Workshop “Removal of Viruses from Water by Flocculation/Filtration”,
Berlin 23 November 2005

PROTOCOL

The workshop was divided into two parts (see attachment). In the morning session (A), five talks were given concerning flocculation/filtration processes, the presence and significance of viruses in raw and finished drinking water as well as the need for indicators for viral pathogens. In the second part of the workshop (B), two working groups were preparing consensus statements for different topics.

A. Short summary of the Presentations

A.1 Removal of Particles from Water by Flocculation/Filtration
Dr. Pia Lipp, Technologiezentrum Wasser (TZW), Karlsruhe

Pia Lipp gave an introduction to the processes during flocculation/filtration as important treatment step for drinking water production. Both processes – filtration and flocculation – have to be optimized to achieve high removal efficiency for particles. Optimization of filtration can be achieved by: filtration velocity < 8 m/h, no changes in velocity of > 3%, state of the art backwash, no start up of dirty filters. For the flocculation step, chemical dosage and mixing have to be optimized for each raw water quality. This can be tested in jar tests in the laboratory. Particle removal has to be optimized to allow for effective disinfection of the filtrate. Drinking water production from surface water always includes several treatment steps and a disinfection step in Germany. Twenty eight per cent of the German drinking water is produced from surface water (1 – 2 % from rivers). Resource protection is an important step in the multi barrier system to obtain raw water with low turbidity and low concentration of indicator microorganisms.

The efficiency of the flocculation/filtration process can be monitored by turbidity measurements (for particles 0.02-10 µm) and particle counting (for particles > 1 µm). Pia Lipp quotes the recommendation for treatment of surface water in Germany (Bundesgesundheitsblatt 1997) as state of the art, which recommends < 0.2 NTU for the filtrate of each single filter prior to disinfection, as well as colony count < 100 cfu/ml and E. coli < 1/100 ml. Rainfall events typically enhance turbidity, and this is often, but not always, associated with elevated levels of microbial indicators.

The discussion after the presentation revealed that in Germany it is not warranted that all plants obtaining drinking water from surface water do include a disinfection step.

A.2 Viruses in Finished Water: Are They There?
Dr. Rosina Gironés, University of Barcelona

Rosina Gironés started with a general introduction to viral pathogens. The principal human enteric viruses are enterovirus, rotavirus, adenovirus, norovirus, hepatitis A and E virus, astrovirus and polyomavirus. Transmission occurs via food, water, fomites, and human to human contact. Enteric viruses may cause gastrointestinal disorders, hepatitis and other diseases (e.g. meningitis, neurological disorders, myocarditis). Infections are often sub-
clinical, and this limits the current understanding of their relevance for human health when occurring in drinking-water.

Rosina Gironés presents two possible candidates for viral indicators in drinking water production: adenoviruses and polyomaviruses (especially JC-Virus and BK-Virus. The initials JC and BK refer to the patients the viruses were originally isolated from). Both are very abundant in sewage independently of season and location as some adenoviruses and polyomaviruses infect children and persist for many years. Porcine adenoviruses can be used to indicate contamination from pig farms. A further advantage of these viruses is that they are DNA-viruses and, therefore, easier to quantify with PCR than RNA-viruses.

Quantitative PCR methods have been developed for the detection of human adenoviruses and JCV. Recovery of the method is high (70 %) in untreated sewage where high concentrations of viruses are present in small volumes. Matrix effects inhibiting enzymes may, however, be a problem for analyses of wastewater. In treated waste water and drinking water viruses need to be concentrated from 50 L or 1000 L, respectively. Recovery of the method drops to 0.1 % - 1 % for these samples.

The treatment efficiency of a water work was investigated using this method. For adenoviruses, virus levels of $10^2$ particles per liter were detected in the raw water (river water). After extensive treatment - including sand filtration, ozonation, active carbon filtration and chlorination – virus levels of 4 particles per liter were detected in the finished drinking water (values not corrected for recovery). The resulting treatment efficiency amounts to about 99 %. JVC was detected in lower concentrations in the river water and in the finished drinking water resulting in comparable treatment efficiencies. No BKV or hepatitis E viruses were detected in finished drinking water.

The discussion focused on the detection of viruses in treated drinking water. Risk assessment would come out with rather high risk of infection with about 4 viruses per L (corresponding to 400 viruses when method efficiency is taken into account) still found in treated water. The problem is that the method cannot differentiate between infectious and inactivated, non infectious virus particles.

A.3 The Risk of Consuming Water Containing a Low Concentration of Viral Pathogens

Dr. Ana Maria de Roda Husman, RIVM, The Netherlands

Ana Maria de Roda Husman explained the strategy for quantitative risk assessment of water borne infections in the Netherlands.

The Dutch DW Act established a maximal annual infectious risk of $10^{-4}$ as standard for (entero)viruses, Cryptosporidium and Giardia. Risk assessment has to be performed for surface waters and vulnerable ground waters as resources for drinking water production. The first step for the risk assessment is the identification of the most important water borne pathogens. Criteria to choose the most important pathogen(s) are: spread via water, extent of health risk, prevention possibilities, epidemiology, prevalence in sewage, resistance to disinfection and others. For instance we know that rotavirus and norovirus are, the most predominant causes for gastroenteritis in The Netherlands. Hepatitis A and E viruses are important because of their epidemic spread. Viruses most prevalent in water are adenoviruses and enteroviruses. Sometimes it is difficult to differentiate waterborne from other transmission routes e.g. unrecognized primary infection may be due to water but then is followed by rapid secondary person-to-person spread, which disguises source. For pathogens considered important data have to be gathered concerning their occurrence in raw water sources and their behaviour during treatment processes. Short term fluctuations (e.g. heavy rainfall) have to be considered when measuring the concentration in the source water. Phages may be used as indicators for testing the efficiency of treatment processes. For coagulation
followed by rapid sand filtration a treatment efficiency of 95 % has been determined for coliphages (somatic and F-specific). Since treatment process efficiency may vary considerably in different plants validation is needed locally. Furthermore, the validity of phages as indicators has to be verified in laboratory experiments using the viruses of interest in comparative investigations. From these data the concentration of viruses to be expected in the finished drinking water can be calculated.

The second step in the risk assessment is the exposure assessment. The dose can be calculated from the concentration of viruses in finished drinking water and the drinking water consumption. It is not meaningful to measure the concentration of viruses in drinking water since the concentrations for acceptable health risks are way below the detection limit of the currently available methods. Concentrations of viruses have to be calculated from raw water concentrations and system assessment (see above). Drinking water consumption data in The Netherlands (data from 1997) have revealed that a mean of 220 ml are consumed per person per day. Using these consumption data, realistic numbers for risk assessment are obtained in contrast to the worst case scenario using a – for most people unrealistic - consumption of two liters per person per day.

The third step in the risk assessment is the hazard characterization. Dose-response relationships are available for some pathogens including some viruses (norovirus, rotavirus, enterovirus). These data can, however, not be generalized because of the very high variability between different virus types.

The fourth and last step in the risk assessment is the risk characterization i.e. the estimation of the infectious risk. The daily infection risk can be calculated using the calculated dose and the infection probability from the dose-response relationship. The annual infection risk is assessed by modelling.

The annual infection risk will never be zero and will increase with higher virus concentrations. The calculations for the risk assessment have to include uncertainty and sensitivity and the risk assessments need to be periodically reviewed and repeated.

A.4 Indicators of Feacal Pollution in Water and Their Significance
Dr. Willi Grabow, University of Pretoria

Willi Grabow emphasized that the golden rule of the 20th Century still remains valid: no fecal pollution → no disease;
no E. coli (fecal indicator bacteria) → no fecal pollution.
The ideal indicator for fecal pollution will be i) present whenever pathogens are present, ii) present in same or higher numbers as the pathogens, iii) specific for fecal pollution, iv) at least as resistant as pathogens, v) non-pathogenic, and vi) detectable by simple and inexpensive techniques.

Shortcomings of the fecal indicator principle are, however,:
  • No ideal indicators complying with all criteria
  • No direct correlation of indicators to any pathogen
  • No quantitative risk assessment possible
  • No indication for non fecal pathogens like Legionella, Mycobacterium, some protozoa.
  • Not suitable as operational indicators for process efficiency, as their resistance may be quite different to that of the pathogens

Due to these shortcomings, endpoint monitoring of drinking water alone is not sufficient. Pathogens including viruses might be present in drinking water that has passed requirements (requirements of absence of fecal indicators). Extensive epidemiological studies are important tools to reveal cases of waterborne disease which might otherwise be overlooked. In Sweden
several outbreaks with *Campylobacter* were detected during a research project while before and after the project hardly any cases were reported. The indicator concept can be improved by better definitions of the indicators (e.g. “coliforms” are a very bad indicator in this respect), by improved sensitivity of the detection methods or by additional indicators. Several alternative indicators have been proposed, e.g. *Clostridium perfringens* due to the high chlorine-resistance of their spores and bacteriophages due to their structural similarity to human pathogenic viruses. Another possibility is to directly use pathogens as indicators (*Cryptosporidium* or different viruses). These are, however, no ideal indicators because they are pathogenic and not easy to measure.

Since no ideal indicators exist for endpoint monitoring and there might, therefore, be an unacceptable risk of infection due to e.g. viruses other approaches are needed. Sterile drinking water is not a practical alternative. The statement in the German Drinking Water Act “Trinkwasser muss frei sein von Krankheitserregern” (drinking water has to be free from pathogens) is, therefore, not practical and never achievable. The concept of risk assessment has to be applied also to drinking water. The WHO proposed in the Water Safety Plans the HACCP-concept (Hazard Assessment Critical Control Points). The HACCP-concept for drinking water production includes a multi barrier treatment system leading to a final quality which complies with the acceptable risk of infection. Indicators are used only to check the Critical Control Points. The acceptable risk of infection was set by the WHO to one infection per 10 000 consumers per year, i.e. 100 per one million. This corresponds to a tolerable Disease Burden of $10^{-6}$ DALYs per year.

To comply with these targets, the numbers of viruses in finished drinking water has to be:

- $< 1$ per 100,000 liters for rotavirus and polio 3
- $< 1$ per 10,000 liters for polio 1 and echovirus 12.

This deduction very clearly shows that end point monitoring is not practical.

The HACCP-concept requires knowledge on the treatment efficiency of the treatment steps applied in drinking water production. Phages can be used for the assessment of treatment efficiencies. The WHO gives some examples for treatment efficiencies for virus removal:

- Flocculation/filtration 30 %
- Slow sand filtration 20 %
- Chlorine, ozone 100 %
  (but residual chlorine levels are too low for virus inactivation)
- UV 99 %
- Microfiltration 90 %
- Ultra-, Nanofiltration 100 %
  (nevertheless, membrane filtration should only be used as part of a multi barrier system)

In the past indicators were used to indicate the absence of pathogens. In the future there will be a need for operational indicators for the control of the Critical Control Points.

Recommendation: We need to avoid drastic changes but assess step by step whether risks in Germany are acceptable with respect to viruses. A substantial amount of research on occurrence of viruses in raw water and during treatment processes would be appropriate. If upgrading is considered necessary new techniques such as membrane filtration may be considered.

**A.5 Viruses in Surface Water and the Means to Detect them**

Dr. Peter Wyn-Jones, University of Wales
Peter Wyn-Jones started his presentation with an overview of potential transmission routes of viruses via water. In the past, evidence for viruses associated with water borne outbreaks were epidemiological, circumstantial or anecdotal. The aetiology of the outbreak remained often unclear. Viruses were not analysed or not detected. An outbreak in Bramham, Yorkshire 1980 caused approximately 3000 cases of illness. From current knowledge norovirus seems to have been the likely cause, but at the time no viral investigation was performed due to lack of suitable techniques. Sewage leaking into a borehole from a cracked sewage pipe and chlorination failure were found as causes for the outbreak.

In a comparable outbreak (pollution of source water, chlorination failure) 1998 in Finland with 1700-3000 cases, norovirus genogroup II was detected in the water and in stools of patients. These examples show the progress over the years in detecting viruses from environmental and clinical samples.

Some enteric viruses grow in cell culture (many enteroviruses and adenoviruses) but many are only detectable by molecular methods (rotavirus, calicivirus, astrovirus, hepatitis E virus, polyomavirus). The ideal detection method for viruses would i) be easy to perform, ii) yield a high recovery, iii) have a small resulting volume after concentration and iv) be not costly. There is currently no ideal detection method available.

The most critical step in the detection of viruses is the concentration step prior to analysis. Samples size varies from 100 ml for irrigation water/sewage effluent to 10 litres for recreational water and 100 – > 1000 litres for drinking water. Concentration can be performed by adsorption to a non reactive matrix (for example, negatively or positively charged membranes, glass wool or glass powder), by entrapment/ultrafiltration, by ultracentrifugation or by immunadsorption. Often, a combination of techniques is used. All concentration methods have advantages but also disadvantages. A method comparison using membrane filtration or glass wool adsorption, both followed by organic flocculation is currently undertaken in the EU-project “Virobathe”. This project is an important step to improve methods and achieve greater consistency in virus detection.

The concentrate can be analysed by cell culture or PCR methods. PCR tests can be tailored to specific viruses or to a whole range of viruses. Results are obtained quicker than with cell culture and at a lower cost. PCR tests may be used for a first screening followed – for positive results - by infectivity assays where possible. Quantitative PCR methods allow quantitative detection by molecular means.

Representative investigations of viruses in raw water have to be performed at different seasons and under different meteorological conditions.

**B Resolutions from the working groups**

**B.1 Working group I: Indicators for viruses surface water**

1. Bacteria are no good indicators for viruses in surface water (and treatment processes).
2. Viral indicators are better indicators for pathogenic viruses due to their comparable structure.
3. Coliphages are easy to detect and may be used as first indication for the presence of pathogenic viruses. They have been shown to correlate with health symptoms in epidemiological studies on bathing waters. Good correlation has also been found to culturable enterovirus. No correlation has been found, however, to many other important pathogenic viruses like norovirus. Coliphages are good indicators for assessing treatment processes because they are small and double stranded.
4. Abundant enteric viruses like adenovirus are ideal candidates to be used as indicators for enteric viruses in surface water. Detection methods are to date, however, only available in few laboratories and are costly.

5. The most critical step in virus detection is the concentration step. The concentrate can in the future easily be analysed by PCR not only for indicator viruses but also for pathogenic viruses of interest if the detection methods are available. This will enable quantitative risk assessment for many pathogenic viruses (worst-case approach because it remains unclear whether or not they are infectious).

6. Research needs:
   Method development for detection of pathogenic viruses.
   Studies on correlation of indicator viruses to pathogenic viruses.
   More information about virus occurrence in raw water also regarding spatial and temporal variation.

B.2 Working group II: Finished water and flocculation/filtration process

1. The WHO approach using risk assessment and HACCP concept for drinking water safety is approved.

2. Routine monitoring of viruses in drinking water is not recommended because concentrations of acceptable risk are way below detection limit of currently available methods. Failure of detecting viruses with currently available methods does not imply compliance with acceptable health risk standards. This is considered a problem because the water works cannot show the consumer by measurement data that the water is “safe”.

3. Risk communication is considered very difficult because the public is not accustomed to the concept of relative risk.

4. Sensitive detection methods have to be validated and standardized to be used primarily in outbreak investigations but also- if possible in the future - for monitoring.

5. The necessity of disinfection after a flocculation/filtration process should be deduced from risk assessment. Disinfection should not be automatically required. Flocculation/filtration may be sufficient for very good raw water quality. The risk assessment approach is better because it triggers thinking and system understanding.

6. Research needs:
   Development and standardisation of sensitive detection methods.
   Optimization of treatment processes using viruses.