Dibutyl phthalate contributes to the thyroid receptor antagonistic activity in the drinking water processes

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Chemical Analysis.

Analysis of organicchlorine pesticides (OCPs). An internal standard of PCNB (pentachloronitrobenzene, Supelco Co., USA) was added to all of the samples before analysis. Analysis of OCPs was performed with an Agilent 6890 series GC equipped with a 63Ni electron capture detector (Agilent, Palo Alto, CA). A 30 m × 0.25 mm i.d. × 0.25 um HP-5 column (J&W, Folsom, CA) was used for pesticide identification and quantification. High purity of nitrogen was used as carrier gas at 2.0 mL/min under the constant flow mode. Splitless injection of a 1-μL sample was performed with a 1.8-min solvent delay time. The temperature programmed was used in the oven. The temperature programmed was used in the oven, started at 85 °C holding for 2 min, then to 180 °C at a rate of 10 °C /min, holding for 15 min, and further to 280 °C at a rate of 20 °C /min holding for 5 min. Injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. OCPs were identified by comparing the GC peak retention times with those of authentic reference standards and quantified by peak area using an internal standard method.

Analysis of phenols and phthalates. Phenols and phthalates were first derivatived by BSTFA + TMCA (99:1, v/v) which was obtained from Supelco (Supelco Park, PA). Internal standard, deuterated pyrene or deuterated bisphenol A, was added respectively. Derivatives of phenols and phthalates were dryness under a low nitrogen flow. Then capped and heated in an air bath at 65°C for 30 min. After cooling, the products of derivative were analyzed directly by GC-MS employing the SCAN mode. Deuterated bisphenol A (> 99%) and deuterated pyrene (> 99%) were purchased from Aldrich Chemistry Corporation (Milwaukee, WI).

Analysis of phenols was performed with an Agilent 6890/MSD5973. The capillary columns used were HP-5 column (J&W, Folsom, CA) (30 m × 0.25 mm i.d. × 0.25 um film thickness). High purity of hellum was used as carrier gas at 1.0 mL/min under the constant flow mode. Splitless injection of a 1-μL sample was performed with a 6-min solvent delay time. The temperature programmed was used in the oven, started at 100 °C then to 180 °C at a rate of 25 °C /min, holding for 15 min, and further to 300 °C at a rate of 20 °C /min holding for 5 min. Injector and detector temperatures were maintained at 280 °C and 300 °C, respectively. All analysis of data and control of GC and MS parameters were performed by the MSD Productivity ChemStation for GC and GC/MSD Systems. Phenols were quantified using an internal standard method.

Analysis of phthalates was performed with an Agilent 6890/MSD5973. The capillary column was HP-5 column (J&W, Folsom, CA) (60 m × 0.25 mm i.d. × 0.25 um film thickness). Details of the pesticides, phenols and phthalates analysis were described in the support information. High purity of hellum was used as carrier gas at 1.0 mL/min under the constant flow mode. Splitless injection of a 1-μL sample was performed with a 6-min solvent delay time. The temperature programmed was used in the oven, started at 100 °C then to 180 °C at a rate of 25 °C /min, holding for 15 min, and further to 300 °C at a rate of 20 °C /min holding for 5 min. Injector and detector temperatures were maintained at 280 °C and 300 °C, respectively. All analysis of data and control of GC and MS parameters were performed by the MSD Productivity

ChemStation for GC and GC/MSD Systems. Phthalates were quantified using an internal standard method.

Quality Control. The contamination of phthalates may occur in any step of the analytical procedure and in the bioassay procedure, resulting in overestimated results. Therefore all equipments were rinsed with chromic acid solution and methanol before use until no significant detection could be found in blank. Concentrations of the analysis were not adjusted by recoveries. The LODs listed in the Table 1 were in the condition of 10000 folds concentration of sample. In the procedure blank, none of the OCPs, phenols or phthalates was detected.

Concentration-dependent relationship of T_3 and amiodarone hydrochloride of thyroid receptor (TR) in the TR yeast assay

The concentration-dependent relationship of T3 and amiodarone hydrochloride of thyroid receptor (TR) in the TR yeast assay in our previous work by Li et al.[1] were shown in Figure S1.

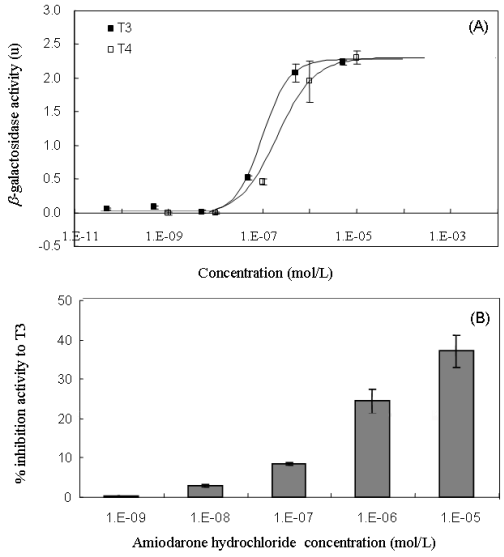

Figure S1. (A) Induction of T3-sensitive β-galactosidase activity by T_3 in yeast strain hTR-GRIP1. (B) Inhibition of T_3-sensitive β-galactosidase activity by amiodarone hydrochloride in yeast strain hTR-GRIP1. T3: 3,3',5-triiodo-L-thyronine.

TR disrupting chemicals

Most study of the TR disrupting chemicals were focus on PCBs, PBBs, PBDEs, TBBPA , TCBPA, phenols, phthalates and organicchlorine pesticides [2-7]. The concentration-dependent relationships of the TR disrupting chemicals detected in our previous work by Li et al.[1] were shown in Figure S2. The REC_{20} and RIC_{20} of the TR disrupting chemicals were shown in Table S1. The chemicals such as PCBs, PBBs, PBDEs, TBBPA and TCBPA may also contribute to the TR agonistic or TR antagonistic activities. However, they have very low water solubility, high n-octanol-water partition coefficients and there were few reports of their existence in the drinking water to our knowledge. We cannot detect these chemicals or they are present in concentrations lower than our detection limits. Therefore no discussion will be given to these groups of chemicals.

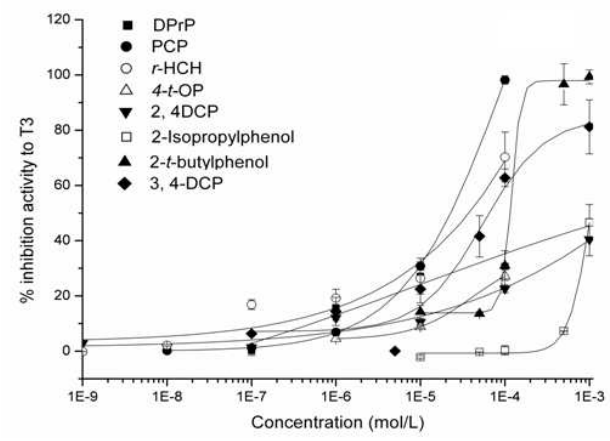

Figure S2. Typical examples of concentration-dependent relationships determined by the yeast strain hTR-GRIP1 for TR agonistic activity. The chemicas' name were shown in Table S1.

Table S1. TR agonistic and antagonistic potency of the tested chemicals in the yeast strain hTR-GRIP1 assay.[a]

	compounds	TR agonistic REC_{20} (mol/L)	TR antagonistic RIC_{20} (mol/L)
Phenols	4-AP	-	>1.0E-04
	2-*t*-butylphenol	2.9E-05	6.2E-05
	2,4-DCP	-	2.0E-05
	3,4-DCP	-	1.6E-06
	4-*t*-OP	-	3.7E-05
	PCP	-	1.1E-06
	4-Phenylphenol	-	>1.0E-05
	2-Isopropylphenol	2.0E-04	3.7E-04
Bisphenol A derivatives	BPA	-	-
	TBBPA	-	7.0E-07
	TCBPA	-	8.6E-07

phthalates	DPrP	-	4.5E-06
pesticides	*p , p′*-DDE	-	-
	p , p′-DDT	-	-
	HCB	-	-
	HCH	-	2.0E-07
Mixtures		REC_{20}(g/L)	RIC_{20}(g/L)
	PCB Mix	-	>5.0E-04
	PBB Mix	-	>5.0E-04
	BDE Mix	-	5.0E-06

[a] n≥3; - : no response

4-AP: 4-aminophenol, BPA: bisphenol A, 2,4-DCP: 2,4-dichlorophenol, 3,4-DCP: 3,4-dichlorophenol, p,p'-DDE: p,p'-dichlorodiphenylethane, p,p'-DDT: 1-chlor-2-(2,2,2-trichlor-1-(4-chlorphenyl)ethyl)benzol, DPrP: dipropyl phthalate, r-HCH: r-hexachlorocyclohexane, HCB: hexachlorobenzene, 4-t-OP: 4-tert-octylphenol, PBBs: polybrominated biphenyls, PBDEs: polybrominated diphenylethers, PCBs: polychlorinated biphenyls, PCP: pentachlorophenol, RIC20: 20% relative inhibitory concentration, REC20: 20% relative induction concentration.

Regression of TEQ_{DBP} and TEQbio

The regression of TEQ_{DBP} (calculated TEQ according to concentrations of DBP) of and TEQbio (calculated TEQ according to TR yeast two-hybrid assay) with a

correlation coefficient of $r = 0.84$ ($p < 0.05$) was shown in Figure S3. It showed that DBP may play the major role in the TR antagonistic activity in drinking water

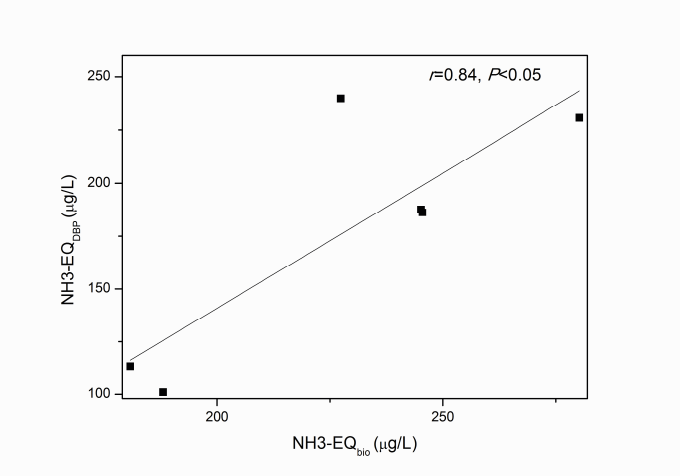

FIGURE S3. Regression of TEQDBP (calculated TEQ according to the concentrations of DBP) and TEQbio (calculated TEQ according to TR yeast two-hybrid assay). DBP: dibutyl phthalate.

Specificity to the TR of the drinking water extracts

It showed that the inhibition activity of drinking water extracts (7.8 fold) increased with decreasing doses of T_3 in Figure S4.

To identify whether the water extracts 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 acivity (u) was nearly the same to the β-galactosidase acivity (u) induced by T_3 (5×10^{-7} mol/L) in the TR yeast assay (Figure S1A). Then the drinking

water extracts (125, 31.2. 7.8 concentration fold of the source water) were incubated with the mixture (β-galactosidase + ONPG) (1/10, v/v). Comparing with the blank (β-galactosidase + ONPG), it showed no inhibition activity (Figure S5). Thus the extracts do not affect β-galactosidase activity. β-galactosidase was purchased from Wako Pure Chemical Idustries, Ltd. (Code No. 072-04141, 10,000 units). O-Nitrophenol-β-galactopyranoside (ONPG, 99%) was purchased from Sigma Chemical (St. Louis, MO, USA).

In our present work, Li et al.[8] have developed a novel screening methods for chemicals with ERRγ disrupting properties using a yeast two-hybrid system. ERRγ shows extremely high constitutive activity without adding ligands. It means that two-hybrid ERRγ yeast can express β-galactosidase by itself. If the water extracts suppress gene expression in the TR yeast assay, it may also suppress the gene expression in the ERRγ yeast assay. However, the drinking water extracts which were tested by ERRγ two-hybrid yeast inhibited no more than 20% of the β-galactosidase activity expressed by the two-hybrid ERRγ yeast itself in the highest concentration (125 fold concentration of the raw water) (Figure S6), which is far lower than the inhibition activity in the two-hybrid TR yeast assay of the drinking water extracts (data not printed). What is more, chemicals like bisphenol A, diethylstilbestrol, 4-nonylphenol and phytoestrogens having ERRγ disrupting activity are detected in many environment samples even in the drinking water, the inhibition activity in the two-hybrid ERRγ yeast assay may caused by the environment chemicals that bind to ERRγ.

Thus, from the three evidences mentioned above, we proposed that inhibition activities of the drinking water extracts were not general suppressive effects on gene expression in the TR yeast assay.

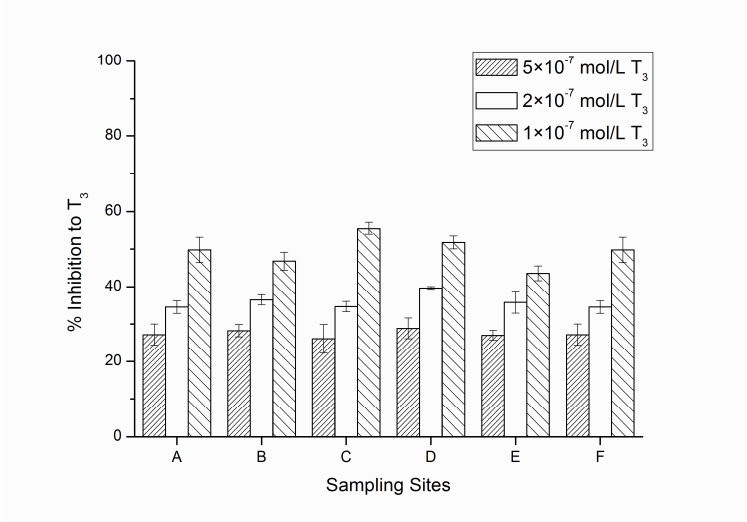

FIGURE S4. Concentration-dependent relationship of thyroid receptor (TR) antagonistic activities of water extracts determined by the TR yeast bioassay. The antagonistic activity of the sample (7.8 fold of the original water) is represented as the percent inhibition activity relative to three concentrations induced by induced by 3,3',5-triiodo-L-thyronine (T3, 5×10^{-7} mol/L, 2×10^{-7} mol/L, 1×10^{-7} mol/L).Values are presented as the average ± standard error (n=3). A: source water, B: effluent of pre-chlorination, C: effluent of coagulation, D: effluent of coal and sand filtration, E: effluent of activated carbon, F: finished water after secondary chlorination.

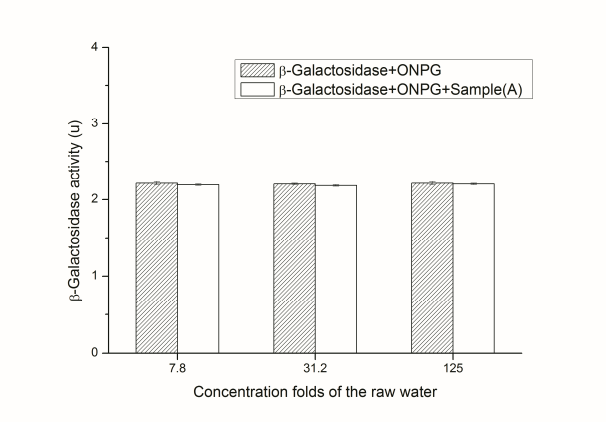

FIGURE S5. Inhibition of β–galactosidase activity by the water sample A .Values are presented as the average ± standard error (n=3). A: source water. ONPG: O-Nitrophenol-β-galactopyranoside.

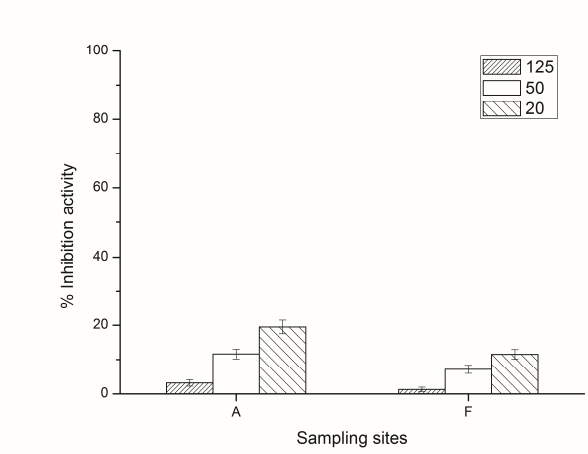

FIGURE S6. ERRγ antagonistic activity of the sample in yeast strain ERRγ-GRIP1. Values are presented as the average ± standard error (n=3). A: source water, F: finished water after secondary chlorination.

Literature Cited

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