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Combined effects of cadmium and tetrabromobisphenol a (TBBPA) on development, antioxidant enzymes activity and thyroid hormones in female rats

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ABSTRACT

Tetrabromobisphenol A (TBBPA) is one of the world's most widely used brominated flame retardants (BFRs) and considered as persistent halogenated contaminant. E-wastes contain a range of toxic chemicals, including BFRs and heavy metals, exerting adverse impacts to human health and environment. Nevertheless, comprehensive evaluation on combined toxicity of these co-existing pollutants is limited. This study conducted a subchronic effects of cadmium and TBBPA on the development and antioxidative defense system as well as thyroid functions in female rats through single and combined exposure at environmentally relevant doses for a 20-day consecutive administration. Body indexes, histopathology, redox status, and thyroid hormones levels were assessed. Slower body weight gains and reduced ovary weight (20.8% and 32.4% for combined and single-Cd exposures, respectively) were observed with significant variation from controls in high dose treatments. Co-exposure resulted in a slight enhancement in TSH levels compared to control (by 7.6% for high dose) without significance. TBBPA-Cd interactions are involved in the changes of kidney weight as well as the induction of SOD activities and MDA levels. The disturbances in the redox status may be a result of an independent effect of Cd and/or TBBPA and also of their interaction. The results implied under these treatment, kidney was more sensitive with significant increased organ coefficient and alteration for antioxidative indices (increasing by 46% for SOD activity). This study represents the toxic effects of Cd and TBBPA co-exposure through oral administration in pubertal rats, which may provide useful information for health risk assessment for young exposed individuals.

1. Introduction

Along with the accelerating update of electronic information technologies, electronic waste (e-waste) has become one of the most rapidly growing environmental problems in the world, especially in China [1] [2]; [3]. E-waste with various kinds of mixture of metal, plastics, glass and ceramics contains a range of toxic chemicals, including brominated flame retardants, heavy metals such as Cd and other potentially hazardous substances, which are harmful to environment and human health [4] [2]; [3]; [5]. Although, their potential toxicological effects have received extensive attention, the combined impacts of TBBPA and Cd remain poorly understood.

Tetrabromobisphenol A, one of the world's highest production volume BFR, is used primarily in resins of electronic circuit boards [6]. Cadmium is widely applied in electroplating and cadmium batteries, therefore, Cd and TBBPA could be both released into environment during e-waste dismantling and cause potential pollution problems. Recently, many studies have shown that Cd and TBBPA were detected simultaneously in water, air, dust samples and various kinds of organism tissues collected from regions surrounded by e-waste recycling sites [7]; [8]. Therefore, occupational workers in e-waste recycling facilities and residents nearby are most likely to be affected through exposure to the mixture of such toxic chemicals.

Heavy metals are also persistent in the environment and could be

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easily accumulated in biota, resulting in a series of chronic toxicities even at relatively low levels, especially for children [9]. Cadmium is one of the most toxic heavy metals used in various industries and has been reported to cause estrogenic effects [10], neurotoxicity [11], immunotoxicity [12], nephrotoxicity and hepatotoxicity [13]. Its exposure also disturbs the calcium metabolism, causing hypercalciuria and stones formation in the kidneys. High Cd exposure can lead to lung cancer and positive associations have been observed between exposure to cadmium and cadmium compounds and cancer of the kidney and prostate. Hence, Cd had been listed in Group 1 carcinogens by WHO International Agency for Research on Cancer (IARC) for its sufficient evidence in humans for the carcinogenicity [14]. TBBPA elicited decreased rat serum thyroxine levels in laboratory study [15]. It has been proven to exert hepatotoxicity and nephrotoxicity [16–19], immunotoxicity [20] and neurotoxicity [16,21], causing critical effects on health. In the past few years, IARC has re-evaluated the toxicity of TBBPA and modified the assessment results continually based on the updating of scientific research. According to the latest classification, TBBPA has been listed in Group 2A carcinogens due to its probably carcinogenic effect to humans.

Since organisms are often exposed to multiple xenobiotics, it is important to study their combined toxicity. However, the joint effect on Cd and TBBPA are rather scarce, except for an aerosol exposure of Cd and TBBPA in CD-1 male mice [22]. Although both Cd and TBBPA are jointly present in the environmental media, food, biota, and human tissues, the toxicity effects of Cd and TBBPA co-exposure through oral exposure has not been reported. In view of these concerns, this study was aimed to investigate the potential combined effects and the possible interaction of Cd and TBBPA under subchronic exposure at environmental relevant dose, with special attention to oxidative damage markers and thyroid functions in rats. To elucidate the influence of TBBPA and cadmium, the present study evaluated the toxicity of single-Cd and co-exposure with TBBPA in pubertal female SD rats during a 20-day consecutive oral administration. Development indexes, histopathology, activities of selected antioxidant enzymes, and thyroid hormones were assessed and data was explored to interpret the potential interactions of these two chemicals. The study could help to better understand the human health risk under co-exposure of heavy metal and brominated flame retardants.

2. Materials and methods

2.1. Chemicals and instruments

Tetrabromobisphenol A (TBBPA, CAS# 79-94-7, purity ≥ 99%), cadmium chloride ($CdCl_2$, CAS# 10108-64-2, purity ≥ 99.9%) and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carboxymethyl cellulose (CMC-Na, CAS# 9004-32-4) was supplied by TCI (Shanghai) Development co. LTD. T3, T4 and TSH ELISA Kit were procured from Shanghai Bangyi biotechnology co. LTD. $CdCl_2$ serial solution for exposure treatment, as well as 0.5% (w/v) sodium carboxymethyl cellulose (CMC-Na) solution were all prepared in ultrapure water. TBBPA working standard was suspended in 0.5% (w/v) CMC solution with 0.1% (w/v) DMSO for exposure treatment.

The following equipment and instruments were used: TB-718 Tissue embedding console system (Sakura Finetek Co. Ltd., Japan), Microtome RM2235 (Leica Microsystems GmbH, Germany), Leica HI1220 Slide drier (Leica Microsystems GmbH, Germany), Leica ST5020 Vacuum tissue processor (Leica Biosystems GmbH, Germany), Olympus BX51 microscope (Olympus corporation, Japan), Synergy HT microplate reader (Bio-Tek Instruments Inc., USA), cooling centrifuge (Sigma, 3–30 K, Germany), AR2130 Electronic balance (OHAUS corporation, USA), and Milli-Q Ultra Pure systerm (Millipore, USA).

2.2. Procurement and maintenance of animals

Specific pathogen free female (56–62 days old) and male (63–70 days old) Sprague-Dawley rats of parental generation were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. (qualified number SCXK (Beijing) 2012–0001), which were quarantined for 1 week prior to use. This study was carried out in Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, and approved by the Institutional Review Board and Human Ethics Committee. Animals were maintained according to the requirements of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) under the standard laboratory conditions of 24 ± 1 °C on a 12/12 dark/light photoperiod with a relative humidity in the range of 45%–65%.

Six or seven healthy young female pups (PND 21) of the parental generation were randomly assigned into controls and treatment groups. The experimental animals were housed 3–4 individuals in each plastic cage (42 cm × 28 cm × 20 cm) with wire mesh top and bedding litters of sawdust. During maintenance, distilled water and standard SPF-grade growth maintenance chow feeds (normal basal rat diet consist of corn, soybean meal, fish meal, wheat bran, flour, salt, calcium powder, vitamins, trace elements, and amino acid with 12.3% lipids, 63.3% carbohydrates, and 24.4% proteins (kcal), supplied by Beijing Keao Xieli Feed Co., Ltd., Beijing) were provided for consumption *ad libitum*. The housing cage was daily disinfected and paddings were daily cleaned or replaced.

2.3. Exposure protocol

The rats were respectively exposed to three scenarios, including TBBPA only, $CdCl_2$ only and TBBPA + $CdCl_2$ combined. During the exposure period, the rats were treated by oral gavage at 50 mg/kg body weight for TBBPA exposure in 0.5% (w/v) CMC solution for 20 consecutive days (from PND 21 to PND 40). $CdCl_2$ exposure was alone or combined with 50 mg/kg·bw TBBPA via drinking water *ad libitum* at 3 levels of 1 mg/L, 10 mg/L and 100 mg/L, respectively. Body weights were recorded daily to adjust the dosing volumes of TBBPA to keep it for 50 mg/kg·bw/d. Two control groups, blank control (CK) and vehicle control (VH) were set. The blank control was treated with ultrapure water and rodent diet without contaminants. The vehicle control (VH) received 0.5% (w/v) CMC solution only (without TBBPA) for substitute as procedure blank treatment. During the experiment, survival, clinical toxic symptoms, behavioral changes were observed and food intake was monitored twice a week to check the appetite of animals. Water consumption was recorded twice a week before drinking bottles refilled with freshly prepared $CdCl_2$ solution. Actual average daily Cd-intake was calculated based on the water consumption, dose of $CdCl_2$ and body weight of rats (data represent mean values in Table 1).

2.4. Sample collection and pretreatment

After 20 days of exposure, all rats were anaesthetized by isoflurane, blood samples were collected via heart puncture into vials without anticoagulant and kept still for 30 min before centrifugation. The subsequent serum was stored in liquid nitrogen until analysis. Thyroid, hypophysis, kidney, liver, uterus, ovary and adrenal were dissected, weighed and immediately frozen with liquid nitrogen after removed.

The thyroid tissue was fixed in 10% buffered formalin for histological analysis. After fixation, thyroid tissue was dehydrated, and embedded in paraffin wax. Sections of 4 μm were stained with haematoxylin and eosin in an automated way. Microscopical assessment was conducted by comparing control and the exposed samples.

2.5. Biochemical analysis

A ground fraction of all samples from each group were subjected to