TOXICOKINETICS OF THE DIASTEREOMER SPECIFIC FLAME RETARDANT HEXABROMOCYCLODODECANE (HBCD): EFFECT OF DOSE, TIME, AND REPEATED EXPOSURE

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Introduction

With EU and North America's increased awareness to find brominated flame retardant (BFR) alternatives, concerns over hexabromocyclododecane's (HBCD's) demand and safety are currently driving regulatory action. HBCD is an additive BFR predicted to increase in global production and use. HBCD's main use is in expanded and extruded polystyrene thermal insulation foam boards widely used in construction industries. With the reduction of greenhouse gas emissions a global priority, energy efficient construction will further increase demand of HBCD. Electronic industries have also been one of the largest consumers of BFRs with e-waste increasing as a source of HBCD exposure worldwide. For this reason HBCD is on the proposed list of substances added to revise the RoHS (Restriction of Hazardous Substances) directive for electronic and electrical equipment. With growing global demands and need for green chemistry, high HBCD levels in indoor dust raise additional concerns to its safety and health risk.

HBCD is a lipophilic compound and is classified as Persistent, Bioaccumulative and Toxic (PBT). Levels of HBCD have been reported in a few human studies to date[1,2]. Recent toxicity studies on commercial HBCD suggest it is a developmental neurotoxicant[3,4], enzyme inducer[5], and endocrine disruptor[6,7] in laboratory animals. On October 28, 2008 the European Chemical Agency included HBCD on the Substances of Very High Concern (SVHC) list. However, commercial HBCD is a mixture of different diastereomers and a shift from the high percentage of gamma (γ) in the mixture and environment to a dominance of alpha (α) in biological samples is observed.[8,9,10] Studies on the toxicokinetics of HBCD are extremely limited and do not provide data for specific stereoisomers in any mammalian system. It is hypothesized that these stereoisomers may have different kinetic behavior as well as toxicity profiles. Considering fat is a major depot for many POPs, this study focused specifically on adipose tissue dosimetry. The data obtained from our dose/response, timing, and repeated exposure studies comparing stereoisomer specific HBCD-γ and -α are necessary in predicting the behavior and risks posed by HBCD for a proper human risk assessment and corrective regulatory action.

Objective

Determine differences in adipose tissue distribution, half life and excretion parameters of HBCD-γ and -α with respect to dose, time and repeated exposure 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. [^{14}C]1,2,5,6,9,10-hexabromocyclododecane alpha (HBCD-α) was thermally converted from purified HBCD-γ at temperatures 170°C for up to 3 hr. It was further dissolved in methyl chloride, checked for isomeric composition by LC/MS, and then purified by flash chromatography on a silica gel column and then by preparative reverse phase HPLC. The non-radioactive commercial mixture was purchased from Sigma-Aldrich (cat#144762, purity of 95%).
Dosing Solutions: Doses were selected based on previous toxicity studies[6,7] and specific activity. A stock solution of [^{14}C]HBCD-γ or -α was made by dissolving 19.23 mg of [^{14}C]HBCD-γ or - α (3.12 μCi/mg) respectively, 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-γ or -α was added to the [^{14}C] HBCD-γ or -α respectively, 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%), 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 at 10ml/kg) was administered by gavage using a curved plastic animal feeding needle. After dosing, mice were held in metabolism cages for 4 days where urine and feces were collected daily. Animals were euthanized by CO_2 asphyxiation followed by exsanguination via cardiac puncture. Tissues were collected and weighed. *Time course:* Same as the dose/response treatment except mice were held for up to 14 days. *Repeated*: Same as the dose/response treatment except mice were dosed 9 days with cold HBCD-γ or –α and on day 10 they were dosed with [^{14}C] HBCD-γ or –α respectively, and held for 4 days (total of 14 days). Both the time course and repeated dose study utilized doses of 3mg/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[12] or (3, 10, 30 mg/kg) [^{14}C]HBCD-α. We focused on adipose tissue since it is a major depot for many POPs. Adipose tissue deposition was analyzed four days after the administration of each diasteriomer. Tissues examined had measurable levels four days after dosing (Figure 1). We found that [^{14}C]HBCD-γ tissue distribution is not a function of dose, its behavior appears to be linear across all doses measured (3-100 mg/kg) and the repeated exposure did not alter from a single dose (data not shown)[12]. These results also demonstrate a lack of tissue-specific sequestration as seen with dioxin and dioxin-like chemicals. This is in contrast to [^{14}C]HBCD-α, where tissue deposition was 5-10 fold higher than [^{14}C]HBCD-γ depending on dose. Adipose tissue concentrations after a single oral exposure for 3, 10 and 30 mg/kg doses were 5.5, 6.2 and 7.9% after 4 days. Increased levels were observed with a 10 day repeated exposure as compared to a single dose of 3mg/kg (Table 1). This is similar to lower brominated PBDEs, BDE-47, where lipid concentration is dictated by dose.[11]

Figure 1. Dose/Response effect on Adipose Deposition

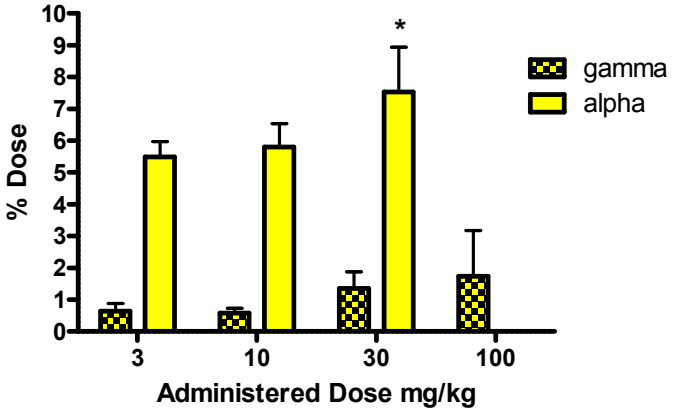


Table 1. Adipose Tissue Concentration of [^{14}C]HBCD-α 4 Days after a Repeated (3mg/kg) vs a Single Oral Dose

Single 3	Repeated	Single 30
5.5 ± 0.7	8.0 ± 1.1	7.9 ± 1.4
(210 ± 24)	(322 ± 55)	(3138 ± 483)

From the dose/response study, the lowest dose was chosen (3mg/kg) for the kinetic study based on environmental relevance. By observing tissue deposition over time, detectable concentrations are present in adipose tissues at all time points investigated (Figure 2). This 14 day time course study shows a biphasic elimination profile for both

diastereomers. There is an initial decline from day 1-2 for [^{14}C]HBCD-γ and day 2-4 for [^{14}C]HBCD-α. The secondary phase is a less steep decline between day 2-14 for [^{14}C]HBCD-γ and day 4-14 for [^{14}C]HBCD-α. Interestingly, [^{14}C]HBCD-γ was found to peak in concentration after 1 day in fat while [^{14}C]HBCD-α peaked by day 2. After 14 days, there is 50 times more [^{14}C]HBCD-α derived radioactivity measured in adipose tissue than [^{14}C]HBCD-γ. The adipose half life of [^{14}C]HBCD-γ and –α are listed for the initial "α" phase and secondary "ß" phase (Table 2). These results demonstrate the biological persistence of [^{14}C]HBCD-α and to a lesser extent [^{14}C]HBCD-γ.

Figure 2. Effect of Time on Adipose Deposition

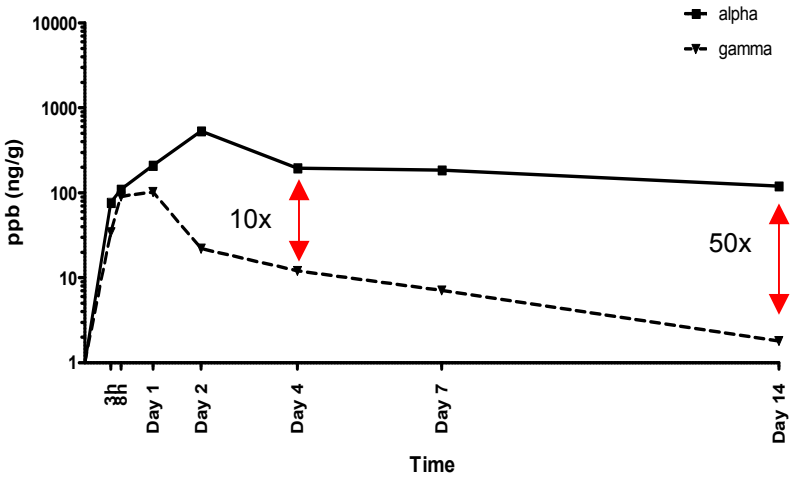
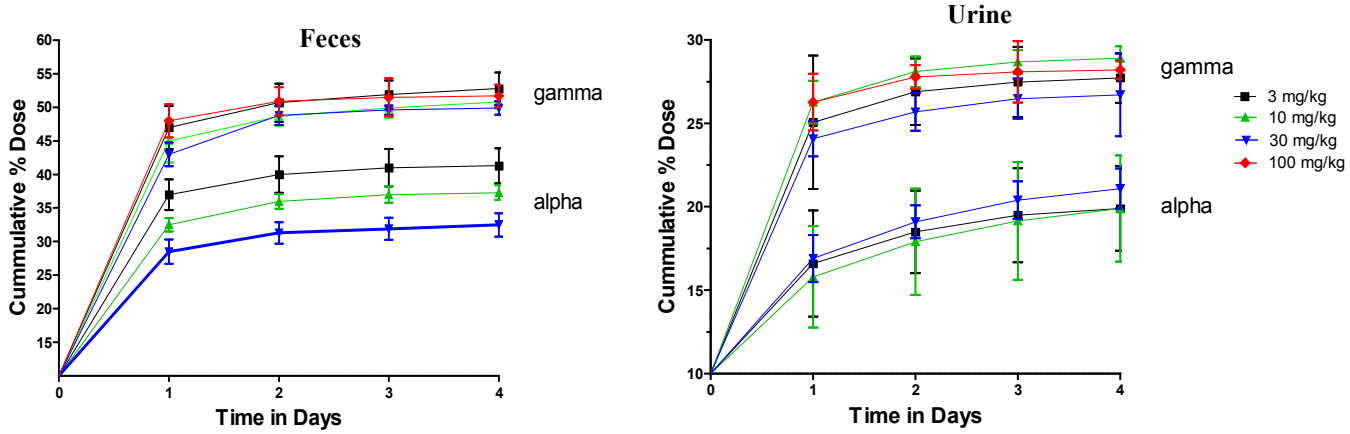


Table 2. Half-life in Adipose Tissue in Days.

	α t1/2	ß t1/2
HBCD-γ	0.78	3.14
HBCD-α	1.5	20.7

By observing elimination over time, a large percentage of the administered dose is excreted in urine and feces by day 1 for [^{14}C]HBCD-γ (~75%) [12] but to a lesser extent for [^{14}C]HBCD-α (~50%) (Figure 3). Results indicate that there is no dose effect in regards to urinary elimination with respect to [^{14}C]HBCD-α or [^{14}C]HBCD-γ. Importantly, less urinary elimination is observed with [^{14}C]HBCD-α than with [^{14}C]HBCD-γ over time. This is the same for fecal elimination; with more being eliminated with [^{14}C]HBCD-γ than [^{14}C]HBCD-α. However, at higher doses, [^{14}C]HBCD-α is less effectively eliminated in feces and suggests increased body burden with dose and possible alteration in hepatic enzymes, efflux transporters and/or enzymatic saturation. These results indicate a biological persistence of [^{14}C]HBCD-α and potential bioaccumulation.

Figure 3. Time Course of Elimination



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