Linköping Studies in Arts and Science • 304

Microbial risk assessment and its implications for risk management in urban water systems

Therese Westrell

Linköping Studies in Arts and Science
In the Faculty of Arts and Science at Linköping University research is pursued and research training is conducted within seven broad problem areas known as themes, in Swedish tema. These are: *Child Studies*, *Communication Studies*, *Gender Studies*, *Health and Society*, *Food Studies*, *Technology and Social Change* and *Water and Environmental Studies*. Jointly they publish the series Linköping Studies in Arts and Science.

Distributed by:
Department of Water and Environmental Studies
Linköping University
S-581 83 Linköping
Sweden

Therese Westrell
Microbial risk assessment and its implications for risk management in urban water systems

Edition 1:1
ISBN 91-85295-98-1
ISSN 0282-9800

Printed by UniTryck, Linköping, 2004.

List of papers

This thesis is based on the following papers that will be referred to in the text by their Roman numerals.

I. **Westrell, T.**, Bergstedt, O., Heinicke, G. and Kärrman, E. (2002). A systems analysis comparing drinking water systems - central physical-chemical treatment and local membrane filtration. *Water Science and Technology: Water Supply* **2**(2): 11-18.

II. **Westrell, T.**, Bergstedt, O., Stenström, T. A. and Ashbolt, N. J. (2003). A theoretical approach to assess microbial risks due to failures in drinking water systems. *International Journal of Environmental Health Research* **13**(2): 181-197.

III. **Westrell, T.**, Teunis, P., van den Berg, H., Lodder, W., Ketelaars, H., Stenström, T. A. and de Roda Husman, A. M. Short and long term fluctuations of norovirus concentrations in surface water and their implications for public health. (*Submitted to Water Research*).

IV. **Westrell, T.**, Andersson, O. and Stenström, T. A. Drinking water consumption patterns in Sweden. (*Submitted to Journal of Water and Health*).

V. Schönning, C., **Westrell, T.**, Stenström, T. A., Arnbjerg-Nielsen, K., Hasling, A. B., Hansen, L. and Carlsen, A. Microbial risk assessment of local handling and reuse of human faeces. (*Submitted to Journal of Water and Health*).

VI. **Westrell, T.**, Schönning, C., Stenström, T. A. and Ashbolt, N. J. (2004). QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Science and Technology* **50**(2): 23-30.

Table of contents

1 Introduction

Sustainable development was defined in the 'Bruntland Report' (WCED, 1987) as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs". Within the water sector sustainability could be described as the ability to plan and manage water resources in such a way that they can be sustained for use by future generations.

All nine Millennium Development Goals set by UN member states in 2000 can be related to water and sanitation, as described in the report from the Joint Monitoring Programme for Water and Sanitation by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) (2004). Ensuring environmental sustainability, for example, demand adequate treatment and disposal of wastewaters to contribute to better ecosystem conservation and less pressure on scarce freshwater resources. Reduced child mortality can be achieved by improved sanitation and drinking water sources. The goal is to halve the proportion of people that are without sustainable access to safe drinking water and basic sanitation by 2015. While the progress in drinking water is good so far, with 83 percent coverage today, the action on sanitation is slow in most developing regions. An estimated 2.6 billion people were still without improved sanitation facilities in 2002 (WHO and UNICEF, 2004).

Today many efforts towards urban water sustainability are being made, for example by reusing treated wastewater for watering of golf courses, irrigation in agriculture, *etc*. However, more sustainable options require water recycling and demand management. One example is NeWater in Singapore where advance-treated wastewater will be returned to raw water reservoirs in what is called indirect potable use (Guendert, 2004). Reuse of treated wastewater for toilet flushing and garden irrigation is practised for example in Tokyo and Kobe, Japan (Ogoshi *et al.*, 2001) and in Sydney, Australia (Anderson, 1996). In order to sustain urban water supply for future generations planning for future population growth and change must be attended to, which will influence the choice of alternative water sources.

It has become clear that sanitation and sanitary systems should not only safely dispose of human residuals, but also provide the option of reusing nutrients in agriculture. Simultaneously, processing of human waste should minimise risks to the human population and the natural environment. Within the Swedish research program Sustainable Urban Water Management Program (hereafter called Urban Water) the objective is to evaluate water and wastewater systems adapted to urban environments, taking into account various stakeholders and interactions (Figure 1-1).

The aim of the research in Urban Water is to develop support for strategic decisions on future sustainable urban water systems in Sweden. The approach consists of a common conceptual framework, a number of sustainability aspects or groups of criteria, indicators and assessment tools.

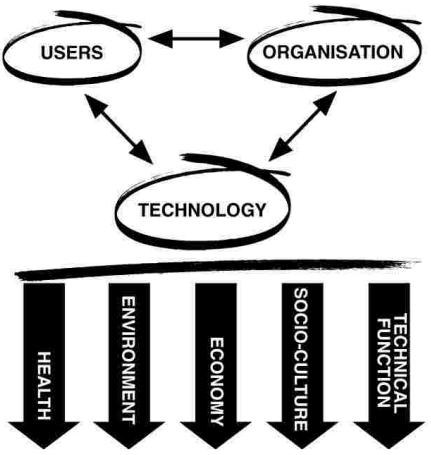

FIGURE 1-1. The conceptual framework of Urban Water with its components: users, organisation and technology and the five sustainability aspects used to evaluate systems (Urban Water, 2002)

The urban water system is more than just the technical system. Users and organisations (*e.g.* water companies, municipal boards, authorities) form the system (Figure 1-1) and the interactions need to be studied, where five sustainability aspects are recognised as central, namely:

- Health and hygiene
- Environment and resource use
- Technology and function
- Economy
- Socio-culture

These aspects must be taken into consideration when assessing urban water systems with respect to sustainable development even though some aspects may be more decisive in each specific application. The criterion for the health and hygiene aspect is 'risk of infection' and the method chosen for evaluation of this criterion is quantitative microbial risk assessment, QMRA. The method of QMRA will be developed into a toolbox for use in comparison of water and wastewater systems (Ashbolt *et al.*, 2004) within Urban Water.

This thesis deals with how to assess hygienic risks in urban water systems as a means to evaluate the future sustainability of the systems, also in comparison with other sustainability aspects. The work is mainly focused on Sweden however the methodology is also applicable to other regions of the world.

1.1 Environmental transmission of infectious diseases

Infectious diseases are transmitted from one person to another and may include various environmental pathways (Figure 1-2). The pathogens excreted in the faeces of an infected person will, in a conventional system, end up in sewage. In Sweden the wastewater from all urban residents (7.7 out of 9 million inhabitants) is treated in municipal wastewater treatment plants (Svenskt Vatten, 2000). Although treatment normally reduces the levels of microorganisms by 90-99.9%, given the potentially high numbers in sewage, substantial loads of pathogens may still remain. When treated wastewater is discharged into receiving waters the pathogens can be transmitted to humans via waters used for recreation or food production. If the water source is used for drinking water production, municipal water treatment must be sufficient to prevent disease transmission to the consumer.

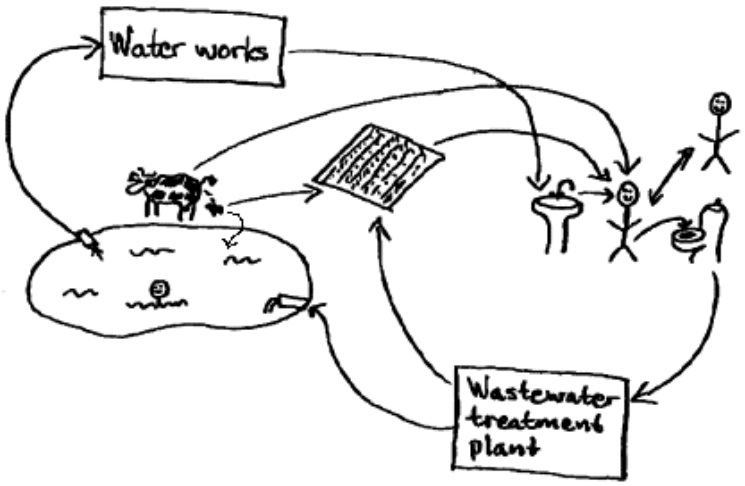

FIGURE 1-2. Circulation of pathogens in the environment

Many pathogens are concentrated in the sewage sludge. When sludge is used in agriculture as a fertiliser, transmission of pathogens via crops could occur if the treatment or storage time has not been sufficient. Some pathogens can be transmitted between animals and humans, so called zoonoses, and can be found in both domestic and wild animals (Wahlström *et al.*, 2003; Hutchison *et al.*, 2004). Heavy rains or snowmelt can cause run-off from pastures and agricultural land

fertilised with animal slurry, manure or sludge, which can contribute substantial loads of pathogens to water courses (Hansen and Ongerth, 1991; Kistemann *et al.*, 2002; Ferguson *et al.*, 2003). Heavy rains can also cause combined sewer overflows (CSO), which means that wastewater mixed with stormwater is discharged, untreated or partially-treated, to the recipient.

Other diffuse sources of microbial pollution to watercourses are also common, for example leaking septic tanks, waterfowl *etc.* In some places crops are irrigated with wastewater and transmission of pathogens to humans (and animals) can occur directly or via crops, aerosols and potentially via groundwater. Transmission of pathogens via the environment can clearly result in infections and diseases and also secondary person-to-person transmission can occur. In this way the circulation of pathogens in the environment will continue. The design of our water and wastewater systems will affect how and where this transmission can occur and how it can be restricted.

1.1.1 Disease outbreaks associated with drinking water

The largest waterborne outbreak of disease in recent time for a developed country occurred in Milwaukee, USA, in 1993 where an estimated 403 000 people fell ill with gastroenteritis caused by the parasitic protozoa *Cryptosporidium* (Mac Kenzie *et al.*, 1994). (The microbial agents are further described in Section 3.1.) About 4 400 people were hospitalised and an uncertain number of people died as a result of the outbreak (Kramer *et al.*, 1996). The reason for the outbreak was deterioration in water quality and decreased effectiveness of the coagulation-filtration process in the waterworks. Although the treated water had turbidity levels more than four times the highest recorded during ten years, it still fulfilled all requested water quality standards at that time (Kramer *et al.*, 1996). Another very severe outbreak occurred in Walkerton, Canada, in 2000 where 2 300 people fell ill and seven died from exposure to the bacteria enterohaemorrhagic *Escherichia coli* (EHEC) and *Campylobacter* in the drinking water (Hrudey *et al.*, 2003). The likely cause of the outbreak was surface water contamination of one of the groundwater wells after heavy rains accompanied by flooding.

Sweden has a long history of surveillance of waterborne disease outbreaks. Every year a few outbreaks are reported, resulting in a few hundred up to several thousand disease cases (Andersson and Bohan, 2001). The two largest outbreaks occurred in 1988 and 1995. In the first of these, approximately 11 000 people became ill due to a chlorination failure in the water treatment plant. The etiological agent, *i.e.* the microorganism that caused the outbreak, could not be established. In 1995 approximately 10 000 people fell ill with *Campylobacter* when a change in pipeline introduced stagnant raw water to the distribution network (Andersson and Bohan, 2001).

More than 70% of Swedish waterborne disease outbreaks are due to unknown agents (Andersson and Bohan, 2001). The most commonly identified agents between 1980 and 1999 were *Campylobacter* and the protozoa *Giardia lamblia*. During the last few years the number of waterborne outbreaks involving noroviruses has increased nationally (Andersson and Bohan, 2001; Carrique-Mas *et al.*, 2003; Nygård *et al.*, 2003), in other Nordic countries, such as Finland (Kukkula *et al.*, 1997; Kukkula *et al.*, 1999) and Norway (Nygård *et al.*, 2004b), and also internationally (Lopman *et al.*, 2003; Blackburn *et al.*, 2004). Surface water systems have been responsible for the largest waterborne outbreaks. Nonetheless most outbreaks occur in groundwater systems where the most common cause is contamination of the water source through (surface) wastewater infiltration (Andersson and Bohan, 2001).

1.1.2 Disease outbreaks associated with recreational waters

In the USA 65 outbreaks in recreational waters occurred during 2001-2002 (Yoder *et al.*, 2004) affecting a total of approximately 2 500 persons. One third occurred in fresh waters. The major agents involved in the outbreaks were *Cryptosporidium*, noroviruses, EHEC, *Giardia* and *Shigella*. In 25% of the outbreaks in fresh waters the etiologic agent was unknown. In Europe the same agents as in the USA are thought to be involved in most recreational waterborne outbreaks.

Outbreaks with *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* have been associated with recreational swimming in lakes (Keene *et al.*, 1994; Ackman *et al.*, 1997; Barwick *et al.*, 2000; McCarthy *et al.*, 2001; Feldman *et al.*, 2002; Samadpour *et al.*, 2002; Bruce *et al.*, 2003). These outbreaks have mainly affected children and the attack rates have been higher when swallowing water and submerging the head. The most likely source has often been infected bathers or run-off from cattle grounds.

The most recent outbreak associated with recreational waters in Sweden occurred in the summer of 2004 when more than 200 people fell ill from swimming in lakes in the Gothenburg area. The causative agent was found to be norovirus. The lake where most people got ill serves as the water source for about half of the city. The performance of the associated drinking water system was evaluated in **Papers I** and **II**. Other norovirus outbreaks in recreational waters have been reported by Hoebe *et al.* (2004) and Maunula *et al.* (2004).

1.1.3 Illness associated with the reuse of wastewater and sludge

Several epidemiological studies have revealed an increased risk for parasitic infestations and other enteric diseases associated with raw wastewater reuse in agricultural irrigation (Katzenelson *et al.*, 1976; Fattal *et al.*, 1986; Cifuentes, 1998;

Srikanth and Naik, 2004). Melloul *et al.* (2002) showed that the incidence of protozoan infections and infections with *Salmonella* were over represented amongst children living in areas with wastewater irrigation compared to control areas (72% compared to 45% and 21% compared to 1%, respectively). Blumenthal *et al.* (2001) noticed in a similar study an increased risk of parasitic helminth infections. Treatment of wastewater, *e.g.* in storage lagoons, however seems to be efficient in reducing the transmission of pathogens (Shuval, 1991; Blumenthal *et al.*, 2001).

Several foodborne outbreaks of disease have been associated with the irrigation of crops with sewage-impacted water (Colley, 1996; Hardy, 1999; Doller *et al.*, 2002). Amahmid *et al.* (1999) investigated the contamination of vegetables after raw sewage irrigation and found high levels of pathogens, *e.g.* 254 cysts of *Giardia* and 2.7 ova of the parasitic roundworm *Ascaris* per kilogram of coriander. Similarly in Israel, Armon *et al.* (2002) demonstrated the presence of *Cryptosporidium* in soil and crops irrigated with wastewater effluents.

There have not been any recorded outbreaks or evidence of transmission of pathogens via wastewater irrigation or sludge application to agricultural land in Sweden (Carlander, 2002). Carlander (2002) however, detected significant numbers of faecal indicator bacteria in groundwater following wastewater irrigation on energy crops, which could imply that transmission of pathogens could occur via this pathway. Furthermore, in Norway, supermarket samples were shown to have *Cryptosporidium* oocysts in 8% of the sprout samples and *Giardia* cysts in 2% of the samples (Robertson *et al.*, 2002).

1.1.4 Epidemiological limitations

Epidemiological tools are often not sensitive enough to detect a few cases arising from exposure to pathogens transmitted via the environment (Eisenberg *et al.*, 2002). As shown in Figure 1-3 infectious diseases may be endemic in the population, *i.e.* a few cases are always present. Large outbreaks are more frequently detected since they draw considerable attention and outbreak investigations can thus be initiated, which can establish the link between the disease and a certain source. For example, the Milwaukee outbreak was only recognised after widespread absence among hospital employees, students, and school teachers, increased numbers of emergency room visits for diarrhoeal illness, and a shortage of anti-diarrhoeal drugs (Kramer *et al.*, 1996). The etiological agent and the waterborne nature of the outbreak were not identified until at least two weeks after its onset. Not until then was a boil-order advisory issued. Minor outbreaks or sporadic cases are unlikely to be reported. Few data are available for evaluation of endemic transmission, and specifically designed blinded randomised studies have given contradictory results, proposing that less than 5% (Hellard *et al.*, 2001; Sinclair, 2003b) or up to 40% (Payment *et al.*, 1997) of community gastrointestinal illness may be waterborne.

Quantitative microbial risk assessment (QMRA) can here serve a purpose for estimating infection risks from low exposure to hazards transmitted via the environment.

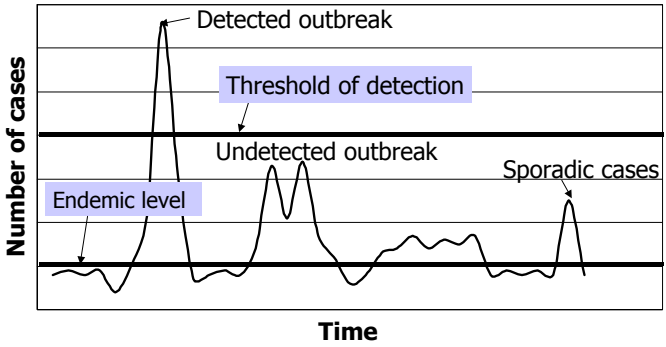

FIGURE 1-3. Difference between endemic background rates, sporadic cases and outbreaks. Adapted from Haas *et al.* (1996).

1.2 What is risk and how is it estimated?

Risk is the likelihood and consequence that something with a negative impact will occur. The 'agent' that causes an adverse effect is a *hazard*. When considering the hazardous agent *Salmonella*, for example, a measure of risk is the probability of getting disease symptoms after exposure to a certain dose of that hazard. Lidskog *et al.* (1997) describe the scientific idea about risk as containing: a cause – effect relationship, negative consequences, probability of occurring and ability to affect. Risk incorporates the probability that an event will occur with the effect it will have on the society and the environment, and also in which socio-political context it takes place (Cutter, 1993).

There are different classifications of risk. One is the separation between dreaded risk, such as nuclear power plant accidents, and more everyday, known risk that most people do not experience as a real danger, *e.g.* accidents with bikes and lawnmowers. Another is the separation between risks of low probability but large negative outcome and risks of high probability but less negative effect. A general consensus is that voluntary risks are much more accepted than non-voluntary risks, *i.e.* people are willing to accept higher risks when they have made the choices themselves compared to risks that they have not chosen and cannot affect (Covello, 1983).

How risks are dealt with within the society is formulated within the area of *risk analysis*, which includes three components: risk assessment, risk management and

risk communication (Haas *et al.*, 1999). These are highly interrelated and should be worked through together for a successful risk analysis.

> *Risk assessment* is defined as the qualitative or quantitative characterisation and estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (here microbial agents).
>
> *Risk management* is the process for controlling risks, weighing alternatives and selecting appropriate action, accounting for risk assessment, values, engineering, economics, and legal and political issues.
>
> *Risk communication* is the communication of risks to managers, stakeholders, public officials, and the public. It includes public perception and the ability to exchange information.

Risk assessment is further divided into four subsequent steps (CAC, 1999):

- *hazard identification*, in which the human health effects of the particular hazard are described;
- *exposure assessment*, which determines the size and nature of the exposed population and the pathways, amount and duration of the exposure;
- *dose-response assessment*, which characterises the relationship between administered dose and incidence of health effect from both human and animal studies; and
- *risk characterisation*, which integrates the information from the previous steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty.

1.3 Microbial risk assessment (MRA)

The first field subjected to risk assessment was that of the risks of chemicals to human health. Microorganisms however differ from chemicals in many ways and the concept has therefore been further developed for assessing microbial hazards. Important characteristics for microorganisms are that they are affected by their environment to a high degree and under unfavourable conditions can be inactivated or die, while under favourable conditions some may multiply. Their transport in the environment differs between different groups of microorganisms. The response in humans and animals after ingestion of pathogenic microorganisms varies widely due to many factors, for example strain or species of the microorganisms, health of humans or animals, prior exposure (immunity), intake together with food *etc.* The risks from microorganisms therefore have to be addressed specifically but can later be integrated in a combined or overall assessment.

Haas *et al.* (1999) define quantitative microbial risk assessment (QMRA) as the application of principles of risk assessment to estimate the consequences from a planned or actual exposure to infectious microorganisms. Risk assessments have also been developed for describing the public health consequences of exposure to pathogens from drinking water, based on its initial use within the food and chemical sectors. QMRA is today applied to establishing standards, guidelines and other recommendations regarding drinking water and consumer health (Rose and Gerba, 1991b; Macler and Regli, 1993; Eisenberg *et al.*, 2002). It has a central role in the new drinking water guidelines from the WHO for assessment of the accomplishment of established health targets and for the evaluation of Water Safety Plans (WHO, 2004). In the latter, it is used to support decisions regarding barriers and treatments necessary to safeguard public health in water supply systems.

One of the most influential QMRA studies was undertaken by Regli *et al.* (1991). They described the quantitative assessment of risk from microorganisms in drinking water, the problems associated with such an analysis as well as the monitoring required to demonstrate that risk levels are met. They also showed how QMRA can be used to determine the level of treatment necessary to ensure that consumers receive a finished drinking water with risks of less than one infection per 10 000 people a year from *Giardia* and enteric viruses in drinking water, a benchmark set up by the US Environmental Protection Agency (USEPA).

Other researchers have assessed the infectious risks in drinking water from viruses (Haas *et al.*, 1993; Gerba *et al.*, 1996b; Crabtree *et al.*, 1997) and protozoa (Haas *et al.*, 1996; Teunis *et al.*, 1997; Gale, 2000; Medema *et al.*, 2003; Pouillot *et al.*, 2004). Microbial risk assessment has both been used to qualitatively (Parkin *et al.*, 2003) and quantitatively (Ashbolt *et al.*, 1997; Soller *et al.*, 2003) assess the health risks of recreational swimming and it is incorporated in the WHO Guidelines for Safe Recreational Waters (WHO, 2003).

The potential adverse health effects associated with the reuse of treated wastewater and sludge is poorly documented (Stenström and Carlander, 1999). QMRA is an appropriate tool for estimating the associated risks and important exposure pathways. This is especially valuable when implementing new reuse strategies. For example, QMRA has been undertaken to assess the health risks of using reclaimed water for irrigation in urban areas, *e.g.* parks and golf courses (Asano and Sakaji, 1990; Rose and Gerba, 1991a; Tanaka *et al.*, 1998; Jolis *et al.*, 1999), use of treated sewage sludge (often referred to as biosolids) in agriculture (Gale, 2003; Eisenberg *et al.*, 2004) and irrigation of crops with raw (Shuval *et al.*, 1997) and treated wastewater (Petterson *et al.*, 2001). In Sweden QMRA have been performed on source-separating sanitary systems, namely the use of urine as fertiliser in agriculture (Höglund *et al.*, 2002) and local greywater treatment (Ottoson and Stenström, 2003).

Many variables in a QMRA are subjected to regional differences. The incidence of diseases in the human and animal population is known to differ between countries, resulting in differences in the occurrence and concentrations of pathogens in surface waters, wastewaters *etc.* The survival of pathogens in the environment is highly affected by climatic factors, *e.g.* temperature and solar irradiation, and hence differs between tropical and temperate regions. The treatment processes used also differ, *e.g.* chlorination is commonly practised on wastewater effluents in the USA while it is seldom used in Sweden; ozonation can occur in several steps in drinking water treatment in the Netherlands while it is still unusual in Swedish waterworks.

QMRA are often focused on a specific pathogen or pathogen group and only consider one exposure pathway. A holistic approach is needed in order to assess the impact on public health of a whole water and wastewater system and in order make comparisons of different systems.

2 Aims, Rationale and Approaches

2.1 Aims

The general aim of this thesis was to investigate health risks from infectious microorganisms transmitted via urban water and wastewater systems. This was undertaken by developing models for microbial risk assessment with particular emphasis on exposure assessment. The specific research questions were:

- Is decentralised drinking water treatment feasible and microbiologically safe from a sustainability point-of-view? (**Paper I**)
- What impacts do failures in drinking water treatment and distribution have on the health of tap water consumers? (**Paper II**)
- What are the concentrations of noroviruses in surfaces waters, do they fluctuate and if so, how can such fluctuations be predicted? (**Paper III**)
- What does tap water consumption look like in Sweden and does it differ between groups in the population? (**Paper IV**)
- What infection risks are associated with local handling and reuse of human faeces? (**Paper V**)
- How can hygienic risks in handling and reuse of wastewater and sewage sludge be controlled? (**Paper VI**)

2.2 Rationale

Paper I

Today the possibility of using different water qualities for different purposes in a household is discussed and sometimes applied (McGann, 2004). Since only about 10 of the 200 litres of water used per person and day (Svenskt Vatten, 2000) are used for drinking and food, it may be possible to supply lower quality water for other uses. As mentioned, reuse of treated wastewater has been realised in *e.g.* Singapore, Australia and Japan, and in China decentralised systems with membrane treatment have been built in order to improve the drinking water quality for the residents (Ma *et al.*, 1998). The health risks however have not been assessed in relation to conventional distribution.

Paper II

Waterborne outbreaks of disease are often associated with different types of failure (Stenström *et al.*, 1994). Teunis *et al.* (1997) concluded that the frequency of failure in treatment processes rather than the average removal would determine the risks of infection with protozoa from drinking water. Payment *et al.* (1997) proposed that contamination in the distribution system was responsible for a major part of waterborne disease. The aim therefore was to assess the type and frequency of failures in drinking water treatment and distribution and their possible health impacts on the population. As mentioned in Section 1.2, most of the waterborne outbreaks in Sweden, where an etiological agent was identified, were caused by *Campylobacter*. No other quantitative assessment of the risks posed by this organism in drinking water has however been published.

Paper III

The numbers of waterborne outbreaks of disease associated with noroviruses appear to have increased in recent years. In order to assess the risk of transmission of noroviruses via drinking water and recreational waters it is essential to know their occurrence and concentration in surface waters or recycled wastewaters. Since these viruses cannot be cultured *in vitro* their detection is dependant on molecular methods, which only result in presence/absence or semi-quantitative real-time PCR data. In order to ensure safe waters for recreation and drinking water production, risk managers are in urgent need of tools to predict the likely occurrence of peak norovirus concentrations in surface waters.

Paper IV

A significant correlation between the risk of becoming ill and the quantity of water consumed have been reported from investigations of waterborne outbreaks (Maurer and Sturchler, 2000; Carrique-Mas *et al.*, 2003). In QMRA it is essential to know the amount of unheated tap water ingested on a daily basis in order to establish the dose of potential pathogens reaching consumers. Water consumption varies between individuals and demographic variables may also be important. A stochastic distribution representing the whole population or certain groups of the population is therefore warranted for risk assessments. Only one such study has been published until now, based on data dating from the end of the 1970's (Roseberry and Burmaster, 1992). More recent and country-specific data are therefore required in order to refine the QMRA models.

Paper V

An increased interest in nutrient recycling has resulted in source-separating systems being implemented at 'eco-villages' in Sweden. In some of these the households are responsible for management of urine and faeces. This arrangement involves new types of exposure than would be encountered in conventional wastewater systems. Recommendations on safe handling of excreta in such systems are still lacking,

especially regarding colder climates. The World Health Organization will publish separate guidelines for the safe reuse of excreta in 2005 (T.A. Stenström, *pers. comm.*). While the health risks associated with the reuse of urine have been assessed (Höglund *et al.*, 2002) the risks associated with storage and reuse of the faecal fraction still need to be evaluated, with emphasis on different exposure scenarios and for situations relevant to Nordic countries.

Paper VI
In order to ensure the safe reuse of wastewater and sludge, proper risk management is needed. Besides posing risks to the end users of food crops or likewise, the handling of these fractions during treatment and reuse should be considered. Proposed here is the application of Hazard Analysis and Critical Control Points (HACCP) for the management of microbial hazards in wastewater and sludge handling and reuse. This has recently also been recommended by Water UK for biosolids treatment and use in agriculture (Water UK, 2004). Other transmission routes besides the consumption of crop however need to be studied, both on an individual basis and on a population basis.

2.3 Approaches

Paper I
A systems analysis comparing the existing drinking water system in the city of Gothenburg with two scenarios with membrane treatment was undertaken (the system structures are presented in Figure 2-1). The methods used were Material Flow Analysis (MFA) for the evaluation of environmental aspects and Quantitative Microbial Risk Assessment (QMRA) for the estimation of health effects. The pathogens chosen for evaluation were *Campylobacter*, rotavirus and *Cryptosporidium*.

Paper II
Incident reports dating several years were reviewed and interviews with key staff were undertaken in order to assemble information regarding failures in treatment and distribution. Based on a compilation of this material a QMRA was carried out with the same pathogens as in Paper I. The QMRA was validated against epidemiological data and included a sensitivity analysis.

Paper III
Norovirus concentrations in a surface water source in the Netherlands were quantitatively described by a most-probable-number (MPN) approach. Statistical distributions were fitted to these data for use in risk assessment. The viruses were monitored monthly over a whole year and daily during part of the winter season. Time series analysis was evaluated as a tool for the prediction of forthcoming concentrations. In addition, samples were analysed for enteroviruses, rotaviruses, F-specific bacteriophages and turbidity.

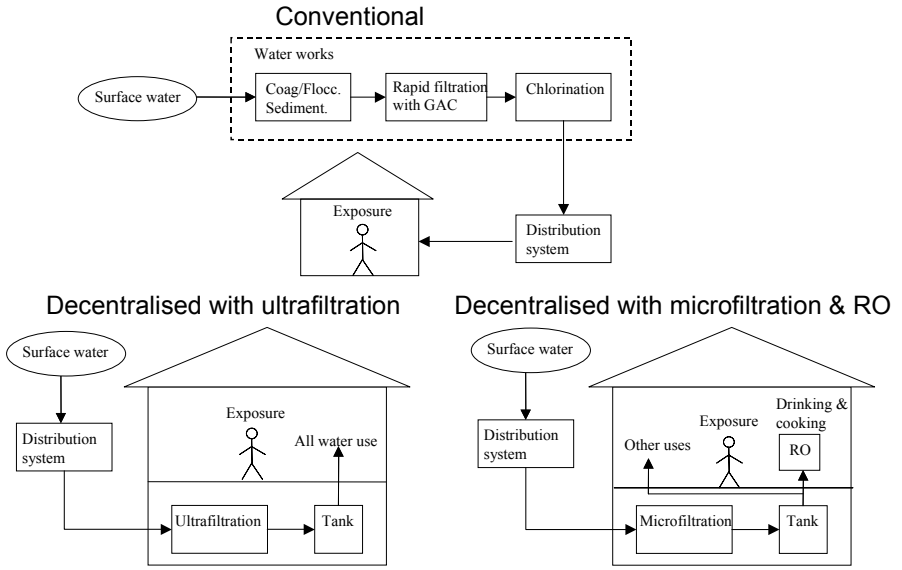

FIGURE 2-1. System structures analysed with MFA and QMRA in **Paper I**. GAC = granular activated carbon. RO = reverse osmosis.

Paper IV

Three sources of information were used to estimate the daily water intake in the Swedish population; a nationwide survey on health and environmental factors from 1999, data from a waterborne disease outbreak investigation in 2002, and a small study on water consumption performed within the Urban Water program in 2003. All three data sets were representative for the Swedish population regarding age and sex distribution. The central issue was the daily consumption of cold tap water however analyses were also made regarding the daily consumption of heated tap water and bottled water.

Paper V

The QMRA dealt with the handling of faeces and possible reuse in the garden as fertiliser. The study was based on a theoretical system where households would use dry toilets in which faeces can be stored for a year before emptying, either for additional storage indoors or for application in the garden. Scenarios were tested for three different exposures; emptying and spreading of the material in the garden, recreational activities in the garden and gardening; and for three different storage times; zero, six or twelve months. Seven pathogens were included in the QMRA, two bacteria, two viruses, two protozoa and one helminth. Two different approaches were used: 1) calculation of the risks of exposure to faeces taking the incidence of the pathogens in the population into consideration ('unconditional') and 2) calculation of the risks when a person in the household was assumed to be infected ('conditional').

Paper VI

This paper integrated QMRA with HACCP for the safe handling and reuse of wastewater and sludge. As a case study a wastewater treatment plant of a medium sized city in the south of Sweden was used. Places where people could be exposed to wastewater or sludge were identified through site visits and in discussions with staff. These included exposure to wastewater and sludge of staff at the treatment plant, visitors to the wetland, people swimming at the bathing place, entrepreneurs collecting and spreading sludge in agriculture and consumers of sludge-fertilised crops. Six pathogens were included in the QMRA; two bacteria, two viruses and two protozoa. The results were expressed as risk per exposure as well as the annual number of infections per exposure route. The latter was also estimated in terms of the resulting increase of endemic disease in the population

3 Quantitative microbial risk assessment (QMRA)

QMRA was the method chosen for assessing risks in water systems. A quantitative approach was preferred since it enables a better comparison of different water and sanitation systems. Substantial amounts of background data have been required for the risk assessments, especially regarding the exposure assessment. Described here are the methods of data collection, the data itself and the data handling in a QMRA framework.

The rationale for the selection of pathogens is described under Hazard identification in Section 3.1 followed by a short description of each pathogen. The Exposure assessment in Section 3.2 deals with how exposures were identified and the information needed to estimate doses of pathogens at exposure. Data availability and quality is addressed, as is the line of thought on how the doses were derived. A separation was made between exposure to 'clean' water, primarily drinking water but also recreational water, and 'dirty' fractions, namely wastewater, sludge and faeces. Groundwater, greywater, urine and stormwater were not dealt with in the thesis.

The Dose-response assessment Section 3.3 mainly deals with how the relationships between dose and response have been derived in previous studies and limitations of the data. Examples of dose-response parameters used in the risk assessments are also given. Issues of data handling, in particular how to address variability and uncertainty, are described in the Hazard characterisation Section 3.4.

3.1 Hazard identification

In hazard identification the microbial agents are identified as well as the spectrum of human illness and diseases associated with each specific pathogen. This also includes pathogenicity and virulence of the microorganism, aspects of acquired immunity and multiple exposures (for example exposure on different days) of the host *etc.*

There is a large range of known waterborne pathogens representing the different groups; bacteria, viruses, protozoa (unicellular organisms) and helminths. Some, like *Salmonella typhi* or *Vibrio cholerae*, have been known for a long time, while others, like noroviruses and *Escherichia coli* O157, have been discovered quite recently (LeChevallier *et al.*, 1999a, 1999b). Since it is not feasible to assess the potential impact of all waterborne pathogens in a risk assessment, a few are chosen as reference pathogens (WHO, 2004). The choice of reference pathogens in this thesis was based on the following criteria:

- The major types of organisms should be represented, *i.e.* bacteria, viruses and parasites;
- The organisms should be occurring in the Swedish population;
- They should have a documented record of being involved in waterborne disease outbreaks or constitute a hazard in sanitation;
- Some of the most persistent organisms should be included;
- Organisms with low infectious doses should be represented;
- Organisms with more serious symptoms should be included if relevant; and
- The organism and its occurrence should be sufficiently well described in the literature.

The pathogens selected for use in QMRA (**Papers I**, **II**, **V** and **VI**) or studied in **Paper III** are described in Sections 3.1.1-3.1.4. Most of the information is from the microbial fact sheets of the recent WHO Guidelines for Drinking Water Quality (WHO, 2004) if not stated otherwise.

The incidence rate of a disease is the yearly number of reported cases divided by the total population, often expressed per 100 000 people (Table 3-1). The reported number of cases is however often substantially underestimated. Several events have to follow upon each other for a case to enter the statistics. The infected person, who excretes pathogens in faeces, may not show any symptoms of disease. People with symptoms must then feel so ill that they seek medical care. The doctor must set the right diagnosis and then report the case further. Not all waterborne diseases are notifiable (have to be reported to an authority) in Sweden and reporting of diseases from laboratories is voluntary (Anonymous, 2004). Estimates of underreporting (*i.e.* how many more cases exist in the community) are presented in Table 3-1. The real incidence of rotavirus is for example estimated to be 21 x 35 = 754 per 100 000 (see Table 3-1). Generally, pathogens causing less severe symptoms are less likely to be reported (Wheeler *et al.*, 1999).

In order to become infected when exposed to a pathogen, pathogens must breach the host's defence mechanisms to reach the target cells where they multiply. ID_{50} is the dose, or number of pathogens, at which 50% of a population will be infected. Dose-response issues are further discussed in Section 3.3. An infected person excretes pathogens, often in very high numbers and for many days (Table 3-1). Not all infections are symptomatic, however. Morbidity is a measure of the percentage of people that will acquire symptoms when infected. As can be seen in Table 3-1 this figure varies considerably.

TABLE 3-1. Different epidemiological statistics of the waterborne pathogens represented in this thesis

	Incidence[a] (per 100 000)	Under-reporting	Morbidity (%)	Excretion[b] (g^{-1} faeces)	Duration[b] (days)	ID_{50}[c]
Salmonella	42-58	3.2[d]	6-80[e]	10^{4-8}	26-51	23 600
Campylobacter	78-97	7.6[d]	25[f]	10^{6-9} [g]	1-77[h]	900
EHEC	0.8-1.4	4.5-8.3[i]	76-89[i]	10^{2-3}	5-12	1 120
Hepatitis A	0.8-7.8	3[k]	70[m]	10^{4-6}	13-30	30
Rotavirus	21[d]	35[d]	50[n]	10^{7-11}	1-39	6
Norovirus	1.2[d]	1562[d]	70[o]	10^{5-9} [p]	5-22[q]	10?
Adenovirus	300[d]	-	54[r]	10^{11} [s]	1-14[r]	1.7
Cryptosporidium	0.3-1.6	4-19[t]	39[e]	10^{7-8}	2-30	165
Giardia	15-26	20[k]	20-40[u]	10^{5-8}	28-284	35
Ascaris	15-25[v]	-	15[g]	10^{4}	107-557	0.7

[a] Based on reporting to Swedish national surveillance 1997-2003 (SMI, 2004) if not stated otherwise. *Cryptosporidium* is from July 2004 a notifiable disease but was until then only included in the voluntary laboratory reporting. [b] Adapted from **Paper V**, with 90% confidence interval, if not stated otherwise. *NB* these are biased to those sick enough to be examined, so it may overestimate the excretion rates in the general population infected with the pathogen. [c] Dose which will infect 50% of exposed individuals. Based on dose-response models reported in Table 3-10. The dose-response for norovirus is still unknown but as few as ten organisms may be sufficient to cause infection (Schaub and Oshiro, 2000). [d] Wheeler *et al.* (1999). The incidence for adenovirus was estimated from a community study and the underreporting is therefore not required. [e] Haas *et al.* (1999). [f] Havelaar *et al.* (2000b). [g] (Feachem *et al.*, 1983). [h] Appendix 3 in Havelaar *et al.* (2000b). [i] Michel *et al.* (2000). [k] Mead *et al.* (1999). [m] Lemon (1997). Children younger than two years rarely manifest symptoms when infected. [n] Gerba *et al.* (1996b). [o] Graham *et al.* (1994). [p] Marshall *et al.* (2001) and K.O. Hedlund, *pers. comm.* [q] Rockx *et al.* (2002). [r] Van *et al.* (1992). [s] Wadell (1984). [t] Calculated from Carrique-Mas (2001) based on the incidence in the first column. [u] Tessier and Davies (1999). [v] Incidence in Denmark, underreporting accounted for (Arnbjerg-Nielsen *et al.*, 2004).

3.1.1 Bacteria

The chosen waterborne bacteria may be zoonotic, *i.e.* can be transmitted from animals to humans and *vice versa*. Bacteria generally have a shorter survival in the environment compared to enteric viruses, parasitic protozoa and helminth ova and are often more easily killed by disinfection. On the other hand the bacteria are the only group that may multiply in the environment (under favourable conditions). The infectious dose is high for many bacteria, but low for some of the selected ones, such as EHEC and *Campylobacter* (see Table 3-1).

Campylobacter

Campylobacter is the most commonly identified cause of waterborne disease outbreaks in Sweden (Andersson and Bohan, 2001) (see Section 1.1.1) and the most important cause of acute gastroenteritis both nationally (Table 3-1) and worldwide. The most frequently isolated species from humans are *C. jejuni* and *C. coli*. The disease outcome is mainly gastrointestinal symptoms (abdominal pains and diarrhoea), but *C. jejuni* can also give rise to reactive arthritis, meningitis and the

severe Guillain-Barré syndrome (an acute immune-mediated disorder of the peripheral nervous system) (Havelaar *et al.*, 2000b; McCarthy and Giesecke, 2001). The principal reservoir of pathogenic *Campylobacter* spp. is the alimentary tract of mammals and birds, commonly found in broilers, cattle, pigs, sheep, wild animals and birds, and domestic pets (Koenraad *et al.*, 1997; Wahlström *et al.*, 2003; WHO, 2004).

Salmonella

Salmonella is second only to *Campylobacter* in terms of the number of annual cases in Sweden regarding reportable enteric diseases (Table 3-1) (SMI, 2004). *Salmonella* are classified into a few species with numerous subspecies or serovars. Many of these are zoonotic and occur frequently in poultry, cattle and swine stocks. Most species cause self-limiting gastroenteritis however *S. typhi* and *S. paratyphi* can cause sepsis and other serious symptoms. Only non-typhoid *Salmonellae* were considered in this thesis. *Salmonella* has the highest infectious dose of the pathogens used in QMRA in this thesis (Table 3-1). It is commonly found in wastewater (Table 3-5) and sewage sludge (Table 3-8) and has been shown to be able to multiply in sludge and sludge-amended soil (Gibbs *et al.*, 1997).

Enterohaemorrhagic *E. coli* (EHEC, VTEC or STEC)

Enterohaemorrhagic *Escherichia coli* (EHEC), verotoxin producing *E. coli* (VTEC) or Shiga-toxin producing *E. coli* (STEC) are different names for the same group of organism, of which the strain O157:H7 is the most commonly recognised. This bacterium can give rise to bloody diarrhoea and 2-7% of cases develop haemolytic uremic syndrome (HUS), which can cause severe kidney syndromes that can be fatal. Cattle are thought to be a primary reservoir for EHEC organisms (AWWA, 1999). The infectious dose is thought to be low, however some discrepancies exist between studies (Haas *et al.*, 2000; Strachan *et al.*, 2001; Teunis *et al.*, 2004). The disease is fairly rare among humans in Sweden (Table 3-1), and therefore the prevalence of EHEC in municipal sewage is expected to be low.

3.1.2 Enteric viruses

Viruses excreted in faeces (enteric viruses) are much more host-specific than bacteria and therefore a certain virus strain will normally only infect a certain host, *e.g.* humans. Viruses need to infect host cells in order to replicate and can therefore not multiply outside of the host. The surface structure of the capsid (the outer "shell" surrounding the viral DNA or RNA) is more resistant than the cell wall and cell membranes of bacteria and viruses therefore survive better in the environment. Non-enveloped viruses are generally more persistent than enveloped viruses. Infected individuals excrete high numbers of viruses (virions) in faeces. Since they generally are very infectious only a few virions are sufficient to cause an infection.

Hepatitis A

The hepatitis A virus can cause infectious hepatitis, which affects the liver and gives rise to the classical symptoms of jaundice. In many cases however, especially in children, the infection is asymptomatic (Koff, 1998). An infection with hepatitis A virus is thought to elicit life-long immunity. The disease is not so common in Sweden (Table 3-1). The transmission of hepatitis via contaminated water and food is well established (WHO, 2004).

Rotavirus

The symptoms of rotavirus infection are gastrointestinal illness with vomiting and diarrhoea. Internationally, rotavirus accounts for nearly half of all cases of diarrhoea in children younger than two years requiring admission to hospital, and may also account for 5-10% of sporadic cases of diarrhoea in adults (Hrdy, 1987). It is also the major cause of gastroenteritis in Swedish children (Uhnoo *et al.*, 1986). The virus has the highest infectivity of any known waterborne virus (Gerba *et al.*, 1996b) and asymptomatic infections occur frequently (Anderson and Weber, 2004). Only one large waterborne outbreak has been reported in Sweden (Stenström *et al.*, 1994).

Norovirus

Norovirus is one of two genera of the human caliciviruses (the other being *Sapovirus*). They were formerly known as Norwalk-like viruses (NLV) or 'small round structured viruses' (SRSV). The symptoms of norovirus infection are acute viral gastroenteritis and vomiting, which generally ceases within a few days. Noroviruses affect all age groups and are today considered to be the most common cause of gastroenteritis in the western world regarding the number of outbreaks and people affected (Koopmans and Duizer, 2004). These viruses can be passed from person-to-person but are also transmitted via contaminated water, foods and fomites (solid surfaces). The number of reported waterborne outbreaks with noroviruses is steadily increasing both in Sweden and internationally (Andersson and Bohan, 2001). In **Paper III** it was shown that the concentration of noroviruses in surface water can be substantial.

Adenoviruses

Human adenoviruses consist of 51 antigenic types associated with a wide range of infections including gastrointestinal, respiratory, urinary tract and eye infections. The types of particular concern for waterborne gastrointestinal illness are adenovirus types 40 and 41, which are excreted in faeces along with other serotypes. Culturable adenoviruses are frequently found in surface waters (Pina *et al.*, 1998), although often in low concentrations (Tani *et al.*, 1995), yet some may come from other animals (de Motes *et al.*, 2004) and therefore will not be infectious in humans. Furthermore, in the control of *Cryptosporidium*, changes in disinfection from chlorine to UV may result in increased adenovirus risk, as they are extremely resistant to UV

disinfection (Meng and Gerba, 1996) and can survive for long periods in water environments (Enriquez *et al.*, 1995).

3.1.3 Parasitic protozoa

Protozoa are unicellular parasitic organisms with complex life cycles. After passing several stages within the host (including sexual reproduction) a transmission stage is formed which is excreted in the faeces. These so called cysts or oocysts are very resistant to different environmental conditions. Many of the protozoa are zoonotic.

Cryptosporidium

The host ranges of different types of *Cryptosporidium* vary. Infections of *Cryptosporidium* in humans are caused by *C. hominis*, previously classified as *C. parvum* genotype 1, or by the animal genotype 2, *C. parvum* (Carey *et al.*, 2004; Xiao *et al.*, 2004). The protozoa cause self-limiting diarrhoea, however cryptosporidiosis can be life threatening in immunocompromised people. *C. parvum* is very common among newborn calves that can excrete oocysts in high numbers, but is also frequently found in adult livestock and other ruminants. The oocysts are extremely resistant to chlorination and have been involved in many waterborne outbreaks, *e.g.* the Milwaukee outbreak (see Section 1.1.1).

Giardia

The flagellated protozoa *Giardia* has been found in a variety of animals. The species infecting humans is *G. intestinalis* (syn. *G. lamblia*, *G. duodenum*), which can also infect numerous mammals (AWWA, 1999). Symptoms generally include diarrhoea and abdominal cramps, however many infections may be asymptomatic. Giardiasis may be chronic in some patients, lasting for more than one year. *Giardia* is the second most common identified etiological agent in waterborne outbreaks of disease in Sweden (Andersson and Bohan, 2001).

3.1.4 Helminths

Parasitic helminths have complex life cycles where the survival stages, ova, are excreted from the host to later be ingested by a new host or a middle host. The ova are extremely resistant to different environmental conditions although they generally cannot withstand higher temperatures (Feachem *et al.*, 1983). In Sweden, helminths are mainly a problem among animal herds.

Ascaris

Most infections with the human roundworm *Ascaris lumbricoides* are asymptomatic although very severe symptoms can occur due to migration of adult worms to the liver, gall bladder or appendix (Feachem *et al.*, 1983). The worms can lead to impaired nutritional status in their host (AWWA, 1999). Ascariasis occurs

worldwide, especially in warm climates and is often associated with poor sanitary conditions. An estimate of the worldwide prevalence is 1 273 million infections (AWWA, 1999). The *Ascaris* ova can survive for several years in moist soils, however they are sensitive to desiccation and are easily killed by high temperature, *e.g.* 100% destruction after one hour at 55 ºC (Feachem *et al.*, 1983).

3.2 Exposure assessment

Exposure assessment is an attempt to determine the frequency, duration and magnitude of pathogen exposure by one or more pathways. The assessment is dependent on adequate methods for recovery, detection, quantification, sensitivity, specificity, virulence and viability of the microorganisms in question and is often dependent on studies and models of transport and fate in the environment.

Exposure assessment uses a wide array of information sources and techniques. Most likely, data will not be available for all aspects of the exposure assessment and those data that are available may sometimes be of questionable or unknown quality. In these situations qualified assumptions must be made. These are based on professional judgments and inferences based on analogy with similar microorganisms or processes *etc.* In the end the exposure assessment will be based on a number of variables with varying degrees of uncertainty. Ideally it is important to capture data uncertainty versus variability in full QMRA models, so these two 'dimensions' can be provided in the final reporting of infection risk.

Exposure to pathogens via the environment can occur through different pathways (partly described in Section 1.1). Pathogenic microorganisms can also enter the body in several ways. The most common is via ingestion but other routes can also be of importance for some microorganisms, like exposure via inhalation, eye or dermal contact (Haas *et al.*, 1999). Standard values for use in risk assessments have been published by the USEPA (USEPA, 1997) and others (McKone and Daniels, 1991; Finley *et al.*, 1994).

In **Paper I** exposure to potential pathogens in drinking water and water ingested during showering was assessed. The former exposure, also used in **Paper II**, was assumed to occur daily with median intakes of 0.96 L as reported by Roseberry and Burmaster (1992), while the latter was assumed to be 10 mL ingested once a week. In **Paper IV** the water consumption in Sweden was assessed and quantified for use in future risk assessments. **Paper V** involved the accidental ingestion of faeces during handling and reuse in the garden where the exposure was based on a survey of studies of daily soil ingestion rates for adults and children. In **Paper IV** exposure to wastewater and sludge during handling and reuse was considered. The exposure volumes, frequencies and number of persons affected were based on a combination

of exposure data described in the literature and assumptions based on site visits and in discussion with people at the site. These are presented in Table 3-2.

TABLE 3-2. Exposure points identified in **Paper VI** with assumptions on volume ingested, frequency and the number of persons affected in a population of 29 000 people.

Type of exposure	Volume ingested (mL or g)	Frequency (times * year^{-1})	Number of persons affected
1. WWTP worker at pre-aeration	1	52	2
2. WWTP worker at belt press	5	208	1
3. (Un)intentional immersion at wetland inlet	30	1	2
4. Child playing at wetland inlet	1	2	30
5. Recreational swimming	50	10	300
6. Child playing at sludge storage	5	1	2
7. Entrepreneur spreading sludge	2	30	2
8. Consumption of raw vegetables	1	2	500

In order to derive the dose of pathogens at each exposure, different points of departure are made. When assessing the risk of infection from drinking water there is the problem that most pathogens cannot be detected in potable waters due to the lack of suitable analytical methods, low concentrations of the pathogens regarded as a cause of concern and potentially large variations with time. A way to circumvent this is to start from the occurrence of the pathogen in the raw water and calculate the concentration in drinking water considering the removal or inactivation during treatment (Teunis *et al.*, 1997). Filtering of large volumes of water is often necessary in order to detect pathogens in surface water (Figure 3-1).

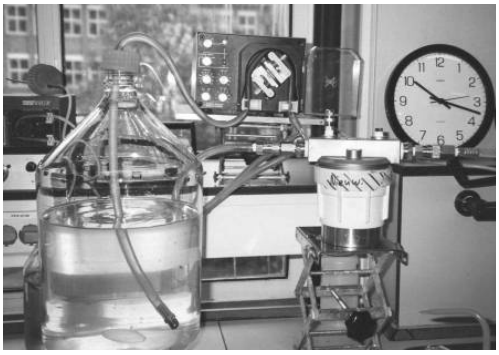

FIGURE 3-1. In **Paper III** large volume water samples of 200-500 L were filtered at the point of sampling while small volume water samples, 10 L, were filtered in the laboratory as shown in the picture.

Exposure to raw or treated wastewater is more straightforward since several studies report the concentration of different pathogens in wastewater. Another approach is to calculate the concentration of pathogens in wastewater from epidemiological data regarding the incidence of the illnesses in the society, adjusted for the rate of

underreporting (*i.e.* the proportion of cases that will not be reported to epidemiological statistics) and morbidity (*i.e.* the proportion of symptomatic cases to infections), the excretion of the pathogens from an infected host and the dilution in wastewater. Such an approach was used in **Paper V** to estimate the potential concentrations of pathogens in faeces.

Due to the problem of detection of many pathogens in water, indicator organisms have been used to imply potential occurrence of pathogens in different water streams. Their concentration and reduction are often used as surrogate values for pathogens. The traditional indicators are bacteria of faecal origin; total coliforms, faecal coliforms (a subset of total coliforms), *Escherichia coli* and Enterococci. During recent years the suitability of indicators has been largely criticised (Ashbolt *et al.*, 2001). The bacterial indicators have been found to be more sensitive to decay than many of the other pathogenic microorganisms and thus do not adequately reflect the survival of more sturdy pathogens such as viruses and protozoa. If risk assessment is based solely on the traditional indicators it will probably underestimate the risks.

There is also no direct correlation between the indicators and the occurrence of a certain pathogen in water (Jiang and Chu, 2004, **Paper III**). The occurrence of pathogens in a water source should therefore be based as much as possible on real pathogen measurements, either from the water of interest or from similar sources described in scientific papers. Indicator organisms can nonetheless be useful as process indicators, *i.e.* to assess the removal of microorganisms in different treatments or model pathogen survival in the environment. Additional indicators, such as bacteriophages and spore-forming bacteria, can be included to better model the behaviour and survival of viruses and protozoa respectively.

Many studies report the reduction of microorganisms by different treatment processes and their die-off or potential re-growth in the environment. Most of these are based on indicator bacteria but some were undertaken with pathogens. There are also differences in the scale of studies, ranging from full-scale to pilot to bench-scale studies. The time and cost of performing microbiological investigations makes it unfeasible to carry out for every system under evaluation. Therefore previous studies reported in the literature are very useful. Many variables included in a microbial risk assessment are however subjected to regional differences, as mentioned in Section 1-3. There is therefore a great need for locally-produced data regarding critical input variables (further dealt with in Section 6.2 and 7.1).

3.2.1 Source water to drinking water

The main pathogen sources of surface water contamination are sewage effluents and agricultural run-off. Concentrations of pathogens in raw water vary substantially

depending on the degree of anthropogenic activities. Wastewater discharge may constitute a significant proportion of the water flow in rivers. Any enteric pathogen that occurs in the population can potentially be found in surface waters impacted by wastewater discharges, though will fluctuate due to seasonality in the incidence of the disease and variability in treatment efficiency. Run-off from agricultural land can contain pathogens from livestock, such as *Cryptosporidium* and EHEC. *Campylobacter* can be frequent in the bird population (Waldenström *et al.*, 2002; Wahlström *et al.*, 2003).

In a survey of Swedish surface water sources, *Giardia* was detected in 26% and *Cryptosporidium* in 32% of the investigated waters (Hansen and Stenström, 1998) (Table 3-3). When screened for *Campylobacter*, 7% of all samples from Swedish surface waters were found positive (SLV, 2002). With repeated sampling in some water sources the bacterium was found in 38% of the samples, often in the absence of faecal indicator bacteria. *Campylobacter* was also detected in groundwater with clear faecal contamination. In Norway *Campylobacter* was isolated from 53% of the samples from a river (Rosef *et al.*, 2001) and from 17% of Finnish lakes and rivers (Hörman *et al.*, 2004).

In **Paper I** and **II** previously unpublished data from Hansen was used where *Cryptosporidium* had been detected in 45% of the samples (Table 3-3). Quantitative data were lacking regarding *Campylobacter* and rotavirus, which instead were taken from Obiri-Danso and Jones (1999) and Gerba *et al.* (1996b), respectively. In **Paper III** the concentrations of noroviruses were estimated from a dilution series of nucleic acid extraction detected with reverse transcriptase polymerase chain reaction (RT-PCR).

Environmental factors such as temperature, UV light, water currents and rainfall affect the concentration of viable pathogens reaching the waterworks, as does pathogen adhesion to particles and sediments (Rao *et al.*, 1984; LeChevallier *et al.*, 1991; Atherholt *et al.*, 1998; Obiri-Danso and Jones, 1999; Payment *et al.*, 2000). Interaction with autochthonous populations of microorganisms will also have a substantial effect on the survival of pathogens in aquatic environments (Medema *et al.*, 1997). The time (in days) for 90% die-off of some pathogens in fresh water can be found in Table 3-7.

Drinking water treatment units act as microbiological barriers. A microbiological barrier is "an appliance or action that counteracts the occurrence of disease-causing viruses, bacteria and parasitic protozoa in the drinking water" (from the Swedish drinking water regulations, SLV, 2003). The number of barriers needed is dependent on the quality of the raw water (SLV, 2003). The barriers could be based either on mechanical removal or inactivation via disinfection. Examples of mechanical barriers are sedimentation, flotation and filtration, which physically (and sometimes

biologically) remove the microorganisms. Through prior addition of chemical coagulants the adsorption of microorganisms to particles is increased and the aggregation of these to form larger particles enhances their removal. In the new national guidelines membrane filtration with pore sizes of 100 nm or less is also accepted as a barrier. These membranes include nanofilters, reverse osmosis and most ultrafilters, however not microfilters with regard to viruses. Disinfection acts by damaging the surface structures or the nucleic acid of the organisms, *i.e.* DNA or RNA. Chlorine for example acts by disrupting cell permeability but also damages nucleic acids and enzymes while UV light mainly causes damages to the nucleic acid (Bitton, 1994).

TABLE 3-3. Concentration (L^{-1}) of different pathogens in surface water

	Mean	Range	Pos[a]	Place	Reference
Campylobacter	Det. in 100 mL	n.a.	7%	Sweden	SLV, 2002
	40-70[b]	<10-600	94%	UK	Obiri-Danso and Jones, 1999
EHEC	Det. in 90 mL	n.r.	1.7	Canada	Gannon *et al.*, 2004
Enterovirus	0.3	0.003-0.90	100%	The Netherlands	**Paper III**
	41	0-190	n.r.	Japan	Tani *et al.*, 1995
Hepatitis A	Det. in 1 L	n.a.	43%	Spain	Pina *et al.*, 1998
Rotavirus	0.2-29	0-41	8-100%	North and South America	Gerba *et al.*, 1996b
Norovirus	Det. in 1 L	n.a.	9.4%	Finland	Hörman *et al.*, 2004
	40	<1-240	38%	The Netherlands	**Paper III**
	390	12- 1 700	100%[c]	The Netherlands	**Paper III**
Adenovirus	Det. in 2 L	n.a.	65%	Spain	Pina *et al.*, 1998
	n.r.	<1-25	24%	Japan	Tani *et al.*, 1995
Cryptosporidium	1.0[b]	<0.01-4.6	32%	Sweden	Hansen and Stenström, 1998
	0.2	<0.01-16	45%	Sweden	Hansen, unpublished. Used in **Papers I** and **II**
	0.1	<0.1-0.4	21%	Norway	Robertson and Gjerde, 2001
Giardia	0.5	<0.01-4.6	26%	Sweden	Hansen and Stenström, 1998
	0.1	<0.1-4	16%	Norway	Robertson and Gjerde, 2001

[a] Percent positive samples. [b] Geometric mean. [c] Winter months.
Det. = detected, n.r. = not reported, n.a. = not applicable

A compilation of studies regarding the removal and inactivation of microorganisms in drinking water treatment processes was undertaken for **Papers I** and **II**. The removal was described as triangular distributions of decimal reduction (*i.e.* expressed as $\log_{10}$ removal) for use in the risk assessment (see further Section 3.4.3). The decimal reduction or $\log_{10}$ removal is:

$$Log_{10}(C_{in}) - Log_{10}(C_{out})$$

where C_{in} is the incoming and C_{out} the outgoing concentration.

TABLE 3-4. Median and range of removal in $\log_{10}$ of microorganisms in drinking water treatment processes. Modified from Hijnen *et al.* (2004), **Paper II** and Kärrman *et al.* (2004).

Process	Bacteria	Viruses	Protozoa
Coagulation/flocculation	1.7 (0.5-3.9)	1.9 (0.2-4.3)	2.0 (0.4-3.7)[a]
Rapid sand or GAC[b] filtration[c]	1.0 (0.3-1.5)	(0.7-1.2)	0.6 (0-1.4)
Slow sand filtration	2.2 (1.3-3.4)	2.1 (0.9-3.5)	n.d.[d] (0.3->6.5)[a]
Chlorination[c]	3.5 (2.5-5.0)	2.0 (1.5-3.0)	0.4 (0-1.0)
UV inactivation[c, e]	(6.5-8)	(3-4)	(3->4.5)
Microfiltration[c]	7 (3-8)	2 (<1-3)	6.5 (>4-7)
Ultrafiltration[c]	8 (7->8)	6 (1->8)	8 (3-8)

[a] *Cryptosporidium*. Lower removal reported for *Giardia*. [b] GAC = granular activated carbon. [c] Compilation adjusted for the process conditions described in **Paper III** and Kärrman *et al.* (2004) regarding the pathogens *Campylobacter*, rotavirus and *Cryptosporidium*. [d] n.d. = not determined. [e] Estimated at a UV dose of 30-40 mWs/cm^2

As shown in Table 3-4 the removal of microorganisms varies between treatment process and organism groups. This is due to different properties of the microorganisms. Viruses are for example more likely to pass barriers that are solely based on mechanical removal (*e.g.* microfiltration) due to their small size, while protozoa and especially *Cryptosporidium*, are very resistant to chlorination. The implications for this regarding doses of pathogens reaching consumers is further discussed in **Paper II**. There can also be large differences between organisms within a certain group. The resistance to UV disinfection for example, varies substantially among the enteric viruses with hepatitis A being the least and adenoviruses the most resistant (Hijnen *et al.*, 2004). Such differences may depend on structural differences between the organisms, for example whether the organisms have DNA or RNA and whether this is single or double stranded (Meng and Gerba, 1996).

After treatment the water is supplied to the consumers via the distribution network. If a chlorine residual is present additional reduction of pathogens is possible. Payment *et al.* (1999) however found that poliovirus and indicators, except *E. coli* were relatively unaffected by the chlorine residuals in the tested systems.

Contamination of treated drinking water with wastewater during distribution is one of the most common causes of waterborne outbreaks (Stenström *et al.*, 1994). Effects of microbial contamination of the distribution network on the health of the consumers were assessed in **Paper II**.

3.2.2 Wastewater and sewage sludge

The occurrence of pathogens in sewage is dependent on the infection levels in the population, which may vary with season (Mounts *et al.*, 2000; Nylen *et al.*, 2002). If slaughterhouses are connected to the municipal sewage they can also contribute substantial amounts of zoonotic pathogens, *e.g. Campylobacter* (Höller, 1988; Koenraad *et al.*, 1996). Some pathogens such as *Salmonella* and adenoviruses are common in the population and are likely to be detected in wastewater (Table 3-5). Others such as noroviruses have a more seasonal occurrence, and are detected in higher concentrations in wintertime (Lodder *et al.*, 1999; Ottoson *et al.*, *submitted*) (Table 3-5). Since EHEC have only been detected with PCR in sewage, the concentration of this organism for use in **Paper VI** was based on the incidence rate, excretion rate and duration, and adjusted with the ratio of calculated and detected numbers of *E. coli* in sewage.

TABLE 3-5. Concentration (L^{-1}) of different pathogens found in wastewater

	Mean	Range	Pos.[a]	Place	Reference
Campylobacter	160 000	500-4 400 000	100%	Germany	Höller, 1988
Salmonella	Det. in 1 mL	n.a.	41%	Sweden	Carlander and Stenström, 2001
	22 000	930-110 000	100%	Finland	Koivunen *et al.*, 2003
EHEC	Det.	n.a.	93%[b]	Germany	Höller *et al.*, 1999
	Det. in 25 g	n.a.	53%[b]	France	Vernozy-Rozand *et al.*, 2002
Enterovirus	28 000	4 200-720 000	100%	Sweden	Ottoson *et al.*, *submitted*
Hepatitis A	Det in 50 µL	n.a.	23%	France	Schvoerer *et al.*, 2000
Rotavirus	215	40-510	100%	USA	Rao *et al.*, 1987
Norovirus	1 900	<800-4 500	78%[c]	Sweden	Ottoson *et al.*, *submitted*
Adenovirus	7 600[d]	250-25 000	100%	Sweden	Bofill-Mas *et al.*, 2000
Giardia	2 000	260-13 000	100%	Sweden	Ottoson *et al.*, *submitted*
Cryptosporidium	20	<8-160	28%	Sweden	Ottoson *et al.*, *submitted*
Ascaris	30	n.r.	n.r.	USA	AWWA, 1999

[a] Percent positive samples. [b] Positive for stx genes by PCR, *E. coli* O157:H7 one of the serotypes detected. [c] 78% positive in winter time, otherwise <100 L^{-1}. [d] Mean calculated from only four samples. Det. = detected, n.a. = not applicable, n.r. = not reported

The mathematical procedure used in **Paper III** to estimate concentrations of noroviruses from dilution series of RT-PCR was used also in Ottoson *et al.* (*submitted*) for the estimation of enterovirus and norovirus concentrations in sewage.

The treatment in Swedish wastewater treatment plants normally consists of sedimentation, chemical precipitation and biological treatment (mostly activated sludge, but also trickling filters). During the 1990's, tertiary treatment for nitrogen reduction was also implemented in many municipalities (MISTRA, 1999), resulting in the introduction of wetland treatment and similar solutions. In an investigation by Stenström (1987) the reduction of indicator organisms in the water phase at different wastewater treatment plants in Sweden was about 99%. A higher reduction would be desirable since the concentration of some organisms is still in the range of hundreds to several thousands per millilitre. The $\log_{10}$ removal of microorganisms in the wastewater treatments used in **Paper VI** is listed in Table 3-6.

TABLE 3-6. Mean and range of removal in $\log_{10}$ of microorganisms in the wastewater treatment processes used in **Paper VI**. Based on previous measurements of faecal coliforms, coliphages and clostridia spores in this system (Stenström *et al.*, 1985) and in a constructed wetland (Stenström and Carlander, 2001).

Process	Bacteria	Viruses	Protozoa
Pre-aeration and sedimentation (chemically aided)	0.2 (0-0.7)	0.2 (0-0.6)	0.2 (0-1.1)
Activated sludge	1.3 (1.1-1.8)	0.6 (0.2-0.7)	0.7 (0.4-0.9)
Chemical precipitation	0.4 (0.1-1.0)	0.7 (0.5-1.0)	0.3 (0-1.2)
Sand filter	0.5 (0.1-1.0)	0.2 (0-0.5)	0.4 (0.3-0.8)
Wetland	2	1	1

The treated wastewater is discharged into the receiving waters. Pathogens can survive for substantial periods in water environments (Table 3-7) and can subsequently contaminate surface waters used for drinking water production and recreational waters (see Section 3.2.1). Numerous epidemiological studies have shown that swimming in wastewater contaminated water results in a greater risk of gastroenteritis (Gerba *et al.*, 1996b).

The die-off of pathogens in water and other materials often follows a first-order decay rate (Tchobanoglous and Burton, 1991):

$$r = C \times k$$

where r is the rate of die-off per unit time per unit volume of water, C is the concentration of the pathogen and k the decay constant. In many studies the time for 90% reduction is instead reported as T_{90}. The relationship between k and T_{90} is

$$T_{90} = \frac{-\ln(0.1)}{k} \approx \frac{2.3}{k}$$

The T_{90}-values used for the survival of pathogens in lake water in **Paper VI** are presented in Table 3-7. The temperatures recorded in the studies ranged from 4 ºC to 25 ºC and the higher temperature the shorter the survival. Although it is unlikely that people will go swimming in water temperatures below 15 ºC, the lower temperatures were included since the survival can be very long, especially for *Cryptosporidium* (up to three months for 1 $\log_{10}$ reduction).

TABLE 3-7 Die-off of pathogens in fresh water expressed as days for 90% reduction, T_{90}. Values used in **Paper VI**

Organism	T_{90}	Reference
EHEC	7-19	Wang and Doyle, 1998
Salmonella	same as for EHEC	Assumption
Rotavirus	5-16	Raphael *et al.*, 1985
Adenovirus	21-29	Enriquez *et al.*, 1995
Giardia	23-30	DeRegnier *et al.*, 1989
Cryptosporidium	40-100	Medema *et al.*, 1997

During wastewater treatment, many of the microorganisms are trapped in, or adsorbed to, particulates and concentrated in the sludge (Chauret *et al.*, 1999). Concentrations of pathogens in untreated sludge are presented in Table 3-8. In **Paper VI** a different approach was however used, in order to be able to utilise national data on the occurrence of some pathogens in sewage. The ratio between concentrations in sewage and in sludge was calculated from the data by Chauret *et al.* (1999) for indicator bacteria, protozoa and bacteriophages, respectively. These ratios were then used to estimate the concentration of pathogens in sludge based on their concentration in sewage.

TABLE 3-8. Concentration (g^{-1} wet weight) of different pathogens found in sewage sludge

	Mean	Range	Pos[a]	Place	Reference
Campylobacter	Det. in 25 g	n.a.	30%	Sweden	Sahlström *et al.*, 2004
Salmonella	Det. in 25 g	n.a.	64%	Sweden	Sahlström *et al.*, 2004
EHEC	Det. in 25 g	n.a.	16%	France	Vernozy-Rozand *et al.*, 2002
	Det in 25 g	n.a.	2%	Sweden	Sahlström *et al.*, 2004
Giardia	441	<25-1180	n.r.	Canada	Chauret *et al.*, 1999
Cryptosporidium	529	<25-381	n.r.	Canada	Chauret *et al.*, 1999
Nematodes[b]	n.r.	0.2-4.5	n.r.	France	Gantzer *et al.*, 2001

[a] Percentage positive samples. [b] *Ascaris* constituted 35% of viable nematode eggs. Figures are reported per gram dry matter. Det. = detected, n.a. = not applicable, n.r. = not reported

The most common sludge treatment in Sweden is mesophilic anaerobic digestion. Chauret *et al.* (1999) found that the reduction or inactivation of microorganisms in mesophilic anaerobic digestions of sludge was low, especially for protozoa. Sahlström *et al.* (2004) also showed that both *Salmonella* and *Campylobacter* were detected in digested sludge. Thermophilic digestion has however shown to be much more efficient. While the removal of total coliforms in mesophilic digestion was 1.5

$\log_{10}$ the corresponding figure for thermophilic digestion was 5.9 $\log_{10}$ (Sahlström *et al.*, 2004). Aerobic digestion has been shown to completely eliminate *Campylobacter* (Koenraad *et al.*, 1997), which may be attributed to the bacteria's inability to cope with aerobic conditions (these bacteria are microaerophilic).

In Sweden, reuse of waste products from wastewater treatment has mainly included sludge. Approximately 35% of all of the sludge from municipal plants was earlier used in agriculture, however the percentage used today has decreased after the directive issued by the Federation of Swedish Farmers (started after discussions on the presence of bromated flame retardants in the sludge) (MISTRA, 1999). The wastewater treatment plant used as a case study in **Paper VI** was still using a part of its sludge in agriculture, however the assumption made in the paper was that all sludge was applied.

3.2.3 Faeces

The faeces fraction is normally disposed of by flushing to municipal sewers. Other systems where the faeces are collected dry however also occur. This type of system was theoretically investigated in **Paper V**. A separate risk assessment has also been performed on a real system in Sweden, which is discussed in Section 7.5.

The survival of pathogens in faeces is poorly investigated. Carlander and Westrell (1998) studied the survival of bacteriophages and *Ascaris* ova in faeces in dry urine-diverting toilets in Vietnam. In **Paper V** an extensive literature survey was undertaken where studies on the survival of pathogens in animal manure, animal slurry and sewage sludge were assumed to be applicable. A similar procedure was used for estimating the survival of pathogens after incorporation into soil. These resulting T_{90}-values are presented in Table 3-9.

TABLE 3-9. Inactivation rates of pathogens in faeces and soil expressed as days for 90% inactivation, compiled for **Paper V**.

	T_{90} faeces (mean ± stdv)	T_{90} soil (mean ± stdv)
Salmonella	30 ± 8	35 ± 6
EHEC	20 ± 4	25 ± 6
Rotavirus	60 ± 16	30 ± 8
Hepatitis A	55 ± 18	75 ± 10
Giardia	27.5 ± 9	30 ± 4
Cryptosporidium	70 ± 20	495 ± 182
Ascaris	125 ± 30	625 ± 150

A potential method for treatment of faeces in small-scale systems of the kind described in **Paper V** is through the addition of urea (Vinnerås *et al.*, 2003b) or by co-composting with organic household waste (Vinnerås *et al.*, 2003a). The latter however requires thorough mixing of the material, which will increase the exposure to potential pathogens in the faeces.

3.3 Dose-response assessment

Dose-response assessment aims at presenting a mathematical relationship between the dose and the probability of infection or illness in exposed persons. Most dose-response studies have been based on human feeding trials, *i.e.* volunteers have been fed with pathogens in different doses and the percentage of subjects seroconverting/excreting the pathogen (or other outcome such as illness) at a certain dose is calculated. Feeding trials using human subjects can provide useful dose-response analysis data, however the doses applied in these studies are usually high, and the subjects are predominantly healthy individuals. Furthermore, these studies often use one or a limited number of strains that may not represent all the virulence characteristics of a species.

Other possible data sets that can be used are those from epidemiological outbreak studies. Epidemiological data, if collected well and if information such as attack rate and ingested dose are provided, can be an ideal data set. These data would essentially provide "real-world' response using subjects that are representative of the population at large. Although epidemiological studies are abundant the enumeration of the disease-causing agent in foods is difficult and often only provide a presence/absence result.

By different mathematical methods dose-response models can be fitted to experimental data (Crockett *et al.*, 1996; Teunis *et al.*, 1996). The risk of becoming infected is dependent on the occurrence of two conditional probabilities: the probability that the organism is ingested and the probability that the organism is able to survive and infect the host once it is ingested. The environment, the pathogen and the host are all variables that play an important role in the probability of infection. Environmental influences include the food vehicle and the stability of the microbiota of the gastrointestinal tract. Pathogen influences include the dose, virulence, and the colonisation potential in the host gastrointestinal tract. Host influences include immune status, age and stomach contents (Coleman and Marks, 1999), which also influence the conditional probability of illness given infection.

It was previously believed that a threshold number of organisms, or minimum infectious dose, had to be ingested before any infection or adverse effects could occur. Today the currently accepted theory is that of the single hit model, *i.e.* that a single pathogen particle has the ability to initiate an infection or illness (Haas, 1983).

The probability of causing infection increases with the dose of the pathogen. The primary non-threshold, single-hit models currently used in microbial risk assessment are the exponential and beta-Poisson dose-response models.

In the exponential model it is assumed that all of the ingested organisms have the same probability, *r*, of causing an infection. The dose ingested is assumed to be Poisson distributed with a mean of D organisms per portion (Haas, 1983; Haas *et al.*, 1999).

$$P_{\text{inf}} = 1 - e^{-rD}$$

where P_{inf} is the probability of infection, r is the probability of one organism initiating an infection and D is the dose.

In the beta-Poisson model, heterogeneity in the organism/host interaction is introduced and r is assumed to follow a beta-distribution (Haas, 1983; Haas *et al.*, 1999). The resulting model is rather complex but can be approximated under the assumption than β is much larger than both a and 1 to:

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

where P_{inf} is the probability of infection, D is the dose ingested and a and β are the dose response parameters.

Several publications are now available on dose-response relations fitted to human feeding trials or outbreak data. The dose-response relations used in the risk assessments of this thesis are presented in Table 3-10. Two different models for EHEC have been used, which vary substantially. In a recent study by Teunis *et al.* (2004) the data from a foodborne outbreak with *E. coli* O157:H7 was shown to agree better with a dose-response model based on *Shigella* than the rabbit model used by Haas *et al.* (2000). For a worst-case evaluation the exact single-hit model ($r = 1$), which represents the maximum risk curve (Teunis and Havelaar, 2000), can be used. This was applied for the parasite *Ascaris* where no dose-response studies are available. *Norovirus* dose-response studies are currently under development, however not yet published. Preliminary results however indicate that as few as 10 PCR-detectable units may cause infection in human adult volunteers (Schaub and Oshiro, 2000). Additional information, *e.g.* regarding the uncertainty of the parameter estimates in dose-response relations, can be found in Teunis *et al.* (1996).

Differences have been discovered regarding pathogenicity and virulence between strains or isolates (Teunis *et al.*, 2002a; Coleman *et al.*, 2004). In the future this

heterogeneity in infectivity should be addressed, however as long as most detection methods used for monitoring pathogens in water do not discriminate between strains or sometimes not even species, such distinctions cannot be made. The incorporation into dose-response relations of differences among hosts in susceptibility to infection (due to immunity) has been proposed by Teunis *et al.* (2002b).

TABLE 3-10. Dose-response parameters for the pathogens used in the risk assessments of **Papers I**, **II**, **V** and **VI**

	Parameters	Comments	Reference
Campylobacter jejuni	$a = 0.145$, $\beta = 7.59$	H.f.t.[a] by Black *et al.* 1988	Medema *et al.*, 1996
Salmonella spp. (non-*typhi*)	$a = 0.3126$, N_{50}[b] = 23 600	H.f.t. by McCullough and Wesley Eisele 1951a, 1951b, 1951c. Several strains	Haas *et al.*, 1999
E. coli O157:H7	$a = 0.2099$, N_{50}[b] = 1 120	Based on h.f.t. on *Shigella* by DuPont *et al.* (1969, 1972) and Levine *et al.* (1973)	Crockett *et al.*, 1996
	$a = 0.49$, N_{50}[b] = 5.96×10^5	Rabbit study by Pai *et al.* 1986	Haas *et al.*, 2000
Hepatitis A	$a = 0.2$, N_{50}[b] = 30	Assumption by Shuval *et al.*, 1997	Shuval *et al.*, 1997
Rotavirus	$a = 0.253$, $\beta = 0.422$	H.f.t. by Ward *et al.* 1986	Teunis *et al.* 1996
Adenovirus 4	k[c] = 2.397	H.f.t. by Couch *et al.* 1966	Haas *et al.*, 1999
Giardia lamblia	$r = 0.0199$	H.f.t. by Rendtorff 1954	Teunis *et al.* 1996
Cryptosporidium parvum	k[c] = 238.6	H.f.t. by DuPont *et al.* 1995	Haas *et al.*, 1996
Ascaris	$r = 1$	None available	Assumption

[a] H.f.t. = human feeding trials. [b] $\beta = N_{50}(2^{1/\alpha}-1)$. [c] $r = 1/k$.

Much is however still unknown regarding the interaction of pathogens in a host. Some of the dose-response relations are based on animal studies and the extrapolation to humans is uncertain. Since low doses are normally encountered in environmental transmission of pathogens there is a necessity to extrapolate the dose-response curve to the low doses in question. Due to ethical reasons dose-response studies have not been made for pathogens that give more severe symptoms. Here, outbreak data can provide the needed information. Other drawbacks are limitations in cultivation technique that can result in either overestimates or underestimates of the correct number of viable organisms. As mentioned earlier, the host defences (immune system) have a large impact on which people get infected and particularly which develop more severe diseases from a certain dose of microorganisms.

3.4 Risk characterisation

The final step of the risk assessment combines the information from the previous steps to estimate the likelihood of an adverse consequence. It should include descriptions of the variability and uncertainty of the hazards and preferably a discussion on the magnitude of the public health problem (Haas *et al.*, 1999).

3.4.1 Stochastic modelling versus point estimates

Point estimates can be used to calculate risks, *i.e.* one value is chosen to represent each variable and the risk is calculated. Mean values of variables are chosen to calculate the average risk while extreme values, such as the 99-percentile, can give an idea of the worst-case situation. Such an approach however does not give a comprehensive picture nor appropriate weight of all combinations. Stochastic modelling where each variable is described as a distribution can instead be used. By random sampling of each distribution with Monte Carlo methods the output risk distribution can be obtained (Figure 3-2).

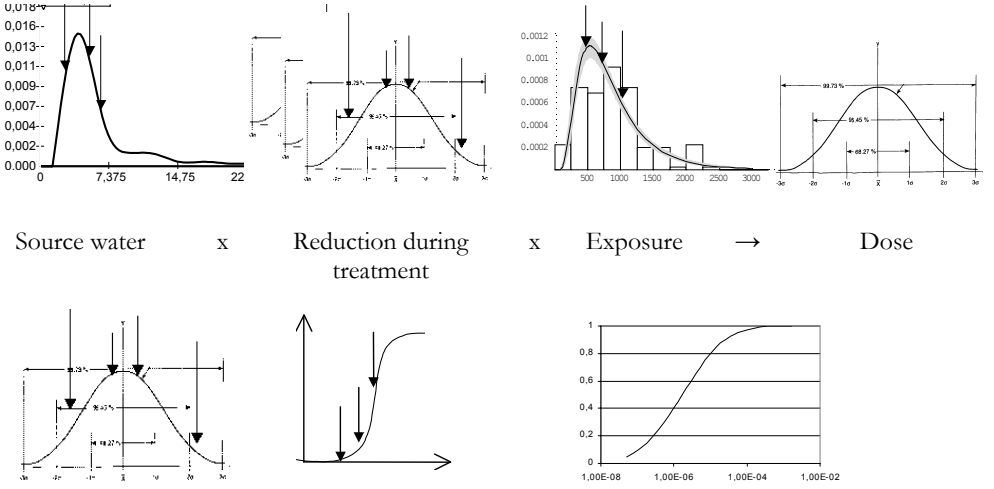

FIGURE 3-2. Schematic picture on the logical sequence of events when calculating the risks of infection, for example from *Cryptosporidium* in drinking water. Arrows indicate random sampling from distributions with Monte Carlo methods. The distributions can be presented either as probability density curves, as shown for the exposure, or as cumulative density curves, as shown for the risk of infection.

3.4.2 Variability and uncertainty

Variability is the inherent variation in the data, which cannot be reduced. The uncertainty reflects flaws in the data collection and can accordingly be reduced by increased investigations. One simple example is the body height of individuals in a population. After measuring a few individuals, statistical measures about the population as a whole can be derived, such as mean, standard deviation *etc.* If more individuals are measured the certainty in the statistical measures will increase, however the variability, for example expressed as the range of heights, will not be reduced.

The USEPA (1997) point out three different types of variability, which can be useful for classifying variability in different variables, *e.g.* concentrations, removals *etc.* These three are:

- spatial variability – variability across locations. Can occur at different levels, *e.g.* regional, local *etc.*
- temporal variability – variability over time whether long or short term (studied in **Paper III**)
- inter-individual variability – variability among individuals (studied in **Paper IV**)

There are also different types of uncertainty, also described by USEPA (1997):

- scenario uncertainty (*e.g.* incorrect or insufficient information, overlooking an important pathway)
- parameter uncertainty (*e.g.* small or unrepresentative samples)
- model uncertainty (*e.g.* excluding relevant variables)

Often the second order uncertainty, *i.e.* the uncertainty in the parameter estimates of the distribution functions, is intended when mentioned in risk assessment papers. Second order uncertainty bounds are presented in the figures of **Paper III** and **IV**. An example from **Paper III** is shown in Figure 3-3.

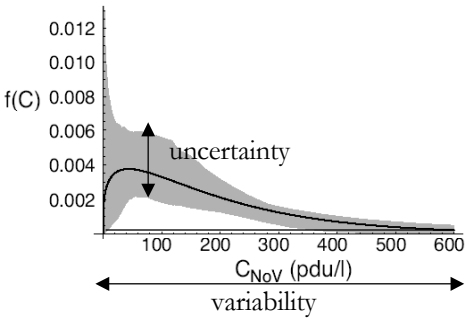

FIGURE 3-3. Variability and parameter uncertainty exemplified by the gamma distribution of the concentrations of noroviruses in surface water during the winter season 2002/2003 from **Paper III**.

3.4.3 Fitting of stochastic distributions

Variability can be taken into consideration in a distribution function. The concentrations of pathogens in source waters are for example usually low but with occasional peaks. Lognormal distributions were therefore chosen to describe this variability, used for example in **Paper II**. In **Paper III** the counts of culturable viruses in a sample were described with a Poisson distribution while the variable concentrations between samples, due to spatial and temporal variations, were described by a Gamma distribution.

Different methods can be used to fit a distribution to data. Frey and Burmaster (1999) describe two equally well-suited methods, maximum likelihood estimation and bootstrap simulation, and also describe how to characterise the uncertainty in the estimates. Maximum likelihood estimation was used in **Papers III** and **IV** and is also one of the methods used in the mathematical package @Risk™ (Palisade Corporation, Newfield, NY) for fitting distributions.

Maximum likelihood estimation is used for finding the parameter values of a distribution that maximise the probability of obtaining a particular set of data. If several distributions are tested the one with the highest likelihood will accordingly have the best fit. The likelihood function is

$$L(x_1, x_2, ..., x_n \mid \theta_1, \theta_2, ..., \theta_k) = L(\theta) = \prod_{i=1}^{n} f(x_i \mid \theta_1, \theta_2, ..., \theta_k)$$

where L is the likelihood, θ the parameter estimates and x the observed sample values. Often the natural logarithm (*ln*) of the likelihood function is used, or even the negative log-likelihood, for computational reasons.

Often though, we do not have access to raw data for all variables in the risk assessment. Instead, the most suitable data can be identified from literature studies. This was the method primarily used for estimating removal efficiencies in **Papers I**, **II**, **V** and **VI**. The values chosen for use in the QMRA were based on a judgement on the quality and applicability of the studies of the specific case. Studies were ranked according to quality of the study (in terms of number of samples, choice of methods, description of set-up *etc.*), scale (lab, pilot, full-scale *etc.*), organisms (real pathogens, indicator organisms, other indicator parameters such as particles *etc.*) and process conditions (processes characteristics should be as similar as possible to those under evaluation in the QMRA). Results from investigations on the removal of human viruses in full-scale operation were for example weighted higher than lab-bench experiments with bacteriophages. Several studies were combined to get an estimation of the removal. Often triangular distributions were used with the most likely value and minimum and maximum values. When even less data were available

uniform distributions were used where all values between minimum and maximum have the same probability of occurring. A good description on how to make this assessment more semi-quantitative can be found in Hijnen *et al.* (2004) where weights for different quality aspects of studies are applied when comparing the results.

3.4.4 Sensitivity analysis

As seen in previous sections many of the input variables in a QMRA have large statistical variability and uncertainties, while the quantitative effect of various phenomena is unknown. With sensitivity analysis the effects of the input variables on the output risk can be assessed. It can therefore be a valuable tool in quantitative risk assessment for examining the main risk-determining phenomena, as well as the variables that mainly determine the inaccuracy (or spread) in the risk estimate, thus identifying where most effort should be placed in reducing uncertainties (Frey and Patil, 2002).

Many mathematical packages used in QMRA allow for sensitivity analysis. One example is @Risk™ where the sensitivity analysis is made with multivariate step-wise regression and/or Spearman rank order correlation. The sensitivity analysis in **Paper II** was adapted from Zwietering and van Gerwen (2000). The impact of the lowest and highest values, respectively, of a distribution (minimum and maximum for closed distributions and 5- and 95-percentiles of open distributions) on the output risk was assessed for each variable with all other variables left unaltered. The resulting 'best-case' and 'worst-case', respectively were compared to the median risk from when all variables were used.

3.4.5 Different ways of expressing risks

The risk of infection can be described or characterised in a number of different ways depending on the purpose, scope, and level of detail of the assessment. One common measure is the risk per person and exposure (used *e.g.* in **Paper I**). This can in turn be described as the mean, the median, or any percentile or confidence interval of the risk. Mean risks are sometimes presented in risk assessments, however since extreme values from the upper or lower percentiles of an output risk distribution can affect the average dramatically (especially when using skewed input distributions) the median risk is often preferable. Both the median and the 95-percentile are presented in all risk assessments in this thesis. In **Papers I** and **II** the 95% confidence interval is depicted in the figures.

By multiplying this figure with the persons affected, the number of infections in this population per exposure is achieved (see *e.g.* **Papers II** and **VI**). This transformation is possible since the dose-response models are 'population-based', *i.e.* based on the

proportion of subjects that will be infected not the probability that each single person is infected. This measure may be more useful in risk communication with the public or specific stakeholders. The risk can also be described as the annual risk of infection per person or population. This is calculated by taking the number of yearly exposures into consideration:

$$P_{yearly} = 1 - (1 - P_{\inf})^n$$

where P_{inf} is the risk per exposure and n the number of exposures per year. The annual risk can be valuable when evaluating risk impacts over longer time periods rather than single exposures, which was the reason for its use in **Paper II**.

4 Epidemiological concerns

4.1 Susceptibility and immunity

Not all individuals in a population are susceptible to infection. Exposure to a certain agent may have resulted in whole or partial immunity against that agent. Many people in Sweden are for example vaccinated against hepatitis A, which may confer lifelong immunity. Prior exposure to *Cryptosporidium* appears to increase the resistance to illness after new exposure (Balbus and Embrey, 2002). Rotaviruses have previously been believed to provide lifelong immunity. Since in principle all children have been infected before the age of two (Hrdy, 1987), no adults would ever be infected. This is however not true (Hrdy, 1987; Svenungsson *et al.*, 2000; Nakajima *et al.*, 2001) and the role of rotaviruses in adult gastroenteritis is now being reassessed (Anderson and Weber, 2004).

Recent evidence suggests that not all persons can be infected by certain pathogens. The 'ability' to become infected with noroviruses for example appears to be dependant on the presence of certain histo-blood types (Huang *et al.*, 2003; Lindesmith *et al.*, 2003) and thus controlled by genetic factors. While some people, estimated at approximately 20% of a population, seem to be resistant to infection (Lindesmith *et al.*, 2003) others have been shown to be re-infected despite high levels of antibodies (Parrino *et al.*, 1977). The latter effect could also be attributed to the numerous strains that are in circulation, *i.e.* immunity only protects for a certain strain. The ability of a pathogen to evolve new surface structures and become more infectious or less infectious must also be considered, *i.e.* a pathogen can change into new forms that the humoral response does not recognise.

4.2 Sensitive subpopulations

Young children, the elderly, pregnant women and the immunocompromised (organ transplants, cancer patients, AIDS patients) are more sensitive to infections than others. The elderly may be less able to build up an effective defence against microbial or chemical contaminants because of a weakened immune system or pre-existing disease. Infants and children are sensitive to microbial and chemical contaminants because their defence mechanisms may not be fully developed. These sensitive populations can represent a rather large part of the total population in a country. In the US for example, this group constitutes almost 20% of the population and is expected to increase significantly, because of increases in life-span and the number of immunocompromised individuals (Gerba *et al.*, 1996a).

If risk assessments are based on dose-response models from studies on healthy adults, they may underestimate the risk for these vulnerable groups. Pouillot *et al.* (2004) however made an attempt to include the immunodeficient population when

assessing the risk from oocysts in drinking water by using dose-response data from a study on immunosuppressed mice. Parkin *et al.* (2003) made a qualitative risk assessment for children exposed to enteroviruses in river water. In **Paper IV** the drinking water intake of sensitive subgroups in the population was assessed.

4.3 Secondary transmission

A person infected with a gastrointestinal pathogen excretes the pathogen in her faeces, regardless of any symptoms of disease. The pathogen can then be transmitted further to other persons, either through direct contact, aerosols or through objects contaminated with faeces. Since some pathogens are zoonotic they can also be further transmitted to animals. Secondary transmission is often specific to the setting (*e.g.* different in homes than in hospitals or day-care centres) and also highly dependent on the infectivity of the pathogen.

4.4 Dynamic modelling

The above-mentioned issues could be taken into consideration in QMRA by using dynamic models. Dynamic models can incorporate the proportion of individuals in a population that are either susceptible to infection, infectious and asymptomatic, infectious and diseased, or immune, and the transition between these states (Eisenberg *et al.*, 1996) (Figure 4-1). In this way the impact of pathogen exposure on a whole population can be assessed, including the role of secondary transmission in sustaining the endemic rate in the population (Chick *et al.*, 2001). Examples on how to use dynamic models have been presented by Chick *et al.* (2001) and Eisenberg *et al.* (2002) regarding the assessment of the impact of waterborne pathogens from drinking water and by Eisenberg *et al.* (2004) regarding the beneficial uses of biosolids. Such models however require considerable amounts of supplementary data.

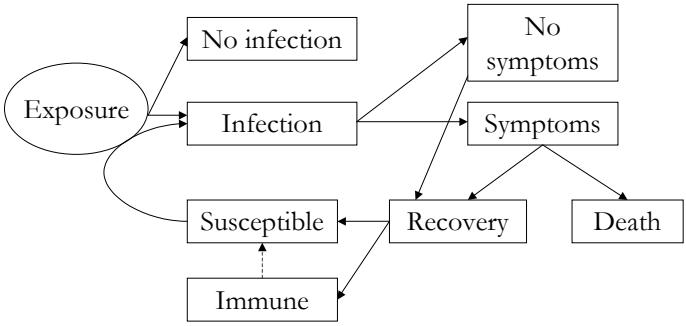

FIGURE 4-1. Possible epidemiological states of a person after the exposure to pathogens

4.5 Health indices

The hazards that may be present in water are associated with a diversity of adverse health outcomes. While most waterborne infections cause acute symptoms such as diarrhoea, some such as hepatitis virus have delayed symptoms. There are also differences in the severity of the symptoms, ranging from mild gastroenteritis to, for example, the severe haemolytic uremic syndrome (HUS) caused by EHEC or Gullain-Barré syndrome caused by *Campylobacter*. In many QMRA the risk of becoming infected with a specific pathogen after a certain exposure is presented, however the outcome of these infections are seldom addressed in terms of illness or fatalities. One way to progress further in the evaluation of health impacts is via the use of a health index. Several health indices exist (McAlearny *et al.*, 1999), among them the Disability Adjusted Life Years (DALY).

4.5.1 Disability adjusted life years (DALY)

In 1992 (with the first stages proposed already in 1988), the Global Burden of Disease (GBD) Study was initiated at the request of the World Bank. The study represents a unique achievement based on the collaboration of over 100 scientists from more than 20 countries and describes the world's disease burden status and trends in the health of populations. The burden from 107 diseases and injuries and 10 major risk factors or risk groups for various age groups and geographical regions were described (Murray and Lopez, 1996; Murray and Lopez, 1997).

To allow comparisons to be made between different health outcomes and allowing quantification of non-fatal outcomes a new unit was introduced: DALY or Disability Adjusted Life Years. DALYs are the sum of life years lost to premature mortality and years lived with disability adjusted for severity (Murray and Lopez, 1997). The basic principle of the DALY is to weight each health effect for its severity from 0 (normal good health) to 1 (death). By multiplying this weight with the duration of the effect and by the number of people affected by a particular outcome, it is then possible to sum the effects of all different outcomes due to a particular agent. In consequence, the DALY is the sum of years of life lost by premature mortality (YLL) and years of healthy life lost in states of less than full health, *i.e.* years lived with a disability (YLD), which are standardised by means of severity weights. In other words, DALY = YLL + YLD.

Although the choice of values incorporated into DALYs have been debated and discussed (Arnesen and Nord, 1999) its key advantages lie in its "aggregation" of different effects and the combining of quality and quantity of life. This metric can be used to promote and enable the setting of rational public health priorities. As such it has been proposed by the WHO for comparing the health impact of different agents in water (WHO, 2004). It can also be used for comparing the health

effects of microbial and chemical risks, for example in order to compare the risks from disinfection by-products in ozonation, with the risk from *Cryptosporidium parvum* in drinking water (Havelaar *et al.*, 2000a). Furthermore, the use of DALY may enhance the understanding of risk in risk communications. Havelaar *et al.* (2000b) used DALY for assessing the total health burden in the Netherlands due to infection with *Campylobacter* species. It could also be useful in a holistic assessment of water systems and in comparisons of water systems. WHO now promote the use of 1 μDALY/y as the benchmark health burden (see Section 5.3).

5 Risk management of water systems

5.1 Guidelines and regulations

Guidelines and regulations exist at different levels for protecting the health of the public when exposed to drinking water, recreational waters, crops amended with sludge *etc*. Many regulations are based on critical levels of indicator organisms that should not be exceeded in order to be classified as acceptable. The frequency of sampling is usually also regulated. Both the drinking water and the recreational water regulations in Sweden are set up in this way (SNV, 1996; SLV, 2001). There are currently no regulations regarding levels of microorganisms in treated wastewater. Upcoming Swedish regulations on the use of sludge for crop fertilisation will include treatment recommendations for use on different types of crop (Schönning, 2003). The current regulations however do not require any sludge treatment, only mixing of sludge into soil within 24 hours.

The revised WHO Guidelines for Drinking Water Quality (WHO, 2004) promote protection of drinking water through use of Water Safety Plans, which incorporates water quality management from the 'source to tap'. A similar approach has also been proposed in the Bonn Charter for Safe Drinking Water by the IWA (2004). The approach in the 3rd edition of the WHO guidelines is based on developing health-based targets. These are based on a consistent framework applicable to all types of hazards and for all types of water supplies. This approach is thought to be more flexible than the previous guideline values since it can account for national priorities and supports a risk–benefit approach. The framework includes different types of health-based targets that differ considerably with respect to the amount of resources needed to develop and implement the targets and in relation to the precision with which the public health benefits of risk management actions can be defined. The different types of targets are: health outcomes, either based on epidemiology or quantitative risk assessment; water quality; performance or specified technology. QMRA has also been incorporated into the WHO Guidelines for Safe Recreational Waters (WHO, 2003) and will be included in their upcoming guidelines for the safe use of wastewater and excreta in agriculture.

5.2 Hazard analysis and critical control points (HACCP)

Within food and drinking water production the risk management system Hazard Analysis and Critical Control Points (HACCP) has been applied (FAO, 1997; WHO, 2004). HACCP offers a preventative management and quality assurance approach rather than random monitoring of the end product. The system involves identification of critical points to control hazards and maintain best management practices throughout production and distribution. Criteria are established for each

control point, which are monitored, and corrective actions are established that should be carried out when critical limits are not met (FAO, 1997).
The working process in the HACCP system consists of seven consecutive steps:

1 Conduct a hazard analysis.
2 Determine the Critical Control Points (location in the process that a certain hazard can be controlled, either through total prevention, elimination or reduction).
3 Establish critical limits (a criterion that separates acceptability from unacceptability, for example a certain temperature, time, moisture level, pH *etc.*).
4 Establish a system to monitor control of the CCP.
5 Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
6 Establish procedures for verification to confirm that the HACCP system is working effectively.
7 Establish documentation concerning all procedures and records appropriate to these principles and their application.

Originally developed for control of microbial hazards during space flights, it is now mandatory within the production and distribution of food. The system facilitates managing and is compatible with other quality management systems, such as the ISO 9000-series (FAO, 1997). Current EU legislation decrees the incorporation of HACCP within drinking water production and it is under implementation in Swedish waterworks (SLV, 2003). The WHO also incorporates HACCP as part of their Water Safety Plans (WHO, 2004). Examples on how to use HACCP in drinking water have been given by Havelaar (1994) and Jagals and Jagals (2004).

As well as controlling microbial risks within drinking water production, HACCP might also be used for safeguarding the quality of different fractions from wastewater and sludge reuse. The approach is in many ways similar to that already utilised in the reuse of wastes within agriculture for reducing the risks for transmission of diseases, namely use of control measures on different levels: waste treatment, crop restrictions, localised application methods, control of human exposure and a combination of the different methods (Blumenthal *et al.*, 1989).

Other researchers have suggested HACCP for water reuse in the food industry (Casani and Knøchel, 2002) and in a wastewater reuse system including groundwater recharge and production of drinking water (Dewettinck *et al.*, 2001). It has recently also been recommended by Water UK for biosolids treatment and use in agriculture (Water UK, 2004).

The HACCP system contains some of the elements of QMRA, including parts of hazard identification and exposure assessment, but could benefit from more

contributions from quantitative microbial risk assessment (Notermans *et al.*, 1994; Haas *et al.*, 1999). In **Paper VI** this idea was developed by trying to adopt HACCP to wastewater and sludge handling and reuse. A major difference in producing safe wastewater or biosolids compared to safe food is that the wastewater already contains the hazards while in the latter the goal is to prevent contamination. The focus must therefore be placed on controlling the *exposure* to wastewater or sludge and eliminate or reduce the hazards through effective treatment.

The hazards or hazardous exposures must be ranked in order to distinguish between important and less important hazards for setting priorities in risk management. The risk associated with each hazard or hazardous event is described in a matrix in HACCP with the likelihood of occurrence (*e.g.*, certain, possible, rare) and the severity of consequences if the hazard occurred (*e.g.*, insignificant, major, catastrophic). In **Paper VI** the occurrence of a hazardous exposure was instead included in the risk estimates and a suggestion of severity of consequences based on the endemic disease in the population was developed (Table 5-1).

TABLE 5-1. Suggested definitions of severity of consequences of hazards based on increase of endemic disease in the community. Developed for use in the ranking of hazardous exposures in **Paper VI**

Item	Definition
Catastrophic	Major increase in diarrhoeal disease >25% or >5% increase in more severe disease or large community outbreak (100 cases) or death
Major	Increase in more severe diseases[a] (0.1-5%) or large increase in diarrhoeal disease (5-<25%)
Moderate	Increase in diarrhoeal disease (1-<5%)
Minor	Slight increase in diarrhoeal diseases (0.1-<1%)
Insignificant	No increase in disease incidence (<0.1%)

[a] Here represented by EHEC

5.3 Tolerable risk

In order to decide a baseline for the infection risks that can be tolerated from a societal point of view there is a need to set up an 'acceptable' or 'tolerable risk' level. The whole concept of acceptable risk can seem controversial. How can we decide which risks are acceptable? Is it acceptable that people get ill or die due to pathogens in drinking water? At the same time we have to balance costs against benefit. Is it defendable or even possible to treat drinking water to the extent that no microorganisms remain?

In the U.S. the Environmental Protection Agency (USEPA) have accepted a yearly risk of 1 infected person in 10 000 from drinking water, which is often expressed as

a risk of 10^{-4} per person. This level has been used by several researchers in risk assessments of drinking water and also to compare risks from other types of exposure (Gerba *et al.*, 1996b; Rose *et al.*, 1996; Tanaka *et al.*, 1998; Jolis *et al.*, 1999). In the 3rd WHO Drinking Water Guidelines a "reference level" of risk of 10^{-6} DALYs per person and year is suggested (WHO, 2004). This is approximately equivalent to a lifetime excess cancer risk of 10^{-5} (*i.e.* one excess case of cancer per 100 000 of the population ingesting drinking water containing the substance at the guideline value over a lifespan). For a pathogen causing watery diarrhoea with a low case fatality rate (*i.e.* the proportion of cases for which the disease would be lethal, *e.g.* 1 in 100 000), this reference level of risk would be equivalent to 1/1000 annual risk of disease to an individual (approximately 1/10 over a lifetime).

The acceptable risk level of 1 in 10 000 has been used for the discussion of risks in **Paper I**, **II**, and **V** and is in **Paper III** referred to in terms of the virus concentration in finished drinking water resulting in this risk level. In **Paper VI** the approach described in the previous section was used for risk classification and prioritisation.

6 Short summary of results

6.1 Paper I – Centralised versus decentralised drinking water treatment

The systems analysis included the evaluation of environmental aspects and microbial health effects. The system structures are shown in Figure 2-1.

Both air and water emissions from the production and transport of chemicals were relatively small. The energy use was dominated by the energy needed for distribution, which was similar for the three systems. The system with two membranes had approximately the same energy use as the conventional system. The decentralised system with ultrafiltration could however reduce total energy use with 25% compared to the other systems.

The conventional treatment exceeded the 10^{-4} median yearly risk of infection primarily regarding viruses but also regarding protozoa and slightly for bacteria (Figure 6-1). Both membrane systems reduced the risks of infection among the consumers substantially, even during partial damages of the membranes (a few broken capillaries, represented by the upper range on the confidence interval in Figure 6-1 and 6-2). Possible growth of bacteria in storage tanks however increases the risk of infection with bacteria.

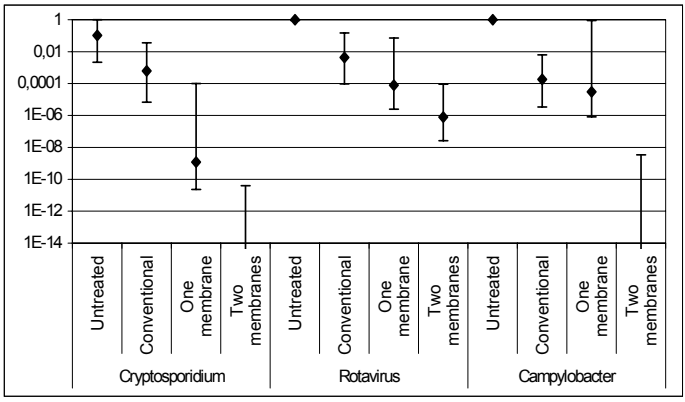

FIGURE 6-1. Yearly risk of infection from drinking water per person, showing median values and 95% confidence intervals. Median values for the two-step membrane alternative is zero regarding *Cryptosporidium* and *Campylobacter*.

Two consecutive membranes gave a good risk reduction regarding the exposure to drinking water since the probability of simultaneous failures was low. Regarding shower exposures, the risks were higher in the second membrane scenario (for rotaviruses also higher than in the conventional system) than the first due to the fact that only microfiltration, which has lower removal capacity (pore size 200 nm) compared to nanofilters (pore size 10 nm), was used for treatment of this water (figure 6-2).

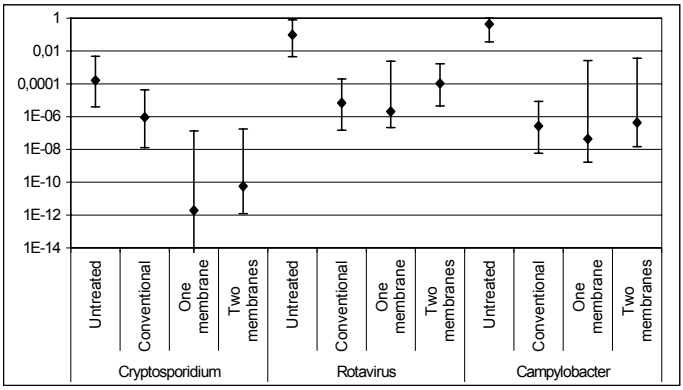

FIGURE 6-2. Yearly risk of infection per person through ingestion of 10 mL water from the shower once a week. Diagram shows median values with 95% confidence intervals.

6.2 Paper II – Failures in drinking water treatment and distribution

The major finding in **Paper II** was that a larger number of people were estimated to be at risk of becoming infected from pathogens passing the treatment under normal operating conditions rather than at failure incidents when calculated on a yearly basis. The incidents in the waterworks were short and did not have a substantial effect on the yearly risk of infection, although the potential for plug-flows was not fully considered. Incidents on the distribution network were less common and only affected parts of the population, however these were of high risk of becoming infected.

Rotavirus, used as a model for viral transmission, caused the largest number of potential infections. The most infections with *Campylobacter* would originate from contamination of reservoirs in the distribution network. The calculated risk of *Cryptosporidium* infections was significantly lower than for the other two pathogens.

The sensitivity analysis pointed out the concentration of pathogens in the raw water, the chlorination regarding *Campylobacter*, and the dose-response functions to be the most critical factors for the risk of infection.

The simulated total number of annual infections in the system was within the range of the equivalent figures estimated from epidemiological data and fraction of gastroenteritis attributable to tap water.

6.3 Paper III – Norovirus fluctuations in surface water

Noroviruses were almost exclusively found during the winter season, while enteroviruses were detected throughout the year with minor peaks in March-April and October-December. Rotaviruses were only detected once in December the subsequent year. The levels of noroviruses were several orders of magnitude higher than those of enteroviruses. All viruses (also bacteriophages) were in low numbers

or non-detectable during the summertime. There was no coincidence in peaks with F-specific bacteriophages or turbidity and human viruses, although some similarities in patterns did occur.

The intensified sampling of noroviruses during the winter season revealed that the winter peak consisted of several shorter peaks of varying duration and magnitude. The peak concentrations were also several orders of magnitude higher than the previous year.

Small volume water samples of 10 litres were as efficient in detecting viruses as large volume water samples of 200-500 litres. They also gave a clearer signal in the gel and blotting in the undiluted RNA extracts due to less concentration of inhibitors.

Norovirus concentrations could be estimated from the results of dilution series in the PCR with an MPN approach. The time series analysis was shown to predict forthcoming sample concentrations accurately and gave a narrower 95% confidence interval than the MPN estimate. Probability distributions were fitted to the norovirus concentrations of the whole year and separately for the winter season.

6.4 Paper IV – Drinking water consumption patterns

Water consumption was shown to differ with demographic and socio-economic factors. Women were for example found to consume higher quantities of cold tap water than did men (on average 0.95 litres compared to 0.79 litres). Tap water consumption was higher in the countryside than in the large cities and consumption was shown to decrease with increasing income. A lognormal distribution was fitted to the quantitative data from the outbreak investigation representing the whole population.

Sensitive subgroups were shown to have higher cold tap water consumption than other groups. The oldest age group, 70 years and above, had the highest daily intake. Although young children had the lowest intake in volume they will have the highest intake in relation to their body weight (Forhammar *et al.*, 1986; USEPA, 2000). In the stratification based on health, people of very poor health had the highest intake.

The heated tap water consumption was somewhat lower than the cold tap water consumption. Men appeared to consume more water in hot beverages than did women (some inconsistency in results between data sets). No differences were detected between age groups, representing 20 years and upward.

Bottled water consumption was generally low with a calculated average of 60 mL per person and day. A slightly higher proportion of women consumed bottled water, however no significant differences were found in consumed volumes

between sexes. The bottled water intake was significantly higher in cities compared to the countryside and increased with increasing income.

6.5 Paper V – Risks associated with local handling of faeces

In approximately 9 out of 10 gardens, the use of stored faeces as a fertiliser would not result in a risk of infection because no pathogens were excreted and collected in the container. One out of 200 containers would contain two pathogens or more. Rotavirus and *Giardia* would be the most frequently-occurring pathogens based on the incidence in the population.

The die-off during storage would be substantial for some of the pathogens, *e.g. Salmonella*, while other pathogens, especially *Ascaris,* have a much higher persistence in faeces. The pathogen with the most severe symptoms, EHEC, was reduced to very low levels already during the storage in the toilet and did not constitute any significant risk in any of the scenarios.

Use of material directly after emptying the toilet container resulted in median risks exceeding 10^{-4} for the unconditional scenario regarding rotavirus and the parasites. After one year of storage however the median risks were below this level for all pathogens, also in the conditional scenario (*i.e.* a family member excreting the pathogen) with the exception of *Ascaris*. The worst-case risks however exceeded the level regarding the viruses and parasites.

The exposure to faeces in terms of ingested amounts was lower during recreational activities or gardening than when emptying the container due to the mixing with soil, however since the frequency of exposure was higher in the former exposure, the annual risks were almost as great.

6.6 Paper VI –HACCP for safe handling and reuse of wastewater and sludge

The highest individual health risk per single exposure was achieved through exposure to droplets and aerosols for workers at the treatment plant, particularly at the belt press for sludge dewatering, and through contact with digested sludge for children or for entrepreneurs when spreading sludge, with a risk of viral infection nearly or equal to 1. The lowest risk was from recreational swimming with infection risks of 10^{-11}-10^{-5} for the range of pathogens evaluated.

The risk entity 'yearly number of infections' took the number of people exposed at each exposure point during a year, into consideration. These values were generally very low for non-viral pathogens ($<<1$) although it reached or nearly reached the

maximum number of infections for both adenovirus and rotavirus in the high-risk exposures mentioned above (which each included two exposed persons). Although several of the exposures only resulted in fractions of infections they had a large impact on the community as a whole in terms of increase in endemic disease level in the community, due to the already low incidence of these pathogens.

Control measures to reduce the hazardous exposure were identified as optimisation of treatment processes for wastewater, use of personal protective equipment for staff, change from mesophilic to thermophilic digestion or prolonged sludge storage times, fencing of sludge storage, and crop restrictions.

7 Discussion

7.1 Use of appropriate data and models

Indicator organisms are used for assessing the faecal contamination of waters. In **Paper III** peaks of F-specific bacteriophages and turbidity did not coincide with those of noroviruses. Since indicator organisms are constantly excreted from humans and animals they would not reflect peaks in pathogen occurrence that were due to varying incidence in the human or animal population.

The sensitivity analysis in **Paper II** pointed out the concentrations of pathogens in the surface water as one of the most critical factors for the risk of infection. The concentrations of *Campylobacter* and rotavirus were based on international data. During recent sampling in the water source in question no *Campylobacter* were detected and the viral contamination seems to be low based on negative findings of noroviruses and enteroviruses. *Cryptosporidium* was however found in concentrations similar to those used in the QMRA. This suggests that the risks of infection with *Campylobacter* and rotavirus may have been overestimated in **Paper II** however they may be realistic at certain events when the raw water concentrations are higher than usual. This highlights the importance of availability to site-specific data regarding the occurrence of pathogens from different sources.

In order to be able to use data compiled from many different studies, the treatment in the waterworks or wastewater treatment plant in **Papers I**, **II** and **VI** was modelled as separate distributions for each process. By using separate distributions it is however assumed that the processes act independently of each other. Hijnen *et al.* (2002) concluded that variability in treatment efficiency in the overall treatment was smaller than expected from the sum of the variation of the processes. This procedure may therefore result in an overestimation of the treatment variability.

In **Paper IV** the drinking water consumption in Sweden was assessed. A log-normal distribution was fitted to one of the data sets for use in future QMRA on drinking water associated health risks. The fitted distribution was shown to be similar to that reported by Roseberry and Burmaster (1992) (Figure 7-1) which justified the use of their model in **Papers I** and **II**. The distribution fitted to the Swedish data was however consistently lower. Besides country-specific differences this effect could be explained by the fact that the data used by Roseberry and Burmaster included all tap water consumption and not only the direct tap water consumption. Our model would therefore be more suitable for the use in QMRA since only the cold tap water constitutes a risk.

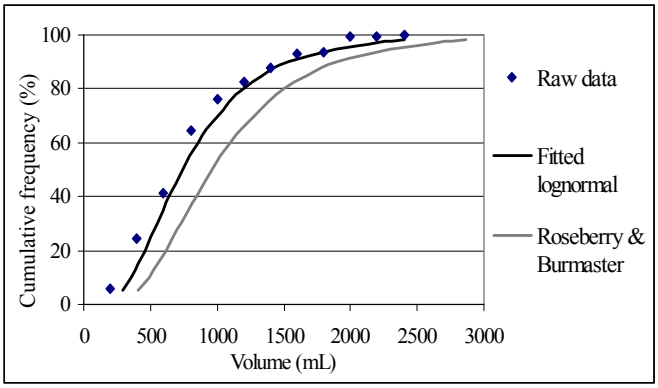

FIGURE 7-1. Cumulative frequencies of daily drinking water consumption. Results from Paper IV compared to the lognormal distribution of Roseberry and Burmaster (1992).

7.2 The importance of different agents

Infections with viruses generally constituted the highest risk in **Papers I**, **II** and **VI**. Only in **Paper V** where the helminth *Ascaris* was included did viruses not present the highest risk, which was due to the maximum-risk dose-response model used for *Ascaris* in the absence of other information. While *Ascaris* is very uncommon in Sweden, viruses are frequently found in both surface water (Table 3-3) and wastewater (Table 3-5) and apart from being very infectious, they are also excreted in high titers from infected persons (Table 3-1). In **Paper II** it was shown that high numbers of people could be infected with rotavirus from drinking water. As mentioned above, the risks may have been overestimated in this case due to the use of non-specific literature data regarding the virus concentrations in raw waters. There is also a chance that a large part of the population is already immune to this pathogen due to early infections in life, however the role of rotavirus in adult gastroenteritis may be largely underestimated (Anderson and Weber, 2004).

When the dose-response models for norovirus are published, norovirus may be the virus of choice for assessing risks associated with drinking water due to the high concentrations detected in sewage and the numerous waterborne outbreaks. Adenoviruses may be useful as a conservative virus index organism due to its persistence in the environment and resistance to some treatment methods. Hepatitis A is uncommon in Sweden but could be appropriate for assessing the more severe symptoms of diseases associated with reuse of wastewater and sludge, especially in high endemic areas.

As mentioned previously, *Campylobacter* causes most waterborne outbreaks of disease in Sweden where a causative agent has been identified. In **Paper II** most infections with *Campylobacter jejuni* would originate from contamination in the distribution network, assumed to originate from sewage. Since most bacteria are sensitive to

chlorine, failures in chlorination or contamination in the distribution network with low chlorine residuals may be the main hazardous events regarding infections with *Campylobacter* via drinking water. This is supported by the study of Nygård *et al.* (2004a) who, in their analysis of the relationship between infections with *Campylobacter* in Sweden and parameters concerning tap water and livestock, concluded that contamination occurring in the water distribution system may be more important than previously considered. Regarding the associated health risks with faeces, wastewater or sludge, *Salmonella* and EHEC were considered to be appropriate. *Salmonella* has a high incidence in the population and are found in high concentrations in wastewater. EHEC is more rare but causes much more severe symptoms. Both *Salmonella* and *E. coli* have been shown to be able to re-grow in sludge (Gibbs *et al.*, 1997; Gantzer *et al.*, 2001).

The calculated risk of *Cryptosporidium* infections was significantly lower than for rotavirus and *Campylobacter* in **Paper II**. This contrasts to the current attention within the water industry where major efforts are placed on minimising the risk of *Cryptosporidium*. One explanation provided above was that the risks with the other pathogens might have been overestimated. While the concentrations of *Cryptosporidium* and *Giardia* (oo)cysts in Swedish surface waters are similar (Table 3-3), *Giardia* is found in much higher concentrations in wastewater (Table 3-5). This suggests that *Cryptosporidium* oocysts in surface waters are more likely to originate from animals than from municipal sewage. It also implies that *Giardia* may be a more suitable agent for assessing the risk of protozoa from wastewater fractions, including faeces.

7.3 Fluctuating pathogen concentrations in surface waters

In **Paper III** it was shown that the occurrence of human viruses had a seasonal variation. Noroviruses had a distinct winter peak, which was even higher in the intensified sampling during the second winter. The latter may be due to the exceptionally high number of outbreaks in the population during the season of 2002/2003 (Lopman *et al.*, 2004). Turbidity was also markedly increased in January 2003, which may be related to the occurrence of an unusual event, *e.g.* a heavy rainfall.

The occurrence of other waterborne pathogens can also be seasonal. Newborn calves can excrete large numbers of oocysts and spring is therefore a time for large potential *Cryptosporidium parvum* contamination. Peak concentrations of both *Cryptosporidium* and *Giardia* (oo)cysts in a watershed have been shown to coincide with calving activity (Ong *et al.*, 1996). During heavy rainfall and snowmelt, run-off from pasture or agricultural land can cause substantial contamination of water bodies (Mawdsley *et al.*, 1995; Kistemann *et al.*, 2002). Such events may also cause combined-sewer overflows and re-suspension of pathogens from bottom sediments

(Atherholt *et al.*, 1998). The pathogen load in an Australian river after one heavy rainfall was estimated to be equivalent to two years of load during 'baseflow' conditions (N. Ashbolt, *pers. comm.*). The need for the development of effective management strategies at urban run-off during storm events was highlighted by Jiang and Chu (2004) due to the increased levels of viruses in rivers following such events.

A question that therefore remains is whether there is a need for additional drinking water treatment during certain seasons or events to avoid waterborne transmission. The norovirus concentrations detected in the source water in wintertime was high (**Paper III**) and even though some proportion of the viruses may not have been infectious, there could be a substantial risk for waterborne transmission in case of insufficient water treatment. In order to reduce the maximum concentration of noroviruses detected in **Paper III** to the acceptable virus level in drinking water suggested by Regli *et al.* (1991) ($<10^{-7}$ viruses per litre) subsequent drinking water treatment must reduce the levels of viruses by 10 $\log_{10}$. The storage reservoirs following on the point from where the water was sampled have been shown to reduce the levels of F-specific bacteriophages by 3 $\log_{10}$ during five months (van Breemen *et al.*, 1998). Additional removal of up to 7 $\log_{10}$ is then still required in the waterworks. While some discrepancies exist between studies on the efficacy of chlorine for disinfection of noroviruses (Keswick *et al.*, 1985; Thurston-Enriquez *et al.*, 2003), these viruses seem to be effectively inactivated with ozone (Shin and Sobsey, 2003). The ordinary treatment conditions may not however be adjusted to cope with such fluctuating concentrations in the raw water.

If discharged wastewater is the most likely source of norovirus peaks, their concentrations in surfaces waters could be reduced by optimising or introducing additional treatment in the wastewater treatment plant. Soller *et al.* (2003) used dynamic disease transmission models to assess the public health benefits of additional wastewater treatment also in winter time, on the risk of acquiring viral gastroenteritis from recreational waters. Although the risk already under the existing treatment scheme was several orders of magnitude below the tolerable illness level set up by the USEPA, the additional winter treatment would further reduce the risk by 15-50%.

Another way to proceed in the management of peaks is by introducing an early warning system. The time series analysis used in **Paper III** was shown to be useful in these respects since it can discriminate an increase or decrease in concentration from random fluctuations. This however requires frequent sampling and rapid processing of samples to allow for the detection of short-term fluctuations. Time series analysis also gives the opportunity to control for events, for example by implementing an action plan to follow when a critical limit is exceeded. This fits

very well into the HACCP system, which nowadays is mandatory for the management of drinking water production within the EU.

The distribution functions developed for noroviruses in **Paper III** could also be valuable in HACCP in order to calculate how probable different virus levels are. This information could then be used to assess the treatment efficiency needed to ensure safe drinking water.

7.4 Use of epidemiology in QMRA

Epidemiology can be integrated into QMRA in different ways and serve different purposes. One is by the use of dynamic models described in Section 4.4 where it can be used to assess the impact of pathogen exposure on a whole population, incorporating susceptibility, immunity and secondary transmission.

The ranking of a hazard or hazardous event in HACCP is usually done by combining the severity of its consequences and the likelihood of its occurrence (Deere *et al.*, 2001). This was in **Paper VI** modified for the application in HACCP of wastewater and sludge treatment and reuse. The ranking was based on the increase of endemic disease expected in the community after a certain hazardous exposure and was estimated on a yearly basis (see Table 5-1). As such it could be applied in different parts of the world with differing endemic rates. It may however be inappropriate to use in areas where the reuse of untreated wastewater and sludge already contributes substantially to the endemic rate. The level at which the exposures were considered to become severe, 0.1% increase in endemic disease, is also the reference level of disease suggested by the WHO for pathogens causing watery diarrhoea (see Section 5.3).

As a means to evaluate the plausibility of the results of the QMRA, a comparison was made in **Paper II** between the simulated number of annual infections and the number of infections estimated from epidemiological data. The proportion of gastrointestinal illness attributable to drinking water used in the calculations, 14-40% from the study by Payment *et al.* (1997), is however debated in the scientific community. In the first Water Evaluation Trial, out of five initiated by the USEPA and the Centers for Disease Control and Prevention (CDC), no excess gastrointestinal illness was detected in households without additional water treatment at the kitchen tap (Sinclair, 2003b). There were however large differences in the chlorine residual in the distribution network between the studies, 2.1 mg L^{-1} in the American study and only 0.5 mg L^{-1} at the first consumers' tap in the Canadian study by Payment *et al.* The system under study in **Paper II** had a chlorine residual of 0.2 mg L^{-1} when leaving the plant. Since Payment *et al.* (1997) concluded that the distribution system appeared to be the major source of contamination, the

chlorine residual is naturally important in reducing the viability of pathogens reaching the consumers.

7.5 Microbial health risks in decentralised systems

The centralised treatment of water and wastewater has been the only solution implemented for cities since the introduction of piped water and sewerage in Sweden by the end of the 19th century. A question remains as to whether this is this the only possible way to go. In this thesis the hygienic risks associated with decentralised treatment of drinking water and local collection of faeces have been investigated.

In **Paper I** it was concluded that decentralised drinking water treatment with membranes was competitive with the centralised conventional treatment regarding environmental impacts and health. Local drinking water treatment may in one sense give a more robust infrastructure, since high concentrations of pathogens in the raw water or sewage ingress and other contamination within the distribution system will be counteracted by the treatment at the point-of-use.

Failures in decentralised systems will, in contrast to failures in centralised systems (**Paper II**), not affect the whole population at the same time. Those failures that could have an impact in the separate systems were identified as indoor cross-connections and membrane failures. The normally negligible risk in an end-point membrane system could be increased by several orders of magnitude if the integrity of the membrane was not maintained, but could still be lower than the risk from the conventional system. Integrity testing is therefore a key component in membrane filtration applications however may be less feasible to carry out in many small units. The number of barriers is also important for safety. As shown in **Paper I**, the decentralised system with only ultrafiltration could constitute a high risk for viruses and bacteria if the membrane was compromised. This highlights the importance of having several subsequent barriers. On the other hand the risks from exposure to water in showers was much higher in the membrane scenario with only microfiltration of the water due to the larger pore size of the membrane.

There is also a risk of cross-connections of water with different qualities within the buildings. This happened in a dual water supply in the Netherlands where partly-treated surface water was supplied to households for garden watering, toilet flushing and laundry use. The cross-connection with potable water was not discovered until after a week and by that time an outbreak of gastroenteritis affecting 200 people had occurred (Sinclair, 2003a). A very important issue is therefore that of operation and maintenance. A system with many small treatment units spread over the city may be difficult to keep under satisfactory control for a centralised organisation, which may suggest that the management would have to be outsourced to sub-contractors.

The microbial health risks in systems with local collection of faeces are highly dependent on the incidence of disease in the population, as shown in **Paper V**. Wastewater from a large city will always contain pathogens, *e.g.* in a population of 100 000 people several hundreds would excrete *Campylobacter* during a year (see Table 3-1). In systems based on a household level however, most households would not have any pathogens in their faeces bins during a year, and in households where a person was infected, the risks for other family members of acquiring an infection would most likely be higher via other transmission routes, *e.g.* person-to-person contact or via fomites.

The study in **Paper V** was based on a theoretical system, however an existing system in Sweden has also been evaluated in a similar way (unpublished). In the latter, in Urban Water called the 'urban enclave', the faeces from each household was emptied into a common outdoor compost (although no composting process has been observed in terms of temperature increase) with sufficient capacity for several years of storage. The possibility of long-term storage will render possible sufficient die-off of potential pathogens in order to reduce risks to tolerable levels. On the other hand, the bins had to be emptied much more frequently than in the system in **Paper V** (on average every month to every third month compared to once a year), which results in more frequent exposure to potentially hazardous material.

A fundamental difference between the systems is that the people in the 'urban enclave' are not only exposed to the faeces from their own family but also to those of thirty other families, which increases the likelihood of exposure to pathogenic microorganisms when emptying bins or reusing the material in the area. In single households knowledge about prior diarrhoeal illnesses could entail increased awareness when handling the faeces – information that would not be available in a system encompassing several households. If systems like this should be implemented in urban areas, it is crucial that they are properly operated and maintained and that routines for handling the hazardous material are set up and followed.

7.6 Outbreaks versus sporadic cases

In **Paper II** it was estimated that a larger number of people were at risk of becoming infected from pathogens passing the treatment under normal operating conditions rather than at failure incidents. It is unlikely that these cases would be reported to epidemiological statistics since the cases would be sporadic both in time and place and the link to drinking water therefore not be evident from a medical viewpoint (see Figure 1-3). While the incidents in the waterworks did not have a substantial effect on the yearly risk of infection due to their short duration and

mixing of water produced during failure with that produced during normal operating conditions, the incidents in the distribution network were more likely to cause evident outbreaks. The reasons for this were that affected persons were of high risk of becoming infected and because a local contamination would affect the residents in a limited area. This geographical cluster of cases could lead to the suspicion of a common source.

To conclude, although the risk of becoming infected during a failure event can be high, the resulting annual number of cases could be higher from tap water produced during normal operating conditions. This issue is very important when it comes to risk management and risk minimisation and the question remains as to whether the focus should be on reduction of the total number of infections or on the prevention of events that can lead to outbreaks.

Risk experts often consider hazards that affect many people at a single occasion and hazards that affect many people but sporadically as comparable (Covello, 1983). From this point-of-view the effort that would have the highest impact on the number of infections would be preferred. Non-experts however often consider hazards that affect many people at the same time as being more dangerous (Covello, 1983). It is therefore probable to believe that the public would want to prevent outbreaks, no matter how small or rare these are.

Considering the impact a waterborne disease outbreak has on society, this may actually be the right priority. Beside the negative health impact for affected people and the possibility of lost consumer confidence in the drinking water, waterborne disease outbreaks also cost the society a substantial amount of money. In the Milwaukee outbreak in 1993, where 400 000 people were estimated to be affected, the total costs of illness has been estimated at US $96 million; $32 million in medical costs and $64 million in productivity losses (Corso *et al.*, 2003). The costs for outbreak investigations and follow-up, *i.e.* trying to determine what pathogens and what technical defects caused the outbreak, how many people were affected, how to prevent further impact and so on, can also be substantial. Andersson *et al.* (1997) estimated the cost of a waterborne outbreak with more than 3 000 cases of *Campylobacter* in Sweden to be 4.8 million SEK (US $675 000). The total cost of waterborne outbreaks in Sweden has not been assessed, however for comparison the annual cost of foodborne illnesses in Sweden has been estimated at 1 082 million SEK (US $152 million) (Lindqvist *et al.*, 2001).

7.7 Sensitive sub-populations

As mentioned in Section 4.2 sensitive populations can constitute a large part of the population and are likely to increase (Gerba *et al.*, 1996a). These groups of individuals would be at the greatest risk of serious illness and mortality from water

and foodborne enteric microorganisms. There may therefore be a need to take these individuals into special consideration in the risk management of drinking water and different reuse practices of wastewater and sludge.

In **Paper IV** it was concluded that the sensitive subpopulations, the young, the elderly and the sick had higher unheated tap water intake than other groups in Sweden. If the drinking water is contaminated with pathogens these groups are at increased risk of becoming infected or to develop more severe symptoms than would healthy individuals. This may call for a raised concern regarding risk assessment and management of drinking water systems. Some countries have special water advice for the immunocompromised population (CDC, 2004) a situation that has mainly arisen as a result of the high sensitivity to and fatality in cryptosporidiosis for HIV patients (Aragón *et al.* 2003). Aragón *et al.* (2003) found that the proportion of cases of cryptosporidiosis in AIDS patients attributable to tap water consumption could be as high as 85%.

Since decentralised treatment of drinking water in **Paper I** was shown to be feasible both from a health risk and from environmental effects perspective, additional water treatment could be provided to sensitive consumer groups such as day-care centres, nursing homes and hospitals. Such decisions should preferably be made in a forum of decision makers and the public in general.

Nwachuku and Gerba (2004) suggest that children should be taken into separate consideration in QMRA. This is because children are of increased risk of infection to enteric pathogens, but also because they may be more environmentally exposed. The hand-to-mouth and object-to-mouth contact is much greater among children than adults (Nwachuku and Gerba, 2004) for example. This was considered in the QMRA in **Papers V** and **VI** where separate soil ingestion rates for children were used. Exposures were identified specifically for children such as playing at the sludge storage and more frequent transfer of water-to-mouth in the wetland compared to adults. Several outbreaks with EHEC from recreational waters have mainly affected children and the attack rates have been higher when swallowing water and submerging the head (Feldman *et al.*, 2002). The same observation was made at the outbreak with noroviruses in Gothenburg in the summer of 2004.

7.8 Application of HACCP in the management of wastewater and biosolids treatment and reuse

HACCP was recently proposed for the management of hazards in biosolids reuse on agricultural land in the UK (Water UK, 2004). The proposed procedure covers different types of hazards, not only microbiological. The focus is however only on the end product and does not include the management of hazards for staff or other

people involved in the treatment and handling of biosolids. These issues were however addressed in **Paper VI**.

In **Paper VI** the calculated risks of infection of staff working at the wastewater treatment plant were exceptionally high. Although staff may have acquired immunity to some of the pathogens encountered in wastewater, this should not be a reason for accepting high risks. Gastrointestinal symptoms, airway symptoms, joint pains, unusual tiredness and toxic pneumonitis have been found in significantly higher frequency among operational staff at sewage treatment plants in Sweden compared to controls (Thorn *et al.*, 2002; Thorn and Beijer, 2004). Although some of these symptoms may be associated with exposure to endotoxins, somewhat higher antibody levels to adenovirus and enterovirus were also found (Thorn and Beijer, 2004). Risk reducing measures would however be relatively easily to implement.

The reuse of sludge or biosolids in agriculture resulted in low risks from crops in **Paper VI**. The use of treated sludge in agriculture probably constitutes lower microbial hazards than the common use of animal slurry and manure, however chemical hazards are most likely higher in sewage sludge. To be on the safe side when reusing wastewater fractions, risks should be reduced substantially at treatment to ensure a safe product for further handling. For this to be possible, wastewater treatment plants must be designed such that the desired effluent quality is consistently achieved (Cooper, 1991).

The ranking of hazardous exposures based on increase in endemic disease was found useful in setting the risks in a public health perspective. The amounts and frequencies of exposure were however uncertain in several of the exposures and need further investigation. The QMRA and HACCP procedure was in **Paper VI** applied to conditions of normal operation of the treatment plant. Worst-case scenarios or hazardous events need to be further evaluated in order to propose a final management system. Such events could be flooding, a major failure in the wastewater treatment or sudden peaks based on treatment variability. The remaining steps in HACCP should also be addressed with the participation of the managers and staff of the treatment plant.

7.9 Risk communication – experts and users

As mentioned in Section 1.2 people generally regard exposures to involuntary risks as less acceptable than voluntary risks. Considering this, people are less likely to accept hazards in their drinking water since that is something they cannot affect and since they are dependent on drinking water for their daily life. People may on the other hand accept high microbial risks from a system they have chosen themselves, as seen for example regarding the faeces collection in the 'urban enclave' mentioned

above. In that system the users appeared to be aware of and take precautionary measures to avoid some of the obvious hazardous exposures such as when emptying bins (H. Krantz, *pers. comm.)*. Other hazardous exposures identified by the risk assessor were however not considered such as children playing at the faeces compost or the occurrence of flies in some apartments originating from the faeces.

Although QMRA require a lot of effort in data collection and skills in modelling, the communication of risks to the public may be the most difficult task in risk analysis. The way that experts use quantitative assessments, with advanced computer programs and models, to identify, estimate and assess risks is very different from the intuitive risk assessment or risk perceptions that people use in everyday life (Slovic, 1987). The question is how experts and the public should communicate with each other when they use such different languages and methods.

One possible way to proceed regarding the communication of microbial risks is by the use of health indices, such as DALYs (described in Section 4.5) since the number of years lost or lived with disability gives more information on the severity of a hazard compared to measures of infection risk. In the Urban Water program multi-criteria decision aid is proposed for use in decision making of urban water systems involving different stakeholders (Söderberg and Kain, 2002; van Moeffaert, 2003). These should take all sustainability aspects into consideration, out of which health is one of the most important.

8 Conclusions

The most important conclusions in relation to the formulated research questions were:

- Decentralised drinking water treatment with membranes can be competitive with centralised conventional treatment regarding environmental impacts and health. Even in systems encompassing treatment processes with very high removal efficiency such as membranes, more than one barrier is required to minimise health risks in case of failures. In order to safeguard the health of consumers in decentralised systems it is crucial that system operation and management is well established.

- Failures in drinking water distribution involving microbial contamination are likely to result in waterborne disease outbreaks. Short-term failures in treatment may not have a large impact on the health of consumers, especially if the water produced during failure is mixed with water produced during normal operating conditions. Averaged over a year, the total number of people at risk of becoming infected with pathogens from drinking water could be higher from water produced during normal operating conditions than during failures.

- The concentrations of noroviruses in surface water can be estimated by a most-probable-number approach. These viruses show substantial fluctuations in concentration over the year with peak concentrations in the wintertime. These peaks could be further resolved into smaller peaks, possibly resulting from outbreaks in the human population or different types of events. Fluctuating concentrations of noroviruses or other pathogens could be predicted by time series analysis and used as an early warning system if complemented by regular monitoring.

- The direct, or cold, tap water consumption in Sweden differs between groups in the population and to that reported from several other countries. Groups already sensitive to infection, *i.e.* the elderly, the sick and children, consume higher volumes of cold tap water than the rest of the population. This may call for special attention in the risk management of drinking water systems.

- The infection risks associated with local handling and reuse of human faeces is highly dependent on the incidence of infectious diseases in the population and would be low for the majority of households in Denmark and Sweden. In order to reduce risks to acceptable levels over the system as a whole, sufficient die-off of the most persistent pathogens must be secured, which

requires facilities for the long-term storage of locally-collected faeces in case no other treatment is provided. Without the assurance of proper maintenance and following of directions, these kinds of systems should not be recommended on a larger scale, especially if the material is to be applied to soil.

- Microbial health risks associated with the handling and reuse of wastewater and sludge can be successfully addressed and controlled within the management system Hazard Analysis and Critical Control Points (HACCP). The infection risks of hazards and hazardous exposure pathways can be put into an epidemiological framework in order to facilitate decision-making regarding hazard prioritisation. Many exposure pathways can be controlled through easy measures. Worst-case scenarios or hazardous events need to be included in a final risk management system.

QMRA has the potential to be used in many different contexts in the future, not the least in the comparison between different water and wastewater systems. Although many important questions have been resolved in this thesis many others remain to be answered. One for example is the resolution needed in the models to make accurate estimations of microbial health risks. As the need for underlying data is great the field would benefit from international co-operation and data exchange. On the other hand, site-specific pathogen monitoring was shown to be very important for the accurate estimation of risks. The implementation of new solutions of water and wastewater/sanitation systems was also shown to incorporate new exposure pathways to pathogens. These need further attention regarding where, when and how often they would occur and whom they will affect.

9 Acknowledgements

I know that many people were surprised when they heard that someone who is personifying the Swedish expression "a little dirt cleans the stomach" would do a PhD in microbial risk assessment. To all of you I can say that I have realised the importance of protecting the population, and especially sensitive persons, from unwanted and unnecessary hazards. I am today also willing to recognise the importance of throwing out food that consists of more mould than original contents...

There are many persons that in different ways have contributed to this thesis with knowledge, ideas and fruitful discussions, but not the least with encouraging words and actions. I would especially like to thank the following persons:

Thor Axel Stenström for supervising a stubborn PhD student like me. You have an exceptional ability of producing good ideas and have supported me when I needed it the most. My co-supervisor *Nick Ashbolt* who came up with the original project idea. Thank you for bringing the latest news from the risk assessment world during you visits to Sweden. It has been inspiring to follow your 'evolution of ideas'.

The water section at SMI, a group of people with enormous hearts, for always caring and sharing. *Görel*, *Anette*, *Emma*, *Anna* and *Sabina*, you are the backbones of our section and the scouts of pathogens in water - always prepared! *Jakob*, *Caroline*, *Anneli*, *Annika* and *Johan* are much appreciated for doing the other strange stuff involving the 'not-so-clean' samples. It's a dirty job but someone's got to do it! To my roommates *Jonas* and *Mick* for freezing and fighting together with me in (I quote Uffe) "the best room at the whole of SMI". A special thanks to Mick for his invaluable language assistance. I also appreciate the pleasant company at lunches and coffee breaks of other people at SMI and MTC.

My home institution, the department of Water and Environmental Studies at Linköping University, for providing an open and stimulating atmosphere. My deepest gratitude to *Ian* -'The rocker'- *Dickson*. Without your computer aid this thesis would certainly have disappeared into cyberspace a long time ago. My fellow PhD students in Linköping and in the Urban Water program for making this journey a lot easier, more enjoyable and more interesting. A particularly big hug to *Helena K* who always lends an ear when I want to complain about the life as a graduate student in general or discuss thoughts about life in particular. When shall we publish our rhymes?

Peter Teunis for his mathematical superiority and humbleness. *Ana Maria de Roda Husman* for her enthusiasm and for being full of new ideas to try out. Thank you both for letting me work with you at RIVM, I had a great time!

I would like to acknowledge the financial support provided by the Swedish research foundation MISTRA within the Sustainable Urban Water Management Program, the Swedish Research Council for Environment, Agricultural Science and Spatial Planning (Formas) and the EU project MicroRisk (contract EVK1-CT-2002-00123). Without their contributions this project would not have been possible. I have especially appreciated the possibilities for scientific exchange at international conferences and national and international collaborations. *Per-Arne Malmqvist* and *Henriette Söderberg* have done a great job running the whole Urban Water program. Thank you both and also *Jan-Olof Drangert* for letting us study such interesting subjects in the research school. The mega city travels have been enormously rewarding and clearly gives another perspective on the conditions of urban water in Sweden compared to other countries.

Rickard who convinced me to apply for the PhD position in the first place and always came up with solutions to my problems. My friends that made sure that I had causes for rejoicing on the side of my studies.

And last a great thanks to my family for all their love. You have inspired me with the feeling that anything is possible! I love you!

10 References

Ackman, D., Marks, S., Mack, P., Caldwell, M., Root, T. and Birkhead, G. (1997). Swimming-associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: evidence of prolonged contamination of a fresh water lake. *Epidemiology and Infection* **119**(1): 1-8.

Amahmid, O., Asmama, S. and Bouhoum, K. (1999). The effect of waste water reuse in irrigation on the contamination level of food crops by *Giardia* cysts and *Ascaris* eggs. *International Journal of Food Microbiology* **49**: 19-26.

Anderson, E. J. and Weber, S. G. (2004). Rotavirus infection in adults. *Lancet Infectious Diseases* **4**(2): 91-99.

Anderson, J. M. (1996). Current water recycling initiatives in Australia: Scenarios for the 21st century. *Water Science and Technology* **33**(10-11): 37-43.

Andersson, Y. and Bohan, P. (2001). Disease surveillance and waterborne outbreaks. In: Water quality: Guidelines, standards and health. L. Fewtrell and J. Bartram (ed.). London, UK, IWA Publishing.

Andersson, Y., Jong, B. d. and Studahl, A. (1997). Waterborne *Campylobacter* in Sweden: the cost of an outbreak. *Water Science and Technology* **35**(11-12): 11-14.

Anonymous (2004). Smittskyddslag. *(The Swedish Infectious Disease Protection Act)*. SFS 2004:168.

Aragón, T. J., Novotny, S., Enanoria, W., Vugia, D. J., Khalakdina, A. and Katz, M. H. (2003). Endemic cryptosporidiosis and exposure to municipal tap water in persons with acquired immunodeficiency syndrome (AIDS): a case-control study. *BMC Public Health* **3**(1): 2.

Armon, R., Gold, D., Brodsky, M. and Oron, G. (2002). Surface and subsurface irrigation with effluents of different qualities and presence of *Cryptosporidium* oocysts in soil and on crops. *Water Science and Technology* **46**(3): 115-122.

Arnbjerg-Nielsen, K., Hansen, L., Kjølholt, J., Stuer-Lauridsen, F., Hasling, A. B., Hansen, N. J., Stenström, T. A., Schönning, C., Westrell, T., Carlsen, A. and Halling-Sørensen, B. (2004). Risikovurdering af anvendelse af helt eller delvist opsamlet komposteret human fæces i private havebrug. *(Risk assessment of partly composted faeces for use in private gardens)*, Miljøstyrelsen, Denmark.

Arnesen, T. and Nord, E. (1999). The value of DALY life: problems with ethics and validity of disability adjusted life years. *British Medical Journal* **319**: 1420-1425.

Asano, T. and Sakaji, R. H. (1990). Virus risk analysis in wastewater reclamation and reuse. In: Chemical Water and Wastewater Treatment. H. H. Hahn and R. Klute (ed.). Berlin Heidelberg, Springer-Verlag**:** 483-496.

Ashbolt, N. J., Grabow, W. O. K. and Snozzi, M. (2001). Indicators of microbial water quality. In: Water quality: Guidelines, standards and health. L. Fewtrell and J. Bartram (ed.). London, UK, IWA Publishing**:** 289-315.

Ashbolt, N. J., Petterson, S. E., Roser, D. J., Westrell, T., Ottoson, J., Schönning, C. and Stenström, T. A. (2004). Microbial risk assessment tool to aid in the selection of sustainable urban water systems. Proceedings from 2nd IWA Leading -Edge Conference on Sustainability in Water Limited Environments, 8 - 10 November 2004, Sydney, Australia.

Ashbolt, N. J., Riedy, C. and Haas, C. N. (1997). Microbial health risk at Sydney's coastal bathing beaches. Proceedings from the 17th Federal Convention of AWWA, Melbourne.

Atherholt, T. B., LeChevallier, M. W., Norton, W. D. and Rosen, J. S. (1998). Effect of rainfall on *Giardia* and *Crypto*. *Journal of American Water Works Association* **90**(9): 66-80.

AWWA (1999). Manual of water supply practices - M48: Waterborne Pathogens. Denver, American Water Works Association.

Balbus, J. M. and Embrey, M. A. (2002). Risk factors for waterborne enteric infections. *Current Opinion in Gastroenterology* **18**(1): 46-50.

Barwick, R. S., Levy, D. A., Craun, G. F., Beach, M. J. and Calderon, R. L. (2000). Surveillance for waterborne-disease outbreaks – United States, 1997-1998. *Morbidity and Mortality Weekly Report* **49**(SS-4): 1-35.

Bitton, G. (1994). Wastewater microbiology. New York, Wiley-Liss.

Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P. and Blaser, M. J. (1988). Experimental *Campylobacter jejuni* infection in humans. *Journal of Infectious Diseases* **157**(3): 472-479.

Blackburn, B. G., Craun, G. F., Yoder, J. S., Hill, V., Calderon, R. L., Chen, N., Lee, S. H., Levy, D. A. and Beach, M. J. (2004). Surveillance for waterborne-disease outbreaks associated with drinking water – United States, 2001-2002. *MMWR Surveillance Summaries* **53**(8): 23-45.

Blumenthal, U. J., Cifuentes, E., Bennett, S., Quigley, M. and Ruiz-Palacios, G. (2001). The risk of enteric infections associated with wastewater reuse: the effect of season and degree of storage of wastewater. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**(2): 131-137.

Blumenthal, U. J., Strauss, M., Mara, D. D. and Cairncross, S. (1989). Generalised model of the effect of different control measures in reducing health risks from waste reuse. *Water Science and Technology* **21**: 567-577.

Bofill-Mas, S., Pina, S. and Girones, R. (2000). Documenting the epidemiologic patterns of polyomaviruses in human populations by studying their presence in urban sewage. *Applied and Environmental Microbiology* **66**(1): 238-245.

Bruce, M. G., Curtis, M. B., Payne, M. M., Gautom, R. K., Thompson, E. C., Bennett, A. L. and Kobayashi, J. M. (2003). Lake-associated outbreak of *Escherichia coli* O157:H7 in Clark County, Washington, August 1999. *Archives of Pediatrics and Adolescent Medicine* **157**(10): 1016-1021.

CAC (1999). Principles and Guidelines for the Conduct of Microbiological Risk Assessment. CAC/GL-30. Codex Alimentarius Commission (FAO/WHO).

Carey, C. M., Lee, H. and Trevors, J. T. (2004). Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. *Water Research* **38**(4): 818-862.

Carlander, A. (2002). Occurrence, reduction and risk of faecal pathogen transmission during wastewater irrigation of willow vegetation filters. Licentiate thesis at the Department of Short Rotation Forestry, Uppsala University, Uppsala.

Carlander, A. and Stenström, T. (2001). Irrigation with pretreated wastewater on short rotation willow croppice - a sanitary study in Sweden. Proceedings from the International Ecological Engineering Conference, Cristchurch, New Zealand.

Carlander, A. and Westrell, T. (1998). A microbiological and sociological evaluation of urine-diverting double-vault latrines in Cam Dum, Vietnam. Uppsala, Swedish University of Agricultural Sciences.

Carrique-Mas, J. (2001). Cryptosporidieinfektion i Sverige - toppen på ett isberg? (*Cryptosporidium infections in Sweden - the tip of an iceberg?) Smittskydd* (1): 6-7.

Carrique-Mas, J., Andersson, Y., Petersen, B., Hedlund, K., Sjögren, N. and Giesecke, J. (2003). A Norwalk-like virus waterborne community outbreak in a Swedish village during peak holiday season. *Epidemiology and Infection* **131**(1): 737-744.

Casani, S. and Knøchel, S. (2002). Application of HACCP to water reuse in the food industry. *Food Control* **13**: 315–327.

CDC (2004). Preventing cryptosporidiosis: a guide for people with compromised immune systems. Fact sheet from U.S. Centers for Disease Control and Prevention.

Chauret, C., Sprinthorpe, S. and Sattar, S. (1999). Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Canadian Journal of Microbiology* **45**(3): 257-262.

Chick, S. E., Koopman, J. S., Soorapanth, S. and Brown, M. E. (2001). Infection transmission system models for microbial risk assessment. *The Science of the Total Environment* **274**(1-3): 197-207.

Cifuentes, E. (1998). The epidemiology of enteric infections in agricultural communities exposed to wastewater irrigation: perspectives for risk control. *International Journal of Environmental Health Research* **8**(3): 203-213.

Coleman, M. E. and Marks, H. M. (1999). Qualitative and quantitative risk assessment. *Food Control* **10**: 289-297.

Coleman, M. E., Marks, H. M., Golden, N. J. and Latimer, H. K. (2004). Discerning strain effects in microbial dose-response data. *Journal of Toxicology and Environmental Health-Part a-Current Issues* **67**(8-10): 667-685.

Colley, D. G. (1996). Widespread foodborne cyclosporiasis outbreaks present major challenges. *Emerging Infectious Diseases* **2**(4): 354-356.

Cooper, R. C. (1991). The hygienic aspects of wastewater reuse. *Waste Management and Research* **9**: 373-377.

Corso, P. S., Kramer, M. H., Blair, K. A., Addiss, D. G., Davis, J. P. and Haddix, A. C. (2003). Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerging Infectious Diseases* **9**(4): 426-431.

Couch, R. B., Cate, T. R., Gerone, P. J., Fleet, W., Lang, D., Griffith, W. and Knight, V. (1966). Production of illness with a small-particle aerosol of adenovirus type 4. *Bacteriological Reviews* **30**: 517-528.

Covello, V. T. (1983). The perception of technological risks: a literature review. *Technological Forecasting and Social Change* **23**: 285-297.

Crabtree, K. D., Gerba, C. P., Rose, J. B. and Haas, C. N. (1997). Waterborne adenoviruses: a risk assessment. *Water Science and Technology* **35**(11-12): 1-6.

Crockett, C. S., Haas, C. N., Fazil, A., Rose, J. B. and Gerba, C. P. (1996). Prevalence of shigellosis in the U.S.: consistency with dose-response information. *International Journal of Food Microbiology* **30**: 87-99.

Cutter, S. L. (1993). Living with risk: the geography of technological hazards. London, Edward Arnold.

de Motes, C. M., Clemente-Casares, P., Hundesa, A., Martin, M. and Girones, R. (2004). Detection of bovine and porcine adenoviruses for tracing the source of fecal contamination. *Applied and Environmental Microbiology* **70**(3): 1448-1454.

Deere, D., Stevens, M., Davison, A., Helm, G. and Dufour, A. (2001). Management strategies. In: Water Quality: Guidelines, Standards and Health. L. Fewtrell and J. Bartram (ed.). London, IWA publishing**:** 257-288.

DeRegnier, D. P., Cole, L., Schupp, D. G. and Erlandsen, S. L. (1989). Viability of *Giardia* cysts suspended in lake, river, and tap water. *Applied and Environmental Microbiology* **55**(5): 1123-1229.

Dewettinck, T., van Houtte, E., Geenens, D., van Hege, K. and Verstraete, W. (2001). HACCP (Hazard Analysis and Critical Control Points) to guarantee safe water reuse and drinking water production - a case study. *Water Science and Technology* **43**(12): 31-38.

Doller, P. C., Dietrich, K., Filipp, N., Brockmann, S., Dreweck, C., Vonthein, R., Wagner-Wiening, C. and Wiedenmann, A. (2002). Cyclosporiasis outbreak in Germany associated with the consumption of salad. *Emerging Infectious Diseases* **8**(9): 992-994.

DuPont, H. L., Chappel, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B. and Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers. *The New England Journal of Medicine* **332**(13): 855-859.

DuPont, H. L., Hornick, R. B., Dawkins, A. T., Snyder, M. J. and Formal, S. B. (1969). The response of man to virulent *Shigella flexneri* 2a. *Journal of Infectious Diseases* **119**(3): 296-299.

DuPont, H. L., Hornick, R. B., Snyder, M. J., Libonati, J. P., Formal, S. B. and Gangarosa, E. J. (1972). Immunity in shigellosis. II. Protection induced by oral live vaccine or primary infection. *Journal of Infectious Diseases* **125**(1): 12-16.

Eisenberg, J. N., Brookhart, M. A., Rice, G., Brown, M. and Colford, J. M., Jr. (2002). Disease transmission models for public health decision making: analysis of epidemic and endemic conditions caused by waterborne pathogens. *Environmental Health Perspectives* **110**(8): 783-790.

Eisenberg, J. N., Seto, E. Y. W., Olivieri, A. W. and Spear, R. C. (1996). Quantifying water pathogen risk in an epidemiological framework. *Risk Analysis* **16**(4): 549-563.

Eisenberg, J. N. S., Soller, J. A., Scott, J., Eisenberg, D. M. and Colford, J. M. (2004). A dynamic model to assess microbial health risks associated with beneficial uses of biosolids. *Risk Analysis* **24**(1): 221-236.

Enriquez, C. E., Hurst, C. J. and Gerba, C. P. (1995). Survival of the enteric adenoviruses 40 and 41 in tap, sea, and waste water. *Water Research* **29**(11): 2548-2553.

FAO (1997). Hazard analysis and critical control point (HACCP) system and guidelines for its application. Annex to CAC/RPC 1-1969, Rev. 3 (1997), Amendment 1 (1999).

Fattal, B., Wax, Y., Davies, M. and Shuval, H. I. (1986). Health risks associated with wastewater irrigation: an epidemiological study. *American Journal of Public Health* **76**(8): 977-979.

Feachem, R. G., Bradley, D. J., Garelick, H. and Mara, D. (1983). Sanitation and disease - health aspects of excreta and wastewater management. Chichester, Wiley & sons.

Feldman, K. A., Mohle-Boetani, J. C., Ward, J., Furst, K., Abbott, S. L., Ferrero, D. V., Olsen, A. and Werner, S. B. (2002). A cluster of *Escherichia coli* O157: nonmotile infections associated with recreational exposure to lake water. *Public Health Reports* **117**(4): 380-385.

Ferguson, C., de Roda Husman, A. M., Altavilla, N., Deere, D. and Ashbolt, N. (2003). Fate and transport of surface water pathogens in watersheds. *Critical Reviews in Environmental Science and Technology* **33**(3): 299-261.

Finley, B., Proctor, D., Scott, P., Harringtoin, N., Paustenbach, D. and Price, P. (1994). Recommended distributions for exposure factors frequently used in health risk assessment. *Risk Analysis* **14**(4): 533-551.

Forhammar, M., Bruce, Å. and Erlandsson, B. (1986). Dricksvattenkonsumtionen i olika åldrar. (*Drinking water consumption by different age groups). Vår Föda* **38**(1): 86-91.

Frey, H. and Burmaster, D. E. (1999). Methods for characterizing variability and uncertainty: comparison of bootstrap simulation and likelihood-based approaches. *Risk Analysis* **19**(1): 109-130.

Frey, H. C. and Patil, S. R. (2002). Identification and review of sensitivity analysis methods. *Risk Analysis* **22**(3): 553-578.

Gale, P. (2000). Risk assessment model for a waterborne outbreak of cryptosporidiosis. *Water Science and Technology* **41**(7): 1-7.

Gale, P. (2003). Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. *Journal of Applied Microbiology* **94**(1): 35-47.

Gannon, V. P., Graham, T. A., Read, S., Ziebell, K., Muckle, A., Mori, J., Thomas, J., Selinger, B., Townshend, I. and Byrne, J. (2004). Bacterial pathogens in rural water supplies in Southern Alberta, Canada. *Journal of Toxicology and Environmental Health A* **67**(20-22): 1643-1653.

Gantzer, C., Gaspard, P., Galvez, L., Huyard, A., Dumouthier, N. and Schwartzbrod, J. (2001). Monitoring of bacterial and parasitological contamination during various treatment of sludge. *Water Research* **35**(16): 3763-3770.

Gerba, C. P., Rose, J. B. and Haas, C. N. (1996a). Sensitive populations: who is at the greatest risk? *International Journal of Food Microbiology* **30**: 113-123.

Gerba, C. P., Rose, J. B., Haas, C. N. and Crabtree, K. D. (1996b). Waterborne rotavirus: a risk assessment. *Water Research* **30**(12): 2929-2940.

Gibbs, R. A., Hu, C. J., Ho, G. E. and Unkovich, I. (1997). Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. *Water Science and Technology* **35**(11-12): 269-275.

Graham, D. Y., Jiang, X., Tanaka, T., Opekun, A. R., Madore, H. P. and Estes, M. K. (1994). Norwalk virus infection of volunteers: new insights based on improved assays. *Journal of Infectious Diseases* **170**(1): 34-43.

Guendert, D. (2004). Singapore success. *Water21* **June 2004**: 23-23.

Haas, C. N. (1983). Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *American Journal of Epidemiology* **118**(4): 573-582.

Haas, C. N., Crockett, C. S., Rose, J. B., Gerba, C. P. and Fazil, A. M. (1996). Assessing the risk posed by oocysts in drinking water. *Journal of American Water Works Association* **88**(9): 131-136.

Haas, C. N., Rose, J. B., Gerba, C. and Regli, S. (1993). Risk assessment of virus in drinking water. *Risk Analysis* **13**(5): 545-552.

Haas, C. N., Rose, J. B. and Gerba, C. P. (1999). Quantitative microbial risk assessment. New York, John Wiley & Sons, Inc.

Haas, C. N., Thayyar-Madabusi, A., Rose, J. B. and Gerba, C. P. (2000). Development of a dose-response relationship for *Escherichia coli* O157:H7. *International Journal of Food Microbiology* **56**(2-3): 153-159.

Hansen, A. and Stenström, T.-A. (1998). Kartläggning av *Giardia* och *Cryptosporidium* i svenska ytvattentäkter. (*Mapping of Giardia and Cryptosporidium in Swedish surface water sources).* The Swedish Institute for Infectious Disease Control (SMI) and the Swedish National Food Administration (SLV).

Hansen, J. S. and Ongerth, J. E. (1991). Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. *Applied and Environmental Microbiology* **57**(10): 2790-2795.

Hardy, M. E. (1999). Norwalk and "Norwalk-like viruses" in epidemic gastroenteritis. *Clinics in Laboratory Medicine* **19**(3): 675-690.

Havelaar, A. H. (1994). Application of HACCP to drinking water supply. *Food Control* **5**(3): 145-152.

Havelaar, A. H., de Hollander, A. E., Teunis, P. F., Evers, E. G., van Kranen, H. J., Versteegh, J. F., van Koten, J. E. and Slob, W. (2000a). Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. *Environmental Health Perspectives* **108**(4): 315-321.

Havelaar, A. H., de Wit, M. A. S. and van Koningsveld, R. (2000b). Health burden in the Netherlands (1900-1995) due to infections with thermophilic *Campylobacter* species. Bilthoven, RIVM.

Hellard, M. E., Sinclair, M. I., Forbes, A. B. and Fairley, C. K. (2001). A randomized, blinded, controlled trial investigating the gastrointestinal health effects of drinking water quality. *Environmental Health Perspectives* **109**(8): 773-778.

Hijnen, W. A. M., Beerendonk, E., Smeets, P. and Medema, G. J. (2004). Elimination of micro-organisms by water treatment processes. Nieuwegein, the Netherlands, Kiwa Water Research.

Hijnen, W. A. M., Medema, G. J. and van der Kooij, D. (2002). Quantitative assessment of the removal of indicator bacteria by full-scale treatment. Proceedings from the International Symposium on Waterborne Pathogens, Cascais/Lisbon, Portugal, IWA/AWWA.

Hoebe, C. J., Vennema, H., de Roda Husman, A. M. and van Duynhoven, Y. T. (2004). Norovirus outbreak among primary schoolchildren who had played in a recreational water fountain. *Journal of Infectious Diseases* **189**(4): 699-705.

Hrdy, D. B. (1987). Epidemiology of rotaviral infection in adults. *Reviews of Infectious Diseases* **9**(3): 461-469.

Hrudey, S. E., Payment, P., Huck, P. M., Gillham, R. W. and Hrudey, E. J. (2003). A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Science and Technology* **47**(3): 7-14.

Huang, P., Farkas, T., Marionneau, S., Zhong, W., Ruvoen-Clouet, N., Morrow, A. L., Altaye, M., Pickering, L. K., Newburg, D. S., LePendu, J. and Jiang, X. (2003). Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. *Journal of Infectious Diseases* **188**(1): 19-31.

Hutchison, M. L., Walters, L. D., Avery, S. M., Synge, B. A. and Moore, A. (2004). Levels of zoonotic agents in British livestock manures. *Letters in Applied Microbiology* **39**(2): 207-214.

Höglund, C., Stenström, T. A. and Ashbolt, N. (2002). Microbial risk assessment of source-separated urine used in agriculture. *Waste Management and Research* **20**(2): 150-161.

Höller, C. (1988). Quantitative and qualitative investigations on *Campylobacter* in the sewage system of a big town. *Zentralblatt Fur Bakteriologie Mikrobiologie Und Hygiene Serie B-Umwelthygiene Krankenhaushygiene Arbeitshygiene Praventive Medizin* **185**(4-5): 307-325.

Höller, C., Koschinsky, S. and Witthuhn, D. (1999). Isolation of enterohaemorrhagic *Escherichia coli* from municipal sewage. *Lancet* **353**(9169): 2039-2039.

Hörman, A., Rimhanen-Finne, R., Maunula, L., von Bonsdorff, C. H., Torvela, N., Heikinheimo, A. and Hänninen, M. L. (2004). *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., noroviruses, and indicator organisms in surface water in southwestern Finland, 2000-2001. *Applied and Environmental Microbiology* **70**(1): 87-95.

IWA (2004). The Bonn Charter for Safe Drinking Water. London, UK, International Water Association.

Jagals, C. and Jagals, P. (2004). Application of HACCP principles as a management tool for monitoring and controlling microbiological hazards in water treatment facilities. *Water Science and Technology* **50**(1): 69-76.

Jiang, S. C. and Chu, W. (2004). PCR detection of pathogenic viruses in southern California urban rivers. *Journal of Applied Microbiology* **97**(1): 17-28.

Jolis, D., Pitt, P. and Hirano, R. (1999). Risk assessment for *Cryptosporidium parvum* in reclaimed water. *Water Research* **33**(13): 3051-3055.

Katzenelson, E., Buium, I. and Shuval, H. I. (1976). Risk of communicable disease infection associated with wastewater irrigation in agricultural settlements. *Science* **194**(4268): 944-946.

Keene, W. E., McAnulty, J. M., Hoesly, F. C., Williams, L. P., Jr., Hedberg, K., Oxman, G. L., Barrett, T. J., Pfaller, M. A. and Fleming, D. W. (1994). A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New England Journal of Medicine* **331**(9): 579-584.

Keswick, B. H., Satterwhite, T. K., Johnson, P. C., DuPont, H. L., Secor, S. L., Bitsura, J. A., Gary, G. W. and Hoff, J. C. (1985). Inactivation of Norwalk virus in drinking water by chlorine. *Applied and Environmental Microbiology* **50**(2): 261-264.

Kistemann, T., Classen, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V. and Exner, M. (2002). Microbial load of drinking water reservoir tributaries during extreme rainfall and runoff. *Applied and Environmental Microbiology* **68**(5): 2188-2197.

Koenraad, P. M. F. J., Jacobs-Reitsma, W. F. and Beumer, R. R. (1996). Short-term evidence of *Campylobacter* in a treatment plant and drain water of a connected poultry abattoir. *Water Environment Research* **68**: 188-193.

Koenraad, P. M. F. J., Rombouts, F. M. and Notermans, S. H. W. (1997). Epidemiological aspects of thermophilic *Campylobacter* in water-related environments: a review. *Water Environment Research* **69**(1): 52-63.

Koff, R. S. (1998). Hepatitis A. *Lancet* **351**(9116): 1643-1649.

Koivunen, J., Siitonen, A. and Heinonen-Tanski, H. (2003). Elimination of enteric bacteria in biological-chemical wastewater treatment and tertiary filtration units. *Water Research* **37**(3): 690-698.

Koopmans, M. and Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology* **90**(1): 23-41.

Kramer, M. H., Herwaldt, B. L., Craun, G. F., Calderon, R. L. and Juranek, D. D. (1996). Surveillance for waterborne-disease outbreaks – United States, 1993-1994. *MMWR Surveillance Summaries* **45**(1): 1-33.

Kukkula, M., Arstila, P., Klossner, M. L., Maunula, L., Bonsdorff, C. H. and Jaatinen, P. (1997). Waterborne outbreak of viral gastroenteritis. *Scandinavian Journal of Infectious Diseases* **29**(4): 415-418.

Kukkula, M., Maunula, L., Silvennoinen, E. and von Bonsdorff, C. H. (1999). Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *Journal of Infectious Diseases* **180**(6): 1771-1776.

Kärrman, E., Bergstedt, O., Westrell, T., Heinicke, G., Stenström, T.-A. and Hedberg, T. (2004). Systemanalys av dricksvattenförsörjning med avseende på mikrobiologiska barriärer och miljöpåverkan. *(Systems analysis of drinking water systems including microbial barriers and environmental impact).* Stockholm, VA-Forsk Report 2004-12.

LeChevallier, M. W., Abbaszadegan, M., Camper, A. K., Hurst, C. J., Izaguirre, G., Marshall, M. M., Naumovitz, D., Payment, P., Rice, E. W., Rose, J., Schaub, S., Slifko, T. R., Smith, D. B., Smith, H. V., Sterling, C. R., Stewart, M. and Cont, A. R. D. M. (1999a). Committee report: Emerging pathogens - bacteria. *Journal of American Water Works Association* **91**(9): 101-109.

LeChevallier, M. W., Abbaszadegan, M., Camper, A. K., Hurst, C. J., Izaguirre, G., Marshall, M. M., Naumovitz, D., Payment, P., Rice, E. W., Rose, J., Schaub, S., Slifko, T. R., Smith, D. B., Smith, H. V., Sterling, C. R., Stewart, M. and Cont, A. R. D. M. (1999b). Committee report: Emerging pathogens - viruses, protozoa, and algal toxins. *Journal of American Water Works Association* **91**(9): 110-121.

LeChevallier, M. W., Norton, W. D. and Lee, R. G. (1991). Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Applied and Environmental Microbiology* **57**(9): 2610-2616.

Lemon, S. M. (1997). Type A viral hepatitis: epidemiology, diagnosis, and prevention. *Clinical Chemistry* **43**(8 Pt 2): 1494-1499.

Levine, M. M., DuPont, H. L., Formal, S. B., Hornick, R. B., Takeuchi, A., Gangarosa, E. J., Snyder, M. J. and Libonati, J. P. (1973). Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. *Journal of Infectious Diseases* **127**(3): 261-270.

Lidskog, R., Sandstedt, E. and Sundqvist, G. (1997). Samhälle, risk och miljö : sociologiska perspektiv på det moderna samhällets miljöproblem. *(Society, risk and environment: sociological perspectives on the environmental problems of modern society).* Lund, Studentlitteratur.

Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindbland, L., Stewart, P., LePendu, J. and Baric, R. (2003). Human susceptibility and resistance to Norwalk virus infection. *Nature Medicine* **9**(5): 548-553.

Lindqvist, R., Andersson, Y., Lindback, J., Wegscheider, M., Eriksson, Y., Tidestrom, L., Lagerqvist-Widh, A., Hedlund, K. O., Lofdahl, S., Svensson, L. and Norinder, A. (2001). A one-year study of foodborne illnesses in the municipality of Uppsala, Sweden. *Emerging Infectious Diseases* **7**(3): 588-592.

Lodder, W. J., Vinjé, J., van de Heide, R., de Roda Husman, A. M., Leenen, E. J. T. M. and Koopmans, M. P. G. (1999). Molecular detection of Norwalk-like caliciviruses in sewage. *Applied and Environmental Microbiology* **65**(12): 5624-5627.

Lopman, B., Vennema, H., Kohli, E., Pothier, P., Sanchez, A., Negredo, A., Buesa, J., Schreier, E., Reacher, M., Brown, D., Gray, J., Iturriza, M., Gallimore, C., Bottiger, B., Hedlund, K., Torven, M., von Bonsdorff, C., Maunula, L., Poljsak-Prijatelj, M., Zimsek, J., Reuter, G., Szucs, G., Melegh, B., Svennson, L., van Duijnhoven, Y. and Koopmans, M. (2004). Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* **363**(9410): 682-688.

Lopman, B. A., Reacher, M. H., van Duijnhoven, Y., Hanon, F. X., Brown, D. and Koopmans, M. (2003). Viral gastroenteritis outbreaks in Europe, 1995-2000. *Emerging Infectious Diseases* **9**(1): 90-96.

Ma, W. T., Sun, Z. C., Wang, Z. X., Feng, Y. B., Wang, T. C., Chan, U. S., Miu, C. H. and Zhu, S. C. (1998). Application of membrane technology for drinking water. *Desalination* **119**(1-3): 127-131.

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B. and Davis, J. P. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water-supply. *New England Journal of Medicine* **331**(3): 161-167.

Macler, B. A. and Regli, S. (1993). Use of microbial risk assessment in setting US drinking water standards. *International Journal of Food Microbiology* **18**(4): 245-256.

Marshall, J. A., Salamone, S., Yuen, L., Catton, M. G. and Wright, J. P. (2001). High level excretion of Norwalk-like virus following resolution of clinical illness. *Pathology* **33**(1): 50-52.

Maunula, L., Kalso, S., Von Bonsdorff, C. H. and Ponka, A. (2004). Wading pool water contaminated with both noroviruses and astroviruses as the source of a gastroenteritis outbreak. *Epidemiology and Infection* **132**(4): 737-743.

Maurer, A. M. and Sturchler, D. (2000). A waterborne outbreak of small round structured virus, *Campylobacter* and *Shigella* co-infections in La Neuveville, Switzerland, 1998. *Epidemiology and Infection* **125**(2): 325-332.

Mawdsley, J. L., Bardgett, R. D., Merry, R. J., Pain, B. F. and Theodorou, M. K. (1995). Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Applied Soil Ecology* **2**(1): 1-15.

McAlearny, A. S., Schweikhart, S. B. and Pathak, D. S. (1999). Quality-Adjusted Life-Years and other health indices: a comparative analysis. *Clinical Therapeutics* **21**(9): 1605-1629.

McCarthy, N. and Giesecke, J. (2001). Incidence of Guillain-Barré syndrome following infection with *Campylobacter jejuni*. *American Journal of Epidemiology* **153**(6): 610-614.

McCarthy, T. A., Barrett, N. L., Hadler, J. L., Salsbury, B., Howard, R. T., Dingman, D. W., Brinkman, C. D., Bibb, W. F. and Cartter, M. L. (2001). Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. *Pediatrics* **108**(4): E59.

McCullough, N. B. and Eisele, C. W. (1951a). Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. *Journal of Infectious Diseases* **88**(3): 278-289.

McCullough, N. and Eisele, C. W. (1951b). Experimental human salmonellosis. III. Pathogenicity of strains of *Salmonella newport*, *Salmonella derby*, and *Salmonella bareilly* obtained from spray-dried whole egg. *Journal of Infectious Diseases* **89**(3): 209-213.

McCullough, N. and Eisele, C. W. (1951c). Experimental human salmonellosis. IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg. *Journal of Infectious Diseases* **89**(3): 259-265.

McGann, B. (2004). Are three water qualities better than one? *Water21* **April 2004**: 35-35.

McKone, T. E. and Daniels, J. I. (1991). Estimating human exposure through multiple pathways from air, water, and soil. *Regulatory Toxicology and Pharmacology* **13**(1): 36-61.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases* **5**(5): 607-625.

Medema, G. J., Bahar, M. and Schets, F. M. (1997). Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochthonous microorganisms. *Water Science and Technology* **35**(11-12): 249-252.

Medema, G. J., Hoogenboezem, W., Veer, A. J. v. d., Ketelaars, H. A. M., Hijnen, W. A. M. and Nobel, P. J. (2003). Quantitative risk assessment of *Cryptosporidium* in surface water treatment. *Water Science and Technology* **47**(3): 241–247.

Medema, G. J., Teunis, P. F. M., Havelaar, A. H. and Haas, C. N. (1996). Assessment of the dose-response relationship of *Campylobacter jejuni*. *International Journal of Food Microbiology* **30**: 101-111.

Melloul, A., Amahmid, O., Hassani, L. and Bouhoum, K. (2002). Health effect of human wastes use in agriculture in El Azzouzia (the wastewater spreading area of Marrakesh city, Morocco). *International Journal of Environmental Health Research* **12**(1): 17-23.

Meng, Q. S. and Gerba, C. P. (1996). Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. *Water Research* **30**(11): 2665-2668.

Michel, P., Wilson, J. B., Martin, S. W., Clarke, R. C., McEwen, S. A. and Gyles, C. L. (2000). Estimation of the under-reporting rate for the surveillance of *Escherichia coli* O157:H7 cases in Ontario, Canada. *Epidemiology and Infection* **125**: 35-45.

MISTRA (1999). Revised programme plan for the MISTRA programme Sustainable Urban Water Management.

Mounts, A. W., Ando, T., Koopmans, M., Bresee, J. S., Noel, J. and Glass, R. I. (2000). Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *Journal of Infectious Diseases* **181** *Suppl 2*: S284-S287.

Murray, C. J. and Lopez, A. D. (1997). Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet* **349**: 1436-1442.

Murray, C. J. L. and Lopez, A. D. (1996). Evidence-based health policy - lessons from the Global Burden of Disease Study. *Science* **274**: 740-743.

Nakajima, H., Nakagomi, T., Kamisawa, T., Sakaki, N., Muramoto, K., Mikami, T., Nara, H. and Nakagomi, O. (2001). Winter seasonality and rotavirus diarrhoea in adults. *Lancet* **357**(9272): 1950.

Notermans, S., Gallhoff, G., Zwietering, M. H. and Mead, G. C. (1994). The HACCP concept: specification of criteria using quantitative risk assessment. *Food Microbiology* **11**: 397-408.

Nwachuku, N. and Gerba, C. P. (2004). Microbial risk assessment: don't forget the children. *Current Opinions in Microbiology* **7**(3): 206-209.

Nygård, K., Andersson, Y., Røttingen, J. A., Svensson, Å., Lindbäck, J., Kistemann, T. and Giesecke, J. (2004a). Association between environmental risk factors and *Campylobacter* infections in Sweden. *Epidemiology and Infection* **132**(2): 317-325.

Nygård, K., Torvén, M., Ancker, C., Knauth, S. B., Hedlund, K. O., Giesecke, J., Andersson, Y. and Svensson, L. (2003). Emerging genotype (GGIIb) of norovirus in drinking water, Sweden. *Emerging Infectious Diseases* **9**(12): 1548-1552.

Nygård, K., Vold, L., Halvorsen, E., Bringeland, E., Rottingen, J. A. and Aavitsland, P. (2004b). Waterborne outbreak of gastroenteritis in a religious summer camp in Norway, 2002. *Epidemiology and Infection* **132**(2): 223-229.

Nylen, G., Dunstan, F., Palmer, S. R., Andersson, Y., Bager, F., Cowden, J., Feierl, G., Galloway, Y., Kapperud, G., Megraud, F., Molbak, K., Petersen, L. R. and Ruutu, P. (2002). The

seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand. *Epidemiology and Infection* **128**(3): 383-390.

Obiri-Danso, K. and Jones, K. (1999). Distribution and seasonality of microbial indicators and thermophilic *Campylobacters* in two freshwater bathing sites on the River Lune in northwest England. *Journal of Applied Microbiology* **87**(6): 822-832.

Ogoshi, M., Suzuki, Y. and Asano, T. (2001). Water reuse in Japan. *Water Science and Technology* **43**(10): 17-23.

Ong, C., Moorehead, W., Ross, A. and Isaac-Renton, J. (1996). Studies of *Giardia* spp. and *Cryptosporidium* spp. in two adjacent watersheds. *Applied Environmental Microbiology* **62**(8): 2798-2805.

Ottoson, J., Hansen, A., Westrell, T., Johansen, K., Norder, H. and Stenström, T. A. (*submitted*). Removal of noro- and enteroviruses, *Giardia* cysts, *Cryptosporidium* oocysts and faecal indicators at four secondary wastewater treatment plants in Sweden.

Ottoson, J. and Stenström, T. A. (2003). Faecal contamination of greywater and associated microbial risks. *Water Research* **37**(3): 645-655.

Pai, C. H., Kelly, J. K. and Meyers, G. L. (1986). Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. *Infection and Immunity* **51**(1): 16-23.

Parkin, R. T., Soller, J. A. and Olivieri, A. W. (2003). Incorporating susceptible subpopulations in microbial risk assessment: pediatric exposures to enteroviruses in river water. *Journal of Exposure Analysis and Environmental Epidemiology* **13**(2): 161-168.

Parrino, T. A., Schreiber, D. S., Trier, J. S., Kapikian, A. Z. and Blacklow, N. R. (1977). Clinical immunity in acute gastroenteritis caused by Norwalk agent. *New England Journal of Medicine* **297**(2): 86-89.

Payment, P. (1999). Poor efficiency of residual chlorine disinfectant in drinking water to inactivate waterborne pathogens in distribution systems. *Canadian Journal of Microbiology* **45**: 709-715.

Payment, P., Berte, A., Prévost, M., Ménard, B. and Barbeau, B. (2000). Occurrence of pathogenic microorganisms in the Saint Lawrence River (Canada) and comparison of health risks for populations using it as their source of drinking water. *Canadian Journal of Microbiology* **46**: 565-575.

Payment, P., Siemiatycki, J., Richardson, L., Renaud, G., Franco, E. and Prévost, M. (1997). A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. *International Journal of Environmental Health Research* **7**(1): 5-31.

Petterson, S. R., Ashbolt, N. J. and Sharma, A. (2001). Microbial risks from wastewater irrigation of salad crops: a screening-level risk assessment. *Water Environment Research* **73**(6): 667-672.

Pina, S., Puig, M., Lucena, F., Jofre, J. and Girones, R. (1998). Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology* **64**(9): 3376-3382.

Pouillot, R., Beaudeau, P., Denis, J. B. and Derouin, F. (2004). A quantitative risk assessment of waterborne cryptosporidiosis in France using second-order Monte Carlo simulation. *Risk Analysis* **24**(1): 1-17.

Rao, V. C., Metcalf, T. G. and Melnick, J. L. (1987). Removal of indigenous rotavirus during primary settling and activated-sludge treatment of raw sewage. *Water Research* **21**(2): 171-177.

Rao, V. C., Seidel, K. M., Goyal, S. M., Metcalf, T. G. and Melnick, J. L. (1984). Isolation of enteroviruses from water, suspended solids and sediments from Galveston bay: survival of poliovirus and rotavirus adsorbed to sediments. *Applied and Environmental Microbiology* **48**(2): 404-409.

Raphael, R. A., Sattar, S. A. and Springthorpe, V. S. (1985). Long-term survival of human rotavirus in raw and treated river water. *Canadian Journal of Microbiology* **31**(2): 124-128.

Regli, S., Rose, J. B., Haas, C. N. and Gerba, C. P. (1991). Modeling the risk from *Giardia* and viruses in drinking water. *Journal of American Water Works Association* **83**(11): 76-84.

Rendtorff, R. C. (1954). The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *American Journal of Hygiene* **59**(2): 209-220.

Robertson, L. J. and Gjerde, B. (2001). Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw waters in Norway. *Scandinavian Journal of Public Health* **29**(3): 200-207.

Robertson, L. J., Johannessen, G. S., Gjerde, B. K. and Loncarevic, S. (2002). Microbiological analysis of seed sprouts in Norway. *International Journal of Food Microbiology* **75**(1-2): 119-126.

Rockx, B., de Wit, M., Vennema, H., Vinje, J., de Bruin, E., van Duynhoven, Y. and Koopmans, M. (2002). Natural history of human calicivirus infection: a prospective cohort study. *Clinical Infectious Diseases* **35**(3): 246-253.

Rose, J. B., Dickson, L. J., Farrah, S. R. and Carnahan, R. P. (1996). Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Water Research* **30**(11): 2785-2797.

Rose, J. B. and Gerba, C. P. (1991a). Assessing potential health risks from viruses and parasites in reclaimed water in Arizona and Florida, USA. *Water Science and Technology* **23**(Kyoto): 2091-2098.

Rose, J. B. and Gerba, C. P. (1991b). Use of risk assessment for development of microbial standards. *Water Science and Technology* **24**(2): 29-34.

Roseberry, A. M. and Burmaster, D. E. (1992). Lognormal distributions for water intake by children and adults. *Risk Analysis* **12**(1): 99-104.

Rosef, O., Rettedal, G. and Lageide, L. (2001). Thermophilic *Campylobacter* in surface water: a potential risk of campylobacteriosis. *International Journal of Environmental Health Research* **11**(4): 321-327.

Sahlström, L., Aspan, A., Bagge, E., Tham, M. L. and Albihn, A. (2004). Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. *Water Research* **38**(8): 1989-1994.

Samadpour, M., Stewart, J., Steingart, K., Addy, C., Louderback, J., McGinn, M., Ellington, J. and Newman, T. (2002). Laboratory investigation of an *E. coli* O157:H7 outbreak associated with swimming in Battle Ground Lake, Vancouver, Washington. *Journal of Environmental Health* **64**(10): 16-20, 26, 25.

Schaub, S. A. and Oshiro, R. K. (2000). Public health concerns about caliciviruses as waterborne contaminants. *Journal of Infectious Diseases* **181** *Suppl 2*: S374-S380.

Schvoerer, E., Bonnet, F., Dubois, V., Cazaux, G., Serceau, R., Fleury, H. J. and Lafon, M. E. (2000). PCR detection of human enteric viruses in bathing areas, waste waters and human stools in Southwestern France. *Research in Microbiology* **151**(8): 693-701.

Schönning, C. (2003). Risker för smittspridning via avloppsslam - redovisning av behandlingsmetoder och föreskrifter. (*Risks of disease transmission via sewage sludge - treatment methods and regulations).* Stockholm, Naturvårdsverket.

Shin, G. A. and Sobsey, M. D. (2003). Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. *Applied and Environmental Microbiology* **69**(7): 3975-3978.

Shuval, H., Lampert, Y. and Fattal, B. (1997). Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Science and Technology* **35**(11-12): 15-20.

Shuval, H. I. (1991). Effects of wastewater irrigation of pastures on the health of farm animals and humans. *Revue Scientifique et Technique de L'Office International de Epizooties* **10**(3): 847-866.

Sinclair, M. (2003a). Setback for Netherlands dual supplies. *Health Stream* (30): 5.

Sinclair, M. (2003b). US waterborne disease study. *Health Stream* (31): 1-3.

Slovic, P. (1987). Perception of risk. *Science* **236**: 280-285.

SLV (2001). Livsmedelsverkets föreskrifter om dricksvatten. (*Regulations and general advices on drinking water from the National Food Administration).* SLVFS 2001:30.

SLV (2002). *Campylobacter* i kött och vatten - kartläggning av *Campylobacter* i rått kött och råvatten till dricksvatten i Sverige år 2000. (*Campylobacter in meat and water – mapping of Campylobacter in raw meat and raw water to drinking water in Sweden 2000).* Uppsala, Swedish National Food Administration.

SLV (2003). Vägledning till Livsmedelsverkets föreskrifter om dricksvatten. (*Guidance to the national regulations on drinking water from the National Food Administration).*

SMI (2004). Epidemiologisk årsrapport 2003. (*Epidemiological yearly report 2003*). Solna, Swedish Institute for Infectious Disease Control (SMI).

SNV (1996). Statens naturvårdsverks föreskrifter om strandbadvatten. (*Regulations on recreational waters from the Swedish Environmental Protection Agency).* SNFS (1996:6).

Soller, J. A., Olivieri, A. W., Crook, J., Cooper, R. C., Tchobanoglous, G., Parkin, R. T., Spear, R. C. and Eisenberg, J. N. S. (2003). Risk-based approach to evaluate the public health benefit of additional wastewater treatment. *Environmental Science and Technology* **37**(9): 1882-1891.

Srikanth, R. and Naik, D. (2004). Prevalence of giardiasis due to wastewater reuse for agriculture in the suburbs of Asmara City, Eritrea. *International Journal of Environmental Health Research* **14**(1): 43-52.

Stenström, T. A. (1987). Kommunalt avloppsvatten från hygienisk synpunkt - mikrobiologiska undersökningar. (*Municipal wastewater from a hygienic point-of-view – microbiological investigations).* Stockholm, Swedish Environmental Protection Agency.

Stenström, T. A., Boisen, F., Georgsson, F., Lahti, K., Lund, V., Andersson, Y. and Ormerod, K. (1994). Vattenburna infektioner i Norden. (*Waterborne infections in the Nordic countries).* Copenhagen, Nordic Councel of Ministers.

Stenström, T. A. and Carlander, A. (1999). Mikrobiella risker för smittspridning och sjukdomsfall - slamspridning och behandling. (*Microbial risks with disease transmission and disease cases - sludge application and treatment*). Stockholm, Swedish Environmental Protection Agency.

Stenström, T. A. and Carlander, A. (2001). Occurrence and die-off of indicator organisms in the sediment in two constructed wetlands. *Water Science and Technology* **44**(11-12): 223-230.

Stenström T. A., Wiman M. and Tullander V. (1985). Hässleholms avloppsreningsverk, intensivundersökning 19-21 april 1982. (*Hässleholm's wastewater treatment plant, intensive investigation April 19-21, 1982).* Report SNV PM 1944, Sewage Works Evaluation Project, Sweden.

Strachan, N. J., Fenlon, D. R. and Ogden, I. D. (2001). Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157. *FEMS Microbiology Letters* **203**(1): 69-73.

Svenskt Vatten (2000). Facts on water supply and sanitation in Sweden. Accessible at http://www.svensktvatten.se/main/main.asp?objectID=376, The Swedish Water and Wastewater Association.

Svenungsson, B., Lagergren, Å., Ekwall, E., Evengård, B., Hedlund, K. O., Kärnell, A., Löfdahl, S., Svensson, L. and Weintraub, A. (2000). Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. *Clinical Infectious Diseases* **30**: 770-778.

Söderberg, H. and Kain, J.-H. (2002). Integrating knowledge or aggregating data - multicriteria approaches for sustainable water and waste systems. Proceedings from the 3rd International Conference on Decision-Making in Urban and Civil Engineering, 6-8 November 2002, London.

Tanaka, H., Asano, T., Shroeder, E. D. and Tchobanoglous, G. (1998). Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data. *Water Environment Research* **70**(1): 39-51.

Tani, N., Dohi, Y., Kurumatani, N. and Yonemasu, K. (1995). Seasonal distribution of adenoviruses, enteroviruses and reoviruses in urban river water. *Microbiology and Immunology* **39**(8): 577-580.

Tchobanoglous, G. and Burton, F. L. (1991). Wastewater engineering: treatment, disposal, and reuse /Metcalf & Eddy, Inc. New York, McGraw-Hill.

Tessier, J. L. and Davies, G. A. L. (1999). Giardiasis. *Primary Care Update for OB/GYNS* **6**(1): 8-11.

Teunis, P., Chappell, C. and Okhuysen, P. (2002a). *Cryptosporidium* dose response studies: variation between isolates. *Risk Analysis* **22**(1): 175-183.

Teunis, P., Takumi, K. and Shinagawa, K. (2004). Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analysis* **24**(2): 401-407.

Teunis, P. F. M., Chappell, C. L. and Okhuysen., P. C. (2002b). *Cryptosporidium* dose-response studies: variation between hosts. *Risk Analysis* **22**(3): 475-486.

Teunis, P. F. M. and Havelaar, A. H. (2000). The Beta-Poisson dose-response model is not a single-hit model. *Risk Analysis* **20**(4): 513-520.

Teunis, P. F. M., van der Heijden, O. G., van der Giessen, J. W. B. and Havelaar, A. H. (1996). The dose-response relation in human volunteers for gastro-intestinal pathogens. Bilthoven, The Netherlands, National Institute of Public Health and the Environment (RIVM).

Teunis, P. F. M., Medema, G. J., Kruidenier, L. and Havelaar, A. H. (1997). Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. *Water Research* **31**(6): 1333-1346.

Thorn, J. and Beijer, L. (2004). Work-related symptoms and inflammation among sewage plant operatives. *International Journal of Occupational and Environmental Health* **10**(1): 84-89.

Thorn, J., Beijer, L. and Rylander, R. (2002). Work related symptoms among sewage workers: a nationwide survey in Sweden. *Occupational and Environmental Medicine* **59**(8): 562-566.

Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J. and Gerba, C. P. (2003). Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Applied and Environmental Microbiology* **69**(7): 3979-3985.

Uhnoo, I., Wadell, G., Svensson, L., Olding-Stenkvist, E., Ekwall, E. and Möllby, R. (1986). Aetiology and epidemiology of acute gastro-enteritis in Swedish children. *Journal of Infection* **13**: 73-89.

Urban Water (2002). Sustainable Urban Water Management: Programme Plan 2003.

USEPA (1997). Exposure factor handbook. Washington, DC, Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency.

USEPA (2000). Estimated per capita water ingestion in the United States. Based on the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals, United States Environmental Protection Agency, Office of Water.

Van, R., Wun, C. C., O'Ryan, M. L., Matson, D. O., Jackson, L. and Pickering, L. K. (1992). Outbreaks of human enteric adenovirus types 40 and 41 in Houston day care centers. *Journal of Pediatrics* **120**(4 Pt 1): 516-521.

van Breemen, L. W. C. A., Ketelaars, H. A. M., Hoogenboezem, W. and Medema, G. (1998). Storage reservoirs - a first barrier for pathogenic micro-organisms in the Netherlands. *Water Science and Technology* **37**(2): 253-260.

van Moeffaert, D. (2003). Multi criteria decision aid in sustainable urban water management. Proceedings from the 2nd International Symposium on Ecological Sanitation, 7-11 April, Lübeck, Germany.

Vernozy-Rozand, C., Montet, M. P., Lequerrec, F., Serillon, E., Tilly, B., Bavai, C., Ray-Gueniot, S., Bouvet, J., Mazuy-Cruchaudet, C. and Richard, Y. (2002). Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in slurry, farmyard manure and sewage sludge in France. *Journal of Applied Microbiology* **93**(3): 473-478.

Vinnerås, B., Björklund, A. and Jönsson, H. (2003a). Thermal composting of faecal matter as treatment and possible disinfection method - laboratory-scale and pilot-scale studies. *Bioresource Technology* **88**(1): 47-54.

Vinnerås, B., Holmqvist, A., Bagge, E., Albihn, A. and Jönsson, H. (2003b). The potential for disinfection of separated faecal matter by urea and by peracetic acid for hygienic nutrient recycling. *Bioresource Technology* **89**(2): 155-161.

Wadell, G. (1984). Molecular epidemiology of human adenoviruses. *Current Topics in Microbiology and Immunology* **110**: 191-220.

Wahlström, H., Tysen, E., Olsson Engvall, E., Brandström, B., Eriksson, E., Mörner, T. and Vågsholm, I. (2003). Survey of *Campylobacter* species, VTEC O157 and *Salmonella* species in Swedish wildlife. *Veterinary Record* **153**(3): 74-80.

Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R. P., Wagenaar, J. A. and Olsen, B. (2002). Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. *Applied and Environmental Microbiology* **68**(12): 5911-5917.

Wang, G. and Doyle, M. P. (1998). Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection* **61**(6): 662-667.

Ward, R. L., Bernstein, D. I., Young, E. C., Sherwood, J. R., Knowlton, D. R. and Schiff, G. M. (1986). Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. *Journal of Infectious Diseases* **154**(5): 871-880.

Water UK (2004). The application of HACCP procedures in the water industry: biosolids treatment and use on agricultural land. London, Water UK, prepared by WRc.

WCED (1987). Our common future, World Commission on Environment and Development, Oxford University Press, Oxford, UK.

Wheeler, J. G., Sethi, D., Cowden, J. M., Wall, P. G., Rodrigues, L. C., Thompkins, D. S., Hudson, M. J. and Roderick, P. J. (1999). Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reporting to national surveillance. *BMJ British Medical Journal* **318**: 1046-1050.

WHO (2003). Guidelines for Safe Recreational Waters: Volume 1 - Coastal and Fresh-Waters. Geneva, World Health Organization.

WHO (2004). Guidelines for Drinking Water Quality, 3rd edition. Geneva, World Health Organization.

WHO and UNICEF (2004). Meeting the MDG drinking-water and sanitation target: a mid-term assessment of progress. Geneva, World Health Organization and United Nations Children's Fund.

Xiao, L. H., Fayer, R., Ryan, U. and Upton, S. J. (2004). *Cryptosporidium* taxonomy: Recent advances and implications for public health. *Clinical Microbiology Reviews* **17**(1): 72-97.

Yoder, J. S., Blackburn, B. G., Craun, G. F., Hill, V., Levy, D. A., Chen, N., Lee, S. H., Calderon, R. L. and Beach, M. J. (2004). Surveillance for waterborne-disease outbreaks associated with recreational water – United States, 2001-2002. *MMWR Surveillance Summaries* **53**(8): 1-22.

Zwietering, M. H. and van Gerwen, S. J. C. (2000). Sensitivity analysis in quantitative microbial risk assessment. *International Journal of Food Microbiology* **58**(3): 213-221.