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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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Supplementary Materials

Appendix A

A1 Inhibition activity of *β*-galactosidase by organic extracts of water samples

To identify whether the water samples affect *β*-galactosidase activity, *β*-galactosidase and o-Nitrophenol-*β*-galactopyranoside (ONPG) were first combined, and showed a significant yellow color. The mixture was diluted until its *β*-galactosidase activity (u) was nearly the same to the *β*-galactosidase acivity (u) induced by T3 in the TR yeast bioassay. Then the organic extracts of soil samples were incubated with the mixture (*β*-galactosidase + ONPG) (1/200, v/v). Comparing with the blank (*β*-galactosidase + ONPG), it showed no inhibition activity (Fig. A1). Thus the water samples do not affect *β*-galactosidase activity.

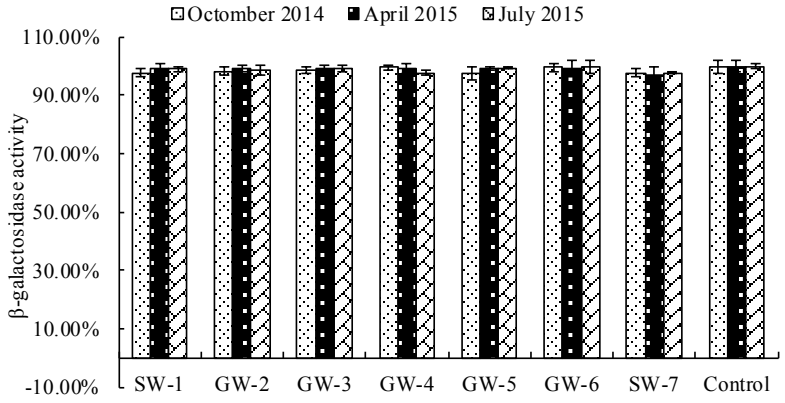

Figure A1 Inhibition of *β*–galactosidase activity by organic extracts of water samples. Data are representative of three independent experiments with three replicates.

A2 Cytotoxicity of the organic extracts of water samples

To ensure that increased/reduced activities in the bioassay were caused by true agonistic/ antagonistic responses and not by cytotoxicity, viability was measured in yeast cells exposed to water samples without T3 (TR agonist activity) and in the presence of T3 (TR antagonistic activity). Yeast cells were plated, and then exposed for 2 h to water samples. And cell viability was determined spectrophotometrically as a change of cell density (OD_{600}) in the assay medium. The cytotoxicity of the samples is represented as the percent inhibition activity (1-$OD_{600\text{-exposure medium}}$/ $OD_{600\text{-blank medium}}$). The results showed that there was no significant change in yeast cell viability (Fig. A2).

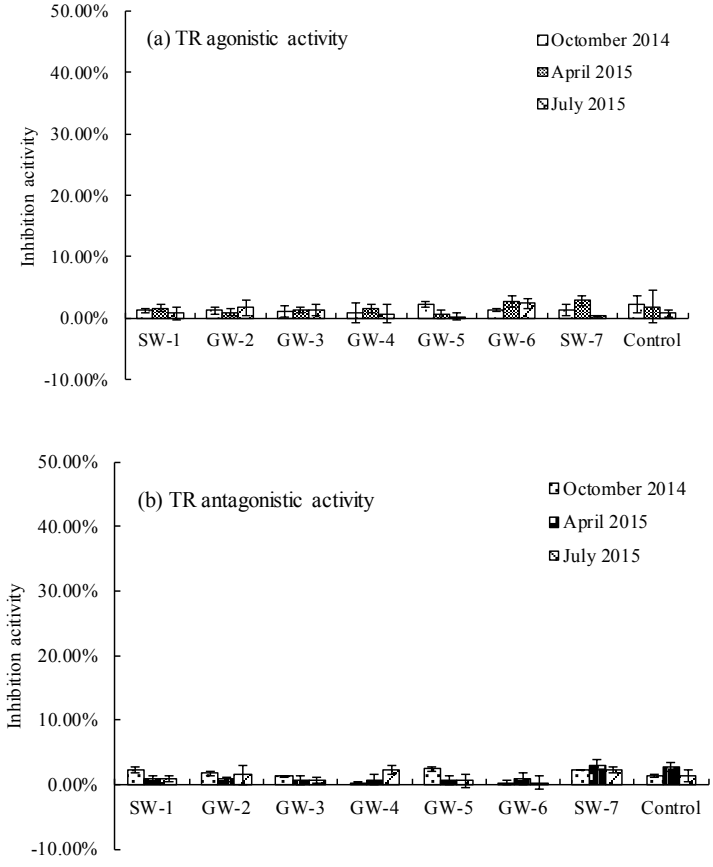

Figure A2 Cytotoxicity of the water samples. The cytotoxicity of the samples is represented as the percent inhibition activity. Data are representative of three independent experiments with three replicates.

A3 The levels of T3 and T4 in different age and gender groups

The levels of T3 and T4 in different age and gender groups were shown in Table A1 [32] and a risk assessment was performed in this study, to compare the levels of exposure in the samples with the suggested sum of T3 and thyroxine (T4) in Table A1.

Table A1 T3 and T4 in different age and gender groups (nmol/L)[32]

	T3		T4		T4 T3 equivalent level[3]	
	minimum	maximum	minimum	maximum	minimum	maximum
Male						
20-44 years	1.41	2.60	74.07	144.69	30.18	58.95
45-59 years	1.34	2.57	71.93	143.50	29.30	58.46
60-90 years	1.38	2.56	69.34	145.46	28.25	59.26
Female						
20-44 years	1.35	2.42	77.37	145.44	31.52	59.25
45-59 years	1.38	2.34	68.82	142.70	28.04	58.14
60-90 years	1.33	2.44	79.68	150.24	32.46	61.21

[3] The calculation from T4 to T3 equivalent level was described as follow.

The recombinant TR gene yeast assay revealed that T3 and T4 acted as TR agonists in concentration-dependent manners with the median effective concentration values of T3 and T4 being 1.10×10^{-7} and 2.70×10^{-7} mol/L, respectively [28]. Based on the above results, the T3 equivalent level of T4 was calculated by the equation as below.

$$\text{T3 equivalent level} = C_{T3\text{-median}}/C_{T4\text{-meidan}} \times C_{T4} \quad \text{(A1)}$$

$C_{T3\text{-median}}$ means the median effective concentration values of T3; $C_{T4\text{-median}}$ means the median effective concentration values of T4; C_{T4} means the concentration values of T4.

The recombinant TR gene yeast assay revealed that amiodarone hydrochloride (AH) and dibutyl phthalate (DBP) acted as TR anti-agonists in concentration-dependent manners with RIC 20 (concentration reducing 20% of the maximum effect) values of AH and DBP being 2.40×10^{-7} and 1.00×10^{-7} mol/L, respectively [9,28]. Above all, AH equivalent level of DBP was calculated by the equation as below.

$$\text{AH equivalent level} = C_{AH\text{-}RIC20}/C_{DBP\text{-}RIC20} \times C_{DBP} \quad \text{(A2)}$$

$C_{AH\text{-}RIC20}$ means the RIC20 values of AH; $C_{DBP\text{-}RIC20}$ means the RIC20 values of DBP; C_{DBP} means the concentration values of DBP.