Chemosphere 178 (2017) 424–431

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Accumulation of α-hexabromocyclododecane (α-HBCDD) in tissues of fast- and slow-growing broilers (*Gallus domesticus*)

Catherine Jondreville [a, *], Ronan Cariou [b], Bertrand Méda [c], Elena Dominguez-Romero [a, c, d], Elsa Omer [b], Gaud Dervilly-Pinel [b], Bruno Le Bizec [b], Angélique Travel [d], Elisabeth Baéza [c]

[a] AFPA, INRA, Université de Lorraine, 54500, Vandoeuvre-lès-Nancy, France
[b] LABERCA, LUNAM Université, Oniris, INRA, 44307, Nantes Cedex, France
[c] URA, INRA, 37380, Nouzilly, France
[d] ITAVI, Centre INRA de Tours, 37380, Nouzilly, France

## HIGHLIGHTS

- A kinetic study of α-HBCDD was conducted in fast- and slow-growing broilers.
- α-HBCDD preferentially accumulated in fatty tissues.
- α-HBCDD was more concentrated in lipids of leg muscles than of breast muscle.
- Muscles were slightly more concentrated in slow- than in fast-growing broilers.
- Depuration half-life was 12–20 d, of which 50% due to dilution through growth.

## ARTICLE INFO

*Article history:*
Received 9 January 2017
Received in revised form
14 March 2017
Accepted 15 March 2017
Available online 22 March 2017

Handling Editor: Myrto Petreas

*Keywords:*
Animal-derived food
Brominated flame retardant
Lipophilic contaminant
Half-life
Elimination

## ABSTRACT

The aim of the current study was to describe the fate of ingested α-hexabromocyclododecane (α-HBCDD) in fast-growing (FG) and slow-growing (SG) broilers, through an exposure to a dietary concentration of 50 ng α-HBCDD $g^{-1}$ feed during 42 and 84 days, respectively. Depuration parameters were assessed in SG broilers successively exposed during 42 days and depurated during 42 days. At market age, SG broilers had ingested 42% more feed than FG broilers, while their body weight gain per g of feed ingested was 34% lower. No isomerization of α- to β- or γ-HBCDD forms occurred, while OH-HBCDD was identified as a product of α-HBCDD metabolism. Irrespective of the strain, abdominal fat displayed the highest α-HBCDD concentration on a lipid weight basis, followed leg muscles and then breast muscle, liver and plasma. The accumulation ratios of α-HBCDD were slightly higher in SG (6.7, 2.1, 2.6 and 9.9 in leg muscles, breast muscle, liver and abdominal fat, respectively) than in FG broilers (5.2, 2.2, 1.1 and 8.4, respectively). The elimination half-lives in SG broilers were 20, 12 and 19 d in leg muscles, breast muscle and abdominal fat, respectively, to which dilution through growth contributed for around 50%. The overall assimilation efficiency of α-HBCDD was estimated at 58 and 50% in FG and SG broilers, respectively, while 22 and 17% of α-HBCDD ingested were estimated to be eliminated in excreta as metabolites.



## 1. Introduction

Hexabromocyclododecane (HBCDD) is a brominated aliphatic cyclic hydrocarbon mainly used as a flame retardant additive in thermal insulation materials, especially in extruded (XPS) and expanded (EPS) polystyrene, which used to represent 80% of uses in Europe (ECHA, 2009). Due to its bioaccumulative, persistent and toxic characteristics, HBCDD has been listed in Annex A of the Stockholm Convention on Persistent Organic Pollutants in November 2014, resulting in a mid-term ban on its manufacture and use. Nevertheless, due to the long lifespan of insulation materials, release of HBCDD into the environment is expected to

*Abbreviations:* AE, assimilation efficiency; AR, accumulation ratio; BW, body weight; FE, feed efficiency; FI, feed intake; HBCDD, hexabromocyclododecane.

* Corresponding author.
*E-mail address:* catherine.jondreville@inra.fr (C. Jondreville).

http://dx.doi.org/10.1016/j.chemosphere.2017.03.064
0045-6535/

continue for a long time (ECHA, 2009).

Although technical HBCDD consists of 70–95% of γ-HBCDD and 5–30% of α- and β-HBCDD, the α-isomer predominates in wildlife as well as in animal-derived products (Marvin et al., 2011; Koch et al., 2015). This shift is generally ascribed to a rapid degradation of β- and γ-HBCDD compared to α-HBCDD, through debromination and hydroxylation (Hakk et al., 2012; Zhang et al., 2014). Moreover, the bio-isomerization of γ-HBCDD into β- and mostly α-HBCDD, as well as the recalcitrance of α-HBCDD to bio-isomerization has been demonstrated in mammals (Szabo et al., 2010, 2011a,b), fishes (Du et al., 2012; Zhang et al., 2014) and birds including laying hens (Fournier et al., 2012; Letcher et al., 2015; Dominguez-Romero et al., 2016). However, the extent to which γ-HBCDD is bio-isomerized to α-HBCDD is not sufficient to explain the predominance of α-HBCDD in products from farm animals exposed to a technical mixture. Especially, although the average levels of HBCDD concentrations in animal-derived food products are generally below 1 ng $g^{-1}$ lipid weight (lw) (EFSA, 2011), national monitoring plans conducted in France revealed concentrations in some samples of poultry eggs and meat reaching 3000 ng $g^{-1}$ lw, mainly as α-HBCDD. For such highly contaminated samples, it was hypothesized that animals may ingest fragments of insulating materials (Hiebl and Vetter, 2007; Zheng et al., 2012). Indeed, Cariou et al. (2014) reported that XPS used for insulating poultry houses contained up to 3.9% HBCDD, of which 75–80% was the α-isomer, and Dominguez-Romero et al. (2016) estimated that laying hens ingesting a quantity as small as 3.2 $mm^3$ of this XPS daily would lay eggs containing 3000 ng α-HBCD $g^{-1}$ lw. This latter study involved laying hens exposed to diets containing 3.6 or 40 ng α-HBCDD $g^{-1}$. By the end of the 18-week exposure period, 19% of ingested α-HBCDD had been eliminated through eggs and 19% was estimated to be retained in body tissues, mainly adipose tissues, yielding concentrations in abdominal fat, expressed as ng $g^{-1}$ lw, 9.2 times higher than in feed. According to the model developed by MacLachlan (2010), the tissues of fast-growing (FG) broilers submitted to the same dietary concentration of α-HBCDD would be around two times more concentrated than those of laying hens mainly due to 1. the absence of egg as an elimination route and 2. the higher relative affinity of non-polar lipophilic compounds to tissues due to the 4 to 5 times lower blood lipid concentration (Walzem et al., 1994; Baéza et al., 2015). In addition to FG broilers, which are an international standard, consumers demand products from alternative systems such as organic systems, involving slow-growing (SG) animals, which, for a raising time almost twice as long, ingest more feed for an often lower market weight, yielding a lower feed efficiency, and display different tissue growth and lipid content (Quentin et al., 2003). All these characteristics may impact the concentration of α-HBCDD in tissues of exposed broilers. The first aim of the current study was thus to compare the content of α-HBCDD in edible tissues of exposed FG and SG broilers at market age. In addition, as knowledge on HBCDD metabolism in birds is still sparse (Law et al., 2014), kinetics of this lipophilic and non-polar contaminant in relation to lipid deposition during growth in different tissues was investigated, the role of excreta as an elimination route was assessed and an attempt was made to identify metabolites of α-HBCDD in several tissues.

## 2. Materials and methods

### 2.1. Experimental feeds

Technical HBCDD, containing 1, 5 and 93% of α-, β- and γ-HBCDD, respectively, was enriched in α-isomer by thermal rearrangement (172 ± 0.4 °C, 6 h), then purified and precipitated at −20 °C as described by Dominguez-Romero et al. (2016). The resulting crystals (99.3% α-HBCDD and traces of η-HBCDD) were dissolved in acetone used to spike soy oil at 10 μg $g^{-1}$.

Experimental feeds were based on maize, wheat and soybean meal and included all the nutrients required by broilers (Table S1). Fast-growing broilers were given a grower diet throughout the 6-week experiment, while SG broilers were given a starter diet up to 3 weeks of age and then a grower diet up to 12 weeks of age. For each of them, one batch of control feed and one batch of spiked feed were prepared. The target concentration of 50 ng α-HBCDD $g^{-1}$ feeds was selected to obtain meat containing several hundreds of ng of HBCDD $g^{-1}$ lw as previously reported in French monitoring plans (DGAL, 2009). Similar environmentally relevant dietary concentrations have been tested by Dominguez-Romero et al. (2016) in laying hens and by Du et al. (2012) in fish. It was achieved by replacing 5 mg of clean oil per g of control feed by 5 mg spiked soy oil. Feeds were pelleted (diameter 2.5 mm).

### 2.2. Birds and experimental design

The experiment was conducted under the application of the Directive 2010/63/EU (2010) in France at the experimental unit (UE PEAT) of INRA Nouzilly (agreement n°C37-175-1). It was approved by the relevant ethics committee (CEEA Val de Loire).

Twenty-nine FG (Ross PM3, weighing 48.7 ± 0.1 g, mean ± SE) and 50 SG (JA657, weighing 42.5 ± 0.3 g) one-day old male chickens were used. Ten one-day old chickens of each strain were slaughtered as control. The remaining ones were raised by groups of 3 or 4 during 1 week to facilitate their adaptation and then placed in individual cages until the end of the experiment. All were vaccinated against Mareck and Gumboro diseases, and infectious bronchitis. Fifteen one-day old FG chickens were given the contaminated feed and were sequentially slaughtered by groups of 4–7 at 2, 4 or 6 weeks of age. The 4 remaining FG broilers were given the control diet up to 6 weeks of age. Thirty-four one-day old SG broilers received the contaminated feed for either 12 weeks, or 6 weeks followed by 6 weeks of depuration with the control feed. They were sequentially slaughtered by groups of 4 or 5 after 3, 6, 9 or 12 weeks of exposure or after 6 weeks of exposure followed by 1, 2, 4 or 6 weeks of depuration. The 6 remaining SG broilers were given the control feed. Two of them were slaughtered at 6 weeks of age and 4 at the end of the experiment (12 weeks). One-day old chickens were slaughtered by decapitation and the older ones by electrical stunning followed by bleeding, after a 12-h fast.

In accordance with the rules for the protection of chickens kept for meat production (Directive 2007/43/EC, 2007), room temperature was progressively reduced from 30 to 31 °C for the first week to 19–21 °C after 3 weeks of age and lighting was progressively reduced from 23 h a day for the first 3 days to 18 h a day after 1 week of age. The daily feed allowance was adjusted according to the official specifications for each strain and given once in the morning. Water was continuously available.

### 2.3. Measurements and sampling

Feed intake (FI) and body weight (BW) after a 12-h fast were individually recorded weekly. Feeds were sampled weekly and stored in the dark at 4 °C. A composite sample of each feed was used for analyses. Blood samples were collected in heparinized tubes in the occipital sinus just before sacrifice, except in control birds. Samples were kept on ice and centrifuged (2000 m $s^{-2}$, 10 min, 4 °C) to obtain plasma. Just after sacrifice, the whole muscles of one leg (*i.e.* thigh + drumstick) including intermuscular fat, one breast muscle (*Pectoralis major*), the liver and the abdominal fat were collected and weighed. Muscles are referred to as leg muscles and breast muscle, respectively. Except in control birds, the excreta