HEXABROMOCYCLODODECANE GAMMA (HBCD-γ): TISSUE DISPOSITION AND ELIMINATION KINETICS IN MICE

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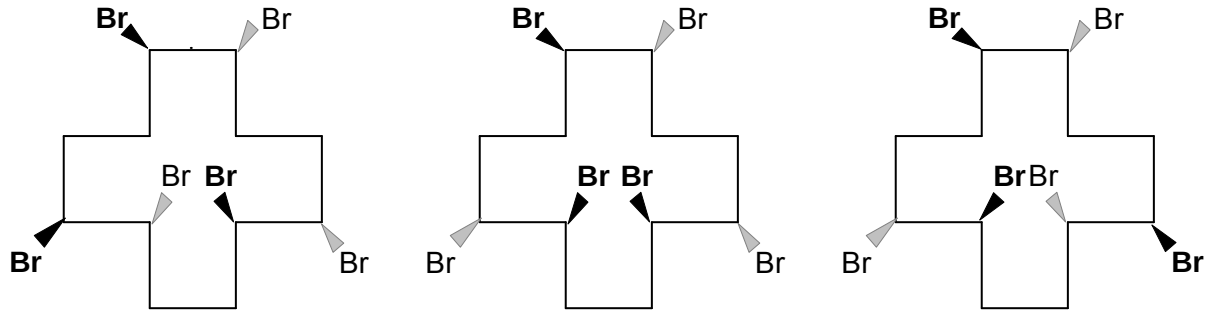
Introduction

Hexabromocyclododecanes (HBCDs) are brominated aliphatic cyclic hydrocarbons and high production volume chemicals used as flame-retardants for plastics and textiles. With the ban of all PBDE commercial mixtures in the European Union and North America's increased awareness to find alternatives, HBCD is predicted to increase in its production and use. It has been implicated as a developmental neurotoxicant[1], enzyme inducer[2], and endocrine disruptor[3] in laboratory animals. Recent studies have suggested HBCD is also highly bioacculmulative.[4,5] Commercial HBCD is a mixture of different 1,2,5,6,9,10-hexabromocyclododecane stereoisomers. Previous literature has focused on the three stereoisomers in the commercial mixture, denoted as alpha, beta and gamma, with gamma predominating (>70%) (Figure 1).[6] Importantly, a shift from the high percentage of gamma in the technical mixture and environmental media to a dominance of alpha in biological samples is observed.[7,8,9]

Figure 1. HBCD Stereoisomers



alpha (α) **beta (β)** **gamma (γ)**

In predicting human health risks posed by HBCD, it is necessary to accurately predict systemic dosimetry and the fate of these compounds. The dose/response relationship of a chemical must be well characterized in order to accurately describe the associated risk. A major focus for HBCD is to establish a basic knowledge of stereoisomer specific absorption, distribution, metabolism, and excretion (ADME). Currently, studies on the toxicokinetics of HBCD mixtures are very limited and provide no data for specific stereoisoforms in any mammalian system. Therefore, based on the limited toxicity profile and the need for basic disposition and elimination kinetics, the major commercial stereoisomer, HBCD-γ, is investigated here in mice.

Objective

To determine basic distribution and excretion parameters of HBCD-γ following an acute exposure with respect to dose and time in mice.

Methods

Chemicals: [^{14}C] 1,2,5,6,9,10-hexabromocyclododecane (HBCD) (2mCi/mmol) was purchased from ARC (St Louis, MO) and [^{14}C] 1,2,5,6,9,10-hexabromocyclododecane gamma (HBCD- γ) was purified at the USDA (Fargo, ND) as determined by reverse-phase high-pressure liquid chromatography (HPLC) using a radioactive flow detector. The non-radioactive commercial mixture of HBCD was purchased from Sigma-Aldrich (cat # 144762, purity of ~ 95%).

Dosing Solutions: Doses were selected based on previous toxicity studies[3] and the specific activity. A stock solution of [^{14}C]HBCD-γ was made by dissolving 19.23 mg of [^{14}C]HBCD-γ (3.12 μCi/mg) in toluene (400ul) until dissolved. Aliquots were used directly from this solution for all dosing regimens. All solutions were subjected to pre and post-dosing radioactivity examination to ensure proper delivered dose. All solutions were designed to deliver approximately 0.2μCi to each mouse; cold HBCD was added to the [^{14}C] HBCD-γ to achieve desired mass (all doses except low dose of 3mg/kg). Unlabeled HBCD was added directly to the dosing solution vial and dissolved in acetone. Corn oil was then added to the vials by weight followed by the evaporation of toluene under vacuum.

Animals: Female C57BL/6 mice (~20 grams) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Animals were maintained on a 12-hour light/dark cycle at ambient temperature (22°C) and relative humidity (55±5%), and were provided with Purina 5001 Rodent Chow (Ralston Purina Co., St. Louis, MO) and tap water *ad libitum*. Prior to the commencement of the study, mice were adapted (3 mice/cage) for one week in Nalgene metabolism cages (Nalgene, Rochester, NY). Mice were then randomly assigned to treatment groups (n=4) and housed individually for the remainder of the study. Mice were ~60 days old at time of treatment.

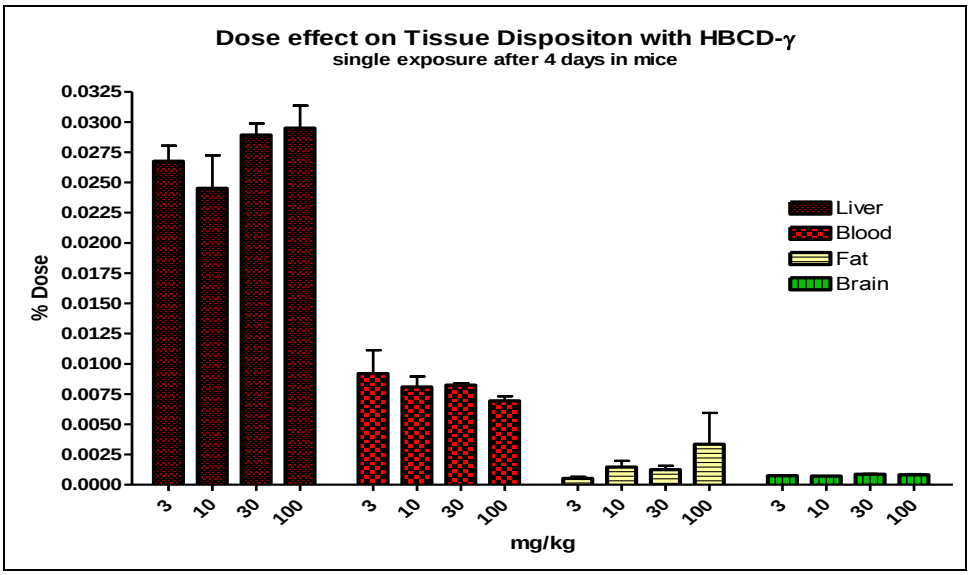
Treatment: *Dose/Response:* A single dose (3, 10, 30, or 100 mg/kg) was administered by oral gavage into the stomach of each mouse using a curved plastic animal feeding needle. After dosing, mice were held in metabolism cages for 4 days and urine and feces were collected daily. Animals were euthanized by CO_2 asphyxiation followed by exsanguinations via cardiac puncture. Tissues were collected and weighed: blood, liver, adipose (abdominal) and brain. *Time course:* Same as the dose/response treatment above except mice were held for 14 days (days 1-6 are reported) after a single dose of 3 mg/kg.

Sample Analysis: Radioactivity in the tissues was determined by combustion (Packard 306B Biological Oxidizer, Downers Grove, IL) of triplicate samples when available (~100mg/sample) followed by liquid scintillation spectrometry (LSS; Beckman Scintillation Counter, Beckman Instruments, Fullerton, CA). All tissue data is reported based on wet weight. Feces were air dried following collection, weighed, and analyzed for radioactivity by combustion and LSS. Daily urine volume was recorded, and 100 μl aliquots (triplicate) were analyzed by direct addition into scintillant for radioactivity determination by LSS.

Results and Conclusions

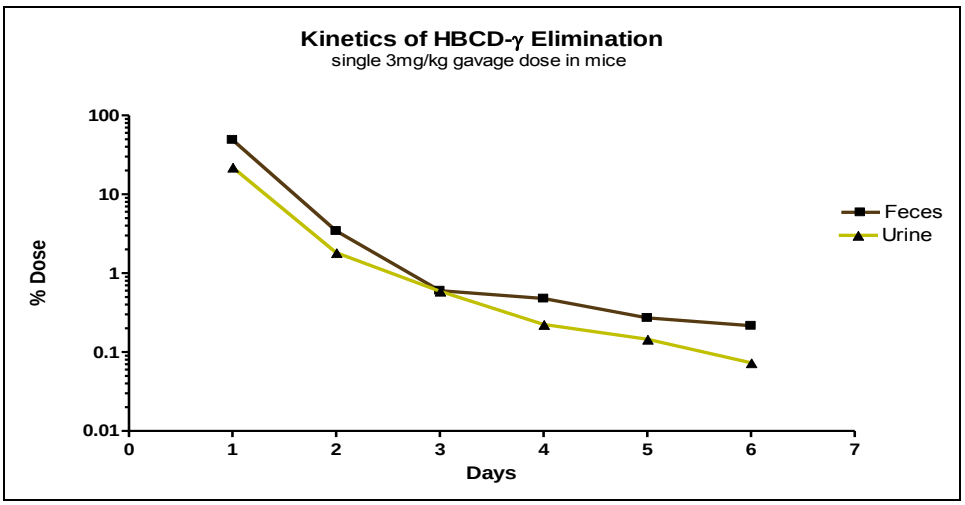
Female C57BL/6 mice were administered a single oral dose (3, 10, 30 or 100 mg/kg) of [^{14}C] HBCD-γ. Tissue distribution was analyzed four days after the administration of HBCD-γ. All tissues examined had low but measurable levels four days after dosing (Figure 2). We found that tissue disposition is not a function of dose and HBCD's behavior appears to be linear across all doses measured (3-100 mg/kg). These results also demonstrate a lack of tissue-specific sequestration as seen with dioxin and dioxin-like chemicals. From highest to lowest, tissue concentrations were highest in liver (0.0275%), followed by blood (0.009%), fat (0.003%), and brain (0.001%). This is unlike the lower brominated PBDEs, such as BDE-47, where the behavior is dictated by lipophilicity.[10] Considering measured levels in fat are lower than blood and liver, the biological persistence of HBCD in mice may be limited.

Figure 2. Dose/Response effect on Tissue Disposition



From the dose/response study, the lowest dose was chosen (3mg/kg) for the kinetic study because its behavior was in the linear range. By observing elimination over time, a large percentage of the administered dose is excreted in urine and feces by day 1 (~75%) (Figure 3). By days four and six, ~85 and 88% are eliminated collectively. Unexpectedly, a large portion of the administered dose is excreted in the urine (~22-28%). This suggests binding to serum proteins and/or active renal transport leading to urinary elimination.[11] Importantly, this evaluation of HBCD-γ is measuring total radioactivity and it is not clear whether its levels are attributed to alpha or gamma. Although there have been reports suggesting isomerization in fish[5], future directives of this study include determining this in a mammalian system.

Figure 3. Time Course of Elimination



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