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Thyroid disrupting effects of polychlorinated biphenyls in ovariectomized rats: A benchmark dose analysis

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ABSTRACT

Polychlorinated biphenyls (PCBs) are proved endocrine disrupting potentials. Reference points (RP) for PCBs are derived from dose-response relationship analysis by using the traditional no observed adverse effect or lowest observed adverse effect level (NOAEL/LOAEL) methods, or a more advanced benchmark dose (BMD) method. In present study, toxicological RP for PCBs' thyroid disruption was established and compared between NOAEL/LOAEL and BMD method in an ovariectomized (OVX) rat model. Sham and OVX controls were given corn oil while other OVX groups were administered with 0.1, 1.0, 5.0, and 10.0 mg/kg bw of PCBs (aroclor 1254) respectively by gavage. Body weight change, liver type I 5′-deiodinase (5′-DI) activity, serum total thyroxine (tT_4), triiodothyroxine (tT_3), thyroid stimulating hormones (TSH), and thyroid histopathological changes were measured and analyzed. In PCBs-treated groups, serum tT_4, tT_3, TSH, and histopathological examinations showed significant changes with a dose-dependent manner compared with those in OVX control ($P < 0.05$). The toxicological RP for PCBs affecting thyroid function of OVX rats was 0.02 mg/kg′bw based on BMD analysis.

1. Introduction

Polychlorinated biphenyls (PCBs) are not restricted to environmental presence such as water, soil, and air, but are routinely discovered in human and animal tissues, producing adverse health effects, including endocrine disruption, neurotoxicity, hepatotoxicity, developmental and reproductive toxicity (Nakai et al., 2004; Robertson and Hansen, 2001; Ulbrich and Stahlmann, 2004). Though PCBs have been banned for use in most industrialized countries, developing countries like China are still under pressure of continuous PCBs pollution; one noticeable new source is the recycled electronic waste (Wang et al., 2011). Researches and risk assessment have emphasized on toxicological threshold of PCBs that are usually used for guidance of regulations. However, the progressive change of environmental presence of PCBs leaves concerns on if the current toxicological threshold will really shield people from harm of PCBs toxicity. One specific concern would be the validity of method used to derive the threshold.

Toxicological threshold, such as Reference point (RP) is of great importance in toxicological studies and risk assessment (Porterfield and Hendry, 1998). Traditionally, a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL) was applied as RP through a human or animal dose-response study. However, the NOAEL/LOAEL method is not flawless in terms of adequately reflecting the information of dose-response relationship, appropriately accounting for the sample size (Crump, 1984). To overcome such limitations, an alternative statistical tool – benchmark dose method – was developed to determine RP for toxic chemicals. The benchmark dose (BMD) is a dose (or an exposure) that causes a prescribed adverse effect in response, known as benchmark response (BMR). A statistical lower bound of the BMD, usually $BMDL_{05}$ or $BMDL_{10}$, is usually used as a point of departure for defining an allowable exposure (EFSA, 2009).

Endocrine disrupting chemicals (EDCs) are natural or synthetic compounds that can alter hormonal and homeostatic systems of organisms through environmental or inappropriate developmental exposures (Diamanti-Kandarakis et al., 2009a). Based on their receptor mechanisms, EDCs were categorized into three types: oestrogen-like hormone, antiandrogen, and thyroid hormone disruptors (Diamanti-Kandarakis et al., 2009b). PCBs, especially those

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structurally similar to thyroxine (T_4), can directly lower concentrations of thyroid hormones in the circulation of experimental animals, particularly serum T_4 levels (Zoeller, 2001). The mechanisms for thyroid disruption of PCBs is not settled, but hypotheses involve aromatic hydrocarbon receptor (AhR) (Van den Berg et al., 1998), thyroid hormone receptors (Chauhan, 2000), as well as liver chromosome enzyme (Kato et al., 2004). Dose-dependent trends of PCBs' thyroid effects have also been identified in animal models with various NOAELs/LOAELs (Crofton et al., 2005; Martin et al., 2010). However, limited number of studies has applied BMD method to quantify the toxic responses of PCBs (Jacobson et al., 2002; Kalantari et al., 2013) and even fewer studies use BMD approach focusing on thyroid disruption of PCBs (Buha et al., 2013; Esteban et al., 2014; Trnovec et al., 2013); the existing studies were conducted either with co-exposure of other EDCs or focusing only on one single endpoint. Thus, there is a urgent need to calculate a more convinciable BMD of PCBs by comprehensively comparing BMD results from different endpoints.

To account for the variability of hormonal tests in vivo, the interfering potential of fluctuating estrogen levels in the organisms are considered by employing an ovariectomized (OVX) rat model. PCBs aroclor 1254 was employed as the test substance because it was one of the commercially available PCB mixtures that are widely used in researches and its potential of thyroid disruption has been proved in both normal and intact rat models (Bansal and Zoeller, 2008; West et al., 1999). Thyroid disruption related endpoints, including total T_4 (tT_4), total triiodothyroxine (tT_3), thyroid stimulating hormones (TSH), liver type I 5′-deiodinase (5′-DI), and thyroid follicular epithelium/colloid ratio were measured and the data was recorded. The dose-response relationship curve for each endpoint was modeled by using BMD approach.

2. Materials and methods

2.1. Chemicals

PCBs aroclor 1254 (CAS No. 11097-69-1) was obtained from AccuStandard Inc. (New Haven, CT, USA). Non-transgenic corn oil was purchased from COFCO Ltd. (Beijing, China). Dowex 50WX2-400 ion exchange resin, sucrose, and propylthiouracil (PTU) were purchased from Sigma-Aldrich (St Louis, MO, USA). [^{125}I] labeled reverse T_3, radioimmunoassay kits for tT_4 and tT_3 were obtained from Beijing North Biotechnology Institute (Beijing, China). Reverse T_3 (rT_3) with purity of 96% was purchased from Toronto Research Chemicals (Toronto, Canada). L-thyroxine sodium with purity of 99% was purchased from Aladdin Reagents (Shanghai, China). Ethylene diamine tetra acetic Acid (EDTA) and HEPEs buffer (pH 7.4) were from Solarbio Company (Beijing, China). Rodent TSH ELISA test kit was from Endocrine Technology (Newark, CA, USA). Pierce BCA protein assay kit was from Thermo Scientific (Rockford, IL, USA)

2.2. Animals and housing

Sixty 10-week old female albino SD rats free of specific pathogens were purchased from Beijing Hua Fu Kang Bioscience Co. Ltd. (Beijing, China). The animals were housed singly in cages under condition of controlled temperature (20–24 °C) and relative humidity (40%–70%), a 12 h light/12 h dark cycle and air change of 10 times/h. All animals were given soy and alfalfa free diet (Hua Fu Kang bioscience, Beijing, China). All animals were provided with unlimited purified water throughout the study.

2.3. Study design

All animals received humane care in compliance with the guiding principles of the Guide for the Care and Use of the Animals Management Rules of the Ministry Health of the People's Republic of China (Documentation NO. 55, 2001, China). This study was approved by the Institutional Animal Care and Use Committee of China National Center for Food Safety Risk Assessment.

After acclimation for seven days, animals were randomly allocated into six groups according to the body weight. Among all rats, 50 received bilateral ovariectomy while the other 10 were only removed with about 2 grams of fat tissue near the ovary as a sham control. After a twelve-day recovery phase, one OVX group and the sham group were treated as controls, given corn oil orally via gavage. The other four OVX groups were dosed with 0.1, 1.0, 5.0, and 10.0 mg/kg bw PCBs aroclor 1254 dissolved in corn oil orally via gavage once per day. The treatment was conducted between 8:00 and 10:00 am for 8 consecutive days. The animals were observed for clinical signs of impairment and recorded body weight every two days. The day after last application, all animals were fasted for 12 h and sacrificed under sodium pentobarbital (50 mg/kg bw) anesthesia. Blood from abdomen aorta was collected in pro-coagulation tubes and centrifuged at 3000 rpm for 10 min to obtain blood serum. Slices of liver tissue from right liver lobe weighting about 1 gram were immediately rinsed with liquid nitrogen. Both blood serum and liver tissues were stored at −80 °C until use. Thyroids of individual animals were preserved in 4% formaldehyde solution.

2.4. 5'DI activity assay

The method was as previously described with minor modifications (Hotz et al., 1996; Ulbrich et al., 2004). Frozen liver tissues were homogenized in precooled buffer (320 mmol/L sucrose, 1.0 mmol/L DTT, 10 mmol/L HEPEs, pH 7.0) at a 1:39 dilution (w/v). The suspensions were centrifuged (3000 × g) at 4 °C for 10 min and the supernatant was decanted into new tubes. 1 mL buffer C was added to the precipitate and suspended thoroughly and re-centrifuged (20,000 × g) at 4 °C for 10 min. The supernatant was collected and mixed with the supernatant from first centrifugation. Aliquots of 100 μL incubation solution (0.005 μmol/L [^{125}I]rT_3, 0.495 μmol/L rT_3, 2 mmol/L DTT, 1mmol/L EDTA, 10 mmol/L potassium phosphate buffer, pH 7.0) were heated at 37 °C for 20 min. 20 μL votexed tissue supernatant was added in the heated incubation solution and returned to 37 °C for another 11 min. The reaction was stopped by adding 200 μL cold T_4/PTU solutions. Centrifuge filter units (Millipore, Billerica, MA, USA) with Dowex acidic ion exchange resin were loaded with 80% of the stopped reaction solution and centrifuged (1500 × g) at 4 °C for 5 min. The units were eluted twice with 1.0 mL 10% acetic acid and centrifuged again as above after each addition. All centrifuged supernatants were collected and used to measure the released $^{125}I^-$ counted in a gamma counter (Xi'an Nuclear Instrument Factory, Shanxi, China). Each sample was run duplicate. Total count per minute (cpm) of 100 μL incubation solution and a blank control (20 μL buffer solution instead of tissue homogenate) were counted. Protein concentration was determined according to the instructions of BCA protein assay kit. 5′ DI activity was expressed as pmol $^{125}I^-$ generated per mg protein per minute.

2.5. Measurement of serum thyroid hormone levels

Serum tT_4 and tT_3 were determined by radioimmunoassay while serum TSH was measured by enzyme-linked immunosorbent assay (ELISA). All experiments were carried out according to the instructions of the manufacturer.

2.6. Histopathology

Thyroids were prepared for histomorphometric analysis with a hematoxylin and eosin stain. Each thyroid gland was paraffin