



Estimation and evaluation of the risks of protozoa infections associated to the water from a treatment plant in southern Brazil using the Quantitative Microbiological Risk Assessment Methodology (QMRA)

Claudia Bauer Visentini

Received: 26 April 2023 / Accepted: 23 March 2024 / Published online: 9 April 2024
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2024

Abstract In this study, the Quantitative Microbial Risk Assessment (QMRA) methodology was applied to estimate the annual risk of *Giardia* and *Cryptosporidium* infection associated with a water treatment plant in southern Brazil. The efficiency of the treatment plant in removing protozoa and the effectiveness of the Brazilian legislation on microbiological protection were evaluated, emphasizing the relevance of implementing the QMRA in this context. Two distinct approaches were employed to estimate the mechanical removal of protozoa: The definitions provided by the United States Environmental Protection Agency (USEPA), and the model proposed by Neminski and Ongerth. Although the raw water collected had a higher concentration of *Giardia* cysts than *Cryptosporidium* oocysts, the estimated values for the annual risk of infection were significantly higher for *Cryptosporidium* than for *Giardia*. From a general perspective, the risk values of protozoa infection were either below or very near the limit set by the World Health Organization (WHO). In contrast, all the risk values of *Cryptosporidium* infection exceeded the threshold established by the USEPA. Ultimately, it was concluded that the implementation of the QMRA methodology should be considered by the Brazilian

authorities, as the requirements and guidelines provided by the Brazilian legislation proved to be insufficient to guarantee the microbiological safety of drinking water. In this context, the QMRA application can effectively contribute to the prevention and investigation of outbreaks of waterborne disease.

Keywords *Giardia* · *Cryptosporidium* · QMRA · Water treatment · Risk of infection · Drinking water

Introduction

Outbreaks of infectious intestinal diseases caused by *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts are evidenced worldwide, in both developed and developing countries (Connors et al., 2021; Costa et al., 2022; Fantinatti et al., 2020; Mac Kenzie et al., 1995). Giardiasis, the disease caused by *Giardia* cysts, has a high incidence, and is considered the main intestinal parasite that affects human beings. These cysts and oocysts are excreted in human and animal feces and, as they are resistant to conventional methods of sewage and water treatment, the main form of transmission is through contact with contaminated water (Efstratiou et al., 2017). Despite this, a considerable part of the protozoa present in raw water can be removed during the water treatment steps, such as coagulation, filtration and disinfection (Betancourt and Rose, 2004). Some studies report that under optimized conditions of coagulation, the conventional treatment can achieve removal of cysts

C. B. Visentini (✉)
Municipal Department of Water and Sewage (DMAE),
St. 24 de Outubro, nº 200, Moinhos de Vento,
Porto Alegre, Rio Grande Do Sul 90510-000, Brazil
e-mail: claudia.bauer.visentini@gmail.com

and oocysts of 1 to 2 logs by decantation and up to 4 logs by filtration (LeChevallier et al., 2004). However, the United States Environmental Protection Agency (USEPA, 2005, 2006) assumes a maximum of only 0.5 logs removal for the decantation step and 2.5 logs for the filtration step. Additionally, some researchers have carried out full-scale experiments and developed equations that relate the removal of protozoa to the efficiency of turbidity removal, such as the Neminski and Ongerth model (Nieminski and Ongerth, 1995). In the disinfection step of the water treatment, a strongly oxidizing chemical agent is used to inactivate (eliminate) the microorganisms. The efficiency of the inactivation of protozoa is determined by the concentration of the disinfectant agent and its contact time with the protozoa. In the water treatment, the contact time refers to the period during which the water remains in the reservoir in contact with the disinfectant agent. *Giardia* cysts can be efficiently inactivated in a relatively short contact time using free chlorine as a disinfectant agent, while *Cryptosporidium* oocysts are more resistant and require the use of stronger oxidizing agents, such as chlorine dioxide and ozone disinfection (Betancourt and Rose, 2004).

In Brazil, the current legislation (Brasil, 2017, 2021) imposes the following requirements for water treatment plants: Samples of filtered water must be collected from each filtration unit every 2 h while the plant is in operation. In at least 95% of the samples, the values of turbidity must be ≤ 0.5 NTU (nephelometric turbidity unit). On the other hand, when the arithmetic mean of *Cryptosporidium* concentration present in the raw water is greater than or equal to 1.0 oocyst/L, the filtration units must produce water with a turbidity value ≤ 0.3 NTU in 95% of the samples.

The Brazilian legislation also requires that, during the chlorination step, water treatment plants adhere to the necessary contact time to promote the inactivation of 1.0 log of *Giardia* cysts. When the treatment plant operates with free chlorine residual, the required contact time is reasonably short, making the inactivation of 1 log of *Giardia* cysts (or more) viable. However, when the unit is operating with residual of chloramines (combined chlorine), the necessary contact time to achieve at least 1 log of cyst inactivation is extremely long and impractical. For this reason, it is advisable for treatment plants to always operate with residual of free chlorine. Regrettably, achieving this objective is not

always possible. The presence of organic matter and ammonia nitrogen causes the natural formation of chloramines during the disinfection stage of water treatment (Yang et al., 2021; Blume et al., 2010). Elevating the dosage of chlorine can eliminate a significant portion of chloramines and maintain the minimum required concentration of free chlorine residual (Tao Hui et al., 2013). Nevertheless, under certain circumstances, the concentration of organic matter may reach levels so elevated that the chlorine dosage needed to operate with free chlorine residual becomes unfeasible. Consequently, the treatment plant is compelled to use residuals of chloramines as agent disinfectant. This unwanted situation is very common in most Brazilian water treatment plants and occurs due to the excessive presence of organic matter, promoted by the improper disposal of industrial effluents and domestic sewage into water bodies (Blume et al., 2010).

Even after undergoing rigorous processes to remove impurities and pathogenic organisms, it is still possible to find small quantities of living microorganisms in the treated water. This is a result of the practical complexity of achieving complete sterilization during the water treatment. Additionally, due to technical limitations of the available methodologies (Jain et al., 2019), there are no reliable indicators that can attest the absence of pathogenic organisms in treated water samples. Therefore, it becomes pertinent to estimate and monitor the risks of microbiological infection that a population may be exposed, which can be carried out through the Quantitative Microbiological Risk Assessment (QMRA) methodology (Owens et al., 2020). This approach has been integrated into standards and criteria for the microbiological quality of water intended for human consumption across various regions worldwide. Notable instances of its adoption include Canada' (Health Canada, 2022), New Zealand (Ministry of Health, 2005), and the USA (USEPA, 2005, 2006). The Environmental Protection Agency of Ground Water and Drinking Water Standard defines a tolerable risk value of $1.0E-4$ per person per year (pppa) for several pathogenic organisms transmissible via water supply for human consumption. That means an annual risk of one infected person per 10,000 people. The World Health Organization (WHO) assumes a tolerable burden of disease of $1.0E-6$ DALY (disability

adjusted life years), which corresponds to a tolerable annual risk of $2.2E-3$ pppa or two infected people for every 1,000 people (WHO, 2016).

The magnitude of the risk of protozoa infection relies on specific variables, including the virulence and susceptibility of the host population, the amount of water consumed during the exposure period, the concentration of (oo)cysts in the collected raw water and the removal of protozoa in the water treatment. (Owens et al., 2020; Health Canada, 2022; Kui et al., 2021; Bataiero et al., 2019). While it presents evident advantages, the QMRA methodology also comes with a notable disadvantage, as its application tends to be more intricate and, possibly, less accessible to the general public. The mathematical model incorporates probabilistic elements, where the input variables are represented by probability distributions (stochastic modeling) (Morgan & Heroin, 2007). Additionally, the data is processed through successive and numerous random samplings (iterations) using specific simulation programs.

As the estimation of infection risk directly relies on the probability distributions of the input variables, the parameters defining the shapes of the distribution curves, namely kurtosis and skewness, also become important factors. Kurtosis characterizes the flatness of the probability distribution curve. The more negative the kurtosis value, the flatter the curve compared with a normal distribution curve, meaning that the data will be more dispersed and distant from the mean. Skewness can also be negative or positive. Negative skewness indicates that the data is more concentrated to the right of the mean, i.e., in the zone of values higher than the mean. The opposite occurs when skewness is positive (Demir, 2022).

Within this context, this study proposed the application of the QMRA methodology to estimate and assess the annual risk of *Giardia* and *Cryptosporidium* infection associated with a water treatment plant located in southern Brazil. The efficiency of the treatment plant was evaluated concerning the removal of protozoa. Additionally, the study analyzed the effectiveness of Brazilian legislation concerning microbiological protection, aiming to assess the relevance of implementing the QMRA methodology. Furthermore, two distinct approaches were employed to estimate the mechanical removal of protozoa and evaluate the impact of each on the estimated values of infection risk.

Materials and methods

The study was carried out using the monitoring data from a Drinking Water Treatment Plant (DWTP) of the Municipal Department of Water and Sewage of the city of Porto Alegre (DMAE), in the state of Rio Grande do Sul, Brazil. The plant operates in conventional treatment with rapid sand filters and a mean flow rate of approximately $77 \text{ m}^3/\text{min}$. It produces water for approximately 167,415 inhabitants, 11.26% of the total population of the municipality.

In the water treatment facilities of Porto Alegre, the presence of protozoa is not common at the points where raw water is collected. However, quantifiable concentrations of *Giardia* and *Cryptosporidium* were observed in one of the city's five water treatment plants during the years 2015, 2016, and 2017. Consequently, the data collected in these specific years were incorporated into the current study. Additionally, the data spanning from 2018 to 2021, obtained from the same treatment plant, were included to offer a more recent and accurate representation of the standard conditions of water treatment in Porto Alegre.

From a general and summarized perspective, the study adhered to the following sequence of execution:

- (1) Estimation of the mechanical removal of protozoa (*Giardia* and *Cryptosporidium*) based on the turbidity data from raw and filtered water provided by the monitoring history of the treatment plant.
- (2) Estimation of inactivation of *Giardia* cyst based on the reservoir water data, such as contact time, residual of free chlorine, pH, and temperature, obtained from the monitoring history of the treatment plant.
- (3) Estimation of the total removal of *Giardia* cysts by combining mechanical removal and inactivation data. The total removal of *Cryptosporidium* oocysts was equivalent to the mechanical removal, given that this protozoan is not inactivated by chlorine.
- (4) Identification of the distribution functions that most accurately represents the behavior of the removal data of protozoa and the (oo)cyst concentration data in raw water.
- (5) Estimation of the risk values of protozoa infection based on the data and results obtained in the previous steps.
- (6) Evaluation of the obtained results.

Mechanical removal of protozoa

The water treatment plant has 12 filtration units. The analysts of the treatment plant collected and analyzed samples of raw water and filtered water from each filter every 2 h. In total, approximately 360 samples of raw water and 4320 samples of filtered water were analyzed per month. All the collected samples were considered in the present study. The method of analysis used was the nephelometric method according to the Standard Methods for the Examination of Water and Wastewater 23rd Edition.

Two different approaches were used to estimate the mechanical removal of protozoa (via filtration): the USEPA definitions, referred to as Approach A, and the

mathematical model of Neminski and Ongerth, referred to as Approach B. Considering the USEPA definitions, a 3 logs removal of protozoa was defined for the months in which at least 95% of the samples of filtered water presented turbidity ≤ 0.3 NTU. As to the months in which the limit of 0.5 NTU was respected in at least 95% of the samples, a removal of 2.5 logs was considered for *Giardia* cysts and 2.0 logs for *Cryptosporidium* cysts. In Table 1, this classification can be observed more clearly. To apply the Neminski and Ongerth model (Eqs. 1 and 2), the percentage of turbidity removal was determined by utilizing the monthly median values from the turbidity data of raw water (approximately 360 samples per month) and the turbidity data of filtered water (approximately 4320 samples per month).

$$\text{Log Giardia removal} = 1,0093 \text{ logs (\%turbidity removal)} + 1,6697 \tag{1}$$

$$\text{Log Cryptosporidium removal} = 0,931 \text{ logs (\% turbidity removal)} + 1,009 \tag{2}$$

Inactivation of *Giardia*

In the disinfection stage, the water treatment plant employs chlorine gas as disinfectant agent. The residuals of free chlorine at the outlet of the reservoirs were monitored every two hours by the treatment plant analysts. The analytical method used was the colorimetric method according to the methodology described in the Standard Methods for the Examination of Water and Wastewater 23rd Edition (4500-Cl-G—SMEWW). The managers of the treatment plant calculated the daily contact time based

on the average water flow in the reservoirs and the dimensions of the pipes and reservoirs, following the guidelines of the LT1ESWTR Disinfection Profiling and Benchmarking Technical Guidance Manual (USEPA, 2003).

After collecting all the necessary data, the Eq. 3 was used to estimate the daily inactivation (measured in logs) of *Giardia* cysts, where, “t” is the daily contact time, “C” is the daily mean of residual free chlorine at the reservoir monitoring point, and “pH” and “T” is the pH and the temperature of the water stored in the reservoir. (USEPA, 1991).

$$\text{Log inactivation of Gardiacyst} = \frac{C \times t}{0.2828 \times pH^{2.69} \times 0.933^{T-5}} \tag{3}$$

The treatment plant has two reservoirs for storing treated water. The reservoirs have different dimensions, and therefore, different contact times and inactivation efficiencies. The longer the contact time, the higher the logs value of inactivation. To organize the evaluation of the results, the author chose to classify the smaller reservoir as “Reservoir 1” and the larger reservoir as “Reservoir 2.”

Table 1 Values of protozoa removal in accordance with the turbidity limits of filtered water and the classification defined by the USEPA (Approach A)

95% of monthly turbidity samples	Removal of <i>Giardia</i> (logs)	Removal of <i>Cryptosporidium</i> (logs)
≤ 0.5 NTU*	2.5	2.0
≤ 0.3 NTU*	3.0	3.0

*Nephelometric turbidity unit (NTU)

Total removal of protozoa

The total daily removal of *Giardia* cysts (logs) was calculated by adding the daily mechanical removal (filtration) to the daily chemical inactivation (chlorination). For the total daily removal of *Cryptosporidium* oocysts, only the daily mechanical removal was considered, since the oocysts are resistant to chlorine. The monthly means of the daily results were subsequently used in the assignment step of the distribution function of the annual data. It is important to highlight that the removal of protozoa was measured in logs.

Concentration of protozoa in raw water

The samples for determining the concentration of cysts and oocysts in raw water were collected monthly from 2015 to 2017 and annually throughout the years 2018 to 2021. The sampling frequency obeyed the Brazilian standard, which requires the protozoa monitoring only when is identified a moving geometric mean ≥ 1000 *Escherichia coli*/100 mL. The concentration of protozoa in raw water was determined by the EPA Method 1623.1:2012, which has a limit of quantification (LQ) of 0.1 cyst or oocyst per liter. This means that this analytical technique is not capable of providing a reliable result for concentrations of (oo)cysts below this value. These analyses were conducted by an external laboratory certified in accordance with the Standard ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories).

Estimation of the probability of infection

The probability of pathogen exposure is characterized by the Poisson distribution, while the probability of infection is described by a binomial process, meaning there are two possible outcomes: infection or non-infection. The literature has established the exponential dose–response model to express the probability of protozoa infection (Haas et al., 2014). This model assumes that all organisms have the same probability of survival and causing infection in the host. Thus, the probability of infection associated with any organism is equal to $1/k$, where k is the dose–response parameter that characterizes the agent–host interaction. The daily dose ingested of protozoa is estimated based on the data of protozoa concentration in the raw water,

the removal of protozoa during the water treatment, and the amount of water ingested daily. Therefore, the probability of infection associated with the ingestion of a daily dose is given by Eq. 4 (Haas et al., 2014; VOSE, 2008).

$$P_i = 1 - e^{(-k \cdot C \cdot I \cdot 10^{-R})} \tag{4}$$

where the input variables are “I” is the daily water ingestion per person, “C” is the pathogen concentration in the raw water, “R” is the removal of the pathogen in the water treatment, and “k” is the dose–response model parameter.

The probability of infection for a daily exposure is given by “ P_i ,” so the probability of not being infected is “ $1 - P_i$.” Therefore, for 365 days of exposure, the probability of infection is given by the Eq. 5 (Haas et al., 2014).

$$P_j = 1 - \prod_{j=1}^{365} [1 - P_i^{(j)}] \tag{5}$$

Before estimating the infection risk values, it was necessary to define the distribution functions of the input variables. For the parameter “k,” referring to the *Giardia*–host interaction, a triangular distribution function was considered with the minimum value of 0.009798, the most likely value of 0.01982 and the maximum value of 0.03582 (Rose et al., 1991). While for the “k” referring to the *Cryptosporidium*–host interaction, a triangular distribution was considered with the minimum value of 0.0022, the most likely value of 0.00419 and the maximum value of 0.0085 (Dupont et al., 1995). For the daily water ingestion per person, a triangular distribution was also considered, with the following values: 0.5 L (minimum value), 1.5 L (most likely value) and 2.0 L (maximum value).

As previously mentioned, *Cryptosporidium* oocysts exhibit high resistance to chlorine. Consequently, in this study, their removal was solely mechanical. The turbidity data of filtered water is characterized by values of low amplitude. Consequently, the estimated data for the removal of *Cryptosporidium* presents an insignificant variation. Due to this, fitting a distribution function to this data is neither necessary nor feasible. Therefore, for each year, the authors opted to use the annual average removal of *Cryptosporidium* as a constant value in the input variable “R” of the Eq. 4.

The estimated values for removal of *Giardia*, as well as the concentration data of protozoa in raw water, were analyzed using Minitab® Statistical Software (Minitab LLC, State College, PA, 2022) to identify the distribution function that best represented (highest p -value) the behavior of these data in each year. Each dataset evaluated had a size of 12 samples, that is, a value representing each month of the year. For the extended periods where there was no detection or quantification of protozoa in raw water, i.e., for periods where the protozoa concentration could be any value below 0.1 (oo)cysts/L (limit of quantification of the analytical technique), the authors defined a uniform distribution function with a mean of 0.05 (oo)cysts/L, a minimum limit of 0.01 (oo)cysts/L, and a maximum limit of 0.09 (oo)cysts/L.

After defining the distribution functions of the input variables, the daily risk of infection was estimated through successive and random sampling (iterations) using the Latin Hypercube simulation technique in the @RISK® 8.0 software (Vose, 2008; Palisade Corporation, 2022). In summary, 1,825,000 values of daily risk of infection were obtained (Eq. 4) through 365 iterations repeated 5000 times. That is, for every 365 daily risk values, an annual risk value was calculated, totaling 5000 values of annual risk of infection (Eq. 5). This operation was performed for each condition investigated, specifically, for each year, each Approach (A or B) and for each type of water reservoir (Reservoirs 1 and 2).

Nomenclature for results presentation

To facilitate the presentation of the results, a nomenclature was established for each condition studied:

Res1A-Considering the water stored in the Reservoir 1 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A).

Res2A-Considering the water stored in the Reservoir 2 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A).

Res1B-Considering the water stored in the Reservoir 1 and the Neminski and Ongerth Model to estimate the mechanical removal of protozoa (Approach B).

Res2B-Considering the water stored in the Reservoir 2 and the Neminski and Ongerth Model to estimate the mechanical removal of protozoa (Approach B).

Summarizing the study in flowcharts

For a clearer understanding of how the study was implemented, Fig. 1 provides flowcharts summarizing the necessary steps to estimate the total removal of *Giardia* (a), the total removal of *Cryptosporidium* (b), and the annual risk of protozoa infection (c).

Statistical tests

The data related to the inactivation of *Giardia*, the total removal of protozoa and the annual infection risk were subjected to statistical analysis using the Student's t -test. The normality of the data was checked using the Shapiro–Wilk and d'Agostino–Pearson tests. The homogeneity of the variances among the datasets was examined using the Levine test with the purpose of adapting the t -test for each type of condition (homoscedastic or heteroscedastic).

The datasets of annual infection risk were also tested concerning the tolerance limits established by the WHO and the USEPA. All calculations were conducted with a significance level set at 0.05. Therefore, for results with p -values greater than 0.05, the “null hypothesis” was not rejected, and the means of the datasets were considered statistically equal.

Additionally, the Student's t -test was used to estimate the margins of error (considering 95% confidence) of the inactivation data of *Giardia* and the total removal data of protozoa.

Results and discussion

Concentration of protozoa in raw water

The graph in Fig. 2 shows the concentrations of cysts and oocysts present in the samples of raw water collected throughout the years 2015 to 2017. In most months, it was not possible to detect or quantify *Cryptosporidium* oocysts. In these cases, the concentration was plotted as zero on the graph.

The peak concentrations of *Giardia* cysts were observed in 2016, reaching maximum values of 21.5 and 17.4 cysts/L during the months of September and October, respectively. As for *Cryptosporidium* oocysts, the highest concentration recorded was 1.3 oocysts/L in July 2016.

Fig. 1 Flowchart summarizing the necessary steps to estimate the total removal of *Giardia* (a), the total removal of *Cryptosporidium* (b), and the annual risk of protozoa infection (c)

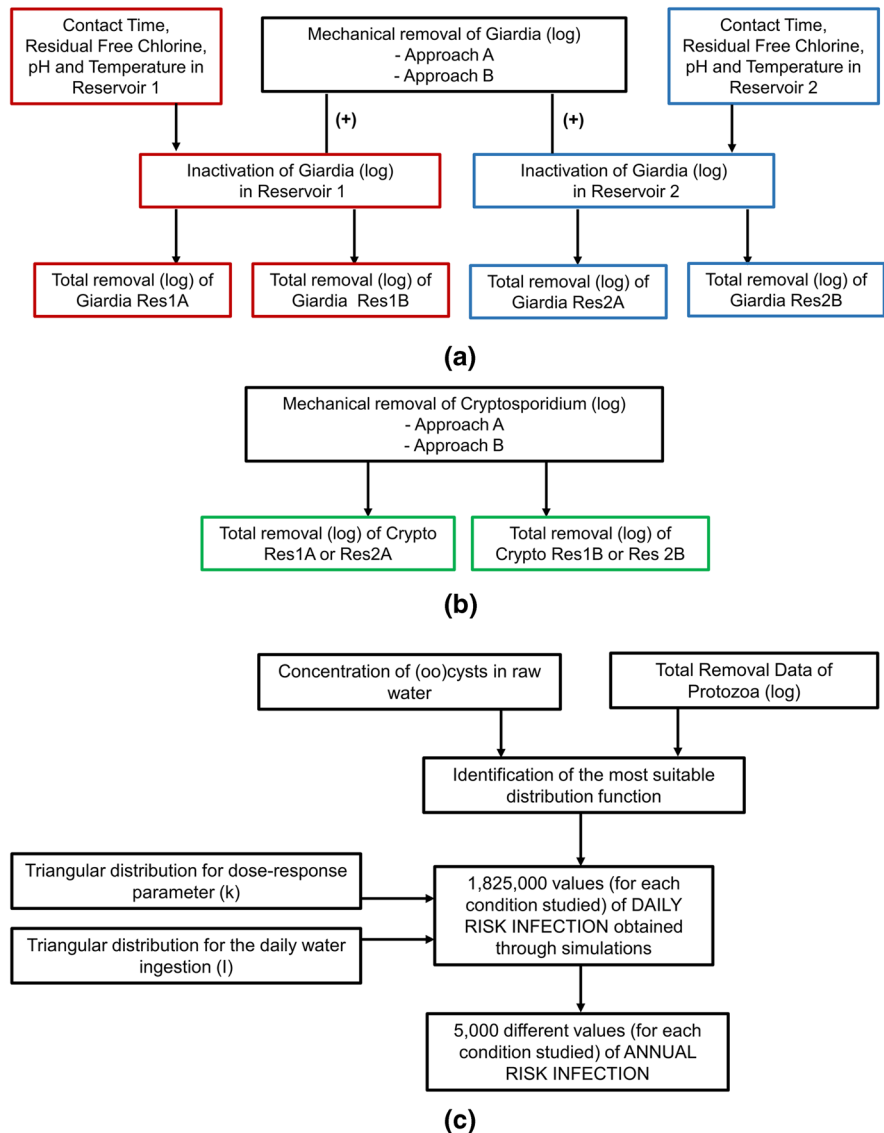


Table 2 shows the annual medians values of the *Giardia* and *Cryptosporidium* concentration and the distribution function that best represented these data. The highest values of protozoa concentration occurred in 2016, when medians of 8.0 cyst/L and 0.6 oocyst/L were obtained.

From January 2018 to December 2021, no cysts and oocysts were detected or quantified in raw water (methodology quantification limit of 0.1 (oo)cyst/L). During this period, the data distribution was considered a uniform distribution with a lower limit of 0.01 and upper limit of 0.09 (oo)cyst/L, in addition to a median value of 0.05 (oo)cyst/L.

Turbidity and mechanical removal of protozoa

Table 3 presents the number of months per year in which at least 95% of the samples collected of filtered water did not exceed the limits of 0.3 and 0.5 NTU (nephelometric turbidity unit). In total, during the period between 2015 and 2021, in 75% of the months the value of 0.3 NTU was not exceeded in at least 95% of the data of turbidity. In the remaining 25%, the amount of data with a turbidity value ≤ 0.3 NTU ranged from 78.5 to 94.6%, with the maximum limit of 0.5 NTU being respected in at least 95% of these data.

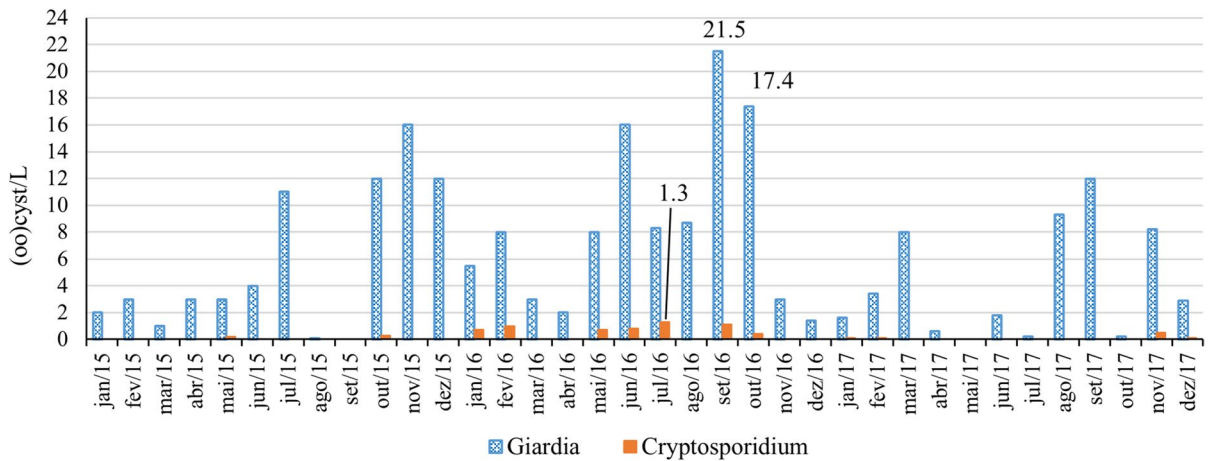


Fig. 2 Concentration of protozoa in raw water throughout the years 2015 to 2017

Table 2 The median values and the type of function distribution of protozoa concentration data throughout the years 2015 to 2021

Year	cyst/L (median)	Distribution function of the data (cyst/L)	oocyst/L (median)	Distribution function of the data (oocyst/L)
2015	3	Gamma (shape 0.70 and scale 8.05) ($p=0.236$)	0.1	Uniform for 10 months ^a
2016	8	Exponential ($p=0.480$)	0.6	Normal ($p=0.852$) and uniform ^b
2017	2	Box Cox ($\lambda=0.5$) ($p=0.328$)	0.1	Uniform for 8 months ^c
2018	0.05	Uniform ^d	0.05	Uniform ^d
2019	0.05	Uniform ^d	0.05	Uniform ^d
2020	0.05	Uniform ^d	0.05	Uniform ^d
2021	0.05	Uniform ^d	0.05	Uniform ^d

^aUniform distribution with lower limit of 0.01 and upper limit of 0.09 oocysts/L for 10 months (303 days), concentration of 0.2 oocysts/L in May and concentration of 0.3 in October

^bUniform distribution with lower limit of 0.01 and upper limit of 0.09 oocysts/L for 5 months (152 days) and normal distribution with mean of 0.85 oocysts/L and standard deviation of 0.3 for 7 months (213 days)

^cUniform distribution with lower limit of 0.01 and upper limit of 0.09 oocysts/L for 8 months (275 days), concentration of 0.1 oocysts/L for 3 months (90 days) and concentration of 0.5 oocysts/L in November

^dUniform distribution with lower limit of 0.01 and upper limit of 0.09 (oo)cysts/L

The removal values of turbidity (%) obtained for each year are also exposed in Table 3. From these values, a mean and a standard deviation of $99.18\% \pm 0.31\%$ were obtained, with a minimum value of 98.00% (in April/2021) and a maximum value of 99.62% (in August/2016).

An excellent performance of filtration was observed during the years 2019 and 2020, where 92% of the months had at least 95% of the values

of turbidity less than or equal to 0.3 NTU. However, in 2020, it was noticed the lowest percentage of removal of turbidity compared with other years (98.94%). This occurred because the percentage of removal of turbidity also depends on the value of the initial turbidity, i.e., the turbidity of the raw water. In other words, the removal of turbidity was lower because the “amount” of turbidity to be removed in 2020 was also lower.

Table 3 The number of months per year in which at least 95% of the turbidity data from filtered water did not exceed the 0.3 and 0.5 NTU limits, and the percentage of turbidity removed per year

Year	≤0.5 NTU* (months)	≤0.3 NTU* (months)	Removal of turbidity (%)
2015	5 (42%)	7 (58%)	99.46
2016	2 (17%)	10 (83%)	99.44
2017	6 (50%)	6 (50%)	99.22
2018	2 (17%)	10 (83%)	99.19
2019	1 (8%)	11 (92%)	99.14
2020	1 (8%)	11 (92%)	98.94
2021	4 (33%)	8 (67%)	99.11
TOTAL	21 (25%)	63 (75%)	-

*Nephelometric turbidity unit (NTU)

In terms of legislation, it can be stated that the filtration units of the treatment plant showed a good performance. In accordance with Brazilian regulations, it is obligatory to maintain turbidity ≤0.3 NTU only when the arithmetic mean of *Cryptosporidium* concentration is greater than or equal to 1.0 oocyst/L. According to the results presented in the previous section, this threshold was not exceeded at any time. This suggests that maintaining turbidity below 0.5 NTU would be sufficient to meet regulatory requirements.

Through the Approach A, the mechanical removal values of protozoa were determined based on the classification presented in Table 1. To exemplify, throughout the year of 2015, the mechanical removal was set at 2.5 logs for *Giardia* and 2.0 logs for *Cryptosporidium* for five months, while for the remaining 7 months, the mechanical removal of protozoa was defined as 3.0 logs.

Therefore, we can state that in the years 2015, 2017, and 2021 the performance in mechanical removal of protozoa was lower than the performance in the years 2016, 2018, 2019, and 2020.

When employing the Approach B (Eqs. 1 and 2), the same removal values of 3.69 logs for *Giardia* and

3.02 logs for *Cryptosporidium* were obtained to all years. In other words, the annual variation among the percentage values of turbidity removal (presented in Table 3) was not significant enough to yield a difference among the values of annual mechanical removal of protozoa obtained through the Approach B.

The maximum value of mechanical removal of *Giardia* achievable under the Approach A is 3.0 logs. Then, in summary, we can attest that the Approach B may produce mechanical removal values of *giardia* greater (3.69 logs) than the maximum possible value obtained via the Approach A (3.0 logs).

Statistical tests (*p*-values)

Statistics of the inactivation data of Giardia

Table 4 displays the *p*-values from *t*-tests conducted to compare the inactivation data of *Giardia* from different reservoirs for each year. Meanwhile, Table 5 presents the *p*-values obtained to compare the inactivation data from different years in each reservoir.

The *p*-values (*t*-test) below 0.05 and approaching zero confirmed the statistical distinctions between the data from the Reservoir 1 and the Reservoir 2. The same was evidenced when comparing the data across the years, with a few exceptions: Considering the data from the same reservoir, the mean of the 2015 dataset can be considered statistically equivalent to the means of the 2017 and 2018 datasets, as *p*-values ranged from 0.16 to 0.23. Similarly, the means of the 2016 and 2021 datasets from Reservoir 2 exhibited a *p*-value of 0.18, supporting the conclusion that they can be deemed statistically equivalent.

Statistics of the removal data of Giardia

Table 6 presents the results of the Student’s *t*-test statistic (*p*-value) determined for the removal data of *Giardia* from all possible year combinations within each studied condition (reservoir and

Table 4 Results of Student’s *t*-test statistics between the datasets from different reservoirs, per year

Test- <i>t</i> between the reservoirs 1e 2	<i>p</i> -value (for each year)						
	2015	2016	2017	2018	2019	2020	2021
	4.5E-131	3.3E-77	5.0E-145	3.7E-116	3.1E-77	2.4E-27	6.8E-52

Table 5 Results of Student's *t*-test statistics between the datasets from different years, per reservoir

Test-t between the years	<i>p</i> -value (for each reservoir)	
	Reservoir 1	Reservoir 2
2015 and 2016	4.5E-19	5.9E-15
2015 and 2017	1.2E-01	1.9E-01
2015 and 2018	2.3E-01	1.6E-01
2015 and 2019	2.9E-04	1.1E-06
2015 and 2020	2.7E-58	4.7E-49
2015 and 2021	3.0E-22	8.5E-14
2016 and 2017	1.4E-23	3.1E-18
2016 and 2018	3.8E-15	1.2E-11
2016 and 2019	5.7E-07	3.3E-03
2016 and 2020	3.5E-18	3.9E-18
2016 and 2021	1.6E-02	1.8E-01
2017 and 2018	3.2E-02	3.1E-02
2017 and 2019	3.5E-06	1.1E-08
2017 and 2020	2.9E-64	2.6E-53
2017 and 2021	4.2E-26	3.3E-16
2018 and 2019	4.3E-03	8.5E-05
2018 and 2020	1.5E-51	3.4E-44
2018 and 2021	8.9E-19	3.0E-11
2019 and 2020	5.3E-36	7.6E-27
2019 and 2021	1.5E-10	6.0E-04
2020 and 2021	4.4E-09	2.4E-12

approach). Some datasets presented *p*-values > 0.05, which means that there is no statistical difference among them. These results will be further discussed in the "Total removal of *Giardia*" section.

To validate the discussions developed in "The impacts of skewness and kurtosis" section, Student's *t*-test were applied to confirm whether the mean of the removal of *Giardia* in the 2021 Res2A condition was statistically different from the means of the 2020 Res2A, 2018 Res1A, and 2019 Res1A conditions. As observed in Table 7, the obtained *p*-values were < 0.05, indicating that these datasets are statistically distinct.

Statistics of infection risk datasets

In all *t*-tests performed with the data of annual infection risk, every obtained *p*-value was found to be below 0.05 and the vast majority of them were

Table 6 Results of Student's *t*-test statistics between the datasets from different years and same condition (reservoir and approach)

Test-t between the years	<i>p</i> -value (for each condition)			
	Res 1A ^a	Res 2A ^b	Res 1B ^c	Res 2B ^d
2015 and 2016	0.07	0.06	0.02 ^e	0.04 ^e
2015 and 2017	0.47	0.46	0.39	0.42
2015 and 2018	0.34	0.48	0.43	0.41
2015 and 2019	0.46	0.20	0.20	0.12
2015 and 2020	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e
2015 and 2021	0.01 ^e	0.02 ^e	0.01 ^e	0.02 ^e
2016 and 2017	0.07	0.06	0.02 ^e	0.03 ^e
2016 and 2018	0.05	0.07	0.05	0.07
2016 and 2019	0.11	0.24	0.14	0.27
2016 and 2020	0.03 ^e	0.02 ^e	0.02 ^e	0.02 ^e
2016 and 2021	0.21	0.34	0.30	0.40
2017 and 2018	0.37	0.48	0.34	0.34
2017 and 2019	0.43	0.19	0.15	0.10
2017 and 2020	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e
2017 and 2021	0.01 ^e	0.02 ^e	0.01 ^e	0.02 ^e
2018 and 2019	0.33	0.21	0.27	0.19
2018 and 2020	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e
2018 and 2021	0.01 ^e	0.03 ^e	0.02 ^e	0.04 ^e
2019 and 2020	0.00 ^e	0.00 ^e	0.00 ^e	0.00 ^e
2019 and 2021	0.02 ^e	0.13	0.06	0.19
2020 and 2021	0.14	0.04 ^e	0.06	0.03 ^e

^aRes1A: considering the water stored in the Reservoir 1 and the Approach A to estimate the mechanical removal of protozoa

^bRes2A: considering the water stored in the Reservoir 2 and the Approach A to estimate the mechanical removal of protozoa

^cRes1B: considering the water stored in the Reservoir 1 and the Approach B to estimate the mechanical removal of protozoa

^dRes2B: considering the water stored in the Reservoir 2 and the Approach B to estimate the mechanical removal of protozoa

^e*p*-values < 0.05 suggests that the observed results are statistically significant, and therefore there is a real difference among the means of the evaluated datasets

equal to absolute zero, confirming the statistical difference among these datasets.

Inactivation of *Giardia*

The inactivation values (in logs) of *Giardia* were calculated (Eq. 3) for each day of each year, meaning

Table 7 Results of *t*-test statistics between data from the 2021 Res2A condition and data from the 2020 Res2A, 2018 Res1A and 2019 Res1A conditions

Test- <i>t</i> between the datasets:	<i>p</i> -value
2021 Res2A ^b and 2020 Res2A ^b	0.042
2021 Res2A ^b and 2018 Res1A ^a	0.002
2021 Res2A ^b and 2019 Res1A ^a	0.001

^aRes1A: considering the water stored in the Reservoir 1 and the Approach A to estimate the mechanical removal of protozoa

^bRes2A: considering the water stored in the Reservoir 2 and the Approach A to estimate the mechanical removal of protozoa

Table 8 The annual mean (\pm margin of error considering 95% confidence) and the standard deviation (SD) values of the inactivation data of *Giardia* (in logs)

Year	Reservoir 1		Reservoir 2	
	Mean \pm margin of error (logs)	SD	Mean \pm margin of error (logs)	SD
2015	2.17 \pm 0.06	0.65	4.80 \pm 0.12	1.37
2016	1.68 \pm 0.07	0.67	3.90 \pm 0.15	1.40
2017	2.23 \pm 0.05	0.50	4.90 \pm 0.11	1.07
2018	2.13 \pm 0.06	0.59	4.71 \pm 0.13	1.21
2019	1.98 \pm 0.07	0.62	4.25 \pm 0.15	1.28
2020	1.10 \pm 0.08	0.62	2.58 \pm 0.20	1.49
2021	1.54 \pm 0.09	0.73	3.77 \pm 0.20	1.40

that 365 inactivation values of *Giardia* were obtained per year (per dataset). Table 8 provides a summary of these values through the annual mean and a margin of error considering 95% confidence, along with the standard deviation (SD) of the samples.

The inactivation values of *Giardia* associated with the Reservoir 2 exceed those of the Reservoir 1 by more than twice. This was expected, since the Reservoir 2 is significantly larger than Reservoir 1 and, therefore, provides a longer contact time between the pathogens and the oxidizing agent.

In general, the years 2015, 2017, and 2018 showed the best performances, with annual means of inactivation of *Giardia* close to 2.2 logs in the Reservoir 1 and 4.8 logs in the Reservoir 2.

The least favorable inactivation results occurred in 2020, with means of 1.10 logs \pm 0.08 logs in the Reservoir 1 and 2.58 logs \pm 0.20 in the Reservoir 2.

In comparison with 2020, the years 2016 and 2021 showed improved inactivation performances. In the Reservoir 1, the means were 1.68 \pm 0.07 logs and 1.54 \pm 0.09 logs for the years 2016 and 2021, respectively. As for the Reservoir 2, the removal mean ranged between 3.57 and 4.05 logs in the years 2016 and 2021.

In the monitoring history of the treatment plant, it was observed that the plant was unable to operate with residuals of free chlorine on approximately 13% of the days in 2016, 40% of the days in 2020, and 25% of the days in 2021. In other words, during these periods, the microbiological disinfection was conducted with chloramine residuals, consequently impairing the performance of inactivation of *Giardia* cysts. As previously mentioned in the “Introduction” section of this study, the formation of chloramines can naturally occur in the presence of organic matter. In addition to the volume and frequency of improper discharge of pollutants, the concentration of organic matter in the raw water can also vary with weather variations, such as temperature, rainfall volume, and sunlight exposure. These variations can directly impact the self-purification capacity of the water body used to supply the water treatment plant. In summary, it is presumed that during the periods when the plant operated with chloramine residuals, the concentration of organic matter and ammonia nitrogen at the collection points of raw water was higher than usual.

Total removal of *Giardia*

The chemical inactivation values of *Giardia* were added to the values of mechanical removal to obtain the total removal values of *Giardia* (in logs). Table 9 presents the values of arithmetic mean and a margin of error considering 95% confidence, the standard deviation (SD), the median, and the type of distribution function of each total removal dataset of *Giardia*, including their shape measurements (skewness and kurtosis).

In summary, the following ascending order of the median values of total removal of *Giardia* was observed: Res1A < Res1B < Res2A < Res2B. This behavior was already expected, since the inactivation values of *Giardia* were higher in the Reservoir 2 (Table 8).

In general, the distributions showed skewness values between -1 and +1, indicating a low degree of skewness. In addition, most of the dataset showed a negative

Table 9 The values of arithmetic mean (\pm margin of error considering 95% confidence), standard deviation (SD), median, skewness, kurtosis, and the type of distribution function of the data of total removal of *Giardia*

Total Removal of <i>Giardia</i> (Log)							
Condition	Mean \pm margin of error (logs)	SD	Median	Skewness	Kurtosis	Distribution function	<i>p</i> -value
2015 Res1A ^a	4.99 \pm 0.07	0.63	4.96	0.26	-1.40	Normal	0.384
2016 Res1A ^a	4.63 \pm 0.08	0.81	4.80	-0.58	-0.67	Box Cox $\lambda=3$	0.530
2017 Res1A ^a	5.00 \pm 0.06	0.46	5.11	-0.59	-0.03	Box Cox $\lambda=5$	0.314
2018 Res1A ^a	5.06 \pm 0.08	0.70	5.18	-0.68	-0.91	Box Cox $\lambda=4$	0.443
2019 Res1A ^a	5.03 \pm 0.09	0.61	5.15	-0.35	-1.48	Box Cox $\lambda=2$	0.184
2020 Res1A ^a	4.10 \pm 0.10	0.79	4.28	-0.14	-1.53	Smallest extreme value Location: 4.47 and scale: 0.65	> 0.250
2021 Res1A ^a	4.29 \pm 0.11	1.01	4.52	-0.20	-1.32	Normal	0.509
2015 Res1B ^b	5.89 \pm 0.07	0.42	5.70	0.79	-0.50	Box Cox $\lambda=-2.714$	0.196
2016 Res1B ^b	5.40 \pm 0.08	0.80	5.74	-0.93	-0.01	Box Cox $\lambda=4$	0.510
2017 Res1B ^b	5.94 \pm 0.06	0.47	5.90	-0.10	-1.21	Normal	0.402
2018 Res1B ^b	5.83 \pm 0.08	0.57	5.87	-0.37	-1.31	Box Cox $\lambda=5$	0.447
2019 Res1B ^b	5.76 \pm 0.09	0.56	5.84	-0.35	-1.23	Box Cox $\lambda=4$	0.473
2020 Res1B ^b	4.84 \pm 0.10	0.79	5.08	-0.32	-1.49	Smallest extreme value Location: 5.2 and scale: 0.63	0.167
2021 Res1B ^b	5.15 \pm 0.11	0.91	5.43	-0.39	-1.15	Box Cox $\lambda=2$	0.435
2015 Res2A ^c	7.67 \pm 0.14	1.11	7.46	0.35	-1.12	Normal	0.549
2016 Res2A ^c	6.83 \pm 0.18	1.73	7.47	-1.00	0.13	Box Cox $\lambda=3$	0.526
2017 Res2A ^c	7.69 \pm 0.13	0.98	7.76	0.03	-1.35	Normal	0.408
2018 Res2A ^c	7.69 \pm 0.15	1.35	7.96	-0.52	-1.18	Box Cox $\lambda=3$	0.505
2019 Res2A ^c	7.40 \pm 0.18	1.23	7.64	-0.32	-1.30	Box Cox $\lambda=3$	0.561
2020 Res2A ^c	5.54 \pm 0.23	1.88	5.74	0.12	-1.13	Logistics Location: 5.53 and scale: 1.09	0.138
2021 Res2A ^c	6.61 \pm 0.23	1.81	6.73	-0.74	0.48	Normal	0.406
2015 Res2B ^d	8.57 \pm 0.14	0.91	8.26	0.56	-0.68	Normal	0.402
2016 Res2B ^d	7.60 \pm 0.18	1.73	8.40	-1.14	0.37	Box Cox $\lambda=4$	0.477
2017 Res2B ^d	8.63 \pm 0.13	1.02	8.44	0.42	-0.95	Normal	0.674
2018 Res2B ^d	8.47 \pm 0.15	1.22	8.65	-0.34	-1.46	Box Cox $\lambda=3$	0.392
2019 Res2B ^d	8.14 \pm 0.18	1.19	8.33	-0.37	-1.51	Normal	0.636
2020 Res2B ^d	6.27 \pm 0.23	1.88	6.65	0.04	-1.17	Smallest extreme value Location: 7.17 and scale: 1.66	0.155
2021 Res2B ^d	7.46 \pm 0.23	1.71	7.68	-0.79	0.30	Normal	0.457

^aRes1A: considering the water stored in the Reservoir 1 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A)

^bRes1B: considering the water stored in the Reservoir 1 and the Neminski and Ongerth Model to estimate the mechanical removal of protozoa (Approach B)

^cRes2A: considering the water stored in the Reservoir 2 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A)

^dRes2B: considering the water stored in the Reservoir 2 and the Neminski and Ongerth Model to estimate the mechanical removal of protozoa (Approach B)

skewness, which indicates that the data is concentrated above its mean. This is possible to confirm by observing the mean and median data in Table 9: The conditions with negative skewness presented median values greater than the mean values. As for kurtosis, the negative values indicated a platykurtic distribution, that is, most of the data shown in Table 9 are distributed in a more dispersed way (flatter curve) than in a normal distribution.

The graph in Fig. 3 displays the values of the mean total removal of *Giardia* on the y-axis and the studied years on the x-axis, with each line representing a studied condition.

Analyzing Fig. 3 together with Table 6 (*p*-values) allowed for the following observations: In all studied conditions, the means of the data of 2020 are indeed lower than the means of the data from other years (*p*-value < 0.05), except for the means of the 2021 data from Reservoir 1, which can be considered equal to those of 2020 (*p*-value > 0.05). Therefore, it is appropriate to assert that the poorest performance in removal of *Giardia* occurred in the years 2020 and 2021, in the Reservoir 1, using the Approach A (mean between 4.00 and 4.40 logs).

The mean values for the years 2015, 2017, 2018, and 2019 are statistically equal within each condition studied (*p*-value > 0.05). Consequently, it is possible to affirm that, considering the same reservoir and approach, the removal performances of *Giardia* of these years can be considered similar to each other and superior to the performances presented in the other years (2016, 2020, and 2021).

The less satisfactory performances of the years 2016, 2020, and 2021 can be explained by their low efficiencies in chemical inactivation, as highlighted in Table 8 and discussed earlier in the corresponding section.

Comparing the obtained values among the different conditions, it was concluded that the best performance

occurred in the Reservoir 2 using the Approach B (Res 2B). In Fig. 3, it is possible to observe that the highest means reached values around 8.5 logs of total removal of *Giardia*. (in the years 2015, 2017, 2018, and 2019).

Annual risk of *Giardia* and *Cryptosporidium* infection

During the period in which protozoa were detected in the raw water (2015 to 2017), the concentration of *Giardia* cysts was significantly higher than the concentration of *Cryptosporidium* oocysts (Fig. 2). Despite this, the annual risk values for *Cryptosporidium* infection were considerably higher than the annual risk values for *Giardia* infection, as observed in Table 10. Table 10 presents the 95th percentile values derived from the data of annual infection risk obtained through the simulations carried out in this study.

These results can be attributed to the fact that *Cryptosporidium* oocysts does not undergo inactivation during the chlorination stage. In some years, the differences between the risk values of *Cryptosporidium* and *Giardia* infection were even more pronounced. In the year 2018, the annual risk values of *Cryptosporidium* infection were approximately 7000 and 59,000 times higher than the annual risk values of *Giardia* infection using the Approach A and the Approach B, respectively. In the year 2019, the annual risk values of *Cryptosporidium* infection were approximately 5000 and 16,000 times higher than those of *Giardia* infection, using the Approach A and the Approach B, respectively. These notable differences can be explained by the high levels of inactivation of *Giardia* in the years 2018 and 2019 (as evidenced in Table 8) combined with *Giardia* concentrations below 0.1 cysts/L (LQ of the method), which contributed to a significant reduction in the estimated values of infection risk.

Fig. 3 The mean of the total removal of *Giardia* (logs) by year and condition

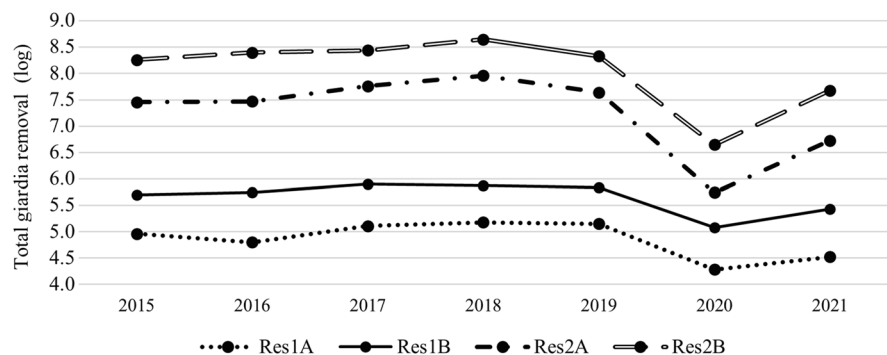


Table 10 The annual risk values of *Giardia* and *Cryptosporidium* infection calculated for the Reservoirs 1 and 2 using the Approach A and the Approach B

Annual Risk of Protozoa Infection (pppa)—P95 ^g					
Approach A ^e			Approach B ^f		
Condition	<i>Giardia</i>	<i>Cryptosporidium</i>	Condition	<i>Giardia</i>	<i>Cryptosporidium</i>
2015 Res1A ^a	7,04E-04	2,37E-03	2015 Res1B ^b	7,79E-05	8,60E-04
2016 Res1A ^a	5,04E-03	9,19E-03	2016 Res1B ^b	8,76E-04	5,31E-03
2017 Res1A ^a	5,82E-04	3,36E-03	2017 Res1B ^b	8,21E-05	1,02E-03
2018 Res1A ^a	8,76E-06	7,96E-04	2018 Res1B ^b	1,09E-06	5,14E-04
2019 Res1A ^a	8,12E-06	6,47E-04	2019 Res1B ^b	1,41E-06	5,14E-04
2020 Res1A ^a	6,38E-05	6,47E-04	2020 Res1B ^b	1,76E-05	5,14E-04
2021 Res1A ^a	1,27E-04	1,15E-03	2021 Res1B ^b	9,90E-06	5,14E-04
2015 Res2A ^c	3,06E-06	2,37E-03	2015 Res2B ^d	2,70E-07	8,60E-04
2016 Res2A ^c	7,27E-04	9,19E-03	2016 Res2B ^d	1,17E-04	5,31E-03
2017 Res2A ^c	3,05E-06	3,36E-03	2017 Res2B ^d	3,18E-07	1,02E-03
2018 Res2A ^c	1,14E-07	7,96E-04	2018 Res2B ^d	8,66E-09	5,14E-04
2019 Res2A ^c	1,22E-07	6,47E-04	2019 Res2B ^d	3,23E-08	5,14E-04
2020 Res2A ^c	1,95E-05	6,47E-04	2020 Res2B ^d	3,28E-06	5,14E-04
2021 Res2A ^c	2,02E-05	1,15E-03	2021 Res2B ^d	1,65E-06	5,14E-04

^aRes1A: considering the water stored in the Reservoir 1 and the Approach A to estimate the mechanical removal of protozoa

^bRes1B: considering the water stored in the Reservoir 1 and the Approach B to estimate the mechanical removal of protozoa

^cRes2A: considering the water stored in the Reservoir 2 and the Approach A to estimate the mechanical removal of protozoa

^dRes2B: considering the water stored in the Reservoir 2 and the Approach B to estimate the mechanical removal of protozoa

^eApproach A: estimation of the mechanical removal of protozoa using USEPA definitions

^fApproach B: estimation of the mechanical removal of protozoa using the Neminski and Ongerth model

^gP95: the 95th percentile of the annual infection risk data obtained through successive and random sampling (iterations) using the Latin Hypercube simulation technique

Table 11 presents the reduction values (in percentage) in the annual risk of protozoa infection when the Approach A was replaced by the Approach B to estimate the removal values of protozoa. In this case, the annual risk of *Giardia* infection reduced by approximately $86\% \pm 6.4\%$. For the annual risk of *Cryptosporidium* infection, the reduction was more pronounced in the years 2015 (-64%), 2017 (-70%), and 2021 (-55%). This is plausible, considering that in these years, the mechanical removal estimated by the Approach A presented the lowest values, according to the observations presented in the “Turbidity and mechanical removal of protozoa” section.

The impacts of skewness and kurtosis

For a better evaluation of the datasets, the annual risk results of *Giardia* infection (obtained by the Approach

A) were ranked in descending order in Table 12. This table also presents the values of median concentration of cysts, total removal mean of cysts, and the parameters that define the shape of the distribution curves of total removal data of *Giardia* cysts (skewness and kurtosis). The highest risk values of *Giardia* infection occurred in the year 2016 ($5.04E-03$), which is probably related to the highest median concentration of cysts in raw water (8 cysts/L).

The influence of the values of skewness and kurtosis on the magnitude of the annual risk values can be evidenced through the conditions classified in the positions 7 to 10 of Table 12.

Although the conditions classified at the positions 8, 9, and 10 presented considerably lower mean values of total removal than the condition 2021 Res2A (position 7), these conditions presented lower risk values of infection. To facilitate the comparison among these results, the data were plotted in a graph

Table 11 The percentage reduction in the infection risk due to the replacement of the Approach A by the Approach B

% Reduction (Approach A-B) ^c		
Condition	<i>Giardia</i>	<i>Cryptosporidium</i>
2015 Res1 ^a	-89%	-64%
2016 Res1 ^a	-83%	-42%
2017 Res1 ^a	-86%	-70%
2018 Res1 ^a	-88%	-35%
2019 Res1 ^a	-83%	-21%
2020 Res1 ^a	-72%	-21%
2021 Res1 ^a	-92%	-55%
2015 Res2 ^b	-91%	-64%
2016 Res2 ^b	-84%	-42%
2017 Res2 ^b	-90%	-70%
2018 Res2 ^b	-92%	-35%
2019 Res2 ^b	-74%	-21%
2020 Res2 ^b	-83%	-21%
2021 Res2 ^b	-92%	-55%

^aRes1: considering the water stored in the Reservoir 1 of the treatment plant

^bRes2: considering the water stored in the Reservoir 2 of the treatment plant

^cPercentage reduction in the infection risk due to the replacement of the Approach A by the Approach B

in the Fig. 4. The distribution curve of the total removal data of cysts for the condition 2020 Res2A (position 8) showed a positive skewness (0.12) and a negative kurtosis (-1.13), while the distribution curve for the condition 2021 Res2A (position 7) showed a negative skewness (-0.74) and a positive kurtosis (0.48). This suggests that the negative kurtosis of -1.13 was the factor responsible for the value of infection risk of the condition 2020 Res2A (position 8) not being higher than the value obtained for the condition 2021 Res2A (position 7), even the latter presenting a higher mean value of total removal of cysts. Furthermore, the results presented for the conditions 2018 Res1A and 2019 Res1A (positions 9 and 10) indicated that the reduction in the risk of infection was even more pronounced when both skewness and kurtosis values were negatives.

As anticipated, the distribution characteristics of the removal data of *Giardia* (skewness and kurtosis) were as crucial for reducing the infection risk values as the magnitude of the mean values of total removal of cysts.

It is well established that a negative kurtosis indicates a flatter distribution curve, meaning the data points are more dispersed than on a normal curve. When a negative kurtosis is combined with a negative skewness, it may indicate that a significant portion of the dataset values is considerably higher than the mean. Consequently, in simulations of such distributions, a majority of iterations will involve values significantly exceeding the mean. This, in turn, can result in a notably lower annual risk value of infection (95th percentile) at the conclusion of the simulations.

The annual infection risks and the tolerable limits

The annual risks of infection were compared with the tolerable annual risks defined by the US Environmental Protection Agency—USEPA (1.0E-4 pppa) and by the World Health Organization -WHO (2.2E-3 pppa), in Table 13.

Conditions in which the annual risk did not exceed the tolerable annual risk were flagged with an “OK.” The others were evaluated based on the magnitude of the risk according to the tolerable values (1.0E-4 and 2.2E-3 pppa), for example: the 2016 Res1A condition presented an annual risk of *Giardia* infection 50 times greater than the tolerable risk stipulated by the EPA and 2.5 times greater than the tolerable risk stipulated by the WHO.

Except for Res1B in 2016, all conditions demonstrated an annual risk of *Giardia* infection within the tolerable limits when utilizing the Approach B. From 2017 to 2021, none of the annual risk values for *Giardia* infection exceeded the WHO tolerable limit, regardless of the Approach applied.

For the annual risk of *Cryptosporidium* infection, none of the conditions studied respected the limit imposed by the USEPA, not even the conditions with concentrations below 0.1 oocysts/L (2018 to 2021). However, considering the WHO limit, only the year 2016 (in both the Approaches) and the years 2015 and 2017 (only in the Approach A) presented risk values considered intolerable.

Table 14 presents the values of annual infection risk obtained under conditions that represent the requirements of the Brazilian legislation (17, 18). In the condition of 3.0 logs of removal of *Cryptosporidium* and 1.0 oocyst/L of concentration of

Table 12 Classification (in descending order of risk) of the conditions studied in the Approach A and the input variables (total removal of cysts, skewness, kurtosis, and *Giardia* concentration)

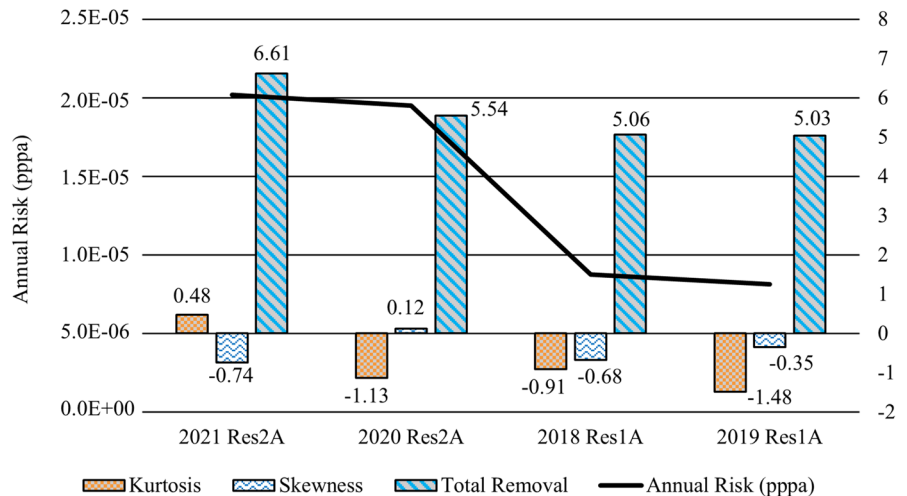
Annual risk of <i>Giardia</i> infection (descending order)						
Position	Conditions	<i>Giardia</i> (annual median in cysts/L)	Total removal (annual mean in logs)	Skewness ^c	Kurtosis ^c	Annual risk (pppa)
1	2016 Res1A ^a	8.00	4.63	-0.58	-0.67	5.04E-03
2	2016 Res2A ^b	8.00	6.83	-1.00	0.13	7.27E-04
3	2015 Res1A ^a	3.00	4.99	0.26	-1.40	7.04E-04
4	2017 Res1A ^a	2.00	5.00	-0.59	-0.03	5.82E-04
5	2021 Res1A ^a	<LQ	4.29	-0.20	-1.32	1.27E-04
6	2020 Res1A ^a	<LQ	4.10	-0.14	-1.53	6.38E-05
7	2021 Res2A ^b	<LQ	6.61	-0.74	0.48	2.02E-05
8	2020 Res2A ^b	<LQ	5.54	0.12	-1.13	1.95E-05
9	2018 Res1A ^a	<LQ	5.06	-0.68	-0.91	8.76E-06
10	2019 Res1A ^a	<LQ	5.03	-0.35	-1.48	8.12E-06
11	2015 Res2A ^b	3.00	7.67	0.35	-1.12	3.06E-06
12	2017 Res2A ^b	2.00	7.69	0.03	-1.35	3.05E-06
13	2019 Res2A ^b	<LQ	7.40	-0.32	-1.30	1.22E-07
14	2018 Res2A ^b	<LQ	7.69	-0.52	-1.18	1.14E-07

^aRes1A: considering the water stored in the Reservoir 1 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A)

^bRes2A: considering the water stored in the Reservoir 2 and the USEPA definitions to estimate the mechanical removal of protozoa (Approach A)

^cSkewness and kurtosis of the distribution curve of protozoa removal data

Fig. 4 Comparison among the results of annual risk of infection, total removal mean of cysts, skewness, and kurtosis of the distribution curves of the cysts removal data; for the conditions 2021 Res2A, 2020 Res2A, 2018 Res1A, and 2019 Res1A



Cryptosporidium, the annual infection risk obtained was higher than the values considered tolerable.

In the other conditions, the values of annual infection risk remained below the tolerable value recommended by the WHO (2.2E-3 pppa), but above the tolerable value recommended by the USEPA (1.0E-4

pppa). Therefore, it is evident that when the concentration of protozoa is greater than 1.0 (oo)cysts/L, the value of annual infection risk must significantly exceed the recommended tolerable value. Due to this observation, it was concluded that the conventional requirements present in the Brazilian legislation are

Table 13 Comparison of the annual risks of infection obtained in this study with the tolerable annual risks defined by the USEPA (1.0E-4 pppa) and WHO (2.2E-3 pppa)

USEPA						
YEAR	<i>Giardia</i>			<i>Cryptosporidium</i>		
	Res1A ^a	Res2A ^c	Res1B ^b	Res2B ^d	Approach A ^e	Approach B ^f
2015	7 x	OK ^g	OK ^g	OK ^g	20 x	9 x
2016	50 x	7 x	8 x	OK ^g	90 x	50 x
2017	5 x	OK ^g	OK ^g	OK ^g	30 x	10 x
2018	OK ^g	OK ^g	OK ^g	OK ^g	8 x	5 x
2019	OK ^g	OK ^g	OK ^g	OK ^g	7 x	5 x
2020	OK ^g	OK ^g	OK ^g	OK ^g	7 x	5 x
2021	1.3 x	OK ^g	OK ^g	OK ^g	10 x	5 x
WHO						
YEAR	<i>Giardia</i>			<i>Cryptosporidium</i>		
	Res1A ^a	Res2A ^c	Res1B ^b	Res2B ^d	Approach A ^e	Approach B ^f
2015	OK ^h	OK ^h	OK ^h	OK ^h	1.1 x	OK ^h
2016	2.5 x	OK ^h	OK ^h	OK ^h	4.5 x	2.5 x
2017	OK ^h	OK ^h	OK ^h	OK ^h	1.5 x	OK ^h
2018	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h
2019	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h
2020	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h
2021	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h	OK ^h

^aRes1A: considering the water stored in the Reservoir 1 and the Approach A to estimate the mechanical removal of protozoa

^bRes1B: considering the water stored in the Reservoir 1 and the Approach B to estimate the mechanical removal of protozoa

^cRes2A: considering the water stored in the Reservoir 2 and the Approach A to estimate the mechanical removal of protozoa

^dRes2B: considering the water stored in the Reservoir 2 and the Approach B to estimate the mechanical removal of protozoa

^eApproach A: estimation of the mechanical removal of protozoa using USEPA definitions

^fApproach B: estimation of the mechanical removal of protozoa using the Neminski and Ongerth model

^gThe annual risk did not exceed the tolerable annual risk defined by the US Environmental Protection Agency – USEPA (1.0E-4 pppa)

^hThe annual risk did not exceed the tolerable annual risk defined by the World Health Organization—WHO (2.2E-3 pppa)

Table 14 The estimated annual infection risks based on the requirements of the Brazilian legislation

<i>Giardia</i>		
Total removal (logs)	cysts/L	Annual risk (pppa)—P95*
3.5	<LQ	1.7E-04
4.0	1.0	1.1E-03
<i>Cryptosporidium</i>		
Total removal (logs)	oocysts/L	Annual risk (pppa)—P95*
2.0	<LQ	1.4E-03
3.0	1.0	2.6E-03

*P95: the 95th percentile of the annual infection risk data obtained through successive and random sampling (iterations) using the Latin Hypercube simulation technique

not sufficient to guarantee the microbiological safety of drinking water.

Conclusion

The water treatment plant examined in this study successfully reached the biological control requirements mandated by the Brazilian legislation. Due to the greater chlorine resistance exhibited by *Cryptosporidium* oocysts, the estimated values for the annual risk of *Cryptosporidium* infection were higher compared with the calculated values for annual risk of *Giardia*

infection. This occurred even when the concentration of *Giardia* cysts in the raw water intake was significantly higher than the concentration of *Cryptosporidium* oocysts. Given that a high level of pollution in water bodies is predominant in the Brazilian context, it is possible to state that the risk values of protozoa infection estimated were satisfactory, closely approaching or falling below the tolerable risk defined by the World Health Organization.

The values of infection risk derived from the Neminski and Ongerth model (Approach B) were notably lower than those obtained using the USEPA definitions (Approach A). This highlights the impact that the approach used to estimate the removal of protozoa can have on the estimation of infection risk. Despite the mathematical complexity associated with the Quantitative Microbial Risk Assessment (QMRA) method, it is imperative for the Brazilian authorities to consider and evaluate its implementation. This is essential given that the requirements and guidance provided by the local legislation have proven to be ineffective, and the QMRA application can effectively contribute to the prevention and investigation of outbreaks of waterborne diseases.

In conclusion, it is important to emphasize that efforts to ensure a low risk of microbiological infection should not solely focus on water treatment. Instead, it is crucial to direct greater attention to resolving the problems related to inappropriate disposal of industrial effluents and domestic sewage into water bodies.

Acknowledgements The author would like to thank her co-workers from Municipal Department of Water and Sewage of Porto Alegre (DMAE) for their encouragement.

Author contributions Claudia Bauer Visentini was responsible for the development of the entire study, including the preparation and revision of the manuscript text, figures, and tables.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The author has read, understood, and has complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors.

Competing interests The author declares no competing interests.

References

- Bataiero, M. O., Araujo, R. S., Nardocci, A. C., Matté, M. H., Sato, M. I. Z., Laureto, M. S., & Razzolini, M. T. P. (2019). Quantification of *Giardia* and *Cryptosporidium* in surface water: A risk assessment and molecular characterization. *Water Supply*, 19(6), 1823–1830.
- Betancourt, W. Q., & Rose, J. B. (2004). Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Veterinary Parasitology*, 126, 219–234.
- Blume, K. K., Macedo, J. C., Meneguzzi, A., Silva, L. B., Quevedo, D. M., & Rodrigues, M. A. S. (2010). Water quality assessment of the Sinos River Southern Brazil Blume. *Brazilian Journal of Biology*, 70(4), 1185–1193.
- Brasil, M. S. (2017). Portaria de consolidação nº 5, de 28 de setembro de 2017. Consolidação das normas sobre as ações e os serviços de saúde do Sistema Único de Saúde. Anexo XX. Do controle e da vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. Ministério da Saúde. Diário Oficial da União. <https://www.gov.br/saude/pt-br/composicao/sectics/daf/pnpmf/orientacao-ao-prescritor/Publicacoes/portaria-de-consolidacao-no-5-de-28-de-setem-bro-de-2017.pdf/view>
- Brasil, M. S. (2021). Portaria GM/MS nº 888. Altera o Anexo XX da Portaria de Consolidação nº 5/GM/MS, de 28 de setembro de 2017, para dispor sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. Ministério da Saúde. Diário Oficial da União. https://bvsms.saude.gov.br/bvs/saudelegis/gm/2021/prt0888_07_05_2021.html
- Connors, E. E., Miller, A. D., Balachandran, N., Robinson, B. M., & Benedict, K. M. (2021). Giardiasis outbreaks—United States, 2012–2017. *Morbidity and Mortality Weekly Report*, 70(9), 304–307.
- Costa, D., Razakandrainibe, R., Basmaciyan, L., Raibaut, J., Delaunay, P., Morio, F., Gargala, G., Villier, V., Mouhajir, A., Levy, B., Riederer, C., Larreche, S., Lesthelle, S., Coron, N., Menu, E., Demar, M., Pommier de Santi, V., Blanc, V., Valot, S., ... Favennec, L. (2022). A summary of cryptosporidiosis outbreaks reported in France and overseas departments, 2017–2020. *Food and Waterborne Parasitology*, 27, 1–7.
- Demir, S. (2022). Comparison of normality tests in terms of sample sizes under different skewness and kurtosis coefficients. *International Journal of Assessment Tools in Education*, 9(2), 397–409.
- Dupont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B., & Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers. *The New England Journal of Medicine*, 332(13), 855–859.
- Efstratiou, A., Ongerth, J. E., & Karanis, P. (2017). Waterborne transmission of protozoa parasites: Review of worldwide outbreaks - An update 2011–2016. *Water Research*, 114, 14–22.
- Fantinatti, M., Pinto, M. G., Lopes-Oliveira, L. A. P., & Da Cruz, A. M. (2020). Epidemiology of *Giardia duodenalis* assemblages in Brazil: There is still a long way to go. *Memorias Do Instituto Oswaldo Cruz*, 115, 1–12.

- Haas, C. N., Rose, J. B., & Gerba, C. P. (2014). *Quantitative microbial risk assessment*. John Wiley & Sons.
- Health Canada. (2022). Guidelines for Canadian drinking water quality—summary tables. Water and air quality bureau, healthy environments and consumer safety branch, health. Canada, Ottawa, Ontario.
- Jain, S., Melo, T. G. C., Dolabella, S. S., & Liu, J. (2019). Current and emerging tools for detecting protozoa cysts and oocysts in water. *Trends in Analytical Chemistry*, *121*, 115695.
- Kui, C. S., Yan, J. Y., Ying, H., & Y.J., Meng, X., Bo, X.J., Hua, T.L., Juan, S.Y., Ping, C.J. (2021). Quantitative microbial risk assessment of *Cryptosporidium* and *Giardia* in public drinking water in China. *Biomedical and Environmental Sciences*, *34*(6), 493–498.
- LeChevallier, M., & Au, K. K. (2004). *Water treatment and pathogen control: Process efficiency in achieving safe drinking water*. IWA Publishing.
- Mac Kenzie, W. R., Schell, W. L., Blair, K. A., Addiss, D. G., Peterson, D. E., Hoxie, N. J., Kazmierczak, J. J., & Davis, J. P. (1995). Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: Recurrence of illness and risk of secondary transmission. *Clinical Cryptosporidium Infection*, *21*, 57–62.
- Ministry of Health. (2005). *Drinking water standards for New Zealand*. Wellington: Ministry of Health.
- Minitab LLC, State College, PA (2022). Minitab® Statistical Software. Desktop application from <https://www.minitab.com/products/minitab/>
- Morgan, M.G., Herion, M. (2007). *Uncertainty: A guide to dealing with uncertainty in quantitative risk and policy analysis*. Cambridge University Press.
- Nieminski, E. C., & Ongerth, J. E. (1995). Removing *Giardia* and *Cryptosporidium* by conventional treatment and direct filtration. *Journal of the American Water Works Association*, *87*(9), 96–112.
- Owens, C. E. L., Angles, M. L., Cox, P. T., Byleveld, P. M., Osborne, N. J., & Rahman, M. B. (2020). Implementation of quantitative microbial risk assessment (QMRA) for public drinking water supplies: Systematic review. *Water Research*, *174*, 1–18.
- Palisade Corporation. (2022). @RISK 8 Industrial. Risk Management Software from https://help.palisade.com/v8_3/en/@RISK/@RISK.htm
- Rose, J. B., Haas, C. N., & Regli, S. (1991). Risk assessment and control of waterborne Giardiasis. *American Journal of Public Health*, *81*(6), 709–713.
- Tao, H., Feng, X., Wei, C., Min, S., Liang, C., & Bo, F. (2013). The effects of glycine on breakpoint chlorination and chlorine dosage control methods for chlorination and chloramination processes in drinking water. *Water Air Soil Pollution*, *224*(10), 224–1686.
- USEPA. (1991). Guidance manual for compliance with the filtration and disinfection requirements for public water systems: using surface water sources. Science and technology branch criteria and standards division office of drinking water U.S Environmental Protection Agency Washington. D.C. EPA-HQ-OW-2002-0061-0150.
- USEPA. (2003). Guidance manual. LT1ESWTR disinfection profiling and benchmarking technical guidance manual. Office of Water (4606M) EPA 816-R-03-004. National Service Center for Environmental Publications (NSCEP).
- USEPA. (2005). Occurrence and exposure assessment for the final long term 2 enhanced surface water treatment rule. Office of water (4606M) EPA 815-R-06-002. National Service Center for Environmental Publications (NSCEP).
- USEPA. (2006). Source water monitoring guidance manual for public water systems. For the final long term 2 enhanced surface water treatment rule. Office of Water (4601M) EPA 815-R06-005. National Service Center for Environmental Publications (NSCEP).
- Vose, D. (2008). *Risk Analysis: A Quantitative Guide* (3rd). John Wiley & Sons.
- WHO. (2016). Quantitative microbial risk assessment: Application for water safety management. Water, Sanitation, Hygiene and Health (WSH). World Health Organization.
- Yang, G., Yang, Q., Xu, J., Bai, X., Han, Q., Nie, J., Zhang, L., Li, H., Gao, H., Zhou, W., & Li, J. (2021). Formation of organic chloramines during chlorination of 18 compounds. *Water Research*, *204*, 117570.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.