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Evaluation and characterization of thyroid-disrupting activities in soil samples along the Second Songhua River, China

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ABSTRACT

In this study, a recombinant thyroid receptor (TR) gene yeast assay combined with Monte Carlo simulation were used to evaluate and characterize soil samples collected from Jilin (China) along the Second Songhua River, for their ant/agonist effect on TR. No TR agonistic activity was found in soils, but many soil samples exhibited TR antagonistic activities, and the bioassay-derived amiodarone hydrochloride equivalents, which was calculated based on Monte Carlo simulation, ranged from not detected (N.D.) to 35.5 μg/g. Hydrophilic substance fractions were determined to be the contributors to TR antagonistic activity in these soil samples. Our results indicate that the novel calculation method is effective for the quantification and characterization of TR antagonists in soil samples, and these data could provide useful information for future management and remediation efforts for contaminated soils.

1. Introduction

The thyroid hormones (THs) are involved in several important physiological processes, including energy metabolism, tissue differentiation and organ growth (Murk et al., 2013). Alterations of circulating TH levels are well-documented consequences of exposure to thyroid disrupting chemicals (TDCs) (Kirkegaard et al., 2011; Levy-Bimbot et al., 2012). A large number of environmental chemicals and stressors are known to disrupt the function of TH system, including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), dioxins, polybrominated flame retardants, phenols, perfluorinated chemicals, phthalates and perchlorate (Boas et al., 2012). These chemicals can interfere with the synthesis, release, transport through blood, metabolism, and clearance of THs, and also thyroid receptors (TRs) and their transcriptional activity by acting as TR agonists or antagonists (Buha et al., 2013; Skipor et al., 2012).

Research on thyroid disruption has been dominated by studies on the aquatic environment, for example, TDCs in samples collected from drinking water, waste water, and surface water have been reported (Li et al., 2014a; Northcott and Jones, 2000; Shi et al., 2016). Kannan et al. (2003) found that the soil extracts elicited estrogenic and androgenic activities in cotton field soils from Georgia and South Carolina, USA. These years, accumulating evidences have documented that a variety of chemical substances, detected in soil, such as PCBs, OCPs, and phenols, have been proved to show the thyroid-disrupting activities (Han et al., 2013; Xu et al., 2015). Once contaminated, the physical and chemical properties of the soil would change, which might lead to soil hardening, the decrease of soil fertility, and detrimental impact on the growth of plants, soil animals and micro-organism (Floch et al., 2011; Robson et al., 2013). Moreover, some TDCs in soil exhibit persistence, bioaccumulation in the tissues of organisms, and biomagnifications through the food chain (Mnif et al., 2011). Further evidences showed that inhalation and contact with TDCs in soil pose a risk to human and wildlife (Burns et al., 2013). However, the data about the thyroid-disrupting activities from soil samples is limited or even unavailable.

Assessing the risks of thyroid disruption in soil will need to improve the detection throughput of TDCs in the environment. The uncertainties of thyroid disrupting potentials varied from the antagonistic or synergistic effects, the possibilities of additives, the difference of TDCs' mechanism(s)-of-action and also the ways of TDCs' results interpretation and extrapolation (Crofton, 2008). Monte Carlo simulation, to calculate the risks of measured TH agonists and antagonists, have been proved to be an effective method to assess the endocrine-disrupting potency of mixtures (Shi et al., 2013; Li et al., 2015).

Data just on the extractable or total concentration of the single chemical may be in some measure inadequate when assessing

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environmental significance. There are also other factors, such as its extractability, sequestration by environmental solids, speciation or 'form', and bioavailability, accounting for the risks (Northcott and Jones, 2000). To guarantee the efficiency of potential TDCs extraction, the mixture of organic extracts from water samples was further classified and separated by polarity, and then the bioassays are conducted to detect their thyroid-disrupting activities, respectively (Ma et al., 2007; Wu et al., 2009; Li et al., 2015). After that, the profiles and identifications of the potential TDCs were conducted by the bioassay-directed fractionation approach.

Jilin City, located along the Second Songhua River (SSR) - the largest tributary of the Songhua River, is one of the most important agricultural and chemical industry centers in northeast China (Li et al., 2015). Literatures indicated that some persistent organic contaminants, such as PCBs and OCPs, entered the soil environment in the SSR basin during the industrial and/or agricultural processes, and some of these compounds may have thyroid-disrupting potential (Xing et al., 2005; Wang et al., 2012). However, information on the thyroid-disrupting activities of soils in Jilin City is limited. The recombinant gene yeast assays have been demonstrated to be suitable tools to detect and quantify the endocrine-disrupting chemicals, and their results could be used for risk assessment (Li et al., 2010a; WHO-UNEP, 2012). Thanks to the features of perform-friendly, time-saving and cost-inexpensively, the bioassays have shown their satisfactory sensitivity and reproducibility in application (Bittner et al., 2015).

A batch of recombinant gene yeast assays were used to investigate the endocrine disrupting activities of soil samples in Jilin City along SSR, including estrogenic, androgenic and thyroid disrupting activities (Li et al., 2015). The present study focused on the detection of thyroid disrupting activities of soil samples from Jilin City along SSR, using the recombinant thyroid receptor gene yeast assay combined with Monte Carlo simulation. The different fractions divided by polarity were also tested and then their results were analyzed to identify the major fraction responsible for the thyroid-disrupting potency. Besides, the anti-estrogenic and anti-androgenic activities in this area have been previously reported (Li et al., 2015).

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 compounds were dissolved in DMSO. HPLC-grade dichloromethane (DCM), methanol and hexane were purchased from Fisher Scientific (Fair Lawn, NJ). Silica (60–200 μm), and Al_2O_3 (50–200 μm) were purchased from Acros Organics (Geel, Belgium).

2.2. Sample collection and processing

Nine soil samples were collected from the surface layer (upper 20–30 cm) in August 2011 (Fig. 1). Each soil sample (500 g) was kept into a glass bottle, which was pre-cleaned with DCM, and then stored at −20 °C for further analysis. All of the samples were under treatment within 72 h.

Soil pH and organic carbon were measured according to the National Environmental Quality Standards for Soils of China (GB15618, 1995).

2.3. Soil organic extract preparation

After being freeze-dried and meshed, 20 g of each soil sample was extracted with 200 mL of DCM via Soxhlet extraction, and the resulting extracts were evaporated to 1 mL under a nitrogen stream. Subsequently, 0.5-mL aliquots were evaporated to near dryness under a gentle flow of nitrogen gas and then redissolved in 0.5 mL of DMSO. The remaining 0.5-mL aliquots were then carefully moved to columns containing 10 g of silica and 10 g of Al_2O_3 (Ma et al., 2007). The column was eluted with 15 mL of hexane to yield a nonpolar fraction, with 70 mL of a hexane/DCM (v/v=7:3) mixture to obtain a medium-polar fraction, and with 30 mL of methanol to acquire a polar fraction (Xiao et al., 2006; Wang et al., 2008). The sub-fractions were also evaporated to dryness under a gentle nitrogen flow and re-dissolved in 0.5 mL of DMSO. The raw organic extracts and three sub-fractions were kept at −20 °C until prior to being used in the bioassay. Test solutions with six different concentrations were obtained by 2-fold dilution

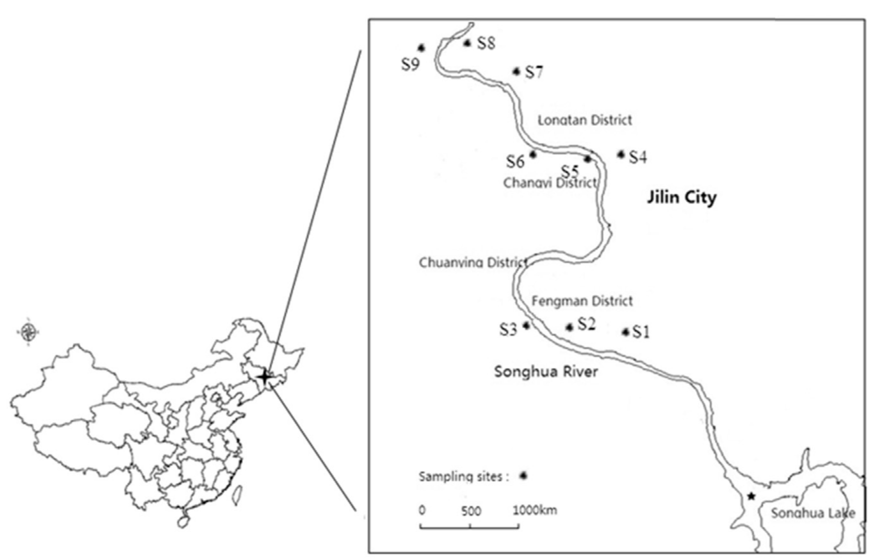

Fig. 1. Sampling sites in Jilin City along the Second Songhua River.