Water Research 158 (2019) 34–45

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

QMRA of adenovirus in drinking water at a drinking water treatment plant using UV and chlorine dioxide disinfection

Jack Schijven [a, b, *], Peter Teunis [c], Trudy Suylen [d], Henk Ketelaars [d], Luc Hornstra [e], Saskia Rutjes [a]

[a] National Institute of Public Health and the Environment, Bilthoven, the Netherlands
[b] Department of Earth Sciences, University of Utrecht, Utrecht, the Netherlands
[c] Emory University, Atlanta, USA
[d] Evides Water Company, the Netherlands
[e] KWR Watercycle Research Institute, Nieuwegein, the Netherlands

ARTICLE INFO

Article history:
Received 22 January 2019
Received in revised form
26 March 2019
Accepted 27 March 2019
Available online 1 April 2019

Keywords:
Adenovirus
Chlorine dioxide disinfection
Drinking water
PCR
QMRA

ABSTRACT

According to the Dutch Drinking Water Act of 2011, Dutch drinking water suppliers must conduct a Quantitative Microbial Risk Assessment (QMRA) for infection by the following index pathogens: enterovirus, *Campylobacter*, *Cryptosporidium* and *Giardia* at least once every four years in order to assess the microbial safety of drinking water. The health-based target for safe drinking water is set at less than one infection per 10 000 persons per year. At Evides Water Company, concern has arisen whether their drinking water treatment, mainly based on UV inactivation and chlorine dioxide, reduces levels of adenovirus (AdV) sufficiently. The main objective was, therefore, to conduct a QMRA for AdV. Estimates of the AdV concentrations in source water were based on enumeration of total AdV by integrated cell culture PCR (iccPCR), most probable number PCR (mpnPCR) and quantitative PCR (qPCR), and on enumeration of AdV40/41 by mpnPCR and qPCR. AdV40/41 represents a large fraction of total AdV and only a small fraction of AdV is infectious (1/1700). By comparison of literature data and plant scale data, somatic coliphages appeared a good, conservative indicator for AdV disinfection by UV irradiation. Similarly, bacteriophage MS2 appeared to be a good, conservative indicator for disinfection by chlorine dioxide. Literature data on the efficiency of chlorine dioxide disinfection were fitted with the extended HOM model. Chlorine dioxide disinfection at low initial concentrations (0.05–0.1 mg/l) was found to be the major treatment step, providing sufficient treatment on its own for compliance with the health-based target. UV disinfection of AdV at 40 mJ/cm^2 or 73 mJ/cm^2 was insufficient without chlorine dioxide disinfection.

1. Introduction

According to the Dutch Drinking Water Act of 2011, Dutch drinking water suppliers must conduct a Quantitative Microbial Risk Assessment (QMRA) for the so-called index pathogens enterovirus, *Campylobacter*, *Cryptosporidium* and *Giardia* at least once every four years in order to assess the microbial safety of drinking water (Anonymous, 2011). In this QMRA, the health-based target for microbiologically safe drinking water is set at less than one infection per 10 000 persons per year, thereby following the World Health Organization (WHO) Guidelines for Drinking-water Quality (WHO, 2017). It is not feasible to conduct a QMRA for every waterborne pathogen, therefore, index pathogens were chosen. If a drinking water treatment is effective in removing the index pathogens, the drinking water is considered to be safe. Nevertheless, in specific situations, this might not be the case. At Evides Water Company, concern has arisen whether their drinking water treatment reduces levels of adenovirus (AdV) sufficiently. In the source water (water from a storage reservoir that is taken in for drinking water production), AdV concentrations are highest from January to April. AdV is known to be more persistent to UV radiation than enteroviruses, the selected index viral pathogen (Hijnen et al., 2006). UV radiation and chlorine dioxide (final treatment step) are the major treatment steps employed by Evides at the

* Corresponding author. National Institute of Public Health and the Environment, Bilthoven, the Netherlands.
E-mail address: jack.schijven@rivm.nl (J. Schijven).

https://doi.org/10.1016/j.watres.2019.03.090
0043-1354/

drinking water treatment plant (DWTP) Berenplaat.

AdV is widespread in nature, infecting birds, mammals and amphibians. To date, 90 genotypes of human adenoviruses have been identified based on whole genome sequencing (Ismail et al., 2018). Human adenoviruses have been classified into seven groups (A–G) on the basis of their physical, chemical and biological properties (Wold and Ison, 2013). Adenoviruses consist of a double-stranded DNA genome in a non-enveloped icosahedral capsid with a diameter of about 80 nm and unique fibres. Human adenoviruses cause a wide range of infections with a spectrum of clinical manifestations, including the gastrointestinal tract, the respiratory tract, the urinary tract and the eyes (Brandt et al., 1969; Bon et al., 1999; Oh et al., 2003; Rodriguez-Baez et al., 2002). Serotypes 40 and 41 are a major cause of gastroenteritis worldwide, notably in developing communities, however, little is known about the presence of these enteric adenoviruses in water sources largely because they are not detectable by conventional cell culture (WHO, 2017). WHO (2017) classifies AdV as having moderate health significance, long persistence, moderate resistance to chlorine and high infectivity.

The main objective was to conduct a QMRA for AdV in the drinking water produced at DWPT Berenplaat.

In general, QMRA for a waterborne pathogen in drinking water consists of determining the pathogen concentration in the source water, its removal by treatment using data on the removal of the pathogen or of a representative indicator organism, using drinking water consumption data and a dose response relationship (WHO, 2017). For the adenovirus QMRA at Berenplaat, estimates of the concentrations in source water were based on total AdV concentrations as determined by integrated cell culture PCR (infectious virus particles) and by most probable number PCR and quantitative PCR (infectious as well as non-infectious virus particles), and on AdV40/41 concentrations as determined by most probable number PCR and quantitative PCR. Here, it was an additional objective to compare PCR data generated as most probable numbers with those generated as genome copies. On the one hand, inhibition of polymerase causes underestimation with quantitative PCR, but not with most probable number PCR. On the other hand, aggregates of virus particles cause underestimation with most probable number PCR, but not with quantitative PCR. In the QMRA for enterovirus, as is conducted in the Netherlands by the drinking water companies, F-specific RNA bacteriophages or somatic coliphages are the default indicator organisms for determining the efficiency of treatment steps in the drinking water production to remove enteroviruses (Schijven et al., 2011). The data on these indicators were used to determine removal efficiency by coagulation followed by sludge blanket clarification and rapid double-layered filtration (sand/anthracite). In order to estimate the efficiency of UV radiation, somatic coliphage concentration measurements were conducted at plant scale and compared with literature data on disinfection of AdV by UV in order to evaluate somatic coliphages as surrogate for UV disinfection of AdV. In addition, the efficiency of medium pressure lamps emitting an UV dose of 40 mJ/cm^2 and 73 mJ/cm^2 were compared. Estimating the efficiency of chlorine dioxide disinfection relied on data from literature and additional laboratory experiments in order to evaluate MS2 bacteriophage as a surrogate for chlorine dioxide disinfection of AdV. Because of a notable seasonal variation of AdV concentrations in the source water and a different dosage of chlorine dioxide during winter time (October–March) and summer time (April–September), all source water concentration data and all treatment data were split into winter and summer time data and distributions were fitted to winter and summer data separately. Effectivity of the drinking water treatment was evaluated on the basis of the contribution of UV irradiation and chlorine dioxide disinfection to the total treatment.

2. Drinking water treatment description

DWTP Berenplaat provides 100 million m^3 of drinking water each year and supplies water to a large proportion of Rotterdam's homes and companies. River Meuse water is stored in the Biesbosch storage reservoirs with an average retention time of five months to improve its chemical and biological quality by natural processes such as degradation and sedimentation. About 12,000 m^3/h of this water is transported to DWTP Berenplaat, where it passes a short water abstraction channel prior to treatment. Pathogen concentrations in Berenplaat-Petrusplaat (the final Biesbosch storage reservoir) water are the starting point for QMRA. Berenplaat water first passes micro-sieves (mesh size 35 μm) and is then treated by coagulation/sludge blanket clarification and rapid double layered filtration. The major disinfection takes place in two parallel arrays of medium pressure UV lamps, one at 40 mJ/cm^2 and the other at 73 mJ/cm^2. It is assumed that the UV lamps provide a constant dose. The setpoint UV-dose for the target disinfection capacity is continuously maintained in the reactor by automatic adjustment of the UV-lamps' power setting i.e. emission intensity, according to variations in operating conditions such as influent water quality (UV-transmission) and flow rate. After UV treatment, the water passes granular activated carbon filters. Hijnen et al. (2010) found no removal of bacteriophage MS2 by granular activated carbon filters at pilot plant scale at this DWTP. Hence, this treatment step is considered to be irrelevant in the QMRA for AdV. The final treatment consists of disinfection with 0.1 mg/l chlorine dioxide in summer and 0.05 mg/l chlorine dioxide in winter. The finished water is stored in closed reservoirs prior to distribution.

3. Materials and methods

3.1. Adenovirus enumeration

Throughout this document, total AdV is referred to as AdVtot, AdV40 and AdV41 are referred to as AdV40/41. All enumerations are PCR methods. Integrated cell culture PCR, most probable number PCR and (real time) quantitative PCR are designated as icc, mpn and q, respectively. The following enumerations were conducted: AdVtoticc, AdVtotmpn, AdVtotq, AdV40/41mpn and AdV40/41q.

3.2. Sampling and concentration by UF

Thirty-five samples of water (approximately 600 L) were collected from the final storage reservoir prior to drinking water treatment and concentrated by a conventional filter adsorption-elution method as previously described (Rutjes et al., 2009). Briefly, magnesium chloride was added to the water sample to a final concentration of 0.05 M to enable the formation of a virus-magnesium complex. By reducing the pH to 3.8 with 0.5 M HCl, these complexes adsorb to a negatively charged cartridge filter with a nominal pore size of 1.2 μm. Viruses were eluted from the filter with an elution buffer of pH 9.0, and were neutralized with a concentrated acetic acid buffer (pH 5.0) resulting in a final eluate with a pH of approximately 7.4. The eluate was further concentrated by ultrafiltration (UF) using a cellulose-acetate filter (NMWL 10 000) under high pressure (3 bar) (Rutjes et al., 2005). The final UF-concentrate volume was usually between 50 and 75 ml and sometimes up to 250 ml. The concentrate was stored at −70 °C until further use.

3.3. AdVtoticc

Human AdV-2 was kindly provided by the group of Dr. Franco M. Ruggeri (Istituto Superiore de Sanitá, Rome, Italy) to use as positive

control virus.

Infectious adenoviruses were enumerated by integrated cell culture PCR. UF-concentrates were quickly thawed at 37 °C. In order to inactivate bacteria, the UF-concentrates were supplemented 1/5 (v/v) with a mixture of antibiotics (final concentrations: 579 μg/ml penicillin G, 4476 U/ml streptomycin sulfate, 72 μg/ml amphotericin B, 2.9 mg/ml kanamycin monosulphate and 576 μg/ml neomycin) and incubated for 1 h at room temperature in the dark.

For each UF-concentrate, A549 cells (ATTC-CCL-185) were grown to confluent monolayers in cell culture flasks (25 cm^2) with 1 × MEM (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen), 1% penicillin/streptomycin and 1% non-essential amino acids (Invitrogen). The numbers of flasks that were inoculated with undiluted UF-concentrate varied from 9 till 31, the numbers of 1/10 diluted samples varied from 3 till 18, and occasionally 1/100 and 1/1000 dilutions were analyzed. UF-concentrate/antibiotic mixtures were incubated on A549 cells for 1 h at 37 °C with 1 ml inoculum of UF-concentrate. Every time, one flask was not inoculated to serve as a negative control. Then, cell cultures were washed 3 times with warmed phosphate buffered saline and 5 ml of warmed 1 × MEM containing 2% fetal calf serum was added. Per sample, three flasks inoculated with undiluted sample were immediately frozen (T_0), the other flasks and the negative control flask were further incubated for 5 days at 37 °C, followed by storage at −70 °C until further use.

For PCR enumeration virus stocks were obtained by three freeze−thaw cycles of infected cells and subsequent centrifugation at 1500*g* for 5 min. Of each supernatant, 140 μl was used for nucleic acid extraction. Nucleic acid was extracted using the NucliSens miniMag magnetic extraction kit (bioMérieux, Zaltbommel, The Netherlands) following the manufacturer's instructions with minor modifications. In each extraction cycle, a negative control containing no target was included. A Lightcycler 480 (Roche Diagnostics, Almere, The Netherlands) was used for real time PCR with TaqMan hydrolysis probes. To control for PCR inhibition, a competitive internal amplification control (IAC) (Yorkshire Bioscience Ltd., York, UK), specific for the AdVtot PCR (Diez-Valcarce et al., 2011), was added to each reaction at a concentration previously found not to influence the detection of the target signal. Instead of FAM, VIC was used as a reporter dye of the IAC. Detection of AdVtot DNA was performed as described by Verhaelen et al. (2012).

3.4. Extraction detection of viral DNA

Genomic material was isolated from 12.5 μl UF-concentrate (corresponding to 30 ml−150 ml of the storage reservoir water). The Nuclisens miniMAG Nucleic Acid Isolation Kit (bioMérieux, Zaltbommel, the Netherlands) was used as described (Rutjes et al., 2005). DNA was eluted in 100 μl elution buffer and stored at −70 °C.

3.5. AdVtotq and AdV40/41q

Quantification of AdVtot and AdV40/41 was performed by real-time quantitative PCR using a Lightcycler 480 (Roche Diagnostics, Almere, The Netherlands). For AdVtot detection, the hexon gene was targeted by forward primer (900 nM) CWT ACA TGC ACA TCK CSG G, reverse primer (900 nM) CRC GGG CRA AYT GCA CCA G and probe (11.25 μM) (FAM)-CCG GGC TCA GGT ACT CCG AGG CGT CCT-(BHQ1) (Hernroth et al., 2002). For AdV40/41 detection, the fiber gene was targeted by forward primer (450 nM) CTT TCT CTC TT (A/C) ATA GAC GCC C, reverse primer (22.5 μM) GAG GGG GCT A (G/C) AAA ACA AAA and probe (450 nM) (FAM)-CGG GCA CTC TTC GCC TTC AAA GTG C-(BHQ-1) (Jothikumar et al., 2005).

AdVtot and AdV40/41 were amplified using the TaqMan Universal PCR Master Mix (Applied Biosystems) combined with TaqMan hydrolysis probes as has been described by Bofill-Mas et al. (2010). Neat and a tenfold dilution of the DNA extracts were run in duplicate (4 reactions/sample).

AdVtot was quantified using plasmid pBR322 containing the HAdV 41 hexon sequence as a qPCR standard to quantify the number of genome copies in the samples (Bofill-Mas et al., 2010). AdV40/41 was quantified as described by Jothikumar et al. (2005).

3.6. AdVtotmpn and AdV40/41mpn

Presence/absence data of AdVtot and AdV40/41 were obtained by qPCR for the hexon and fiber gene, respectively.

3.7. Somatic coliphages enumeration

Influent and effluent samples of the first three treatment steps, coagulation/sludge blanket clarification, rapid sand filtration and UV disinfection (40 and 73 mJ/cm^2) were collected weekly to monthly from 2009 to 2012 for enumeration of somatic coliphages according to ISO 10705-2 (2000). Sample sizes varied from 0.03 L to 40 L for coagulation/sludge blanket clarification, from 0.1 L to 160 L for rapid sand filtration and from 0.1 L to 1900 L for UV disinfection.

4. Data analysis

All computational analyses were conducted using Mathematica (version 11.1.1.0, Wolfram Inc, Illinois).

4.1. Scenarios

Twelve risk assessments were conducted, namely using the virus source concentrations of AdVtoticc, AdV40/41mpn and AdV40/41q, applying a UV dose of 40 mJ/cm^2 or 73 mJ/cm^2, and with or without chlorine dioxide disinfection.

4.2. Virus source concentration estimation

Best estimates of most probable numbers per sample were used to calculate sample concentrations, to which, subsequently, Gamma distributions were fitted (Schijven et al., 2011). To the data from qPCR enumeration (genome copies per litre) a negative binomial distribution was fitted from which a Gamma distributed concentration was derived as described by Schijven et al. (2011).

To the paired positive AdVtoticc and AdVtotmpn samples, as well as to the paired positive AdVtoticc and AdVtotq samples, Beta distributions were fitted as described for determining recovery efficiency by Schijven et al. (2011). These Beta distributions represent the fractions of infectious virus particles, based on either mpnPCR or qPCR data. For each Gamma distribution (winter and summer), ten thousand Monte Carlo (MC) samples were generated. MC samples of infectious AdV40/41 virus particles were calculated by multiplying the Gamma-distributed concentration data of AdV40/41mpn and AdV40/41q with the Beta-distributed fractions of infectious virus particles based on mpnPCR and qPCR data, respectively.

For comparing with other fractions of infectious virus particles, literature data on enumeration of rotavirus by means of iccPCR and mpnPCR in samples from the River Meuse (Rutjes et al., 2009) and enterovirus (Lodder et al., 2015) by means of tissue culture and mpnPCR were collected and their ratio was determined by fitting a Beta distribution as well as described here above.

4.3. Estimation of treatment efficiency using plant scale somatic coliphage data

For the first three treatment steps: coagulation/sludge blanket clarification, rapid sand filtration and UV disinfection (40 and 73 mJ/cm^2), Beta distributions were fitted to the winter and summer influent and effluent unpaired raw data for each of these treatments to characterize the fraction of virus particles passing treatment, as described in detail for QMRAspot (Schijven et al., 2011).

4.4. UV disinfection

In order to evaluate somatic coliphage as a surrogate for AdV, the plant scale disinfection of somatic coliphages was compared with literature data on disinfection of AdV. The literature data were taken from Guo et al. (2010) and Linden et al. (2007) for medium pressure UV lamps. The data from Guo et al. (2010) encompass the UV doses in the range of 65–90 mJ/cm^2 to obtain 4 $\log_{10}$ disinfection. The data from Linden et al. (2007) add $\log_{10}$ disinfection data at the lower UV doses of 13 and 28 mJ/cm^2. The relation between UV dose and $\log_{10}$ disinfection is horizontally asymptotic, hence, a logistic function was fitted to these data using NonlinearModelFit in Mathematica. Here, it was assumed that the uncertainty of the $\log_{10}$ disinfection followed a normal distribution. Model prediction provided the distribution parameters for $\log_{10}$ disinfection at UV doses of 40 and 73 mJ/cm^2.

4.5. Chlorine dioxide disinfection parameter estimation

Data for virus disinfection by chlorine dioxide were available from Thurston-Enriquez et al. (2005) for AdV40/41 and from Hornstra et al. (2011) and from Hornstra (2014) for MS2. The data from Thurston-Enriquez et al. (2005) were obtained by reading the normalised concentration data from the published graph, because the original data were not available anymore. To all these data, the extended HOM model (Haas and Joffe, 1994) was fitted:

$$log_{10}N_t = log_{10}N_0 - kC_0^n t^m \eta / ln10 + \varepsilon \quad (1)$$

where, N_t is the virus concentration [litre^{-1}] at time t [min], N_0 is the initial virus concentration [litre^{-1}], C_0 is the initial chlorine dioxide concentration [mg/l], k is the virus inactivation rate coefficient [min^{-1}], m determines the time-dependency of the virus disinfection, n is the dependency on the disinfectant concentration and ε represents uncertainty. Efficiency factor η corrects for the loss of disinfectant:

$$\eta = \frac{m}{\psi^m}\gamma[m, 0, \psi] \quad (2)$$

where γ is the generalised incomplete Gamma function and ψ is:

$$\psi = nk^* t \quad (3)$$

where, k^* is the first-order rate coefficient of chlorine dioxide decay [min^{-1}].

All experiments on disinfection of MS2 by Hornstra et al. (2011) were conducted under identical physico-chemical conditions, therefore, it was assumed that the values for k, m and n were the same for all these experiments, only C_0 varied (0.005 mg/l – 0.5 mg/l). The experimental temperature was 0 °C. In addition, Hornstra (2014) conducted disinfection experiments with a C_0 of 0.06 mg/l and 0.16 mg/l at 5 °C using water from the Berenplaat. The Thurston data for AdV40 were conducted at C_0 values of about 0.5 mg/l at 5 °C and 15 °C and at pH 6 and 8.

For a given C_0 and k^*, one needs to estimate values for k, m and n by fitting the extended HOM model to virus concentration data decreasing non-linearly in time. It can easily be shown that there is no unique solution, therefore some assumptions needed to be made. It is common knowledge that virus inactivation is very much determined by virus type, temperature and pH (*e.g.* Bertrand et al., 2012; Schijven et al., 2016), hence, k was assumed to depend on virus type (MS2 and AdV), on temperature and on pH. Accordingly, a common k value was estimated for MS2 from the data of Hornstra et al. (2011), another common k value for MS2 from the data of Hornstra (2014), and four k values for the disinfection experiments with AdV40 by Thurston-Enriquez et al. (2005). Furthermore, it was assumed that m was dependent only on virus type. Both viruses have a protein mantle of which the outer chemical groups mainly consist of carboxyl and amino groups, suggesting that the reaction mechanism between chlorine dioxide and the virus particles was the same for both viruses and that, consequently, a common value of n was applicable to all disinfection data. Subsequently, having estimated all these parameter values, MS2 was evaluated as a surrogate for chlorine dioxide disinfection of AdV by predicting disinfection of both MS2 and AdV under the same conditions (pH, temperature and ClO_2-concentration). Finally, multivariate regression analysis with temperature and pH as covariates was conducted to develop an empirical formula to predict k for MS2 at 15 °C.

4.6. Prediction of chlorine dioxide disinfection at plant scale

The prediction of virus reduction by chlorine dioxide disinfection at plant scale was based on the decay rate of the chlorine dioxide residual at plant scale including its variability. At plant scale, chlorine dioxide is continuously injected into the effluent of activated carbon filtration. The time before the water enters the drinking water storage tanks is on average 4.4 min in summer and 6.3 min in winter. Because of the continuous injection, it is assumed that the chlorine dioxide concentration in the activated carbon effluent remained constant prior to entering the storage tanks. In the storage tanks, the water follows a labyrinth, i.e. passes the tanks in pulse flow with a shortest residence time of 154 min in summer and 226 min in winter. In the storage tanks, chlorine dioxide gradually decays.

Chlorine dioxide decay was monitored in Berenplaat water at 20 °C, representing summer time, and a C_0 value of 0.103 mg/l and proceeded first order:

$$lnC_t = lnC_0 - k^* t \quad (4)$$

Parameter k^* was estimated by means of linear regression.

Chlorine dioxide decay in Berenplaat water was also monitored at 5 °C to represent winter time. This was done for three C_0 values: 0.052, 0.103 and 0.151 mg/l. The chlorine dioxide decay proceeded initially faster than thereafter and, hence, was fitted to a Weibull distribution:

$$lnC_t = lnC_0 - (k^* t)^\beta \quad (5)$$

where β is a shape parameter. Parameter values were obtained from maximum likelihood estimation.

For the summer period, the logarithmic concentration reduction of virus by chlorine dioxide disinfection was predicted using equations (1)–(3). For the winter period, the logarithmic concentration reduction of virus by chlorine dioxide disinfection was predicted using equations (1), (2) and (6).

$$\psi = n(k^* t)^{\beta} \quad (6)$$

Minimal exposure times to chlorine dioxide in summer and in winter were used to predict the logarithmic concentration reduction of virus, including uncertainty ε.

4.7. Exposure and risk of infection

Exposure to the index pathogens is given as the dose D, the number of ingested adenoviruses per person per day and was calculated by multiplying the Monte Carlo samples of their concentration in Berenplaat water, C_{source}, treatment z_i (four treatment steps) and consumption data W, divided by the recovery of the samples R:

$$D = C_{source}\frac{1}{R}\prod_{i=1}^{4} z_i W \quad (7)$$

Drinking water consumption W was assumed to be lognormal distributed with parameters $\mu = -1.85779$ and $\sigma = 1.07487$ for the Netherlands, corresponding to a mean of 0.27 L per person per day (Schijven et al., 2011). Thus, doses were calculated for the winter and summer period.

The exact beta-Poisson dose response model was used with sets of parameter values representing uncertainty and variability in the infectivity of ingested AdV (Teunis et al., 2016). It was assumed that infectivity of total AdV and of AdV40 and 41 fit into this generalised dose-response relationship.

$$P_{inf,person,day} = 1 - {}_1F_1(\alpha, \alpha+\beta; -D) \quad (8)$$

where α and β are infectivity parameters and ${}_1F_1$ is the confluent hypergeometric function. Parameters α and β are Monte Carlo sample pairs (joint distribution), reflecting uncertainty and variability of infectivity. Infection risk per person per year was calculated from the Monte Carlo samples of the daily infection risk by first combining the winter and summer daily infection risks applying equation (7) 10,000 times for each day in a year random sample from the combined daily infection risks to obtain 365 Monte Carlo sample distributions, which are then multiplied (Teunis et al., 1997):

$$P_{inf,person,year} = 1 - \prod_{i=1}^{365}\left(1 - P_{inf,person,day,i}\right) \quad (9)$$

5. Results

5.1. Estimation of virus concentration in the source water

Fig. 1a, d, e, g and h show the observed concentrations of total AdV as enumerated with iccPCR, mpnPCR and qPCR as well as of AdV40/41 as enumerated with mpnPCR and qPCR. Clearly, in the first quarter of the year (2012), virus concentrations reached peak values. During the second and third quarter of the year, denoted as summer, total AdV was detected 4 times using cell culture, but was below detection limit for the molecular based detection methods; in all summer samples, AdVtot was not detected. In only one summer sample, viruses were detected using the AdV40/41 specific PCR and is line with the theoretical limit of detection (LOD) for these methods based on differences in analyzed volumes. Detection probability will increase when analyzed volumes increase. The theoretical LOD by molecular methods is 1 viral DNA genome per analyzed volume of sample, which varied in our case between 9 ml and 113 ml of source water. The volumes studied by iccPCR varied from 52 L to 391 L, resulting in a theoretical LOD of 1 infectious particle in several hundreds of litres of source waters, indicating that the theoretical LOD may be orders of magnitude lower, also depending on the ratio of infectious particle/viral genome.

Also, variability between concentrations in qPCR samples is larger than in mpnPCR. The qPCR data contain occasionally very high concentration values, not found by mpn. The latter does not discriminate between a high or a low virus count in a single PCR-reaction well. Table 1 summarizes the corresponding distribution parameter values that describe the Gamma-distributed virus concentrations for the winter and summer period.

Table 2 lists the distribution parameter values of the Beta distributions that describe the fraction of infectious virus particles as determined from the total AdV data. The fraction of infectious virus particles was on average 5.9×10^{-4} (-3.2 $\log_{10}$ or 1/1700) of the virus particles enumerated by mpnPCR as well as by qPCR (see also Fig. 1b and c). Table 2 also includes the concentrations of infectious AdV40/41 as predicted from the total AdV concentrations and the corresponding fraction of infectious virus particles. For comparison, Table 2 also includes the parameters of the Beta distribution describing the fraction of infectious rotavirus particles (iccPCR and mpnPCR) as determined by Rutjes et al. (2009) and of enterovirus (cell culture and mpnPCR) as determined by Lodder et al. (2015). On average 1/100 of enumerated RV particles was infectious. This is 17 times higher than as estimated for AdV. The distribution describing the fraction of infectious RV is wider than that of AdV. On average 1/25 of enumerated enterovirus particles was infectious with distribution similarly wide as that for RV. Note that the enterovirus samples were collected from different river water locations from 1987 till 2012. Table 2 also includes the parameters of the Beta distribution describing the fraction of AdV40/41 of AdVtot particles (see also Fig. 1j and k). According to the mpnPCR enumeration, this fraction was on average 0.5 (-0.3 $\log_{10}$ varying from 0.2 to 0.8) and according to the qPCR enumeration this fraction was on average 0.36 (-0.44 $\log_{10}$, varying from 0.008 to 0.9). For being able to fit the Beta distribution, those data, where AdV40/41 was detected but AdVtot not and where the concentration of Ad40/41 was higher than AdVtot, were omitted (see footnote Table 2). Near the detection limit (a few viruses detected), differences in sensitivity of the qPCR (using different primers for AdV40/41 and AdVtot) may result in finding apparently more AdV40/41 than AdVtot.When comparing mpnPCR with qPCR (see Fig. 1f and i), it appears only when peak values in the qPCR enumeration occur, qPCR concentration values are higher than mpnPCR concentrations. Otherwise, mpnPCR concentration values are about a factor two higher than qPCR concentration values.

5.2. Coagulation, rapid sand filtration

Table 3 lists the parameter values of the Beta distributions that represent the fraction of somatic coliphages that were able to pass these treatments based on enumeration data of somatic coliphages in plant scale samples. The first two treatment steps contribute little to virus removal. Coagulation/sludge blanket clarification was more efficient in winter, whereas rapid filtration was inefficient in winter.

5.3. UV disinfection

Table 3 also lists the parameter values of the Beta distributions that represent the fraction of somatic coliphages that were able to pass UV disinfection, either at 40 mJ/cm^2 or 73 mJ/cm^2, based on enumeration data of somatic coliphages in plant scale samples. The

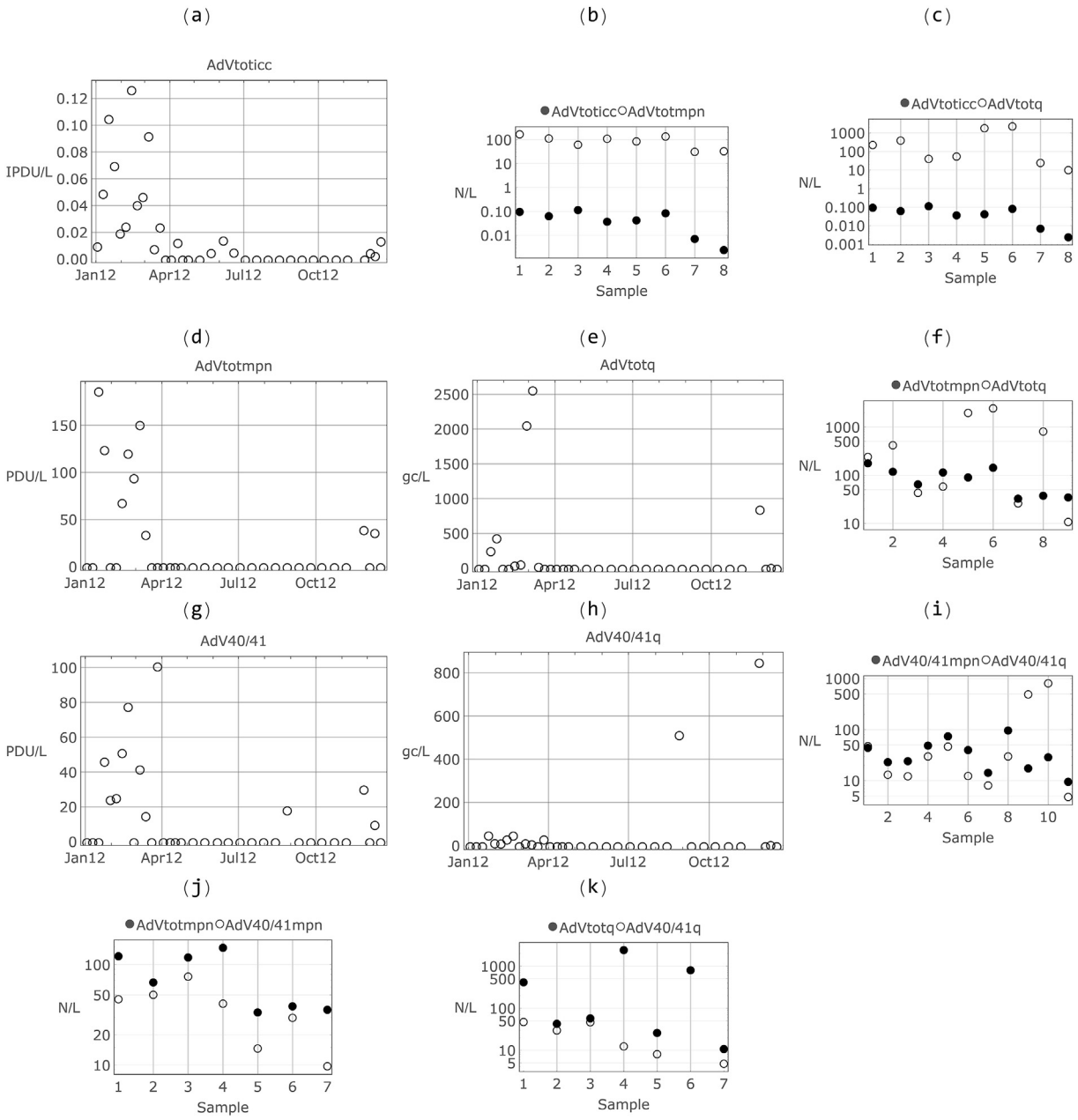

Fig. 1. Observed concentrations of total AdV as enumerated with integrated cell culture PCR (a), mpnPCR (d) and qPCR (e) as well as of AdV40/41 as enumerated with mpnPCR (g) and qPCR (h). For comparison pairs of concentrations (>0): (b) for (a) and (d), (c) for (a) and (e), (f) for (d) and (e), (i) for (g) and (h), (j) for (d) and (g) and (k) for (e) and (h).

distribution for the summer data with 73 mJ/cm^2 is very skewed to the left due to more non-detects in the effluent water.

Fig. 2 predicts the $\log_{10}$ reduction of AdV by UV disinfection based on the literature data listed in Table 4. At 40 mJ/cm^2 and 73 mJ/cm^2, the predicted $\log_{10}$ reductions are 3.3 ± 0.1 and 4.0 ± 0.1. The reductions of somatic coliphages as determined at plant scale, were on average a bit lower, suggesting that somatic coliphages may be less sensitive towards UV disinfection than AdV, or that plant scale conditions were less optimal for UV treatment, indicating that the fluence may have been lower than anticipated. The Beta distribution for the reductions of the somatic coliphages by UV disinfection at plant scale reflects variability. The latter in combination with the lower sensitivity towards UV disinfection as well as the uncertainty inherent to the sparse literature data on UV disinfection of AdV justified the use of the plant scale somatic coliphage data for the risk assessment.

5.4. Chlorine dioxide disinfection

The conditions and estimated model parameter values for each disinfection experiment are included in Fig. 3. Fig. 3 shows the observed and fitted $\log_{10}$ reductions by chlorine dioxide from Hornstra et al. (2011) that were aimed at determining disinfection of bacteriophage MS2 at low initial chlorine dioxide concentrations (c0 is 0.0049 mg/l − 0.45 mg/l), all at 0 °C and pH 7.2 (3a − 3h), of bacteriophage MS2 at two low initial chlorine dioxide concentrations (0.059 mg/l and 0.16 mg/l) and at 5 °C and pH 7.8 to mimic the plant scale conditions in winter (3i and 3j, Hornstra (2014)), and of AdV at higher chlorine dioxide concentrations (0.47 mg/l − 0.53 mg/l) at 5 °C and 15 °C and pH 6 and pH 8 (3k − 3n) from

Table 1
Gamma distribution parameter values and $\log_{10}$ concentrations of AdVtot and AdV40/41 by iccPCR, mpnPCR and qPCR.

Season	Number of samples		Gamma distribution		$\log_{10}$ concentration (L^{-1})		
	Detects	Non detects	r	λ	Mean	95%	5%
AdVtoticc							
Winter	15	5	0.57	0.056	−1.5	−0.92	−3.6
Summer	4	11	0.64	0.0057	−2.4	−1.9	−4.4
AdVtotmpn							
Winter	9	11	0.61	70	1.6	2.2	−0.38
Summer	0	15	0.01	2.6	−1.6	−2.1	−130
AdVtotq							
Winter	9	11	0.14	2300	2.5	3.2	−6.7
Summer	0	15	0.01	2.6	−1.6	−2.1	−130
AdV40/41mpn							
Winter	10	10	1.8	18	1.5	1.9	0.68
Summer	1	14	0.36	6.6	0.36	0.99	−2.9
AdV40/41q							
Winter	10	10	0.23	240	1.7	2.4	−3.2
Summer	1	14	0.015	2300	1.5	1.6	−83

Thurston-Enriquez et al. (2005). As shown, the data were fitted satisfactorily by maximum likelihood estimation. At higher temperature, disinfection of MS2 increased. The two bottom left figures compare disinfection of MS2 with that of AdV by predicting MS2 disinfection at the conditions observed for AdV and vice versa. It shows that MS2 was more persistent to chlorine dioxide than AdV. Extrapolation of AdV disinfection at the plant scale conditions was considered too uncertain, therefore, prediction of disinfection of MS2 as a conservative indicator was applied. Standard deviation ε was 0.44. The predicted value of k of MS2 at 15 °C was 13.7 min^{-1}. In the winter time, chlorine dioxide decay in Berenplaat water followed a Weibull distribution with $k^* = 0.065\ min^{-1}$ and $\beta = 0.42$. In the summer time, the decay proceeded first order with $k^* = 0.017$. This led to a predicted $\log_{10}$ reduction by chlorine dioxide disinfection of 6.8 (5.9–7.6) in winter time and of 14.4 (13.5–15.2) in summer time (Table 3).

5.5. Total treatment

Table 3 also includes the means, 5-percentiles and 95-percentiles of the $\log_{10}$ reduction of the four total treatment scenarios. It clearly shows that chlorine dioxide disinfection is the major treatment step for AdV removal.

5.6. Infection risks

Fig. 4 shows the box-whisker plots of the infection risks per person per year for all twelve scenarios (four treatments scenarios with AdVtoticc, AdV40/41mpn and AdV40/41q). For the treatment scenarios including chlorine dioxide disinfection, risks were far below 10^{-4}. The infection risks with the treatment scenario that includes a UV dose of 40 mJ/cm^2 as well as chlorine dioxide disinfection are for all viral methods at least five orders in magnitude below the 10^{-4} risk. Switching to a UV dose of 73 mJ/cm^2 shifts the risks another 1.5 $\log_{10}$ further down. In the treatment scenarios without chlorine dioxide disinfection, the 10^{-4} risk level is always exceeded. The large range of the box whiskers with the treatment of 73 mJ/cm^2 is a consequence of the wider Beta distribution for UV disinfection at this dose. With respect to the multibarrier principle, on the one hand, this indicates that total failure of chlorine dioxide disinfection leads to non-compliance with the health-based target. On the other hand, chlorine dioxide disinfection alone provides adequate treatment.

6. Discussion

6.1. Source concentration estimation

Detection of infectious viral pathogens in environmental samples usually uses mammalian cell culture methods. It is generally known that cell culture assays will underestimate the viral concentration in the sample, since not all viral strains will be detected with similar efficiencies if detected at all (Jiang, 2006). Detecting adenoviruses by cell culture infectivity is usually challenging due to the inconsistent onset of viral induced cytopathic effect, which is overcome by combining the use of cell culture with PCR (iccPCR) as has been used in the current paper. Although in several papers cell lines have been described that aim to improve detection of enteric adenoviruses (Polston et al., 2015), detection of environmental isolates is still challenging, which made us decide to use the approach described in current paper.

It was estimated that the fraction of infectious AdV particles was on average 1/1700 by mpnPCR and qPCR. By comparison, the fraction of infectious RV was on average 1/100 in mpnPCR (Rutjes et al., 2009) and that for infectious enterovirus was on average 1/

Table 2
Beta distribution, $\log_{10}$ fraction and $\log_{10}$ concentration of infectious AdV40/41 particles.

Fraction	Number of	Beta distribution		-$\log_{10}$ fraction		
	paired samples	α	β	Mean	95%	5%
AdVtoticc/AdVtotmpn	8	1.6	2800	3.2	2.8	4.1
AdVtoticc/AdVtotq	8	0.65	1100	3.2	2.7	5.1
RVicc/RVmpn[a]	11	0.38	41	2.0	1.4	5.2
EVcc/EVmpn[b]	11	0.35	8.5	1.4	0.76	4.7
AdV40/41mpn/AdVtotmpn	7[c]	3.1	3	−0.3	−0.089	−0.7
AdV40/41q/AdVtotq	6[d]	0.63	1.1	−0.44	−0.048	−2.1

		$\log_{10}$ concentration (L^{-1})		
		Mean	95%	5%
Infectious AdV40/41mpn	Winter	−1.7	−1.2	−3.0
	Summer	−2.9	−2.2	−6.4
Infectious AdV40/41q	Winter	−1.5	−0.79	−7.3
	Summer	−1.7	−1.2	−31

[a] Rotavirus (Rutjes et al., 2009).
[b] Enterovirus (Lodder et al., 2015).
[c] In 4 samples AdV40/41 was detected, but AdVtot not.
[d] In 5 samples AdV40/41 was detected, but AdVtot not, and in one sample the estimated concentration of AdV40/41 was higher than that of AdVtot.

Table 3
Distribution parameters and log_{10} reduction values derived from plant scale somatic coliphage data for the first three treatment steps and model predictions for chlorine dioxide disinfection of bacteriophage MS2.

Treatment	Season	Number of samples				Beta distribution		-log_{10} reduction		
		Influent		Effluent		α	β	Mean	95%	5%
		Detects	Non detects	Detects	Non detects					
Coagulation/Sludge blanket clarification										
	Winter	28	0	21	0	7.3	33	0.74	0.54	1.0
	Summer	16	1	14	0	1.3	0.95	0.24	0.015	1.0
Rapid filtration										
	Winter	21	0	32	0	3.9	0.21	0.023	0.	0.13
	Summer	14	0	18	0	7.9	6.9	0.27	0.13	0.27
UV disinfection medium pressure 40 mJ/cm^2										
	Winter	32	0	7	8	0.076	25	2.6	1.8	19
	Summer	18	0	3	6	0.17	180	3.0	2.3	10
UV disinfection medium pressure 73 mJ/cm^2										
	Winter	32	0	6	11	0.12	1900	4.2	3.4	14
	Summer	18	0	0	7	0.0024	0.13	1.7	5.0	550

Treatment	Season	Normal distribution		-log_{10} reduction		
		μ	σ	Mean	95%	5%
Chlorine dioxide disinfection						
	Winter	−8.6	0.44	6.8	5.9	7.6
	Summer	−13.8	0.44	14.4	13.5	15.2
Total treatment with UV40, without chlorine dioxide						
	Winter			3.3	2.5	19
	Summer			3.5	2.8	11
Total treatment with UV73, without chlorine dioxide						
	Winter			5	4.2	15
	Summer			2.2	6.6	550
Total treatment with UV40 and chlorine dioxide						
	Winter			12	11	28
	Summer			17	16	25
Total treatment with UV73 and chlorine dioxide						
	Winter			13	13	24
	Summer			16	20	570

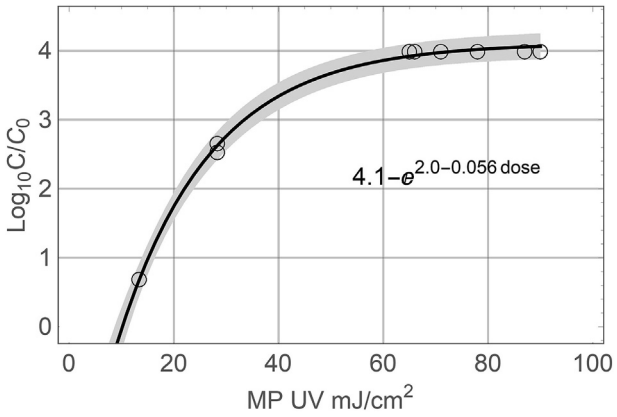

Fig. 2. Predicted log_{10} reduction based on the literature data of AdV by UV disinfection as a function of UV dose (mJ/cm^2) from medium pressure lamps.

25 in mpnPCR (Lodder et al., 2015). The fraction of infectious virus particles may depend on virus type, water matrix and time. RV was enumerated in samples from the River Meuse, whereas AdV was enumerated in water from a storage reservoir fed with water from the River Meuse. Residence time in the three successive Biesbosch storage reservoirs is five months on average. The detected AdV may, therefore, have been longer in the environment following discharge with wastewater than the detected RV, and, hence, there was more time for virus inactivation. Another comparison between qPCR and tissue culture enumeration was made by De Roda Husman et al. (2009), where inactivation of poliovirus-1 and a Coxsackie virus

Table 4
Literature data on disinfection of AdV strains by medium pressure UV lamps.

	AdV	Cell line	UV dose mJ/cm^2	log_{10} disinfection
1	AdV5	HEK293	90	4.0
2	AdV5	PLC/PRF/5	87	4.0
3	AdV40	HEK293	66	4.0
4	AdV40	PLC/PRF/5	65	4.0
5	AdV41	HEK293	78	4.0
6	AdV41	PLC/PRF/5	71	4.0
7	AdV40	PLC/PRF/5	13	0.7
8	AdV40	PLC/PRF/5	28	2.5
9	AdV40	PLC/PRF/5	28	2.7

Data 1–6 from Guo et al. (2010); data 7–9 from Linden et al. (2007).

B4 was monitored for a period of one year in artificial groundwater and artificial surface water at 4 °C and at 22 °C. The fraction of infectious virus was initially about 1/100 and decreased to 1/10000 or less depending on virus type, matrix, temperature and time. This range encompasses that of the 90%-interval estimated for the fraction of infectious total AdV. QMRA based on genome copy concentrations of a virus in the source water may, therefore, overestimate the risk by two to four orders in magnitude. In this study, the fraction of infectious total AdV particles in total AdV particles was used to estimate infectious AdV40/41 particle concentrations. Depending on mpnPCR or qPCR, the fraction AdV40/41 particles in total AdV was estimated to average 0.36 ($10^{-0.44}$) – 0.50 ($10^{-0.30}$) for the observed Ad40/41 concentrations that were lower than the observed total AdV concentrations (Table 2). Although this fraction varies, given the fact that 90 types of adenoviruses based on whole genome sequencing have been described (Ismail et al.,

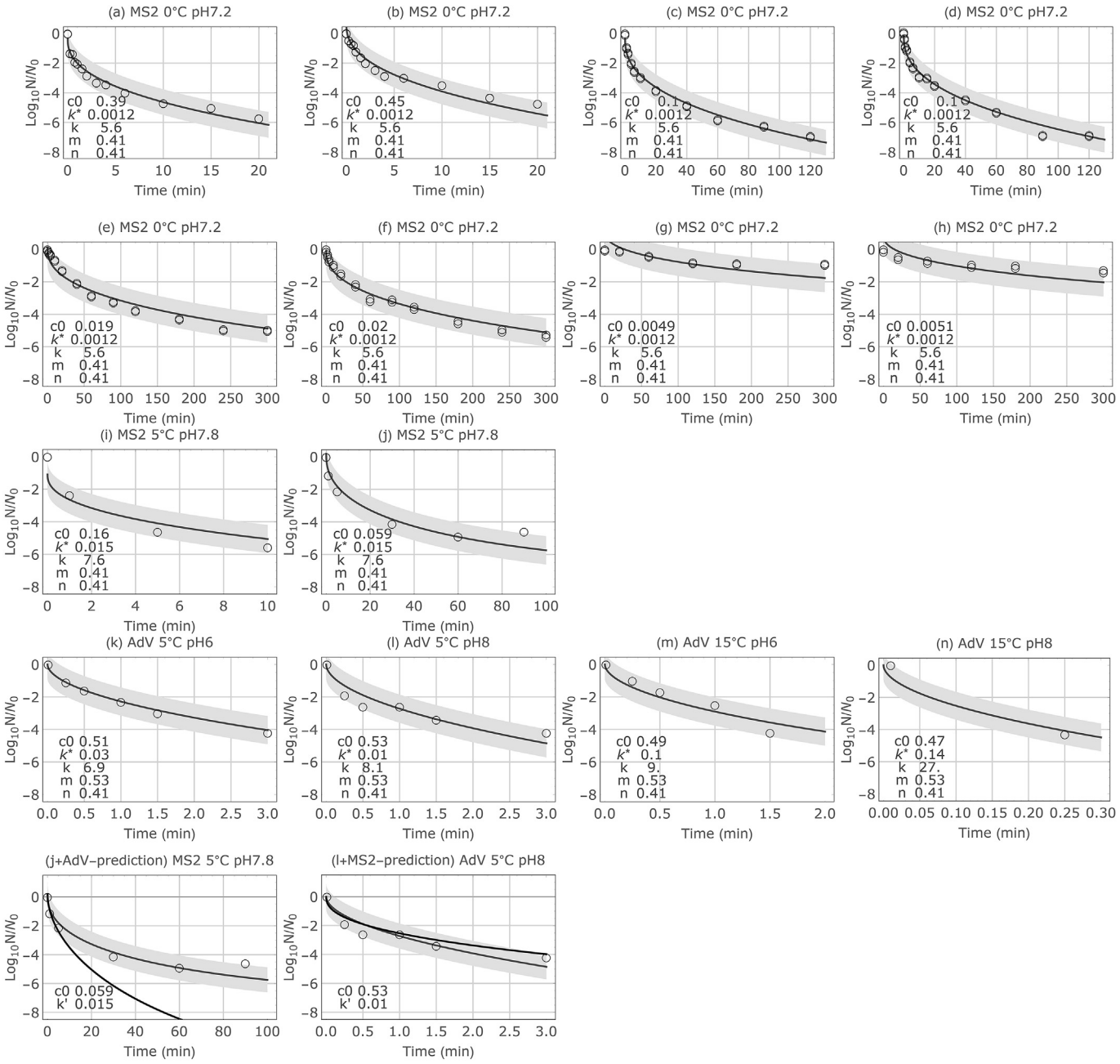

Fig. 3. Observed and fitted $\log_{10}$ reductions by chlorine dioxide from Hornstra et al. (2011) that were aimed at determining disinfection of bacteriophage MS2 at low initial chlorine dioxide concentrations all at 0 °C and pH 7.2 (3a – 3h), of bacteriophage MS2 at two low initial chlorine dioxide concentrations and at 5 °C and pH 7.8 to mimic the plant scale conditions in winter (3i and 3j, Hornstra (2014)), and of AdV at higher chlorine concentrations at 5 °C and 15 °C and pH 6 and pH 8 (3k – 3n) from Thurston-Enriquez et al. (2005). See equations (1)–(3) for the meaning of the model parameters.

2018), the fraction of 0.36–0.50 seems surprisingly high. The relative high fraction of AdV40/41 may be explained by the fact that the enteric group F adenoviruses may be more stable than viruses in other AdV groups. AdV40/41 have been shown to be the dominating serotypes in environmental water samples by Hartmann et al. (2013), although their results indicate that some other serotypes may be stable enough to be detected up to weeks after contamination as well. The fact that in some samples AdV40/41 were detected in the absence of AdVtot may be explained by a combination of the low virus concentrations in the source waters approaching the detection limit and the high fraction of AdV40/41. Differences in detection limit may also be ascribed to using different target genes for detection of AdVtot (hexon gene) and AdV40/41 (fiber gene).

Quantification of viruses in surface waters or other environmental samples is challenging, because of the complex character of the matrix. Virus concentrations in source waters for drinking water production are known to be generally low, and may be close to or below detection levels. Moreover, as reviewed by Schrader et al. (2012), environmental samples are known to contain substances that may inhibit nucleic acid extraction and PCR. Inhibitors are a very heterogeneous group of chemical substances. One matrix may contain different inhibitory substances and the same inhibitors can be found in many different environmental but also

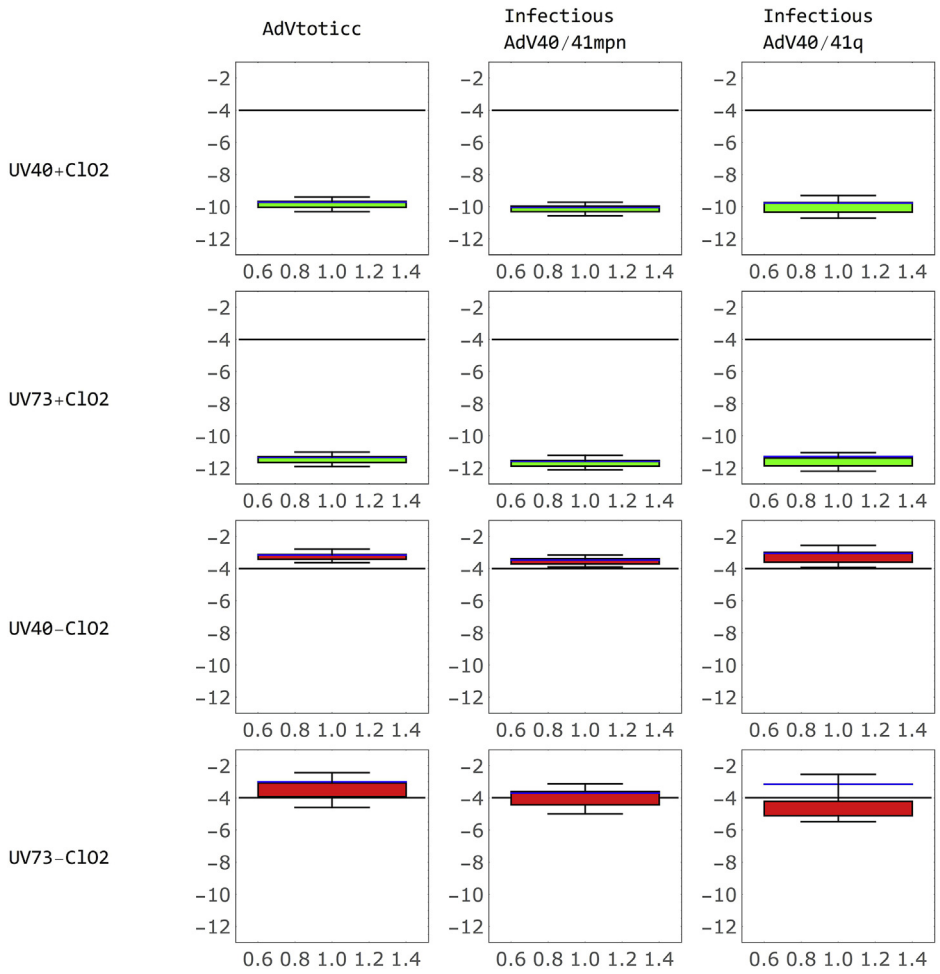

Fig. 4. Box-whisker plots of the infection risk per person per year for 12 scenarios: AdVtoticc, infectious AdV40/41mpn and infectious AdV40/41q with each UV40 or UV73 and with or without ClO_2. If the 95-percentile exceeds the 10^{-4} risk level, the box turns from green to red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

clinical matrices. Organic as well as inorganic substances, which may be dissolved or solid, can appear as extraction of PCR inhibitors. Nucleases may degrade template nucleic acids (Schrader et al., 2012), having a negative effect on the extraction, or nucleic acids present in the sample may compete for target RNA or DNA attachment to the isolating raisin (Rutjes et al., 2005). Calcium ions are an example of inorganic substances with inhibitory effects on the PCR. However, most of the known inhibitors are organic compounds. Examples are polysaccharides, humic acids, tannic acid as well as different proteins, such as proteinases (Schrader et al., 2012). The presence of these inhibitory substances may complicate quantification based upon a standard curve amplified by PCR in optimal reaction conditions. Although in theory, inhibition could be quantified using proper amplification controls (D'Agostino et al., 2011), a qPCR HAdV validation study for quantitation of HAdV genome copies in berry fruit demonstrated that the level of between-laboratory variation was too great to be able to describe the performance characteristics of the method in quantitative terms. This is despite the fact that nucleic acid standard solutions were supplied along with the trial materials. The high between-laboratory variation may be caused by several factors, such as the condition in which the standard solutions have reached the partner institutes, operational differences between thermocyclers used in the various laboratories but also the differences in berries (D'Agostino et al., 2012). This study highlighted the vulnerability of quantification based on an external standard.

In addition, viruses may not be homogeneously distributed in the sample, but be present (partly) as aggregates. The occasional high concentrations detected by qPCR suggest this. When such aggregates are sampled, qPCR may lead to overestimation of virus concentrations. Although qPCR data appeared to have occasional high outliers not present in the mpnPCR data (Fig. 1d–i), these outliers appeared to have little or no effect on the final distributions of the infection risks.

6.2. UV disinfection

Comparing literature data on UV disinfection of AdV with those of somatic coliphages as determined from plant scale data, indicated that the latter were more persistent, suggesting that somatic coliphages are a good indicator for UV disinfection of AdV.

Although a UV dose of 73 mJ/cm^2 is more efficient than a UV dose of 40 mJ/cm^2, a failing chlorine dioxide disinfection would still lead to insufficient treatment.

6.3. Chlorine dioxide disinfection

Literature data on the efficiency of chlorine dioxide to disinfect AdV and bacteriophage MS2 were used to predict disinfection of AdV at the low concentrations of chlorine dioxide as applied at the

DWTP. It showed that under the assumption of the same kinetics that disinfection is more efficient at higher temperature and, obviously, with higher disinfectant concentration, and also at higher pH. MS2 appeared to be less sensitive than AdV. Because the disinfection data of MS2 were near to the plant scale conditions, it is more appropriate to predict MS2 disinfection than AdV disinfection and thus to use MS2 as a process indicator. Clearly, a low chlorine dioxide concentration as applied here appeared to be highly efficient and is the major treatment step at this DWTP for the reduction of AdV.

6.4. Risk and uncertainty

It may be stated that the risk estimates were conservative because somatic coliphages and bacteriophage MS2 appeared to be more persistent to UV and chlorine dioxide disinfection, respectively, than AdV.

7. Conclusions

AdV40/41 represents a large fraction of total AdV and only a small fraction of AdV is infectious (on average 1/1700).

Somatic coliphages have been shown to be a good indicator for UV disinfection of AdV, and bacteriophage MS2 for chlorine dioxide disinfection of AdV.

According to the QMRA, the drinking water at DWTP Berenplaat is compliant with the legal health-based target for AdV.

This paper provides the first description of the application of chlorine dioxide in drinking water production at the low initial concentrations of 0.05 mg/l and 0.1 mg/l, which, nevertheless, appeared to be very effective in the reduction of AdV. Chlorine dioxide was the dominant treatment step for AdV at this DWTP.

Acknowledgments

Arno Swart and Harold van den Berg from the National Institute of Public Health and the Environment, Bilthoven, the Netherlands, are greatly acknowledged for their useful comments. This research was funded by Evides Water Company and the Ministry of Infrastructure and Water Management.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.03.090.

References

Anonymous, 2011. Besluit van 23 mei 2011, houdende bepalingen inzake de productie en distributie van drinkwater en de organisatie van de openbare drinkwatervoorziening (Drinkwaterbesluit). Staatsblad van het Koninkrijk der Nederlanden 293, 1–90.

Bertrand, I., Schijven, J.F., Sanchez, G., Wyn-Jones, P., Ottoson, J., Morin, T., et al., 2012. The impact of temperature on the inactivation of enteric viruses in food and water: a review. J. Appl. Microbiol. 112 (6), 1059–1074.

Bofill-Mas, S., Calgua, B., Clemente-Casares, P., La Rosa, G., Iaconelli, M., Muscillo, M., et al., 2010. Quantification of human adenoviruses in European recreational waters. Food Environ. Virol. 2 (2), 101–109.

Bon, F., Fascia, P., Dauvergne, M., Tenenbaum, D., Planson, H., Petion, A.M., et al., 1999. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. J. Clin. Microbiol. 37 (9), 3055–3058.

Brandt, C.D., Kim, H.W., Vargosko, A.J., Jeffries, B.C., Arrobio, J.O., Rindge, B., et al., 1969. Infections in 18, 000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. Am. J. Epidemiol. 90 (6), 484–500.

D'Agostino, M., Cook, N., Rodriguez-Lazaro, D., Rutjes, S., 2011. Nucleic acid amplification-based methods for detection of enteric viruses: definition of controls and interpretation of results. Food Environ. Virol. 3 (2), 55–60.

D'Agostino, M., Cook, N., Di Bartolo, I., Ruggeri, F.M., Berto, A., Martelli, F., et al., 2012. Multicenter collaborative trial evaluation of a method for detection of human adenoviruses in berry fruit. Food Anal. Methods 5 (1), 1–7.

De Roda Husman, A.M., Lodder, W.J., Rutjes, S.A., Schijven, J.F., Teunis, P.F.M., 2009. Long-term inactivation study of three enteroviruses in artificial surface and groundwaters, using PCR and cell culture. Appl. Environ. Microbiol. 75 (4), 1050–1057.

Diez-Valcarce, M., Kovač, K., Cook, N., Rodríguez-Lázaro, D., Hernández, M., 2011. Construction and analytical application of internal amplification controls (IAC) for detection of food supply chain-relevant viruses by real-time PCR-based assays. Food Anal. Methods 4 (3), 437–445.

Guo, H., Chu, X., Hu, J., 2010. Effect of host cells on low-and medium-pressure UV inactivation of adenoviruses. Appl. Environ. Microbiol. 76 (21), 7068–7075.

Haas, C.N., Joffe, J., 1994. Disinfection under dynamic conditions: modification of Hom's model for decay. Environ. Sci. Technol. 28 (7), 1367–1369.

Hartmann, N.M., Dartscht, M., Szewzyk, R., Selinka, H.C., 2013. Monitoring of adenovirus serotypes in environmental samples by combined PCR and melting point analyses. Virol. J. 10 (1), 190.

Hernroth, B.E., Conden-Hansson, A.C., Rehnstam-Holm, A.S., Girones, R., Allard, A.K., 2002. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. Appl. Environ. Microbiol. 68 (9), 4523–4533.

Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. Water Res. 40 (1), 3–22.

Hijnen, W.A.M., Suylen, G.M.H., Bahlman, J.A., Brouwer-Hanzens, A., Medema, G.J., 2010. GAC adsorption filters as barriers for viruses, bacteria and protozoan (oo) cysts in water treatment. Water Res. 44 (4), 1224–1234.

Hornstra, L., 2014. Desinfectie van E. coli, Campylobacter en MS2 door chloordioxide in het actief kool filtraat van drinkwaterproductielocatie Berenplaat. KWR 2014.029, Nieuwegein, the Netherlands.

Hornstra, L.M., Smeets, P.W.M.H., Medema, G.J., 2011. Inactivation of bacteriophage MS2 upon exposure to very low concentrations of chlorine dioxide. Water Res. 45 (4), 1847–1855.

Ismail, A.M., Lee, J.S., Lee, J.Y., Singh, G., Dyer, D.W., Seto, D., Chodosh, J., Rajaiya, J., 2018. Adenoviromics: mining the human adenovirus species D genome. Front. Microbiol. 9 article 2178. https://doi.org/10.3389/fmicb.2018.02178.

ISO, I., 2000. 10705–2: Water Quality. Detection and Enumeration of Bacteriophages–Part 2: Enumeration of Somatic. Coliphages. International Organization for Standardization, Geneva, Switzerland.

Jiang, S.C., 2006. Human adenoviruses in water: occurrence and health implications: a critical review. Environ. Sci. Technol. 40, 7132–7140.

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D., Erdman, D.D., 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Appl. Environ. Microbiol. 71 (6), 3131–3136.

Linden, K.G., Thurston, J., Schaefer, R., Malley, J.P., 2007. Enhanced UV inactivation of adenoviruses under polychromatic UV lamps. Appl. Environ. Microbiol. 73 (23), 7571–7574.

Lodder, W.J., Schijven, J.F., Rutjes, S.A., de Roda Husman, A.M., Teunis, P.F.M., 2015. Entero-and parechovirus distributions in surface water and probabilities of exposure to these viruses during water recreation. Water Res. 75, 25–32.

Oh, D.Y., Gaedicke, G., Schreier, E., 2003. Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. J. Med. Virol. 71 (1), 82–93.

Polston, P.M., Rodríguez, R.A., Seo, K.J., Kim, M., Ko, G.P., Sobsey, M.D., 2015. Field evaluation of an improved cell line for the detection of human adenoviruses in environmental samples. J. Virol. Methods 205, 68–74.

Rodriguez-Baez, N., O'brien, R., Qiu, S., Bass, D.M., 2002. Astrovirus, adenovirus, and rotavirus in hospitalized children: prevalence and association with gastroenteritis. J. Pediatr. Gastroenterol. Nutr. 35 (1), 64–68.

Rutjes, S.A., Lodder, W.J., Van Leeuwen, A.D., de Roda Husman, A.M., 2009. Detection of infectious rotavirus in naturally contaminated source waters for drinking water production. J. Appl. Microbiol. 107 (1), 97–105.

Rutjes, S.A., Italiaander, R., van den Berg, H.H., Lodder, W.J., de Roda Husman, A.M., 2005. Isolation and detection of enterovirus RNA from large-volume water samples by using the NucliSens miniMAG system and real-time nucleic acid sequence-based amplification. Appl. Environ. Microbiol. 71 (7), 3734–3740.

Schijven, J.F., Sadeghi, G., Hassanizadeh, S.M., 2016. Long-term inactivation of bacteriophage PRD1 as a function of temperature, pH, sodium and calcium concentration. Water Res. 103, 66–73.

Schijven, J.F., Teunis, P.F., Rutjes, S.A., Bouwknegt, M., de Roda Husman, A.M., 2011. QMRAspot: a tool for quantitative microbial risk assessment from surface water to potable water. Water Res. 45 (17), 5564–5576.

Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors–occurrence, properties and removal. J. Appl. Microbiol. 113 (5), 1014–1026.

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

Teunis, P., Schijven, J., Rutjes, S., 2016. A generalized dose-response relationship for adenovirus infection and illness by exposure pathway. Epidemiol. Infect. 144 (16), 3461–3473.

Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., Gerba, C.P., 2005. Inactivation of enteric adenovirus and feline calicivirus by chlorine dioxide. Appl. Environ. Microbiol. 71 (6), 3100–3105.

Verhaelen, K., Bouwknegt, M., Lodder-Verschoor, F., Rutjes, S.A., de Roda

Husman, A.M., 2012. Persistence of human norovirus GII. 4 and GI. 4, murine norovirus, and human adenovirus on soft berries as compared with PBS at commonly applied storage conditions. Int. J. Food Microbiol. 160 (2), 137–144.
WHO, 2017. Guidelines for Drinking-Water Quality, fourth ed. incorporating the 1st addendum World Health Organisation, Geneva, Zwitserland. ISBN: 978-92-4-154995-0.
Wold, W.S.M., Ison, M.G., 2013. Adenoviruses. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, seventh ed. Lippincott, Williams & Wilkins, Philadelphia (Chapter 56).