aerosolisation process (dimensionless). These factors are multiplicative and are described by:

$$Q_a = F \times q \times A \times I$$

Typical values for A for small rotating impact sprinklers is 0.003 to 0.01, with the value increasing with increasing temperature and wind speed (Camann, 1980). Typical values for I vary with the type of pathogen ranging from 0.13 for faecal coliforms, 0.7 for coliphage, and >>1 for enteric viruses. Values of $I > 1$ suggest

micro-organism clumping in the effluent which are disaggregated during the aerosolisation process (Camann, 1980).

Other factors which require evaluating are the Dispersion Factor, $D_{(x)}$, and microbial die off in the aerosol, $M_{(x)}$. $D_{(x)}$ is common to all Gaussian dispersion models and its value is a complex analytical function of atmospheric stability, downwind distance, windspeed and aerosol plume height (Camann, 1980; Turner, 1994). $M_{(x)}$ is based on a simple first order kinetic equation driven by aerosol age and a pathogen die off rate, λ ($.s^{-1}$) due to environmental stress. λ varies with the organism (typical value –0.02 to –0.05 for faecal coliform and 0 for enteric viruses) and atmospheric conditions, becoming more negative with high solar radiation and temperature, and low relative humidities (Camann, 1980).

Application

The Gaussian model was used to calculate the atmospheric concentrations of pathogens ($pfu.m^{-3}$) and then calculate the probability of infection as a function of downwind distance, pathogen density in the effluent and pathogen type. For the investigation focussing on enteric viruses and *Giardia*, the following assumptions were made:

Human respiration intake was assumed to be 30 $L.minute^{-1}$ for a 10 minute exposure.
Eflluent Flow Rate: $50L.s^{-1}$;
Plume Height: 3m;
Windspeed: $3m.s^{-1}$;
Atmospheric condition: E class (stable);
Aerosolisation Efficiency: 0.0033;
Impaction Factor: 1.0 for *Giardia*, 1.2 for viruses;
Die off Rate: 0.0 for *Giardia* and viruses; and
Exposure per year: 26

The results showed that for Echovirus there was little health risk from aerosols even at distances as close as 20m to the irrigation source when the effluent concentration was $\leq$ 1 $pfu.L^{-1}$. However as the virus concentration increased, acceptable separation distance (irrigation plume to receptor) increased to 400m for 100 $pfu.L^{-1}$ and $\geq$ 600m for $\geq$1,000 $pfu.L^{-1}$. The acceptable separation distance was defined as the distance at which the calculated infection risk was $\leq 10^{-4}$ per year. For highly infectious Rotavirus and *Giardia*, the acceptable separation distances increased substantially.

The authors noted that while the approach taken provided great promise, more work is required to define the aerosol drift physics as affected by gravity settling and evaporation; pathogen die-off rates due to time, temperature and sunlight; and pathogen virulence in small aerosols after droplet evaporation.

2.1.5 Pathogen Inactivation/growth in the environment

A wide variety of studies have been undertaken to investigate the rate of decay of micro-organisms on food crops (reviewed by Faechem *et al.*, 1983; Yates *et al.*,

1987). These studies have provided some ranges of potential survival times that may be expected under field conditions, along with identifying how environmental conditions influence pathogen survival.

Development of an exposure profile requires the use of microbial inactivation curves that describe the persistence of microorganisms over time. By using such a model, the fraction of the pathogen population that remains infectious can be calculated at any time following irrigation.

The inactivation of microorganisms has generally been regarded as a first order logarithmic process since Chick proposed the first order kinetic model for log-linear survival curves based on the analogy of the first order chemical reaction (Chick, 1908). A first order inactivation model was used by Asano *et al.* (1992) to describe the inactivation of enteric viruses in the environment for risk assessment in wastewater reuse. An inactivation coefficient of 0.69 d^{-1} was applied for a range of environmental conditions based on experimental data collected for a range of crops using poliovirus (Engineering Science, 1987).

The available data for assessing the most representative decay coefficient for different pathogens under field conditions is extremely limited. A brief summary of reported values is included in Table 5 Many of the studies are based on few data points and were conducted under specific environmental conditions that are difficult to translate to different climates around the world. In addition, there is still question as to whether the first order inactivation model is the most appropriate to be used in risk assessment. Since the 1960s with an increase in available data, evidence for deviations from single phase log-linear inactivation including shouldering and tailing-off phenomena has become apparent (Hiatt, 1964; Cerf, 1977; Rennecker *et al.*, 2000). *Ascaris* inactivation in sludge also has been sown to initially show an initial period of roughly first-order inactivation, and a tailing region, with the initial first-order rate constant being greater than 0.002 d^{-1}, the average, long-term rate constant was closer to 0.001 d^{-1} (Nelson and Darby, 2002). Deviation from single-phase decay may result from the influence of internal factors, such as variation of sensitivity (frailty) within the microbial population (Yates *et al.*, 1987; Grant *et al.*, 1993), and external factors including differential exposure to detrimental environmental factors.

Petterson *et al.* (2001a) modelled the inactivation of enteric viruses on lettuce and carrots, using data collected on crops grown under glasshouse conditions and irrigated with wastewater seeded with a model virus *Bacteroides fragilis* B40-8. The results showed evidence for bi-phasic inactivation, and notably the presence of a persistent sub-population of viruses.

The estimation of exposure to viruses from the consumption of food crops irrigated with wastewater has been shown to be highly sensitive both to the shape of the inactivation curve (Petterson and Ashbolt, 2001) and the value of the inactivation coefficient (Petterson *et al.*, 2001b). This sensitivity may also be expected when modelling the risk from other pathogens. Collection and modelling of additional data in this area is therefore a high priority for improving the performance of exposure models.

2.2 Indicator and Model Organisms

The use of human pathogens for experimentation is often not possible due to difficulty in laboratory culture, enumeration and occupation health risk to researchers. It is therefore usually necessary to use model organisms for undertaking experimentation. A model organism is essentially a tracer, which has behavioural characteristics similar to those of the pathogen of interest. In particular, a model organism should have the same or greater resistance to environmental stressors. The most common faecal indicators used with environmental samples are bacteria belonging to the coliform group, however, numerous studies have demonstrated that coliforms are inadequate as models for many pathogens, in particular viruses and parasitic protozoa (Goyal, 1983; Payment and Armon, 1989; Ashbolt *et al.*, 2001). Current research suggests that bacteriophages and *Clostridium perfringens* could be better suited as models for human pathogens (Payment and Franco, 1993; Ashbolt *et al.*, 2001). A summary of model organisms appropriate to each pathogen group is provided in Table 6.

When developing an exposure profile, data must be drawn from a wide array of sources as is evidenced by the previous sections. Interpretation of experimental results by the risk analyst must take into consideration the model organism(s) used. The results of the investigation should only be used in the exposure profile if the model organism is appropriate to the hazard being modelled (Table 6), for example removal rates of thermotolerant coliforms through a wastewater treatment process will not provide an appropriate rate to use for enteric viruses or parasites.

Table 6: Summary of appropriate model organisms for human pathogens

2.3 Variability and Uncertainty

The quantitative values for each component of the exposure profile will vary spatially and temporally depending on a range of biophysical and anthropogenic influences. In addition, there is uncertainty regarding the nature and extent of this variability. A crucial question to be asked of any exposure profile is therefore: How reliable are the exposure estimates? and; Under what conditions are they expected to be representative? A meaningful answer can only be provided if appropriate consideration has been given to accounting for variability and uncertainty in the exposure modelling.

There are two mathematical approaches used to describe variable values and propagate uncertainty in exposure assessment:

> **Deterministic:** A single “best guess” value is assumed for each variable in the model. Variability or uncertainty may be propagated using an estimate of the standard deviation. Worst case scenarios may be investigated using a single worst case estimate of the variable value.

Stochastic: Each variable may be described using a probability density function (PDF) to describe variability or uncertainty. The exposure analysis may then be undertaken by Monte Carlo simulation.

Undertaking public health risk assessment using a deterministic approach with a series of average, conservative and worst-case values has three potential limitations as highlighted by Thompson *et al.* (1992). Firstly, by selecting a combination of moderate, conservative and worst-case assumptions, risk assessors and risk managers have no way of knowing the degree of conservatism in an assessment. When risk assessments lack sufficient uncertainty analysis, risk managers and the public are unable to put the point estimates into some kind of perspective. Secondly, by setting the bias high enough to swamp the uncertainty for each of many variables - but not necessarily all the variables - risk assessments may consider scenarios that will rarely (if ever) happen. Thirdly, it is fundamentally meaningless to run traditional sensitivity analyses (e.g. to make calculations at ±10% or ±25% for each input value) to determine the uncertainties in the final point estimates because many of the input variables are at or near their maxima.

Stochastic analysis using Monte Carlo simulation allows for these limitations to be overcome, provided appropriate parameters are available for the pathogen-scenario of interest. Within this framework each variable takes on a range of values with a known probability. The Monte Carlo simulation then randomly selects a value from each input probability density function (PDF), and calculates a single result. This is repeated a large number of times to produce a complete distribution of outcomes (ultimately of infection/illness for the given pathway).

The most significant danger in adopting a stochastic approach to undertaking the exposure analysis, is that a level of precision may be inferred that is not justified by the data. Additional effort must be taken to ensure transparency of all assumptions and in particular to highlight those PDFs that are based on adequate data, and those that have been postulated by the risk analyst. There are many circumstances when a transparent deterministic approach to uncertainty analysis would provide a clearer and arguably more informative result.

2.4 Sensitivity Analysis

An extensive review undertaken by Frey and Patil (2002) highlighted the following roles for sensitivity analysis as an invaluable tool for risk assessment: Sensitivity analysis may be used to:

- Identify the most significant exposure or risk factors and aid in developing priorities for risk mitigation
- Identify important uncertainties for the purpose of prioritising additional data collection or research
- Verify and validate models
- Provide insight into the robustness of model results when making decisions.

Ten methods for undertaking sensitivity analysis from a range of disciplines were identified, reviewed and evaluated for their applicability to risk assessment by Frey and Patil (2002). Each method was characterised individually and the methods were compared on the basis of four criteria including applicability to risk assessment; computational intensiveness; ease and clarity in representation of sensitivity; and the purpose of analysis. No single method was identified as superior, however each was noted to have its own key assumptions and limitations (see Box 5). The primary recommendation was to use two or more methods, to increase confidence in the identification of key inputs.

BOX 5: Methods available for undertaking sensitivity analysis in risk assessment

Frey and Patil (2002) reviewed 10 methods for undertaking sensitivity analysis and evaluated their applicability for risk assessment. The methods were classified as 1) Mathematical: 2) Statistical; or 3) Graphical. The main features of each of these classifications as presented by Frey and Patil (2002) are summarised.

1) **Mathematical :** Mathematical methods assess sensitivity of a model output to the range of variation of an input. These methods typically involve calculating the output for a few values of an input that represent the possible range of the input. These methods do not address the variance in the output due to the variance in the input, but they can assess the impact of range of variation in the input values on the output. Applications for mathematical methods include screening the most important inputs, verification and validation, and to identify inputs that require further data acquisition or research. Examples of mathematical methods include nominal range sensitivity analysis, break- even analysis, difference in log-odds ration and automatic differentiation.

2) **Statistical Methods:** Statistical methods involve running simulations in which inputs are assigned probability distributions and assessing the effect of variance in inputs on the output distribution. Depending on the method, one or more inputs are varied at a time. Statistical methods allow the effect of interactions among multiple inputs to be identified. Examples of statistical methods include regression analysis, analysis of variance, response surface methods, Fourier amplitude sensitivity test, and mutual information index.

3) **Graphical Methods:** Graphical methods give representation of sensitivity in the form of graphs, and charts or surfaces. Generally, graphical methods are used to give visual indication of how an output is affected by variation in inputs. Graphical methods can provide a screening tool before further analysis of the model, or to represent complex dependencies between inputs and outputs. An example of a graphical method is the use of scatter plots.

A simple deterministic approach for undertaking sensitivity analysis in QMRA used in the food industry has been presented by Zwietering and Gerwen (2000). Three stages of sensitivity analysis were presented and included deterministic sensitivity,

worst case sensitivity and stochastic analysis. The first two mathematical measures have been applied for undertaking sensitivity analysis of a wastewater reuse model. The calculations and interpretation of these mathematical measures of sensitivity are included in Box 6 to illustrate the usefulness of some simple measures of sensitivity in evaluating the risk model.

BOX 6: Application of two simple measures of sensitivity

1. Characterisation of the main determinants of risk

For a first selection of the most relevant determinants of risk, a step characteristic can be used. The step characteristic indicates the log reduction or increase in the number of organisms relative to the previous step in the model and is given by:

$$SC_K = Log\left(\frac{N_k}{N_{k-1}}\right)$$

where N_k is the number of organisms at step *k*.

2. Worst case sensitivity

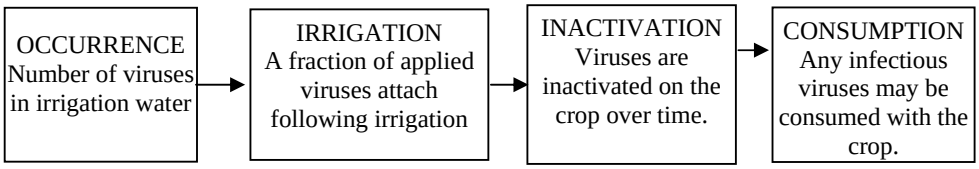
Using a similar measure, the factor sensitivity shows the relevance of variations of a factor for each process step.

$$FS_K = Log\left(\frac{N_k(extreme)}{N_k(average)}\right)$$

A high FS value means high sensitivity to variations and shows that changes to the assumptions/conditions at that process step has profound effects on the model outputs.

EXAMPLE: Risks from the consumption of spray irrigated lettuce crops

A model was constructed for evaluating the risks associated with the consumption of wastewater irrigated lettuce crops (Petterson 2002) This model consisted of a series of process steps and is illustrated by Figure below:



Process steps for wastewater irrigation of lettuce crops model

Exposure to viruses was therefore calculated as:

$$Exposure = N \times f \times S(t) \times q$$

Where:

N is the number of viruses in the irrigation water applied to the crop
f is the fraction of those viruses that survive the irrigation process and attach to the lettuce plant
S(t) represents the fraction of viruses remaining infectious at consumption
q is the quantity of crop consumed.

At each point in the model a best estimate of the model parameters and an extreme estimate were selected. A summary of parameters is included in the following Table

Model parameters used for undertaking sensitivity analysis

Model Component	**"Best" Estimate**	**"Extreme" Estimate**
Virus Occurrence	2.6 $(vu \cdot L^{-1})$[1]	470 000 $(vu \cdot L^{-1})$[2]
Virus Attachment (*f*)	0.024[3]	0.071[3]
Virus Inactivation: S(t) Bi-phasic inactivation $C_t = aC_0 * h_1 + (1-a)C_0 h_2$	$h_1 = 2.5d^{-1}$ $h_2 = 0.5d^{-1}$ a = 0.12%[3]	$h_1 = 2.0d^{-1}$ $h_2 = 0.3d^{-1}$ a = 0.96%[3]
Consumption per event *q*	100g[3]	300g[3]

Sources: [1]Californian dataset used by Asano *et al.* (1992) [2]Yates (1998) [3]Petterson (2002)

The step characteristic and factor sensitivity were calculated for each component of the process. The results are included in table *.

Table *. Results from sensitivity analysis

Process Step	**SC** Step Characteristic	**FS** Factor Sensitivity
Occurrence		5.49
Attachment	-1.6	0.45
Inactivation	-6.2	2.2
Consumption		0.48

The step characteristic (SC) indicated the importance of each component to the calculated exposure. The value of the SC shows the magnitude of reduction in

virus numbers at each stage in the model. For the lettuce model, virus attachment resulted in only a 1.6 log reduction in virus numbers, whereas virus inactivation led to a 6.2 log reduction. The influence of virus inactivation was therefore much more important for reducing risk, and had a greater influence on the calculated exposure.

The factor sensitivity (FS) may be used to evaluate the sensitivity of the model to variation within each component of the model. The highest factor sensitivity was for virus occurrence (FS = 5.49). This indicated that the estimation of exposure was very sensitive to changes in the initial virus concentration; the uncertainty associated with virus numbers is very high (1.5 $vu \cdot L^{-1}$ compared with 470 000 $vu \cdot L^{-1}$).

The lettuce model was also sensitive to variation in the value of the inactivation parameters (FS = 2.2). The uncertainty associated with parameter estimation therefore has important implications for calculating risk. The uncertainty associated with the estimation of the attaching fraction (FS = 0.45) and the quantity consumed (FS = 0.48) were less important.

The calculation of step characteristics (SC) for each component in the risk models indicated which variables were the most important for reducing risk. For the lettuce model, virus inactivation was the most important component for reducing risk. Once outside the host and exposed to environmental conditions, viruses inactivate. On the surface of the lettuce leaf, conditions greatly promote virus inactivation due to exposure to sunlight, high temperatures and desiccation. Inactivation rates were therefore very high (best estimates of 2.5 d^{-1} fast phase, and 0.5 d^{-1} slow phase) and if sufficient time is allowed between final irrigation and consumption (for example 14 days) then exposure of the consumer to infectious viruses would be very low. Some removal was also achieved through the irrigation process as viruses were inactivated and others did not attach to the crop, however this removal was small in comparison to removal by inactivation. Through the calculation of factor sensitivities (FS) the sensitivity of the model to variation in the value of the two inactivation rates and the sub-population size were found to be high. Uncertainty associated with the rate of virus inactivation may therefore be expected to have an important effect on risk estimation.

In this example, a simple mathematical approach to sensitivity analysis allowed for the most important model components to be identified within a standard framework. This provides valuable input for identifying further research needs and for risk management.

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3 Dose-response analysis

Dose-response modelling is the key to microbial risk assessment as it provides a link between exposure and the probability of potential infection. The primary source of data for undertaking dose-response analysis is based on human feeding trials. Human feeding trials are undertaken using healthy volunteers who are given a known dose of

a particular pathogen under controlled conditions. The response of each individual in the study is then followed to determine the numbers who become infected.

Infection occurs when the pathogenic organism multiplies within the host (it is important to note here that illness is a related but essentially separate outcome). For this to take place three conditions need to be fulfilled: the organism must have been ingested or inhaled, the organism must have survived to reach a suitable site for colonisation in the host and finally the organism needs to be infectious and therefore able to multiply.

Models for infection have been developed based the 'single hit' theory. The assumptions of the single hit model are: that the inoculum is known but for Poisson uncertainty, that organisms act independently, individual probabilities of success do not depend on their numbers (independence), and that any single organism can start infection (Teunis *et al.*, 2002).

The simplest form of the single-hit model is the exponential relationship ($P_{\text{inf}}(n;r) = 1-(1-r)^n$ after ingestion of n dose where r is the probability of a single hit of an organism overcoming host barriers to reach a site for infection) (Haas, 1983). In this relationship, r is constant for the population and susceptibility is assumed to be constant.

The value of r is however likely to vary, between pathogens and hosts. When r is assumed to have a beta-distributed probability, a very complicated dose-response relationship emerges containing a confluent hypogeometric function. Furumoto and Mickey (1967) made some simplifying assumptions to this relationship, and derived a simple dose-response relationship referred to as the β-Poisson:

$$P_{\text{inf}}(D;\alpha,\beta) = 1-\left(1+\frac{D}{\beta}\right)^{-\alpha}$$

which holds when $\beta \geq 1$ and $\alpha \leq \beta$

The β-Poisson model has been fitted to Rotavirus data on infection from Ward *et al.* (1986) with maximum likelihood parameters of (α = 0.253, β = 0.422). The performance of the β-Poisson approximation with Rotavirus data has been evaluated by Teunis and Havelaar (2000). While this model produces a good fit for the data, it has been shown to produce misleading results during uncertainty analysis since the parameter conditions of the approximation are not met, and the β-Poisson is strictly not a single hit model (Teunis and Havelaar, 2000).

An important property of the single-hit relationship is that it has a maximum risk curve that limits the upper confidence level of the dose-response relation. This occurs when the probability that an ingested organism will pass the host's defence mechanisms and find a site suitable for colonisation is equal to 1 (r=1). This property is not retained by the β-Poisson approximation, where the upper confidence level of the dose-response relation may exceed the maximum risk curve (Teunis and Havelaar, 2000). The maximum risk curve or maximum possible response curve is therefore

important for uncertainty analysis and for risk assessment of pathogens with unknown properties.

An assumption of the exponential and β-Poisson dose-response models is that individuals ingest a number of microorganisms that is a random sample from a Poisson distribution. In many circumstances, however, micro-organisms have been shown to be overdispersed in environmental samples (Pipes *et al.*, 1977; El-Shaarawi *et al.*, 1981; Haas and Heller, 1988; Maul *et al.*, 1990; Petterson *et al.*, 2001a). In response to this conflict, Haas (2002) presented a conditional dose-response function that could be combined with any theoretical or empirical distribution function for the number of microorganisms ingested to determine the risk associated with exposure. Development of a dose-response relationship based on an overdispersed distribution of microorganisms led to a reduced estimated population risk for the same mean dose (Haas, 2002). The assumption of a Poisson distribution from a public health point of view, therefore represents a conservative upper bound to the actual risk resulting from an estimated mean dose (Haas, 2002).

Alternative models for describing variability in *r*, particularly in relation to a covariate such as immune status, have been suggested (Teunis *et al.*, 2002). These models retain the properties of the single hit model (notably the maximum risk curve) and provide potential for QMRA as a means of accounting for variation in susceptibility to infection in the general population.

A summary of dose-response models that have been fit to human feeding experiment data is given in Table 7.

4 Risk characterisation

The exposure profile and dose-response information are combined during the process of risk characterisation, and probability of infection rates are calculated for the exposed population. Single calculated values of risk (probability of infection) are essentially meaningless unless they are interpreted within the framework of the model assumptions and the circumstances of the exposed population. In its simplest form, interpretation of risk estimates is undertaken by comparing the calculated value to some benchmark of tolerable risk. By far the most commonly applied benchmark in risk assessment has been the USEPA's 10^{-4} risk of infection per annum from drinking water (Regli *et al.*, 1991). It may however be argued that the tolerable risk of infection from a particular disease should be dependent upon the duration and severity of the symptoms (e.g. Rotavirus may be a far more tolerable illness than Hepatitis A).

The burden of disease may be described using Disability Adjusted Life Years (DALYs), which is an integrated measure combining years of life lost by premature mortality (YLL) with years lived with a disability (YLD). Havelaar *et al.* (2000) applied the DALY measure for evaluating the total health burden of infection from *Campylobacter* spp. in the Dutch Population. This example is included in BOX 7. DALYs may also be used to compare the health effects of different agents or conditions and to inform the debate on levels of acceptable risk.

The need to establish some measure of acceptable or tolerable risk for wastewater reuse in agriculture has been widely acknowledged. In addition, any reference level of acceptable risk should be expressed in DALYs to avoid confusion between

different health outcomes, and comparison between the effects of different pathogenic agents.

BOX 7: Health burden in the Netherlands due to infection with thermophylic Campylobacter (Havelaar et al., 2000): An example of the application of DALYs

Infection with thermophilic *Campylobacter* spp. usually leads to an episode of acute gastroenteritis. Occasionally, more severe diseases may be induced, notably Guillain-Barré syndrome and reactive arthritis. For some, the disease may be fatal. The total disease burden was evaluated using the DALY, a summary of the results is included in following table:

Health burden due to infection with thermophilic *Campylobacter* spp. in the Netherlands, assuming no age-weighting or discounting

Population	Number of cases	Duration (years)	Severity weight	YLD/YLL
Morbidity				
General population: gastroenteritis	311,000	0.014	0.067	291
General practitioner: gastroenteritis	17,500	0.023	0.393	159
Clinical phase Guillain-Barré	58.3	1	0.281	16
Residual symptoms: Guillain-Barré	57.0	37.1	0.158	334
Reactive arthritis	6570	0.115	0.210	159
Mortality				
Gastroenteritis	31.7	13.2	1.0	419
Guillain-Barré	1.3	18.7	1.0	25
TOTAL				1403

Source: Summary of results from Havelaar *et al.* (2000) presented by Prüss and Havelaar (2001)

The results show an annual loss of approximately 1400 DALYs per year in the Dutch population of 15 million. The most significant impact on public health is from gastroenteritis-related mortality and the residual symptoms of Guillain-Barré Syndrome, despite the fact that the incidence is low. Acute gastroenteritis (both patients who do and do not visit their GP) is an additional important source of disease burden (Prüss and Havelaar, 2001).

5 Interactions with Risk Management (monitoring and auditing)

Management of microbial risk involves identifying sources of contamination and managing barriers to prevent contamination from reaching the consumer or exposed population. The focus of risk management is to take a systems approach examining the entire process as a whole including environmental and human elements. Figure 2 illustrates a generic flow diagram for the sources of microbial risk from the use of wastewater and excreta in agriculture, and the environmental/human factors that influence the magnitude of risk. This generic diagram is applicable for risks resulting from the consumption of crops and exposure to microbial aerosols.

Hazard Analysis Critical Control Points (HACCP) is a framework for managing risk that seeks to control hazards as close as possible to their source. While the HACCP framework was largely developed by the food industry for managing food safety, the framework has been successfully applied for the management of drinking water supplies (Deere *et al.*, 2001), and provides potential for the management of risks associated with wastewater reuse. The important components of the HACCP framework are:

Hazard analysis: The potential hazards associated with the wastewater reuse scenario are identified and assessed. The hazard analysis should focus on the entire system from the source of the hazard to exposure with the aid of process flow charts (Figure 2). The magnitude of different hazards is often assessed within a qualitative framework to assist in the assigning of priorities to different pathways. When undertaking a qualitative risk assessment, dose-response models and risk characterisation steps (Table 7, Section 4), are usually replaced with risk rankings. These rankings are generally derived from expert opinion summarising: 1) likelihood of possible risk pathways, 2) severity of outcome from each pathway, and 3) numbers of people that may be impacted.

Identification of critical control points (CCPs): CCPs are defined as points, steps or procedures at which control can be applied and a hazard can be prevented, eliminated or reduced to acceptable levels.

Establishment of critical limits for each CCP: A prescribed limit or tolerance (of a hazard or surrogate) is defined for each CCP to ensure that the health hazard is effectively controlled.

Monitoring and corrective action: Appropriate monitoring procedures are chosen to ensure that critical limits are met. Appropriate and immediate action is needed if the results of monitoring indicate that the criteria are not met at a certain CCP.

The results of QMRA can provide an important input into the HACCP process particularly in the identification of CCPs and the evaluation of critical limits. An example of the use of a QMRA model within a HACCPs approach to management is included in Box 8.

Figure 2 Generic flow diagram for sources of microbiological risk in wastewater/excreta reuse for agriculture

Box 8: Possible qualitative risk assessment approach to rank or scale hazardous scenarios	
Step	**Comment**
1. Hazard scenario	Identification of hazardous scenarios, such as massive rainfall induced contamination of source water, filter breakthrough or loss breakdown of chemical disinfection system; *i.e.* not necessarily limited to a single pathogen.
2. Likelihood	Ranking or scaling of how likely the event is, *e.g.* # events per year
3. Consequence	Ranking or scaling of the consequence, *e.g.* short-term injury or ill-health through to permanent disability or death.
4. Scale of effect	Consideration of the number of people affected by the hazard scenario
5. Risk score	Different weightings may be given to (2) to (4) and summed to give a value for each hazard scenario
6. Rank	Each hazard scenario is then ranked, to provide a priority list for risk management.

Targets [not yet thought through – nor sure we should go this way?]

To define targets we need to have tolerable risk defined (say by DALYs) then work backwards through each pathway considered to provide pathogen numbers at various points. Then look at how we may control pathogens below these target numbers.

No generally appropriate to have pathogen numbers, such as in PHLS (2000) for bacterial pathogens to not be detected in 25g of fresh vegetables, as nobody is going out to measure them, nor for the real issues, such as viruses and parasites.

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Table 1: Summary of model inputs and assumptions for QMRA undertaken for wastewater reuse scenarios

	Asano *et al.*, 1992	**Tanaka *et al.*, 1998**	**Shuval *et al.*, 1997**	**Petterson, 2002**	**Storey and Ashbolt, 2002**	**Dowd *et al.*, 2000**	**Gardner *et al.*, 1998**
Country	**United States**	**Israel**	**Israel**	**Australia**	**Australia**	**United States**	**Australia**
Identified Hazards	Enteric viruses	Enteric Viruses	Hepatitis A Rotavirus Cholera	Enteric Viruses	Enteric Viruses	Salmonella Enteric Viruses	Echovirus Rotavirus Giardia Cryptosporidium
Exposure Route	1. Landscape Irrigation of Golf Course 2. Consumption of Spray Irrigated food crops 3. Swimming in recreational impoundments 4. Groundwater Recharge	As for Asano *et al.*, 1992	Consumption of spray irrigated crops	Consumption of spray irrigated lettuce and carrot crops	Exposure to reclaimed water intended for non-potable use. Investigation of the incorporation of viruses in distribution pipe biofilms and subsequent sloughing events.	Inhalation of aerosols downwind of biosolids placement	Inhalation of wastewater aerosols by the local community
Data Sources Number of organisms in wastewater	Virus concentrations of 500 and 73 400 vu/100L in unchlorinated secondary effluent representing the 90th percentile and the maximum value for activated sludge effluents 1 and 111vu/100L for	Enteroviruses enumerated from 377 unchlorinated secondary effluent samples (242 positive), from 4 treatment plants in California, U.S.. Virus concentration was modelled stochastically using log-normal	A constant enteric virus: faecal coliform ratio was assumed of 1:10^{5} for wastewater meeting guideline of 1000FC/100mL Cholera concentration = 10^{5}-10^{6}/100mL (estimated by authors)	Log-normal distribution fit to enteroviruses concentrations from California (Tanaka *et al.*, 1998)	Three virus concentrations were tested: 0.01 vu/L 1 vu/L 10 vu/L Chosen to represent the range of virus concentrations expected depending on reclaimed water facility performance (normal,	Airborne Salmonella and Coliphage (Male-specific[F+} enumerated on *E. coli* Dowd *et al.* (1997)	F-specific coliphages [enteric virus concentration estimated using a phage:virus ratio of 10:1 and 100:1 based on Havelaar *et al.* (1993) and Kott *et al.* (1978)] *Giardia* and *Cryptosporidium* measured from 10

	tertiary effluent representing the limit of detection and the maximum concentration found in tertiary effluents	distributions fit to the positive data points from each treatment plant.			sub-optimal and worst case).		sewage plants (bi-monthly for 18 months) in Queensland, Australia.
Pathogen Transport:	2. Organism attachment to food crops: All organisms contained in 10 ml of irrigation water per consumed portion of crop. 3. No dilution 4. Removal via percolation: $f=C/C_0=10^{-0.007L}$ where L= Depth of unsaturated zone, cm = 300	As for Asano *et al.*, 1992	Organism attachment to food crops: The quantity of wastewater clinging to crop following irrigation was estimated to be: 10.8mL/100g Lettuce 0.36mL/100g Cucumber based on unspecified laboratory experiments. All organisms contained in this wastewater were assumed to have attached.	Lettuce: A fraction (best estimate = 0.024) of viruses applied were assumed to attach to the lettuce crop. Carrot: Virus attachment to the carrot tuba assumed to follow an adsorption isotherm. The density of viruses on the carrot was related to the density of viruses in the irrigation water using a constant of adsorption (*k*: best estimate =$7.2\ 10^{-4}$)	1% of viruses in bulk water assumed to be incorporated into biofilm. Biofilm thickness of 100, 200 and 300 μm tested. 10%, 50% and 90% sloughing events tested.	Point-source and Area source model applied to simulate the generation of aerosols.	Aerosol modelling: A Gaussian dispersion model was applied for evaluating aerosol dispersion (Camann, 1980).
Pathogen inactivation in the environment	Single phase log-linear inactivation with constant =$0.69\ d^{-1}$ 1. time = 1 day 2. time = 14 days 3. no decay 4. 6 months from recharge to	As for Asano *et al.*, 1992	Total virus inactivation from the wastewater source until ingestion was assumed to be 99.9%	Bi-phasic loglinear inactivation over time $C_t = aC_0e^{-h_1t} + (1-$ where C_t is the virus concentration at time t (time= 14	0.01% of incorporated viruses assumed to persist for 100 days.	??	First order kinetic inactivation incorporated into the dispersion equation, however constant assumed = 0 i.e no inactivation.

	consumption.			days), C_0 is the initial virus concentration and Lettuce: h_1 =0.5 (slow phase) h_2 =2.5 (fast phase) sub-population size (a) = 0.12% Carrot: h_1 =0.05 (slow phase) h_2 =0.8 (fast phase) sub-population size (a) = 2%			

Consumption patterns	1. 0.001L (1ml) per event, twice weekly over 30years. Lifetime exposure = 3 120 events 2. 0.01L (10ml) per event, frequency of events unspecified. 3. 0.1L (100ml) per event, twice weekly for 5 months of the year, over 40 years = 1 600 events 4. 2 L per day (50% dilution with groundwater) for 70 years.	As for Asano *et al.*, 1992	100g lettuce or cucumber on 150 days per year.	100g or 300g per event. Consumption on 100 days per year.	1ml, 100ml and 1000ml		300 Liters of air during a 10 minute exposure event. Annual risk based on 26 events per year.
Dose-Response Model	β-Poisson: Parameters from Haas (1983) for Echovirus 12 Poliovirus 1 Poliovirus 3	β-Poisson: Rotavirus Rose and Gerba (1991)	β-Poisson: a=5 N_{50}=30 and 1000 (estimated by authors to represent the range of expected responses). Cholera: $N_{50}=10^4$ vibrios (estimated by authors)	β-Poisson: Rotavirus (Ward *et al.* 1986) and Echovirus (Shiff *et al.* 1984) Exponential: Adenovirus (Couch *et al.* 1966) and Maximum risk curve (Teunis and Havelaar, 2000)	Maximum risk curve (exponential with r=1) (Teunis and Havelaar, 2000).	β-Poisson: *Salmonella typhi* Exponential: Coxsackievirus B3 (Haas *et al.*, 1999)	β-Poisson: Echovirus Rotavirus Exponential: *Giardia* Cryptosporidium

Risk Characterisation	Daily, Annual and Lifetime risks of infection for minimum and maximum virus concentrations.	The risk model was used to evaluate: *Reliability:* probability of meeting an acceptable risk; and *Expectation:* the average risk for many exposure events. For each exposure scenario the acceptable level of risk was the USEPA benchmark of $<10^{-4}$ infections per year.	Annual risk of illness compared with the USEPA benchmark of $< 10^{-4}$ infections per year.	Risk estimates compared with the USEPA benchmark of $< 10^{-4}$ infections per year and used to identify critical control points, and critical limits within a HACCP framework for risk management.	Daily and Annual risk (only calculated for 1mL exposure) compared with the USEPA benchmark of $< 10^{-4}$ infections per year.		Acceptable separation distance for each pathogen defined as the distance at which the calculated infection risk was = to 10^{-4} per year.

Table 2. Advantages and limitations of reference pathogens selected/required for modelling in risk assessment

Pathogen Group	Ref. pathogen	Advantages	Limitations
Viruses	Rotavirus	• Highly infectious and therefore a conservative model • Dose-response relationship available • Endemic throughout the world, and maybe particularly important in developed countries	• High infectivity may result in overestimation of risk for less infectious viruses such as echovirus
	Hepatitis A	• Persistent in the environment and to disinfection • Important disease throughout the world with serious health consequences	• Not necessarily reflective of more infectious viruses that cause gastroenteritis • No dose-response model available • Not as prevalent in sewage as rotavirus, NLV or adenovirus
	Adenovirus	• One of the most numerous culturable virus groups in wastewater • Not as virulent as rotavirus and may better represent enteric viruses in dose-response models	•
		•	•
Bacteria	ETEC (e.g. *E. coli* O157:H7)	• Highly infectious and relatively persistent in the environment • Resulted in a number of waterborne outbreaks	• Generally not isolated by standard methods for *E. coli*. Numerous enterotoxigenic (ET) strains possible and regionally variable. • Limited dose-response model available
	Campylobacter jejuni	• Major water and food borne pathogen • Sequelae described	• Difficult to culture from environmental waters, as may form dormant cells • Many *Campylobacter*-like environmental organism of unknown health impact
	Salmonella spp. (non-typhoid)	• Major water and food borne pathogen • Relatively easy to detect in water	• Complex methods to enumerate from waters • Vast range of serogroups, many may not be human pathogens
	Vibrio cholerae (Cholera-types)	• Major pathogen of wet developing regions of the world	• No dose-response model available • Difficult to culture from environmental waters, as may

			form dormant cells or grow in waters • Various environmental strains are not human pathogens
	Helicobacter pylori	• Potential waterborne pathogen, although weak epi evidence to date	• Environmental growth & forms of this pathogen are poorly understood • No occurrence data in sewage/excreta • No dose-response model available
		•	•
Protozoa	*Cryptosporidium*		
	Giardia lamblia		
Helminths	*Ascaris lumbricoides*	• Major helminth pathogen • Highly persistent in soil/excreta • Good methods for estimating numbers	• No dose-response model available

Table 3. Reported concentrations of reference pathogens in raw sewage (from Yates and Gerba, 1998, see references cited within)

Organism	Disease	Numbers per Litre
Viral Pathogens		
Adenovirus	Respiratory illness, conjunctivitis, vomiting, diarrhea	
Enteroviruses (polio)	Paralysis, meningitis, fever	182-492,000
Hepatitis A virus	Infectious hepatitis	Not reported*
Norwalk virus	Epidemic vomiting and diarrhea	ND – 10^{4} **
Rotavirus	Diarrhea, vomiting	400-85 000
Bacterial Pathogens		
Salmonella	Typhoid, paratyphoid, salmonellosis	20-80 000
Shigella	Bacillary dysentery	10-10 000
Campylobacter spp.	Gastroenteritis	37 000
Vibrio cholerae	Cholera	100-100 000
Protozoan Pathogens		
Entamoeba histolytica	Amoebic dysentery	4
Giardia lamblia	Diarrhea, malabsorption	125-200 000
Balantidium coli	Mild diarrhea, colonic ulceration	28-52
Cryptosporidium parvum	Diarrhea	0.3-4 000
Helminths**		
Ascaris (roundworm)	Ascariasis	5-111
Ancylostoma (hookworm)	Anemia	6-188
Trichuris (whipworm)	Diarrhea, abdominal pain	10-41

ND – not detected, * Hepatitis A virus by PCR various from non-detected to positive in up to 50% of samples in some regions (Pinta *et al*., 2001). ** PCR positive particles (Lodder *et al*., 1999). *** Human infectious helminths may largely be absent in sewage from highly developed regions or many fold high in local regions where they are endemic (Bouhoum and Schwartzbrod, 1998; Bouhoum *et al*., 2000).

Table 4a Pathogen removal in treated wastewater (from Yates and Gerba, 1998) [suggest Log red rather than % as latter implies too many sig. figs. in the table]

	Enteric Viruses	***Salmonella***	***Giardia***	***Cryptosporidium***
Concentration in raw wastewater (no. L^{-1})	100 000-1 000 000	5 000-80 000	9 000-200 000	1-4 000
Removal during:				
Primary treatment[a]				
% removal	50-98.3	95.5-99.8	27-64	0.7
No. remaining L^{-1}	1,700-500,000	160-3,360	72,000-146,000	
Secondary treatment[b]				
% removal	53-99.92	98.65-99.996	45-96.7	
No remaining L^{-1}	80-470,000	3-1,075	6,480-109,500	
Tertiary treatment[c]				
% removal	99.983-99.9999998	99.99-99.9999995	98.5-99.99995	2-7[d]
No. remaining L^{-1}	0.007-170	0.000004-7	0.099-2,951	

[a] Primary sedimentation, and disinfection
[b] Primary sedimentation, trickling filter/activated sludge and disinfection,
[c] Primary sedimentation, trickling filter/activated sludge, disinfection, coagulation, filtration, and disinfection
[d] Filtration only

Table 4b Percent removal of pathogens/index organisms by wastewater treatment wetlands and ponds

Treatment	Coliphage	*Giardia*	*Cryptosporidium*	Thermo-tolerant Coliforms	*Ascaris*	Country	Reference
Artificial wetland systems (detention – 4-6 days)	40-95	73-98	58-89	61-98		United States	Gerba *et al.* (1999)
Sub-surface flow wetland (detention: 4 days)	95.2	87.8	64.2	98.2		United States	Thurston *et al.* (2001)
Stabilisation ponds (detention – 12 days)				99.99	100	Colombia	Madera *et al*. (2002)
Stabilisation ponds (detention – 16 days)		100			100	Morocco	Bouhoum *et al*. (2000)
Stabilisation ponds (detention – 25 days)		99.1				Kenya	Grimason *et al.* (1996b)
Stabilisation ponds (detention – 40 days)		99.7				France	Grimason *et al.* (1996b)
Stabilisation ponds(detention: 30-40 days)		99.68-100				France	Wiandt *et al.* (1995)
Stabilisation pond sludge (1 year)					50-60 (average, long-term rate constant 0.001 d^{-1})	Mexico	Nelson and Darby (2002)

Table 5. Pathogen inactivation in the environment

a) Survival in Faeces[1]	**T_{90} (Days for 90% inactivation)**	
Organisms	**4°C/low temp range**	**20°C/high temp range**
Indicator organisms		
*E. coli**	70-100	15-35
Enterococci*	100-200	100-200
Bacteriophages	20-200	10-100
Bacterial pathogens		
*Salmonella**	10-50	
EHEC*	10-30 days	10-30
Virus		
Rotavirus	100-300 (conservative model – no reduction)	20-100
Parasitic protozoa		
Giardia	15-100	5-50
Cryptosporidium	30-200	20-120
Parasitic helminths		
Ascaris	100-400	50-200

*Possible growth not taken into consideration.

[1] Compiled by Thor Axel Stenström, Caroline Schönning and Therese Westrell

B) Survival in Soil[1]	**T_{90} (Days for 90% inactivation)**	
Organism to be modelled	**4°C/low temp range**	**20°C/high temp range**
Indicator organisms		
*E. coli**	20-100	15-70
Enterococci*	20-80	15-50
Bacteriophages	10-100	5-50
Bacterial pathogens		
*Salmonella**	20-50	15-35
EHEC*	10-40	10-40
Virus		
Rotavirus	10-50	5-30
Parasitic protozoa		
Giardia	20-40	5-20
Cryptosporidium	40-950	30-400
Parasitic helminths		
Ascaris	250-1000(in soil)	15-100(on soil)

[1] **Compiled by Thor Axel Stenström, Caroline Schönning and Therese Westrell**

C) Survival on Crops	Inactivation Coefficient (d^{-1})	Data Source	Reference
Artichoke, Broccoli, Celery and Lettuce	0.69	Seeded Poliovirus inactivation over 4 days in an environmental chamber (Engineering Science, 1992)	Asano *et al.*, 1992
Celery (Environmental Chamber) Iceberg Lettuce (Environmental Chamber) Romaine Lettuce (Field Conditions) Butter Lettuce (Field Conditions)	0.55[1] 0.3[1] 0.8[1] 0.6[1]	Poliovirus seeded onto plants and time for 99 percent removal was recorded in both an environmental chamber and under field conditions.	Sheikh *et al.* (1999)
Winter Triumph Lettuce	2.5 (fast phase) 0.5 (slow phase) sub-population size 0.12%[2]	Plants spray irrigated at maturity with *B.fragilis* bacteriophage B40-8seeded wastewater. Experiment undertaken in uncontrolled glasshouse conditions.	Petterson *et al.*, (2001b)
Carrot	0.8 (fast phase) 0.05 (slow phase) sub-population size 2%[2]	Plants grown in pots and irrigated at maturity with *B.fragilis* bacteriophage B40-8 seeded wastewater Experiment undertaken in uncontrolled glasshouse conditions.	Petterson *et al.*, (2001b)

1Estimated value of inactivation coefficient assuming log-linear relationship ($C_t=C_0e^{-ht}$) and time for 2 log virus removal. Added here for the purpose of comparison, not included in cited paper.

[2] The data showed evidence of bi-phasic decay [$C_t = aC_0e^{-h_1t} + (1-a)C_0e^{-h_2t}$, where C_t is the virus concentration at time t, C_0 is the initial virus concentration, *a* is the size of the sub-population, h_1 is the inactivation coefficient of the sub-population (slow phase) and h_2 is the inactivation coefficient of the remaining population (fast phase)].

Table 6: Summary of appropriate model organisms for human pathogens

Human Pathogen	Appropriate Model Organism	Comment
Bacteria *Shigella*, enterotoxigenic *E. coli*, *Campylobacter*, *Vibrio cholerae* (Cholera)	*E. coli*, intestinal enterococci	The thermotolerant coliform/*E. coli* group of bacteria have been used for more than 100 years as a model for pathogenic bacteria. Behaviour of *E. coli*, intestinal enterococci (not total coliforms) under environmental conditions is expected to reflect enteric pathogens, but not environmental bacteria such as *Legionella*.
Viruses e.g Adenovirus, Rotavirus, Enteroviruses, Hepatitis A, NLV	Bacteriophages – somatic coliphages or F-RNA coliphages	Bacteriophages are viruses that infect bacteria, and considered non-pathogenic to humans, and can be readily cultured and enumerated in the laboratory. Generally present in faeces of warm-blooded animals, but certain strains may be human specific.
Protozoa e.g. *Cryptosporidium* oocysts, *Giardia* cysts	*Clostridium perfringens* Particle counter	*Clostridium perfringens* is a spore forming bacteria, which is highly resistant to environmental conditions. It has been shown to be a useful model for *Cryptosporidium* oocysts and *Giardia* cysts. Aerobic (*Bacillus*) spores could also be used, but likely to grow in treatment systems and slough off surfaces providing misleading numbers. Protozoan pathogens are generally larger in size than those belonging to the other groups. Studies have been successfully undertaken using particles of similar size (e.g fluorescent beads, or total particles 5-20µm) as a models for oo/cysts.
Helminths e.g. *Ascaris lumbricoides* and *Trichuris trichiura* ova	*Ascaris suum* ova/embryos	Viability of *A. suum* is readily assayed and generally available for addition prior to treatment, as endemic numbers of helminths are generally too low to measure many logs of process reduction.

Table 7 Summary of best-fit dose response parameters (Reproduced from Haas and Eisenberg (2001))

Organism	**Exponential**	**Beta-Poisson**		**Reference**
	k	*N50*	α	
Poliovirus I (Minor)	109.87			Minor *et al.* 1981
Rotavirus		6.17	0.2531	Haas *et al.*1993
Hepatitis A virus [a]	1.8229			Ward *et al.* 1986
Adenovirus 4	2.397			Couch *et al.* 1966
Echovirus 12	78.3			Akin 1981
Coxsackie [b]	69.1			Couch *et al.* 1965
				Suptel, 1963
Salmonella [c]		23,600	0.3126	Haas *et al.* 1999
Salmonella typhosa		3.6 106	0.1086	Hornick *et al.* 1966
Shigella [d]		1120	0.2100	Haas *et al.* 1999
Escherichia coli [e]		8.6 107	0.1778	Haas *et al.* 1999
Campylobacter jejuni		896	0.145	Medema *et al.* 1996
Vibrio cholera		243	0.25	Haas *et al.* 1996
Entamoeba coli		341	0.1008	Rendtorff 1954
Cryptosporidium parvum	238			Haas *et al.* 1996
				Dupont *et al.* 1995
Giardia lamblia	50.23			Rose *et al.* 1991

[a] dose in grams of faeces (of excreting infected individuals)
[b] B4 and A21 strains pooled
[c] Multiple (non-typhoid) pathogenic strains (*S. pullorum* excluded)
[d] Flexnerii and dysenteriae pooled
[e] Nonenterohaemorrhagic strains (except 0111)