Influencing factors and health risk assessment of microcystins in the Yongjiang river (China) by the Monte Carlo simulation method

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ABSTRACT

A total of 90 water samples and associated environmental data were collected from three sample points from March–December 2017 from river Yongjiang. The relationship between the environmental factors and the microcystins(MCs) concentration were investigated in this article. The results showed that total phosphate (TP) is the dominant factor affecting the contents of MC-LR and the total content of MCs (TMCs), while pH and total nitrogen/total phosphate(TN/TP) ratio are the main factors affecting the content of MC-RR and MC-YR, respectively. Professional risk assessment software @Risk7.5 was used to evaluate the risk of dietary intake of microcystins (MCs). The result indicated that the health risks of MC-RR from drinking water source were higher than those of MC-LR and MC-YR, and the pollution of MCs would lead to high potential health risks, especially in children. Health risks for children found for MC-RR from drinking water sources were higher than the maximum allowance levels recommended by the US Environmental Protection Agency (USEPA) ($1x10^{-4}$), and health risks for adults found for MC-RR from drinking water sources were higher than the maximum allowance levels recommended by the International Commission on Radiological Protection (ICRP) ($5x10^{-5}$). Therefore, it is necessary to strengthen the protection and monitoring of drinking water source for effective control of water pollution and safeguarding of human health.

Subjects Health Ecotoxicology and Environmental Impacts

Keywords Microcystins; Environmental factors; @Risk7.5; Monte Carlo method; Health risk assessment

1. INTRODUCTION

Eutrophication of freshwater bodies can result in algal blooms, especially those caused by cyanobacteria. The outbreak of Cyanobacteria blooms secretes a secondary metabolite of the algal toxin that inhibits the growth of other species in water and ensures the growth advantage of algae itself (Ame et al. 2003, Cao et al. 2017). Microcystin is a type of algal toxin consisting of monocyclic peptides with more than 90 isomers(Yang et al. 2017). Of these, the most widely distributed and highly toxic are microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR) (Zegura 2016). These toxins are synthesized in the cells and released after cell rupture, resulting in the appearance of microcystins in the water source.

Cyanobacteria blooms appear in eutrophicated waters worldwide, and MCs can be bioaccumulated by aquatic animals and can reach humans, severely endangering human health and leading to illness or death. In 1975, the drinking water source in the small town of Pennsylvania was contaminated with MCs, which resulted in more than half of the local population suffering from acute gastroenteritis (Keleti et al. 1982). In 1996, 116/131 patients, who received routine treatment at the Brazil hemodialysis center, presented abnormal symptoms such as blurred vision, nausea, and vomiting due to MCs contamination, and >50 individuals succumbed to mortality (Pouria et al. 1998). Studies on drinking water showed that in Haimen and Fu Sui, where liver cancer were high incidence in China, the content of MCs in the water were high as well (Ueno et al. 1996). In 2010, the International Agency for Research on Cancer (IARC) listed microcystins as a "possible human carcinogen" (Group 2B) based on its potential carcinogenicity (2010).

Although MCs have been confirmed to cause acute and chronic damage to the human body, reports on MC-LR, MC-RR, and MC-YR and their risk assessment in Yongjiang river of China are yet lacking. The environmental conditions of water source are crucial in toxin production. However, the factors affecting the production of MCs (nutrient levels and climatic conditions) in Yongjiang have not yet been elucidated. Therefore, the present study investigated the concentration and distribution of MCs in Yongjiang river as influenced by seasonal changes in water quality and the related parameters.

Owing to the requirement of prolonged duration and inoperability of toxicological health risk assessments, the present study could not be conducted in a population. Thus, the Monte Carlo simulation mathematical and logical model was used. In recent years, this model has been used in order to understand the behavior of water systems. This would aid in decision-making due to the main advantage of the ability to model any appropriate assumptions of the problem or the system (Clausen et al. 2017). Moreover, the Monte Carlo simulation of uncertainties was applied in the risk assessment model by collecting limited samples to predict the overall situation. In this regard, the risk uncertainty is intuitively expressed, which is in agreement with the nature of the uncertainty and predictability of the risk; the risk managers and policymakers can provide a favorable intuitive and scientific basis (Paladino et al. 2017, Sasi et al. 2017). The US

Environmental Protection Agency (USEPA) has set Monte Carlo simulation as a basic method in the risk analysis policy (Moolenaar 1996). Currently, American Palisade developed @Risk Monte Carlo software that is loaded into Excel simulation technology for risk analysis based on the professional software. The software is mainly based on the analysis of stochastic simulation method of Monte Carlo that provides various potential results using a variety of probabilistic simulations, the probability of occurrence of events that constitute a risk, forecast the risk of uncertainty quantitatively, and summarize the characterization results. Li et al. (2017) used the Monte Carlo model to assess the quantitative risk of aluminum in Youtiao, which was not found to exceed the provisional tolerable weekly intake (PTWI) set by the joint FAO/WHO Expert Committee on Food Additives (JECFA) for the public. Jia et al.(2018) used the Monte Carlo simulation to evaluate the trace elements in four freshwater fish from a mine-impacted river, and found that consumption did not exert any appreciable adverse impact on human health due to the exposure to trace elements in fish muscle.

The present study was conducted to investigate the current status of drinking water sources in the Yongjiang river in China regarding the contamination of MCs. A risk assessment was conducted using the Monte Carlo simulation. These findings provide a basis to undergo an effective control of water pollution and quality in order to protect the human health of the area.

2. MATERIALS AND METHODS

2.1. Sampling location

Yongjiang river (108°06’-108°49’E, 22°50’-22°52’N) is a major water resource with an average annual flow of 1292 m^3/s in the Nanning City area. The nutrient enrichment is the causative for the reproduction of algae. It is the main urban water source in Nanning city, China, and the tributary channel is also a vital transportation route. The surface area of the lake is 2676 ha with a maximum depth of 23 m and is used for entertainment as well as a source of water for domestic use, agriculture, fishery, and industry. The nutrients entering the Yongjiang River come from the cultivated land and farms. The nutrient enrichment is causative for the reproduction of algae.

2.2. Sampling

Specifically, water samples were collected from the Yongning river in Nanning City from March to December in 2017. For this, the river section was set in a total of three sampling points: Qingxiu District, Jiangnan District, and Yongning District, respectively. The water samples were collected 3 times/month from each sampling point, i.e., a total of 90 samples were collected in 10 months. Sample collection, containers, stabilization, and transportation to the laboratory were in accordance with the methods described in Pesce et al. (Pesce et al. 2002).

2.3. Water quality analysis

Water parameters [for example, χ^1=Water Temperature, χ^2=pH, χ^6=Dissolved Oxygen (DO)] were measured in situ and [for example, χ^3=Total Phosphate (TP), χ^4=PO_4^{3-}-P, χ^5=Total Nitrogen (TN)] were measured in laboratory. The data represented the results obtained from three experiments. The analysis of all the above water samples was in reference to the standard methods (Wang et al. 2002).

2.4. Determination of MCs

Water samples were collected from the sampling sites and passed through the sand funnel to remove large particles of impurities and were stored at 4°C and protected from light. A total of 2000-ml water sample was collected, and 500-mL water sample is passed through the 0.45-μm filter under reduced pressure filtration and extracted two times with 5mL ultra-pure water after five times freezing-thawing at -80 °C/37 °C. After filtering through the 0.45-μm hybrid filter for removing the algal cells, the solution was passed through solid phase extraction (SPE) (500 mg/6 mL) (SUPELCO, USA). The SPE was rinsed with 20 mL of 20% methanol and 10 mL deionized distilled water. The toxin was eluted from the stationary phase with 80% methanol (containing 0.05% TFA), and each sample was dried in a water bath at 60 °C). The air-dried samples were suspended into 1 mL deionized distilled water for high performance liquid chromatography (HPLC) (Shimadzu LC-10A, Japan) analysis. The solubilized toxin samples were analyzed using HPLC with UV detector at 238 nm and symmetrical C_{18} column (3.9×150 mm^2) (Waters, USA). The mobile phase constituted of 33% acetonitrile and 67% deionized distilled water in 0.1% phosphate buffer (pH=3). The flow rate was set at 1 mL/min. MC-LR and MC-RR (Solarbio, approximate purity, 95%) and MC-YR (Alexis, approximate purity 98%) were used as standards. Furthermore, the concentrations of MCs were determined by calibrating such area under the peak with the corresponding standard curves.

2.5. Method of risk assessment

2.5.1. Construction of exposure assessment model

The three main sources of exposure to pollutants were intake, inhalation, and skin absorption; the proportion of each was different due to the variations in the pollutants. The present study assessed the risk exposure caused by drinking water. The daily exposure to MC-RR, MC-YR, and MC-LR by direct drinking water was assessed using the Monte Carlo simulation by @Risk7.5 software operating platform; the Bootstrap sampling method was used for the quantification of uncertainty. Each Bootstrap sample was simulated with 10,000 Monte Carlo simulations to obtain different percentile values (P5–P95) for determining its uncertainty. The probabilistic assessment method was used to construct the exposure evaluation model (Zobitz et al. 2011).

The mechanism underlying the different exposures and various exposure routes with different exposure dose formula were employed for the exposure assessment model of the health risk of chemical pollutants from the US environmental protection agency. Chronic daily intake (CDI) evaluated the safety of MCs in drinking water and the health risks of different routes in different populations. The chronic daily intake (μg/kg/d)] formula is as follows.

Daily exposure of drinking water (Duy et al. 2000, Funari et al. 2008):

$$CDI= \frac{Cw \times IR \times EF \times E}{BW \times AT}$$

Here, Cw is the concentration of pollutants in the water, μg/L; IR is the amount of drinking water, L/d; EF is the frequency of exposure, drinking water for daily necessities, 365d/a; ED is exposure duration, a; BW is the average body weight, kg; AT is the average time, ED×365 d/a. According to the WHO, the standard weight of adults is 70 kg, and the daily drinking amount is 2 L/d; while the weight of children is 16 kg and the daily drinking amount is 1 L/d (van et al. 2009).

2.5.2. Construction of risk description model

The characteristics of the pollutants in a water environment are generally divided into genetic, toxic substances (including radioactive pollutants and chemical carcinogens) and somatic toxic substances (for instance, non-carcinogens) as recommended by the USEPA water environmental health risk assessment model. The risk and non-cancer risk MCs from carcinogenic exposure from drinking water is calculated.

(1) Health hazard risk model of chemical carcinogens

The formula of health hazard risk caused by chemical carcinogens recommended by USEPA (Duy et al. 2000):

$$R_i^{\,c} = \frac{1\text{-}\exp(-D_i q_i)}{70}$$

In the formula, $R_i^{\,c}$ is the average personal carcinogenic annual risk of chemical carcinogen i through drinking water, a^{-1}; 70 indicates the average life expectancy of Chinese population, a;

D_i is the daily average exposure to chemical carcinogen i through drinking water, i.e., CDI, μg/kg/d; q_i is the carcinogenic strength coefficient of the chemical carcinogen i through the drinking water, and currently, there is no recognized carcinogenic intensity coefficient of MCs. Based on the formula of carcinogenic strength coefficient of carcinogens (Hitzfeld et al. 2000), this study deduced the formula as follows:

$$CPI = \frac{(OR-1) \times LR}{D}$$

Where, CPI is the coefficient of carcinogenic strength estimated from the population data, namely q_i, kg/d/μg/L. According to the population of 80,000 inhabitants, the study showed that the person who drank the river water presented a liver cancer OR of 1.246 (Falconer and Buckley 1989, Yeh et al. 1989). MC-LR indicated the risk of cancer in an individual in the whole local population. This parameter was not dimensionless and was calculated according to the risk of cancer during the individual's lifespan in the population of China: 6.2×10^{-3} (Yeh et al. 1989). D indicated the calculation of the average daily life exposure dose, μg/kg/d. According to the results of the study by Wolf et al., the carcinogenic strength of MC-RR was set to one-tenth of that of MC-LR, while the strength of carcinogenicity of MC-YR and MC -LR was equivalent(Wolf and Frank 2002).

(2) Non-carcinogenic health risk assessment model

The health risk assessment model recommended by USEPA was used to evaluate the non-carcinogenic health risk of MCs in the Yongjiang river source water. The non-carcinogenic risk was described using a hazard index (HI) by the following formula:

$$HI = \frac{CDI}{RfD}$$

Here, *RfD* is the reference dose for MCs, the internationally accepted *TDI* instead of MC-LR *RfD* was 0.04 μg/kg/d. According to the equivalent toxicity relationship among MC-RR, MC-YR and MC-LR, the *RfD* values of MC-RR and MC-YR were 0.4 and 0.04 μg/kg/d, respectively (Wolf and Frank 2002, Lee et al. 2017).

The HI to 1 is usually calculated for the benchmark: HI >1 indicates harm to the human body; the value higher than the reference dose for exposure may be dangerous; HI≤1 indicates that the extent of damage is smaller and the exposure level is lower than the reference dose, which is unlikely to be detrimental (Younes 1999).

2.6. Statistical analysis

IBM SPSS Statistics 22.0 software was used to perform all descriptive statistical analysis included minimum value, maximum value, mean value, Pearson's correlation analysis and stepwise multiple linear regression. Moreover, the risk assessment of MC-RR, MC-YR, and MC-LR exposure in water source was carried out using @Risk7.5 probabilistic evaluation software based on the Monte Carlo simulation technique.

3. RESULTS

3.1. Analysis of MCs by HPLC

Based on the high-performance liquid chromatography (HPLC) analysis method recommended by the national standard (GB/T 20466-2006), the separation conditions were optimized, and the methodological test study conducted according to the experimental apparatus. The order of the peaks and time of each standard substance were as follows: MC-RR (7.599 min), MC-YR (14.125 min), and MC-LR (17.601 min). The test sample was analyzed in 18 min (Figure 1).

3.2. Concentration level, distribution characteristics, and environmental impact factors of MCs in source water

3.2.1. Concentration distribution characteristics of MCs in source water

The concentration of MC-LR, MC-RR, and MC-YR in water samples were detected, the total content of MCs (TMCs) in water is the sum of the content of extracellular MCs (EMCs) and intracellular MCs (IMCs) dissolved in the water, and the results summarized in Table 1.

3.2.2. Seasonal distribution characteristics of MCs in water samples

The seasonal distribution of MCs in Yongjiang river source is shown in Figure 2, which demonstrated that the concentration of MC-YR (refers to the sum of intra and extra and the same below) was significantly lower than the other two MCs. The concentrations of MC-RR and MC-YR peaked in October, respectively.

3.2.3. Pearson's correlation analysis of environmental factors and MCs concentration

Pearson's correlation analysis of environmental factors and MCs concentration was analyzed and the results shown in Table 2. According to the correlation analysis, the concentration of MC-RR was positively correlated with water temperature and TN (p<0.01), with significant negative correlation with pH (p<0.01); The concentration of MC-YR was negative correlated with TN:TP ratio (p<0.05); The concentration of MC-LR was positively correlated with water temperature, TP and $PO4^{3-}$-P (p<0.01), with significant negative correlation with DO, TN:TP ratio (p<0.01); The concentration of TMCs was positively correlated with water temperature and TP (p<0.01), with significant negative correlation with pH, DO and TN:TP ratio (p <0.01).

3.2.4. Stepwise multiple linear regression analysis of MCs concentration and environmental factors

Stepwise multiple linear regression analysis of MCs concentration and environmental factors was shown in Table 3. The results indicate that TP (χ^3) is the dominant factor affecting the contents of MC-LR and TMCs. pH (χ^2) and TN:TP ratio (χ^7) are the primary factors affecting the content of MC-RR and MC-YR, respectively. These findings were in agreement with the results of the correlation analysis.

3.3. Assessment of MCs exposure in source waters

The risk assessment of MC-RR, MC-YR, and MC-LR exposure in water source was carried out using @Risk7.5 probabilistic evaluation software based on the Monte Carlo simulation technique (Sasaki et al. 2017). The corresponding probability distribution of various parameters used in the assessment was based on @Risk7.5 provided by the standard distribution function.

3.3.1. Distribution fitting of the concentration of MCs in source water

The present study used @Risk7.5 software to fit the processed samples. The sample data of MCs concentration in source water was characterized as continuous data. The fitting results were Gamma, Invgauss, Lognorm, Expon, and Loglogistic, followed by the optimal fitting distribution model. Three main methods were used to test the goodness of fit: Chi-Sq (Chi-squared) test, K-S (Kolmogorov–Smirnov) test, and A-D (Anderson–Darling) test (Lipton et al. 1995, Cummins et al. 2009). Taken together, the sample fitting results (Table 4) were used to determine the fitting distribution types of the optimal probability of the pollution data: MC-RR had Gamma and Invgauss distribution, MC-YR had Lognorm, Expon, and Loglogistic distribution, and MC-LR had Gamma and Expon distribution. Furthermore, the results of the distribution parameters after fitting and comparison with the sample data parameters are summarized in Table 5.

The abscissa in Figures 3, 4, and 5 represent the concentrations of MC-RR, MC-YR, or MC-LR; The concentrations are partitioned, the length of each interval is the group distance, the rectangular area is the frequency of the group, and the ratio of the total sample. The vertical axis is the frequency divided by the group distance obtained, and the curve is the most suitable distribution probability distribution. The fitting result can be visually observed from the coincidence of the rectangle shape and the figure.

3.3.2. Daily exposure calculation

The @Risk7.5 software was utilized for the random extraction of the MCs concentration profiles from the water to calculate the daily exposure of direct drinking water by different populations to MC-RR, MC-YR, and MC-LR. Each simulation cycle was performed for 10,000 cycles, and the simulation results are shown in Table 6. In adults and children, a significant difference was observed in daily exposure. P50, P85, P90, and P95 (Table 6) represent the high exposure sites of each population. The MCs exposed to drinking water showed that the children's daily intake was 2-fold higher than that of the adults, suggesting that children are more susceptible to the pollution of MCs than adults.

3.4. Risk characterization of MCs in source water

3.4.1. Carcinogenic risk of MCs in source water

Based on the exposure parameters and carcinogenic risk formula, @Risk7. 5 risk analysis software was used to extract the numerical value of MCs concentration in water randomly and calculate the carcinogenic risk of MC-RR, MC-YR, and MC-LR intake by different groups of individuals through direct drinking water. Each simulation cycle of 10,000 displayed the statistical simulation results summarized in Table 7. The carcinogenic annual risk of MC-YR was less than that of MC-LR and MC-RR, and MC-RR was the primary hazard in the source water. The maximum acceptable level and the negligible level of carcinogenic risk for the population recommended by some institutions is listed in Table 8. The annual risk of carcinogenesis of MCs in a water source is 10^{-6}–10^{-4}. The carcinogenic risk of MC-YR and MC-

LR in adults and children is lower than the maximum acceptable risk level designated by USEPA (1×10^{-4}) and ICRP (5×10^{-5}), and the risk of cancer in children is higher than that in adults. The health risks caused by MC-RR from drinking water source for children were significantly higher than the maximum allowance levels recommended by USEPA (1×10^{-4}). Also, the health risks caused by the MC-RR from drinking water source for adults were significantly higher than the maximum allowance levels recommended by ICRP (5×10^{-5}). These statistical details indicated that MC-RR in water bodies exhibited a significant carcinogenic risk to the health of adults and children.

3.4.2. Non-carcinogenic risk of MCs in source water

The exposure parameters and non-carcinogenic hazards index (*HI*) formula was used to calculate the value of different populations through direct drinking water intake of MC-RR, MC-YR, and MC-LR, using @Risk7.5 risk analysis software. Each simulation cycle for a total of 10,000 times provided the statistical simulation results (Table 9), which demonstrated that the average non-carcinogenic risk index of MCs in different populations through drinking water intake and the non-carcinogenic risk index of P90 and P95 at high levels of exposure were <1. This suggested that MCs, which are ingested through drinking water, pose less risk to health. In particular, the non-carcinogenic risk index of MC-RR was less than that of MC-YR, and the non-carcinogenic risk index of MC-YR was less than that of MC-LR, indicating that MC-LR was more hazardous to human health than MC-YR and MC- RR. The MC-RR, MC-YR, and MC-LR display a non-carcinogenic index in children than adults, and thus, MCs are detrimental to children.

4. DISCUSSION

World Health Organization (WHO) suggested that the concentration of MC-LR in drinking water was <1 μg/L(Ibelings, Backer et al. 2015, Menezes, Churro et al. 2017). Australia has developed a long-term safe drinking water guideline with a safety standard of 500 Microcystis cells/mL. The short-term drinking water index was <0.1 μg/L and 5000 Microcystis cells/mL(Nishiwaki-Matsushima, Ohta et al. 1992). Another study suggested that the maximum acceptable level in drinking water was 0.5 μg/L of MC-LR, or a lack of equivalent values for other MCs, 1 μg/L of total MCs(Dittmann, Neilan et al. 1997).

The water quality monitoring in Yongjiang river demonstrated that although no major algal bloom occurred, MC-RR, MC-YR, and MC-LR were present in the water column during the monitoring period. These pollutants indicated that the concentration of MCs in the Yongjiang river was influenced by seasonal changes in water quality-related parameters. Furthermore, the concentration of MC-YR was significantly lower than the other two MCs; This finding was similar to that from others studies, which suggested that the MCs are primarily dominated by MC-RR and MC-LR(Yang, Xie et al. 2006, Bi, Dai et al. 2017). The temperatures in March and April rise gradually; however, the level of MC-LR and MC-RR began to increase in May, which might be attributed to the slow growth of algae. With the significant increase in the growth conditions such as temperature, algal cell growth, accelerated cell aging, the autolysis phenomenon also increased significantly, as well as, the released concentration of MCs in water with respect to MC-RR and MC-YR was elevated. The peak of TMCs content appeared in October, which was related to the higher water temperature in the same period and the suitable climatic conditions. The significant decrease in the TMCs content in November and December might occur due to the decrease in temperature, the variable growth of Microcystis, and death of algal cells. These phenomena were similar to those described previously in Tai, Yang-cheng, and Xuanwu lakes in China(Xu, Wang et al. 2010, Li, Gu et al. 2014).

Previous studies demonstrated that the concentration of MCs was associated with the nutrient level and climate conditions. Water temperature played a major role in the growth of Microcystis. Reportedly, under low temperature, Microcystis grew slowly, and its ability to adapt to low temperature was weaker than that of the other algae (Wang et al. 2010). However, the study also found that the influence of water temperature on 3 types of MCs was identical; MC-LR and MC-RR exhibited significant effects depending on the water temperature, while the MC-YR is correlated to the temperature of water had little effect (Table 2). Before October, the water content of MC-RR is low that peaked in October when the water temperature began to decline; Thus, the changes in the process and temperature is not synchronized, indicating the absence of a marked correlation with the water temperature. Intriguingly, water pH was also shown to influence the toxin production in Nanning Yongjiang river. The maximum toxicity was detected at a pH below or above the medium level. As a result, MC-RR and TMCs were negatively correlated with pH (Table 3), which was similar to the results from other studies. Notably, the pH value cannot be used as an appropriate parameter to determine the toxin production. A majority of the blue-green algae can grow well in the water at pH 6.5–7.9 (Wang et al. 2002). The pH of Yongjiang river was within this range. DO showed a negative correlation of MC-LR with TMCs in Yongjiang river. However, some researchers indicated that the increased level of dissolved oxygen is related to the concentration of MCs (Wang et al. 2002). The relationship between toxin and DO could be attributed to the relative stable DO values measured in Yongjiang river. Correlation analysis results indicated that increasing the TP concentration could increase the MCs production, especially that of MC-LR. The current observations were in agreement with those from a study conducted in the large eutrophic Lake Erie in the USA (Harke et al. 2016) that demonstrated positive correlations between TP and the abundance of toxic Microcystis and MCs. Consistent with the trend, Vézie et al. (2002) also found that higher P concentrations were beneficial to the growth of toxic Microcystis. Although TP was a dominant explanatory variable, the effect of TN on the production of MCs could not be ignored. The concentration of MC-YR was negatively correlated with the TN/TP ratio. Previous studies also demonstrated that decreasing the TN/TP ratio concentration could promote the growth and toxicity of Microcystis (Yu et al. 2014, Lei et al. 2015). According to stepwise multiple linear regression, showed TP was found to be the dominant factor affecting the contents of MC-LR and TMCs, and pH and TN/TP ratio as the main factors affecting the content of MC-RR and MC-YR, respectively. These findings were in agreement with the results of correlation analysis.

The Monte Carlo simulation model determined the risk levels and putative human exposure scenarios associated with the blooms in Yongjiang river used for drinking. The whole process of security risk assessment was always accompanied by the uncertainty. The entire process of risk assessment was conducted two steps: exposure assessment and hazard characterization. Although the extrapolation of the experimental results did not lead to certainty, such an extrapolation could be carried out from experimental animals to the general population and from the general population to specific populations (sensitive populations). The variations in human individuals involved parameters such as genetics, age, sex, and environmental (nutritional status) and other factors. On the other hand, missing data or limitations led to uncertainties, including NOVEL, time differences, and lack of exposure data. Recent studies have gradually established superior methods, such as benchmark dose (BMD) and chemical-specific adjustment factor (CSAF), to address and reduce the uncertainty in the risk assessment (Ibelings et al. 2015). The USEPA and Health Canada have gradually started utilizing the BMD and CSAF methods to develop the health guidance values (Zeller et al. 2017).

Many countries that regulate cyanotoxins in drinking water use a parametric value based on the WHO Guidelines for 1 μg/L MC-LR (Ibelings et al. 2015, Menezes et al. 2017). With respect to the drinking source waters, most countries use guidance values based on cyanobacterial biomass (cell density, chlorophyll-a, biovolume) indirectly reflecting the potential hazardous MCs concentrations (Valerio et al. 2009). The risk of health issues due to the presence of other cyanotoxins in drinking waters is deduced; however, the adoption of guidance values for Cyanotoxins in water bodies used for drinking is yet to be investigated (Lee et al. 2017).

5. CONCLUSION

This study analyzed the influencing factors and the health risk assessment of MCs by Monte Carlo simulation method in Yongjiang river, China. The results showed that TP is the dominant factor affecting the contents of MC-LR and TMCs, while PH and TN/TP ratio are the main factors affecting the content of MC-RR and MC-YR, respectively. The health risk assessment results showed that the risk of MC-RR for human health hazards is higher than that of MC-LR and MC-YR, and children are more vulnerable to MCs contamination than the adults. The risk of carcinogenicity of MC-RR to children health under high exposure was greater than the maximum acceptable risk level recommended by USEPA. The annual risk of carcinogenic exposure in adults with MC-RR was greater than the maximum acceptable risk level recommended by the ICRP. The non-carcinogenic risk index for MCs was <1. Therefore, MCs in the water bodies should be monitored in regards to the carcinogenic risk to human health.

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Figure 1

HPLC chromatogram of MCs.

(A) HPLC chromatogram of MCs standards, the order of the peaks and time of each standard substance were as follows: MC-RR (7.599 min), MC-YR (14.125 min), and MC-LR (17.601 min). (B) HPLC chromatogram of MCs water samples, the same time represents the same substance, like A.

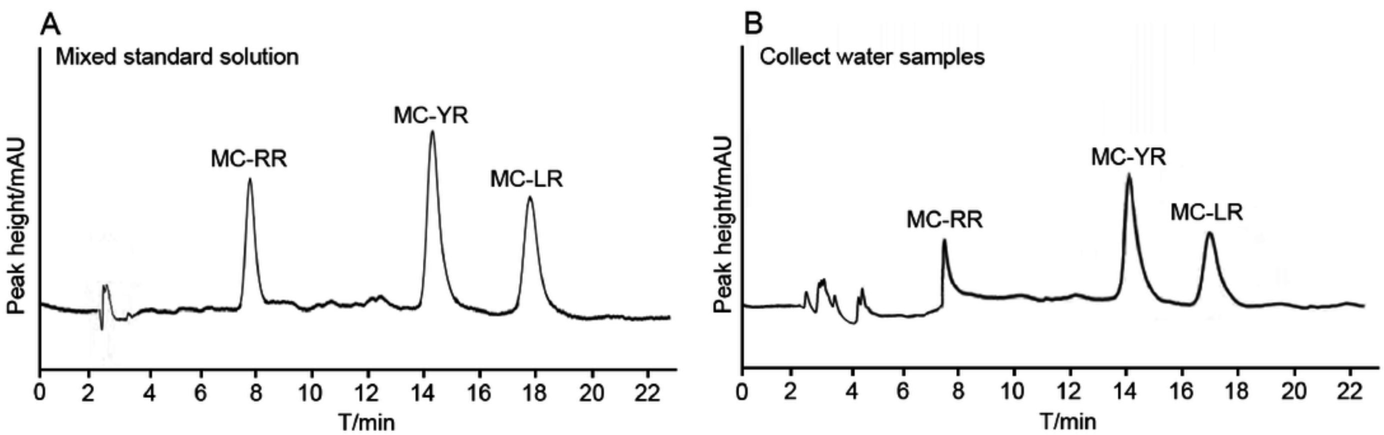

Figure 2

Concentration of MCs in source water in various seasons.

The total content of MCs (TMcs) in water is the sum of the content of extracellular MCs (EMCs) and intracellular MCs (IMCs) dissolved in the water.

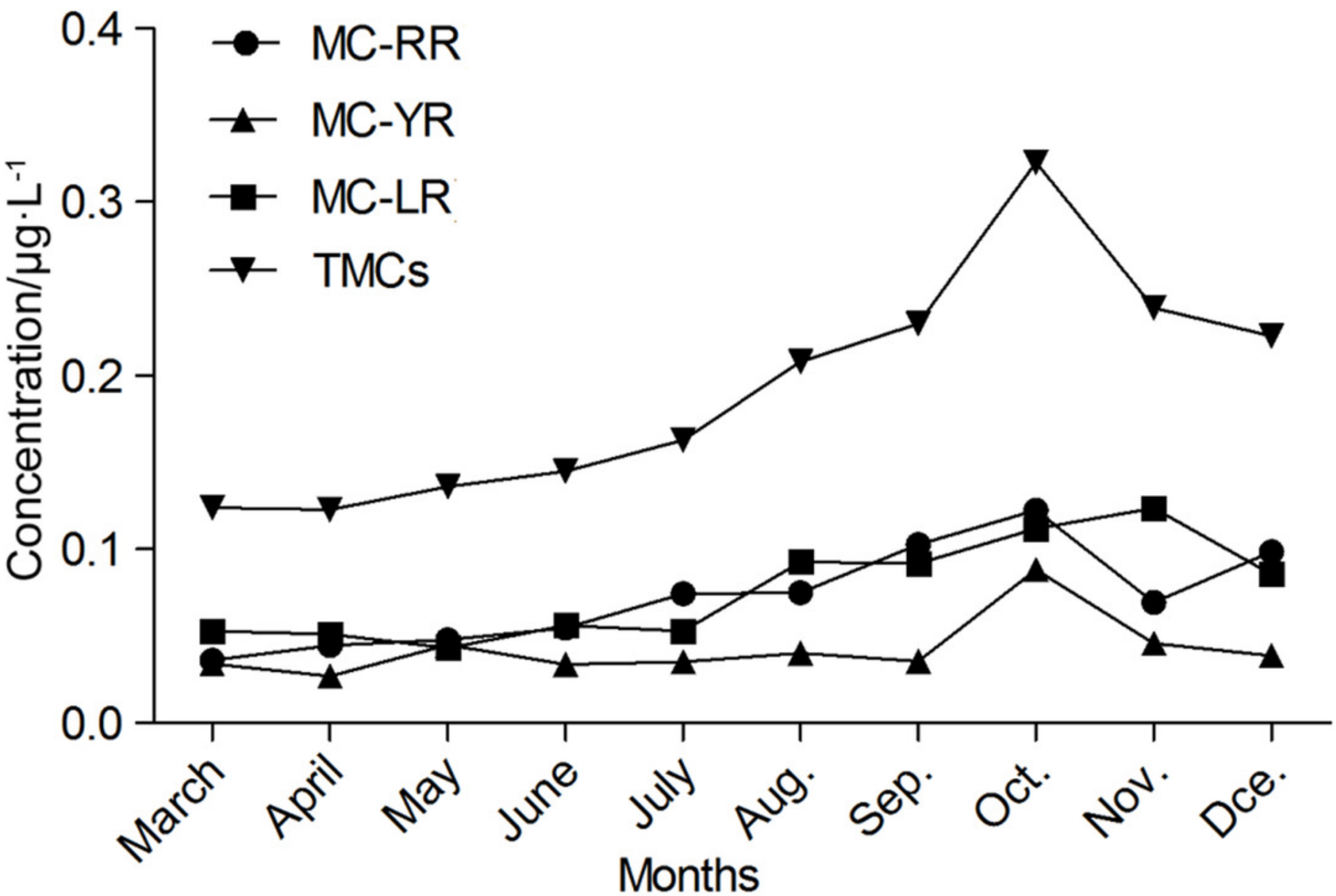

Figure 3

Probability distribution graph after fitting of MC-RR in source water.

The data comparison revealed that the optimal fitting distribution of the most suitable concentration of MC-RR in source water was Gamma (μ 0.073, s 0.061) (first number μ as the position parameter and the second number s as the scale parameter.

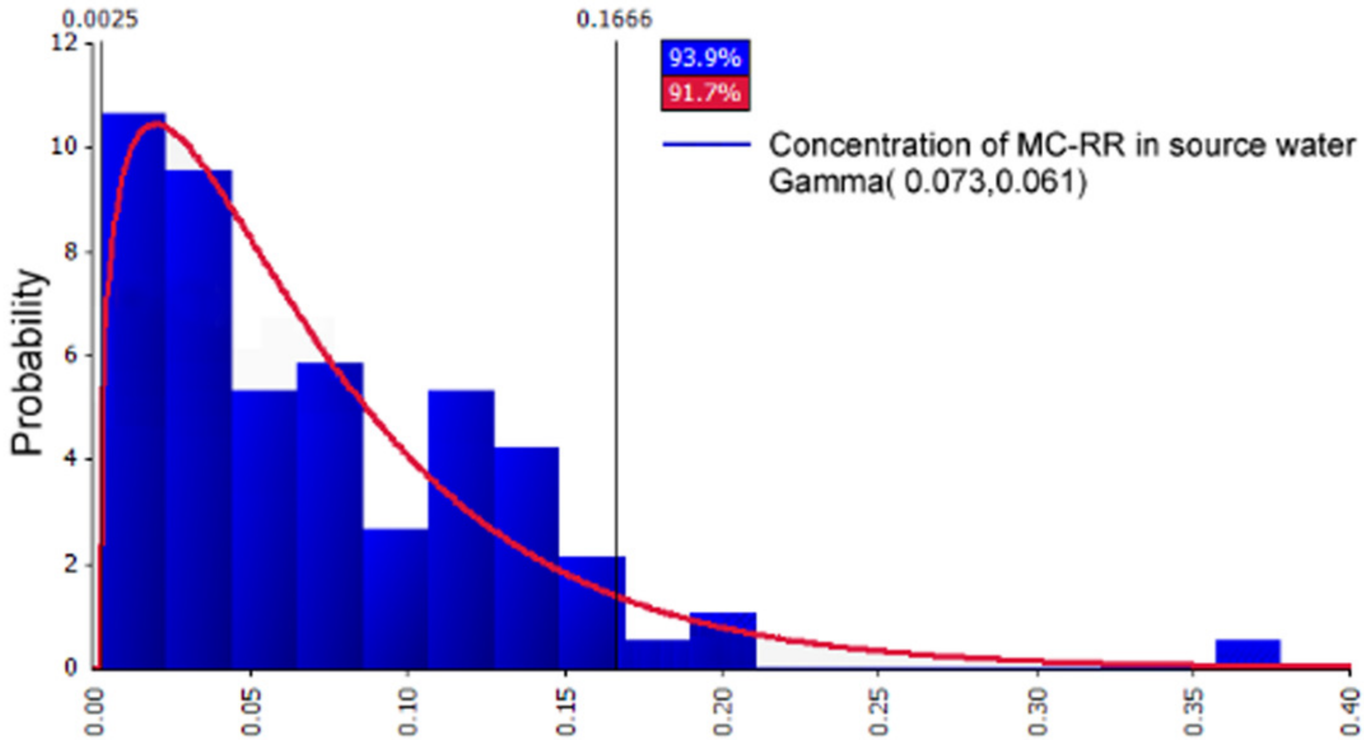

Figure 4

Probability distribution graph after fitting of MC-YR in source water.

The data comparison showed that the best-fitted distribution of MC-YR concentration was Lognorm (*μ* 0.047, *s* 0.071) .

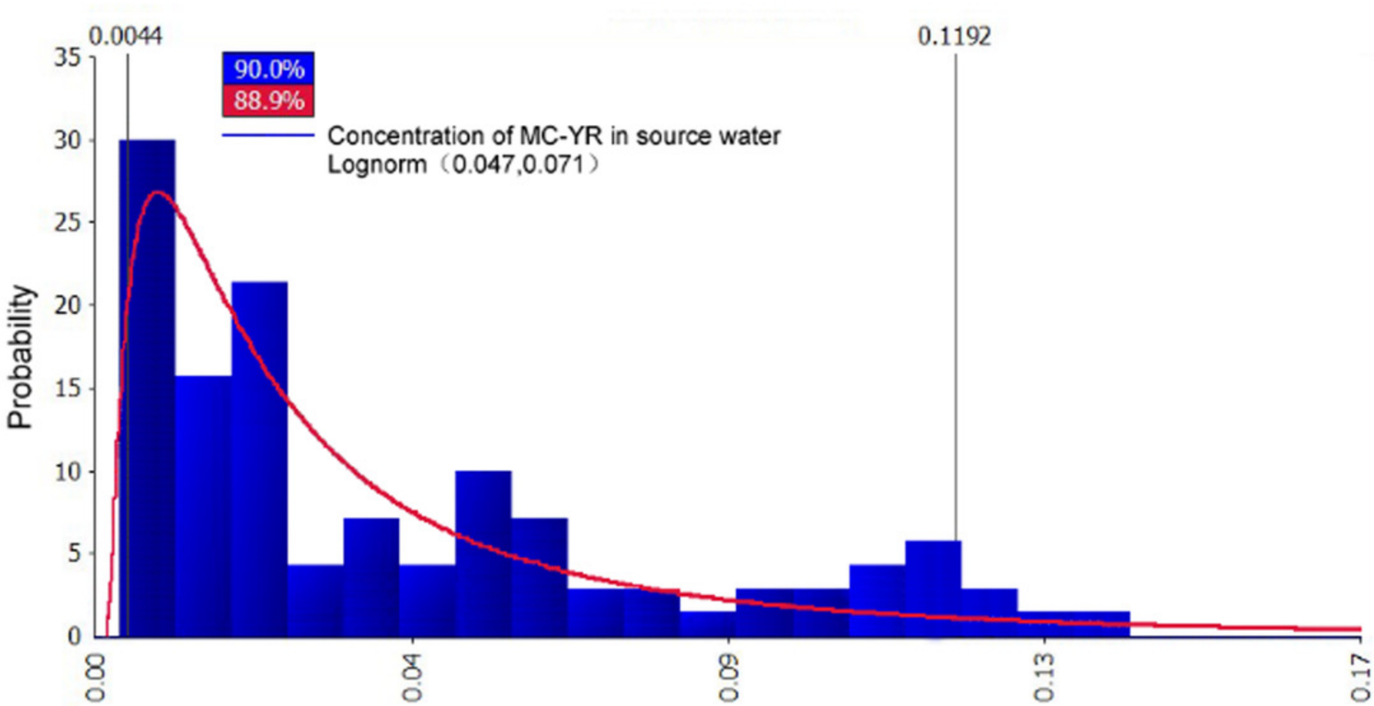

Figure 5

Probability distribution graph after fitting of MC-LR in source water.

The data comparison showed that the best-fitted distribution of MC-LR concentration was Gamma (μ 0.076, s 0.070).

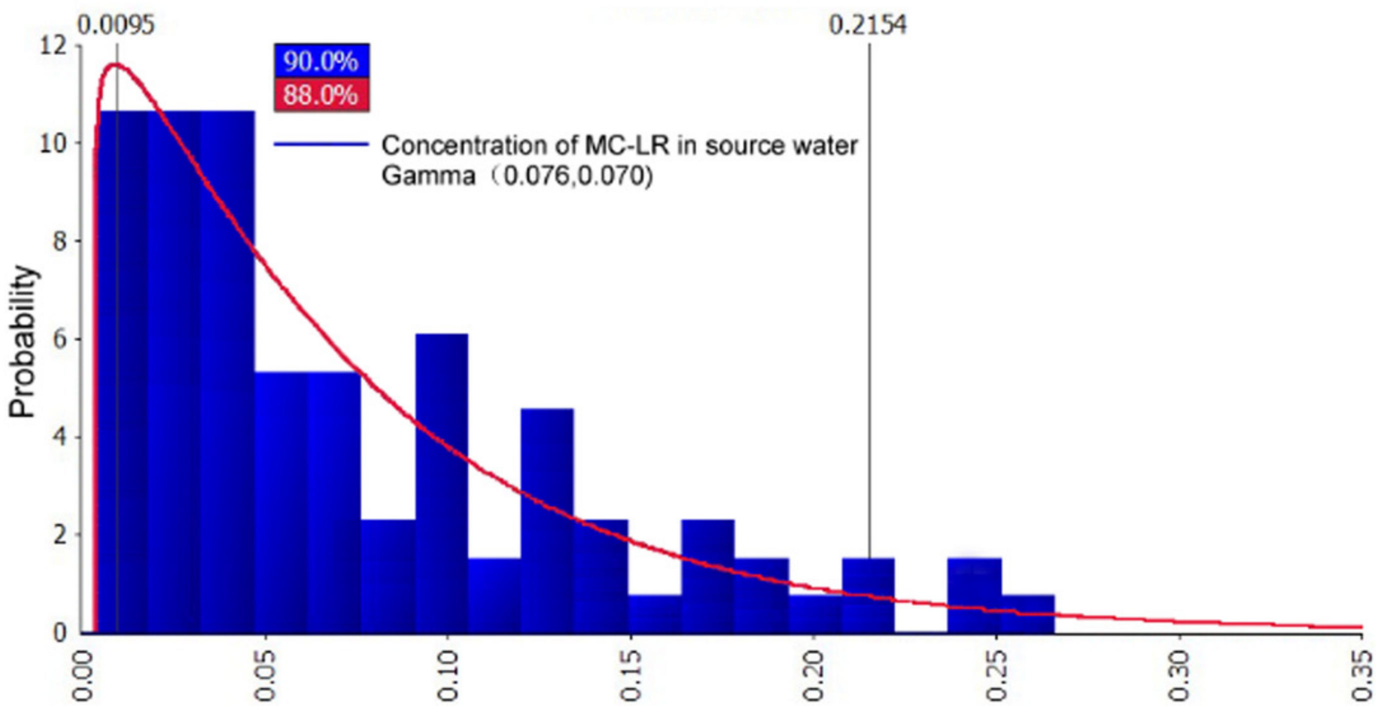

Table 1 (on next page)

Results of analysis of MCs in water samples

Toxin types	Number of samples	EMCs	IMCs	TMCs (EMCs+IMCs) concentration (μg/L)	
		Detection Rate/%	Detection Rate/%	Range	Average
MC-LR	90	77.78	76.67	0.0341–0.2663	0.0763
MC-RR	90	74.44	77.78	0.0224–0.3783	0.0727
MC-YR	90	64.44	56.67	0.0329–0.1433	0.0424

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Table 2(on next page)

Correlation coefficients between MCs and the influencing factors

***Correlation is significant at the 0.05 level (2-tailed).**

****Correlation is significant at the 0.01 level (2-tailed).**

Environmental Factors	Correlation Coefficient			
	MC-RR	MC-YR	MC-LR	TMCs
Water Temperature	0.436**	0.085	0.480**	0.614**
PH	−0.729**	−0.029	−0.164	−0.566**
DO	−0.063	−0.076	−0.768**	−0.570**
TN	0.286**	−0.179	−0.079	0.055
TP	0.043	0.073	0.851**	0.610**
PO_4^{3-}-P	−0.047	−0.071	0.399**	0.204
TN:TP ratio	0.040	−0.229*	−0.434**	−0.347**

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Table 3(on next page)

Results of stepwise multiple linear regression

MCs	Fitting equation	Correlation coefficient R	Adjusted R^2	Tolerance	VIF
TMCs	$y=116.848+46.449\chi3$ (1)	0.581	0.337	1	1
	$y=805.526+42.979\chi3-92.886\chi2$ (2)	0.716	0.513	0.989	1.001
	$y=538.069+35.557\chi3-75.968\chi2+6.452\chi1$ (3)	0.741	0.549	0.693	1.443
MC-RR	$y=799.874-99.263\chi2$ (4)	0.718	0.515	1	1
	$y=676.095-91.169\chi2+3.42\chi1$ (5)	0.759	0.576	0.846	1.181
MC-YR	$y=49.805-1.024\chi7$ (6)	0.246	0.060	1	1
MC-LR	$y=11.216+41.829\chi3$ (7)	0.806	0.650	1	1
	$y=63.349+32.481\chi3-6.653\chi6$ (8)	0.848	0.718	0.678	1.476

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Table 4 (on next page)

Fitting distribution and related parameters of MCs in source water (μg/L)

MCs	Fitting of Distribution	Distributed parameters		Fit test sort			50% Confidence value	90% Confidence value	95% Confidence value
		Mean	Std Dev	K-S	A-D	Chi-Sq			
MC-RR	Gamma	0.073	0.061	1	1	1	0.056	0.154	0.194
	Invgauss	0.073	0.066	5	2	2	0.053	0.154	0.202
MC-YR	Lognorm	0.047	0.071	4	2	1	0.026	0.103	0.154
	Expon	0.042	0.039	2	1	4	0.030	0.093	0.120
	Loglogistic	0.081	-	1	4	6	0.026	0.124	0.216
MC-LR	Gamma	0.076	0.070	1	1	2	0.055	0.168	0.216
	Expon	0.075	0.073	2	2	1	0.053	0.170	0.221

Table 5 (on next page)

Estimated value of the quantile for overall sample in different theoretical distributions

Projects	Pollutant	Real value(μg/L)	Types	Predicted value (μg/L)	Relative difference of real value (%)
P50	MC-RR	0.058	Gamma	0.056	3.01
			Invgauss	0.053	7.81
	MC-YR	0.024	Lognorm	0.026	8.82
			Expon	0.030	25.17
			Loglogistc	0.026	10.03
	MC-LR	0.057	Gamma	0.055	2.02
			Expon	0.053	6.10
P75	MC-RR	0.011	Gamma	0.099	13.00
			Invgauss	0.095	16.91
	MC-YR	0.062	Lognorm	0.053	13.88
			Expon	0.057	8.17
			Loglogistc	0.056	9.41
	MC-LR	0.112	Gamma	0.104	6.79
			Expon	0.104	7.46
P90	MC-RR	0.136	Gamma	0.154	12.93
			Invgauss	0.154	13.09
	MC-YR	0.108	Lognorm	0.103	4.31
			Expon	0.093	14.10

			Loglogistc	0.124	15.12
	MC-LR	0.166	Gamma	0.168	1.09
			Expon	0.170	2.46
P95	MC-RR	0.167	Gamma	0.194	16.26
			Invgauss	0.202	21.06
			Lognorm	0.154	29.08
	MC-YR	0.119	Expon	0.120	0.58
			Loglogistc	0.216	80.78
	MC-LR	0.215	Gamma	0.216	0.26
			Expon	0.221	2.56
P99	MC-RR	0.378	Gamma	0.285	24.71
			Invgauss	0.321	15.02
			Lognorm	0.325	127.11
	MC-YR	0.143	Expon	0.183	27.56
			Loglogistc	0.735	412.77
	MC-LR	0.266	Gamma	0.326	22.62
			Expon	0.338	27.03

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Table 6 (on next page)

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Daily exposure to MC intake through drinking water (μg/kg/d)

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	0.002	0.005	0.001	0.003	0.002	0.005
P50	0.002	0.004	0.001	0.001	0.002	0.004
P85	0.004	0.008	0.003	0.006	0.004	0.009
P90	0.004	0.009	0.003	0.007	0.005	0.010
P95	0.005	0.010	0.003	0.007	0.006	0.013

Table 7 (on next page)

Carcinogenic exposure of MCs from source water

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	$1.27x10^{-5}$	$1.37x10^{-5}$	$3.34x10^{-6}$	$5.76x10^{-6}$	$5.20x10^{-6}$	$8.10x10^{-6}$
P50	$1.40x10^{-5}$	$1.43x10^{-5}$	$2.21x10^{-6}$	$4.40x10^{-6}$	$4.69x10^{-6}$	$8.30x10^{-6}$
P85	$5.43x10^{-5}$	$1.13x10^{-4}$	$6.93x10^{-6}$	$1.09x10^{-5}$	$9.18x10^{-6}$	$1.28x10^{-5}$
P90	$5.47x10^{-5}$	$1.45x10^{-4}$	$7.60x10^{-6}$	$1.16x10^{-5}$	$9.85x10^{-6}$	$1.32x10^{-5}$
P95	$8.63x10^{-5}$	$1.83x10^{-4}$	$8.11x10^{-6}$	$1.20x10^{-5}$	$1.11x10^{-5}$	$1.38x10^{-5}$

Table 8(on next page)

Maximal acceptable level and negligible level recommended by different institutions

Institutions	Maximum risk level	Ignore the level of risk	Remarks
USEPA	$1x10^{-4}$	--	Radiation
ICRP	$5x10^{-5}$	--	Radiation
Royal Association of England	$1x10^{-6}$	$1x10^{-7}$	--
Holland Environmental Protection Agency	$1x10^{-6}$	$1x10^{-8}$	Chemical contaminants
Swedish Environmental Protection Agency	$1x10^{-6}$	--	Chemical contaminants

Table 9 (on next page)

Non-carcinogenic exposure risk of MCs using source water

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	0.005190	0.011352	0.030260	0.066190	0.054490	0.119200
P50	0.004123	0.009019	0.017090	0.037380	0.040400	0.088380
P85	0.009157	0.020031	0.067480	0.147610	0.104530	0.228660
P90	0.009722	0.021267	0.077190	0.168840	0.118790	0.259860
P95	0.011903	0.026038	0.085150	0.186270	0.153860	0.336580

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