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Single and 14-day repeated dose inhalation toxicity studies of hexabromocyclododecane in rats

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ABSTRACT

Limited toxicological information is available for hexabromocyclododecane (HBCD),a widely used additive brominated flame retardant. Inhalation is a major route of human exposure to HBCD. The aim of this study was to determine the acute inhalation toxicity and potential subchronic inhalation toxicity of HBCD in Sprague-Dawley rats exposed to HBCD only through inhalation. The acute inhalation toxicity of HBCD was determined using the limit test method on five male and five female Sprague-Dawley rats at a HBCD concentration of 5000 mg/m^3. Repeated-dose toxicity tests were also performed, with 20 males and 20 females randomly assigned to four experimental groups (five rats of each sex in each group). There were three treatment groups (exposed to HBCD concentrations of 125,500, and 2000 mg/m^3) and a blank control group (exposed to fresh air). In the acute inhalation toxicity study, no significant clinical signs were observed either immediately after exposure or during the recovery period. Gross pathology examination revealed no evidence of organ-specific toxicity in any rat. The inhalation $LC_{50(4\ h)}$ for HBCD was higher than 5312 ± 278 mg/m3 for both males and females. In the repeated dose inhalation study, daily head/nose-only exposure to HBCD at 132 ± 8.8, 545.8 ± 35.3, and 2166.0 ± 235.9 mg/m^3 for 14 days caused no adverse effects. No treatment-related clinical signs were observed at any of the test doses. The NOAEL for 14-day repeated dose inhalation toxicity study of HBCD is 2000 mg/m^3.

1. Introduction

Hexabromocyclododecane (HBCD) is a brominated cyclic alkane that is used as an additive flame retardant. It is the third most used brominated flame retardant worldwide, after tetrabromobisphenol A and the polybrominated diphenyl ethers. HBCD is an effective flame retardant at low doses, so it is widely used in a wide range of polystyrene resins, textiles, and household electrical equipment (Covaci et al., 2006). The banning of all commercial polybrominated diphenyl ether mixtures in the European Union and North America has led to more HBCD being produced and used (Szabo et al., 2010). The total global market for HBCD in 2001 was 16,700 t, 9500 t of which were consumed in the European Union, which is the largest market for HBCD (Alaee et al., 2003).

The wide use of HBCD as an alternative to polybrominated diphenyl ethers has led to certain environmental and health problems occurring. HBCD is an additive flame retardant, so it can enter the environment during the production, use, and disposal of products. HBCD can persist in the environment, bioaccumulate, and migrate over long distances (Covaci et al., 2006). HBCD has been detected in a wide range of biotic and abiotic environmental samples (Law et al., 2006), and even in samples from the Arctic (Lindberg et al., 2004). It has been found in toxicological studies that exposure to HBCD can have sub-chronic toxic effects (Birnbaum and Staskal, 2004; Covaci et al., 2006; Marvin et al., 2011). The main toxic effects of HBCD that have been found are cytotoxicity (Debenest et al., 2010), endocrine disturbances (interference with thyroid function) (Huang et al., 2013; van der Ven et al., 2006; Yamada-Okabe et al., 2005), hepatotoxicity (Canton et al., 2008; Szymanska et al., 2000), neurotoxicity and immunotoxicity (Eljarrat et al., 2009; Ema et al., 2008; Nakajima et al., 2009), reproductive developmental toxicity (Mariussen and Fonnum, 2003; McCormick et al., 2011), and teratogenicity (Shi et al., 2010). However, there is still a very limited amount of toxicological data for HBCD available. HBCD has been included in the

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Stockholm Convention on persistent organic pollutants (Sindiku et al., 2015).

A large amount of research on HBCD in indoor air and dust has been performed in recent years (Abdallah et al., 2008; Harrad et al., 2010a, 2010b; Takigami et al., 2009a, 2009b). It has been shown in that research that HBCD concentrations are higher in indoor than outdoor air. Exposure through the dermal and inhalation routes may be quantitatively important (in addition to intake in food) to the uptake of HBCD by humans (Thomsen et al., 2007). The results of a small number of studies of dermal exposure to HBCD have been published, but there is a lack of data on the toxicological effects of inhaled HBCD.

The aim of this study was to determine the acute toxicity and potential subchronic toxicity of HBCD in Sprague-Dawley rats exposed only through inhalation. The study was carried out in compliance with Organization for Economic Cooperation and Development guidelines for testing chemicals.

2. Materials and methods

2.1. Animal husbandry and maintenance

Healthy male and female specific pathogen-free Sprague-Dawley rats, about 7 weeks old, were purchased from Vital River Laboratory Animal Technology Co. (Beijing, China), and they were quarantined for 1 week and acclimatized before use. The animals were housed in a specific pathogen-free room that was maintained at 22 ± 3 °C and a relative humidity of 50 ± 20%, with artificial lighting from 08:00 to 20:00 and 10–20 air changes per hour, until the tests were performed. The animals were housed individually in independent ventilated cages under the same conditions during the exposure-free period. The animals received formula feed sterilized with ^{60}Co radiation, supplied by Beijing Keaoxieli (Beijing, China), and tap water ad libitum.

2.2. Experimental groups

Head/nose-only exposure tests were performed to minimize the exposure of the rats to HBCD through dermal contact and oral ingestion. Animals were selected based on weight and sex after 7 days of quarantine, and then randomly allocated to the test groups. The animals were marked at different body positions with picric acid to allow them to be identified.

The limit test method was used to evaluate the acute inhalation toxicity of HBCD in five male and five female Sprague-Dawley rats. The tests were performed at a HBCD concentration of 5000 mg/m^3.

A repeated-dose toxicity study was performed on 20 males and 20 females randomly assigned to four experimental groups, each group consisting of five rats of each sex. There were three treatment groups, which were exposed to HBCD at concentrations of 125, 500, and 2000 mg/m^3, and a blank control group (exposed to fresh air only).

2.3. Dose selection

A range-finding study was performed in which four groups of five rats of each sex were exposed, through head/nose-only inhalation, to HBCD dust aerosol target concentrations of 125, 250, and 500 mg/m^3 for 6 h/d for 5 consecutive days. Five control animals of each sex were exposed to clean air. No treatment-related effects (clinical signs, body weight changes, or clinical chemical, hematological, or pathological changes) were found even in rats exposed to the highest HBCD concentration. These results led us to choose target concentrations of 125, 500, and 2000 mg/m^3 for the next study. The highest test concentration of 2000 mg/m^3 was selected because it was expected to cause at least some overt systemic effects. Four groups of five animals of each sex were exposed to HBCD at the test concentrations for 6 h/d for 14 consecutive days, and 10 control animals were exposed to clean air.

2.4. Test substance

The HBCD (CAS No: 3194-55-6; >99% pure) that was used in the tests was purchased from Beijing Hongjie Technologies Co. (Beijing, China). The HBCD was ground in a ball mill and then passed through a screen mesh before being used to generate a dust aerosol for use in the tests.

2.5. Exposure of rats to HBCD

A TSE head/nose-only exposure system (TSE Systems, Bad Homburg, Germany) was used for the tests. The system consisted of a compressed air generator, a dust aerosol generator, a head/nose-only exposure unit with individual exposure cages, a humidity generator (for maintaining constant humidity), filters for purifying the incoming and exhaust air, and an exhaust pump. The desired flow rates were maintained using electronic mass flow controllers and control system software. Test parameters (such as the flow rate, humidity, pressure, and temperature) were continuously monitored by sensors.

The head/nose-only exposure unit was constructed so that each individual animal was exposed to the optimal HBCD concentration consistently throughout a test with the lowest possible air flow (therefore using the smallest possible amount of HBCD). The test atmosphere in an inner cylinder was forced toward the outside of the unit directly into openings in the exposure cages. This arrangement ensured that each individual animal inhaled constantly refreshed air containing a uniform HBCD aerosol concentration and particle size. The direct-flow design (using a controlled flow direction within the inhalation system) effectively prevented the re-inhalation of exhaled air. During the exposure tests, the animals were placed in exposure cages constructed to prevent the animals from avoiding inhaling the test aerosol and to prevent the test aerosol from escaping from the system (by using a negative pressure inside the system during a test).

A dust aerosol of HBCD was generated using compressed air, and the aerosol was sprayed into the exposure unit. Different HBCD concentrations were achieved by adjusting the dust generator parameters and the compressed-air flow rate. The air flow rate, the relative humidity, and the temperature were continuously monitored. The tests were performed keeping the temperature and humidity within the range specified in Organization for Economic Cooperation and Development guidelines (20–24 °C and a relative humidity of 30–70%). Once the HBCD concentration in the exposure system had reached the required concentration (5000 mg/m^3), the rats were transferred to glass exposure tubes, with only the animals' snouts protruding into the inhalation system. The animals were then exposed to HBCD for 4 h. The exhaust air was filtered and purified before being released to the outdoor atmosphere. The HBCD concentration in the air in the system during a test was determined by performing a membrane filter gravimetric analysis of the air in a test chamber. Three samples were collected in parallel every hour during a test, using a sampling flow rate of 2 L/min and a sampling time of 60 s. The mean concentration in the three replicate samples collected at each time point was calculated. The membrane filter was dried for 24 h before use. The mass distributions were measured using a TSE SpectroPan instrument (TSE Systems), and these distributions were used to calculate the mass-based median aerodynamic diameter and the geometric standard deviation.