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Determination of endocrine-disrupting potencies of agricultural soils in China via a battery of steroid receptor bioassays☆

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ABSTRACT

Pollution of agricultural soils by pesticides, such as organochlorine pesticides (OCPs), can be a significant issue since high detection rates of these compounds were reported in our previous studies. However, more uncertain kinds, quantities and density of pollutants remained in soil samples were unidentified. In this study, the total hormonal activities of complex mixtures of both known and unknown contaminants in agricultural soils in mainland China were measured by applying highly sensitive reporter gene assays for detecting agonists/antagonists for estrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). High detection rates of estrogenic activities and anti-progestogenic activities were observed among the 123 soil samples, reaching 79% and 73%, respectively. More than half of the soil samples showed obvious antagonistic effects against AR and GR. Approximately a third of tested samples exhibited androgenic, progestogenic and glucocorticoidic effects. A total of 72% and 78% soil extracts had mineralocorticoid-like and anti-mineralocorticoid activities, respectively. Significant positive correlations were observed between estrogenic activity and the concentrations of Σdichlorodiphenyltrichloroethanes (DDTs), Σendosulfans, Σchlordanes, heptachlor and Σdrins, respectively, but not other receptors. As a rapid and convenient precaution method, determination of endocrine-disrupting potencies of contaminated soils via bioassay could help to identify and define sites that required further attention for ecological risk assessments.

1. Introduction

Soil is an important reservoir for complex mixtures of pollutants. Contaminated soils affect human and wildlife health through direct contact with soil or via the infiltration of soil contamination into groundwater, surface water and atmosphere. In China, the rapidly developing industrial and agricultural activities cause increased use of chemicals, which have deteriorated the soil quality. During the last few decades, the occurrence and ecological risk of persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), in soils of China have been continuously paid attention (Cai et al., 2008; Niu et al., 2016a, 2016b).

Current assessments on soil were mainly focused on the determination of pollutants using analytical method (Xiao et al., 2006a; Sidlova et al., 2009). Our recent studies also indicated that currently and historically used pesticides were observed in agricultural soils in China with high detection frequency (Niu et al., 2013; Tang et al., 2014). Like most current environmental risk assessments, these studies routinely analyzed the concentrations of selected kinds of pollutants and further studies were hampered by high cost and limited sampling size. However, considering the complicated situation of environmental samples, only a small

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proportion of pollutants can be identified and characterized, let alone the potential interactions among the various chemicals on biological effects.

Many soil contaminants identified in the chemical analysis of soil samples were also reported to be endocrine-disrupting chemicals (EDCs). An increasing number of studies have shown that OCPs were capable of binding estrogen receptor (ER) and androgen receptor (AR) as xenoestrogens or anti-androgens (Hotchkiss et al., 2008; Kojima et al., 2004). ER and AR belong to the group of steroid hormone receptors (SHRs), which regulate a large number of physiological processes in human and wildlife equipped with the corresponding steroid hormones. Other members of the SHR family, including progesterone receptor (PR), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), also play important roles in endocrine systems that may similarly be disturbed if agonists and antagonists for PR, GR and MR are present in the environment (Odermatt and Gumy, 2008; Sargis et al., 2010). Previous work reported the binding capacity and antagonistic activity of various chemicals including OCPs to PR (Li et al., 2008; Tran et al., 1996; Scippo et al., 2004). Our recent studies showed that OCPs could interfere with GR and MR (Zhang et al., 2016, 2017). The comprehensive investigations of multiple hormonal activities for SHRs disrupted by natural origin complex mixture at environmental level were still particularly sparse.

To better understand potential detrimental effects of soil pollution, the application of rapid *in vitro* bioassays to determine the total toxic effects of the mixtures derived from environmental samples may provide an adequate and overall assessment despite the uncertainty of the kinds, quantities and density of pollutants (Xiao et al., 2006b; Citterio et al., 2002). There is an increasing interest in using a battery of various bioassays to assess ecological risks for polluted environmental compartments (Van der Linden et al., 2008; Xiao et al., 2006a, 2006b; Suzuki et al., 2013). Several studies evaluated the genotoxicity and endocrine-disrupting effects of field soils in some regions in China (Li et al., 2015; Xiao et al., 2006a, 2006b). However, there is still a paucity of data on multiple hormonal activities in agricultural soils in China.

In this study, the total hormonal activities of complex mixtures of both known and unknown contaminants in agricultural soils across mainland China were measured by applying highly sensitive reporter gene assays for detecting ER, AR, PR, GR and MR agonists/antagonists. Since these assays all use the same cell line CHO-K1 and the same method procedure, results among assays can be easily and reliably compared. Ecological risk assessment based on the total endocrine activity of soil extracts can provide information on the integrated distribution patterns of EDCs and their adverse impact on ecosystems and humans, and also offer ecotoxic data for further identification of culprit chemicals and effective risk management in China.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO) and 17β-estradiol (E2; purity, >97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cortisol (COR >98%) was purchased from Aladdin Industrial Inc. (ShangHai, China). Mifepristone (RU486; >98%) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Aldosterone (ALD; >99%) was purchased from J&K Scientific Ltd. (Beijing, China). Progesterone (PRO; 100%), spironolactone (SPI; >97%), flutamide (FLU; >99%) and tamoxifen (TAM; ≥99%) were purchased from Selleckchem (Houston, TX, USA). 5α-Dihydrotestosterone (DHT, >98%) was purchased from Axygen Scientific Inc. (Union City, CA. USA). All chemicals were dissolved in DMSO and stored at −20 °C (Yang et al., 2014).

2.2. Sample collection

A total of 123 surface soil samples (0–20 cm) were collected from agricultural fields in 31 provinces or regions across China during May of 2013 (Niu et al., 2016a). The sampling locations were set according to the distribution of farmland soils in China (Niu et al., 2016a) and distribution of soil sampling sites were mapped in Fig. 1. At each sampling site, five samples were collected using acid-cleaned stainless steel scoops, thoroughly mixed to form a composite sample and then transferred to pre-cleaned aluminum foil bags. All samples were immediately transported to the laboratory and stored at −20 °C before analysis.

2.3. Sample extraction and cleanup

The soil samples were freeze-dried, homogenized and sieved (0.154 mm mesh). Each soil sample (25 g) was immersed in dichloromethane and Soxlet extracted for 24 h with a copper sheet to remove sulfur. The extracts were concentrated, solvent-exchanged to hexane and then eluted through a column containing (from bottom to top) Na_2SO_4, Florisil, silica gel, aluminum, and Na_2SO_4 using 70 mL of hexane/DCM (7/3, v/v) (Niu et al., 2016a). The surrogate standards were added into the soil samples prior extraction and the recoveries for OCPs were higher than 75.6% (Niu et al., 2016a, 2016b). The concentrated extracts were evaporated to near dryness under a gentle stream of nitrogen, redissolved in 25 μL of DMSO, and stored in the dark at −20 °C as a bulk solution.

2.4. Cell culture

Chinese hamster ovary K1 (CHO-K1) cells were grown in Dulbecco's modified eagle medium (DMEM) (Hyclone, Logan, UT) with 10% fetal bovine serum (FBS) (Hyclone) and 100 U/mL streptomycin-penicillin (Hyclone) under the condition of an atmosphere of 5% CO_2 at 37 °C with saturating humidity. In the dual-luciferase reporter gene assay, the cells were seeded with fresh phenol red-free DMEM supplemented with charcoal/dextran-treated FBS (CD-FBS).

2.5. Plasmid constructs

The rat ERα expression plasmid rERα/pCI and the estrogen-responsive element (ERE) containing reporter plasmid pERE-AUG-Luc+ were kindly provided by Dr. M. Takeyoshi (Chemicals Assessment Center, Chemicals Evaluation and Research Institute, Oita, Japan) (Takeyoshi et al., 2002). The human AR expression plasmid pSG5-hAR was kindly provided by Dr. John Isaacs (The Johns Hopkins School of Medicine, USA) (Alen et al., 1999). The human PR expression plasmid pSG5-hPR was kindly supplied by Dr. Pierre Chambon (Institute for Genetics and Cellular and Molecular Biology, France) (Garcia et al., 1992). The human GRα expression plasmid pF25GFP-hGRα, and the reporter plasmid pMMTV-luc that contains androgen responsive element (ARE), progesterone responsive element (PRE), glucocorticoid response element (GRE) and mineralocorticoid response element (MRE), were kindly provided by Dr. Evangelia Charmandari (Biomedical Research Foundation of the Academy of Athens, Greece) (Nicolaides et al., 2014). The human MRα expression plasmid pEGFP-C1-hMRα was kindly provided by Dr. Claudia Grossmann (Universität Halle-Wittenberg, Germany) (Grossmann et al., 2005). pRL-TK (Promega, Madison, WI, USA) was used as an internal control in the dual-luciferase reporter assays according to the manufacturer's instructions.