

Research Article

Thyroid-Disrupting Activities of Groundwater from a Riverbank Filtration System in Wuchang City, China: Seasonal Distribution and Human Health Risk Assessment

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The recombinant thyroid hormone receptor (TR) gene yeast assay was used to evaluate thyroid disruption caused by groundwater from the riverbank filtration (RBF) system in Wuchang City, China. To investigate seasonal fluctuations, groundwater was collected during three seasons. Although no TR agonistic activity was found, many water samples exhibited TR antagonistic activity. The bioassay-derived amiodarone hydrochloride (AH) equivalents ranged from 2.99 to 274.40 $\mu\text{g/L}$. Water samples collected from the riverbank filtration system during the dry season had higher TR antagonistic activity. All samples presented adverse 3,3',5-triiodo-L-thyronine (T3) equivalent levels, ranging from -2.00 to $-2.12 \mu\text{g/kg}$. Following exposure to water samples with substantial TR antagonist activity, predicted hormonal changes in humans of different gender and age ranged from 0.65 to 1.48 $\mu\text{g/kg}$ of T3, being 47% to 231% of normal. No obvious difference was found between genders or among age groups. Overall, the results revealed that the RBF system could remove the thyroid-disrupting chemicals in the river water to some extent. Considering the varying degrees of risk to human health, further treatment is needed to remove the potential thyroid-disrupting chemicals in pumping water after riverbank filtration to ensure drinking water safety.

1. Introduction

Thyroid hormones (THs) are essential for normal growth, development, and metabolism of organisms; an imbalance in TH levels may have significant consequences for human health [1]. There is accumulating evidence that thyroid-disrupting chemicals (TDCs) disrupt TH homeostasis by interfering with the synthesis, release, transport, metabolism, and clearance of THs [2–6]. There are numerous potential routes of human exposure to TDCs, including contaminated air, water, soil, and diet [7]. TH-disrupting activity has been found in industrial effluents, sediment extracts, water sources, and even in drinking water in China [8–12]. Therefore, TDCs have become a potential threat to human health and aquatic ecosystems in China.

A riverbank filtration (RBF) system refers to a primary water treatment method in which river water filters across the riverbed and transports as the underlying groundwater toward the production wells [13, 14]. The water is decontaminated naturally via chemical, biological, and physical processes, including filtration, sorption, and biodegradation during its subsurface passage [15]. Thus, water from pumping wells is generally cleaner than water collected directly from the river in terms of reduced turbidity, microbial contaminants, natural organic matter, organic trace pollutants, and pharmaceutical residues [14, 16, 17]. The RBF system, as a reliable and cost-efficient approach for groundwater supplies, has been promoted worldwide over the past several decades [18], especially in cases where river water is unsuitable or directly contaminated (e.g., accidental contaminant release).

In an RBF system, the quality of groundwater from the collector well can be significantly affected by the river water because of variation in river discharge and quality [19]. Significant seasonal water quality changes have been documented with saturated riverine groundwater infiltration flow paths [14]. Currently, the criteria for the quality of groundwater derived from an RBF system focus on the redox milieu and related geochemical processes [20]. Knowledge is lacking regarding the toxicological effects of water samples, as well as public health risk (i.e., thyroidal and estrogenic endocrine disruptions).

To date, there is no definitive risk assessment (RA) tool for endocrine-disrupting chemicals (EDCs) [21]. There are challenges to RA in hazard characterization, exposure assessment, dose-response assessment, and risk characterization [22, 23]. More specifically, effective exposure to EDCs is complex to calculate; limitations in epidemiological and toxicological studies restrict reliable data for RAs [21]. Plotan et al. implemented a new RA method for human health by comparing the levels of exposure from samples with the established 17β -estradiol acceptable daily intake (ADI) and estimated daily consumption of estradiol through drinking water and an omnivorous diet [7]. Using this approach, new toxicological findings (i.e., endocrine disruption and mixture toxicity) could be integrated; RA values can be calculated with respect to different receptors by gender and age, which is effective in the assessment of estrogenic chemicals in the environmental medium [7].

Despite the wide RBF application for groundwater supplies in developed countries, China has few RBF systems and mainly relies on surface sources for drinking water [13, 24, 25]. Considering the substantial potential of RBF use in many large cities along major rivers, the Chinese government recently initiated a feasibility study of RBF treatment in several demonstration cities in the framework of improving the safety of the drinking water supply in China. Wuchang City was chosen as one of the pilot sites because of its location adjacent to the Lalin and Mangniu Rivers, which are tributaries of the Songhua River. As such, it satisfied the requirements for RBF system installation. The objectives of this study were to (1) quantify and characterize thyroid-disrupting activity and (2) perform a human health risk assessment for groundwater samples from the pumping wells in an RBF system in Wuchang City, China.

2. Materials and Methods

2.1. Chemicals. 3,3',5-Triiodo-L-thyronine (T3, 95%) and dimethylsulfoxide (DMSO, 99.5%) were purchased from Sigma Chemical (St. Louis, MO, USA). Amiodarone hydrochloride (AH) was purchased from Shanghai Pharmaceutical (Shanghai, China). The stock solutions of all chemicals were prepared in DMSO.

2.2. Sample Collection and Processing. There was significant seasonality in the Lalin and Mangniu Rivers caused by summer (June to September) precipitation, which accounted for approximately 70% of total annual rainfall. In winter

(December to March), the frozen period lasted for almost four months [26, 27].

Based on the seasonal variability in the targeted rivers, seven sampling locations were established for water sample collection during the dry season (October 2014), thawing period (April 2015), and wet season (July 2015). SW1 and SW7, located in the Lalin River and Mangniu River, respectively, represented the water quality of these rivers. GW2-GW6, collected from shallow groundwater, represented the groundwater conditions (Figure 1).

Each water sample (4L) was collected in a precleaned amber glass bottle and stored at 4°C until further analysis. All samples were treated within 24 h. After being filtered through glass fiber filters (0.7 μ m, Millipore, MA, USA), the water sample was extracted via solid-phase extraction using an HLB cartridge (500 mg, Waters Corp., MA, USA) [9]. Then, the resultant extracts were dried under a nitrogen stream and redissolved in 0.2 mL DMSO. Three concentration levels of test solutions were obtained by 2-fold dilution of extracts and the residues were stored at -20°C before performing the bioassay. Milli-Q water with a conductivity of 18.2 Ω was used as procedural blanks.

2.3. The Recombined TR Gene Yeast Assay. The yeast strains transfected by the TR gene were developed in our laboratory. The yeast was produced with a yeast two-hybrid assay system and selected by growth on synthetic dextrose (SD) medium (lacking tryptophan and leucine, SD/-Leu/-Trp) according to the methods previously described [28].

The recombined TR gene yeast assays, including agonistic and antagonistic activity tests, were conducted as described in the previous report [9, 29]. We followed the methods of Kong et al. [29]. T3 and AH were selected as the positive controls for agonistic activity and antagonistic activity against TR, respectively. Each experimental group included the sample, positive control, negative control (DMSO), and procedural blank. All experiments were performed in triplicate, and means of results were used for optimization. The β -galactosidase activity was calculated by equations reported by Gaido et al. [30].

2.4. Cytotoxicity. In order to ensure that the obtained results from bioassays were caused by real agonistic/antagonistic responses rather than cytotoxicity, viability was also measured in cells exposed to water samples at the maximum assay concentration. We followed the methods of Kong et al. [29]. Yeast cells were plated similarly to that in the original assay and exposed for 2 h to the medium in the presence of water samples. The change in cell density (OD_{600}) in the assay medium was used to represent cell viability spectrophotometrically. The results were assumed as noncytotoxic when the ratio ($OD_{600\text{-exposure medium}}/OD_{600\text{-blank medium}}$) ranged from 80% to 120%.

2.5. Data Analysis. The bioassay-derived equivalent concentration was calculated by comparing the agonistic/antagonistic activity of the water samples with the concentration of the standard chemicals [31]. Exposure assessment was based on the equation in the previous report [7]:

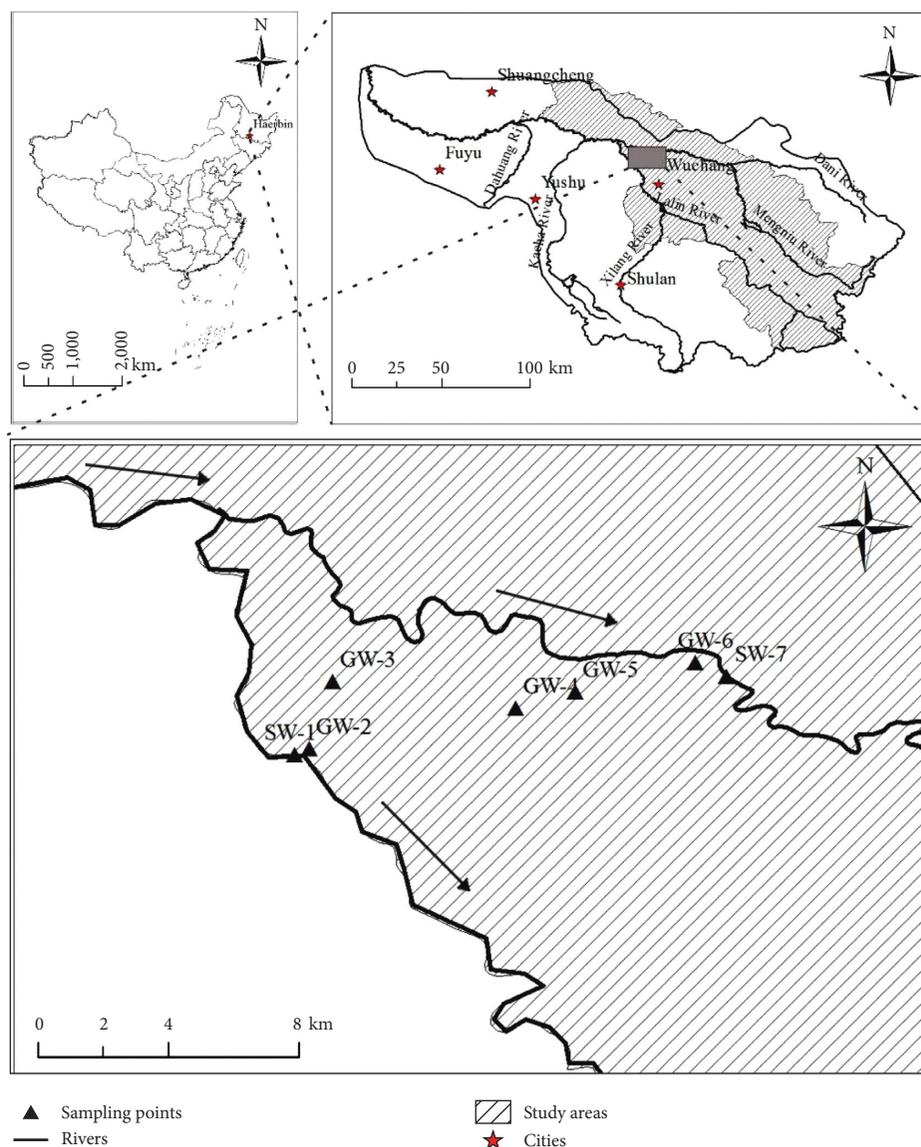


FIGURE 1: Sampling locations in Wuchang City, China.

TR agonist activity (ng/kg body weight, BW/day)

= Sample equivalent T3 concentration (ng/L)

$$\times \text{Dilution factor} \times \frac{\text{Daily dose (L/day)}}{\text{Average body weight, ABW (kg)}} \quad (1)$$

TR antagonistic activity (ng/kg BW/day)

= (Sample equivalent T3 concentration ng/L

– Concentration of added T3 ng/L) (2)

$$\times \text{Dilution factor} \times \frac{\text{Daily dose (L/day)}}{\text{ABW (kg)}}$$

It was assumed that the average adult body weight was 60 kg and the average daily dose of drinking water was 2 L/

day, as suggested by the United States Environmental Protection Agency. The T3 spike concentration was set at 5.00×10^{-6} mol/L, which is equivalent to 3.25 ng/L on the plate and was sufficient to determine potential antagonistic effects. The dilution factor was 0.02 in this study. Statistical analysis was performed using SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) by *t*-test. A *p* value less than 0.05 was reported as significant.

3. Results

3.1. Cell Viability and System Credibility. To examine the β -galactosidase inhibition induced by the interaction of TDCs with TR, we tested the proficiency of the bioassay system as described in Appendix A. These results suggested that the water samples did not suppress the TR gene expression (Appendix A, Figure A1). To account for stimulatory or toxic matrix effects on yeast, we determined cytotoxicity values. The results revealed no significant

changes in cell viability, indicating that noncytotoxicity was found in these samples (Appendix A, Figure A2). The blank samples did not disrupt the TR.

3.2. TR Agonistic/Antagonistic Activity. TR agonistic activity of the water samples was not detected by the yeast assay (Figure 2). All water samples from the targeted area in different seasons exhibited TR antagonist potency (Figure 3). Higher concentrations of TR antagonists were found in river water (SW-1 and SW-7) samples during every sampling season. The TR inhibition rating of SW-7 was 44.32% during the wet season, 31.71% during the thawing period, and 28.54% during the dry season, which was higher than that of other samples. Similar to SW-7, other samples of river water exhibited higher TR antagonist potency. TR antagonistic activities were also found in groundwater samples, ranging from 9.00% to 25.91%, although the maximum inhibition rate of GW-6 during the wet season was 40.41%. The corresponding AH equivalents (AEQ) ranged from 2.99 to 274.40 $\mu\text{g/L}$ AH (Figure 4).

In addition, significant seasonal changes in TR antagonistic activity were found in the collected water samples. In the SW-1, SW-7, and GW-6 samples collected during the wet season, TR antagonist potency values were higher than those in the other two seasons ($p < 0.05$), whereas the GW-2, GW-3, GW-4, and GW-5 samples collected during the dry season had higher TR inhibition rates ($p < 0.05$, Figure 3).

3.3. RA Results. Based on the RA procedure, a risk assessment was performed that compared the levels of exposure in the samples with the suggested sum of T3 and thyroxine (T4) as shown in Table A1 (Appendix A) [32]. The potential effect on human daily exposure to T3 antagonistic activity in various groups, by gender and age, was also determined.

All samples collected from the targeted area exhibited adverse T3 equivalent levels, ranging from -2.00 to $-2.12 \mu\text{g/kg}$ T3 (Figure 5). Exposure to the water samples presented the greatest TR antagonist activity and predicted hormonal changes in various groups ranged from 0.65 to 1.48 $\mu\text{g/kg}$ T3 (Table 1) with the percentage value ranging from 47% to 231% (Table 2). The river water samples from wet season had the highest mean TR antagonist activity, accounting for 61–231% times the normal T3 and T4 levels in humans. Compared with the samples of river water, the water samples after riverbank filtration had lower TR antagonist effects. However, no obvious difference was observed in the TR antagonist effects between genders or among age groups ($p > 0.05$).

4. Discussion

4.1. TR-Disrupting Activities. Although no TR agonist activity was detected, most of the water samples from the targeted area showed TR antagonistic activity in a concentration-dependent manner, which was similar to the results obtained in other studies [9, 12, 33, 34]. Thus, TR antagonist activities in the water samples were notable and more common than TR agonist activities [12]. The AH

equivalent of water samples ranged from 2.99 to 274.40 $\mu\text{g/L}$, which was comparable with the results of the previous analysis of TR antagonist activity using recombinant yeast assays in aquatic environments in China (Table 3).

Higher TR inhibiting activities were found in the river water (SW-1 and SW-7). Some compounds have been reported to be strong TR antagonists, such as phthalate esters and phenols [35], which have been detected in rivers and drinking water supplies [11, 36]. These might be important sources of thyroid-disrupting activities in the targeted river. Previous studies demonstrated that di-n-butyl phthalate (DBP) was the predominant contributor to thyroid antagonist activity in drinking water in China [9, 11, 37]. DBP has also been detected in the Songhuajiang River with concentrations ranging from 1.69 to 11.80 $\mu\text{g/L}$ [38], which is higher than the lowest observed effective concentration.

Water quality monitoring in riverbank filtration systems has concentrated on common chemical indices, and relevant toxicological data are limited. As far as we know it was the first time to investigate the thyroid-disrupting activities of groundwater after riverbank filtration in China. The results showed that water samples from pumping wells exhibited lower TR antagonist activity than those from river, which indicated that riverbank filtration removes potential TR antagonists to some extent. Many studies have demonstrated that riverbank filtration technology was efficient in reducing turbidity, microcystins, inorganic chemical constituents, and synthetic organic compounds [16, 19], some of which have been identified as potential TDCs [35]. For example, it has been reported that the nitrate and turbidity concentrations were lower in the riverbank-filtered water than in the river water at a riverbank filtration site in the Daesan-Myeon area in the Republic of Korea [19].

Furthermore, the TR antagonist activities of groundwater exhibited a positive correlation with the distance from the pumping wells to the river. The further from the river, the lower the TR antagonist activity, which is in agreement with findings from the previous study on riverbank filtered water quality [39]. The distance between the pumping well and the river affects the travel time in the riverbank filtration system, with prolonged travel times for farther distances and shortened travel times for nearby wells. Previous research demonstrated the positive effect of increased travel time on contaminant removal [13, 40]. For instance, study regarding flooding impacts on RBF systems showed that increased abstraction rates and a high transmissivity aquifer facilitate rapid water quality recoveries [13]. The above results revealed that the RBF system could remove TDCs in the river water to some extent and their removal efficiency was positively related to the distance from the river. This study could serve as a reference for addressing the optimal positions of riverbank filtration wells before initiating field studies.

4.2. Seasonal Variation. The TR antagonistic activity of the water samples showed obvious seasonal variation. Factors, such as precipitation and temperature, varied among seasons and could impact the quantity and quality of river

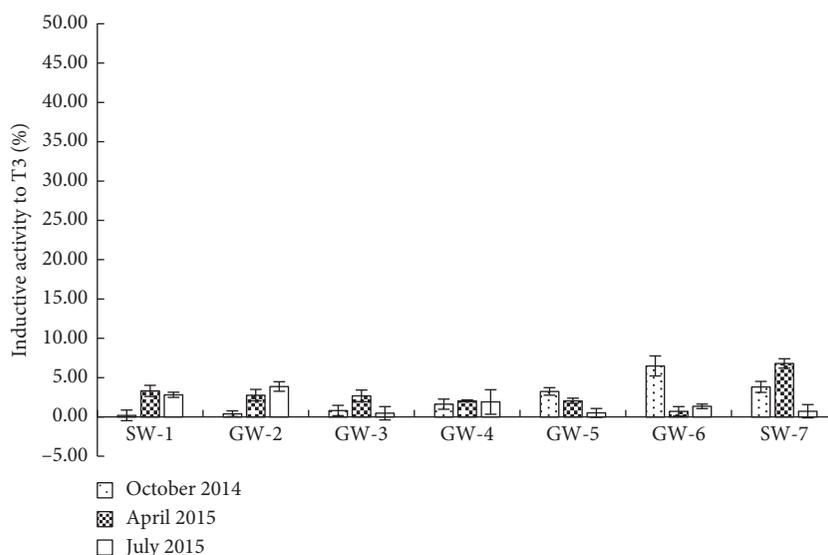


FIGURE 2: Induction of β -galactosidase activity by water samples using recombinant TR gene yeast assay.

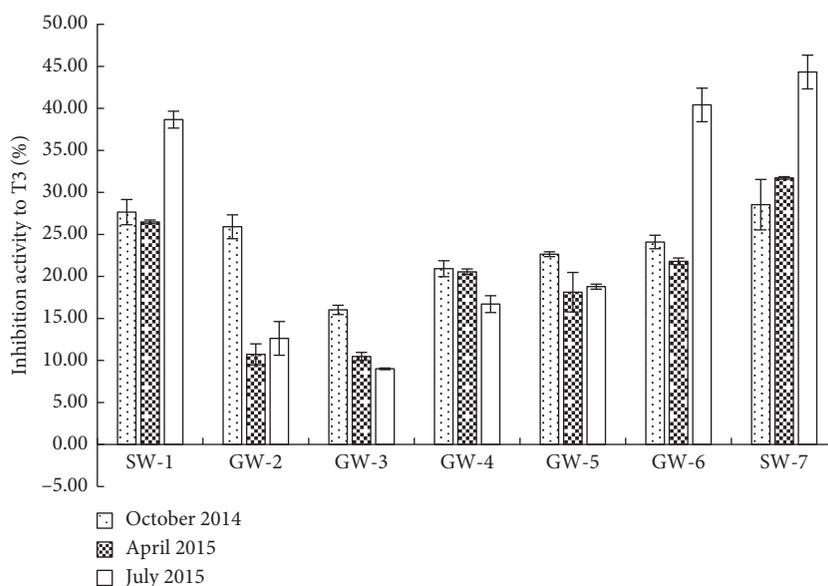


FIGURE 3: Inhibition of β -galactosidase activity by water samples using recombinant TR gene yeast assay.

water and corresponding groundwater by influencing the flow convergence process, contaminant inputs into water bodies, and redox conditions during underground passage [41].

The RBF samples GW-2, GW-3, GW-4, and GW-5, collected in the dry season, had higher TR antagonistic activity, which corroborated the scenario developed by Sprenger et al. [40]. The sensitive climatic factors influenced contaminant removal by modifying the redox conditions and travel time during underground passage. Droughts promoted anaerobic conditions during RBF passage and caused the breakthrough of pathogens, metals, suspended solids, dissolved oxygen content, and organic micro-pollutants [40]. Some EDCs were thought to experience

significant degradation under aerobic conditions [42]. Bisphenol A, atypical TDC, was found to degrade effectively under aerobic conditions during RBF passage. Our findings regarding oxidation-reduction potential of water samples tested in situ (data not shown), in addition to flow data from the literature [43, 44], support the hypothesis that under drought conditions, TDC concentration is expected to increase in the pumping water because of decreased removal efficiency under anoxic conditions [40].

In addition, high TR antagonist activity was also found in water samples collected during the thawing period. Therefore, it is necessary to investigate water samples during this time, especially for rivers with a long thawing period.

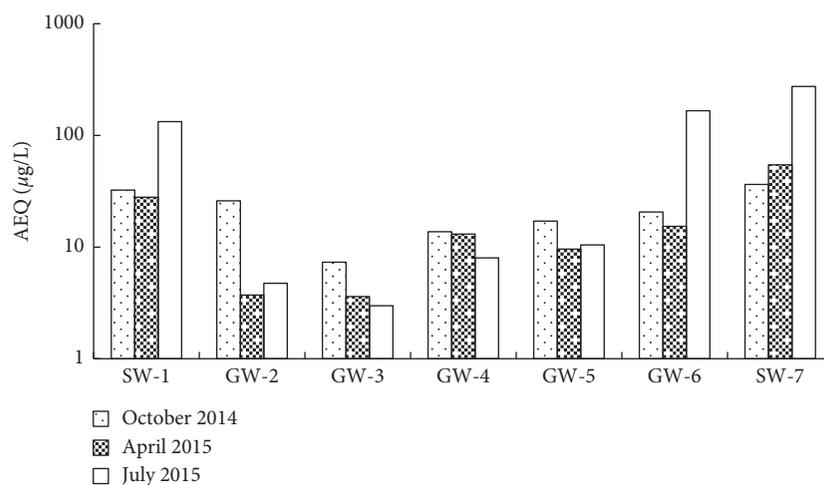


FIGURE 4: Thyroid-disrupting potency of water samples collected from the riverbank filtration system in Wuchang City, China. AEQ: amiodarone hydrochloride equivalent level.

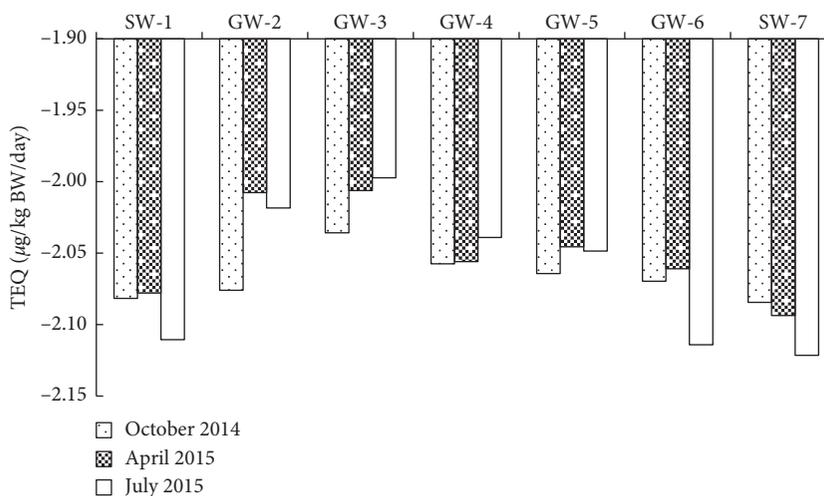


FIGURE 5: Thyroid-disrupting potency of water samples collected from the riverbank filtration system in Wuchang City, China. TEQ: T3 equivalent activity, BW: body weight.

TABLE 1: Predicted hormonal changes in various groups caused by exposure to the mean level of detected thyroidal antagonist activity in river water and groundwater samples ($\mu\text{g}/\text{kg}$).

		Male			Female			
		20–44 years	45–59 years	60–90 years	20–44 years	45–59 years	60–90 years	
River water	October 2014	Min	-0.75	-0.76	-0.74	-0.74	-0.77	-0.70
		Max	-1.40	-1.42	-1.42	-1.37	-1.44	-1.35
	April 2015	Min	-0.75	-0.76	-0.74	-0.75	-0.77	-0.70
		Max	-1.40	-1.42	-1.42	-1.37	-1.45	-1.35
	July 2015	Min	-0.78	-0.79	-0.77	-0.78	-0.80	-0.73
		Max	-1.43	-1.45	-1.45	-1.40	-1.48	-1.38
Groundwater	October 2014	Min	-0.73	-0.74	-0.72	-0.72	-0.75	-0.68
		Max	-1.38	-1.40	-1.39	-1.35	-1.42	-1.33
	April 2015	Min	-0.70	-0.71	-0.69	-0.70	-0.72	-0.65
		Max	-1.35	-1.37	-1.37	-1.32	-1.40	-1.30
	July 2015	Min	-0.71	-0.72	-0.70	-0.71	-0.73	-0.66
		Max	-1.36	-1.38	-1.38	-1.33	-1.41	-1.31

TABLE 2: Percentage of predicted effects on T3 levels through exposure to the mean level of detected thyroidal antagonist activity in the river water and groundwater samples.

Percentage	Antagonistic activity predicted effect (%)					
	River water			Groundwater		
	October 2014	April 2015	July 2015	October 2014	April 2015	July 2015
<i>Male</i>						
20–44 years	56–204	56–204	58–209	54–200	52–197	53–198
45–59 years	57–213	57–214	60–218	56–210	54–206	54–207
60–90 years	55–213	55–213	58–218	54–209	52–206	52–207
<i>Female</i>						
20–44 years	56–192	56–192	58–197	54–189	52–185	53–186
45–59 years	59–226	59–227	61–231	57–223	55–219	56–220
60–90 years	51–184	51–184	53–189	49–181	47–178	48–179

TABLE 3: Analysis of TR antagonist activities using recombinant yeast assay in an aquifer in China.

	AEQ ($\mu\text{g/L}$)	Reference
This study	2.99~274.40	
Datansha WWTP	ND~170.00	[8]
Gaobeidian WWTP	16.20~425.00	[10]
Beixiaohe WWTP	3.29~175.00	[10]
Guanting Reservoir	21.00~2150.00	[12]
Yangtze River	123.60~1332.00 ²	[34]
Yangtze River	166.08~684.00 ²	[33]

²The TR antagonist activities were described as DBP equivalent level in the original article. The calculation from DBP equivalent level to AH equivalent level was described in Section 3 of Supporting Information. AEQ: amiodarone hydrochloride (AH) equivalent level.

4.3. RA Based on the Toxicological Data. During traditional RA, concentrations of the identified contaminants from chemical analysis were used to characterize the potential risk, which does not directly reflect the real toxicological effects of these chemicals on human health [21–23, 45]. In this study, the RA was performed based on toxicological data rather than concentrations of individual chemicals, which provided a new methodology to calculate the risks of environmental mixtures. Results indicated that this novel RA method was effective for the quantification and characterization of TR antagonists in water samples and these data could provide useful information on drinking water safety [7]. Moreover, it should be pointed out that TR antagonist potency was found in the water samples from the river and pumping wells in the targeted area, which posed varying degrees of risk to human health, based on gender and age class. Because riverbank filtration wells are often the first step in a multibarrier concept to provide drinking water, further treatment is needed to remove the potential TDCs to ensure drinking water safety [40, 46].

There are some uncertainties in the RA approach. Firstly, some compounds could cause thyroid hormone disruption through different modes of action [4]; however, in this study, only the disrupting effect caused by TR activation was evaluated by the *in vitro* bioassay. As a result, the real biological effect in the environmental medium might be underestimated. Moreover, there were significant differences between the *in vitro* and *in vivo* potency of individual TDCs because of metabolic stability. Thus, their *in vitro* thyroid-

disrupting activities might not necessarily correspond to adverse *in vivo* effects. In addition, physiological data regarding thyroid hormones are limited. In this study, the recommended T3 and T4 concentrations reported by Ma et al. [32] were used to compare with the possible intake of TDCs from the water samples through the direct drinking pathway. Because of the above limitations, it should be noted that the main purpose of our study was not to derive strict guideline values, but to better understand the possible risk to human health from the groundwater in these regions.

5. Conclusions

This study revealed the presence of thyroid-disrupting activities in the groundwater from riverbank filtration systems, as well as in the river water by a recombinant TR gene yeast assay, and presents their hypothetical impact on thyroid hormones in humans using a novel risk assessment approach. The main conclusions include (1) the RBF system removed the TDCs from the river water to some extent and their removal efficiency was positively related to the distance from the river; (2) the RBF samples collected during the dry season had higher TR antagonistic activity because of decreased removal efficiency under anoxic conditions; (3) the novel RA approach, comparing the T3 equivalent levels with the normal T3 and T4 levels in humans, could be promoted as an effective method to assess the EDCs in the environmental matrix. These findings are highly relevant to environmental safety and human health. It warrants further research on drinking water use from riverbank filtration systems.

Data Availability

The data used to support the findings of this study are included within the article as well as the supplementary information files.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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Supplementary Materials

A1: inhibition activity of β -galactosidase by organic extracts of water samples. A2: cytotoxicity of the organic extracts of water samples. A3: the levels of T3 and T4 in different age and gender groups. (*Supplementary Materials*)

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