Investigation of Removal-inactivation ratio of *Cryptosporidium* for QMRA

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Abstract: We studied removal ratios and other data pertaining to *Cryptosporidium*, a chlorine-resistant pathogenic microorganism, at water purification plants (WPPs) to confirm the safety of tap water. Then we evaluated risk using the quantitative microbial risk assessment (QMRA) method. We focused on aerobic spore-forming bacteria as a surrogate for *Cryptosporidium* to calculate the removal ratio for each process, and for ozone treatment we calculated ozone inactivation ratios from dissolved ozone concentrations under multiple conditions. We estimated concentrations in raw water based on *Cryptosporidium* detection results, and we evaluated risk by comparing the removal-inactivation ratios determined in this study with the treatment performance values satisfying the health outcome targets in the WHO Guidelines for drinking-water quality. These results confirmed that target treatment performance values were met and tap water was safe.

Keywords: Advanced water treatment; Aerobic spores; *Cryptosporidium*; Ozone treatment; Risk assessment

Introduction

Cryptosporidium is a protozoan with a wide range of hosts including mammals. When *Cryptosporidium* infects humans, it is known to cause diarrhea, abdominal pain, vomiting, and other symptoms. *Cryptosporidium* is reported to be chronically present in Japanese water resources, [1)] and it is a pathogenic microbe in need of attention from water utilities due to its high chlorine resistance.

By FY 2013, Tokyo Metropolitan Waterworks Bureau (hereinafter called “TMWB”) had completed implementation of advanced water treatment in the form of ozone and biological activated carbon (hereinafter called “BAC”) adsorption treatment at all water purification plants (hereinafter called “WPPs”) on the Tone-gawa River / Arakawa River system and the Tone-gawa River / Edo-gawa River system. [2)] We then began conducting surveys regarding *Cryptosporidium* treatment performance and risk assessments at the WPPs at which advanced water treatment had been introduced.

In this study, TMWB calculates removal ratios using aerobic spore-forming bacteria as a surrogate for *Cryptosporidium.* Aerobic spore-forming bacteria are microbes that form chlorine-resistant spores and are also present in ordinary raw water at WPPs. Additionally, it has been reported that conservative estimates of *Cryptosporidium* removal performance can be obtained from results of monitoring aerobic spore-forming bacteria removal ratios. [3)] However, it is conceivable for inactivation ratios through ozone treatment to differ between *Cryptosporidium* and aerobic spore-forming bacteria. Thus, it was decided to calculate *Cryptosporidium*'s inactivation ratio from dissolved ozone concentrations and contact time.

For *Cryptosporidium* risk assessment, we decided to use the Quantitative Microbial Risk Assessment (hereinafter called "QMRA") method in the WHO Guidelines for drinking-water quality, fourth edition (hereinafter called "GDWQ").[4)] QMRA is a mathematical method of assessing infection risk from human pathogens, and it is useful for understanding and managing risks related to waterborne microbial hazards.

Study 1: Factual surveys of aerobic spore-forming bacteria in WPPs

We conducted surveys of aerobic spore-forming bacteria in water purification processes at a frequency of once per month from FY 2016 to FY 2017. These surveys were conducted at Asaka WPP on the Tone-gawa River / Arakawa River system and Misato WPP on the Tone-gawa River / Edo-gawa River system. The locations of WPPs and rivers are shown on Figure 1, while sample sites at WPPs are shown in Figure 2.

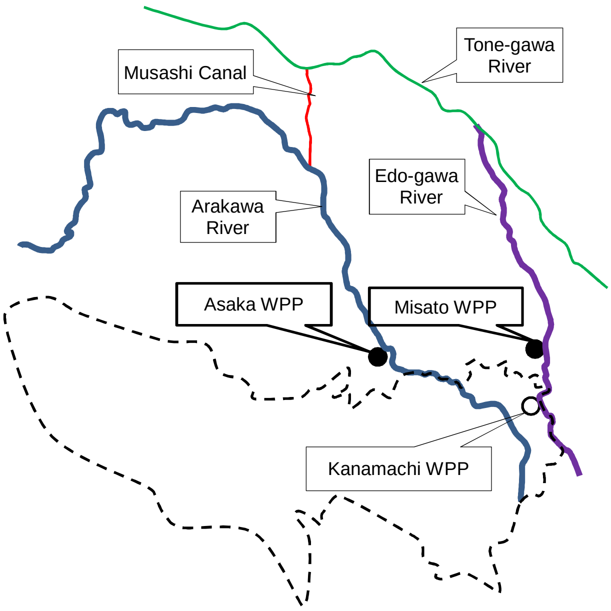

Figure 1: Locations of WPPs and rivers

At each sample site, water is sampled from the WPP's internal sampling tap, and aerobic spore-forming bacteria were measured.

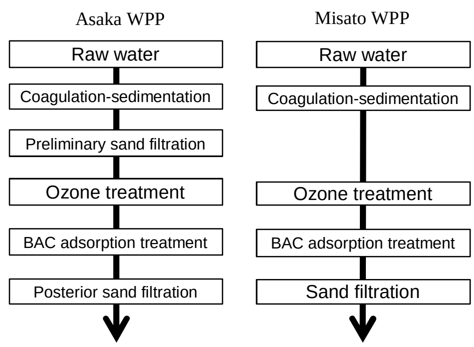

Figure 2: Sample sites in WPPs

Study 2: Surveys of inactivation ratios in ozone treatment

Figure 3 portrays a schematic diagram of the 3-level vertical channel bends countercurrent contact method and BAC adsorption pond, which are used in ozone treatment at Asaka and Misato WPPs.

We used Formula 1 below to calculate inactivation ratios with reference to the United States

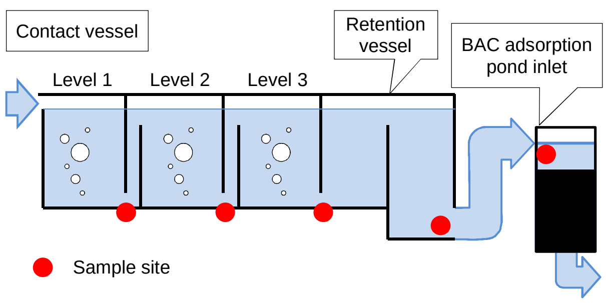

Figure 3: Ozone treatment and BAC adsorption pond

Environmental Protection Agency (US EPA)'s "Long Term 2 Enhanced Surface Water Treatment Rule" Toolbox Guidance Manual. [5]

$$-\text{Log}\ (I/I_0) = \text{Log}\ (1 + 2.303 \times k_{10} \times C \times HDT) \quad - \quad \text{Formula 1}$$

Here, k_{10} represents the *Cryptosporidium* inactivation coefficient. It is calculated as per Formula 2 below.

$$k_{10} = 0.0397 \times (1.09757)^T - \quad \text{Formula 2}$$

T represents the water temperature (°C). C represents dissolved ozone concentration per vessel, converted into concentration within the complete mixing vessel (mg/L) as per the formulae in Table 1. *HDT* represents the hydraulic detention time (minutes). However, for Level 1, no calculations were conducted due to the US EPA's recommendation that it not be included in the *Cryptosporidium* inactivation ratio calculations. [5]

Table 1: Dissolved ozone concentration conversion

	Level 2	Level 3	Retention vessel	BAC inlet
Conversion formulae	$C = \frac{C_{out}}{2}$	$C = \frac{C_{out}}{2}$	$C = C_{out}$	$C = C_{out}$

Due to facility design and management restrictions of Asaka WPP or Misato WPP, we decided to conduct surveys at Kanamachi WPP on the Tone-gawa River / Edo-gawa River system. There are no major differences between the various elements of the ozone treatment performed at these three WPPs as shown in Table 2.

Table 2: Ozone treatment facilities at WPPs

WPP		Asaka	Misato	Kanamachi
Method		3-level vertical channel bends countercurrent contact		
Water depth		6.0 m	6.35 m	6.3 m
Injection method		Diffuser method		
Contact time	Contact vessel	Approx. 12 min.	Approx. 12 min.	Approx. 12 min.
	Retention vessel	Approx. 7 min.	Approx. 10 min.	Approx. 6 min.

The surveys of dissolved ozone concentrations at Kanamachi WPP were conducted six times from winter to summer in 2016 to account for the effects of water temperatures. In each survey, the target values of dissolved ozone concentrations at retention vessel outlets were changed at three levels: 0.1 mg/L, 0.2 mg/L, and 0.3 mg/L. Dissolved ozone concentrations were then measured using water samples from each of the vessels in Figure 3. Actual *HDT* was calculated from Kanamachi's ozone treatment vessels and pipe capacities and treated water volumes.

Study 3: QMRA on *Cryptosporidium* in WPPs

Estimates of *Cryptosporidium* concentrations in raw water

We estimated *Cryptosporidium* concentration distributions in raw water based on detection results from FY 1997 through 2016 at Asaka and Misato WPPs. Based on the assumption that a log-normal distribution is appropriate for distributions of microbe concentrations in water, [6] we estimated *Cryptosporidium* concentration distributions to fit a log-normal distribution.

For the number of *Cryptosporidium*, we took the logarithm of non-detections (0 oocysts / 10 L) as "detections of less than 1 oocyst / 10 L," and fit it to a normal distribution with an assumed mean and standard deviation. Based on this, we sought a probability distribution for *Cryptosporidium* concentrations in raw water at Asaka and Misato WPPs. Next, using an estimated log-normal distribution, we sought the 97.5th percentile for *Cryptosporidium* concentration distribution.

Risk assessment

We calculated the treatment performance necessary to achieve the per-person annual 10^{-6} DALY of the health outcome target as per GDWQ (hereinafter called "the target treatment performance (*PT*)"). For this calculation, we used the 97.5th percentile for *Cryptosporidium* concentration distributions in raw water.

The calculation process for target treatment performance (*PT*) is shown in Table 3. GDWQ values were used for disease burden (*db*), post-infection onset probability ($P_{ill/inf}$), and other elements. However, calculations assumed daily unheated drinking-water intake (*V*) to be 300 mL. [7]

Table 3: Calculation process for target treatment

Item	Formulae, etc.	Value
Health outcome targets (*HT*)		1.0×10^{-6} DALY / year
Percentage of susceptible people (f_s)		100% (ratio of population)
Disease burden per patient（*db*）		1.5×10^{-3} DALY
Tolerated annual onset risk（P_{ill}）	$P_{ill} = \dfrac{HT \times 100}{db \times f_s}$	6.7×10^{-4} / year
Post-infection onset probability（$P_{ill/inf}$）		0.7
Tolerated daily infection risk （$P_{inf,d}$）	$P_{inf,d} = \dfrac{P_{ill}}{P_{ill/inf}} \div 365$	2.6×10^{-6} / day
Dose – response relationship（*r*）		2.0×10^{-1}
Tolerated daily exposure （*E*）	$E = \dfrac{P_{inf,d}}{r}$	1.3×10^{-5} units / day
Daily unheated drinking-water intake（*V*）	Assumed 300 mL	0.3 L
Tolerated *Cryptosporidium* count in 1 L of drinking water（C_D）	$C_D = \dfrac{E}{V}$	4.3×10^{-5} units / L
Cryptosporidium count in 1 L of raw water（C_R）	97.5 percentile	Estimated 97.5 percentile
Target treatment performance（*PT*）	$PT = \log\dfrac{C_R}{C_D}$	

Finally, we compared the calculated target treatment performance (*PT*) with the summed removal-inactivation ratios consisting of the removal ratio from aerobic spore-forming bacteria measurements and the inactivation ratio obtained from surveys of inactivation ratios in ozone treatment.

Results and considerations 1: Factual survey of aerobic spore-forming bacteria in WPPs

Survey results from Asaka and Misato WPPs are shown in Figure 4 and 5 below. We noticed that aerobic spore-forming bacteria in processed water changes seasonally due to the bacteria multiplication of high-water-temperature seasons. We decided to calculate removal ratios of treatment processes using survey results from January through April and October through December because *Cryptosporidium* does not multiply within the environment.

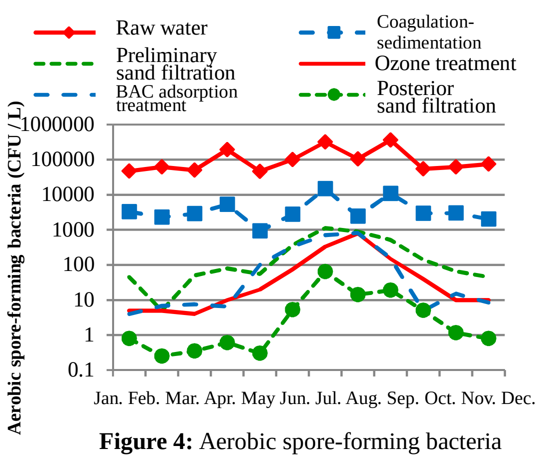

Figure 4: Aerobic spore-forming bacteria (Asaka WPP)

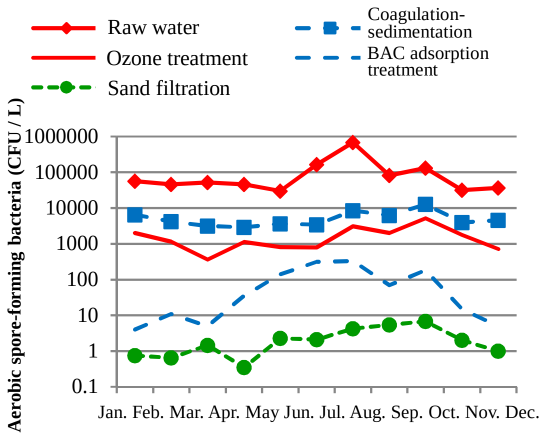

Figure 5: Aerobic spore-forming bacteria (Misato WPP)

Aerobic spore-forming bacteria concentrations and removal ratios (means of January through April and October through December) for each sample site are shown in Figures 6 and 7. Total removal ratios (means) for all water purification processes at each WPP, excepting ozone treatment, were calculated as 4.31 Log for Asaka WPP and 4.10 Log for Misato WPP.

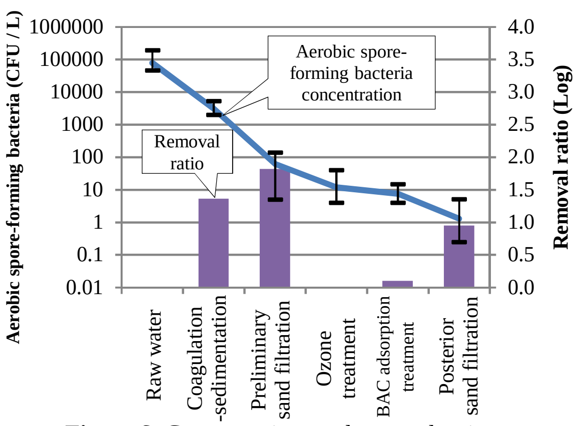

Figure 6: Concentrations and removal ratios (Asaka WPP)

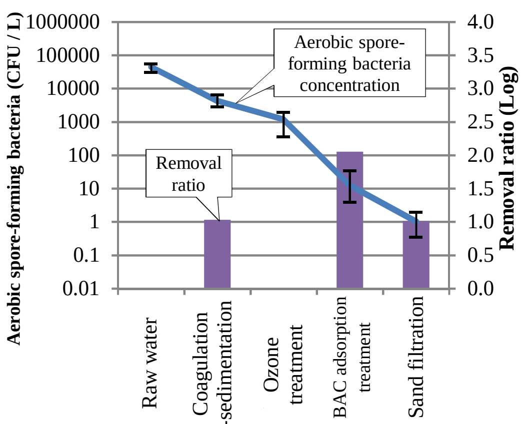

Figure 7: Concentrations and removal ratios (Misato WPP)

Results and considerations 2: Survey of inactivation ratios in ozone treatment

For the six surveys conducted at Kanamachi WPP, water temperatures were measured at 8.9°C, 10.0°C, 17.3°C, 22.5°C, 24.4°C, and 28.0°C. *HDT* were calculated from facility capacities and treated water volumes at Kanamachi WPP as follows: approx. 5.4 minutes for each level of contact vessel, approx. 8.5 minutes for retention vessel, and approx. 35 minutes from ozone treatment outlet to BAC adsorption pond inlet.

A graph of *Cryptosporidium* inactivation ratios calculated using Formulae 1 and 2 is shown in Figure 8. As is apparent from the numerical formulae, as well, *Cryptosporidium* inactivation ratios increased in tandem with water temperature and ozone concentration increases.

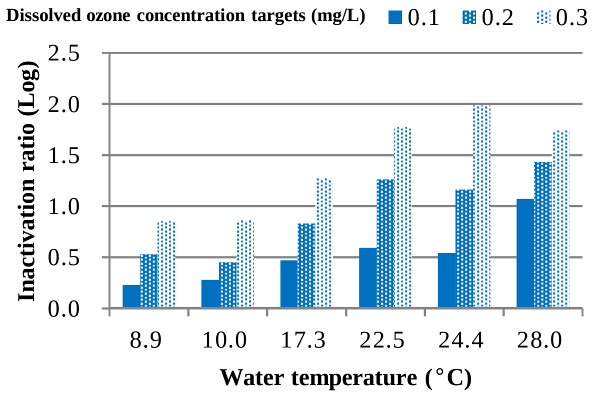

Figure 8: Inactivation ratios in ozone teratment

For the annual range of water temperatures, we investigated what *Cryptosporidium* inactivation ratios were attainable, even at the very minimum, from Figure 8. In actual advanced water treatment processes, high concentrations of dissolved ozone result in the occurrence of the byproduct: bromate. For this reason, it is difficult to perform ozone treatment at consistently high concentrations in the range of water temperatures spanning from winter to summer.

At WPPs of TMWB, taking into account the generation of bromate, dissolved ozone concentrations at Level 3 contact vessels or retention vessel outlets are generally managed based on the target values 0.3 mg/L for winter and 0.1 mg/L for summer. At water temperatures of 17.3°C or more, *Cryptosporidium* inactivation ratios of around 0.5 Log were achieved even at target dissolved ozone concentration values of 0.1 mg/L. At low water temperatures of 10.0°C or less, bromate generation is suppressed and it is therefore believed possible to manage dissolved ozone concentration targets of 0.2 mg/L or more. Based on these elements, it was believed that *Cryptosporidium* inactivation ratios in ozone treatment at actual WPPs are maintained around 0.5 Log at least throughout the year.

Results and considerations 3: QMRA on *Cryptosporidium* in WPPs

Estimates of *Cryptosporidium* concentrations in raw water

Figure 9 shows *Cryptosporidium* concentrations as proportions to the

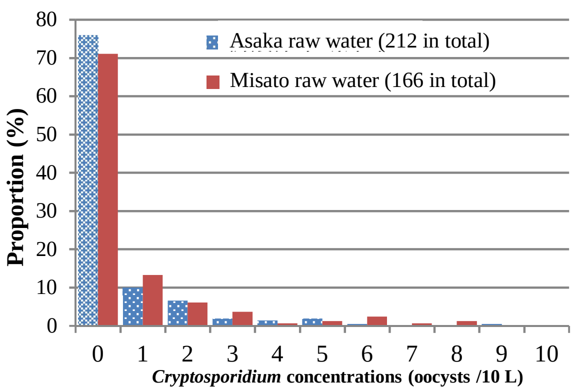

Figure 9: Proportions of *Cryptosporidium* concentrations

number of tests performed on Asaka WPP raw water (hereinafter called "Asaka raw water") and on Misato WPP raw water (hereinafter called "Misato raw water") from FY 1997 to FY 2016.

For estimation, non-detections (0 oocysts / 10 L) of *Cryptosporidium* were assumed to be distributed as in Table 4. For each type of raw water, we calculated the natural logarithm of *Cryptosporidium* concentrations, and we sought the mean and standard deviation. The results of this are displayed in Table 5. Based on these results, we estimated probability distributions for *Cryptosporidium* concentrations in Asaka raw water and Misato raw water. *Cryptosporidium* concentration estimates are shown in Figures 10 and 11, rounded to the nearest integer. We calculated the 97.5th percentile of estimated *Cryptosporidium* concentration distributions to be 5.8 oocysts / 10 L for Asaka raw water and 7.0 oocysts / 10 L for Misato raw water.

Table 4: Assumed breakdown of non-detections

/10 L	0.1 oocysts	0.01 oocysts	Total number of non-detections
Asaka	81	80	161
Misato	59	59	118

Table 5: Estimated means and standard deviations

	Mean natural log	Standard deviation
Asaka raw water	-2.43	2.13
Misato raw water	-2.26	2.15

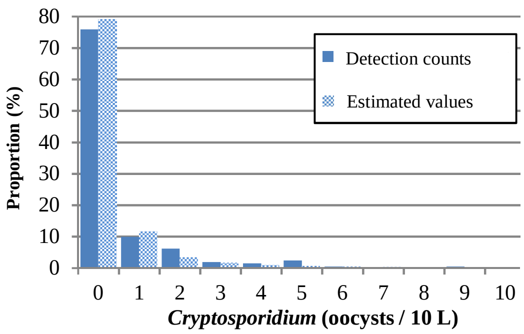

Figure 10: Estimated concentration distribution (Asaka raw water)

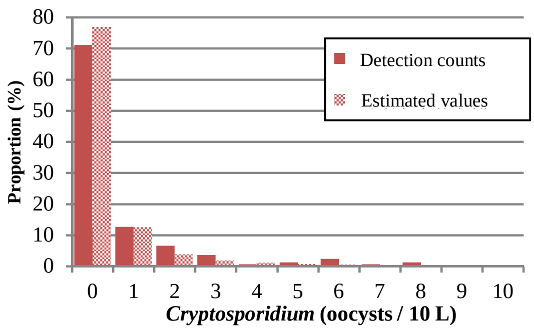

Figure 11: Estimated concentration distribution (Misato raw water)

Risk assessment

We calculated target treatment performance (*PT*) at Asaka and Misato WPPs as per the calculation process in Table 3. However, the 97.5th percentile (*Cryptosporidium* count in 1 L) used for each WPP were 0.6 oocysts / L for Asaka raw water and 0.7 oocysts / L for Misato raw water because *Cryptosporidium* count in 1 L of raw water is used for target treatment performance calculations. Calculations yielded target treatment performance values of 4.14 Log for Asaka WPP and 4.21 Log for Misato WPP.

We calculated the removal-inactivation ratio for each WPP by adding the removal ratios obtained from aerobic spore-forming bacteria measurements and the inactivation ratios obtained from surveys of inactivation ratios in ozone treatment. However, based on survey results, ozone inactivation ratios were taken as 0.5 Log and added to removal ratios. This resulted in removal-inactivation ratios of 4.81 Log for

Asaka WPP and 4.60 Log for Misato WPP.

Figure 12 presents a comparison of acquired target treatment performance values and removal-inactivation ratios. These results showed that removal-inactivation ratios were higher than target treatment performance values for both Asaka and Misato WPPs. From this result, we confirmed that tap water satisfies the per-person annual 10^{-6} DALY, the health outcome target required in GDWQ.

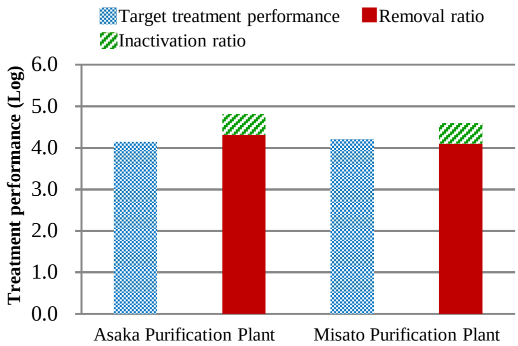

Figure 12: Target treatment performance values and removal-inactivation ratios

Conclusion

In our studies, we focused on aerobic spore-forming bacteria as a surrogate for *Cryptosporidium* removal ratios. By calculating removal ratios for aerobic spore-forming bacteria in purification processes at actual WPPs, we were able to understand *Cryptosporidium* removal ratios. For inactivation ratios in ozone treatment, we studied dissolved ozone concentrations in ozone treatment vessels at actual WPPs and determined these ratios by utilizing the US EPA's Toolbox Guidance Manual. We showed that by applying the above methods, we could determine *Cryptosporidium* removal-inactivation ratios at actual WPPs.

We found target treatment performance values that satisfy health outcomes targets in GDWQ. By comparing target treatment performance values and removal-inactivation ratios from our studies, we performed QMRA on *Cryptosporidium* at WPPs. Our results showed that actual WPPs satisfied target treatment performance values, and therefore that there is a reliable supply of tap water that satisfies the per-person annual 10^{-6} DALY health outcome targets.

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