Paper prepared for NJF Seminar no. 327, Copenhagen 20-21 August 2001

Hygienic aspects on the reuse of source-separated human urine

Caroline Schönning

Water and Environmental Microbiology
Swedish Institute for Infectious Disease Control
SE-171 82 Solna, Sweden
caroline.schonning@smi.ki.se

Introduction

Human excreta contain plant nutrients and 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. Urine is the fraction that contains the major part of the nutrients in domestic wastewater, approximately 80% of the nitrogen, 55% of the phosphorous and 60% of the potassium (Swedish EPA, 1995). 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 contribute a smaller amount of nutrients 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.

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 ecological sanitation alternatives being introduced are small-scale systems that demand more personal involvement of the users, including handling of the waste. Thus if not sufficiently treated the possible exposure points for pathogens are increasing compared to conventional piped systems. With the main goals of recycling nutrients and minimising utilisation of natural resources, hygiene aspects must be prioritised. To successfully introduce and optimise alternative wastewater systems it is necessary to evaluate hygiene risks and sanitary aspects in accordance with sustainability criteria. Thereafter recommendations for e.g. storage, treatment or reuse practises should be formulated.

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). Urinary tract infections result in significantly higher amounts of excreted bacteria. These have not been reported to be transmitted to other individuals through the environment. The pathogens traditionally known to be excreted in urine are *Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi* and *Schistosoma haematobium* (Feachem *et al.*, 1983). These are rarely sufficiently common in urine 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 where a freshwater snail is needed as an intermediate host. Furthermore, the inactivation of urinary excreted pathogens in the environment reduces their ability for transmission.

Aim of the study

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 aim of this study was to investigate and evaluate health risks from infectious diseases related to handling and reuse of source-separated urine in agriculture.

The specific objectives 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 (urine + flushwater);
- to quantify microbial health risks in urine-separating sanitation systems by using Quantitative Microbial Risk Assessment (QMRA).

Faecal contamination

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. Any faecal cross-contamination that may occur by misplacement of faeces in the urine-separating toilet is therefore regarded as a possible health risk. 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.

Analysis of faecal indicator bacteria

Analysis of various indicator bacteria implied different degrees of faecal contamination if evaluated according to their normal abundance in faeces, which in further investigations could partly be explained by different growth and survival characteristics. *E. coli* had a rapid inactivation in the urine and faecal streptococci were found to grow within the urine pipes. It was concluded that none of the commonly used indicator bacteria were suitable to quantify faecal cross-contamination in source-separated urine.

Analysis of faecal sterols
As an alternative or complement to microorganisms in detecting faecal contamination chemical biomarkers have been suggested as indicators. The most widely studied biomarkers are probably coprostanol and structurally related faecal sterols (Vivian, 1986). These compounds are metabolites of cholesterol formed in the intestine and excreted in faeces. One advantage compared to bacterial analysis is that samples can be analysed after some time of storage. However, rather sophisticated and complex equipment is needed.

The presence of human faeces in urine samples was successfully determined by analysing for faecal sterols. Cross-contamination was evident in 28% of the samples from urine collection tanks. In tanks where the urine was found to be contaminated, it was possible to calculate the amount of faecal matter still in suspension. Using an average value of 4 μg coprostanol per mg faeces, contamination was calculated to vary between 1.6 and 18.5 mg of faeces per l urine mixture with a mean of 9.1 ± 5.6 mg/l.

Survival of microorganisms in urine
The fate of the enteric pathogens entering the urine tank is of vital importance for the health 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 was therefore necessary to estimate the survival of various microorganisms in urine as a function of time.

Survival of bacteria
Survival studies of bacteria in urine were performed at 4°C and 20°C. Their persistence was also investigated at some different dilutions of the urine and at different pH-values. Bacteria were added or originally present in the urine mixture. At different time intervals the bacteria were enumerated and T_{90}-values (time for 90% inactivation) for the different organisms were then estimated.

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 (Figure 1). A lower temperature and a higher dilution involved a longer survival of most bacteria. pH-values the furthest from neutral had the most negative effect on survival of the organisms. At pH 6 most of the bacteria had a better survival than at pH 9. The reduction of bacteria at high pH-values may be an effect partly of the pH and partly of the presence of ammonia.

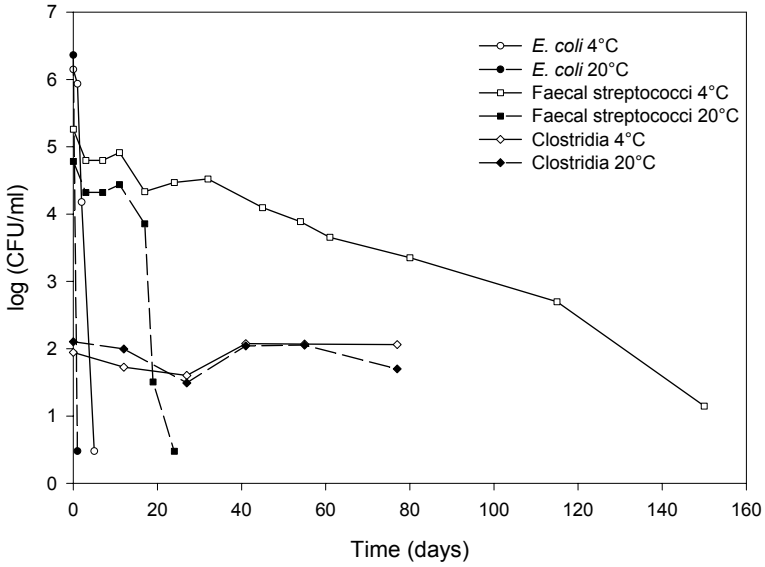


Figure 1. Inactivation of *E. coli*, faecal streptococci and *C. perfringens* spores (clostridia) in source-separated human urine (pH 9) at 4°C and 20°C.

Survival of protozoa

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

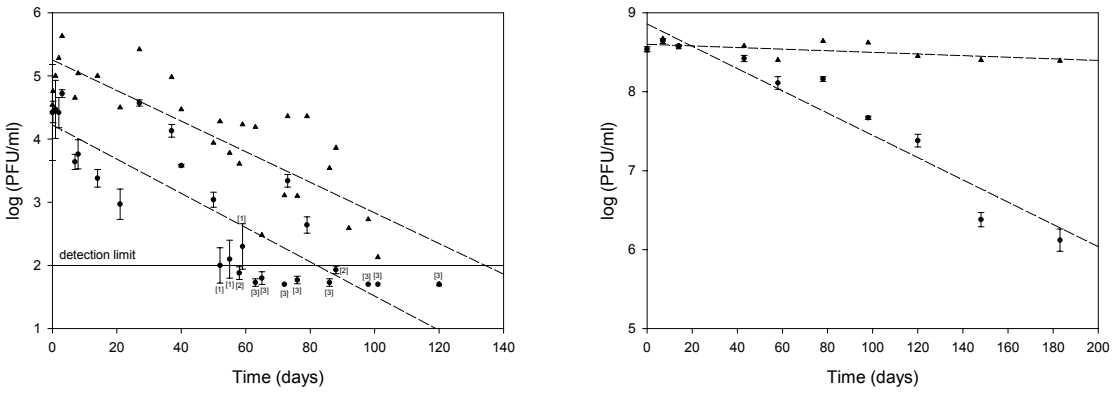
In urine mixture at pH 9 and 4°C, oocysts of the protozoa *Cryptosporidium parvum* were inactivated to below the detection limit (<1/300) within 63 days. The T_{90}-value for *Cryptosporidium* was determined at 29 days (Table 1). At 20°C the T_{90} was estimated at 5 days.

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 and in urine at pH 5 and pH 7, according to the dye permeability assay. In buffer solutions (pH 5, 7 and 9) there were no significant differences in inactivation rate between pH-values for 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.

Survival of viruses

To investigate virus survival rotavirus and *Salmonella typhimurium* phage 28B were chosen as model organisms. Rotaviruses were enumerated as peroxidase stained plaques in infected MA-104 cell monolayers (reported as PFU/ml). Phage 28B was quantified by the double agar layer method. 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.

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, respectively (Figure 2, Table 1). 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).



(a) (b)

Figure 2. Inactivation of (a) *rhesus* rotavirus and (b) *Salmonella typhimurium* phage 28B in diverted 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.

Discussion

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. 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 diverted urine.

Cryptosporidium oocysts were reduced by approximately 90% per month in the urine mixture and was considered to be the most resistant of all the protozoa. Thus other protozoa like *Giardia* and *Entamoeba* do not imply a higher risk than *Cryptosporidium*.

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. 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. Rotavirus has been reported to be as resistant or more resistant than several other enteric viruses (Ward *et al.*, 1989; Pesaro *et al.*, 1995).

For source-separated human urine mainly temperature, pH and ammonia were considered as affecting inactivation. 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. Temperature seemed to affect all microorganisms investigated. For bacteria further dilution of the urine prolonged the survival, which may be 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.

Table 1. Summarised results from the survival experiments, given as T_{90}-values (time for 90% reduction)

	Gram-negative bacteria	Gram-positive bacteria	*C. parvum*	Rotavirus	*S. typhimurium* phage 28B
4°C	1	30	29	172[a]	1 466[a]
20°C	1	5	5	35	71

[a] survival experiments performed at 5°C

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.

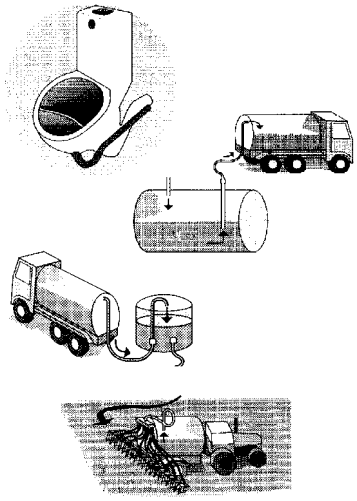
Microbial risk assessment of urine-separating systems

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). 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.

Exposure scenarios

The transmission pathways investigated in the QMRA included accidental ingestion of unstored urine (1 ml); accidental ingestion of stored urine (1 ml); inhalation of aerosols while spreading the urine; and ingestion of crops contaminated by urine (Figure 3). 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.

Calculations of the doses ingested were based on the measured faecal contamination, the incidence of infection by *Campylobacter jejuni*, *Cryptosporidium parvum* and rotavirus in the population, the excretion of these pathogens and their inactivation in urine mixture. Finally the risks for infection were calculated by using dose-response models.



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

Quantitative risks

Except for rotavirus, calculated risks were below 10^{-3} (1:1 000) for all exposure routes independent of the urine storage time and temperature evaluated (Table 2). Due to the persistence of rotavirus at low temperatures (≤5°C) and a low infectious dose risks for rotavirus infection were up to 0.56 by ingestion of unstored and stored (4°C) urine. If stored at a higher temperature (20°C) for six months, risk for rotavirus infection decreased to below 10^{-3}. 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 2. Calculated risks, means and (standard deviations), for a single exposure by accidental ingestion of urine and inhalation of aerosols in the worst-case scenario

Pathways	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 ($<10^{-15}$)

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 4, 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.

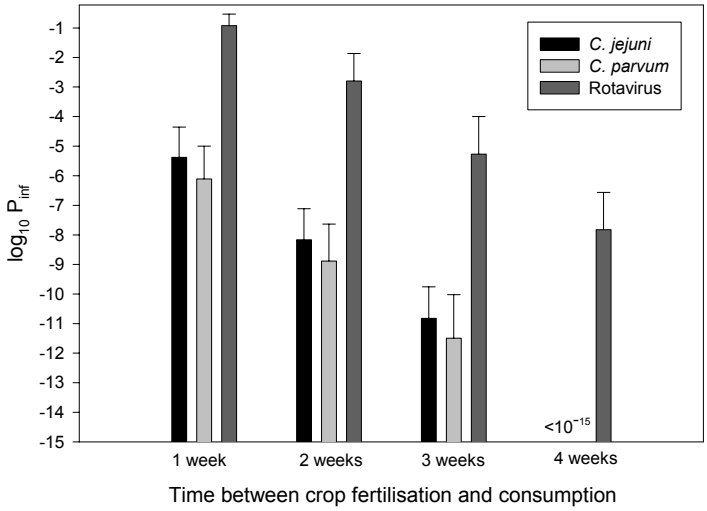


Figure 4. Mean probability of infection by pathogens following ingestion of 100 g crop fertilised with unstored urine with varying time between fertilisation and consumption. Error bars indicate one standard deviation.

Acceptable risk and risk management

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. 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).

Assuming that the acceptable risk for infection is 1:1 000 per year 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 2, Figure 4). 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). 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 for example by wearing gloves and mouth protection when handling urine. 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.

Guidelines for the reuse of human urine

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 3). 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. Regulatory standards or guidelines have yet to be determined by the agency responsible.

These guidelines were set based on the inactivation of microorganisms in urine and the results from the risk assessment do not imply that the recommendations need to be modified. Under conditions (i.e. regarding temperature, pH and nitrogen concentration) other than those given, the inactivation may be different.

Processing of crops, using e.g. heat, 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.

Table 3. 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.
[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.

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.

For diverted 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 and also leads to high nitrogen losses. Harrowing directly after spreading would further decrease the exposure for both humans and animals. 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.

Future perspectives

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.

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 applied to non-food crops the foodborne route of transmission is eliminated, but there is still an infection risk for people involved in the production and processing of crops as well as for humans and animals in the surroundings. By following suggested 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. Urine-separation and the reuse of human urine could thus be appropriate parts of a sustainable future regarding sanitation.

This paper is a summarised version of a doctoral thesis published by the author in February 2001: Höglund, C. (2001). *Evaluation of microbial health risks associated with the reuse of source separated human urine*. PhD thesis, Department of Biotechnology, Royal Institute of Technology, Stockholm, Sweden. The full thesis summary (87 pages, without papers) can be downloaded from: http://www.lib.kth.se/Sammanfattningar/hoglund010223.pdf

References

Asano, T. and Levine, A.D. (1998). Wastewater reclamation, recycling, and reuse: An introduction. In: Asano, T. (ed.) Wastewater reclamation and reuse. Technomic Publishing Company, Inc., Lancaster, PA, USA. p. 1-56.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G. and Stott, R. (2000). Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bulletin of the World Health Organization **78**(9):1104-1116.

Campbell, A.T., Robertson, L.J. and Smith, H.V. (1992). Viability of *Cryptosporidium parvum* oocysts: Correlation of in vitro excystation with inclusion or exclusion of fluorogenic vital dyes. Applied and Environmental Microbiology **58**(11):3488-3493.

Cooper, R.C. and Olivieri, A.W. (1998). Infectious disease concerns in wastewater reuse. In: Asano, T. (ed.) Wastewater reclamation and reuse. Technomic Publishing Company, Inc., Lancaster, PA, USA. p. 489-520.

Feachem, R.G., Bradley, D.J., Garelick, H. and Mara, D.D. (1983). Sanitation and Disease - Health aspects of excreta and wastewater management. John Wiley and Sons, Chichester, UK.

Haas, C.N. (1996). Acceptable Microbial Risk. Journal of the American Water Works Association **88**(12):8.

Haas, C.N., Rose, J.B. and Gerba, C.P. (1999). Quantitative Microbial Risk Assessment. John Wiley and Sons, Inc., New York, NY, USA.

Hamdy, E.I. (1970). Urine as an Ascaris lumbricoides ovicide. Journal of the EgyptianMedical Association **53**:261-264. In: Feachem, R.G., Bradley, D.J., Garelick, H. and Mara, D.D. 1983. Sanitation and Disease - Health aspects of excreta and wastewater management. John Wiley and Sons, Chichester, UK.

Lewis-Jones, R. and Winkler, M. (1991). Sludge parasites and other pathogens. Ellis Horwood Limited, Chichester, UK.

Meinhardt, P.L., Casemore, D.P., Miller, K.B. (1996). Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. Epidemiologic Reviews **18**(2):118-136.

Olsson, A. (1995). Source separated human urine - occurrence and survival of faecal microorganisms and chemical composition. (Källsorterad humanurin - förekomst och överlevnad av fekala mikroorganismer samt kemisk sammansättning). Report 208, Department of Agricultural Engineering, Swedish University of Agricultural Sciences, Uppsala, Sweden. (In Swedish, English summary).

Pesaro, F., Sorg, I. and Metzler, A. (1995). In situ inactivation of animal viruses and a coliphage in nonaerated liquid and semiliquid animal wastes. Applied and Environmental Microbiology **61**(1):92-97.

Porter, A. (1938). The larval Trematoda found in certain South African Mollusca with special reference to schistosomiasis (bilharziasis). Publications of the South African Institute for Medical Research **8**:1-492. In: Feachem, R.G., Bradley, D.J., Garelick, H. and Mara, D.D. 1983. Sanitation and Disease - Health aspects of excreta and wastewater management. John Wiley and Sons, Chichester, UK.

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

Robertson, L.J., Campbell, A.T. and Smith, H.V. (1993). In vitro excystation of Cryptosporidium parvum. Parasitology **106**:13-19.

Swedish EPA. (1995). What does household wastewater contain? (Vad innehåller avlopp från hushåll?). Report 4425, Swedish Environmental Protection Agency, Stockholm, Sweden. (In Swedish).

Tortora, G.J., Funke, B.R. and Case, C.L. (1992). Microbiology: an introduction. The Benjamin/Cummings Publishing Company, Inc., Redwood City, California, USA.

Vivian, C.M.G. (1986). Tracers of sewage sludge in the marine environment: A review. The Science of the Total Environment **53**:5-40.

Ward, R.L., Knowlton, D.R., Stober, J., Jakubowski, W., Mills, T., Graham, P. and Camann, D.E. (1989). Effect of wastewater spray irrigation on rotavirus infection rates in an exposed population. Water Research **23**(12):1503-1509.