10) Zhou, L., Nakanishi, T., Matsudate, K., Echigo, S., Ohkouchi, Y., Itoh, S. Infection Risk Assessment of *Campylobacter jejuni* in Drinking Water Treated with Water Treatment Process Reducing Chlorinous Odor, Asian Core Program 第３回包括シンポジウム，京都大学宇治キャンパス，2013.10.28-29.
11) 周靚，Infection Risk Assessment of *Campylobacter jejuni* in Drinking Water Treated with Water Treatment Process Reducing Chlorinous Odor（カルキ臭低減型浄水処理プロセスにおけるCampylobacter jejuniの感染リスク評価に関する研究），八大学工学系連合会「博士学生交流フォーラム」，京大桂キャンパス船井記念講堂，2013.11.8.
12) 浅田安廣，大河内由美子，松館圭太，伊藤禎彦，障害調整生存年数推定を目的とした河川水中*Campylobacter jejuni*の実態調査，第16回日本水環境学会シンポジウム講演集，391-392，2013.
13) 浅田安廣，大河内由美子，越後信哉，伊藤禎彦，河川水中*Campylobacter jejuni*に対するギラン・バレー症候群発症関連菌株の推定，第50回環境工学研究フォーラム講演集，116-118，2013.
14) 大坂幸弘，春日郁朗，栗栖太，古米弘明（2013）水道水中の細菌再増殖に与える水温および残留塩素の影響評価，第29回日本微生物生態学会大会，p.89.
15) 松館圭太，周 靚，越後信哉，伊藤禎彦，カルキ臭低減型浄水処理プロセスにおける陽・陰イオン交換処理による細菌除去，第48回日本水環境学会年会，2014（発表予定）

研究成果の刊行物・別刷

Effect of the Ratio of Illness to Infection of *Campylobacter* on the Uncertainty of DALYs in Drinking Water

Sadahiko ITOH

Department of Environmental Engineering, Kyoto University, C-1, Kyotodaigaku Katsura, Nishikyo, Kyoto 615-8540, Japan

ABSTRACT

The yearly risk of *Campylobacter* infection was estimated for a treatment plant in the Netherlands. The median and mean values for the overall removal efficacy of the four treatment steps at the plant were estimated to be 7.46 $\log_{10}$ and 6.22 $\log_{10}$, respectively. The mean yearly risk of infection was estimated to be 1.68×10^{-3} infection/person/yr. The uncertainty analysis demonstrated that the following items had large impacts on the yearly risk of infection: the ratio of *Campylobacter* to *E. coli* in the source water, the method of pairing the concentration data before and after treatment, and the variation in the removal efficacy of slow sand filtration depending on the water temperature. Based on these results, the important components that are required to improve the accuracy of the estimates were identified. Disability Adjusted Life Years (DALYs) and costs-of-illness in the distribution area were estimated. The uncertainty analyses of DALYs showed that the ratio of illness to infection is the most important factor that affects the DALYs. It should be noted that it is important to estimate the ratio of illness to infection to decrease the uncertainty of the DALYs.

Keywords: *Campylobacter*, disability adjusted life years, quantitative microbial risk assessment

INTRODUCTION

Since the 1980s, Quantitative Microbial Risk Assessment (QMRA) has been used to quantify the microbial safety of drinking water (Haas *et al.*, 1999; Medema *et al.*, 2006). The microbial exposure or dose is calculated based on the pathogen concentration in the drinking water and the consumption of unboiled drinking water. The risk of infection is calculated based on the chance of ingesting pathogens and developing an infection from this exposure (dose-response relation). In many studies, the variability of each element, such as pathogen concentration in the source water and the removal and inactivation efficacy of the water treatment steps, is described by a Probability Density Function (PDF). The yearly risk of infection is quantitatively estimated by Monte Carlo simulation. To date, the methodology of QMRA has been developed and improved by several studies.

Drinking water is supplied without chlorination in the Netherlands. The Dutch legislation requires that several microbes are assessed, notably enteric viruses, *Cryptosporidium*, *Giardia* and other relevant pathogens (Smeets *et al.*, 2009). Although *Campylobacter* is not specified in this list, this pathogen is considered to be one of the most important bacteria that cause waterborne diseases in many European countries (Medema *et al.*, 2006). The removal and inactivation efficacy required by the water treatment plants was estimated based on the monitored concentrations of pathogens in raw water and the acceptable risk level (10^{-4} infection/person/yr) in the Netherlands (Smeets *et al.*, 2009). These results suggest that the required log reduction for

Address correspondence to Sadahiko Itoh, Department of Environmental Engineering, Kyoto University, Email: itoh@urban.env.kyoto-u.ac.jp
Received September 26, 2012, Accepted December 25, 2012.

Campylobacter is the largest among the four index pathogens listed above. To date, however, the accuracy of estimating the risk of *Campylobacter* infection via drinking water and the adverse health outcomes has not been clarified.

First, many techniques that were developed by previous QMRA studies were applied in this study, and the yearly risk of *Campylobacter* infection was estimated in a case study. Second, Disability Adjusted Life Years (DALYs) as adverse health outcomes and costs-of-illness were approximated. Thus, one of the aims of this study is to perform a complete QMRA. Third, uncertainty analyses of the estimates were conducted. As a result, the variable that has the greatest impact on decreasing the yearly risk of infection was identified, and components or variables that can help improve the accuracy of the estimates were determined. In addition, the uncertainty analyses of DALYs were conducted to find important factors that affect the DALYs.

METHODS

Case description

The Weesperkarspel treatment plant of Waternet (water cycle company for Amsterdam and the surrounding areas) located in the western Netherlands was used as a case study. With a production average of 115,000 m^3/day, the plant supplies drinking water to the eastern part of Amsterdam and the suburbs. The source water for this plant is abstracted from a polder (the 5.4 km^2 Bethune Polder) that is a land reclaimed for agriculture and stock raising. The water is pumped out as surface water from the neighboring watercourses and is mainly seepage water flowing through a good permeable underground of the polder. When water demand is high, such as in the summer, water can also be abstracted from the nearby Amsterdam-Rhine Canal (ARK-water). The volume of ARK-water is 5% of the total annual produced volume. The Amsterdam-Rhine Canal water comes from the Rhine River, and is more polluted than the water from the Bethune Polder (Hijnen *et al.*, 2005).

Figure 1 shows the treatment process of the plant. The raw water is pre-treated by coagulation, stored in the lake water reservoir, and then undergoes rapid sand filtration (RSF) at the Loenderveen plant. The average time in the reservoir (123 ha, 6.9×10^6 m^3) is 89 days. After the RSF, the water is transported to the Weesperkarspel plant where it undergoes several treatment steps such as ozonation, softening, granular activated carbon (GAC) filtration and slow sand filtration (SSF).

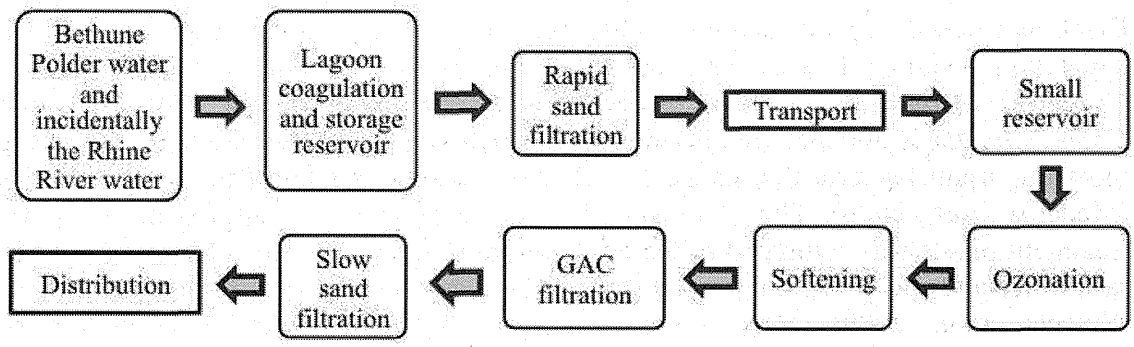

Fig. 1 - Treatment process of Weesperkarspel.

Pathogen and its indicator

Generally, it is not easy to measure the concentrations of pathogens in source water, treatment plant water or drinking water, and often there are not a large number of monitored concentrations. For pathogenic bacteria like *Campylobacter*, indicator bacteria like *E. coli* and enterococci have been proposed as process indicators to assess the elimination capacity of water treatment processes (Hijnen and Medema, 2010). For SSF, *E. coli* is a conservative surrogate for removal of *Campylobacter* as demonstrated by Hijnen *et al.* (2004) and Dullemont *et al.* (2006). For RSF, Hijnen *et al.* (1998) showed that *E. coli* is removed slightly better than environmental *Campylobacter* bacteria. It has been found that *E. coli* and *Campylobacter* can be similarly inactivated by ozonation (Smeets *et al.*, 2005). Based on these information, this study estimated the efficacy of removing and inactivating *E. coli* by the treatment plant. Then, the *E.coli* concentration was translated into a *Campylobacter* concentration using the ratio of *Campylobacter* to *E. coli* in the surface water.

Data on *E. coli* in the raw water from the Bethune Polder were provided by Waternet. The analyses included *E. coli* concentrations that were measured 46 times from April, 2002 to December, 2004.

Non-parametric validation of treatment efficacy

First, the method to determine the removal and inactivation efficacy of the water treatment process was selected. Pairing by date has been widely used to determine the reduction efficacy. In this method, influent and effluent samples taken on the same day are compared and the reduction efficacy is calculated for each pair. This assumes that samples before and after treatment are correlated in time. On the other hand, pairing by rank assumes that there is a complete correlation between the influent and effluent concentrations (lowest influent concentrations correlate to lowest effluent concentrations, etc.). To enable pairing by rank, the samples of the influent and effluent concentrations are sorted by concentration in descending order before determining the treatment efficacy. The other pairing method is the random method, which assumes no correlation by date or rank. In this method, the influent and effluent sample concentrations are paired randomly. Smeets *et al.* (2008) demonstrated that the date and random methods resulted in similar removal, while the rank method proved to be the best method to validate the treatment efficacy in a water treatment process in the Netherlands. However, the applicability of the rank method to different situations is not known. Therefore, the rank, date, and random methods were compared in this study.

The confidence interval for the monitored *E. coli* concentrations was determined by adapting a standard nonparametric bootstrapping procedure. Thus, bootstrap samples of *E. coli* concentrations were produced for the monitored water before and after each treatment step, such as coagulation-storage, RSF, and ozonation. The observed treatment efficacy was calculated from the bootstrap samples paired by date and rank. A difference of 89 days (average time in the reservoir) was given even when the samples were paired by date before and after coagulation-storage. By comparing the predicted concentrations to the monitored effluent concentrations, the accuracy of the rank, date, and random methods was compared.

Parametric treatment model

Coagulation-storage, RSF, ozonation and SSF are considered the main microbial barriers at the water treatment plant examined in the case study. Therefore, the removal and inactivation efficacy were estimated for each of these four steps. The *E. coli* concentrations after coagulation-storage were measured 91 times from January, 2002 to August, 2005. The *E. coli* concentrations after RSF and after ozonation were measured 556 and 326 times, respectively, from January, 2003 to December, 2004. Thirty of the 326 measurements in ozonated water were performed with large volume samples ranging from 10 L to 100 L.

For each step of coagulation-storage, RSF and ozonation, the parametric distributions, which fit the distributions of the treatment efficacy estimated by the rank method, were selected for the QMRA.

Water treated with SSF is the drinking water that is supplied to the city. The available *E. coli* data include concentrations monitored daily from January, 2003 to December, 2004 for each of two parallel lines. *E. coli* was detected only once (0.2 *E. coli*/100 mL) over a total of 1393 measurements, and all of the other samples were negative for *E. coli*. Therefore, the removal efficacy of SSF cannot be determined based only on the monitored data. In order to estimate the removal efficacy, a pilot-scale plant was dosed with *E. coli* in order to detect *E. coli* in the effluent (Dullemont *et al.*, 2006). A maximum value of 4.2 $\log_{10}$, mean elimination capacity (MEC) of 2.4 $\log_{10}$ and minimum value of 2.0 $\log_{10}$ were obtained when determining the removal efficacy six times under conditions where the water temperature was below 13°C. A triangular distribution with these parameters was constructed. In addition, the removal efficacy under conditions where the water temperature was above 13°C was determined three times. The impact of these results will be examined by an uncertainty analysis in this study.

The overall removal efficacy with the four treatment steps was calculated by Monte Carlo simulation, which was performed by drawing random values from each PDF that was given to the four steps. This procedure was repeated 100,000 times to achieve stable results. The *E. coli* concentration in the finished (treated) water can be estimated by multiplying the overall removal efficacy with the source water concentration by Monte Carlo simulation. Crystal Ball 7® (Oracle Corporation, USA) was used to select the parametric PDFs fitted to the variables and to perform the Monte Carlo simulation.

Risk calculation

Daily exposure (dose) (*E. coli*/day) was calculated by multiplying the estimated concentration in the treated water with the amount of unboiled drinking water consumed per day in the Netherlands. To account for the variability in water consumption within the population, a Poisson distribution with a mean value (rate in the Poisson model) of 0.706 glass/day was recommended to use with the QMRA (Mons *et al.*, 2007). Assuming that a glass contains 250 mL, the mean value is equivalent to 177 mL/day.

The *E. coli* dose (*E. coli*/day) was translated into the *Campylobacter* dose (*Campylobacter*/day) using the ratio of *Campylobacter* to *E. coli* (C/E ratio) in the

surface water. It is needed to use C/E ratio for the Bethune Polder water or the source water after the ARK-water was added to the Bethune Polder water. *Campylobacter*, however, is not a pathogen measured in the routine practice of water quality monitoring. There is very limited data of *Campylobacter* concentrations in the source water that can produce C/E ratios with corresponding *E. coli* concentrations (Hijnen *et al.*, 2005). On the other hand, there is a data set of concentrations of *E. coli* and *Campylobacter* in the Meuse River at the intake site of a water company in the Netherlands (Medema *et al.*, 2006). These data measured 22 times in 1994 were used to determine the C/E ratio in this study. After calculating the ratios from the concentrations of *E. coli* and *Campylobacter*, a PDF that fits the distribution of the C/E ratios was chosen.

The daily risk of infection P_d (infection/person/d) was calculated from the *Campylobacter* dose using a dose-response model. The dose-response relationship of *Campylobacter jejuni* presented by Tenuis *et al.* (2005) is a Beta-Poisson model where $\alpha = 0.024$ and $\beta = 0.011$. Although the Beta-Poisson approximation should retain the criteria of ≥ 1 and $\alpha \leq \beta$, the above α and β do not satisfy these criteria. Actually, when the aforementioned Beta-Poisson model was applied, it was noted that the Beta-Poisson model can exceed the maximum risk curve at low doses (Medema *et al.*, 2006). This means that the dose-response model predicts a theoretically impossible probability of infection. Therefore, the Beta-Poisson model is not appropriate for the calculation. Alternatively, the exact Beta-Poisson model can be approximated for low doses (< 0.1 organisms/L) by setting γ of the exponential model equal to the expected value of the Beta distribution (α / (α + β)), thus avoiding this complication. Consequently, the Beta-Poisson model was approximated by the exponential model ($P_d = 1 - \exp(-0.686 \times D)$, D: dose) with $\gamma = 0.686$, which was used in this study. The effects of using the maximum risk curve or the Beta-Poisson model were examined by the uncertainty analysis.

The individual health risk is represented by the average yearly risk of infection. Under the assumptions of a binomial process, the yearly risk of one or more infections is calculated using the following equation (1).

$$P_y = 1 - (1 - P_d)^{365} \quad (1)$$

Monte Carlo simulation was performed by drawing random values from each PDF of the *E. coli* concentration in the source water, four treatment steps, water consumption, and the C/E ratio to calculate the yearly infection risk P_y. This simulation assumes that there are no correlations between the variables. Stable results were achieved with acceptable calculations 100,000 times.

Uncertainty analysis

It is natural that the estimated values of target variables and yearly risk of infection have large uncertainty. Uncertainty analyses were performed to examine the impact of using ARK-water, the impact of the removal efficacy of SSF under conditions with high water temperatures, the impact of the C/E ratio, the impact of dose-response models, and the impact of data pairing methods such as the rank and date methods. The uncertainty analyses of DALYs were also performed to find important factors that affect the DALYs and examine the impact of the ratio of illness to infection.

RESULTS AND DISCUSSION

Comparison of the data pairing methods

The *E. coli* concentrations in the treated water that were predicted by the date, rank and random methods were compared to the monitored *E. coli* concentrations. Figures 2(a) and 2(b) show examples of the predicted and monitored *E. coli* concentrations after coagulation and storage. The distributions of the concentrations were presented by CCDFs (Complementary Cumulative Distribution Functions) on a double log scale, which is well-suited for magnifying data from rare events (Smeets *et al.*, 2008). It was found that the pairing method by date resulted in an overestimation of effluent concentrations, indicating that the date method tends to assess the removal efficacy at a lower value. This reduced value is because the date method often yielded a low removal efficacy and could even predict "negative removal". "Negative removal" would imply that microbes were occasionally "produced" by the treatment, which is unlikely. However, the rank method did not allow for negative removal. The rank method provided an appropriate estimate of the removal efficacy for Monte Carlo simulation since the monitored concentrations in Fig. 2(a) were consistent with the predicted concentrations. The random and the date method resulted in a similar estimate of the removal efficacy (data are not shown). Consequently, the rank method was used in the following analyses.

Application of distribution type

PDFs were selected to describe the distributions of the *E. coli* concentrations in the source water, the removal and inactivation efficacy by coagulation-storage, RSF, ozonation and SSF, and the C/E ratio. In general, extreme events can dominate the average health risk. Therefore, the PDF should fit the extremes (tail) of the observed variations. From the point of emphasizing the fit to rare events, the results of the Anderson-Darling test were more emphasized than the results of the chi-square test and the Kolmogorov-Smirnov test when selecting a distribution type. The selected PDFs

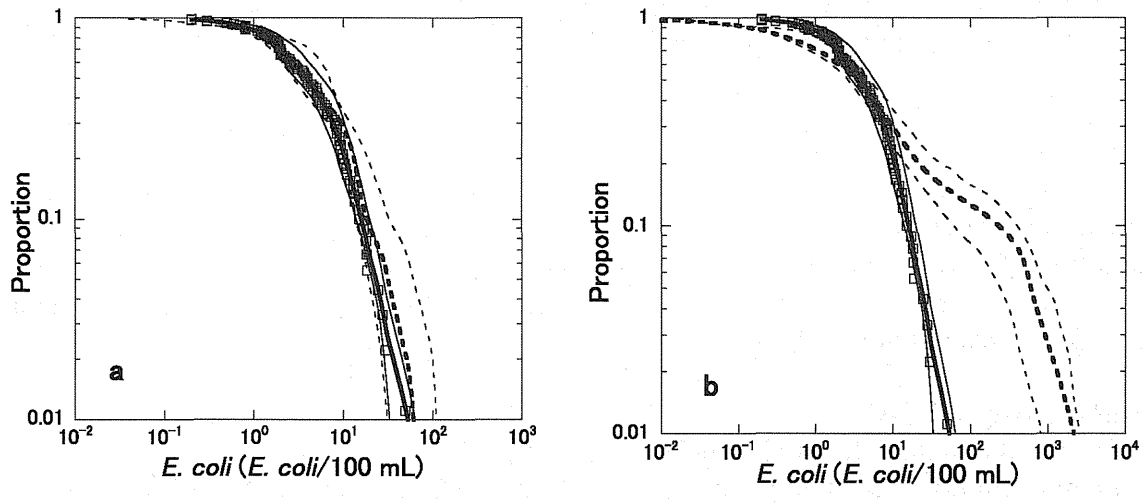

Fig. 2 - *E. coli* concentration after coagulation and storage calculated with the non-parametric model validated by the rank (a) and date (b) methods. Calculated concentration by the rank and date methods (dashed) are compared to monitored concentrations (line and markers). Median concentration (thick) and 95% CI (fine) are shown.

and estimated parameters are summarized in Table 1.

The data used to determine the C/E ratio were the *E. coli* and *Campylobacter* concentrations in the Meuse River that were measured 22 times in 1994 (Medema *et al.*, 2006). In general, there is a large variation in pathogen to *E. coli* ratios. Therefore, PDFs were applied to the distribution of these ratios. As a result, the Lognormal distribution was best fitted as shown in Table 1. The variation of C/E ratio given by the Lognormal distribution includes all factors such as faecal contamination by livestock and waterfowl, possible contamination caused by combined sewer overflows, agricultural activity, rainfall, snow melting, water temperature, and so on (Hijnen *et al.*, 2005). However, this is not the C/E ratio in the Bethune Polder water. Since it is assumed that the C/E ratio has large uncertainty, the impact of the C/E ratio on the yearly risk of infection was analyzed.

Overall removal efficacy and yearly risk of infection

The mean and median of the overall log reduction were estimated to be 7.46 $\log_{10}$ and 6.22 $\log_{10}$, respectively, by the Monte Carlo simulation as shown in Table 2. It is noteworthy that the overall log reduction was estimated to be 7.46 $\log_{10}$ (median) instead of setting the overall removal to 100%, although most of the *E. coli* concentrations in SSF-treated water are 0. Since this calculated overall reduction is applied to the *E. coli* concentrations in the source water, the *E. coli* concentrations in the treated water were not estimated to be 0 as shown in Table 2. As a result, the mean was calculated to be 1.64×10^{-4} *E. coli*/100 mL, and the median was 4.35×10^{-6} *E. coli*/100 mL. The *E. coli* dose is calculated by multiplying the *E. coli* concentration in the drinking water by water consumption. The mean and median were estimated to be 2.99×10^{-4} and 1.24×10^{-8} *E. coli*/day, respectively.

After translating the *E. coli* dose into the *Campylobacter* dose using the C/E ratio, the *Campylobacter* dose-response relationship was applied. As a result, the daily risk of *Campylobacter* infection was estimated to be a mean of 6.51×10^{-6} infection/person/d and a median of 9.24×10^{-11} infection/person/d. As a result of calculating the yearly risk of infection from the daily risk of infection, a mean of 1.68×10^{-3} infection/person/yr and a median of 3.37×10^{-8} infection/person/yr were obtained. This estimate is called the base case (shown in Table 3). The Dutch drinking water regulations require water companies to comply with a 10^{-4} yearly risk of infection

Table 1 - Probability density functions (PDF) fitted to the target variables.

		PDF type	Estimated parameters
E.coli in the source water (*E.coli*/100 mL)		Gamma	$\mu = -2.50$; $\lambda = 383$; $\rho = 0.674$
Treatment efficacy (log reduction) of *E.coli*	Coagulation-storage	Logistic	$\mu = 1.48$; $\lambda = 0.15$
	RSF	Weibull	$\mu = 1.74$; $\lambda = 0.59$; $\rho = 2.38$
	Ozonation	Normal	$\mu = 1.91$; $\sigma = 0.88$
	SSF	Triangular	Min. = 2.00; MEC (Mean Elimination Capacity) = 2.40; Max. = 4.20
C/E ratio		Lognormal	$\mu = 0.0415$; $\sigma = 0.104$

Table 2 - Statistics estimated in the QMRA.

	Lower 95% CI boundary	Median	Mean	Upper 95% CI boundary
Overall log reduction	5.41	7.46	6.22	9.58
E. coli in the treated water (*E. coli*/100 mL)	1.07×10^{-8}	4.35×10^{-6}	1.64×10^{-4}	9.25×10^{-4}
E. coli dose (*E. coli*/day)	0	1.24×10^{-8}	2.99×10^{-4}	1.36×10^{-3}
Campylobacter dose (*Campylobacter*/day)	0	1.35×10^{-10}	9.52×10^{-6}	3.36×10^{-5}
Daily risk of infection (infection/person/d)	0	9.24×10^{-11}	6.51×10^{-6}	2.30×10^{-5}
Yearly risk of infection (infection/person/yr)	0	3.37×10^{-8}	1.68×10^{-3}	9.06×10^{-3}
DALYs (yr)	0	6.37×10^{-5}	2.71	1.49×10

Table 3 - Uncertainty analysis of the yearly risk of infection.

		Yearly risk of infection (infection/person/yr)			Note
		Lower 95% CI boundary	Mean	Upper 95% CI boundary	
Base case		0	1.68×10^{-3}	9.06×10^{-3}	
Amsterdam-Rhine canal water (ARK-water)		0	1.72×10^{-3}	8.60×10^{-3}	
Removal efficacy by SSF with high temperatures		0	1.01×10^{-3}	3.72×10^{-3}	
C/E ratio	0.001 (0.1%)	0	6.59×10^{-5}	3.54×10^{-4}	Min. (WHO, 2004)
	1 (100%)	0	2.53×10^{-2}	2.84×10^{-1}	Max. (Smeets, 2008)
Dose-response model	Maximum risk curve	0	2.30×10^{-3}	1.27×10^{-2}	
	Beta-Poisson	0	4.24×10^{-3}	2.77×10^{-2}	$\alpha = 0.024$, $\beta = 0.011$ (Tenuis *et al.*, 2005)
Date method		0	3.18×10^{-2}	4.72×10^{-1}	

target by a site-specific QMRA. Therefore, a mean of 1.68×10^{-3} infection/person/yr would not meet this requirement.

Sensitivity analysis

Sensitivity analyses of the estimated yearly risk of infection were performed (Itoh, 2010). For a sensitivity analysis, Spearman's rank correlation coefficients are computed between the assumed variables and predicted variables. The contribution to variance that is calculated by squaring the rank correlation coefficients indicates the relative importance by showing the percentage of the variance of the predicted variable contributed by each variable in the model.

It was found that the statistical methods used to analyze the water consumption data have large impacts on the results of the sensitivity analysis, although they do not have large effects on the probability of infection. It should be noted that statistical methods

used to analyze water consumption data may complicate the results of sensitivity analysis if the water consumption data are not analyzed by an appropriate statistical method. To avoid this problem, it is preferable to apply a continuous model like the Exponential model rather than a discrete model like the Poisson model.

Among the four treatment steps of coagulation-storage, RSF, ozonation and SSF, ozonation has the greatest impact, which means that the rank correlation coefficient between the inactivation efficacy by ozonation and the yearly risk of infection was the largest. This is because the *E. coli* inactivation efficacy by ozonation varies greatly from 0 to 4 $\log_{10}$. Therefore, the stable performance of ozonation inactivation is the most important to stably produce safe drinking water at the water treatment plant. The mean value of the yearly risk of infection of this treatment plant was estimated to be 1.68×10^{-3} infection/person/yr, which was larger than the required probability of infection ($< 10^{-4}$ infection/person/yr). In order to improve the protection against *Campylobacter* infection among consumers, it can be suggested that it is most effective to stably inactivate organisms by ozonation.

Uncertainty analysis

Impact of ARK-water intake

The volume of ARK-water is 5% of the annual total production at the plant, and the ARK-water is more polluted than the Bethune Polder water. However, detailed data regarding the daily quantity of ARK-water intake, fluctuations in microbe concentrations, etc. are insufficient. Therefore, only *E. coli* concentrations in the Bethune Polder water were used to analyze the base case, and the effects of ARK-water intake were examined in the uncertainty analysis. ARK-water is used mainly in the summer for three months from June to August. Therefore, it is assumed that 20% of ARK-water is added to water from the Bethune Polder during this time frame. The *E. coli* concentration in ARK-water was set to 1.75 times higher than the concentrations in Bethune Polder water (Hijnen *et al.*, 2005). The results of calculating the yearly risk of infection are shown in Table 3. Although the mean value slightly increased to 1.72×10^{-3} infection/person/yr, ARK-water intake did not greatly affect the risk of infection.

Impact of the removal efficacy by SSF with high water temperatures

The removal efficacy of SSF under conditions with water temperatures above 13°C was determined three times using a pilot-scale plant, while the removal efficacy under conditions with water temperatures below 13°C was determined six times (Dullemont *et al.*, 2006). When incorporating this data, it is possible to expect that the removal efficacy by SSF is not constant but can change in a year, depending on the water temperature. However, the influence of water temperature on the removal efficacy has not been investigated for the three other treatment steps. Therefore, the removal efficacy by SSF with high water temperatures was examined in the uncertainty analysis.

A maximum value of 5.6 $\log_{10}$, MEC of 3.6 $\log_{10}$ and minimum value of 3.1 $\log_{10}$ were obtained when the removal efficacy was determined three times. A triangular distribution with these parameters was constructed. This means that the removal efficacy increases when water temperatures are high compared to the removal efficacy when water temperatures are below 13°C (MEC 2.4 $\log_{10}$). The water temperature is

above 13°C for approximately five months from May to September. Therefore, the removal efficacy by SSF during this time period was set to the above values. The results of calculating the yearly risk of infection are shown in Table 3. The mean value slightly decreased to 1.01×10^{-3} infection/person/yr. The effect of increasing the removal efficacy of SSF more than one $\log_{10}$ over five months within one year is apparent.

Impact of the C/E ratio

For the base case, a Lognormal distribution with a mean value of 0.0415 and a standard deviation of 0.104 was assigned to the distribution of the C/E ratio obtained from measurements from the Meuse River. On the other hand, the concentration ranges of enteric pathogens and faecal indicators in different types of source water are described by reviewing scientific literatures in the WHO Guidelines for Drinking Water Quality (2004). Based on thisinformation, the minimum C/E ratio was set at 0.001 (0.1%). In contrast, recontamination by waterfowl, etc. is observed in the reservoir where the water is stored for an average of 89 days. At another treatment plant, Leiduin of Waternet, the water abstracted from the dunes is stored in an open pond. It has been reported that recontamination occurs in this pond. The yearly variation in the *Campylobacter* and *E. coli* concentrations in this pond shows that the mean *Campylobacter* concentration was approximately 50% of the mean *E. coli* concentration (Smeets, 2008). Based on these data, the maximum C/E ratio was set to 1 (100%).

The results of calculating the yearly risk of infection are summarized in Table 3. When the C/E ratio was set at 0.001 (0.1%), the mean value was estimated to be a very low at 6.59×10^{-5} infection/person/yr. On the other hand, when the C/E ratio was set at 1 (100%), the mean value increased to 2.53×10^{-2} infection/person/yr. Therefore, it is clear that C/E ratio significantly affects the estimated yearly risk of infection. The C/E ratio used for the base model is the value measured in the Meuse River, and not the value for the actual source water. The data of *Campylobacter* concentrations in the Bethune Polder water or the source water after the ARK-water was added to the Bethune Polder water were not available for this study. It should be noted that a strategic monitoring of *Campylobacter* concentration in the source water that can be used to perform the QMRA is necessary. It could help improve the accuracy of the risk calculation model.

Impact of the dose-response model

The maximum risk curve was applied instead of the Exponential model. The maximum risk curve is calculated when the probability that an ingested organism will pass the host's defense mechanisms and find a site suitable for colonisation is maximized and assumed equal to 1. The results of calculating the yearly risk of infection are shown in Table 3. The mean value slightly increased to 2.30×10^{-3} infection/person/yr. Since the maximum risk curve is given by the exponential dose-response function with r = 1, the daily probability of infection P_d is described as $P_d = 1 - \exp(-D)$. This can be approximated by $P_d \approx D$ at low doses. On the other hand, the Exponential model is approximated by $P_d = 1 - \exp(-\gamma D) \approx \gamma D = 0.686 \times D$ at low doses. It can be verified that the ratio between the mean value of 1.68×10^{-3} infection/person/yr by the base model and the mean value of 2.30×10^{-3} infection/person/yr by the maximum risk

curve is close to 0.686. Thus, the maximum risk curve is also an important tool for the uncertainty analysis, providing the upper limit for the possible infection response.

It was described in **Risk calculation** that it is not appropriate to use the Beta-Poisson model (Tenuis *et al.*, 2005). As a trial, the yearly risk of infection was calculated using the Beta-Poisson model. As shown in Table 3, the estimated mean value was 4.24×10^{-3} infection/person/yr, which is larger than the mean value of 2.30×10^{-3} infection/person/yr by the maximum risk curve. Therefore, it was confirmed that the Beta-Poisson model is not appropriate for these analyses.

Comparison between the rank method and date method

It was confirmed that it was appropriate to use the rank method rather than the date and random methods for pairing the microbe concentrations before and after treatment. The removal and inactivation efficacy by coagulation-storage, RSF and ozonation was estimated by the date method for reference. The median value of the overall removal efficacy was 6.18 $\log_{10}$, which was smaller than the median value of 7.46 $\log_{10}$ obtained by the rank method by 1.28 $\log_{10}$. Moreover, the mean value was 2.16 $\log_{10}$ and was much smaller than the mean value of 6.22 $\log_{10}$ by the rank method. This is because a low removal efficacy was often found with the date method, and even "negative removal" could occur. The results of calculating the yearly risk of infection are shown in Table 3. The mean value was estimated to be 3.18×10^{-2} infection/person/yr and was 19 times larger than that obtained by the rank method. Therefore, it is necessary to select an appropriate data pairing method before performing a QMRA.

Summary of the uncertainty analyses

The removal efficacy by SSF increases during seasons with high water temperature. When incorporating this increased removal efficacy, the yearly risk of infection slightly decreased. It is recommended that the influence of water temperature on removal efficacy is investigated for the treatment steps.

It was found that the C/E ratio significantly affects the yearly risk of infection. The C/E ratio used for the base model is the value measured in the Meuse River and not that of the treatment plant source water. It is highly important to determine the C/E ratio in the source water in order to improve the accuracy of the QMRA. In addition, it is preferable to directly monitor the *Campylobacter* concentration, when possible.

With respect to data pairing methods before and after treatment, the estimated yearly risk of infection for the rank method and date method differed 19 times. Therefore, it should be emphasized that it is necessary to select an appropriate data pairing method before performing the QMRA.

Disease burden and costs-of-illness

Estimation of DALYs and costs-of-illness

QMRA primarily focuses on estimating the risk of infection. Waterborne diseases, however, differ in nature, severity and duration. Therefore, it is necessary to use a metric that measures the overall health burden of waterborne diseases, such as the DALYs (Havelaar and Melse, 2003). DALYs are the sum of Years of Life Lost (YLL) by

premature mortality and Years Lived with a Disability (YLD), weighted with a factor between 0 and 1 for the illness severity. Since the health outcomes associated with *Campylobacter* infection are not only diarrhoeal illness but also critical health effects, such as Guillain-Barré syndrome or reactive arthritis, estimations using DALYs are meaningful and important. Havelaar and Melse (2003) determined the DALYs associated with *Campylobacter* infection in developed countries. Since YLD was estimated to be 3.2 and YLL was estimated to be 1.4 per 1000 cases of gastroenteritis, the DALYs were calculated as follows.

$$3.2 + 1.4 = 4.6 \text{ DALYs} \quad (2)$$

The drinking water produced in the Weesperkarspel treatment plant is supplied mainly to the eastern part of the city of Amsterdam and the suburbs. The service population is estimated to be approximately 360,000 persons based on the volume produced (de Moel *et al.*, 2006). Since the mean yearly risk of infection was calculated to be 1.68×10^{-3} infection/person/yr, the number of persons infected yearly in the supply area is:

$$1.68 \times 10^{-3} \times 360{,}000 = 605 \text{ persons} \quad (3)$$

Although infection is necessary to cause disease, not all infections induce symptoms. The process of developing an illness due to an infection is a dynamic phenomenon (Havelaar *et al.*, 2009). The ratio of illness to infection is not simple and can be affected by protective immunity, pathogen dose, area, country, and so on. Setting the ratio of illness to infection is discussed in ***Uncertainty analysis of DALYs***. For this estimation, a conservative ratio of 1 is assumed. In this case, the disease burden based on DALYs would be calculated using the expected number of infections per year for the population multiplied by the DALY contribution per infection:

$$605 \times 4.6 / 1{,}000 = 2.78 \text{ DALYs} \quad (4)$$

The cost of one case of gastroenteritis by *Campylobacter* was estimated to approximately 370 € (if the case is not discounted), which is the sum of direct health care costs, direct non-health care costs, and indirect non-health care costs (Kemmeren *et al.*, 2006). Using this estimate, the costs-of-illness in the distribution area per year are calculated as follows:

$$605 \times 370 \text{ €} = 220{,}000 \text{ €} \quad (5)$$

The DALYs and costs-of-illness demonstrated here were calculated based on values given tentatively and assumptions because of insufficient information. Factors affecting the accuracy of estimated DALYs are severity weights and durations for different outcomes of *Campylobacter* infection for calculating YLD, the probability to develop severe diseases such as reactive arthritis (ReA) after infection, the case-fatality ratio for calculating YLL, and so on (Havelaar and Melse, 2003). A scenario set to estimate the DALYs and costs-of-illness also has assumptions because of the absence of information with respect to use of medical services. For example, only patients visiting a general practitioner would be at risk for ReA (Kemmeren *et al.*, 2006). To improve the accuracy of the estimates, more research is needed, and data collection should focus

on quantitative data. The estimates shown here can be updated if new information becomes available.

Uncertainty analysis of DALYs

The result of calculating the DALYs by the Monte Carlo simulation is shown in Table 2. The mean and median were estimated to be 2.71 and 6.37×10^{-5} year, respectively. The ratio of illness to infection was set at 1 as a conservative value. As discussed above, however, it seems this ratio has a large uncertainty.

Dose-response models applied in microbial risk assessment are established based on some evidence such as that obtained by volunteer experiments. As most dose-response models reach a maximum probability of illness of 1, it is also assumed that the ratio of illness to infection is 1 (Havelaar *et al.*, 2009). These infectious disease models do not usually distinguish between asymptomatic and symptomatic infections.

The best fitting parameter estimates of the unconditional model for illness given dose show that 69% of subjects exposed to a single colony forming unit would become ill (Tenuis *et al.*, 2005), although the second outbreak occurred among schoolchildren during a visit to a daily farm was analyzed. A volunteer experiment conducted by Black *et al.* (1988) demonstrated that the ratio of diarrhoeal illness to infection ranged from 0% to 60%. In developed countries, it has been reported that approximately one-third of all infected patients develop watery diarrhoea or more severe health outcomes (Havelaar and Melse, 2003). A risk assessment study conducted in the Netherlands gave an estimate of more than 9 million infections and approximately 3 million clinical cases of campylobacteriosis per year (Evers *et al.*, 2008). These studies are the examples supporting relatively high ratios of illness to infection.

An exposure, however, would frequently result in asymptomatic infections. It was reported that healthy carriers outnumbered diseased persons by a factor of approximately 80, or in other words, only 1.2% of all excreters of *Campylobacter* are actually experiencing an episode of illness (Tompkins *et al.*, 1999). Similar calculations for the Netherlands (de Wit *et al.*, 2001) suggest the ratio of asymptomatic to symptomatic shedders is 120 : 1.

There are also reported conflicts between estimates of infection with *Campylobacter* from theoretical and empirical studies (Havelaar *et al.*, 2009). As described above, a risk assessment gave an estimate of more than 9 million infections and approximately 3 million clinical cases per year in the Netherlands (Evers *et al.*, 2008). In contrast, a population-based study from the same country indicated that in 1999 the incidence of acute *Campylobacter*-associated gastroenteritis was only 80,000 cases (Mangen *et al.*, 2005). In the latter case, only 1 out of 100 of infections leads to symptoms of campylobacteriosis. Thus, there is an approximately 40-fold difference between the risk-based and the epidemiological estimates of disease incidence. Normal risk assessment models do not take the influence of acquired protective immunity into account. *Campylobacter* infection, however, can develop protective immunity. As a consequence, not every individual in the population is fully susceptible to illness from a *Campylobacter* infection. A high level of exposure would frequently result in asymptomatic infections. A large difference shown above could be explained mainly by

the impact of protective immunity that contributes to reduce the risk of infection and increase the rate of asymptomatic infections. This is accord with the high prevalence of antibodies detected by sero-surveillance (Havelaar *et al.*, 2009).

Based on these information, the minimum ratio of illness to infection can be set at 0.01. A mean obtained by the Monte Carlo simulation was 2.76×10^{-2}, which was 98 times smaller than that obtained in the base case (2.71 shown in Table 2).

DALYs can be basically calculated from the yearly risk of infection shown in Table 3 using the formulas (3) and (4). As a result of comparing the impacts of factors shown in Table 3, it was found that the ratio of illness to infection is the most important factor that affects the DALYs. Currently, both 0.01 and 1 given in estimating DALYs are the ratios having some evidence that can be found in literatures as discussed above. It should be noted that it is important to estimate the ratio of illness to infection in a target area or country to decrease the uncertainty of the DALYs.

CONCLUSIONS

The yearly risk of infection of *Campylobacter* was estimated for a treatment plant in the Netherlands as a model case. First, it was confirmed that it was appropriate to use the rank method rather than the date and random methods for pairing the microbe concentration data before and after the treatment steps. Next, the median and mean overall removal efficacy by the four treatment steps at the plant was estimated to be 7.46 $\log_{10}$ and 6.22 $\log_{10}$, respectively. The mean value of the yearly risk of infection was estimated to be 1.68×10^{-3} infection/person/yr. From the sensitivity analysis, it was noted that it is most effective to stably inactivate organisms by ozonation to stably produce safe drinking water at the water treatment plant.

The uncertainty analysis demonstrated that the factors with large impacts on the yearly risk of infection were the C/E ratio in the source water, the method of pairing the microbe concentration data, and the variation in the removal efficacy of SSF depending on the water temperature. From the obtained results, several important components or variables were identified that can help improve the accuracy of the QMRA estimates, and data collections where the priority should be given were suggested.

Based on the yearly risk of *Campylobacter* infection at the treatment plant, DALYs and costs-of-illness in the distribution area per year were estimated to be 2.78 DALYs and 220,000 €, respectively. The uncertainty analyses of DALYs showed that the ratio of illness to infection is the most important factor that affects the DALYs. It should be noted that it is important to estimate the ratio of illness to infection to decrease the uncertainty of the DALYs.

ACKNOWLEDGEMENT

The author would like to thank Dr. Gertjan Medema and Dr. Partick Smeets from KWR Watercycle Research Institute in the Netherlands for their instruction in performing QMRA.

REFERENCES

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

de Moel P. J., Verberk J. Q. J. C. and van Dijk J. C. (2006) Drinking Water: Principles and Practices. World Scientific Publishing Co. Pte. Ltd., Singapore.

de Wit M. A. S., Koopmans M. P. G., Kortbeek L. M., Wannet W. J. B., Vinjé J., van Leusden F., Bartelds A. I. M. and van Duynhoven Y. T. H. P. (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. *Am. J. Epidemiol.*, **154**(7), 666-674.

Dullemont Y. J., Schijven J. F., Hijnen W. A. M., Colin M., Magic-Knezev A. and Oorthuizen W. A. (2006) Chapter 2 Removal of microorganisms by slow sand filtration. In: *Recent Progress in Slow Sand and Alternative Biofiltration Processes*, Gimbel R., Graham N. J. D. and Collins M. R. (eds.), IWA Publishing, London, UK, pp.12-20.

Evers E. G., van der Fels-Klerx H. J., Nauta M. J., Schijven J. F. and Havelaar A. H. (2008) *Campylobacter* source attribution by exposure assessment. *Int. J. Risk Ass. Mgt.*, **8**(1-2), 174-190.

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

Havelaar A. H. and Melse J. M. (2003) *Quantifying public health risk in the WHO Guidelines for Drinking-Water Quality. A burden of disease approach.* Report 734301022, RIVM, Bilthoven, The Netherlands.

Havelaar A. H., van Pelt W., Ang C. W., Wagenaar J. A., van Putten J. P. M., Gross U. and Newell D. G. (2009) Immunity to *Campylobacter*: its role in risk assessment and epidemiology. *Critical Reviews in Microbiol.*, **35**(1), 1-22.

Hijnen W. A. M. and Medema G. J. (2010) Elimination of Micro-organisms by Water Treatment Processes. IWA Publishing, London, UK.

Hijnen W. A. M., Dullemont Y. J. and Medema G. J. (2005) Weesperkarspel (The Netherlands) site report. Catchment survey & monitoring program, Report on the EU MicroRisk Project, June 2005, EC, Brussels.

Hijnen W. A. M., Schijven J. F., Bonné P., Visser A. and Medema G. J. (2004) Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. *Water Sci. Technol.*, **50**(1), 147-154.

Hijnen W. A. M., Visser A. and Medema G. J. (1998) Removal of indicator bacteria during drinking water production at location Scheveningen. Water Company South-Holland, *Kiwa report* 98.216. (in Dutch)

Itoh S. (2010) Effects on the sensitivity analysis of unboiled water consumption data in quantitative microbial risk assessment. *J. Water and Waste*, **52** (8), 55-65. (in Japanese)

Kemmeren J. M., Mangen M. J. J., van Duynhoven Y. T. H. P. and Havelaar A. H. (2006) *Priority setting of foodborne pathogens: Disease burden and costs of selected enteric pathogens*. Report 330080001, RIVM, Bilthoven, The Netherlands.

Mangen M. -J. J., Havelaar A. H., Bernsen R. A. J. A. M., van Koningsveld R., de Wit G. A. (2005) The costs of human *Campylobacter* infections and sequelae in the Netherlands: A DALY and cost-of-illness approach. *Acta Agriculturae Scandi., Section C -- Economy*, **2**(1), 35-51.

Medema G. J., Loret J. F., Stenstrom T. and Ashbolt N. (eds.) (2006) *MICRORISK;*

Quantitative microbial risk assessment in the water safety plan. Final Report on the EU MicroRisk Project, EC, Brussels.

Mons M. N., van der Wielen J. M. L., Blokker E. J. M., Sinclair M. I., Hulshof K. F. A. M., Dangendorf F., Hunter P. R. and Medema G. J. (2007) Estimation of the consumption of cold tap water for microbiological risk assessment: an overview of studies and statistical analysis of data. *J. Water Health*, **5**(Suppl. 1), 151-170.

Smeets P. W. M. H. (2008) *Stochastic modeling of drinking water treatment in quantitative microbial risk assessment.* Water Management Academic Press, Delft, The Netherlands.

Smeets P. W. M. H., Dullemont Y. J. and Medema G. J. (2005) *E. coli* as surrogate for *Campylobacter* inactivation at bench-scale and full-scale and high ozone resistance of environmental *E. coli* and *Campylobacter*. *Proc. 17th IOA Conference,* 22-26 August 2005, Strasbourg, France.

Smeets P. W. M. H., Dullemont Y. J., van Gelder P. H. A. J. M., van Dijk J. C. and Medema G. J. (2008) Improved methods for modeling drinking water treatment in quantitative microbial risk assessment; a case study of *Campylobacter* reduction by filtration and ozonation. *J. Wat. Health*, **6**(3), 301-314.

Smeets P. W. M. H., Medema G. J. and van Dijk J. C. (2009) The Dutch secret: how to provide safe drinking water without chlorine in the Netherlands. *Drink. Water Eng. Sci.*, **2**, 1-14.

Tenuis P. F. M., van den Brandhof W., Nauta M., Wagenaar J., van den Kerkhof H. and van Pelt W. (2005) A reconsideration of the *Campylobacter* dose-response relation. *Epidemiol. Infect.*, **133**(4), 583-592.

Tompkins D. S., Hudson M. J., Smith H. R., Eglin R. P., Wheeler J. G., Brett M. M., Owen R. J., Brazier J. S., Cumberland P., King V. and Cook P. E. (1999) A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Commun. Dis. Public Health*, **2**(2), 108-113.

World Health Organization (2004) *Guidelines for Drinking Water Quality*, third edn, WHO, Geneva, Switzerland.

銅を用いた水中の微生物の不活化技術の現状と課題

岸田 直裕* 島崎 大* 小坂 浩司*
小菅 瑠香* 秋葉 道宏* 林 謙治*

目的　近年注目を集めている銅を用いた水中の微生物の不活化技術の現状および課題を明らかとする。

方法　国内外の学術雑誌等に掲載された文献情報を基に，銅を用いた微生物の不活化技術の歴史，不活化機構，不活化効果が確認されている微生物，水中の微生物の不活化技術について整理した。

結果　銅を用いた微生物の不活化技術は古くから利用されていたが，1930年代より抗生物質の利用が広まったことから，銅を用いた不活化技術は使用されなくなった。一方で，近年は抗生物質耐性菌の存在が問題視されており，抗生物質に代わる微生物の制御アプローチの1つとして，銅を用いた微生物の不活化技術が再認識され始めている。不活化機構については，その詳細はいまだ明らかとなっていないものの，銅イオン自体の毒性と銅表面に生成される活性酸素による強力な酸化作用によって不活化が起こると推測されている。*Legionella pneumophila*, *Salmonella enterica*, *Mycobacterium tuberculosis* 等の公衆衛生上問題となる多くの病原微生物に対して不活化効果が確認されている。建物内の給水管を中心に多くの水関連設備において，近年銅を用いた不活化技術の導入が検討されており，人への健康影響がほとんど発生しないと推測される水道水質基準を満たす濃度範囲であっても，水中の微生物を不活化可能であることが一部の研究でわかってきた。一方で，不活化効果が短期間に留まることも多く，効果を長期間持続させる技術を開発することが今後の課題であるといえる。また，銅管は残留塩素の低減や消毒副生成の生成にも影響を及ぼしていると報告されており，このようなリスクと不活化効果というベネフィットのアセスメントが今後必要であろう。

結論　銅を用いた水中の微生物の不活化技術には，実用上の課題は残るものの，その有用性は十分に明らかとなっており，病院施設の給水設備等での利用が今後期待される。

Key words：銅，消毒，抗菌性，活性酸素，水衛生

Ⅰ　緒　　言

水の安全性を鑑みた上で，最も重要な要素の1つとして微生物の管理が挙げられる。人体に重大な健康影響を及ぼす病原微生物の制御は，水衛生の歴史の中でも常に最も重要な課題であった。我が国をはじめとする先進国においては，公衆衛生の普及に伴い水系感染症の発生は劇的に減少したが，2010年11月にスウェーデンの Östersund 市で水道経由のクリプトスポリジウムの集団感染症が発生し，約10,000人の推定患者が発生する等[1]，依然として水系感染症が散発している現状にある。

* 国立保健医療科学院
連絡先：〒351-0197 埼玉県和光市南 2-3-6
国立保健医療科学院　別館212室　岸田直裕

水の微生物学的安全性を確保する上で最も重要な要素は，水中の微生物の不活化(消毒)技術である。我が国の水道システムにおいては，水道法で義務付けられているように，塩素による微生物の不活化によって，微生物学的に安全な飲料水を供給しているが，クリプトスポリジウムやジアルジア等，塩素に耐性のある微生物も存在しており，塩素消毒以外の様々な不活化技術が提案されている。本稿ではその中でも近年注目を集めている銅を利用した不活化技術について述べる。

Ⅱ　方　　法

銅を用いた微生物の不活化技術については，ドアノブ等の銅固体表面に存在する微生物の不活化効果に関する総説論文が過去に発表されているが[2]，水

中の微生物の不活化効果については報告されていない。そこで本稿では，国内外の学術雑誌等に掲載された文献情報を基に，銅を用いた水中の微生物の不活化技術の現状と課題について整理した。また，銅を用いた微生物の不活化技術の歴史，不活化機構，不活化効果が確認されている微生物の種類についても詳解する。

国内外の文献情報の検索には，㈱科学技術振興機構が運営するJ Dream II（JST文献検索サービス）およびエルゼビア社が運営するScopus（文献検索ツール）を用いた。J Dream IIは，科学技術や医学・薬学関係の国内文献および一部の海外文献を網羅的に検索可能な日本最大級の科学技術文献データベースである。またScopusは，世界の5,000以上の出版社から出版される20,500以上の科学技術・医学等のタイトルを網羅する世界最大級の抄録・引用文献データベースであり，海外文献の網羅的検索に適している。さらに，一般のインターネット検索等によって学術文献以外の情報も収集した。銅（Copper），不活化（Inactivation），消毒（Disinfection），水（Water），水道（Water supply），飲料水（Drinking water）等のキーワードを組み合わせて検索を用い，ヒットした文献の題名・抄録内容から，銅を用いた微生物の不活化技術に関連していると思われる文献を選別した。これらの検索により，学術論文24報，報告書・学会講演集等のその他の文献9件を収集し，それらの情報を整理した。

Ⅲ　結果および考察

1.　銅を用いた微生物の不活化技術の歴史

銅を用いた微生物の不活化技術の歴史は古く，紀元前2,600～2,200年頃には，エジプトにおいて胸部裂傷や飲料水の消毒に利用されており，ギリシャやローマ等でも同様に火傷の処置等に使用されていたと記録されている[3]。19世紀になると，銅工業の従事者がコレラに対する免疫を有していることがフランスで確認されたことから，医学での有効性が認識され，19世紀から20世紀初頭に掛けて，結核や梅毒等の幅広い疾病の処置に無機銅の製剤が使用された[3]。1930年代より，抗生物質の利用が広まったことから，銅を用いた消毒技術は使用されなくなったが，一方で，抗生物質耐性菌の存在が問題視されており，抗生物質に代わる微生物の制御アプローチの1つとして，銅を用いた微生物の不活化技術が再認識され始めている[2]。近年では，ドアノブ[4,5]やシャワーヘッド等の給水装置[6]，貯水瓶[7]等にも抗菌材料として銅の利用が検討されており，幅広い分野での利用が期待されている。米国環境保護庁（EPA）は2008年に銅合金を正式に抗菌材料として登録しており，安全性についても評価している[8]。また硫酸銅は藻類の制御に有効であることが知られており，湖沼や貯水池等では殺藻剤として100年以上前から現在に至るまで世界各地で使用されている[9]。

2.　銅による微生物の不活化機構

銅による微生物の不活化機構の詳細はいまだに明らかとなっていないのが現状であるが，いくつかの不活化機構・ルートが推測されており，本稿では代表的なものを紹介する。

銅イオンから各種の反応を経て活性酸素が発生することが知られており，活性酸素による強力な酸化作用によって微生物の不活化が起こると考えられている。はじめに銅板等から溶出した2価の銅イオンは，次式の通り，システインやグルタチオン等のチオール（スルフドリル）基と反応し，1価の銅イオンを生成する。そして1価の銅イオンは酸素と反応し，2価の銅イオンへと戻るとともに，過酸化水素を生成する。さらに生成された過酸化水素はフェントン反応と類似した反応(式3)によって1価の銅イオンと反応し，2価の銅イオンを生成するとともに，より強力な酸化作用を持つヒドロキシラジカルを生成する。これらの反応によって生成されたヒドロキシラジカルがたんぱく質や脂質を酸化すること等によって，細胞分子に損傷を与えると考えられている[2]。

$$Cu^{2+} + 2RSH \longrightarrow 2Cu^{+} + RSSR + 2H^{+} \quad (1)$$

$$2Cu^{+} + 2H^{+} + O_2 \longrightarrow 2Cu^{2+} + H_2O_2 \quad (2)$$

$$Cu^{+} + 2H_2O_2 \longrightarrow Cu^{2+} + OH^{-} + \bullet OH \quad (3)$$

*RSH：チオール；RSSR：ジスルフィド

また，銅イオンは微生物の恒常性（ホメオスタシス）に影響を与えることが知られており，この作用も銅を用いた微生物の不活化に関与していると考えられている。このことは，実際に *copA*, *cueO* 遺伝子欠損株を用いた実験で実証されており，とくに細胞内損傷の一次機構として重要であると報告されている[10,11]。

これらの情報を踏まえてGrassらは，図1に示す作用順序を提案している[2]。はじめに銅イオン自体の毒性によって細胞の損傷が起こり，損傷部位から細胞質が流出し，その後，銅イオンによって生成されたヒドロキシラジカル等の活性酸素が細胞へさらなる損傷を与える。そして最終的にDNAまで損傷されると推測されている。

3.　銅による不活化効果が確認されている微生物種

表1に示すとおり，これまでに数多くの病原微生物に対して，銅を用いた不活化技術が有効であるこ