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

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Short title: Accumulation of α -HBCDD in broilers

Abbreviations

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

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.

Keywords: animal-derived food, brominated flame retardant, lipophilic contaminant, half-life, elimination

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 continue for a long time (ECHA, 2009).

Although technical HBCDD consists of 70 to 95% of γ -HBCDD and 5 to 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, 2011), 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 3,000 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 et al., 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 to 80% was the α -isomer, and Dominguez-Romero et al. (2016) estimated that laying hens ingesting a quantity as small as 3.2 mm³ of this XPS daily would lay eggs containing 3,000 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 et al. (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 $\pm$ 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 μ g 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 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 to 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-hour fast.

In accordance with the rules for the protection of chickens kept for meat production (Directive 2007/43/EC), room temperature was progressively reduced from 30-31 °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-hour 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 ($2,000\text{ m s}^{-2}$, 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 (faeces + urine) produced during 72 h before slaughter were totally collected and mixed. All the samples corresponded to individuals except those from the 10 control chickens sacrificed the first day of experiment, which were pooled to ensure sufficient sample sizes, and the excreta and plasma, which were pooled per date of slaughter. All samples except livers were stored at -20 °C. Liver samples, which were also used for metabolomics studies, were immersed in liquid nitrogen, wrapped in aluminum foil, vacuum packed and stored at -80 °C before being ground in liquid nitrogen.

2.4. Chemical analyses

HBCDD isomers (α -, β - and γ -) were analyzed in feed, muscle tissue, abdominal fat, liver tissue, plasma and excreta according to ISO/IEC 17025:2005 accredited methods, as described by Bichon et al. (2015) pending slight modifications (plasma digestion and extraction) and by Dominguez-Romero et al. (2016) (solid matrices extraction, all matrices purification and analysis). Feed was dried in an oven at 80 °C and other solid matrices, including excreta, were lyophilized prior to extraction by pressurized liquid extraction (PLE). Plasma was digested with one volume of formic acid at room temperature during 20 min, diluted with one volume of ultrapure water, loaded on a C18 SPE preconditioned with 5 mL acetonitrile and 5 mL ultrapure water, washed with 20 mL ultrapure water, dried under vacuum during 2 h and eluted with 20 mL *n*-hexane. For all matrices, purification steps involved multilayer (neutral and H₂SO₄ modified) silica gel SPE columns, followed by partitioning between *n*-hexane and 1 N NaOH. Final extracts were analyzed by LC-ESI(-)-MS/MS (6410, Agilent Technologies), monitoring $[M - H]^- > [Br]^-$ transitions. Quantification was achieved according to the isotopic dilution principle (¹³C-labeled isomers as internal standards and ²H₁₈- β -HCBDD as external standard). Limits of quantification (LOQ) were determined individually for each sample and isomer. A reporting limit (LoR) higher than each LOQ was established by matrix at 0.03 ng g⁻¹ feed (at 12% moisture), at 0.3 ng g⁻¹ lw for muscle tissue, at 0.2 ng g⁻¹ lw for liver tissue and abdominal fat, at 0.02 ng g⁻¹ dry weight (dw) in excreta and at 0.006 ng mL⁻¹ in plasma.

Control and contaminated feeds given to SG broilers and matrix pools of samples collected in SG broilers slaughtered after 12 weeks of exposure or on the control feed (leg and breast muscles, liver, abdominal fat) were analyzed using LC-HRMS to screen for brominated compounds, impurities or metabolites, as described by Cariou et al. (2016). Briefly, sample PLE extracts were partitioned between *n*-hexane and H₂SO₄, and fingerprints acquired by LC-ESI(-)-HRMS (Q-Exactive, Thermo Scientific) over the *m/z* range 400–750 at a resolving power of 70,000 (fwhm @200). Then, a filtering script was applied to acquired data to highlight brominated clusters.

Lipid concentration in abdominal fat was determined gravimetrically after the PLE step of HBCDD isomers analysis. In muscle and in liver, it was determined according to the chloroform/methanol method described by Folch et al. (1957). For these latter tissues, the classes of lipids were determined using Iatroscan (Iatron, Tokyo, Japan) thin layer chromatography with a flame ionisation detector system (TLC-FID) permitting to determine the concentrations of triglycerides (TG), esterified cholesterol (EC), free cholesterol (FC) and phospholipids (PL) according to Baéza et al. (2010). Lipid classes in plasma were determined according to 4 enzymatic kits (Biolabo, Maizy, France) (Marchand et al., 2010) and the total lipid content (g L^{-1}) was then calculated as $\text{TG} + \text{EC} + \text{FC} + \text{PL}$.

2.5. Calculations and statistical analyses

Feed intake (FI, g) and body weight gain (BWG, g) from the first day of experiment were individually calculated each week. Daily feed intake relative to BW ($\text{g feed kg}^{-1} \text{ BW d}^{-1}$) and feed efficiency (FE, $\text{g BWG g}^{-1} \text{ FI}$) were calculated as the ratio of daily FI (g d^{-1}) to the mean BW (kg) and of BWG (g) to FI (g) on the considered period, respectively. Neutral lipids concentration in tissues was calculated as the sum of TG, EC and 30% PL (Poulin and Haddad, 2012).

Within each strain, the accumulation ratio (AR) and the assimilation efficiency (AE) of α -HBCDD in each tissue were calculated at market age, i.e. 6 and 12 weeks in FG and SG broilers, respectively. AR was calculated as $C_{\text{tissue}}/C_{\text{feed}}$, with C_{tissue} , the concentration of α -HBCDD in the tissue ($\text{ng g}^{-1} \text{ lw}$) and C_{feed} its concentration in feed ($\text{ng g}^{-1} \text{ fresh weight, fw}$). AE was calculated as $(C_{\text{tissue}} \times \text{lip}_{\text{tissue}} \times 100)/(C_{\text{feed}} \times \text{FI})$, with $\text{lip}_{\text{tissue}}$ the lipids content of the tissue (g), accounting for two legs and two breasts.

Over each balance study of 72 hours, the amount of α -HBCDD excreted was calculated as the concentration of α -HBCDD in excreta, expressed in $\text{ng g}^{-1} \text{ dw}$, multiplied by the amount of dry matter excreted, assuming that 30% of ingested dry matter is excreted through feces (Jurjanz et al., 2014). Then, the cumulative excretion of α -HBCDD was calculated considering that the retention coefficient of α -HBCDD is constant over each 3 or 2-week period between two slaughter dates.

Statistical analyses were performed by means of the Statistical Analysis Systems software package (SAS, version 9.1, SAS Institute, Cary, NC, USA), using the broiler as experimental unit. Differences were considered significant for P -values < 0.05 . First, the absence of impact of exposure to α -HBCDD on performance (BW, FI, FE) and on the weight and lipid content of tissues was checked within each strain, as described in supporting information (tables S2, S3, S4 and S5).

Control broilers were excluded from the following analyses. Within each strain, concentrations of α -HBCDD in tissues ($\text{ng g}^{-1} \text{lw}$) were analysed as repeated measures using the MIXED procedure, as described by Dominguez-Romero et al. (2016). The model included the tissue as repeated measure in order to account for the correlation between tissues collected from the same broiler, the treatment, which corresponds to the duration of exposure for FG broilers ($n=3$) and to the combined exposure and depuration durations for SG broilers ($n=8$), and the interaction between the tissue and the treatment. A Scheffe test was used for comparison of means. Strains were compared at market age for lipid concentration and content in tissues as well as AR and AE of α -HBCDD, according to a model similar to the one described above, including the tissue as repeated measure, the strain and their interaction.

Kinetic parameters of α -HBCDD depuration were estimated in each tissue of SG broilers as described in OECD (2012) by adjusting first order kinetics models to lipid deposition (g) and to α -HBCDD concentration ($\text{ng g}^{-1} \text{lw}$) in tissues of broilers exposed during 6 weeks and depurated during 0 to 6 weeks. In each tissue, the lipid deposition constant rate ($k_{\text{glip}}, \text{d}^{-1}$) was estimated through a linear regression of the following form: $\ln(\text{lip}_{\text{tissue}}) = a + k_{\text{glip}} \times t_d$ with t_d the depuration duration (d). Similarly, the overall depuration rate constant (k, d^{-1}) was estimated through the regression $\ln(C_{\text{tissue}}) = b - k \times t_d$. The lipid deposition-corrected depuration rate constant k_d was calculated as $k - k_{\text{glip}}$.

3. Results and discussion

No β - and no γ -HBCDD were detected in any of the feeds. No α -HBCDD was detected in the control feeds, while the assayed concentrations in spiked feeds were $35.7 \text{ ng } \alpha\text{-HBCDD g}^{-1}$ in the grower feed

given to FG broilers, and 37.2 and 38.7 ng α -HBCDD g⁻¹ in the starter and grower feeds given to SG broilers, respectively.

3.1. Feed ingested, growth and tissue characteristics

Within each strain, BW, FI, FE, daily FI relative to BW, as well as the weight and the lipid concentration in tissues were overall not influenced by exposure to α -HBCDD (tables S2, S3, S4 and S5). Similarly, exposure of laying hens to diets containing up to 40 ng α -HBCDD g⁻¹ during 18 weeks (Dominguez-Romero et al., 2016), of zebrafish to diets containing up to 50 ng α -HBCDD g⁻¹ during 42 days (Du et al., 2012) or of Atlantic salmon to diets containing 280 ng α -HBCDD g⁻¹ (Bertnssen et al., 2011a) did not elicit any disturbance in their growth or lipid deposition in tissues.

As expected (Lewis et al., 1997; Quentin et al., 2003), SG broilers exhibited lower growth performance compared to FG broilers. Within 6 weeks, the BW of FG broilers increased by 2,762 $\pm$ 44 g to a final BW of 2,810 $\pm$ 44 g. Body weight gain and final BW reached within 12 weeks by SG broilers were 7% lower (2,574 $\pm$ 20 g and 2,616 $\pm$ 20, respectively) (Fig. 1). At the end of the experiment, SG broilers had ingested 42% more feed than FG broilers (6,543 $\pm$ 14 vs. 4,609 $\pm$ 55 g) and their FE was 34% lower (0.393 $\pm$ 0.003 vs. 0.599 $\pm$ 0.007 g BWG g⁻¹ FI). The total amount of α -HBCDD ingested by FG and SG and broilers was 167 $\pm$ 2 and 253 $\pm$ 1 μ g, and 59.6 $\pm$ 0.9 and 98.8 $\pm$ 1.0 μ g kg⁻¹ BWG, respectively (Fig. 2, Figure S1). Conversely, the amount of α -HBCDD daily ingested relative to BW was higher in FG than in SG broilers (2.74 $\pm$ 0.04 and 2.28 $\pm$ 0.02 μ g kg⁻¹ BW d⁻¹, respectively; Figure S1).

Lipid concentration in leg muscles at market age was higher in SG than in FG broilers (52.5 $\pm$ 6.3 vs. 31.0 $\pm$ 3.3 g kg⁻¹ fw, P < 0.05), while the two strains displayed similar breast muscle lipid concentrations (13.1 $\pm$ 1.2 vs. 13.2 $\pm$ 0.9 g kg⁻¹ fw, P > 0.05) (Table 1). However, in accordance with Quentin et al. (2003), the amount of lipids deposited in leg muscles were higher in SG aged over 8 weeks than in FG broilers aged over 4 weeks (over 1,425 g BW), while the contrary was observed in breast muscle (Figure S2). At market age, lipids deposited in leg muscles of SG and FG broilers were 19.9 $\pm$ 2.4 and 10.2 $\pm$ 1.3 g (P < 0.05), respectively, while breast muscle contained 3.8 $\pm$ 0.2 and 6.8 $\pm$

0.5 g lipids ($P < 0.05$), respectively (Table 1). This kinetics also highlights a slow lipid deposition compared to BW gain in leg muscles and abdominal fat of SG broilers aged less than 3 weeks (Figure S2). Slightly fewer lipids were deposited in abdominal fat of SG compared to FG broilers (34.2 ± 3.8 g vs. 40.4 ± 2.5 , $P < 0.05$), although SG broilers are expected to deposit more abdominal fat than FG broilers (Quentin et al., 2003).

In accordance with previous observations (Marion and Woodroof, 1965; Santoso et al., 1995; Baéza et al., 2015), $83 \pm 1\%$ of leg muscles lipids were neutral, due to the presence of intermuscular fat, while this proportion was $71 \pm 1\%$ in breast muscle, $65 \pm 1\%$ in liver and $49 \pm 1\%$ in plasma, irrespective of the strain (Table S6). Although not assessed in the current study, lipids profile in abdominal fat are almost totally made of neutral lipids (Sinanoglou et al., 2011).

3.2. Concentration of HBCDD in tissues

The γ - isomer was quantified in 42% and 47% of tissue samples from control and exposed broilers, respectively. For the β - isomer, these proportions were 17 and 10%, respectively. The α - isomer was quantified in 75% of tissue samples from control broilers, resulting in a fairly constant profile (43, 14 and 14% of α -, β - and γ -HBCDD, respectively) (results not shown). Similar β - and γ -HBCDD concentrations in tissues from control and exposed broilers suggest that no isomerization of α - to γ - or β -HBCDD occurred as previously reported in mammals, in fishes and in birds (e.g. Szabo et al., 2011a; Zhang et al., 2014; Dominguez-Romero et al., 2016). In order to account for the overall procedural contamination, α -HBCDD concentrations in tissues of exposed broilers were individually corrected using the HBCDD profile observed in controls.

In the two strains, the concentration of α -HBCDD ($\text{ng g}^{-1} \text{lw}$) was higher in abdominal fat than in breast muscle and in liver, while leg muscles displayed intermediate concentrations (Tissue, $P < 0.001$) (Table S7, Fig. 3). Plasma pooled samples displayed concentrations in the order of the ones in liver. Irrespective of the strain, the AR of α -HBCDD in leg muscles and breast muscle at market age was around 1.6 and 4.3 times lower than in abdominal fat, respectively (Table 1, Table 2). On a fresh

weight basis, these AR were 40 and 260 times lower than in abdominal fat, respectively (Table 2). Thus the consumption of leg muscles leads to an exposure about 7 times higher than the consumption of breast muscle. Fatty tissues were also reported to be the major tissue depot for α -HBCDD in mice (Szabo et al., 2011a, 2011b), in mirror carp (Zhang et al., 2014), in American kestrels (Letcher et al., 2015), as well as in laying hens (Dominguez-Romero et al., 2016). The affinity of non-polar lipophilic contaminants for neutral rather than for total lipids (Poulin and Haddad, 2012) partly explains this ranking of tissues.

The AR in abdominal fat of FG broilers was 8.4 ± 0.6 (Table 2), which is very similar to 9.2 ± 0.6 estimated in laying hens at steady state (Dominguez-Romero et al., 2016). This result is at variance with a higher AR in FG broilers than in laying hens predicted by MacLachlan (2010). Overall, ARs were higher in SG than in FG broilers ($P < 0.05$) (Table 2). However, the difference between the two strains greatly varied between tissues, as the ARs in leg muscles, liver and abdominal fat were 30, 113 and 18% higher, respectively, in SG than in FG broilers ($P < 0.05$), while they were similar in breast muscle ($P > 0.1$). These differences between the two strains are coherent with the aforementioned differences in lipid concentrations in their respective tissues (Table 1). In contrast, in accordance with lower amounts of lipids deposited in breast muscle and in abdominal fat, the AE of α -HBCDD in SG broilers was 59 and 19% lower, respectively, than in FG broilers (0.14 ± 0.01 vs. $0.33 \pm 0.07\%$ and 5.9 ± 0.7 vs. $7.2 \pm 0.3\%$, respectively, $P < 0.05$), while, in relation to a greater amount of lipids deposited, the AE of α -HBCDD in leg muscles tended to be higher in SG broilers than in FG broilers (1.7 ± 0.4 vs. $1.1 \pm 0.2\%$, $P > 0.05$) (Table 1, Table 2).

In addition to their overall ranking, tissues displayed variable kinetics within each strain (treatment $\times$ tissue, $P < 0.001$) (Table S7, Fig. 3). In animals having completed their growth, α -HBCDD is first distributed to highly perfused tissues such as liver and then to poorly perfused tissues such as abdominal fat (Szabo et al., 2011a; Zhang et al., 2014; Dominguez-Romero et al., 2016). In the current study, α -HBCDD concentration in tissues also depends on the kinetics of exposure relative to BW and of tissue lipid deposition.

Within 6 weeks of depuration, α -HBCDD concentration in tissues of SG broilers was divided by 4.1 in leg muscles, 11.4 in breast muscle, 4.5 in liver and in plasma and 5.3 in abdominal fat. First order kinetics models could be adjusted to lipid deposition (g) and α -HBCDD concentration ($\text{ng g}^{-1} \text{lw}$) in all tissues except liver (Table 3, Fig. 3). The overall depuration rate constants were 0.0340 ± 0.0029 , 0.0593 ± 0.0050 , $0.0374 \pm 0.0024 \text{ d}^{-1}$, corresponding to overall half-lives of 20 ± 2 , 12 ± 1 and $19 \pm 1 \text{ d}$ in leg muscles, breast muscle and abdominal fat, respectively. Corrected for lipid deposition in tissues, depuration rate constants were divided by 2.6, 2.0 and 2.6 and, in turn, half-lives multiplied by the same factors, to reach the values of 53, 24 and 47 d, respectively, in accordance with the more active irrigation of intramuscular fat, compared to intermuscular and peripheral fats. Elimination half-lives of similar order were reported in viscera of mirror carps (12 d) and in their muscle and skin (28-30 d) (Zhang et al., 2014). In laying hens, the elimination half-life in abdominal fat was shorter (35 d), probably due to the contribution of egg laying to α -HBCDD elimination (Dominguez-Romero et al., 2016). Thus, with a high capacity of accumulation in body fat and intermediate half-life, α -HBCDD may be classified as highly to moderately cumulative among the persistent organic pollutants possibly transferred to animal-derived food. Indeed, according to Kan and Meijer (2007), the former, such as DDT, display an AR from feed to body fat around 10 and a half-life of 50 to 70 days, while the latter, such as lindane, an AR in body fat of around 2 and a half-life of 7 to 14 days.

It was expected that the estimated lipid deposition-corrected depuration rate constants could be used for estimating the uptake rate constant and the bioaccumulation factor of α -HBCDD in SG broilers (OECD, 2012). Unfortunately, the number of sampling points between 0 and 6 weeks was insufficient to properly describe the slow lipid deposition in leg muscles and in abdominal fat of SG broilers (Figure S2), which probably reflects an overall slow body fat deposition. Nevertheless, associated to a high level of exposure relative to BW at the beginning of the experiment (Figure S1), it may explain the peak of α -HBCDD concentration up to 347 ± 15 and $608 \pm 35 \text{ ng g}^{-1} \text{lw}$ at 3 weeks of exposure, respectively (Fig. 3, Table S7).

3.3. Detection of other brominated compounds in feed and in tissues

In the control feeds and pooled control sample of tissues, no brominated compound other than HBCDD (procedural contamination in tissues) was detected. In contrast, three pentabrominated compounds were detected in the contaminated feeds. The tentative chemical formulas were $C_{12}H_{19}Br_5$ (pentabromocyclododecane, PeBCDD), $C_{12}H_{17}Br_5$ (pentabromocyclododecene, PeBCDDe) and $C_{12}H_{18}Br_5Cl$ (pentabromochlorocyclododecane, PeBCICDD), with relative signal areas representing 0.016, 0.11 and 0.32% of that of α -HBCDD, respectively. The same pentabrominated compounds were detected in all pooled samples from individuals after 12 weeks of exposure, with relative signal areas to that of α -HBCDD in the 0.01-0.06, 0.18-0.72 and 0.15-0.30% ranges, respectively, depending on the matrix. Additionally, signals matching monohydroxylated HBCDD (OH-HBCDD) were detected in leg muscle and liver pools of exposed individuals only, with relative signal areas representing 0.004 and 0.014% of that of α -HBCDD, respectively. Theoretically, OH-HBCDD shows six isomers, but only one chromatographic peak was detected. The detection of OH-HBCDD in animal matrices only suggests that it would be the only product from α -HBCDD metabolism. Similarly, OH-HBCDD was reported as the only metabolic product in tissues of mice (Hakk et al., 2012) and of laying hens (Dominguez-Romero et al., 2016) exposed to α -HBCDD.

3.4. Balance of α -HBCDD

According to equations predicting the body lipid content of broilers of different strains from their BW (Henn et al., 2014), FG and SG broilers at slaughter would contain 118 and 131 g lipid kg^{-1} BW at slaughter. Assuming that most of the body lipids not included in the assessed tissues are made of neutral lipids (osseous, intermuscular, subcutaneous and visceral fat) and, thus, are as concentrated in α -HBCDD as abdominal fat, the body burden of α -HBCDD would be 31% higher in SG than in FG broilers (126 vs. 96 μg), for α -HBCDD intake 51% higher (253 vs. 167 μg), yielding an assimilation efficiency 8 percentage units lower (50 vs. 58%) (Table S8). The lower feed efficiency of SG broilers

compared to FG broilers (-34%) would thus be partly modulated by 1. their higher body lipid deposition and 2. their lower daily exposure to α -HBCDD relative to BW (-15%).

These AEs are in the order of 56% of ingested α -HBCDD recovered in tissues in Atlantic Salmon, considering that lipids present in fillets represent 62% of body lipids and that AE in fillet is 35% (Berntssen et al., 2011a, 2011b). In laying hens exposed during 18 weeks to α -HBCDD, 19% of ingested α -HBCDD was eliminated through eggs and 19% was estimated to be retained in body tissues, reaching a total of 38%. This lower value compared to broilers may be due to the high plasma neutral lipids concentration in laying hens, which would limit the distribution of α -HBCDD to tissues and thereby stimulate its elimination through metabolism.

Neither γ - nor β - HBCDD was detected in excreta. During exposure, the concentration of α -HBCDD in excreta increased from 22 to 27 ng g⁻¹ dw and from 21 to 54 ng g⁻¹ dw in FG and SG broilers, respectively, corresponding to the excretion as intact compound of 17 to 20% and of 16 to 33% of α -HBCDD ingested, respectively (Table S9). Considering that 10% of α -HBCDD ingested is not absorbed (Szabo et al., 2011a), 7 to 10% and 6 to 23% of α -HBCDD ingested would thus be absorbed and re-excreted intact in excreta, respectively. This is in the order of the results of Szabo et al. (2011a, 2011b) who reported that 24% of ingested α -HBCDD was absorbed and re-excreted intact in feces of mice, through non biliary processes. In birds including chicken, non biliary fecal excretion of polychlorinated biphenyls was reported as a result of a significant partitioning capacity of excreta for these lipophilic compounds (Polin et al., 1989; Drouillard and Norstrom, 2003). At the end of the exposure period, it was estimated that 80 and 67% of α -HBCDD ingested are not excreted intact, while the AE estimated through body burden is 58 and 50%, respectively in FG and SG broiler. This discrepancy may be due to the elimination of α -HBCD as polar metabolites, which would thus amount 22 and 17% of α -HBCDD ingested, respectively (Fig. 4). This proportion is in the order of 22% of α -HBCDD eliminated as polar metabolites through feces and urine by mouse (Szabo et al., 2011a, 2011b). The concentration of α -HBCDD in excreta was divided by 100 within 1 week of depuration in SG broilers, so that elimination as intact α -HBCD during the depuration period did not reach 1% of α -

HBCD ingested (Table S9). This indicates that the aforementioned diffusion process would be strongly limited during depuration which may be linked to the drastic reduction in plasma α -HBCD concentration promptly after the cessation of exposure.

4. Conclusion

The current study confirms the absence of isomerization of α - to β - or γ -HBCDD, the production of OH-HBCDD as metabolic product and the significant accumulation and persistence of α -HBCDD in fatty tissues. It highlights that the consumption of contaminated leg muscles would elicit a higher exposure to α -HBCDD than breast muscle. This results from the greater concentration of lipids particularly rich in neutral lipids and therefore more concentrated in α -HBCDD in leg muscles compared to breast muscle. Despite a markedly lower feed efficiency and a longer raising time, the assimilation efficiency of α -HBCDD in tissues of SG broilers is only slightly lower than in FG broilers and the concentration in tissues, especially in the main edible muscles, only slightly higher.

ASSOCIATED CONTENT

Supporting information available

Feeds composition (Table S1); body weight, feed ingested and tissue characteristics of broilers (Tables S2, S3, S4, S5, S6; Figures S1, S2); α -HBCDD concentration in animal tissues (Table S7); estimates of α -HBCDD assimilation efficiency (Table S8); balance of α -HBCDD (Table S9). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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547

Table 1

Lipid concentration and content in tissues of slow-growing (SG) and fast-growing (FG) broilers at market age¹

	Lipid (g kg ⁻¹ fw)		Lipid (g) ²	
	FG	SG	FG	SG
Leg muscles	31.0 ± 3.3a	52.5 ± 6.3b	10.2 ± 1.3a	19.9 ± 2.4b
Breast muscle	13.2 ± 0.9	13.1 ± 1.2	6.8 ± 0.5b	3.8 ± 0.2a
Liver	47.8 ± 1.3	59.0 ± 2.6	2.2 ± 0.1	2.8 ± 0.2
Abdominal fat	883 ± 9a	906 ± 8b	40.4 ± 2.5b	34.2 ± 3.8a
Probability ³				
Strain	< 0.01		0.87	
Tissue	< 0.001		< 0.001	
Strain*tissue	0.10		< 0.01	
RSD	17.9		7.0	

¹Market age is 6 and 12 weeks in fast- and slow-growing broilers, respectively

²Accounting for two legs and two breasts

³Data were analyzed as repeated measures as specified in the materials and methods section. Values in the same row not followed by the same letter (a, b) differ ($P < 0.05$). RSD is the residual standard deviation of the model.

Table 2

Accumulation ratio (AR) and assimilation efficiency (AE) of α -HBCDD in tissues of slow-growing (SG) and fast-growing (FG) broilers at market age¹

	AR ⁴		AE ^{2,5} (%)	
	FG	SG	FG	SG
Leg muscles	5.2 ± 0.3a	6.7 ± 0.7b	1.1 ± 0.2	1.7 ± 0.4
Breast muscle	2.2 ± 0.3	2.1 ± 0.1	0.33 ± 0.07b	0.14 ± 0.01a
Liver	1.2 ± 0.1	2.6 ± 0.4	0.06 ± 0.00	0.10 ± 0.03
Abdominal fat	8.4 ± 0.6a	9.9 ± 0.9b	7.2 ± 0.3b	5.9 ± 0.7a
Probability ³				
Strain	< 0.03		0.30	
Tissue	< 0.001		< 0.001	
Strain*tissue	0.17		0.01	
RSD	1.0		0.68	

^{1, 2, 3}See table 1

⁴AR, accumulation ratio calculated as the ratio of the concentration of α -HBCDD in the tissue (ng g⁻¹ lw) to its concentration in feed (ng g⁻¹ fw)

⁵AE, assimilation efficiency calculated as the percentage of ingested α -HBCDD recovered in each tissue

Table 3. Parameters of the kinetics of depuration of α -HBCDD in tissues¹ in slow-growing broilers

Tissue	Leg muscles	Breast muscle	Abdominal fat
k_{glip} (d^{-1})	0.0210 ± 0.0036	0.0300 ± 0.0019	0.0228 ± 0.0037
R^2	0.90	0.97	0.98
RSD	2.05	0.17	1.94
k (d^{-1})	0.0340 ± 0.0029	0.0593 ± 0.0050	0.0374 ± 0.0024
R^2	0.96	0.98	0.93
RSD	8.7	3.1	22
Overall $t_{1/2}$ (d)	20 ± 2	12 ± 1	19 ± 1
$k_d \times 10^{-3}$ (d^{-1})	13.0	29.3	14.6
$t_{1/2}$ (d)	53	24	47

¹ No first order kinetics model could be adjusted to liver depuration data

Estimates ($\pm$ standard error) of the lipid deposition rate constant (k_{glip}) and of the overall depuration rate constant (k) were obtained as described in the materials and methods section; R^2 and RSD are the coefficient of determination and the residual standard deviation of the corresponding regression analysis, respectively. The lipid deposition-corrected depuration rate constant (k_d) was calculated as $k - k_{\text{glip}}$. The overall and lipid-deposition-corrected half-lives are calculated as $\ln(2)/k$ and $\ln(2)/k_d$, respectively.

Fig. 1. Age-dependent body weight of fast- and slow-growing broilers

Values are means

Fig. 2. Ingestion of α -HBCDD by fast- and slow-growing broilers

Values are means; labels correspond to the age (week) of broilers

Fig. 3. Age-dependent concentrations of α -HBCDD (ng g⁻¹ lw) in tissues fast-growing (FG) and slow-growing (SG) broilers during exposure and depuration

LM, leg muscles; BM, breast muscle; L, liver; AF, abdominal fat; P, plasma

The data points are means $\pm$ standard error as presented in Table S7. Within each strain, values at the same age not followed by the same letter (a, b, c, d) differ ($P < 0.05$). Concentrations in plasma, obtained on pooled samples, were not introduced in the statistical analysis.

On the graph representing depuration in SG broilers, the lines correspond to the first order kinetics models presented in Table 3.

602 **Fig. 4.** Estimates of the balance of α -HBCDD in fast-growing (FG) and slow-growing (SG) broilers at
 603 the end of the experiment

Table 1

Lipid concentration and content in tissues of slow-growing (SG) and fast-growing (FG) broilers at market age¹

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Table 2

Accumulation ratio (AR) and assimilation efficiency (AE) of α -HBCDD in tissues of slow-growing (SG) and fast-growing (FG) broilers at market age¹

	AR ⁴		AE ^{2,5} (%)	
	FG	SG	FG	SG
Leg muscles	5.2 ± 0.3a	6.7 ± 0.7b	1.1 ± 0.2	1.7 ± 0.4
Breast muscle	2.2 ± 0.3	2.1 ± 0.1	0.33 ± 0.07b	0.14 ± 0.01a
Liver	1.2 ± 0.1	2.6 ± 0.4	0.06 ± 0.00	0.10 ± 0.03
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^{1, 2, 3}See table 1

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RSD	2.05	0.17	1.94
k (d^{-1})	0.0340 ± 0.0029	0.0593 ± 0.0050	0.0374 ± 0.0024
R^2	0.96	0.98	0.93
RSD	8.7	3.1	22
Overall $t_{1/2}$ (d)	20 ± 2	12 ± 1	19 ± 1
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