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Evaluation of microbial health risks associated with the reuse of source-separated human urine

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Doctoral thesis

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ABSTRACT

Human excreta contain plant nutrients and have the potential to be used as a fertiliser in agriculture. Urine contributes the major proportion of the nutrients (N, P and K) in domestic wastewater whereas faeces contribute a smaller amount and involves greater health risks if reused due to the possible presence of enteric pathogens. Human urine does not generally contain pathogens that can be transmitted through the environment.

Source-separation of urine and faeces is possible by using urine-separating (or urine-diverting) toilets, available as simple dry toilets or porcelain flush toilets with divided bowls. The risk for transmission of disease when handling and reusing the urine is largely dependent on the cross-contamination by faeces. In this research, the presence of human faeces in urine samples was successfully determined by analysing for faecal sterols. Cross-contamination was evident in 22% of the samples from urine collection tanks, and in these quantified to a mean (± SD) of 9.1 ± 5.6 mg faeces per litre urine mixture. Testing for indicator bacteria was shown to be an unsuitable method for determining faecal contamination in human urine since *E. coli* had a rapid inactivation in the urine and faecal streptococci were found to grow within the system.

The fate of any enteric pathogens present in urine is crucial for the risk for transmission of infectious diseases. Gram-negative bacteria (e.g. *Salmonella* and *E. coli*) were rapidly inactivated (time for 90% reduction, T_{90} <5 days) in source-separated urine at its natural pH-value of 9. Gram-positive faecal streptococci were more persistent with a T_{90} of approximately 30 days at 4°C. Clostridia spore numbers were not reduced at all during 80 days. Similarly, *rhesus* rotavirus and *Salmonella typhimurium* phage 28B were not inactivated in urine at low temperature (5°C), whereas at 20°C their T_{90}-values were 35 and 71 days, respectively. *Cryptosporidium* oocysts were less persistent with a T_{90} of 29 days at 4°C. Factors that affect the persistence of microorganisms in source-separated human urine include temperature, pH, dilution and presence of ammonia.

By using Quantitative Microbial Risk Assessment (QMRA), the risks for bacterial and protozoan infections related to handling and reuse of urine were calculated to be $<10^{-3}$ for all exposure routes independent of the urine storage time and temperature evaluated. The risk for viral infection was higher, calculated at 0.56 for accidental ingestion of 1 ml of unstored urine. If the urine mixture was stored at 20°C for six months the risk for viral infection was reduced to 5.4×10^{-4}.

By following recommendations for storage and reuse, which are dependent on the type of crop to be fertilised, it is possible to significantly decrease the risk for infections. So far, the level of risk that is acceptable is unknown. The acceptable risk will be one of the main factors determining the future utilisation of source-separated human urine in agriculture.

Key words: urine-separation, urine, wastewater systems, wastewater reuse, recycling, enteric pathogens, faecal sterols, indicator bacteria, hygiene risks, microbial persistence, microbial risk assessment, QMRA, fertiliser, crop.

Muir's law:

When we try to pick out anything by itself,

we find it hitched to everything else in the universe

LIST OF PAPERS

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

I. Höglund, C., Stenström, T.A., Jönsson, H. and Sundin, A. 1998. Evaluation of faecal contamination and microbial die-off in urine separating sewage systems. *Water Science and Technology* **38**(6):17-25.

II. Höglund, C. and Stenström, T.A. 1999. Survival of *Cryptosporidium parvum* oocysts in source separated human urine. *Canadian Journal of Microbiology* **45**(9):740-746.

III. Höglund, C., Ashbolt, N., Stenström, T.A. and Svensson, L. 2000. Viral persistence in source-separated human urine. Accepted for publication in *Advances in Environmental Research*.

IV. Höglund, C., Leeming, R. and Stenström, T.A. 2001. Faecal contamination of source-separated human urine based on the content of faecal sterols. Submitted to *Water Research*.

V. Höglund, C., Ashbolt, N. and Stenström, T.A. 2000. Microbial risk assessment of source-separated urine used for reuse in agriculture. Submitted to *Waste Management and Research*.

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1. INTRODUCTION

1.1 History

1.1.1 Early reuse of human excreta

Human excreta have traditionally been used for crop fertilisation in many countries. In Japan the recycling of urine and faeces was introduced in the 12th Century and in China human and animal excreta have been composted for thousands of years (Esrey *et al.* 1998). In Swedish cities, organised collection and transportation of latrine products to farmers started in the 18th Century (Tingsten 1911). As the population grew, quantities increased and treatment alternatives to facilitate the handling of excreta were developed. Pudrett, a mixture of latrine products and peat provided a fertiliser without smell that could be transported long distances (Tingsten 1911). Decreasing the risk for transmission of disease was another implication for further refinement of the latrine products (Wetterberg and Axelsson 1995). The latrine products were also mixed with lime to produce limed ammonium nitrate and ammonium sulphate (Tingsten 1911). Another product used as fertiliser was urat, consisting only of urine mixed with peat litter (Bachér *et al.* 1944). During the 19th Century urine was stored and used as a detergent for washing clothes in Denmark (Hansen 1928, in Drangert 1998). In Sweden urine has been used to smear wounds and dry skin and to some extent to drink as therapy (Frode-Kristensen 1966). Other historic uses of urine include tanning of hides and production of gunpowder (Stenström 1996).

1.1.2 The introduction of the water closet

Public water supplies were introduced for several reasons in Sweden, among them to improve sanitary conditions and fight epidemics of cholera (Isgård 1998). Access to water was a necessity for the use of water closets (WC), which during the middle of the 19th Century were introduced in Europe (Cronström 1986). In Sweden the first "official" WC was installed in 1883 but the introduction was quite slow due to the prohibition against using water for flushing purposes (Cronström 1986). There was also an intense debate ongoing at that time in which the health authorities and physicians argued for WCs whereas those against feared clogging and pollution of waters (Lundgren 1994). The implementation of WCs would also end the recycling and utilisation of plant nutrients from urine and faeces in agriculture. Representatives for the farmers therefore argued against the implementation of WCs (Lundgren 1994). The effect of plant nutrients and the need for recycling of manure and latrine products was known according to Liebig's mineral theory (Mårald 2000). The latrine contents produced in the cities were considered "a mine of wealth" (Goddard 1996). At the same time the role of hygienic practices was more widely realised in Europe. Bacteria were recognised as spreading disease and connected to uncleanliness and immorality, not considered to belong in a modern society (Mårald 2000). The use of latrine products in agriculture was questioned. In England Chadwick tried to combine hygiene and reuse of nutrients. One solution he advocated was the application of wastewater on agricultural fields, removing microorganisms through soil filtration (Chadwick 1842, in Mårald 2000). The economic value of latrine products was gradually outweighed by the demands for hygienic

and aesthetic conditions in cities and the WC was introduced on a larger scale (Pitkä-Kangas 1995). Furthermore, mineral fertilisers were developed at the turn of the century (Mårald 1999).

When finally accepted, the water closet was soon considered to be the ideal solution. Indeed, safe water and piped sewage have improved public health. However, several factors including increased availability and quality of food had an effect on health during the 19th and 20th Centuries, and it is difficult to determine how much the implementation of sanitary systems contributed (Castensson *et al.* 1988; Fogel 1988; Wetterberg and Axelsson 1995). In developing countries the lack of safe water and sanitation is still considered to be responsible for a large proportion of illnesses and deaths. Combined with personal and domestic hygiene it is considered the second most important risk factor after malnutrition (Murray and Lopez 1996).

1.1.3 Wastewater treatment

Sewers were first constructed only for drainage and toilet waste from WCs was collected in cesspools in many cities in Sweden (Isgård 1995). The emptying of these was complicated and unhygienic, and a centralised system was advocated. From 1909, toilet wastewater was allowed into the sewerage system in Stockholm which then was extended (Cronström 1986). The closed pipes minimised human contact with the wastewater and quickly removed toilet waste and other sewage, providing a cleaner city.

Other problems soon arose. The people who had feared pollution of water streams were correct. Near residential areas, waters were full of dirt and the smell was obvious. As a first step, mechanical treatment of the wastewater was introduced (Cronström 1986). The discharge from sewage treatment plants caused oxygen depletion due to the large content of organic material that was not fully degraded. Therefore, chemical or biological treatment was introduced at the plants (Isgård 1998). However, the oxygen depletion and eutrophication in receiving waters continued due to the supply of phosphorous, which caused algal blooms and depletion of oxygen when the algae were degraded. Strict regulations on phosphorous discharge were adopted and chemical treatment was introduced (Cronström 1986). In other parts of the world nitrogen removal was implemented along with phosphorous removal (Isgård 1998). However in Sweden it was not until the 1980s that eutrophication of the Baltic Sea was suggested to be caused by excessive nitrogen discharges (Swedish EPA 1997; Isgård 1998). Sewage treatment plants now had to take action against the problem. The largest anthropogenic source of nitrogen to water is agriculture, and restrictions are being introduced to deal with this (Swedish EPA 1997).

1.1.4 Recycling of nutrients

Over the past decade the existing (or conventional) wastewater treatment systems have been criticised for their non-sustainability. Toilet waste contains virtually all the plant nutrient humans ingest through food and drink and could theoretically be recycled to plants. Phosphorous is a finite resource, with present recoverable reserves calculated to last for less

than 200 years (Larsson *et al.* 1997), whereas potassium is assumed to last for 300 years (Crowson 1992, in Lindfors *et al.*1995). Production of nitrogen fertilisers requires energy, as does the reduction of nitrogen in sewage treatment plants. Oil and gas, the most important energy resources for production of nitrogen fertilisers, have been calculated to last for 40 and 60 years, respectively (WRI 1992, in Lindfors *et al*. 1995). Even though the reduction of phosphorous is high (94% on average) in Swedish sewage treatment plants (SCB 1999), approximately 20% of the Swedish anthropogenic load to the Baltic Sea originates from the treatment plants (Larsson *et al.* 1997). Individual households in rural areas in Sweden are estimated to contribute an approximate equal amount (Larsson *et al.* 1997). The conventional system has even been considered to be based on a principal error in its use of large volumes of clean water to dilute and transport small volumes of human waste (Niemczynowicz 1994; Swedish EPA 1995a; Esrey 2000).

Sewage sludge from treatment plants is often used in agriculture, mainly due to its large content of precipitated phosphorous. It can be debated whether this practice is mainly for disposal of a waste product or for utilisation of the plant nutrients. In Sweden approximately 30% of the sludge has been used in recent years (SCB 2000). Since October 1999 however, the Federation of Swedish Farmers do not recommend the use of sludge on agricultural land due to the high content of organic contaminants (Eksvärd 1999). Risks from PCBs and other organic contaminants, heavy metals and pathogens have also been discussed (Lewis-Jones and Winkler 1991; Dumontet *et al.* 1999; Eksvärd 1999).

To increase sustainability (recycle nutrients, decrease outlets etc.; Section 1.4) alternatives to conventional treatment have been suggested. These can be called complementary, alternative or ecological wastewater systems (Kärrman *et al.* 1999) and the aim is often to reuse the plant nutrients of the wastewater as a fertiliser. One concept is source-separating wastewater systems which include blackwater systems, where the wastewater from toilets is treated separately, urine-separating systems with separate handling of urine and different types of dry systems where the toilet waste is handled without the use of flushwater (Jenssen 1999; Kärrman *et al.* 1999). Blackwater can be mixed with organic household waste and treated by liquid composting or digestion (Jenssen 1999; Kärrman *et al.* 1999). The collection and periodic release of urine into existing sewers under the term anthropogenic nutrient solution (ANS) has been suggested (Larsen and Gujer 1996).

1.2 Nutrient content and volume of domestic wastewater

In Sweden approximately 200 litres of wastewater are produced per person and day (Swedish EPA 1995b). The major plant nutrients nitrogen (N), phosphorous (P) and potassium (K) can all be found in human excreta and thus in domestic wastewater (Table 1). The nutrient content in urine and faeces will vary depending on the food intake, e.g. on protein intake (Drangert 2000; Jönsson *et al.* 2000). The main proportion of the phosphorous in greywater (all domestic wastewater except that from toilets) originates from detergents (Swedish EPA 1995b).

Table 1. Composition and volume of domestic wastewater expressed per person and day (pd) (Swedish EPA 1995b)

Parameter	Unit	Urine	Faeces	Greywater	Total
Volume	kg/pd	1.5[a, b]	0.1[a]	150	200[c]
Dry substance	g/pd	60	35	80	175
Nitrogen	g/pd	11.0	1.5	1.0	13.5
Phosphorous	g/pd	1.0	0.5	0.3[d]	1.8
Potassium	g/pd	2.5	1.0	0.5	4.0
BOD_7	g/pd	6[e]	14	28	48

[a] Excluding flushwater.
[b] Hellström and Kärrman 1996.
[c] Including flushwater, conventional WC.
[d] Value for 1998 (Jönsson *et al*. 2000).
[e] Measured by Jönsson *et al.* (1998).

Urine is the fraction that contains the major part of the plant nutrients in domestic wastewater, approximately 80% of the nitrogen, 55% of the phosphorous and 60% of the potassium (Figure 1). At the same time it constitutes less than 1% of the total wastewater volume. Thus it is possible to collect a relatively concentrated fertiliser by separating urine from the wastewater. Faeces also contain relatively large proportions of N, P and K whereas their content in greywater is lower and also diluted with large amounts of water. Furthermore, the content of metals in urine is very low (Kirchmann and Pettersson 1995; Jönsson *et al.* 2000).

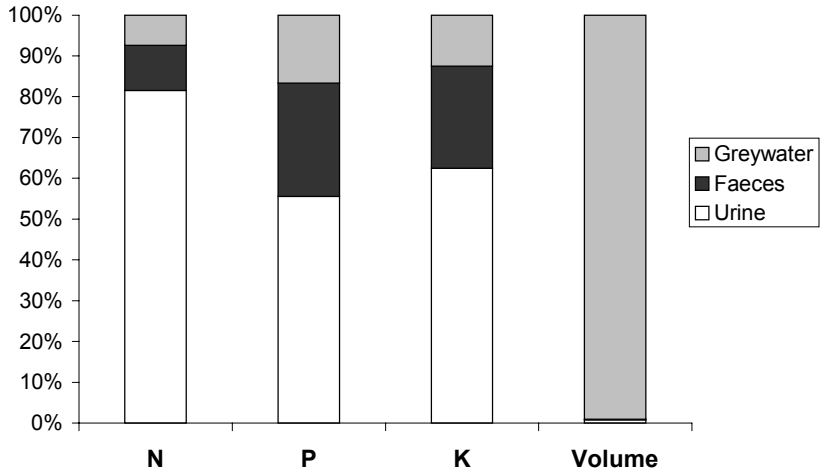


Figure 1. Content of major plant nutrients and volume in domestic wastewater.

1.3 Wastewater systems that separate urine and faeces

At the time when latrine contents were collected in buckets from each household in Swedish cities, urine was often collected separately and poured into the drain to avoid smells and to prevent the latrine from filling too quickly (Sondén 1889). Already in 1867 it was known that

"the proportion of value of the fertilising ingredients held in solution in urine to that contained in faeces is as six to one" (Krepp 1867, in Drangert 1999) while Müller, a German scientist at that time, saw it as a necessity to separate the urine from the faeces in order to produce a fertiliser that was of manageable proportions (Müller 1860, in Mårald 2000). The separation, or diversion, of urine and faeces was made possible by the use of Marino's toilet (Figure 2). In many other parts of the world it is also a tradition to keep the urine and faeces apart. The old Japanese practice of nightsoil recovery from urban areas separated urine and faeces, since urine was regarded as a valuable fertiliser (Matsui 1997). In Yemen the urine is drained away and evaporated on the outer face of multistorey buildings to obtain the faeces as a dry fraction without smell for later use as fuel, a system that has been in use for hundreds of years (Esrey *et al.* 1998).

Today the alternatives to the conventional wastewater system include systems that separate urine and faeces in order to utilise the nutrients more efficiently. In regions without piped sewerage, nutrient utilisation as well as improved sanitation is possible to achieve by avoiding mixing the fractions. If the faecal fraction is kept dry there will be less leaching from e.g. pit latrines and in many places the faeces are also reused. Thus, the two main reasons to separate urine and faeces are to recycle the plant nutrients in urine and to obtain a faecal fraction that is more practical and safer to handle.

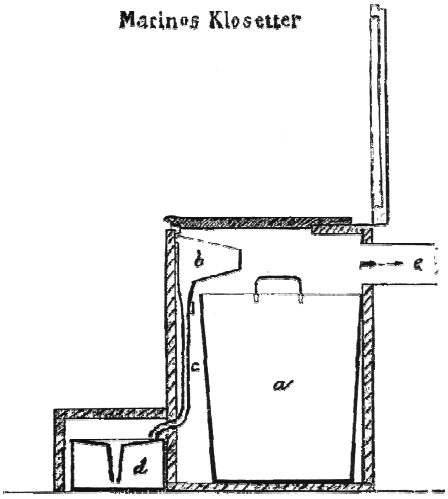


Figure 2. Marino's toilet.

Facilitating the composting of faeces might be another reason for keeping the liquid separate from the solids. In the Clivus Multrum™ toilet the urine and leachate drains to the bottom of the composting chamber where it is collected in a separate tank (Del Porto and Steinfeld 2000). This system is manufactured around the world but has been criticised for wasting the urine resource (Esrey *et al.* 1998; Drangert 1999). Another system, the Aquatron™ can either be connected to a conventional toilet or a urine-separating toilet (see below). Through centrifugal forces the liquid is separated from the solids, which drop down into a composter.

The liquid, containing flushwater, possibly urine and suspended solids from faecal matter, is treated in an UV-unit and disposed of as greywater (Del Porto and Steinfeld 2000).

The latter are examples of techniques that literally first mix the two fractions urine and faeces, and then separate them. The term *urine diversion* has been used when the fractions are never mixed (Esrey *et al.* 1998). In Sweden however, the English term *source-separation* has been used analogously with source-separation of solid waste. Thus, the term used throughout this thesis is *source-separation of urine*, leading to the term *urine-separating toilets*.

1.3.1 Source-separation of urine in Sweden

As mentioned (Section 1.3) separation and reuse of urine and faeces was conducted about a century ago in Sweden but also in other countries like Germany and Denmark (Hösel 1987, in Heinicke 2000). The revival of the idea occurred in the beginning of the 1990s when the urine-separating toilet from WM-Ekologen AB (now Wost Man Ecology AB; Figure 3a) and Dubbletten™ from BB Innovation & Co AB (Figure 3b) were presented. At present there is one more separating porcelain toilet on the Swedish market that uses flushwater, the Gustavsberg model Nordic 393U (Figure 3c). A number of dry toilets that separate urine from faeces and add-ons to simple dry toilets used in summer houses are also available (Swedish Consumer Agency 2000).

(a) (b) (c)

Figure 3. Urine-separating toilets originating from Sweden. (a) Model DS from Wost Man Ecology AB; (b) Dubbletten™ from BB Innovation & Co AB; (c) Nordic 393U from Gustavsberg.

At the time of introduction, the new types of separating toilets were mainly installed in so-called eco-villages. These are housing areas where building materials, energy sources and wastewater systems have been chosen to comply with sustainability. More recently a variety of housing areas and institutions have installed urine-separating toilets. The toilets can be

completely dry or flushwater can be used for either fraction. In Dubbletten™, there is separate flushing mechanism for the urine that uses about 0.1 l of water per flush. The faeces are flushed with approximately 4 l of water. Wost Man Ecology markets one dry toilet (ES) where only the urine is flushed with 0.1-0.2 l, and a model (DS) where the flush rinses both bowls using 0.8 l and 4-6 l for flushing the urine and faeces bowls, respectively (Jönsson *et al.* 2000). The toilet from Gustavsberg uses 2-4 l flushwater with 0.2-0.4 l entering the urine bowl. Since piped solutions are generally more accepted, dry handling of faeces mainly occurs in eco-villages and summer houses.

The urine is usually collected in a tank placed underground or inside in the basement. When the tank is full it is emptied and the urine transported to a farm for later use as a fertiliser on agricultural land. Before its utilisation the urine is stored either in the housing area or near the field (Figure 4). For individual households the urine may also be utilised in the garden directly from the collection tank, without separate storage (see also Section 6).

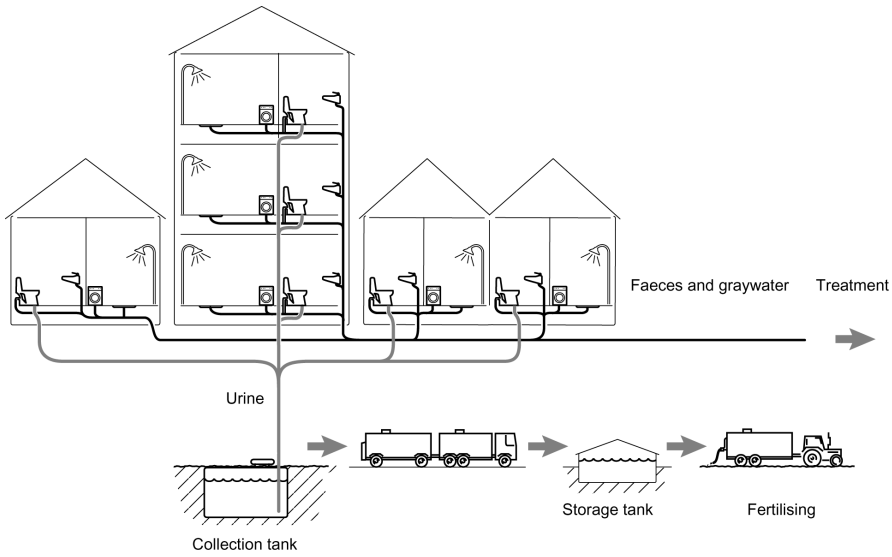


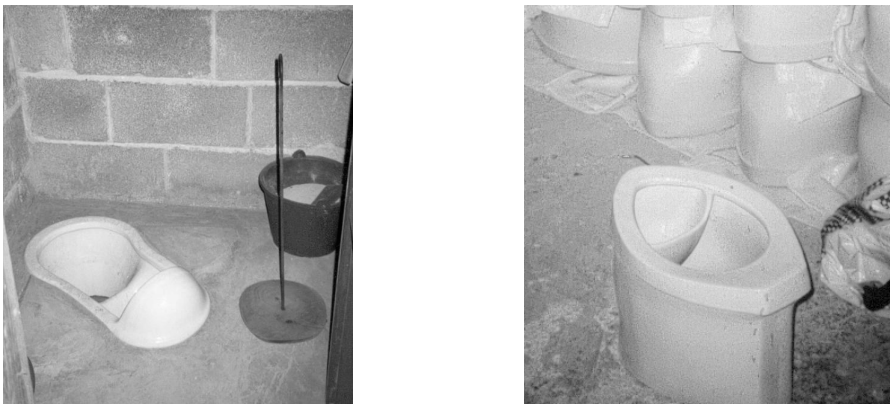
Figure 4. A large-scale urine-separating wastewater system (from Jönsson *et al.* 2000).

Research presented in this thesis was conducted on both small-scale and large-scale systems using the toilets Dubbletten™ or Wost Man Ecology DS or ES.

1.3.2 Source-separation of urine in other parts of the world

Dubbletten™ and Wost Man Ecology's toilets are marketed in several European countries and in the USA. The old tradition of using human excreta in agriculture is still practised in China and new types of toilets that separate urine from faeces are also being introduced on a large scale (Shunchang *et al.* 1997; Stenström pers. comm.). In Vietnam various types of dry latrines (double-vault and bucket latrines) with urine-separation are in use, although without complete utilisation of the urine (Carlander and Westrell 1998; Esrey *et al.* 1998). These have been promoted since 1956 followed by health education programmes to ensure safe reuse of the faeces (Esrey *et al.* 1998). In India a demonstration area has been built in Kerala with

toilets that separately collect the urine and the water used for anal cleaning into an evapo-transpiration reedbed (Esrey *et al.* 1998; Calvert 1999). In Mexico around 100 000 separating toilets made of cement have been distributed, however only part of them along with necessary education in health and maintenance for the users (Clark 1997). Dry urine-separating toilets are also in use in Central America, often provided with a separate urinal (Gough 1997; Esrey *et al.* 1998). Several projects are on-going in Africa, e.g. in Zimbabwe, in Kenya and in Ethiopia (Ahlgren and Evjen 1999; Faul-Doyle 1999; Morgan 1999; Sundin 1999; SUDEA 2000).



(a) (b)

Figure 5. Urine-separating toilets in (a) China and (b) Mexico.

Ecological sanitation is a term not yet defined, but the approach involves treating human excreta as a resource, sanitising them and then recycling the nutrients contained in the excreta (Esrey *et al.* 1998). In developing countries ecological sanitation often refers to a dry system where the urine is diverted from the faeces. The Swedish International Development Cooperation Agency (Sida) is promoting ecological sanitation including the "don't mix" approach to human excreta, since a lot can be gained in health and nutrient resources compared to e.g. traditional pit latrines (Esrey *et al.* 1998). Furthermore, it is impossible to provide the whole world with piped sewers and wastewater treatment (WRI 1996; Drangert 1998). Currently it is estimated that more than three billion people are lacking safe sewage disposal (WHO 1997) and that less than 10% of the wastewater in developing countries are treated (WRI 1996). With the current growth in World population (UNFPA 1999) the sewage problem will increase and rapid solutions are necessary.

1.4 Sustainable wastewater systems

At a minimum, sustainable development should not threat the natural systems constituting the basics of life, i.e. the atmosphere, the waters, the soils and the organisms (WCED 1987). These systems are all to a greater or lesser extent affected by water and wastewater systems. The Swedish government formulated the goals for a sustainable future as protection of the

environment while efficiently using and managing natural resources, especially renewable resources (SOU 1997), all of which could be attributable to water and wastewater systems.

Recent definitions of sustainable water management for cities include "A sustainable urban water system should over a long time perspective provide required services while protecting human health and the environment, with a minimum use of scarce resources" (Lundin 1999) and the vision of the Swedish research programme Sustainable Urban Water Management (Malmqvist 1999) "Every human has a right to clean water. For urban areas, our vision is water management where water and its constituents can be safely used, reused and returned to nature". Urban areas have received more attention since a dense settlement and population usually involves more complex systems and larger environmental and sanitary problems. However, in Sweden a large proportion of eutrophicating pollution origins from sanitary systems for individual households in rural areas (Larsson *et al.* 1997) and efforts for technical development of these systems have also been made (Hellström 1999).

According to VISION 21 (WSSCC 2000) new approaches to meet future water and sanitary system demands should be based on the principle that human faeces and urine can not be considered as waste products, but rather as resources to be recycled in safe ways, such as to agriculture. Hence, these products should be managed as close as possible to their source of production and water should be minimally used to transport waste (WSSCC 2000). The VISION 21 approaches are being generally accepted, considering sanitation and health on a global scale, but mainly addressing the problems in developing regions. However, developed regions also require adaptations for local solutions to wastewater treatment and recycling of human wastes. The urine-separation technique adapts well to the purposes and scope of VISION 21.

Systems analysis and other quantitative evaluation methods have often been used to compare water and wastewater systems. Either a range of criteria including different aspects can be used (Malmqvist 1997, Malmqvist and Stenberg 1997; Kärrman *et al.* 1999) or a specific aspect, e.g. hygiene, can be evaluated (Albihn and Stenström 1998). Urine-separating systems have been included as a part of several of the systems investigated in Sweden (Malmqvist 1997; Malmqvist and Stenberg 1997; Albihn and Stenström 1998; Kärrman *et al.* 1999; Jönsson *et al.* 2000).

1.4.1 Criteria for wastewater systems

To be able to compare and individually evaluate water and wastewater systems, the Swedish Environmental Protection Agency (Swedish EPA) (1995a) developed a set of criteria that was sorted into the following categories:

- Transmission of disease and sanitary conditions
- Environmental impact and efficient use of resources
- Technical and socio-economic criteria

It was further concluded that a sustainable wastewater system needs to include recycling of resources such as plant nutrients, whereas to fulfil the health criteria the risk for transmission of pathogens should be minimised. Within the concept sanitary conditions, occupational health and animal protection were included.

Hellström *et al.* (2000) further subdivided the above criteria and defined five main categories for sustainable urban systems, with health and hygiene listed first. Each category included several sub-criteria where access to clean water, risk for infection, exposure to toxic compounds and working environment were the sub-criteria for health and hygiene.

In many cases the term sustainability has been used only from an ecological perspective, including environmental protection and minimising the use of natural resources (Larsen and Gujer 1996; Otterpohl *et al.* 1996). Health aspects are important to include especially since the conventional water and wastewater system has provided a high sanitary standard and recycling alternatives may enable the spread of pathogens in the environment (Swedish EPA 1995a).

This thesis focuses on the first health criteria, specifically applied to urine-separation and mainly considering transmission of disease to humans. Occupational health and transmission of disease to animals are partly included in Sections 5 and 6, whereas the other main criteria are dealt with in the general discussion (Section 7).

1.5 Pathogenic microorganisms in wastewater systems

The findings of John Snow during the cholera epidemics in London in the middle of the 19th Century were the start of epidemiology and the first recorded waterborne outbreak (Beaglehole 1993). Snow recognised that consumption of water implied an increased risk of contracting the disease, but the disease-causing agent (*Vibrio cholera*) had yet to be isolated and identified. In 1857 Pasteur established the theory that infectious disease is caused by germs or bacteria (Kirby *et al.* 1956). Early recorded waterborne diseases also include typhoid, dysentery and polio (Stenström 1996). Some of the epidemics were either due to the consumption of contaminated surface water or due to the fact that wastewater contaminated groundwater sources. Still today, new emerging pathogens are being identified and recognised as causing a significant amount of disease. Many of these originate from faeces and have the ability to be transmitted via food and water. One example is *Cryptosporidium parvum*, for which the first cases were reported in 1976 (Marshall *et al.* 1997). It has later been found to be widely distributed in water and caused the largest waterborne outbreak documented in 1993, with an estimated 400 000 infected (MacKenzie *et al.* 1994; Marshall *et al.* 1997).

Four major groups of microorganisms can be transmitted through the environment and cause infectious diseases: bacteria, protozoa, viruses and helminths (worms that infect humans) (Feachem *et al.* 1983). In addition fungi are capable of causing disease in humans and animals, even though only a fraction of species are parasitic or opportunistic (Dixon and

Fromtling 1991; Haug 1993). Regarding wastewater systems and sewage, the pathogens infecting the gastrointestinal (GI) tract causing diarrhoeas have a major significance. However, many other clinical manifestations are also important and GI infections may cause other severe symptoms in a later stage of the disease (WCED 1997; Havelaar *et al.* 2000). Feachem *et al.* (1983) divided the excreta-related pathogens into six categories depending on their infectious dose, transmission route (e.g. the need for an intermediate host), latency, persistence and multiplication. The impact improved sanitation had on the prevalence of a disease caused by the pathogen was considered to depend on category (Feachem *et al.* 1983; Cairncross and Feachem 1993). This perspective was mainly applicable to areas with no or low-cost sanitation in the tropics while in this thesis pathogens are instead discussed and evaluated within the four groups above. The present investigations mainly considered bacteria, protozoa and viruses.

1.5.1 Transmission routes

Transmission of infections can either be *direct* through different means of person to person contact, including short-distance airborne, or *indirect* (secondary), which includes vehicle-borne (food, water, fomites etc.), vector-borne, airborne long-distance and parenteral (injections with contaminated syringes) transmission (Beaglehole *et al.* 1993). The secondary transmission routes for pathogens emanating from faeces and urine are illustrated in Figure 6. These routes can largely be defined as *faecal-oral* (or *urine-oral*), since most involve either ingestion or inhalation through the oral cavity. Diarrhoeal diseases are also transmitted directly, especially those caused by viruses (Cairncross and Feachem 1993; Ryan *et al.* 1997).

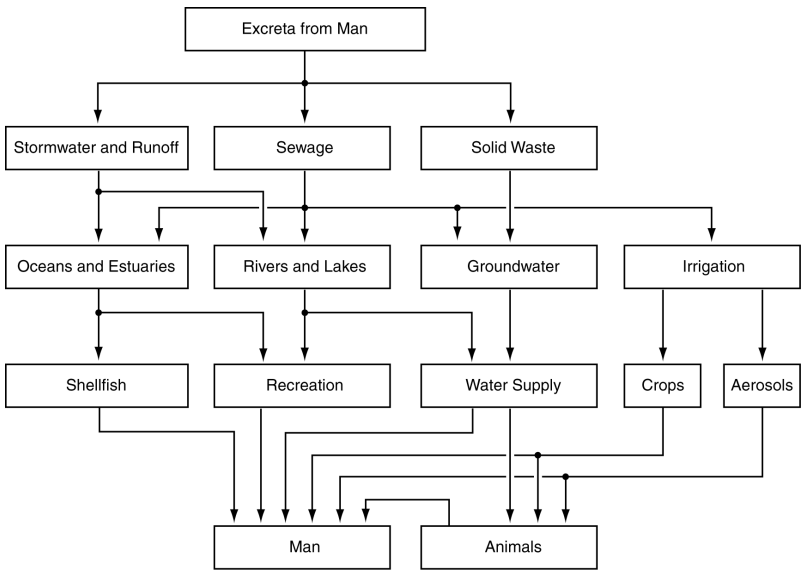


Figure 6. Secondary transmission routes for pathogens originating from human urine and faeces (modified from Haas *et al.* 1999).

An infection is defined as the entry of the pathogen via e.g. the GI tract, the respiratory tract or the skin and its multiplication and establishment inside the host (Jawetz *et al.* 1987).

Healthy carriers have an infection that is subclinical with no apparent symptoms (asymptomatic infections), but which confers the same degree of immunity as an overt infection (Bitton 1994). Furthermore, carriers constitute a potential source of infection for others in the community since they shed infectious organisms e.g. in faeces (Haas *et al.* 1999).

1.5.2 Pathogenic microorganisms in urine

In a healthy individual the urine is sterile in the bladder. When transported out of the body different types of dermal bacteria are picked up and freshly excreted urine normally contains <10 000 bacteria per ml (Tortora *et al.* 1992). By urinary tract infections, which in more than 80% of cases are caused by *E. coli* (Murray *et al.* 1990), significantly higher amounts of bacteria are excreted. However, these have not been reported to be transmitted to other individuals through the environment. Pathogens causing venereal diseases may occasionally be excreted in urine but there is no evidence that their potential survival outside the body would be of health significance (Feachem *et al.* 1983).

The pathogens traditionally known to be excreted in urine are *Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi* and *Schistosoma haematobium* (Feachem *et al.* 1983). Leptospirosis is a bacterial infection causing influenza-like symptoms and is in general transmitted by urine from infected animals (Feachem *et al.* 1983; CDC 2000a). It is considered an occupational hazard e.g. for sewage workers and for farm workers in developing countries (CDC 2000a). Human urine is not considered to be an important route for transmission since the prevalence of the infection is low (Feachem *et al.* 1983; CDC 2000a). Infections by *S. typhi* and *S. paratyphi* only cause excretion in urine during the phase of typhoid and paratyphoid fevers when bacteria are disseminated in the blood (Feachem *et al.* 1983). This condition is rare in developed countries (Lewis-Jones and Winkler 1991). Even though the infection is endemic in several developing countries with an estimated 16 million cases per year, urine-oral transmission is probably unusual compared to faecal-oral transmission (Feachem *et al.* 1983; CDC 2000b). Schistosomiasis, or bilharziasis, is one of the major human parasitic infections mainly occurring in Africa (Feachem *et al.* 1983). When infected with urinary schistosomiasis caused by *Schistosoma haematobium*, the eggs are excreted in urine, sometimes during the whole life of the host. The eggs hatch in the environment and the larvae infect specific aquatic snail species, living in fresh water. After a series of developmental stages aquatic larvae emerge from the snail, ready to infect humans through penetration of the skin (Feachem *et al.* 1983). The disease does not occur in Europe or in the US (CDC 2000c).

Mycobacterium tuberculosis and *Mycobacterium bovis* may be excreted in urine (Bentz *et al.* 1975; Grange and Yates 1992) but tuberculosis is not considered to be significantly transmitted by other means than by air from person to person (CDC 1999a). *M. tuberculosis* is exceptionally isolated in nature, but was identified in wastewater coming from hospitals (Dailloux *et al.* 1999). Humans are able to infect cattle with both the bovine strain and the human strain and it has been reported that individuals on farms have transmitted bovine

tuberculosis to cattle by urinating in the cowsheds (Huitema 1969; Collins and Grange 1987). Feachem *et al.* (1983) doubts that transmission of either human or bovine tuberculosis is significantly affected by exposure to wastes or polluted water. Other mycobacterial species (atypical or environmental mycobacteria) may also be isolated from urine. They are also widely distributed in the environment and commonly found in waters, including as contaminants in drinking water (Grange and Yates 1992; Dailloux *et al.* 1999).

Microsporidia are a group of protozoa recently implicated in human disease, mainly in HIV-positive individuals (Marshall *et al.* 1997; Cotte *et al.* 1999). The infective spores are shed in faeces and urine, and urine is a possible environmental transmission route (Haas *et al.* 1999). Microsporidia have been identified in sewage and in waters, but no water- or foodborne outbreaks have been documented although they have been suspected (Cotte *et al.* 1999; Haas *et al.* 1999).

Cytomegalovirus (CMV) is excreted in urine, but the transmission of CMV occurs person to person and the virus is not considered to be spread by food and water (Jawetz *et al.* 1987; CDC 1999b). CMV infects a large proportion of the population, 50-85% by the age of 40 was reported for the USA (CDC 1999b). Two polyomavirus, JCV and BKV, are excreted in urine (Bofill-Mas *et al.* 2000). Both have been found in sewage in various countries, including Sweden. Even though the occurrence in waste products enables transmission to other humans in the environment, a majority of the population will be infected by close contact within the family or outside the family at a young age (Kunitake *et al.* 1995; Bofill-Mas *et al.* 2000). In one Japanese investigation it was found that 46% of persons aged 20-29 years excreted urinary JCV (Kitamura *et al.* 1994). One foodborne outbreak of hepatitis A caused by lettuce contaminated by urine has been reported (Ollinger-Snyder and Matthews 1996). Hepatitis B was also found in human urine and urine was suggested as a potential route of transmission in hyperendemic areas (Knutsson and Kidd-Ljunggren 2000). Adenovirus may also be excreted in urine, especially from children with hemorrhagic cystitis, transplant patients and HIV-positive individuals (Mufson and Belshe 1976; Shields *et al.* 1985; Echavarria *et al.* 1998). However, the public health concern from urinary transmission has not been recognised.

It can be concluded that pathogens that may be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are thus not considered to constitute a health risk related to the reuse of human urine in temperate climates. An exception in tropical areas is *Schistosoma haematobium*, which however implies a low risk due to its lifecycle. Furthermore, the inactivation of urinary excreted pathogens in the environment reduces their ability for transmission (Papers I-III).

1.5.3 Pathogenic microorganisms in faeces

The most prevalent enteric diseases in developed countries are probably caused by viruses (Tauxe and Cohen 1995; Mead *et al.* 1999). However, bacteria are generally the leading cause of gastrointestinal diseases in surveillance systems since many of the enteric viruses are reported only on a voluntary basis (Mead *et al.* 1999; SMI 2000). The ratio of community

incidence to reported cases is also higher for viral diseases than for bacterial (Wheeler *et al.* 1999). In Sweden *Campylobacter*, *Salmonella*, *Giardia*, *Yersinia*, *Shigella*, *Entamoeba* and enterohaemorrhagic *E. coli* O157 (EHEC O157) in descending order caused most of the reported cases in 1999 (SMI 2000). In a community cohort incidence study in the UK, small round structured viruses (SRSVs, Norwalk-like viruses) were the most prevalent cause of GI disease, followed by *Aeromonas* spp., *Campylobacter* spp. and rotavirus (Wheeler *et al.* 1999). Surveillance systems are generally considered to underestimate the number of cases (Mead *et al.* 1999; Wheeler *et al.* 1999; Barwick *et al.* 2000). In many cases the aetiological agent also remains unknown (Tauxe and Cohen 1995; Wheeler *et al.* 1999).

Of the bacteria identified as being a common cause of GI infections *Campylobacter*, *Salmonella* and EHEC are zoonoses, able to infect both humans and animals. Zoonotic diseases having many different animal reservoirs may have complex transmission routes in the environment and these diseases can be more difficult to control (Yu and Meissner 1989; Slifko *et al.* 2000).

In the US, the most frequent parasitic causes of diarrhoeal diseases are *Giardia lamblia* and *Cryptosporidium parvum* (Tauxe and Cohen 1995). In Sweden *G. lamblia* was the third most, and *Entamoeba histolytica* the sixth most, common reported cause of GI infections, whereas *C. parvum* only is reported voluntarily (SMI 2000). The yearly incidence of *C. parvum* has in other countries been reported to vary between 2.5 and 24.2 cases per 100 000 (Duncanson *et al.* 2000). Both *G. lamblia* and *C. parvum* are zoonoses and transmitted by food and water as well as directly from humans and animals (Tauxe and Cohen 1995; Olson *et al.* 1999). Recently other protozoa like microsporidia and *Cyclospora* have been recognised as causes of diarrhoeal diseases in humans, although their importance as pathogens still remains unclear (Tauxe and Cohen 1995; Marshall *et al.* 1997).

More than 120 different types of viruses may be excreted in faeces (Haas *et al.* 1999). Enteric viruses are a major cause of gastrointestinal infections in humans in developed countries, estimated to be responsible for 80% of the cases in the US (Mead *et al.* 1999). They are also assumed to be responsible for many cases where no aetiological agent is found (Schwartzbrod 1995). The most commonly identified viral pathogens are rotavirus, enteric adenoviruses and the Norwalk-like viruses (Tauxe and Cohen 1995). Other important viruses excreted in faeces include enteroviruses, reoviruses and hepatitis A virus (Feachem *et al.* 1983). Enteric viruses are mainly transmitted person to person but waterborne outbreaks of rotavirus and Norwalk-like viruses have been documented (Gerba *et al.* 1985; Stenström *et al.* 1994). Human enteric viruses are traditionally not considered to be transmitted to animals. However, more recently genetically related viruses have been found in humans and different animal species, neither proving or excluding zoonotic transmission (Bishop 1996; van der Poel *et al.* 2000).

Human helminth infections are a major cause of morbidity and mortality, particularly in developing countries. Ascariasis is one of the most common helminthic infections globally, with an estimated one billion cases (Feachem *et al.* 1983; Gopinath and Keystone 1995).

Humans excrete fertilised eggs that are developed in the soil and cause disease by ingestion. The distribution of infection is among other factors related to the level of sanitation (Gopinath and Keystone 1995). Infection by hookworms (ancylostomiasis) and whipworms (trichriasis) are the other two intestinal worm infections globally distributed and causing severe clinical manifestations in heavily infected individuals (Feachem *et al.* 1983). Enterobiasis is the most common helminth in Europe and the US (Gopinath and Keystone 1995) as well as worldwide (Feachem *et al*. 1983). It is, however, considered to be of minor public health concern since it does not cause severe disease and it is generally not excreted in faeces (Feachem *et al.* 1983).

Faeces do not always contain pathogens. However, from a risk perspective their presence should always be considered since there are so many different types of enteric infections and the prevalence is unknown for several of them. To ensure a reduction in pathogens, faeces need to be treated or stored under controlled conditions.

1.5.4 Diseases related to sanitation

Although implemented for the protection of public health, the conventional wastewater treatment systems are not primarily designed for reducing pathogens. Still, they have been fairly effective in reducing transmission of pathogenic microorganisms. This owes both to minimised human contact with wastewater, the different treatment processes and to dilution of the treated wastewater in the recipient (Stenström 1996). Waterborne outbreaks are, however, often caused by sewage-contaminated drinking water (Stenström *et al.* 1994; SMI 1998). In the Nordic countries known aetiological agents were identified in 36% of waterborne outbreaks in 1975-1992. Of these 46% were bacterial, 43% viral and 11% protozoan (Stenström *et al.* 1994). In the US 76% of reported outbreaks in 1997-1998 had an identified agent, with 32% bacterial, 8% viral and 60% protozoan (Barwick *et al.* 2000). Viruses are considered to be responsible for a major proportion of the outbreaks where no aetiological agent is found (Hedberg and Osterholm 1993). Waterborne and foodborne transmission illustrate a pathogen's ability to survive in, and spread through, the environment.

In many countries that lack piped sewerage, traditional pit latrines are used for defecation (Esrey *et al.* 1998). These are simple and can be built at low cost but there are risks for disease transmission either through contamination of groundwater or due to overflow during heavy rains (Esrey *et al.* 1998). In these areas traditional waterborne diseases like cholera are still prevalent and the direct contact with human excreta causes infections of helminths, e.g. hookworms (Feachem *et al.* 1983). Contamination of groundwater by nitrate may also cause methemoglobinemia (Lagerstedt *et al.* 1994; Esrey 2000).

1.5.5 Transmission of pathogens in reuse systems

Higher incidences of enteric infections in the population have been recorded in epidemiological investigations in areas where wastewater was used on crops (Katzenelson *et al.* 1976; Cifuentes 1998; Bouhoum and Amahmid 2000). In some studies however, no increased risk for infectious diseases could be detected (Ward *et al.* 1989; Devaux *et al.* 2000). Foodborne outbreaks caused by wastewater irrigation of vegetables and fruits have

been documented (Yates and Gerba 1998). Risk assessments (Section 1.9) have also evaluated the increased risk from wastewater irrigated crops (Rose and Gerba 1991; Shuval *et al.* 1997; Blumenthal *et al.* 2000). Irrigation with wastewater on crops used for energy or industrial purposes may be safer but still involves risks for transmission of disease to humans and animals in the surroundings and transport of pathogens to the groundwater (Carlander *et al.* 2000). The safety of using sewage sludge in agriculture has been debated due to both chemical and microbial risks. The microbial risks are poorly characterised (Lewis-Jones and Winkler 1991; Dumontet *et al.* 1999; Sidhu *et al.* 1999) but there are no evident cases where sludge has caused infectious disease (Cooper and Olivieri 1998; Stenström and Carlander 1999). Animal manure has traditionally been used as a fertiliser in agriculture and health aspects have only recently been acknowledged due to the recognition of zoonotic agents like EHEC causing serious illness in humans (Strauch 1991; Pesaro *et al.* 1995; Olson *et al.* 1999; Gagliardi and Karns 2000).

The handling and reuse of all different types of waste products with human or animal origins involve hygiene risks. Whether human excreta (faeces and/or urine) are reused directly, diluted in wastewater (treated or untreated) that is reused, or are a constituent of sewage sludge used in agriculture, enteric pathogens will be present and able to cause infections by ingestion of the waste product or by consumption of crops that have been fertilised. Cysts and oocysts of protozoa and helminth ova are considered to be of great public health concern since they remain viable for extended periods outside their human host (Cooper and Olivieri 1998), and viruses have received attention due to low infectious doses and difficulties in analysing their presence in waste products (Asano and Levine 1998).

Many of the new wastewater solutions being introduced are small-scale systems that demand more personal involvement of the users, including handling of the waste. Thus the possible exposure points for pathogens are increasing compared to conventional systems. With the main goals of recycling nutrients and minimising utilisation of natural resources, hygiene aspects may also be less prioritised. To successfully introduce and optimise alternative wastewater systems and the utilisation of waste products it is necessary to evaluate hygiene risks and sanitary aspects in accordance with sustainability criteria (Section 1.4.1). Thereafter recommendations for e.g. storage, treatment or reuse practises should be deduced.

1.6 Indicators of faecal contamination and microbial behaviour

For the past 100 years the hygienic quality of water has been determined by enumeration of intestinal microorganisms, particularly those of the coliform group (Walker *et al.* 1982). These organisms have been used as *indicators* of faecal contamination and possible health risks related to the water. Analysing samples for a few faecal indicator organisms is generally more efficient than looking for a range of enteric pathogens possibly present. Important criteria for an ideal indicator are 1) that it be present only when faecal contamination is present, 2) that it exhibits the same or greater survival characteristics as the pathogen for which is it a surrogate and does not multiply in the environment, and 3) that its presence can

be detectable by means of easy, rapid and inexpensive methods (Cooper and Olivieri 1998). Indicator organisms are also used as surrogates or models for the behaviour of pathogens e.g. to determine the efficiency of treatment processes. Even though still in use, the accuracy, specificity and validity of indicator organisms have been questioned in recent years (Walker 1982; Nichols *et al.* 1993). This is largely due to the variability in survival of indicator bacteria, greater resistance of pathogens than indicators and a poor correlation to specific pathogens (Nichols *et al.* 1993; Haas *et al.* 1999).

As an alternative or complement to microorganisms in detecting faecal contamination chemical biomarkers have been suggested as indicators. Biomarkers can be defined as organic compounds that have maintained sufficient structural integrity for their source to be recognised (Leeming *et al.* 1996). The most widely studied biomarkers are probably coprostanol and structurally related faecal sterols and stanols (e.g. Vivian 1986; Leeming *et al.* 1996; Chan *et al.* 1998). These compounds are metabolites of cholesterol formed in the intestine and excreted in faeces (Lichtenstein 1990).

1.6.1 Indicator bacteria

Coliforms (total coliforms) are the historic indicators of faecal contamination (Berg 1978; Cooper and Olivieri 1998). It has long been known that many of the bacteria in this group are not of faecal origin, but still the group continues to be used as an indicator in several areas, primarily in drinking waters (WHO 1993). They are, however, not suitable as sole indicators of the hygienic quality of reuse products since other groups of microorganisms are considered more resistant (Cooper and Olivieri 1998). Coliforms may grow in the environment and regrowth, e.g. in composted material, makes it difficult to determine the efficiency of a treatment process (Bitton 1994; Cooper and Olivieri 1998). The coliform group includes *E. coli* and other faecal (or thermotolerant) coliforms and presence of these more accurately correlates with warm-blooded animal faecal discharges (Geldreich 1978). The density of *E. coli* in faeces is 10^5-10^8 CFU/g (Geldreich 1978; Stenström 1996).

Faecal streptococci, mainly including species of *Enterococcus* and more recently referred to as enterococci (WHO 1993), are present in human faeces at densities of 10^5-10^7 CFU/g (Geldreich 1978; Stenström 1996). They are considered more resistant than coliforms and are able to grow at both 10°C and 45°C, in medium at pH 9.6 and in medium containing 6.5% NaCl (Kenner 1978; WHO 1993; Cooper and Olivieri 1998). Ratios between faecal coliforms and faecal streptococci were previously used for determining sources of recent contamination (Geldreich 1976), but lately the usefulness of these ratios have been questioned due to their different survival characteristics (Lewis-Jones and Winkler 1991; Cooper and Olivieri 1998). Enterococci have been suggested for indicating the presence of viruses, particularly in sludge and seawater (Bitton 1994). Health risks from exposure to recreational waters have been reported to correlate with densities of faecal streptococci (Kay 1994; Prüss 1998) and they are widely used for monitoring such waters (Cooper and Olivieri 1998).

Bacterial spores are extremely resistant and might be too persistent to indicate the presence of pathogens (Berg 1978). Spores from sulphite reducing clostridia (mainly *Clostridium perfringens*) have, however, been used as indicators of past contamination and as tracers to follow the fate of pathogens, e.g. for estimating the inactivation of protozoan cysts and viruses in treatment processes (Payment and Franco 1993; Bitton 1994; Venczel *et al.* 1997). Clostridia are only excreted by 13-35% of individuals in densities of 10^5-10^6 CFU/g (Geldreich 1978; Stenström 1996; Leeming *et al.* 1998a) and can also be derived from other environmental sources (WHO 1993).

Other bacteria that have been suggested as indicators of faecal contamination include the anaerobes *Bacteroides* and *Bifidobacterium* but they are more rapidly inactivated in water than coliforms and methods have not been standardised (Geldreich 1978; WHO 1993; Bitton 1994).

1.6.2 Bacteriophages

Bacteriophages are viruses that infect bacteria, and are thus not harmful for humans. Many enteric viruses are more resistant in the environment than bacteria. Viruses are also smaller than bacteria, which assigns them different transport features. Therefore bacteriophages have been suggested as model organisms to better predict the presence and behaviour (including survival and transport) of human enteric viruses in the environment (Havelaar *et al.* 1991; Baker and Herson 1999). Coliphages naturally occurring in faeces have also been used as general indicators of faecal contamination. Compared to human viruses, bacteriophages are easily quantified. Animals and humans excrete different types of phages, making it possible to distinguish between animal and human contamination (Bitton 1994).

Three groups of bacteriophages have mainly been suggested as models for enteric viruses; somatic coliphages, F-specific bacteriophages and the phages of the anaerobic bacterium *Bacteroides fragilis* (Havelaar *et al.* 1991). These phages can both be used for detection of faecal or sewage contamination and as indicators of treatment efficiency (WHO 1993; Cooper and Olivieri 1998). In Sweden the *Salmonella typhimurium* phage 28B (Lilleengen 1948) has been frequently used in studies of viral behaviour. Phage 28B has not been shown to occur naturally either in environmental samples or in faeces. It has previously been used as a tracer organism to determine the source of faecal contamination, to model groundwater flow and transport of viruses within soil columns and for inactivation studies in liquid composting systems and latrines (faeces) (Eller 1995; Norin *et al.* 1996; Stenström 1996; Johansson *et al.* 1998; Carlander and Westrell 1999; Carlander *et al.* 2000).

1.6.3 Faecal sterols

Faecal sterols is a collective name for the sterols and stanols excreted in faeces. Coprostanol (5β-cholestan-3β-ol) is the principal faecal sterol in human faeces, constituting approximately 40-60% of the total sterol content (Walker *et al.* 1982). It is produced by the hydrogenation of cholesterol (cholest-5-en-3β-ol) by anaerobic bacteria in the digestive tract (Figure 7). The composition of faecal sterols in faeces depends on diet, endogenous synthesising and

biohydrogenation in the digestive tract, but also on age; children excrete less coprostanol than adults (Ponz de Leon *et al.* 1987; Leeming *et al.* 1996). Analysis of faecal sterols, especially coprostanol, has been used as an alternative to indicator bacteria to determine faecal contamination (Hatcher and McGillivary 1979; Walker *et al.* 1982; Vivian 1986; Nichols *et al.* 1996). One advantage is that samples can be analysed after some time of storage. However, rather sophisticated and complex equipment is needed (McCalley *et al.* 1981).

Coprostanol has since the beginning of the 1960s been used as an indicator of faecal contamination (Walker *et al.* 1982). It has been used for tracing contamination in sediment (Hatcher and McGillivary 1979; Chan *et al.* 1998; Edwards *et al.* 1998) and for distinguishing animal and human sources of contamination (Venkatesan and Santiago 1989; Leeming *et al.* 1996). Coprostanol is however degraded rather quickly (90-95% degradation in 1-2 weeks at 20°C) in sewage sludge and in seawater by bacteria present in sewage and natural waters as well as in soil (Switzer-Howse and Dutka 1978; Walker *et al.* 1982; Bartlett 1987; Leeming 1996). According to Canule and Martens (1996) biomarkers are only semi-quantitative indicators of organic matter in aquatic environments if decay rates are not known. In some studies concentrations of coprostanol were related to indicator bacteria, whereas in others they were not, probably due to variability in inactivation of indicator bacteria and the degradation of coprostanol that might be occurring at a different rate (Walker *et al.* 1982; Düreth *et al.* 1986; Vivian 1986; Nichols *et al.* 1993). Coprostanol was found to be stable in source-separated urine, and suggested as an indicator of faecal contamination of urine (Sundin *et al.* 1999).

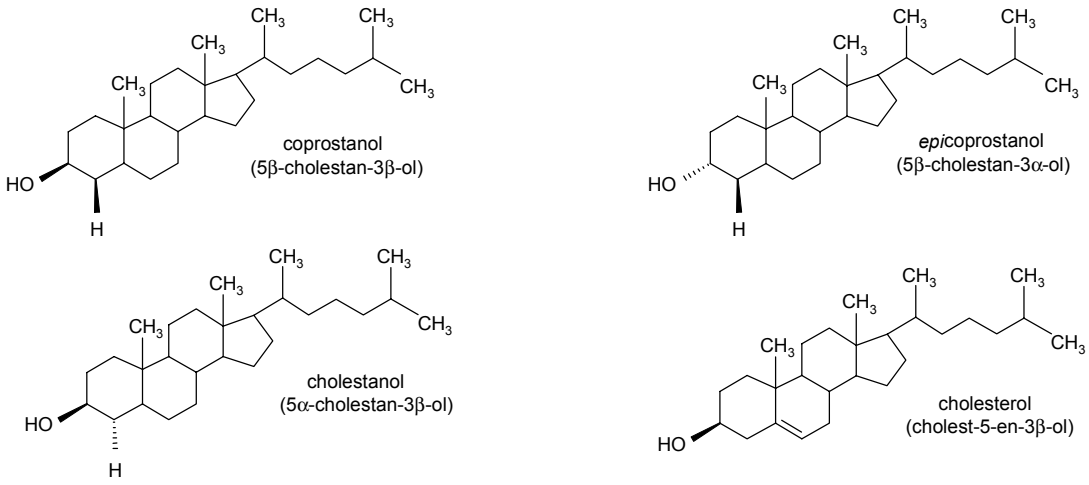


Figure 7. Faecal sterols quantified to determine human faecal contamination.

The measurement of coprostanol alone is considered to be a presumptive valid indicator of faecal contamination, but by including the measurement of other faecal sterols a unique fingerprint of human faecal contamination can be obtained (Grimalt *et al.* 1990; Leming *et al.* 1998a). In humans, intestinal microorganisms preferentially hydrogenate cholesterol to the 5β-cholestan-3β-ol (i.e. coprostanol) (Parmentier and Eyssen 1974), and little if any of the 5β-cholestan-3α-ol (*epi*coprostanol) or 5α-cholestan-3β-ol (cholestanol) is produced *in vivo*

(Figure 7; McCalley *et al.* 1981). In contrast, in microbial populations outside the mammalian gut, coprostanol and *epi*coprostanol are minor products compared to the thermodynamically more stable cholestanol. These interrelationships were first used in environmental studies by Grimalt *et al.* (1990), and later used by Leeming *et al.* (1998b) to conclude that the coprostanol in stormwater was of faecal origin rather than produced via *in situ* hydrogenation of cholesterol present in anaerobic sediments and subsequently re-mobilised. Furthermore, animals such as dogs and birds either do not have coprostanol in their faeces or it is present in trace amounts relative to other sterols (Leeming *et al.* 1996). Herbivores excrete coprostanol but the dominating faecal sterol is 24-ethyl-5β-cholestan-3β-ol (24-ethylcoprostanol) (Leeming *et al.* 1996). The contamination can still be quantified by the concentration of coprostanol once the source has been determined (Leeming *et al.* 1998a).

Analysis of sterols involves organic extraction. Chloroform has traditionally been used as a solvent (Bligh and Dyer 1959; Leeming *et al.* 1996). In Sweden a method using methyl-tert-buthyl-ether (MTBE) was developed with the specific application to urine (Börjesson *et al.* 1998). Thereafter saponification, heptane extraction and derivatisation of the sterols with a silating agent are conducted (Börjesson *et al.* 1998). The final analysis is performed using gas chromatography (GC).

Neither coprostanol nor a single indicator bacteria alone is probably sufficient to discern faecal contamination (Leeming *et al.* 1998b). The combined use of several faecal sterols and bacterial indicators, however, offers a new way to estimate sources of faecal contamination that may be appropriate for many aquatic systems and parts of wastewater systems, e.g. coastal waters and stormwater (Leeming and Nichols 1998; Leeming *et al.* 1998b).

1.7 Survival of microorganisms in the environment

From the time of excretion, the concentration of enteric pathogens usually declines by the death or loss of infectivity of a proportion of the organisms. Protozoa and viruses are unable to grow in the environment, thus numbers will always decrease, whereas bacteria may multiply under favourable environmental conditions. The ability of a microorganism to survive is defined as its persistence (Feachem *et al.* 1983). The persistence of microorganisms in the environment is a field that has been widely investigated. Often the persistence has been related to various features of the environment like pH or temperature (e.g. Hurst and Goyke 1986; Fayer and Nerad 1996) or to the environment itself, e.g. water or soil (e.g. Raphael *et al.* 1985; Sobsey *et al.* 1986).

Bacterial inactivation as well as viral inactivation has often been reported to follow first-order kinetics (Mitchell and Chamberlain 1978; Cramer *et al.* 1983; Gray *et al.* 1993; Deng and Cliver 1995). Initial increases or a plateau before the onset of inactivation have been noted as well as a fast inactivation of the major part of the microbial population with a few organisms persisting for longer periods (Mitchell and Chamberlain 1978; Burge *et al.* 1981; Feachem *et al.* 1983). Much of the literature has focused on the total inactivation of microorganisms

(Feachem *et al.* 1983; Strauch 1991) but the inactivation curve or e.g. T_{90}-values (time for a 90% inactivation of organisms) are needed to predict the health risks from human excreta through different transmission routes.

1.7.1 Physiochemical and biological factors that may affect the survival in excreta and reuse systems

The elapse of time is the overall feature affecting survival of microorganisms in the environment. Several physiochemical and biological factors will have an impact but it is difficult to draw any general conclusions regarding their effect since it varies depending on the microorganism. Furthermore, the factors all act together, yielding different survival conditions at any particular location. Factors that are especially recognised as having an effect on enteric microorganisms include:

Temperature. Most microorganisms survive well at low temperatures (5°C) and rapidly die at high temperatures (>40°C) (Feachem *et al.* 1983). This is the case for various types of media including water, soil, sewage and crops (Feachem *et al.* 1983). However, to ensure inactivation in e.g. composting processes, temperatures around 55-65°C are needed to kill all types of pathogens (except bacterial spores) within hours (Burge *et al.* 1981; Haug 1993; Eller 1995; Norin *et al.* 1996). The hardiest organisms in this respect include cysts of *Entamoebae histolytica*, *Ascaris* eggs and *Mycobacterium tuberculosis* (Haug 1983). Viruses, such as bovine parvovirus and *Salmonella typhimurium* phage 28B (Section 1.6.2) are also considered to be heat resistant (Eller 1995; Norin *et al.* 1996). Temperature is often considered to be the predominant factor determining viral inactivation (Yates *et al.* 1985; Snowdon *et al.* 1989a; Deng and Cliver 1995). Temperature effects might especially be of concern in temperate regions where the temperatures are quite low during a large part of the year. Freezing is considered to inactivate bacteria and protozoa to some extent whereas viruses are unaffected (Gerba 1996).

pH. Many microorganisms are generally adapted to a neutral pH (7) even though enteric pathogens need to withstand the acidic conditions in the stomach to cause an infection (Thea and Keusch 1989). Highly acidic or highly alkaline conditions will have an inactivating effect on most microorganisms by the hydrolysation of cell components or denaturation of enzymes (Atlas and Bartha 1998). Bacterial survival is shorter in acid soils (pH 3-5) than in alkaline soils (Feachem *et al.* 1983). However, most enteric viruses are resistant to inactivation at both low (3.5) and high (10.0) pH (Snowdon *et al.* 1989a; Gerba *et al.* 1996). pH affects the adsorption of viruses to particles, which in turn affects survival (Yates and Gerba 1998). Addition of lime to excreta in dry latrines and to sewage sludge increases pH to around 12 and will inactivate microorganisms (Lewis-Jones and Winkler 1991). Robertson *et al.* (1992) suggested that both low (1.5) and high (10.5) pH have a significant impact on oocyst viability.

Ammonia. In natural environments ammonia (NH_3) produced by bacterial populations may be deleterious to other populations (Atlas and Bartha 1998). Inactivation by ammonia is however mainly related to human excreta and sewage. Ammonia generated at high pHs may act as an

inactivating agent for viruses (Ward and Ashley 1977; Cramer *et al.* 1983; Pesaro *et al.* 1995). Inactivation mechanisms suggested include protein denaturation and cleavage of nucleic acids (Ward 1978; Cramer *et al.* 1983). Ammonia has also been demonstrated to affect *Cryptosporidium* oocysts (Jenkins *et al.* 1998). The mechanism suggested by Jenkins *et al.* (1998) is that ammonia, which is uncharged and non-polar, can penetrate the oocyst and raise the internal pH to deleterious levels, whereas H^+ and OH^- are unable to inactivate oocysts. The viability of *Ascaris* eggs was shown to be reduced in ammonia-treated sewage sludge (Ghigletti *et al.* 1997).

Moisture. Moisture content is mainly applicable to the survival in soil and in faeces. A moist soil favours the survival of microorganisms (Yates and Gerba 1998) and drying may be used as a process to sanitise excreta in dry latrines (Esrey *et al.* 1998). Virus survival is prolonged under moist conditions (Lewis-Jones and Winkler 1991). Protozoan cysts are highly sensitive to desiccation which may also affect their survival on plant surfaces (Snowdon *et al.* 1989a; Yates and Gerba 1998). For *Ascaris* eggs to be inactivated moisture levels below 5% is needed (Feachem *et al.* 1983).

Solar radiation and UV-light. The inactivating effect of solar radiation and UV-light has been long known (Mitchell and Chamberlin 1978; Bitton 1994). Disinfection by UV-radiation is used for the treatment of both drinking water and wastewater (Westrell 1998; Kolch 2000; Sommer *et al.* 2000). Microbial inactivation is caused by inducing damage to nucleic acids and is proportional to the UV-dose (Ws/m^2) (Bitton 1994; Yates and Gerba 1998). The effect of solar radiation is important in the environment, e.g. in marine waters (Sinton *et al.* 1999). Out in the field there is a shorter survival time on the soil and crop surface where sunlight can affect the organisms (Yates and Gerba 1998).

Presence of other microorganisms. The survival of microorganisms is often longer in material that has been sterilized than in an environmental sample containing other organisms, although contradicting results have also been reported for both soil and water (Snowdon *et al.* 1989a; Lewis-Jones and Winkler 1991; Deng and Cliver 1992; Yates and Gerba 1998). Bacteria affect the survival of viruses since they may produce metabolites that adversely affect virus particles or may use the virus capsid as a nutrient source (Snowdon *et al.* 1989a; Lewis-Jones and Winkler 1991). Protozoa are known predators of bacteria (Habte and Alexander 1975; Mallory *et al.* 1983).

Nutrients. If nutrition is available and other conditions are favourable bacteria may grow in the environment (Feachem *et al.* 1983). Nutrient deficiencies thus only affect bacteria. Enteric bacteria adapted to the GI tract are not always capable of competing with indigenous bacteria for the scarce nutrients available and the ability to reproduce and even survive in the environment therefore tends to be limited (Yates and Gerba 1998).

Ionic strength. High salt concentrations affect the osmotic pressure and denaturate proteins in microorganisms (Atlas and Bartha 1999). Faecal streptococci were reported to have a greater

tolerance than faecal coliforms to salinity in seawater (Sinton *et al.* 1994). Early studies showed no increase in virus inactivation in waters with high salt concentrations (Katzenelson 1978), whereas Yates and Gerba (1998) reported that virus inactivation may be prevented or increased depending on type of salt and concentration.

Microbial activity is strongly dependent on oxygen availability (Lewis-Jones and Winkler 1991; Atlas and Bartha 1998). This activity might in turn have an effect on virus inactivation and according to Snowdon *et al.* (1989a) viruses are inactivated more rapidly in aerated systems. Faecal coliform bacteria have been reported to be both affected and unaffected by dissolved oxygen in wastewater stabilisation ponds (Feachem *et al.* 1983; Almasi and Pescod 2000). The free oxygen in a soil environment largely determines the bacterial metabolism that can occur and thus the survival of bacteria (Atlas and Bartha 1998). In soil the particle size and permeability will also have an impact on microbial survival, and in soil as well as in sewage and water environments various organic and inorganic chemical compounds may affect the microorganisms (Yaeger and O'Brien 1979; Yates *et al.* 1985; Lewis-Jones and Winkler 1991; Gerba 1996). Sedimentation may be responsible for a significant fraction of observed inactivation (Mitchell and Chamberlin 1978; Carlander *et al.* 2000). An apparent reduction of microorganisms may also be caused by adsorption to particles and by aggregation of microorganisms and materials (Mitchell and Chamberlin 1978; Burge *et al.* 1981).

1.7.2 Relative persistence of enteric microorganisms

It is difficult to draw general conclusions regarding the relative persistence of microorganisms. The persistence of an organism depends on e.g. strain or type, climatic factors and differences between laboratory conditions and a specific environment investigated *in situ*. For chemical disinfectants the general order of persistence protozoan cysts > spore-forming bacteria > enteric viruses > vegetative bacteria was reported (Bitton 1994). Lewis-Jones and Winkler (1991) state that bacteria are generally more easily inactivated than helminth eggs or protozoan cysts and according to Crook (1998) viruses are generally more resistant than bacteria to environmental stresses whereas parasitic cysts survive longer than both bacteria and viruses in the environment. The bacterial spores, however, are the most resistant organisms known.

Gram-positive bacteria are generally more persistent than Gram-negative under environmental pressures, e.g. desiccation and salinity, due to differences in their cell wall construction (Sinton *et al.* 1994; Madigan *et al.* 1997). Bacterial indicators have been questioned since they are often considered less persistent than non-bacterial pathogens (Nichols *et al.* 1993; Crook 1998; Haas *et al.* 1999).

Parasites resistant to a wide range of environmental conditions include eggs of helminths and the cysts of some protozoa (Snowdon *et al.* 1989a). These parasites, which have stages in their life cycle which are adapted to long survival in the environment outside the GI tract, are

particularly considered to be potentially transmitted through the application of waste products to land (Snowdon *et al.* 1989a; Cooper and Olivieri 1998).

The oocysts of *Cryptosporidium parvum* are thick-walled and it is assumed that their resistance to various environmental pressures as well as to disinfectants is related to the metabolic dormancy and impermeable wall of the oocyst (Jenkins *et al.* 1997). *Giardia* cysts are considered to be less resistant than *Cryptosporidium* oocysts, which has been supported by the shorter survival in water, soil and cattle faeces (Olson *et al.* 1999).

Several studies have compared the survival of bacteriophages and enteric viruses (e.g. Cramer *et al.* 1983; Yates *et al.* 1985; Pesaro *et al.* 1995). Sometimes the phages were more persistent than the human viruses, whereas in other instances they were not, making it difficult to assign a general indicator for viral persistence useful in various environments. The *Salmonella typhimurium* phage 28B was, for example, found to be more resistant to high temperatures than bovine parvovirus (Eller 1995). Viruses have a wide range of properties and will thus have a wide range in their persistence (Lewis-Jones and Winkler 1991). Hepatitis A virus has been reported to have a greater resistance than other viruses to temperature, UV-radiation and low pH (Yates and Gerba 1998).

According to Wheeler and Carroll (1989) helminth ova and possibly some enteric viruses are the hardiest of the pathogens related to reuse of faecal waste. *Ascaris* eggs are amongst the most resistant of all helminth ova due to their multilayered structure of chitin and lipid and they have been recovered from soil after several years (Snowdon *et al.* 1989a; Lewis-Jones and Winkler 1991). They have along with Taenia been used as an indicator for hygienic quality in the WHO guidelines for the reuse of wastewater (WHO 1989; Blumenthal *et al.* 2000).

1.8 Quantification of microorganisms

1.8.1 Bacteria

Cultivation on agar media is the traditional and still the most extensively used method of enumerating bacteria (Mason *et al.* 1986; Wang and Doyle 1998). Colony-forming units (CFU) are counted under the assumption that each colony originates from one bacterial cell. However, it is known that only a small fraction of the total bacteria present in an environmental sample are able to grow on an agar surface; 0.1-10% according to a review by Mason *et al.* (1986). Direct counts of total cell numbers are mostly carried out by epifluorescence microscopy (Mason *et al.* 1986; Kepner and Pratt 1994). Fluorescent compounds that bind to specific parts of the cells, e.g. to nucleic acids, enables the differentiation of viable and dead bacteria (Boulos *et al.* 1999). Measurements of the metabolic activity e.g. by the rate of oxygen consumption or metabolisation of substrates allow for quantification on a statistical level (Pepper *et al.* 1995).

Faecal indicator bacteria are generally enumerated by cultivation or by less exact MPN (most probable number) methods (ISO 1990; Pepper *et al.* 1995). For survival studies cultivation methods are generally used (e.g. Rose *et al.* 1996; Sinton *et al.* 1999) since a specific bacterial species rather than a total population is to be enumerated. If directly cultivated from excreted faeces, enteric bacteria will probably be in a healthy condition, whereas after some time in a different environment they can be affected by various stress factors (Troussellier *et al.* 1998). Taking the viable but non-culturable bacteria (VBNC, Oliver 1993) into consideration could possibly yield slower inactivation rates but would require significantly more detailed investigations.

1.8.2 Protozoa – Cryptosporidium oocysts

A range of methods has been suggested to assess the viability of *Cryptosporidium* oocysts including excystation (Robertson *et al.* 1993), dye exclusion (Campbell *et al.* 1992), fluorescence *in situ* hybridisation (FISH) (Vesey *et al.* 1998) and cell culturing (Slifko *et al.* 1997). Correlations between assessment methods and true infectivity have been debated (Fayer 1995; Black *et al.* 1996; Jenkins *et al.* 1997; Bukhari *et al.* 2000). Infectivity studies in mouse models are probably the most reliable measure even though they involve a great deal of variability (Bukhari *et al.* 2000). Yet such studies are very expensive and time consuming, and in most studies alternative methods are used.

Excystation measures the ability of the oocysts to release their sporozoites (Robertson *et al.* 1993). Inclusion or exclusion of vital dyes measures the integrity of the sporozoite's cell membrane, and generally 4´-6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) are used to assess viability of *Cryptosporidium parvum* (Campbell *et al.* 1992). More recently dyes that bind to nucleic acids has been developed, e.g. SYTO-9 and SYTO-59 (Belosevic *et al.* 1997) and FISH-techniques (Vesey *et al.* 1998), but have not yet been used as widely as DAPI/PI and excystation. Contradicting results on the correlations between different methods have been reported (e.g. Bukhari *et al.* 2000; Neumann *et al.* 2000). A drawback for all quantitative microscopic procedures is the limited number of organisms that can be counted due to time constraints (Sinton *et al.* 1999), with the result that only a 99% reduction can be determined in survival and disinfection studies (Finch *et al.* 1997; Slifko *et al.* 1997; Paper II). Still, these methods have been widely used in survival studies (Medema *et al.* 1997; Merry *et al.* 1997; Jenkins *et al.* 1998). In cell cultures infectivity rather than viability can be measured and a higher reduction may be possible to detect (Slifko *et al.* 1997).

1.8.3 Viruses

There are several approaches to virus detection and enumeration (Bitton 1994). Animal inoculation was the traditional method for detecting viruses prior to tissue cultures. Tissue cultures can also be used for quantification of viruses. Many viruses infect host cells and display a cytopathic effect (destruction of individual cells) while others need to be confirmed by further tests, including immunological procedures, monoclonal antibodies, or nucleic acid probes (Bitton 1994). Infected host cells are either directly counted or less exact methods such as $TCID_{50}$ (the dilution that will infect 50% of the tissue culture units challenged) and most

probable number (MPN) can be determined (Block and Schwartzbrod 1989; Bitton 1994). The daily variability in the sensitivity of viral assay techniques can contribute a substantial amount of variation to the data (Snowdon *et al.* 1989b; Hurst 1991). There are still some viruses which cannot be detected by tissue cultures (e.g. Norwalk and Norwalk-like viruses) (Estes *et al.* 2000).

Bacteriophages are generally simpler to analyse than human viruses, which is one reason for their indicator status. Plaque assays using soft or overlay agar along with the bacterial host are the general method used for bacteriophages (Pepper *et al.* 1995). Virus replication within the bacterial host cell will lead to localised areas of cell destruction (plaques) that can be counted and expressed as plaque-forming units (PFU).

1.9 Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) is a tool used to predict the consequences of potential or actual exposure to infectious microorganisms (Haas *et al.* 1999). The methodology is based on the chemical risk assessment concept for which the National Academy of Sciences published recommended definitions and main principles (National Research Council 1983). Microbial risk assessments were first developed for drinking waters (Regli *et al.* 1991) and have later been applied to practices such as irrigation of crops and discharge to recreational impoundments (Ashbolt *et al.* 1997; Shuval *et al.* 1997; Tanaka *et al.* 1998).

Like epidemiological studies, microbial risk assessments can be used for prevention of disease (Beaglehole *et al.* 1993; Yates and Gerba 1998). By knowing the magnitude of risk, preventive measures can be taken through risk management. Blumenthal *et al.* (2000) recently suggested the new World Health Organization (WHO) guidelines for the reuse of wastewater to be based partly on epidemiological investigations and partly on microbial risk assessments. According to Haas *et al.* (1999) direct measurement of pathogens in combination with QMRA can be used to develop guidelines for food, water and other vehicles. For these applications QMRA is an alternative or complement to indicator microorganisms (Haas *et al.* 1999; Blumenthal *et al.* 2000). An advantage of QMRA is that it allows prospective studies rather than retrospective which epidemiological studies are (Haas *et al.* 1999; Blumenthal *et al.* 2000). The validity of epidemiological studies lies in their focus on actual health effects. However, waterborne outbreaks and reported cases are often underestimated (Mead *et al.* 1999; Barwick *et al.* 2000) and QMRA may be a way to circumvent this (Haas *et al.* 1999).

1.9.1 The method

Risk assessment is a part of risk analysis that also includes risk management and risk communication. The definitions of the terms are (National Research Council 1983; Haas *et al.* 1999):

Risk assessment – the qualitative or quantitative characterisation and estimation of potential adverse health effects associated with exposure of individuals to hazards (materials or situations, physical, chemical and/or microbial agents).

Risk management – the process for controlling risks; weighing policy alternatives and selecting the most appropriate action taking into account risk assessment, values, engineering, economics and legal, social and political issues.

Risk communication – the communication of risks to managers, stakeholders, public officials and the public; includes public perception and ability to exchange scientific information.

The same framework as has previously been used for chemicals can be applied to microorganisms, even though they have many different features. Microbial risks are often related to one occasion of exposure, corresponding to an acutely high dose of a chemical. The repeated long-term exposures to low doses are not applicable to microorganisms. The outcome of the exposure is dependent on the virulence of the pathogen and on the individual's response. If the exposure causes an asymptomatic infection, illness or mortality is dependent on age, immunity, nutritional status etc. Furthermore, secondary transmission of disease needs to be included in microbial risk assessments to obtain the total effect on public health (Craun *et al.* 1996; Haas *et al.* 1999). The risk assessment provides the scientific process and involves the following steps (Rodier and Zeeman 1994; Haas *et al.* 1999):

Hazard identification – identifies pathogens and routes of transmission, e.g. in a specific wastewater system, and describes acute and chronic health effects of the agents.

Dose-response assessment – characterises the relationship between doses and incidences of adverse health effects in exposed populations.

Exposure assessment – measures or estimates the route, amount, frequency and duration of human exposures to agents.

Risk characterisation – integrates the information from exposure, dose-response and health hazard steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty.

QMRA thus starts by a problem formulation where all the transmission routes and pathogens of interest are identified. It then assesses the dose of a certain pathogen to which an individual may be exposed and uses this dose in a dose-response model to calculate the probability of infection. Risks are finally characterised by taking into consideration the frequency of the exposure events for the range of pathogens studied, to estimate a total risk.

Dose-response experiments for bacteria, protozoa and viruses have been conducted with healthy volunteers where they are fed known doses of a pathogen (e.g. Ward *et al.* 1986;

DuPont *et al.* 1995). The resulting infected and unaffected individuals were used to create dose-response models (Figure 8). Haas (1983) first demonstrated that the exponential (Equation 1) and Beta-Poisson (Equation 2) dose-response models could describe the probability of infection (P_{inf}) in humans from waterborne pathogens (Teunis *et al.* 1996; Haas *et al.* 1999). These types of studies are difficult to perform and are thus not available for all pathogens of interest. The dose-response models challenge the concept from epidemiological studies that a threshold dose is required to cause infection. Thus, a single pathogen can cause infection, although the probability of this is very low (Teunis *et al.* 1996).

$$P_{inf} = 1 - e^{-rD} \qquad [1]$$

where D = dose; r = the probability of infection for any organism;
median infectious dose, $N_{50} = \ln(2)/r$

$$P_{inf} = 1 - (1 + (D/\beta))^{-\alpha} \qquad [2]$$

where D = dose; α and β = parameters describing the probability (Beta) distribution of r;
median infectious dose, $N_{50} = \beta\,(2^{1/\alpha} - 1)$

Exposure assumptions involve the volume ingested, e.g. from drinking water or from swallowing water while swimming, exposure frequency and the duration of exposure, e.g. how many hours of swimming during how many days of the year. US EPA set some standard values including 2 l of water consumed 365 days per year for an adult (Regli *et al.* 1991). In the risk characterisation all these assumptions are included and the risk is expressed e.g. as infections per year and individual, or in a population.

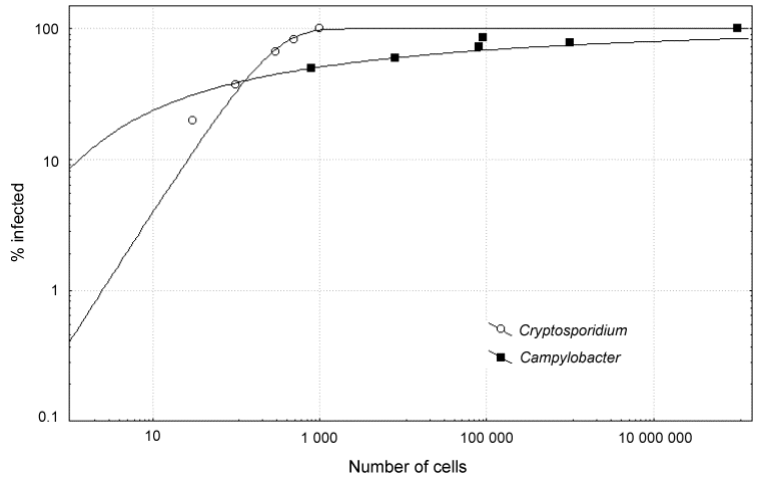


Figure 8. Examples of dose-response models for *Cryptosporidium* and *Campylobacter* (modified from Teunis pers. comm.). A specific number of cells ingested (dose) results in a certain percentage of the volunteers being infected (response).

The information is often incomplete, hence the assumptions used in the terminology. To account for some of the variability, probability density functions (PDFs) are often used rather than point estimates or constant values (Eisenberg *et al.* 1996; Teunis *et al.* 1997; Haas *et al.* 1999). Monte Carlo simulations are then used to sample the PDFs in risk calculations. The assumptions also call for uncertainty and variability analysis.

Blumenthal *et al.* (2000) consider risk assessments using point estimates to be valuable in determining the magnitude of risk but stress the need for more thorough risk assessments as performed for e.g. water (Eisenberg *et al.* 1996), where secondary transmission and immune status are included and the risk is characterised at the population level. Ashbolt (1999) on the other hand discusses screening-level microbial risk assessments as an initial tool to estimate risks when all data needed are not available. Such QMRAs can also be valuable to identify where further data collection is needed. Haas *et al.* (1999) also points out that even though assessments are made using assumptions and results in quantification with a large range of variation and uncertainty, the method is useful for ranking risks, comparing different environmental problems and proposing different solutions. Currently risk assessments are often requested and, depending on the purpose and resources, they will probably continue to result in varying degrees of complexity.

1.9.2 Acceptable risk

The acceptable risk is a key point in risk management. If a risk is considered to be too high preventive measures can be taken to decrease the risk to the acceptable level or the practice may not be approved, e.g. the reuse of wastewater or human urine in agriculture. Acceptable risks can also be used to design treatment processes (Regli *et al.* 1991; Haas *et al.* 1999). In Sweden there have not yet been discussions on acceptable risks related to infectious microorganisms. The US EPA, however, has proposed a level of 1:10 000 per year for the consumption of drinking water (Regli *et al.* 1991). This limit has been debated and Haas (1996) argued that it should be lowered to 1:1 000 per year. The proposal of an acceptable risk limit needs to involve representatives from various parts of society and the proposal may vary depending on the present health status of the population concerned (Blumenthal *et al.* 2000).

2. OBJECTIVES AND SCOPE OF THE PRESENT INVESTIGATIONS

The overall aim of this thesis was to investigate and evaluate health risks from infectious diseases related to urine-separating wastewater systems and the reuse of human urine in agriculture.

The specific objectives of the present investigations were:

- to determine the faecal contamination that occurs in urine-separating toilets;
- to determine the inactivation rates of different groups of microorganisms in source-separated human urine and relate the inactivation to some of the characteristics of the urine mixture;
- to quantify microbial health risks in urine-separating wastewater systems by using Quantitative Microbial Risk Assessment (QMRA).

Field samples were collected from several urine collection tanks (Section 3.2). Faecal contamination was first studied using traditional methods, i.e. by analysis of various groups of indicator bacteria (**Paper I**; Section 3.3). As a complement to the indicator bacteria coprostanol was quantified in urine mixture samples (**Paper I**; Section 3.4). To further refine the quantification of faecal contamination a range of faecal sterols were analysed and ratios between them were used to determine if the coprostanol was of faecal origin (**Paper IV**; Section 3.5). In **Paper I** the inactivation of different bacteria in urine mixtures was studied and related to the effect of temperature, pH and dilution (Section 4.2). *Cryptosporidium parvum* was studied to estimate the inactivation rates of protozoa at various pH-levels in urine mixtures at low temperature (**Paper II**; Section 4.3). Rotavirus and a bacteriophage were chosen as models to estimate viral inactivation at two different temperatures (**Paper III**; Section 4.4). In **Paper V** previous results were summarised using microbial risk assessment methods to estimate quantitative risks from the exposure of pathogens in urine mixtures (Sections 5.1-5.3).

3. FAECAL CONTAMINATION (I, IV)

3.1 Faecal contamination constitutes the risk in urine-separating systems

From literature studies it was concluded that pathogens excreted in urine constitute a minor risk in relation to the reuse of human urine (Section 1.5.2). Only a few pathogens, mainly found in tropical areas, are known to use urine as a significant route for transmission (Papers I and IV). Environmental transmission of urinary excreted pathogens is therefore of limited concern in temperate climates, but any faecal cross-contamination that may occur by misplacement of faeces in the urine-separating toilet is regarded as a possible health risk (Papers I and IV). To estimate the risk of pathogen transmission during handling,

transportation and reuse of source-separated urine, the amount of faecal material contaminating the urine fraction was determined by analysing various indicators in the urine mixture, i.e. the collected urine and flushwater (Papers I and IV).

3.2 Sampled urine-separating systems

Urine mixtures from twelve different separating wastewater systems were analysed. In two of the systems there was more than one collection tank connected to the system, and in total fifteen tanks were sampled. Two of the areas, Understenshöjden (~160 inhabitants) and Åkesta (~100 inhabitants, two tanks sampled), are eco-villages where the inhabitants have chosen the system themselves. In Understenshöjden the faeces are treated locally together with the greywater, whereas in Åkesta the faeces are collected dry and later used as a soil conditioner in the housing area. Åkesta was the first eco-village implementing urine-separation and at start this was done by using two separate toilets, one for urine and one for faeces. At the time of sampling some of these had been replaced by a urine-separating toilet. A further large housing area, Palsternackan (~160 inhabitants, three tanks sampled), was investigated. In Öster Färnebo urine was collected from a residential house for boarding school students. Stensund is also a boarding school for adults where two separating toilets are placed in a greenhouse building that has many visitors. Weleda is a company where urine is collected from the office building. Urine from six collection tanks connected to individual households was also analysed. All tanks were placed underground and samples were collected from the upper part of the liquid (referred to as urine samples) and from the bottom, where a sludge layer had formed (referred to as sludge samples), using a syringe fastened to a metal pole. Samples were collected one to three times from each tank and the number of samples included in the investigations (Sections 3.3-3.5) was dependent on the time of the investigation.

3.3 Analysis of indicator bacteria to determine faecal contamination

The concentrations of different groups of indicator bacteria were determined in samples from urine collection tanks as a first step towards investigating the faecal contamination of source-separated urine (Paper I). Standard methods used for water quality analysis were used to determine colony-forming units per ml of urine mixture (CFU/ml).

Total coliforms were found in varying concentrations in the collection tanks with a mean of 260 CFU/ml (median 21 CFU/ml). *E. coli* was seldom found; in 84% of the samples the concentration was below the applied detection limit of 10 CFU/ml. Clostridia spores were also found in varying concentrations, ranging from <1 CFU/ml to 2 000 CFU/ml. Faecal streptococci occurred in high and varying concentrations with 76% of the samples having concentrations above 1 000 CFU/ml and 16% >100 000 CFU/ml. The concentrations in the sludge samples were generally higher than in the urine samples from the upper part of the tanks. In Figure 9 the median and maximum concentrations for the different indicator bacteria in urine and sludge samples are compared. The results from the different sampling rounds

gave comparable results, indicating unit specific variation. Further sampling of the urine collection tanks resulted in similar results, with *E. coli* absent in a majority of samples and with high concentrations of faecal streptococci (Paper IV).

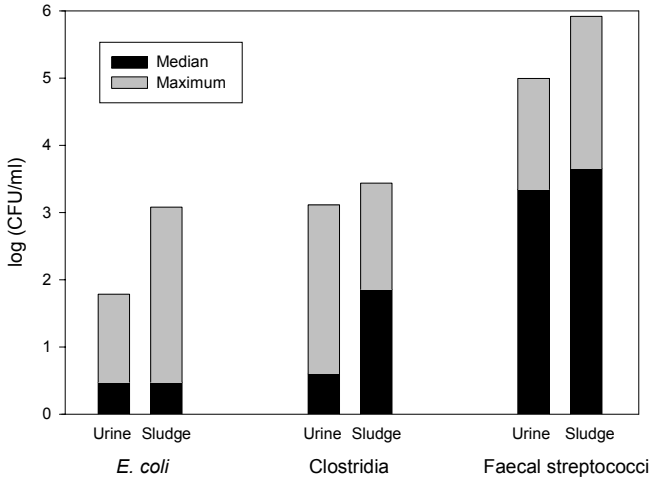


Figure 9. Median and maximum concentrations of *E. coli*, clostridia and faecal streptococci in urine and sludge samples. The median values for *E. coli* were below the detection limit (<10 CFU/ml).

The results implied that indicator organisms normally used in water quality analysis are not suitable for this type of sample. The value of total coliforms as a faecal indicator is small since the bacteria may emanate from sources other than faecal contamination, e.g. from the technical system itself. *E. coli* was seldom found, which was later explained by its short survival in urine, making it unsuitable as an indicator for faecal contamination (Paper I; Section 4.2). Clostridia spores were persistent but are only excreted by 13-35% of the population (Figure 9; Section 1.6.1) and are therefore not suitable for quantification of faecal contamination. In some samples the concentration of faecal streptococci corresponded to 100% faeces (Section 1.6.1), a degree of contamination that would be impossible and not indicated by *E. coli* or clostridia. The high concentration of faecal streptococci in the collection tanks may be explained by a growth within the system or in the biofilm in the pipes. Faecal streptococci are known to be able to grow at a pH of 9.6 and at high salt concentrations (Section 1.6.1), and may thus be favoured by the conditions prevailing, especially regarding pH (Section 4.1). This assumption was also supported by the results from the biochemical phenotyping, where the respective toilets showed a high homogeneity between strains and where few isolates from the faecal part of the wastewater system were retained in the collection tanks (Paper I). Several strains were found to grow in fresh urine (Nordling 1998) and high counts of faecal streptococci were found in sludge samples from urine pipes (Vinnerås *et al.* submitted). However, inactivation of a mixed population occurred in urine mixture (Section 4.2).

3.4 Faecal contamination based on the analysis of coprostanol

As an alternative to indicator bacteria the concentration of coprostanol in urine mixture was analysed (Paper I). The samples were extracted with MTBE and finally analysed by GC (Section 1.6.3; Börjesson *et al.* 1998). The amount of coprostanol in the samples was used to calculate the concentration of faecal material in the collected urine mixture using the reference value 5.6 μg coprostanol per mg of faeces (Table 2; Ferezou *et al.* 1978).

Table 2. Frequency of faecal contamination levels in urine samples and sludge samples based on the analysis of coprostanol (Ferezou *et al.* 1978)

Faecal contamination	Unit	Frequency
Urine samples		
nd-0.20	ppm (mg/l urine mixture)	41% (12 samples)
0.21-0.50	”	21% (6)
0.51-1.0	”	7% (2)
1.1-5.0	”	14% (4)
5.1-10.0	”	7% (2)
>10	”	10% (3)
Sludge samples		
nd-5	ppm (mg/kg sludge wet weight)	46% (11 samples)
6-10	”	4% (1)
11-50	”	17% (4)
51-100	”	4% (1)
>100	”	29% (7)

The two highest values recorded in the urine samples were 10.6 ppm and 13.3 ppm and these were both found in collection tanks containing only small amounts of urine mixture. The two highest values recorded in the sludge samples were 355.5 ppm and 417.5 ppm.

Overall, the concentration of coprostanol was higher in the sludge samples than in the corresponding urine samples. This was in general consistent with the bacteriological analyses (Paper I; Section 3.3), but within samples there was no correlation between levels of coprostanol and amounts of bacteria.

3.5 Faecal contamination based on the analysis of a range of faecal sterols

The potential for small amounts of coprostanol to be formed in sludges and biofilms within the system by metabolisation of cholesterol by non-intestinal bacteria means that the measurement of coprostanol alone might infer contamination in systems that are not contaminated. To eliminate this possible source of error, concentrations and ratios of both the precursor (i.e. cholesterol) and all its hydrogenation stanol products were determined, thereby obtaining a sterol profile. By re-evaluation of samples previously analysed (Paper I; Section

3.4) and the addition of new samples, a range of faecal sterols was used to determine faecal contamination of source-separated urine.

Two methods that mainly differed in the organic solvents used for extraction were used (Section 1.6.3; Leeming *et al.* 1996; Börjesson *et al.* 1998). The difference in measurement of coprostanol and cholesterol by the two methods was less than 1.5% and the coefficients of variation were less than 6% for replicates (Börjesson *et al.* 1998).

Urine and sludge samples that contained faecal matter from cross-contamination were identified on the basis of the set of ratio criteria shown in Table 3. The ratios were derived from concentrations of coprostanol, cholestanol, *epi*coprostanol and cholesterol measured in the samples. The criteria values were partly defined from previous studies (Leeming *et al.* 1998a; Leeming and Nichols 1998). For samples to be identified as being faecally cross-contaminated, they needed to fulfil all the criteria in Group A (Table 3). On the other hand, if only one of the criteria set out for Group B was satisfied, then the sample was considered to be uncontaminated. Group C contained samples with sterol profiles that were difficult to confidently assign to either Group A or B.

Table 3. Criteria used to determine whether samples were cross-contaminated by faeces

	Group	**A** contaminated	**B** uncontaminated	**C** indeterminate
Criteria	Ratios	All criteria must be fulfilled	Either criteria fulfilled	If Group B criteria not fulfilled, and not all Group A criteria fulfilled, then sample is designated indeterminate and may require further investigation.
1	coprostanol/cholestanol	≥ 0.5	≤ 0.3	
2	*epi*coprostanol/coprostanol	≤ 0.4	≥ 0.5	
3	coprostanol/cholesterol	≥ 0.1	≤ 0.05	

Eight out of 36 (22%) urine samples were assigned to Group A indicating that episodes of faecal cross-contamination had occurred (Figure 10). In tanks where the urine was found to be contaminated (Group A), it was possible to calculate the amount of faecal matter still in suspension. Using an average value of 4 µg coprostanol per mg faeces derived from Ferezou *et al.* (1978), Walker *et al.* (1982) and Leeming *et al.* (1998a), contamination was calculated to vary between 1.6 and 18.5 mg of faeces per l urine mixture (Table 4) with a mean of 9.1 ± 5.6 mg/l.

Faecal contamination was more often evident in sludge samples collected from the bottom of the tanks, which probably reflects accumulation of past contamination. Eleven out of 30 sludge samples (37%) were assigned to Group A. However, it is not feasible to calculate an accurate average faecal contamination over time because the sludge layer is partly retained within the tanks after emptying. To use the concentration of coprostanol in the sludge samples would probably lead to an overestimation of the amount of faeces that had entered the tank. Therefore to more accurately quantify the faecal contamination it is recommended to analyse the urine collected from the middle of the tanks.

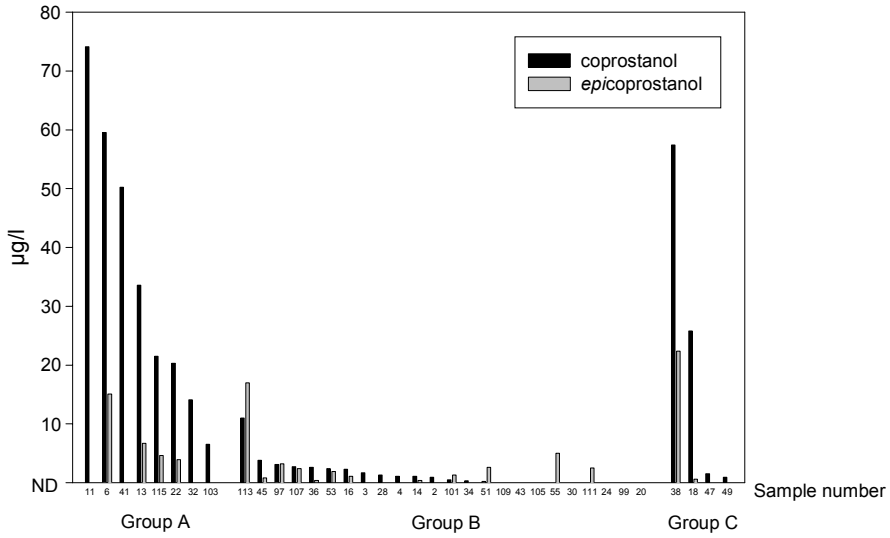


Figure 10. Coprostanol and *epi*coprostanol concentrations in urine samples from collection tanks sorted by criteria given in Table 3 and by concentrations of coprostanol.

Using the criteria in Table 3 to categorise samples, it is clear from the results in Figure 10 that once faecal contamination became apparent via the sterol profile, coprostanol concentrations were all above 5 µg/l. Similarly in the sludge samples, the faecal sterol profile became evident at coprostanol concentrations over 155 µg/g. There was only one instance in each of the urine and sludge samples where a Group B sample had higher coprostanol concentration than a Group A sample (Figure 10). In both cases the accompanying *epi*coprostanol concentrations gave a clear indication that the sterol profile was from a non-faecal source. These results suggest that in the majority of cases a coprostanol threshold might be sufficient to determine faecal cross-contamination. However, it is also evident that in order to avoid false positives, the ratio criteria remains the most reliable method for determining the presence of human faecal contamination and a valuable check for borderline samples.

Six out of fifteen systems showed signs of recent faecal contamination on at least one of the sampling occasions (urine samples; Table 4) and a further three systems had had previous cross-contamination episodes significant enough to be detected (sludge samples). Since a larger proportion of the samples collected from eco-village and public places were contaminated compared to individual households it is suggested that there is a greater risk of contamination the more users that are connected to a tank.

Table 4. Concentrations, in increasing order, of coprostanol in contaminated urine samples (Group A) and indeterminate samples (Group C) that were re-assigned to contaminated, and the corresponding faecal contamination calculated according to Ferezou *et al.* (1978), Walker *et al.* (1982) and Leeming *et al.* (1998a)

	Sample #	Site	Type of site	Coprostanol [µg/l]	Faeces [mg/l]
Group A	103	Åkesta	eco-village	6.5	1.6
	32	Palsternackan	eco-village	14.1	3.5
	22	Åkesta	eco-village	20.3	5.1
	115	Weleda	workplace	21.5	5.4
	13	Gustavsberg	individual household	33.6	8.4
	41	Gustavsberg	individual household	50.2	12.5
	6	Stensund	workplace/school	59.5	14.9
	11	Weleda	workplace	74.1	18.5
Group C	18	Färnebo	school	25.8	6.5
	38	Stensund	workplace/school	57.4	14.4

3.6 Discussion

In summary, the various indicator bacteria implied different degrees of faecal contamination if evaluated according to their normal abundance in faeces (Section 1.6.1), which in further investigations could partly be explained by different growth and survival characteristics (Section 4.2; Jönsson *et al.* 2000). It was concluded that none of the commonly used indicator bacteria were suitable to quantify faecal cross-contamination in source-separated urine. Coliphages have previously been analysed for, but not found in urine collection tanks (Olsson 1995), which was also the case for *E. coli*. Faecal sterols seem to be more suitable for detecting and quantifying faecal cross-contamination. If only coprostanol is analysed there will be an overestimation of contamination whereas if the coprostanol concentration is compared to other faecal sterols false positives can be avoided. Comparing the results from Paper I and Paper IV, the threshold concentration could be applied to Paper I and only the top 31% of urine samples would be considered to be contaminated. In Paper IV several studies were compiled to estimate the concentration of coprostanol in faeces, yielding a slightly lower concentration than that used in Paper I (4 µg/mg compared to 5.6 µg/mg). No correlation was found between coprostanol and indicator bacteria, which is probably a consequence of the varying survival and growth characteristics of the indicator bacteria in contrast to the stability of coprostanol.

Sundin *et al.* (1999) suggested cumulative contamination to be measured, using sediments (sludge) rather than urine samples. However, it has not been feasible to measure the exact volume of urine and flushwater that were added in more than a couple of urine tanks (Section 4.1; Jönsson *et al.* 2000). It was also unknown how much sludge was left on the bottom each time the tanks were emptied, which makes it difficult to calculate the contamination over time. The sedimentation of sterols will have an impact on the estimate of faecal contamination

and by collecting grab-samples, a snapshot of the present contamination in the collection tanks will be obtained. However, the results from several samples and tanks can be considered as a representative average. The actual risk will be dependent on whether pathogens in the faecal material sediment at the same rate as the sterols. This will vary depending on the size of the pathogen and the adsorption to particles (Medema *et al.* 1998). Even though sludge samples may overestimate the faecal contamination, the risk of using the sludge may be higher than that of using the liquid phase due to a higher concentration of pathogens.

It is suggested that the degree of faecal contamination might be related to the number and type of users (e.g. children or adults, regular or irregular) of the separating toilets. It is also possible that cross-contamination may be related to the type of toilet used. This was not specifically investigated since comparisons would demand a larger number of toilets of the same model to be used under the same circumstances, i.e. by the same type of users.

4. PERSISTENCE OF MICROORGANISMS (I, II, III)

The fate of the enteric pathogens entering the urine tank is of vital importance for the hygiene risks related to the handling and reuse of the urine. To determine the duration and conditions for sufficient storage of the urine mixture before its use as a fertiliser, it is therefore necessary to estimate the survival of various microorganisms in urine as a function of time.

4.1 Characteristics of source-separated human urine

Collected source-separated human urine (urine mixture) has different characteristics than freshly excreted urine since it is mixed with flushwater and transported through pipes to a tank. Most collection tanks are placed outside, usually buried underground. The temperature thus varies depending on climate and season. In Sweden the temperature of the urine mixture ranged from 3°C to 19°C (Höglund *et al.* 1999). The pH of fresh urine is normally between 4.8 and 7.5 (Diem and Lentner 1970) but in all samples collected it was around 9.0 (Höglund *et al.* 1999, Jönsson *et al.* 2000). When urine is excreted, the major proportion of the nitrogen is present as urea and in the pipes this is converted to ammonium which is in equilibrium with ammonia (Equations 3-5). The pH is related to the concentration of ammonium (Jönsson *et al.* 2000).

$$CO(NH_2)_2 + 3\,H_2O \xrightarrow{\text{urease}} 2\,NH_4^+ + OH^- + HCO_3^- \quad [3]$$

$$NH_4^+ + OH^- \leftrightarrow NH_3\,(aq) + H_2O \quad [4]$$

$$NH_3\,(aq) \leftrightarrow NH_3\,(g) \quad [5]$$

The amount of flushwater used for the urine bowl depends on the type of toilet used (Table 5; Section 1.3.1). In three housing areas the volume of collected urine mixture (urine + flushwater) was measured along with the concentration of plant nutrients (Table 5; Jönsson *et al.* 2000). Since the concentration of nitrogen in fresh urine is around 7 g/l (Table 1) it can be concluded that water is also added from cleaning of the toilet and perhaps from leakage of groundwater into the system, resulting in approximately 1-2 parts of water per part urine.

Table 5. Characteristics of collected urine mixture in three housing areas (modified from Jönsson *et al.* 2000)

Parameter	Unit	Understenshöjden	Palsternackan	Hushagen
Toilet model		Dubbletten™	Dubbletten™	Wost Man Ecology DS
Urine mixture[a]	l/pd[b]	1.34	1.30	2.24
Urine flushwater	l/pd[b]	0.34	0.43	1.20
pH		9.1	9.2	9.0
Conductivity	mS/cm	25.4	24.1	14.8
N total	g/l	3.63	3.31	2.35
N NH_4^+	g/l	3.58	3.28	2.13
P	g/l	0.31	0.31	0.14[c]
K	g/l	1.00	0.89	0.48
N/P		11.6	10.7	16.4[c]

[a] Urine, flushwater, water for cleaning the toilet and possibly groundwater leaking into the system.
[b] Litre per person and day.
[c] Some of the phosphorous attached to the tank surface and was thus lost from the analysis, yielding a non-representative result.

Urine collected from other types of urine-separating toilets will have different characteristics. Several of the dry latrines used in developing countries use no flushwater. The urea also seems to be degraded in short pipes, which are often used for the dry systems, yielding a pH of around 9 (Franzén and Skott 1999; Sundin 1999).

Temperature, dilution, pH and ammonia were the parameters considered that may affect the persistence of microorganisms in urine tanks. These parameters are known to affect the inactivation or survival of microorganisms (Section 1.7.1). The technical design of the urine-separating system, e.g. flushing and storage procedures, may influence the parameters.

4.2 Survival of bacteria

Survival studies of bacteria in source-separated urine were performed at two temperatures (4°C and 20°C), at three different dilutions of the urine mixture (undiluted, 1:1 and 1:9), that was already diluted with flushwater (Section 4.1), and at four different pH-values (4.5, 6.0, 8.9 and 10.5). The bacteria studied were *Escherichia coli*, *Salmonella senftenberg*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, faecal streptococci and *Clostridium perfringens* (Paper I). These are either indicator bacteria (*E. coli*, faecal

streptococci and *C. perfringens*), models for enteric pathogens (*S. senftenberg* and *S. typhimurium*) or considered opportunistic pathogens (*P. aeruginosa* and *A. hydrophila*).

Bacteria were added or originally present in concentrations of 10^6 CFU/ml, except for clostridia (10^2 CFU/ml), in urine mixture. At different time intervals the bacteria were assayed by the spread-plate method on their respective selective media. Clostridia were analysed using the pour plate method. T_{90}-values (or D-values; time for 90% inactivation) for the different organisms were then estimated.

E. coli, *S. senftenberg*, *S. typhimurium*, *P. aeruginosa* and *A. hydrophila* died off more or less immediately ($T_{90} < 1$ day) at pH 4.5 and pH 10.5. At pH 6.0 and pH 8.9 these bacteria had $T_{90} < 3$ days, except *E. coli* at pH 6.0 where T_{90} was 5 days at 4°C. In undiluted and 1:1 diluted urine (pH 9) *S. senftenberg*, *S. typhimurium*, *P. aeruginosa* and *A. hydrophila* also died off immediately ($T_{90} < 1$ day) at both 4°C and 20°C. In the 1:9 dilution these organisms all had $T_{90} < 20$ days. *E. coli* had an approximate fivefold longer survival in 1:9 dilution than in undiluted urine mixture; $T_{90} \approx 14$ days compared to $T_{90} \approx 3$ days at 4°C and $T_{90} \approx 5$ days compared to $T_{90} \approx 1$ day at 20°C.

At 4°C faecal streptococci had $T_{90} < 1$ day at pH 10.5 and $T_{90} < 4$ days at pH 4.5. At pH 6.0 and 8.9 almost no reduction occurred during 40 days after an initial one $\log_{10}$-reduction. In 1:9 dilution (pH 9) the T_{90}-value for faecal streptococci was approximately three times higher than in undiluted urine mixture, 19 days compared to 6.5 days at 20°C, whereas the difference at 4°C was less, all T_{90}-values being < 35 days. Gram-positive faecal streptococci were given special attention since they were found to potentially grow within the pipes (Paper I; Section 3.3) and in a second survival experiment T_{90}-values were calculated to 30 days at 4°C and to 5 days at 20°C (Figure 11; Jönsson *et al.* 2000).

C. perfringens spores showed no reduction during 35 days under any conditions, and in a second experiment conducted at conditions normally prevailing in urine tanks (pH 9) there was no reduction during 80 days at either temperature (Figure 11; Jönsson *et al.* 2000).

A lower temperature involved a longer survival of most bacteria and the more diluted the urine, the longer the survival of the bacteria examined. The pH was approximately the same in all dilutions. pH-values the furthest from neutral had the most negative effect on survival of the organisms. At pH 6.0 most of the bacteria had a better survival than at pH 8.9. The reduction of bacteria at high pH-values may be an effect partly of the pH and partly of the presence of ammonia.

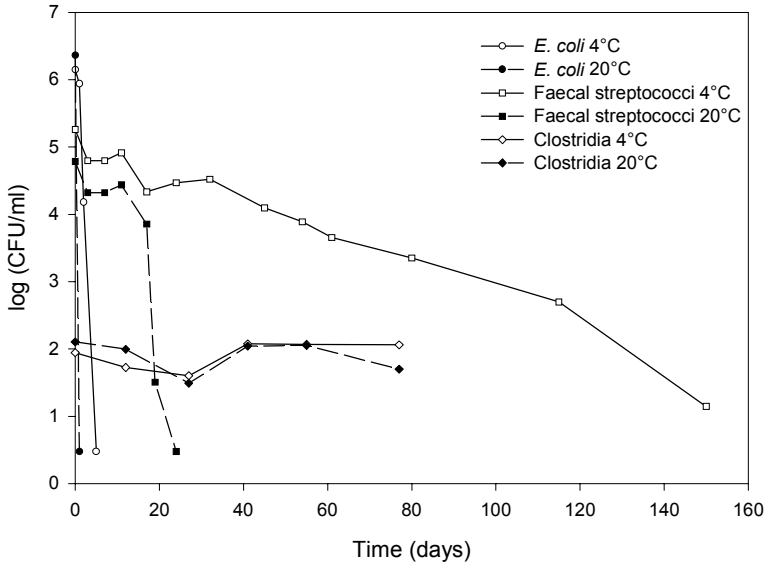


Figure 11. Inactivation of *E. coli*, faecal streptococci and *C. perfringens* spores (clostridia) in source-separated human urine (pH 9) at 4°C and 20°C.

The short survival of *E. coli* in urine makes it unsuitable as an indicator for faecal contamination. Gram-negative bacteria such as *Campylobacter* and *Salmonella* cause a majority of gastrointestinal infections (Section 1.5.3). All bacteria belonging to this group were inactivated rapidly in urine mixture, indicating a low risk for transmission of gastrointestinal infections caused by bacteria when handling source-separated urine.

4.3 Survival of protozoa

Cryptosporidium parvum was chosen as a representative to study the survival of protozoa in urine since it is known to be persistent in waste products as well as in water and to be resistant to disinfectants (Meinhardt *et al.* 1996). The inactivation in buffers was investigated as a comparison to evaluate the effect of pH. The two different *in vitro* viability methods (Section 1.8.2) used were excystation (Robertson *et al.* 1993), and inclusion or exclusion of the vital dyes (4′-6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) (Campbell *et al.* 1992).

In urine mixture at pH 9 and 4°C, the oocysts were inactivated to below the detection limit (<1/300) within 63 days according to both dye exclusion (Figure 12) and excystation. It was not possible to determine the time needed for oocysts to be *fully* inactivated, since that is beyond the scope of the methods used, i.e. the microscopic procedure (Section 1.8.2). With these limitations in sensitivity, it was only possible to determine an approximate 2 $\log_{10}$ (99%) reduction in oocyst viability. The T_{90}-value for *Cryptosporidium* was determined at 29 days according to the dye permeability assay. At 20°C the T_{90} was estimated to 5 days.

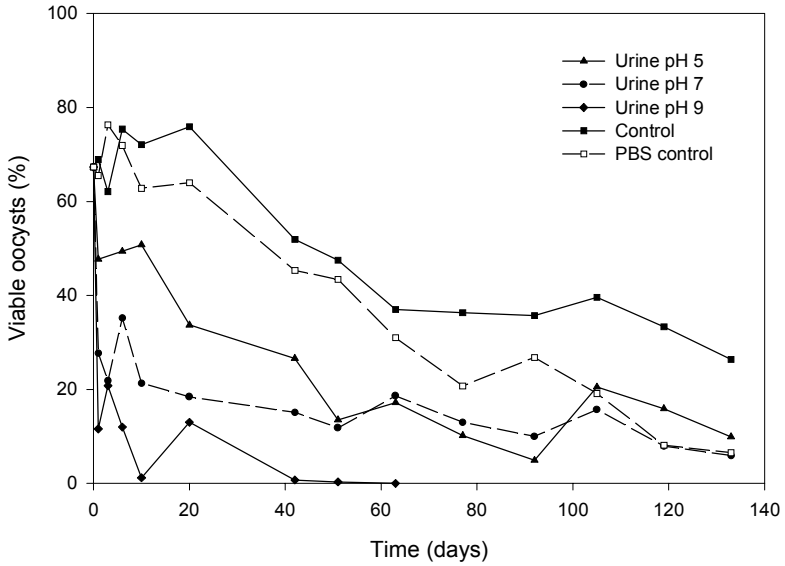


Figure 12. Inactivation of *Cryptosporidium parvum* oocysts in source-separated human urine with different pH-values at 4°C, assessment of viability performed by dye permeability assay with DAPI and PI. Control is oocyst stock solution and PBS control is oocysts in Phosphate Buffered Saline, pH 7.4.

By pairwise comparisons of the inactivation coefficients (*K*) it was shown that the inactivation rate of *Cryptosporidium* oocysts in urine at pH 9 was significantly higher ($p < 0.01$) than in the controls (oocyst stock solution and PBS (pH 7.4)) and in urine at pH 5 and pH 7, according to the dye permeability assay. In Sörensen buffer solutions (pH 5, 7 and 9) there were no significant differences in inactivation rate between pH-values for either dye permeability or excystation methods and T_{90}-values ranged from 115 to 168 days. When correlating urine samples with the corresponding buffer samples it was only at pH 9 that the inactivation rate was significantly higher ($p < 0.01$) in urine, according to the dye permeability assay. Pearson correlation showed no significant correlation between excystation and the dye exclusion method in any stored urine sample or control.

The overall results thus indicated that the inactivation of *Cryptosporidium* oocysts is more rapid in source-separated urine than in controls and that pH alone does not have an effect on oocyst viability, similarly to previously reported studies (Jenkins *et al.* 1998). The results thus indicated that the antiprotozoan effect of urine at pH 9 is mediated by other factors besides the actual pH. Ammonia (NH_3) has been demonstrated to act as an inactivating agent for *Cryptosporidium* (Jenkins *et al.* 1998). The concentration of free ammonia (NH_3) in the urine (pH=9; T=4°C) was around 0.03 mol/l (Paper III; Svensson 1993; Jönsson *et al.* 1998). Thus it is likely that ammonia present in the urine mixture may have an effect on the inactivation of the oocysts.

Even though the correlation of *in vitro* viability methods to infectivity can be questioned, *Cryptosporidium* oocysts were found to be inactivated by two methods measuring different features. We consider *Cryptosporidium* to be one of the most persistent protozoan parasites. Thus, when *Cryptosporidium* oocysts are inactivated in urine other protozoa are not expected to remain in considerable amounts.

4.4 Survival of viruses

Since infectious doses for viruses are generally low and they are shed in higher numbers than bacteria and protozoa, environmentally persistent viruses may constitute the major risk associated with recycling of human waste.

To investigate virus inactivation during storage of source-separated human urine, *rhesus* rotavirus (RRV) and *Salmonella typhimurium* phage 28B (phage 28B) were chosen as viral models and their persistence was followed during a period of six months at 5°C and 20°C (Paper IV). Rotaviruses were enumerated as peroxidase stained plaques in infected MA-104 cell monolayers (reported as PFU/ml). The persistence of RRV was analysed in two separate experiments using the same method (Experiment 1 and Experiment 2). Phage 28B was quantified by the agar layer method (Adams 1959). The inactivation of RRV and phage 28B were assumed to follow first order kinetics and the inactivation rate, k ($\log_{10}$ inactivation per day), was determined as the slope of the inactivation curves.

The inactivation of RRV in the two experiments was similar ($p < 0.05$), hence the data were combined in Figure 13. Inactivation rates were approximately four to ten times higher at 20ºC compared to 5ºC in both urine mixture and controls. The time for a 90% reduction of infectious virus (T_{90}-value) was calculated for each experiment (Table 6), yielding an average of 35 days at 20ºC and 172 days at 5ºC. At 20ºC infectious viruses were eliminated to below the theoretical detection limit of 100 PFU/ml within the period of investigation, whereas at 5ºC a large number of infectious viruses were still detected at the end of the study. The persistence of rotavirus in the pH 7 controls was consistently higher, but inactivation rates were within the same range as in stored urine mixture (Table 6).

The counts of RRV varied over time, especially at 5°C, where the trend in the decay was statistical but not obvious to the eye. This led to difficulties in determining whether there was an inactivation of RRV or not. The variation over time for the control mirrored the variation in the urine mixture, thus indicating that at least some of the variation in the results was caused by the method used, e.g. the variability of the cell line. Even though the regression line indicated a slow decay in both experiments at 5°C (T_{90} = 240 and 104 days, respectively), for the purposes of risk assessment, it may be assumed that there was no decay of RRV during six months of storage at ≤5°C. The inactivation of RRV in the control medium was almost as rapid as in the urine mixture at both 5°C and 20°C, implying that temperature was more important than other conditions prevailing in the urine mixture.

Table 6. Persistence of *rhesus* rotavirus in source-separated human urine and controls at 5°C and 20°C

Experiment	Temperature [°C]	Medium	Inactivation rate (k) [day^{-1}][a]	95% confidence interval [day^{-1}][a]	r^2	T_{90} [days]
1	5	urine	0.0042	0.0015-0.0069	0.12	240[b]
		MEM[c]	0.0031	-0.0015-0.0077	0.08	326[b]
	20	urine	0.0264	0.0228-0.0300	0.81	38
		MEM[c]	0.0256	0.0196-0.0316	0.82	39
2	5	urine	0.0097	0.0068-0.0125	0.51	104[b]
		MEM[c]	0.0023	-0.0020-0.0067	0.08	426[b]
	20	urine	0.0308	0.0244-0.0372	0.72	32
		MEM[c]	0.0189	0.0121-0.0256	0.78	53

[a] Log_{10} PFU/ml.
[b] Data do not necessarily support inactivation.
[c] Eagle's Minimal Essential Medium (pH 7.2).

As observed for rotavirus, phage 28B was highly persistent in urine at 5ºC with an estimated T_{90}-value of 1 466 days, and in the PBS control the estimated inactivation was even lower (T_{90} = 2 431 days; Table 7). At 20ºC the T_{90}-value was calculated at 71 days. There was minimal phage inactivation in the pH 7.4 control at 20ºC (Figure 13). Comparing inactivation rates, phage 28B inactivation in urine was approximately twenty times higher at 20ºC than at 5ºC, whereas in the control the inactivation was about twice as high at 20ºC compared to 5ºC (Table 7).

Table 7. Persistence of *Salmonella typhimurium* phage 28B in source-separated human urine and controls at 5°C and 20°C

Temperature [°C]	Medium	Inactivation rate (k) [day^{-1}][a]	95% confidence interval [day^{-1}][a]	r^2	T_{90} [days]
5	urine	0.0007	0.0002-0.0012	0.24	1 466
	PBS[b]	0.0004	0.0004-0.0012	0.17	2 431
20	urine	0.0141	0.0125-0.0157	0.92	71
	PBS[b]	0.0010	-0.0001-0.0022	0.35	971

[a] Log_{10} PFU/ml.
[b] Phosphate Buffered Saline (pH 7.4).

The phage model was more persistent than RRV at both temperatures (Tables 6 and 7), and thus the phage was an appropriate conservative model organism in this environment. Furthermore, the bacteriophage counts exhibited less variation than the RRV counts, indicating that the phage analysis was more robust as well as being a simpler technique.

No obvious effect of ammonia was observed either for RRV or phage 28B in urine (pH 9) at 5ºC. The collected urine mixture contained approximately 3.3 g NH_4^+/l (Jönsson *et al.* 1998), which at pH 9 would yield an ammonia concentration of 0.026 mol/l at 5ºC and 0.066 mol/l at

20ºC (Svensson 1993). The higher ammonia concentration might be one explanation for the faster inactivation of phage 28B at 20ºC. The inactivation of RRV, however, was similar in urine and control media at 20ºC, with no additional urine-related virucidal effect evident.

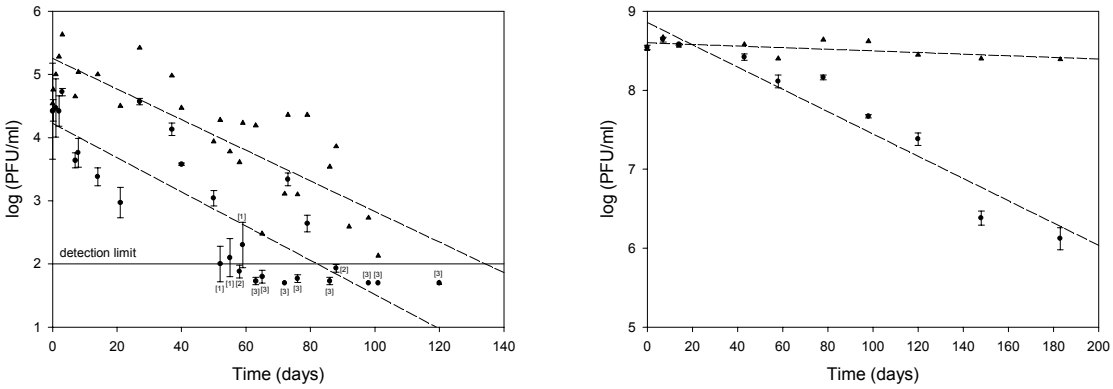


Figure 13. Inactivation of (a) *rhesus* rotavirus and (b) *Salmonella typhimurium* phage 28B in source-separated human urine (●) and control medium (▲) at 20°C. For urine each data point is a mean of triplicate samples (three counts for each sample), error bars represent one standard deviation. For the control the data points represent one sample (mean of three counts). The dashed lines are generated from linear regression. Numbers in brackets (a) indicate the number of samples that were below the detection limit on the day of analysis.

In summary, no significant inactivation of either rotavirus or the phage occurred at 5ºC during six months of storage, while the mean T_{90}-values at 20ºC were estimated at 35 and 71 days for rotavirus and the phage, respectively. In pH-controls (pH 7), the inactivation of rotavirus was similar to that in urine at both temperatures, whereas no decay of the phage occurred at either 5ºC or 20ºC. Therefore, rotavirus inactivation appeared to be largely temperature dependent, whereas there was an additional virucidal effect on the phage in urine at 20ºC (pH 9).

In previous studies (Paper I; Franzén and Skott 1999) the *Salmonella* phage was found to be more persistent in urine than reported here. At various pH-values and dilutions there was no inactivation of phage 28B during 50 days, except at pH 4.5 at 20°C (Paper I). In a study in Mexico no inactivation was recorded during six weeks at a temperature varying from 14°C to 22°C (Franzén and Skott 1999).

Since viruses are such a heterogeneous group, their resistance or removal cannot be universally defined. It also seems difficult to find one model organism, i.e. a bacteriophage, suitable to replace more than a few human enteric viruses in one specific environment or process. Therefore, every system concerned with viral inactivation or removal needs to be specifically investigated. Both rotavirus and phage 28B were more persistent in urine than the

enteric bacteria (clostridia spores excluded) and protozoa (*Cryptosporidium parvum*) investigated (Papers I-II).

4.5 Discussion

The processes of survival and death of microorganisms are complex. Apparent dead bacteria include those that have lost the ability to reproduce (non-viable) and those that are dormant either as spores or as temporarily inactive units (Mason *et al.* 1986). Mechanical processes such as aggregation may also cause an apparent reduction in the numbers of organisms (Section 1.7.1; Burge *et al.* 1981). As described in Section 1.7, there are several factors that may affect the persistence of microorganisms in the environment. For source-separated human urine mainly temperature, pH and ammonia were considered. The presence of other microbes, available oxygen and, for bacteria, available nutrients, will most certainly have an effect on microbial behaviour in the urine as well. A complete understanding of the processes involved in microbial inactivation in source-separated urine was not obtained but it can be summarised as follows. Gram-negative bacteria were rapidly inactivated in source-separated human urine (Paper I; Section 4.2). Oocysts of the protozoa *Cryptosporidium parvum*, which are known to be resistant to environmental pressures, were reduced by approximately 90% per month in the urine mixture (Paper II; Section 4.3). Viruses were the most persistent group of microorganisms with no inactivation in urine at 5°C and T_{90}-values of 35-71 days at 20°C (Paper III; Section 4.4). Temperature seemed to affect all microorganisms investigated. For bacteria further dilution of the urine prolonged the survival, which was probably due to lower concentrations of harmful compounds. The effect of pH is difficult to separate from the effect of ammonia, except for *C. parvum* oocysts where there was no difference in inactivation in buffer solutions with pH 5, 7 and 9 and thus an additional impact of ammonia or other compound in the urine was verified. Rotavirus was neither affected by pH nor ammonia since the inactivation in buffer (pH 7) was similar to that in urine. According to Hamdy *et al.* (1970, in Feachem *et al.* 1983) urine is ovicidal and *Ascaris* eggs are killed within hours. Olsson (1995) however reported the reduction of *Ascaris suum* in urine to be minor. The investigations of *Ascaris suum* in 4°C and 20°C indicated a reduction of 15-20% during a 21-day period. Early studies also reported inactivation of *Schistosoma haematobium* in urine (Porter 1938, in Feachem *et al.* 1983). Further studies of helminths including *Ascaris* is necessary, especially if the system is to be promoted in developing countries.

Sedimentation of microorganisms may in reality account for part of an observed inactivation (Höglund *et al.* 2000). In the survival studies sedimented material could be observed on the bottom of laboratory vessels. This material was resuspended before each analysis and sedimentation should thus not have had an impact on the results observed in Papers I-III.

5. RISK ASSESSMENT (V)

5.1 Objectives for a QMRA

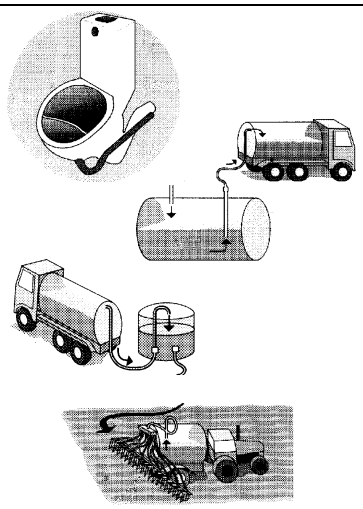
The objectives of performing a Quantitative Microbial Risk Assessment for urine-separating systems were:

- to summarise previous results on faecal contamination and inactivation of microorganisms in source-separated human urine;
- to obtain quantitative results that could be communicated to stakeholders, researchers and managers in other fields of profession;
- to provide results that could be used for future comparisons between risks related to urine-separating systems and other wastewater systems.

5.2 Exposure scenarios

The QMRA performed could be defined as on a screening-level, since many assumptions rather than actual measurements were included, e.g. the concentration of pathogens in urine mixture (referred to as urine in this section; Paper V). The assumptions were often conservative, i.e. values giving a higher risk were chosen. Thus, results include maximum risks, which is a way to apply to the precautionary principle. *Campylobacter jejuni*, *Cryptosporidium parvum* and rotavirus were chosen as representatives for various microbial groups.

The transmission pathways investigated included accidental ingestion of unstored urine, either by cleaning blocked urine pipes or from urine in the collection tank; accidental ingestion of stored urine; inhalation of aerosols while spreading the urine; and ingestion of crops contaminated by urine (Figure 14). Persons at risk include inhabitants in the housing area, workers handling the urine, including farmers applying the urine to arable land, persons in the surroundings of the field and persons consuming fertilised crops. The volume accidentally ingested was assumed to be 1 ml based on assumptions by Asano *et al.* (1992) and pathogens ingested through contaminated crops corresponded to 10 ml of urine per 100 g of crop (Asano *et al.* 1992; Shuval *et al.* 1997). Risk from the aerosol exposure was estimated for a person 100 m away inhaling 0.83 m^3 during an hour (Dowd *et al*. 2000; Paper V). A spray type fertilising technique was assumed.



Exposure	Risk
Cleaning of blocked pipes	Ingestion of pathogens
Accidental ingestion when handling unstored urine	Ingestion of pathogens
Accidental ingestion when handling stored urine	Ingestion of pathogens
Inhalation of aerosols created when applying urine	Inhalation of pathogens
Consumption of crops fertilised with urine	Ingestion of pathogens

Figure 14. Exposure pathways in the urine-separating system investigated in the microbial risk assessment.

The eco-village Understenshöjden was used as a model and the mean faecal contamination based on the faecal sterol analysis (Paper IV) was used as an estimate (lognormal PDF) of faeces entering the urine tank. The collection of urine was assumed to take place for a year, and reported or estimated cases of infection per year (incidence) were used together with PDFs for excretion times and excretion densities to calculate the concentration of pathogens in the faeces, i.e. in the urine. Two different scenarios were investigated:

- *Worst-case* or epidemic, where all infections were assumed to occur during the same time period, just before the collection tank was emptied. Thus no inactivation took place in the collection tank.
- *Normal* case or sporadic, where infections in the population were assumed to be evenly spread out over a year. Collection of urine either occurred at 4°C or 20°C.

The inactivation results from Papers I-III were used to estimate the concentration of pathogens in the urine after storage at 4°C or 20°C.

5.3 Quantitative risks

Risks were calculated per exposure, which corresponded to a yearly risk for some of the exposures. Blockage of pipes is likely to occur about once a year per household (Jönsson *et al.* 2000) and the tank was assumed to be emptied after a year. Fertilising with urine usually also takes place once a year in Sweden. Consumption of crop on the other hand might result in repeated exposures (Paper V).

The risks for the exposure pathways in the worst-case scenario are summarised in Table 8. The risks in the normal scenario where infections occurred sporadically were generally around one $\log_{10}$ lower (Paper V). Except for rotavirus, calculated risks were all below 10^{-3}

(1:1 000). Due to the persistence of rotavirus at low temperatures (≤5°C) and a low infectious dose (median infectious dose, N_{50} = 5.6) risks for rotavirus infection were up to 0.56 by ingestion of unstored and stored (4°C) urine (Table 8). If stored at a higher temperature (20°C) for six months, risk for rotavirus infection decreased to below 10^{-3} (Table 8). The risk for *Campylobacter* infection was negligible ($<10^{-15}$) except if unstored urine was handled or used for fertilising. *Cryptosporidium* constituted a lower risk in unstored urine than *Campylobacter* but six months storage at 20°C was needed for risks to be negligible.

Table 8. Calculated risks, mean and (standard deviation), for a single exposure by accidental ingestion of urine and inhalation of aerosols in the worst-case scenario

Pathway	Storage conditions	*C. jejuni*	*C. parvum*	Rotavirus
accidental ingestion	unstored	4.8×10^{-4} (3.7×10^{-3})	8.7×10^{-5} (8.4×10^{-4})	5.6×10^{-1} (2.2×10^{-1})
	1 month 4°C	nr	1.6×10^{-5} (1.8×10^{-4})	5.6×10^{-1} (2.2×10^{-1})
	6 months 4°C	nr	2.6×10^{-8} (5.5×10^{-7})	5.6×10^{-1} (2.2×10^{-1})
	1 month 20°C	nr	6.9×10^{-11} (6.8×10^{-10})	3.3×10^{-1} (2.4×10^{-1})
	6 months 20°C	nr	nr	5.4×10^{-4} (5.7×10^{-3})
aerosol inhalation	unstored	1.2×10^{-4} (9.6×10^{-4})	2.0×10^{-5} (2.0×10^{-4})	4.2×10^{-1} (2.4×10^{-1})
	1 month 4°C	nr	3.6×10^{-6} (4.3×10^{-5})	4.2×10^{-1} (2.4×10^{-1})
	6 months 4°C	nr	6.0×10^{-9} (1.3×10^{-7})	4.2×10^{-1} (2.4×10^{-1})
	1 month 20°C	nr	1.6×10^{-11} (1.6×10^{-10})	2.0×10^{-1} (2.0×10^{-1})
	6 months 20°C	nr	nr	1.4×10^{-4} (2.2×10^{-3})

nr = negligible risk ($<10^{-15}$)

Aerosol inhalation involved similar risks as direct ingestion of urine. This was calculated for the case when a fine nozzle spray-type fertilising technique was used, and a spray-type fertilising technique can thus be considered inappropriate. Farmers often use other types of equipment (i.e. a spread plate) with minimal aerosolisation (Fernholm 1999).

The risk from ingestion of contaminated crops will be dependent on the time that passes between fertilisation and harvest of the crop, i.e. consumption, since pathogen inactivation will continue on the crop due to UV-radiation, desiccation etc. In Figure 15, the risks from consumption of crops one to four weeks after fertilising with unstored urine are presented. The risk for bacterial or protozoan infection was $<10^{-5}$ after one week, whereas three weeks were needed for the risk of viral infection to be of the same magnitude. The rate of inactivation on crop was estimated from previous studies (Asano and Sakaji 1990; Asano *et al.* 1992; Petterson *et al.* 1999), but will vary due to weather conditions and the estimate is thus uncertain. Still, the risks were low ($<10^{-7}$ after 4 weeks) even when unstored urine and the worst-case scenario was used, and risks may be acceptable even if a significantly slower inactivation occurs and the exposure occurs repeatedly.

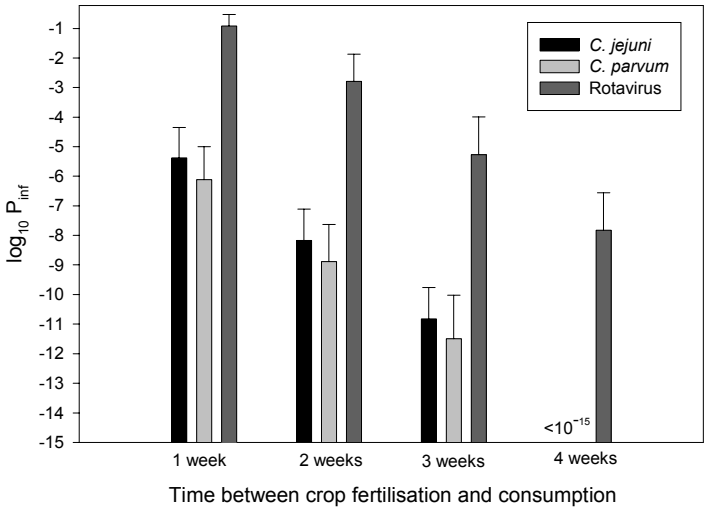


Figure 15. Mean probability of infection by pathogens following ingestion of 100 g crop fertilised with unstored urine with varying time between fertilisation and consumption. Error bars indicate one standard deviation.

5.4 Discussion

There is usually a large variability in the results from risk assessments due to the range in the PDFs used. The uncertainties of assumptions when data are lacking also need to be considered when interpreting the calculated risks. Using worst-case scenarios may avoid underestimation of risks, even though sometimes being less realistic. Dose-response models are obtained from trials with healthy adults. A large part of the population (20%) may however be referred to as immunocompromised (Gerba *et al.* 1996) and thus require lower doses to evolve infection and illness. People exposed in a urine-separating system will usually not belong to this group, with the exception that the contaminated crops may be consumed by anyone. To adequately assess risks of an infectious disease in a population, issues including secondary spread and short- and long-term immunity need to be considered (Craun *et al.* 1996; Eisenberg *et al.* 1996). In this study only primary infection on an individual level was considered. More sophisticated risk assessments require further assumptions and more complex mathematical models.

Animals in the surroundings could be exposed to pathogens in the urine by treading the fertilised field, inhaling aerosols or ingesting contaminated crops (further discussed in Sections 6 and 7). Risks for transmission of zoonotic diseases were not specifically investigated even though *Campylobacter* and *Cryptosporidium* may be transmitted from humans to animals. The rapid inactivation of *Campylobacter* in urine implies that there is usually no risk of animals getting infected. Human rotavirus, like other human enteric viruses, is not considered to be transmitted to animals. The risk for transportation of pathogens from urine to groundwater or recreational water (due to run-off) was considered negligible due to

the limited amount of liquid (corresponding to <5 mm of rain) that is applied when fertilising with urine (Jönsson *et al.* 2000).

Assuming that the acceptable risk for infection is 1:1 000 per year (Section 1.9.2) then all practices would be considered safe if occurring once a year, except for viruses. For viral risks to be less than 1:1 000 a storage time of six months at 20°C or a period of three weeks between fertilisation and consumption would be needed (Table 8; Figure 15). The 1:1 000 and 1:10 000 risks were discussed in relation to drinking water (Regli *et al.* 1991; Haas 1996). For wastewater systems higher risks may be considered acceptable. Furthermore, several of the exposures will be partly on a voluntary basis that may allow for higher risks compared to involuntary exposure. If individuals are aware of the exposure they also have the possibility to protect themselves.

Risk assessment is a valid tool for evaluating new systems, for which there are no possibilities to conduct epidemiological studies. In the eco-village considered here, and in general, there is at this stage no other possible way to quantify risks for the urine-separating system. Results from the QMRA are important for evaluating the system even though it was performed at a screening-level.

5.5 Risk minimisation

Assuming that storage of urine mainly occurs during the cold season in temperate climates, minimal inactivation of viruses would occur. Transmission of viral disease due to cross-contamination of faecal material might therefore be considered as the greatest risk when reusing human urine. Independent of what is considered to be an acceptable risk, it would be valuable to reduce the overall risk by introducing other safety barriers beside storage. If the workers handling the urine were to wear gloves and mouth protection the risk for accidental ingestion could almost be eliminated and chemical clearing of urine pipe blockages instead of mechanical would decrease the risk for splashing. A close-to-ground fertilising technique would minimise the formation of aerosols when applying urine as a fertiliser and there would probably be no risk for surrounding people and only a low risk for the farmer in the tractor. Harrowing directly after spreading would further decrease the exposure for both humans and animals. Restriction of crops is another important means of control (Section 6). Cereal crops among others may be processed before consumption, implying no risk due to effective pathogen inactivation. Furthermore, collection and reuse of urine from hospitals, homes for the elderly and also from day-care centres could be avoided since the prevalence of enteric diseases is often higher at such institutions than in the normal population.

6. GUIDELINES FOR THE REUSE OF HUMAN URINE

Guidelines are tools for regulatory agencies with the purpose of protecting public health. If they are enforceable by law they are generally called regulations (Crook 1998). Since urine-separating systems are being implemented in Sweden, it was decided to set reuse conditions based on the parameters urine storage time and temperature (Table 9; Jönsson *et al.* 2000). Guidelines may in this context be seen as recommendations on how to use source-separated urine in agriculture in order to minimise the risks for transmission of infectious diseases and as a part of risk management (Section 5.5). Regulatory guidelines have yet to be determined by the agency responsible.

Table 9. Relationship between storage conditions, pathogen content[a] of the urine mixture and recommended crop for larger systems[b]. It is assumed that the urine mixture has at least pH 8.8 and a nitrogen concentration of at least 1 g/l

Storage temperature	Storage time	Possible pathogens in the urine mixture	Recommended crops
4°C	≥1 month	viruses, protozoa	food and fodder crops that are to be processed
4°C	≥6 months	viruses	food crops that are to be processed, fodder crops[c]
20°C	≥1 month	viruses	food crops that are to be processed, fodder crops[c]
20°C	≥6 months	probably none	all crops[d]

[a] Gram-positive bacteria and spore-forming bacteria are not included.
[b] A larger system in this case is a system where the urine mixture is used to fertilise crops that will be consumed by individuals other than members of the household from which the urine was collected.
[c] Not grasslands for production of fodder. Use of straw is also discouraged, further discussed below.
[d] For food crops that are consumed raw it is recommended that the urine be applied at least one month before harvesting and that it be incorporated into the ground if the edible parts grow above the soil surface.

These guidelines were set based on the inactivation of microorganisms in urine (Papers I-III) and correspond well with the results of the QMRA. Under conditions (i.e. regarding temperature, pH and nitrogen concentration) other than those given, the inactivation may be different. The Gram-negative bacteria are the major bacterial group causing gastrointestinal infections (Sections 4.2). Gram-positive bacteria (faecal streptococci) have a slower inactivation rate than Gram-negative bacteria (Paper I; Section 4.2) and may be present after one month's storage at 4°C. Bacteria belonging to this group are, however, considered to be less of a health concern in the urine-separating systems. If initially present in high concentrations, faecal streptococci may be used as an indicator of the effects of storage. Bacterial spores will be present since they were persistent in urine (Paper I). This group of bacteria is also of less concern in relation to urine-separation.

Processing of crops in this case means commercial processing, using e.g. heat, which will inactivate all pathogens potentially present except bacterial spores. Fertilising grasslands used for fodder to cattle with urine is not recommended since grazing animals may consume

substantial amounts of soil. Similarly the use of urine on straw to be used as bedding material is discouraged since animals may consume part of the material and since the lower parts of the plant are more exposed to microorganisms in urine and contaminated soil than the upper parts, e.g. grain.

For single households the urine mixture is recommended for all type of crops, provided that the crop is intended for the household's own consumption and that one month passes between fertilising and harvesting, i.e. consumption. Incorporating the urine into the ground is also recommended, but only for crops where the edible parts grow above the soil surface. For crops growing under the surface it is, from a hygiene point of view, more beneficial not to work the urine into the ground since inactivation of potential pathogens by heat, UV-radiation and desiccation is faster on the surface.

The results from the risk assessment (Paper V) do not imply that the recommendations need to be modified. In this paper, risks for infection with one to four weeks between fertilisation and harvest (i.e. consumption) were calculated, but even if the crop were to be consumed raw right after fertilising with urine the risks are quite low. In the worst-case scenario (Section 5.1) the risk for bacterial infections from ingestion of 100 g crop is negligible ($<10^{-15}$) if urine stored for one month at 4°C is applied (unpublished data). After six months' storage at 4°C, or one month's storage at 20°C, the mean risk for protozoan infections is $<10^{-7}$. This was calculated for infection of humans, but the risk for infection of animals is also considered to be sufficiently low for the urine to be used on fodder crops. However, in Denmark viable *Cryptosporidium* oocysts were found in urine storage tanks after six months (Bagge pers. comm.). This may be a reason for re-evaluating the recommendations for fodder crops. On the other hand *Cryptosporidium* infections are more prevalent in cattle (Viring *et al*. 1993; Wade *et al.* 2000) than in humans (Paper V; Section 1.5.3) and the risk for transmission from urine-fertilised fodder crops is probably low compared to the risk from transmission within herds. If the urine is stored for six months at 20°C the risk for viral infection is around 10^{-3} without any time passing between fertilisation and consumption, which might be on the border of the acceptable. However, it is likely that some time passes before harvesting as recommended, since urine is applied as a fertiliser rather than for irrigation and after a week the risk for viral infection is $<10^{-5}$.

Due to concentration of sedimented pathogens the sludge formed at the bottom of urine tanks may involve a higher risk than the urine (liquid phase) as discussed in Sections 3.6 and 4.5. The sludge constitutes a minor part of the volume and if mixed with the rest of the contents of the tank risks may only be slightly altered (Höglund *et al.* 2000). Furthermore, often only a fraction of the sludge is collected when tanks are emptied. However, if handled separately other precautions may need to be considered.

Guidelines and regulations to ensure hygienic quality of waters generally include limits for indicator organisms and/or pathogen concentrations. Guidelines may also include specified treatment processes, e.g. in relation to the utilisation of sewage sludge (Stenström 1996). The

guidelines may be specific for a country or apply to internationally set boundaries. WHO has established guidelines for the reuse of wastewater (WHO 1989) that are mainly applicable to developing countries. These are currently under revision and the new guidelines have been suggested to be based on indicator organisms and epidemiological studies as well as results from microbial risk assessments (Blumenthal *et al.* 2000). The WHO guidelines include reuse conditions, i.e. depending on the microbial quality of the wastewater, different crops can be irrigated, which is similar to our approach. Specific irrigation techniques are also recommended and specific treatments with known reductions of microorganisms may eliminate the need for routine monitoring (WHO 1989; Blumenthal *et al.* 2000). For source-separated urine it is recommended to use a fertilising technique that applies the urine close to the ground, not creating aerosols, since spray application implies quite high risks for viral infections (Paper V; Section 5.3) and also leads to high nitrogen losses. Relying on treatment (in this case storage) is a simpler method than monitoring by the analysis of microbiological parameters. To control the process (i.e. the storage conditions), pH and the nitrogen concentration could be measured.

7. GENERAL DISCUSSION

7.1 Health risks

Whether urine-separation and the reuse of urine can be recommended depends on whether the associated health risks are considered to be acceptable. These risks can be balanced against benefits like the fertiliser value of human urine. Higher risks from reuse of waste products may be acceptable in areas where enteric disease is endemic and where it is more often transmitted through poor hygiene and sanitation (Blumenthal *et al.* 2000). In areas where food is scarce, benefits from larger harvests may reduce other risks such as malnutrition, which otherwise causes immunosuppression and makes the individual more susceptible to infections. As discussed in Section 5.4, the awareness of risks and the voluntary aspect are issues that also need to be considered when establishing an acceptable risk.

Hygienisation or sanitation may refer to a treatment that reduces the number of microorganisms in a waste product to prevent negative impacts on humans and the environment (Herbst 2000). For source-separated urine the only sanitising treatment that has been discussed is storage. This is a simple means of controlling pathogen spread, and a non-complex system is often preferable. If storage takes place inside a building it is possible to have a more even and higher temperature. Temperatures above 20°C would probably increase the inactivation of microorganisms, and further investigations at higher temperatures could also be of interest for systems in tropical climates. In Sweden the urine mixture may freeze during storage if the tank is placed outside and above ground. Viruses survive freezing whereas protozoa (*Cryptosporidium*) are considered to be sensitive to freezing and the number of bacteria may be reduced (Lewis-Jones and Winkler 1991; Gerba 1996). Fayer and Nerad (1996), however, reported *Cryptosporidium* oocysts to still be infectious after freezing. Repeated episodes of freeze-thawing will probably have an increased inactivating effect even

on bacteria (Morley *et al.* 1983; Sanin *et al.* 1994). The high concentration of ammonium in combination with a pH of 9 causes inactivation of microorganisms due to the formation of undissociated ammonia in the mixture. If NaOH is used for cleaning blocked urine pipes (Section 7.2) this may further increase the pH and the level of undissociated ammonia and thus have a positive effect on the hygienisation. A lower dilution of the urine increases the rate of pathogen inactivation, probably due to the higher concentration of ammonia and ions.

The results reported were obtained for selected non-pathogenic and pathogenic microorganisms. Whether the inactivation obtained is applicable to other pathogens depends on their resistance to the physiochemical parameters discussed. The risk for infection furthermore depends on the abundance of various pathogens in the urine mixture (i.e. in faeces) and the infectious dose determined by host-parasite relationships (Jawetz *et al.*1987). Several Gram-negative bacteria were investigated and all were found to be rapidly inactivated, which resulted in the conclusion that pathogens belonging to this group constitute a low risk. *Vibrio* species are, however, known to be persistent at alkaline pH-values (Feachem *et al.* 1983) and may have a longer survival in urine. The main concern is *Vibrio cholerae*, especially in communities with low sanitary standards (Cooper and Olivieri 1998). Further investigations may be justified for evaluation of sanitary systems in developing countries even though short survival times were reported in faeces and sewage (Feachem *et al.* 1983). *Cryptosporidium* was considered to be the most resistant of all the protozoa based on results from e.g. disinfection studies, and its infectious dose is low. Thus *Giardia*, *Entamoeba*, microsporidia and *Cyclospora* do not imply a higher risk than *Cryptosporidium*. The Gram-positive faecal streptococci had a similar inactivation rate to the protozoa but other Gram-positives would, if pathogenic, result in a lower risk than for *Cryptosporidium*, since infectious doses for bacteria are generally higher than those for protozoa. At low temperatures there was no reduction of the viruses investigated. With the high excretion and low infectious dose of rotavirus, there is probably no other enteric virus that constitutes a higher risk. However at 20°C, phage 28B was more resistant than rotavirus and other viruses could be equally persistent in urine. As discussed in Paper III, rotavirus has been reported to be as resistant or more resistant than several other enteric viruses (Ward *et al.* 1989; Pesaro *et al.*1995). Hepatitis A viruses are also known to be resistant, e.g. to heat and UV-radiation, and have been recognised as foodborne and waterborne pathogens that could be of concern when applying wastes to land (Sobsey *et al.* 1986; Deng and Cliver 1995; Yates and Gerba 1998). In various mixed animal and human wastes at a pH around 7, the inactivation rate of hepatitis A was more rapid than that of rotavirus in source-separated urine (Deng and Cliver 1995). Comparisons to the SRSVs (or Norwalk-like viruses) that are a prevalent cause of viral gastroenteritis have, however, not been possible since SRSVs are not yet culturable (Section 1.8.3). Since virus survival has been recognised as highly influencing health risks in reuse systems (Paper V; Snowdon *et al.* 1989a; Deng and Cliver 1995) further investigations in urine as well as in other waste products may be relevant. Helminth eggs are very persistent in the environment. Due to the lifecycles of helminths often including development to the infectious stage outside the host, the transmission routes and risks for infections need to be evaluated separately, especially in relation to conditions prevailing in areas where the

infections are endemic. Pathogens yet to be recognised could theoretically be present in the urine. It is, however, unlikely that they would occur in higher concentrations than those calculated for in the risk assessment or be more persistent than rotavirus, which constituted the highest risk (Paper V).

Certainly, the inactivation of pathogens will continue after the urine has been applied to the soil. Inactivation in soil and on crops is hard to predict since local conditions always will vary regarding climate (e.g. temperature, sunlight, moisture), type of soil (e.g. particle size, water holding capacity) and type of crop (e.g. cereals, leafy/root vegetables) (Snowdon *et al.* 1989a). Many studies on inactivation have been conducted, but few report T_{90}-values (Badawy *et al.* 1990; Petterson *et al.* 1999). Inactivation on crops is generally considered to be faster, with a total inactivation ranging from days to weeks, than inactivation in soil and on the soil surface, which ranges from weeks to months (Feachem *et al.* 1983; Lewis-Jones and Winkler 1991). According to a review by Yates and Gerba (1998) enteric viruses are likely to survive less than two weeks on crops during the summer and less than six weeks during spring and fall. As in other environments bacteria are probably less persistent than viruses (Section 1.7) whereas parasitic cysts may remain viable for long periods if not desiccated. Regarding the risk for pathogen transmission, there is a choice of whether to store the urine at conditions that virtually eliminates pathogens or to account for further inactivation in the field. If appropriately stored, risks for potential transmission of zoonotic diseases to animals in the fields will be lower. Zoonotic agents may infect e.g. cattle or birds, and either cause disease or the animal may function as a reservoir or vector, with the possibility of further transmission of the pathogen. Bacterial spores have been recognised as a potential problem for animals in relation to the reuse of other organic wastes (Albihn pers. comm.). These spores will be present in any organic waste, and will also withstand harsh treatments such as pasteurisation. That clostridia spores were present in urine after six months of storage is thus not surprising. Their impact on animal health needs to be clarified but if appropriate application techniques are used, the risks related to urine can be considered low. Run-off and contamination of groundwater is unlikely at the volumes of urine applied (Section 5.4; Jönsson *et al.* 2000). Another risk that has been recognised is that of the uptake and growth of pathogens in plants. This has been reported for bacteria that were present in the seed (Mahon *et al*. 1997; Itoh *et al*. 1998), for plants grown hydroponically (Oron *et al*. 1995; Rababah and Ashbolt 2000) and for damaged plants (Crook 1998), but probably does not occur to a sufficiently large extent in the field to be a risk. If applied to non-food crops the foodborne route of transmission is eliminated, but there is still an infection risk for for people involved in the production and processing of crops as well as for humans and animals in the surroundings. In Sweden urine has been used on short-rotation willow coppice grown for energy production (EC 2000).

Epidemiological studies on people in contact with source-separated urine would be a reliable way to investigate whether the practice of reusing urine affects public health. This type of study would hardly be feasible with the small numbers of people in Sweden who handle urine. Several investigations regarding the impact of wastewater reuse on the health of people in the

immediate vicinity have been conducted. These have often focused on parasites that are endemic in the area of investigation and that are known to be persistent in the environment (Blumenthal *et al.* 1996; Cifuentes 1998). Clear evidence of increased infection rates was found in several of the investigations, some of them involving irrigation with untreated or poorly treated wastewater (Katzenelson *et al.* 1976; Fattal *et al.* 1986). According to Cooper and Olivieri (1998) there are no recorded incidents of infectious disease transmission associated with reuse of appropriately treated wastewater, possibly because the risk is too low for detection by epidemiological methods. In the risk assessment method, used as an alternative in predicting risks for infection, viruses constituted the highest risk (Paper V). The transmission of e.g. rotavirus commonly occurs person to person, which implies that handling of waste products would only marginally affect the prevalence of such diseases in a society (Lewis-Jones and Winkler 1991). Even though individual cases of viral infections theoretically could arise from handling urine, they would probably not be recognised by any surveillance system. The risk for an outbreak caused by direct contact with urine is low, since few persons are exposed, e.g. compared to a drinking water supply or recreational water. The risk for foodborne outbreaks could be minimised by following the guidelines for storage in combination with choice of crop (Section 6) and risks for infections caused by unsafe handling of food produce should be similarly considered (Cifuentes 1998; Yates and Gerba 1998).

Urine is a clean product if not contaminated by faeces. Most other waste products will contain the faecal fraction as well and will need further treatment. The safety of reusing wastewater or sewage sludge is much more dependent on the pathogen-reducing efficiency of the treatment process. Organic waste that is treated by adequate pasteurisation, in Sweden at 70°C for one hour, involves less risk than urine. Compared to conventional wastewater systems, the separate handling of urine may be seen as a slight increase in the *total* risk since the other wastewater fractions, containing the majority of pathogens, continue to be treated in a sewage treatment plant. Whether a small-scale urine-separating system with local treatment of all the wastewater involves a lower or higher risk depends on the actual construction of the system in use.

The environmental fate of consumed pharmaceuticals and their effect on humans, animals and microorganisms is heavily debated. Antibiotic resistance and the presence of hormones are issues which are probably related to sewage (Swedish EPA 1996). The risks related to pharmaceuticals is an aspect of public health that has not been considered in this thesis. According to the Federation of Swedish Farmers, it is the pharmaceutical issue that is currently hindering the promotion of urine as a fertiliser in organic farming in the EU (Eksvärd pers. comm), where it is not approved. Microorganisms often have the ability to degrade e.g. steroids and the most commonly used antibiotics also exist naturally and thus could be expected to be degraded in the environment. Furthermore, effluent from conventional sewage treatment ends up in an aquatic environment where organisms such as fish are likely to be affected by e.g. hormones, whereas reuse products applied to soil involve less potential harm. A terrestrial system may also degrade pharmceutical substances faster

than an aquatic system. However, this area needs to be investigated, not only in relation to the reuse of urine, but in relation to all types of wastewater systems and waste products.

7.2 The present situation in Sweden

With phosphorous being a limited resource and with the need for water conservation in many areas, urine-separation is an appealing complement to conventional wastewater collection and treatment or small-scale infiltrating units. However, the system needs to be looked at in a wider perspective, including all the criteria in 1.4.1 as has been done in several Swedish studies (Bengtsson *et al.* 1997; Malmqvist and Stenberg 1997; Kärrman *et al.* 1999; Jönsson *et al.* 2000). In these studies the sustainability of the system in comparison to other wastewater systems as regards energy, nutrient recycling and, to some extent, sociological aspects and health has been investigated. Life Cycle Assessments (LCAs) and other types of environmental systems analysis have resulted in the conclusion that supplementing the conventional system with urine-separation is a favourable alternative, decreasing both eutrophication and use of energy (Bengtsson *et al.* 1997; Malmqvist and Stenberg 1997; Kärrman *et al.* 1999; Jönsson *et al.* 2000). Urine-separating systems are suitable in urban as well as in rural areas and the possible benefits will depend on local conditions and on the future situation regarding chemical fertilisers, sludge management etc.

The different parts of the system have also been investigated in detail (Jönsson *et al.* 2000; Jönsson *et al.* manuscript; Vinnerås *et al.* submitted). The toilets may require some changes in behaviour and the separation is better if this is accepted by the users. From a hygiene point of view the toilet should be constructed so that the risk of faecal material entering the urine part of the bowl is as small as possible. Any flushwater that has been in contact with faeces should not enter the urine bowl since enteric pathogens may follow. The smaller the volume of flushwater used the better since microorganisms will be inactivated faster when the dilution is low. It is also an advantage to keep the dilution low for the transportation and spreading of urine. Water savings of approximately 50% compared to a conventional low flush toilet (4-6 l/flush) are possible (Jönsson *et al.* 2000). Blockages in the urine water lock of the toilets can be a problem, but by choosing proper materials and construction their occurrence can be minimised. Furthermore, the blockages are easily removed by cleansing with NaOH (Jönsson *et al.* 2000). A correct construction with pipes that are completely sealed, have a sufficiently large diameter (>75 mm) and are set at a positive slope is also necessary to avoid blockages further on in the piping system. All metals should be avoided in the system since they may be dissolved by ammonia and added to the urine (Vinnerås *et al.* submitted). Even though the urea in the urine is converted to ammonium and ammonia, nitrogen losses will be low if the urine tanks are filled from the bottom (Figure 4), closed and have a minimal air exchange (Jönsson *et al.* 2000; Jönsson *et al.* manuscript).

The effect on crops is crucial in order for farmers to be interested in using human urine as a fertiliser. Urine contains the major plant nutrients (N, P and K) in proportions suitable for plants and the nutrients are readily available since the major proportion is present in mineral

form. Urine also contains other nutrients like sulphur and magnesium (Kirchmann and Pettersson 1995; Kvarmo 1998). Field investigations as well as pot experiments have revealed that the fertilising effect of urine is comparable to that of mineral fertilisers (Kirchmann and Pettersson 1995; Kvarmo 1998; Richert Stintzing *et al.* 2001). Nitrogen losses in the field were <10% (Jönsson *et al.* 2000), less than those reported for animal urine and slurry (Rodhe and Johansson 1996; Weslien *et al.* 1998). Used on grassland, the nitrogen losses would be larger and the urine used less efficiently (Rodhe and Johansson 1996; Rodhe *et al.* 1997). Salts, including chlorides, may be toxic to some plants (Holliman 1998) and there has been concern about the toxicity of urine. However, no toxic effects have been reported for urine-fertilised crops (Kvarmo 1998; Jönsson *et al.* 2000). Urine is easy to spread but the volume is substantially larger than that of mineral fertilisers, which may result in negative effects due to soil compaction. Of crucial importance also is the acceptance of urine as a fertiliser by the market. At present the EU does not include human urine on the list for approved fertilisers in organic farming (EEC 1991; EC 1997), where it would probably be of best use. The Swedish association for organic farming (KRAV) follows the EU regulations (KRAV 2000), whereas the International Federation of Organic Agriculture Movements (IFOAM) allows urine (and faeces) if sanitary requirements are met (IFOAM 2000). These requirements should be established by standardising organisations (IFOAM 2000). Until recently, most of the companies in the Swedish food industry had not considered the reuse of human waste products, including urine, in agriculture (Berglund 2001). Their policies are often aimed at closing the loops but few of them have made any decisions regarding recycling of plant nutrients. Regarding urine, two companies claimed to disapprove of its use whereas one of the larger producers of dairy products would approve of using urine on agricultural products (fodder crops), on condition that there is no risk for faecal contamination (Berglund 2000), which is of course impossible to guarantee and not necessary according to our risk estimates. Furthermore, the possibility of enteric pathogens originating from urine passing all the barriers involved in the production of dairy products is negligible. A similar low risk is involved in the production of other foods, with the only exception being vegetables intended for raw consumption (Section 6).

Research has been carried out on methods to concentrate the nutrients in urine, nitrogen in particular. The methods tested include reverse osmosis, nitrification, drying (in combination with nitrification), stripping of ammonia followed by precipitation with acid, crystallisation of struvite by adding MgO, and mineral adsorption of ammonia, the latter two also in combination with freeze-thawing (Ban *et al.* 1999; Jönsson *et al.* 2000; Lind *et al.* 2000; Johansson and Hellström submitted). Energy is required for several of the processes and some of them have only been tested on fresh or synthetic urine. So far, none of the methods have been introduced on a large scale. There have also been investigations on how to prevent the conversion to ammonium and keep the nitrogen as urea by adding acid (Hellström *et al.* 1999). Solutions like this will, however, bring about a more complex system that uses chemicals. Several of the treatment processes will probably have a positive effect on pathogen inactivation, but this needs to be investigated. Diluted urine has also been used in aquaculture systems to produce algae, zooplankton and vegetables (Adamsson 2000).

The economics involved have not been thoroughly investigated, most certainly because it is a difficult task. Environmental gains need to be evaluated e.g. in relation to the extra cost of installing urine-separating toilets. The rebuilding of a well-functioning wastewater system is probably not economically viable. There are, however, many not so well-functioning systems, mainly individual systems in rural areas that need to be modified. In addition, for new housing areas urine-separation might be an attractive alternative. There are already municipalities in Sweden which require that urine from all newly built houses not connected to the communal wastewater system be collected for reuse (Tanum Municipal Council 1998). In 1997, 12% of the municipalities in Sweden had at least some installations for urine-separation and 25% of them expressed an interest in the system (Söderberg 1999). The number of urine-separating toilets that have been installed and the volume of urine that is collected and used in agriculture in Sweden is, however, unknown.

7.3 The present situation in developing countries

The most common sanitation system in the world is the pit latrine, which provides on-site sanitation for 20% of the population in developing countries (WHO 1997; Esrey *et al.* 1998). Even though these latrines protect the population from infectious diseases compared to on-the-ground excretion, the excreta collected in the pits may contaminate the groundwater due to transport of pathogens and nitrate leaching (Lagerstedt *et al.* 1994; Stenström 1996). Exclusion of the urine would significantly decrease the leaching, and urine-separation has been suggested for preventing transmission of disease, as well as for avoiding nitrate contamination (Esrey *et al.* 1998; Jacks *et al.* 1999). As discussed in Sections 4.5 and 7.1, further research on pathogens common to developing countries, especially in tropical areas, would be valuable. The risks from handling and reusing the faecal fraction are probably in more acute need of control than the risks related to urine. If the faecal fraction is kept dry and the pH is raised by adding lime or ash, enteric pathogens will be inactivated (Lewis-Jones and Winkler 1991; Stenström 1996; Carlander and Westrell 1997; Esrey *et al.* 1998). Education is also crucial in order to get the systems to function hygienically. Apart from decreasing diarrhoeal diseases, the management of water and sanitation is also important for reducing cases of e.g. malaria and schistosomiasis (WCED 1987).

Much can also be gained if a cheap fertiliser is available. For sustainable agriculture in developing countries it is important to increase the use of organic fertilisers as a complement to mineral fertilisers (WCED 1987). Only animal manure and crop residues were mentioned earlier, whereas later reports have emphasised human urine and faeces as being important resources as well (WSSCC 2000). Compared to developed countries, agriculture in developing countries may obtain an even larger benefit from utilising the plant nutrients found within human excreta, since this contributes a larger proportion of plant nutrients than animal manure, due to the lower intake of animal protein in developing countries (FAO 1999a). It would be especially beneficial to use human excreta in urban agriculture (FAO 1999b; Drangert 2000). In combination, improved sanitation and higher nutritional status

could significantly improve public health in developing countries (WCED 1987). The low risks for transmission of infections through urine further support the implementation of urine-separation. The higher temperature in many of the developing regions would probably be beneficial for the inactivation of enteric pathogens in the urine (Papers I-III). In addition, the more concentrated urine obtained from toilets or latrines that do not use flushwater would probably increase the inactivation rate (Paper I).

7.4 Future perspectives

The discussion about sustainability is ongoing. However the EU does not yet include urine on their list of approved fertilisers in organic farming, where it would be a large resource. Urine-separation has been evaluated and will be further compared as part of a water and wastewater system both regarding health and other criteria (e.g. Hellström *et al.* 2000). If hygiene and health are included in sustainability criteria, then these criteria will be included in the choice of future systems, but perhaps not directly compared. Microbial risk assessment is often used but acceptable risks and risk management need to be further discussed in order for the society to use results from QMRA investigations.

In 1997 there was a political suggestion that conventional flush toilets should be replaced by urine-separating toilets in all rental apartments in Sweden (Eriksson 1996). This was before the research on microbial risks and other issues related to the systems had given any results, indicating that politics rather than research results may decide what the wastewater systems of the future will look like. Today urine-separating wastewater systems have been thoroughly investigated and some problems related to the systems have been solved. Research still needs to be conducted on specific crops and on the environmental fate and effects of pharmaceutical compounds. The hygiene risks in relation to grazing lands or in relation to pathogens common in developing countries also need further investigation. There is, however, a need of research on the conventional system and other alternative wastewater systems as well. For example, risks from pharmaceuticals and pathogens are unclear in relation to the agricultural reuse of sewage sludge.

How risks and acceptable risks will be managed will probably be one issue that decides the future of recycling wastewater systems. Other factors include the economic value placed on natural resources, user acceptance and political decisions related to sustainability and agriculture, e.g. acceptance and permissibility of waste products in organic farming.

8. CONCLUSIONS

The risk for transmission of infectious diseases in relation to source-separated urine is largely dependent on the cross-contamination by faeces.

- Human urine does not generally contain pathogens that will be transmitted through the environment.
- By analysis of faecal sterols, faecal contamination corresponding to a mean of 10 ppm was detected in approximately half of the sampled systems on at least one occasion and in 22% of all urine samples.
- Indicator bacteria are not suitable for determining faecal contamination of source-separated urine due to a rapid inactivation of *E. coli* in urine mixtures and to growth of faecal streptococci within the systems.

The risk for transmission of infectious diseases is dependent on the storage temperature and duration of storage of the urine mixture before it is used as a fertiliser.

- The enteric bacteria of main concern (Gram-negative bacteria) were rapidly inactivated in source-separated urine at both 4°C and 20°C (T_{90} <5 days).
- The inactivation rate of *Cryptosporidium* oocysts at 4°C corresponded to a T_{90} of 29 days and was estimated to 5 days at 20°C. These rates are expected to be higher for other protozoa.
- Viruses were, with the exception of clostridia spores, the most persistent microbial group investigated. During six months of storage, the numbers of rotavirus and a bacteriophage were not reduced in urine at a low temperature (5°C). At 20°C T_{90}-values were 35 and 71 days, respectively.
- The elevated pH (pH 9) caused by the conversion of urea to ammonium is beneficial for the inactivation of microorganisms in the urine.
- A shorter storage time at a lower temperature will involve higher risks for individuals handling the urine and for those in contact with the fertilised field or crop, including animals. Further inactivation of pathogens is expected in the field and the risk for infection by ingestion of crop will be reduced during the time between fertilisation and consumption.

Guidelines including reuse conditions and other recommendations may ensure a minimal risk for exposure to pathogens in source-separated urine.

- Protection (e.g. wearing gloves) and awareness of risks is important, especially for those handling unstored urine.
- Using suitable fertilising techniques and working the urine into the soil, as well as letting some time pass between fertilisation and harvesting, will decrease the exposure of humans and animals to potential pathogens.
- If urine is used on crops that are to be commercially processed, e.g. cereal crops, the risk for infection through food consumption is negligible.

- Urine collected from individual households and used for the household's own consumption involves less risk than large-scale systems and is suitable for fertilising all types of crops if one month is allowed between fertilisation and consumption.

Urine-separating wastewater systems may contribute to sustainable development in both developed and developing countries and in both rural and urban areas.

- Health criteria for wastewater systems will be fulfilled if urine is handled and reused according to recommended guidelines. The microbial health risks from urine-separating systems are considered to be low, also if compared to conventional systems.
- In Sweden, systems analyses of urine-separating systems have resulted in decreased emissions of eutrophying nitrogen and phosphorous and energy consumption compared to the conventional system. Small-scale treatment systems may be especially suitable for supplementation with urine-separation since their treatment efficiency is often low and their contribution to eutrophication significant.
- As a fertiliser, urine would probably be of best use in organic farming, but its fertilising effect is comparable also to that of mineral fertilisers.
- In developing countries there is an urgent need for safer handling of excreta as well as for fertilisers. International organisations promote dry sanitation and the concept of regarding human excreta as a resource. The survival in urine of pathogens common to these areas might require further investigations even though the reuse of urine is a safe alternative compared to the reuse of faeces or poorly treated wastewater.

How health risks and acceptable risks are to be managed will probably be one of the main issues that decides the future of urine-separating and other recycling wastewater systems.

- Acceptable risks of 1:1 000 - 1:10 000 per year have been discussed in relation to drinking water. According to the Quantitative Microbial Risk Assessment (QMRA) results presented in this thesis, the risk for infections through urine mixtures will often be in this range or lower. Furthermore, higher risks may be more acceptable for a wastewater system since the exposed population is smaller and is also aware of the risks associated with waste products.
- Reuse of human excreta and wastewater in developing countries, especially in tropical areas, may involve higher risks due to the higher prevalence of enteric diseases. However, the risks need to be balanced against possible soil fertilisation benefits and the risks from current sanitary systems.

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