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# Enantiomer-specific accumulation and depuration of $\alpha$ -hexabromocyclododecane ( $\alpha$ -HBCDD) in chicken (*Gallus gallus*) as a tool to identify contamination sources

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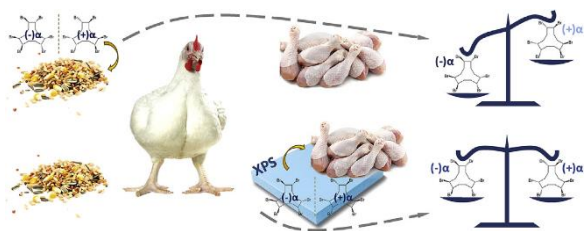
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## Keywords

Hexabromocyclododecane, chiral chromatography, enantiomeric enrichment, livestock, broiler, chemical risk assessment.

## Abstract

A LC-ESI(-)-HRMS method dedicated to the analysis of 6 HBCDD enantiomers at trace levels in animal matrices was developed, using a cellulose based stationary phase with a particle size of 2.5 mm. This method was applied to a sample set derived from a kinetic study of  $\alpha$ -HBCDD previously conducted in fast- and slow-growing chickens (*Gallus gallus domesticus*, n = 49, plus controls), in order to study the enantiomer specific accumulation and depuration of  $\alpha$ -HBCDD in various tissues. Regarding abdominal adipose tissue, muscle and liver, the average enantiomeric fractions of  $\alpha$ -HBCDD (EF $\alpha$ ) for continuously exposed groups ranged between 0.434 and 0.467, with standard deviations below 0.014, showing a significant enrichment in (-) $\alpha$  enantiomer even accentuated for slow growing individuals during depuration with EF $\alpha$  reduced by about 0.020. Similar trends were observed for pooled plasma. Then, EF $\alpha$  of circulating plasma  $\alpha$ -HBCDD appeared to closely reflect EF $\alpha$  in storage tissues and liver, suggesting some equilibrium. The racemic elimination of  $\alpha$  enantiomer in excreta during the contamination phase indicated that no preferential gastrointestinal absorption took place. By contrast, preferential excretion of (-)  $\alpha$ -HBCDD from the circulating compartment to the intestinal lumen occurred during the depuration. Finally, the method was applied to samples collected in three chicken farms, selected for total HBCDD levels in muscle in the ng/g range, as a tool to determine whether the contamination occurred ante- or post-mortem, according to the chiral signature. Ante-mortem contamination was hypothesised for 2 farms, with feed being excluded as contamination source.



## 1. Introduction

1,2,5,6,9,10-Hexabromocyclododecane (HBCDD) is a brominated flame-retardant aiming at decreasing the flammability of polymeric consumer products. This aliphatic cyclic hydrocarbon is mainly used in building as an additive for thermal insulation material in extruded (XPS) and expanded (EPS) polystyrene foam (ECHA, 2009). Despite its interesting industrial applications, HBCDD proved to be persistent, transportable over long distances, bio-accumulative, biomagnificative and toxic to both human and wildlife. Therefore, in 2014, HBCDD was listed in Annex A of the Stockholm Convention on Persistent Organic Pollutants, resulting in a ban on its production and use. Nevertheless, due to the long lifespan of insulation materials, release of HBCDD into the environment and the human food chain is expected to continue for a long time (ECHA, 2009).

Technical HBCDD consists of a racemic mixture of 3 main enantiomer pairs:  $\gamma$ -HBCDD (75-89%),  $\beta$ -HBCDD (5-13%) and  $\alpha$ -HBCDD (1-12%) (Heeb et al., 2005, 2007; Köppen et al., 2007). Although  $\gamma$ -HBCDD dominates in the technical mixture,  $\alpha$ -HBCDD dominated in biota (Tomy et al., 2004; Morris et al., 2004; Janak et al., 2005) and most processed food (Barghi et al., 2016). This shift is generally ascribed to (i) rapid biodegradation of  $\beta$ - and  $\gamma$ -HBCDD compared to  $\alpha$ -HBCDD (Hakk et al., 2012), (ii) bio-isomerisation of  $\gamma$ -HBCDD into  $\beta$ - and mostly  $\alpha$ -HBCDD (Szabo et al., 2011; Fournier et al., 2012) as well as (iii) thermodynamic rearrangement of  $\gamma$ -HBCDD into profile dominated by  $\alpha$ -HBCDD above 160 °C, perhaps during the extruding process (Jeannerat et al., 2016).

Diet is considered as a major route of exposure for human through foodstuffs of animal origin including livestock animals (EFSA, 2011). Consequently, it is important to identify the mechanisms underlying HBCDD entry into the food chain for subsequent implementation of risk management options. With regard to possible contamination sources, HBCDD could be transmitted to animals via animal feed (Ortiz et al., 2011; Fernandes et al., 2016) or through ingestion of insulation materials debris. Contamination might also occur during food processing (post-mortem) by direct or indirect contact with a contaminated material (Rani et al., 2014). We recently described the fate of  $\alpha$ -HBCDD in dietary exposed chicken (Jondreville et al., 2017):  $\alpha$ -HBCDD preferentially accumulated in fatty tissues and was more concentrated in leg muscles than in breast muscle. The elimination half-lives in slow-growing chicken were 20 and 12 days in leg and breast muscles, respectively, to which dilution through growth contributed around 50%. The edible muscles were similarly or slightly more concentrated in  $\alpha$ -HBCDD in slow- compared to fast-growing chickens.

## 2. Material and Methods

### 2.1. Chemicals

Reference solutions of native (a-, b-, g-, h-) as well as  $^{13}\text{C}_{12}$ -labelled (a-, b-, g-) and  $^2\text{H}_{18}$ - $\beta$ -HBCDD stable isotopologues were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Silica gel (70/230 mesh) was obtained from

Due to stereospecific biological processes, enantiomeric enrichments have been reported in biota, depending on species. Indeed, enrichments in (+) $\alpha$  or (-) $\alpha$  enantiomer were reported in various crustacean, fish, bird and marine mammal and human species (Janak et al., 2005; Köppen et al., 2010; He et al., 2010; Abdallah and Harrad, 2011; Zhang et al., 2014). Furthermore, even if enantiomeric pairs exhibit identical physical-chemical properties, they mostly display different toxicological activities (Müller and Kohler, 2004). Indeed, Zhang et al. (2008), reported that (-) $\alpha$ -, (-) $\beta$ - and (-) $\gamma$ -HBCDD enantiomers were significantly less cytotoxic toward Hep G2 cells compared to corresponding (+)-enantiomers.

Therefore, chiral signature appears as a marker of biological processes and a useful tool to understand if a food from animal origin was contaminated ante- or post-mortem, thereby revealing sources of contamination. During the past decade, instrumental developments in enantioselective trace analysis have brought about the development of powerful tools for environmental risk assessment and source apportionment. To date, studies relative to the chiral analysis of HBCDDs have been performed by liquid chromatography (LC) using a permethylated  $\beta$ -cyclodextrin stationary phase (Nucleodex b-PM, 4 mm x 200 mm, 5 mm) (Heeb et al., 2005; Köppen et al., 2007), which is preferred to other chiral columns of similar particle size (Guerra et al., 2008). However, such particle size appears relatively high as regard to recent advances and practices in analytical chemistry, thus limiting chromatographic resolution. Additionally, the optimal flow, close to 1 mL min<sup>-1</sup>, does not ideally fit with electrospray ionisation (ESI) prior to mass spectrometry (MS) analysis, the most suited technique for detection. Indeed, most authors use triple quadrupole mass analysers and high resolution Orbitrap analysers have been also successfully used (Zacs et al., 2014). Recently, alternative modified cellulose chiral columns with 2.5 mm particle size became commercially available and were reported to suit for HBCDD enantiomers separation using supercritical fluid chromatography prior to MS detection (Riddell et al., 2016).

The aim of the present study was (i) to develop a new LC method dedicated to HBCDD enantiomers compatible with ESI in terms of optimal flows, (ii) to study the enantiomer specific accumulation and depuration of  $\alpha$ -HBCDD in different tissues of livestock chicken and (iii) to apply this knowledge as a tool to hypothesise antemortem (e.g. feed or insulation materials ingestion) or postmortem contamination sources at the farm scale based on the specific chiral signature.

Fluka (Buchs, Switzerland), sodium sulfate and ammonium acetate from Merck (Darmstadt, Germany). Sulfuric acid (98%) was purchased from Panreac (Barcelona, Spain) and 1 N sodium hydroxide from Rea-gecon (Clare, Ireland). Toluene, acetone and n-hexane were provided by LGC Promochem (Picograde®, Wesel, Germany) and dichloromethane by Biosolve (Valkenswaard, The Netherlands). LC-MS grade water, acetonitrile and methanol were obtained from Sigma-Aldrich (ChromaSolv, St. Louis, MO, USA).

## 2.2. Feed preparation and experimental design

Synthesis and purification of  $\alpha$ -HBCDD (99.3% with traces of h-HBCDD) obtained by thermal rearrangement from technical HBCDD (Fluka) was described elsewhere (Dominguez-Romero et al., 2016). Experimental feed preparation and design were also previously described (Jondreville et al., 2017). The experiment was conducted in France at the experimental unit (UE PEAT) of INRA Nouzilly (France, agreement n°C37-175-1). It was approved by the relevant ethics committee (agreement CELMEA-2012-0015) and compliant with the Directive 2010/63/EU on the protection of animals used for scientific purposes.

Briefly, feeds, based on maize, wheat and soybean meal, were steam pelleted (diameter 2.5 mm) and included all required nutrients for chickens. The assayed concentration of  $\alpha$ -HBCDD in composite contaminated feed samples were 35.7 and 38.7 ng g<sup>-1</sup> fw, respectively. Twenty-nine fast-growing (FG, Ross PM3) and 50 slow-growing (SG, JA657) one-day old male chickens (*Gallus gallus domesticus*) were raised up to market age, i.e. up to 6 and 12 weeks, respectively. Ten one-day old chickens of each strain were slaughtered as control (beginning of experiment). The remaining ones were placed in individual cages until the end of the experiment. Fifteen FG broilers were given the contaminated feed and were sequentially slaughtered in groups 2-6 weeks after experiment begun. The 4 remaining FG broilers were given the control diet and slaughtered at 6 weeks of experiment. Thirty-four SG chickens 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 3-12 weeks after exposure begun. The 6 remaining SG chickens were fed with the control feed and slaughtered at 6 or 12 weeks of experiment.

The excreta (faeces + urine) produced during 72 h before sacrifice were collected and mixed. Plasma was prepared from blood samples collected just before sacrifice. Just after sacrifice, the whole muscles of one leg (thigh + drumstick), one breast muscle (*Pectoralis major*), the liver and the abdominal adipose tissue were collected. All the analysed samples were comprised of individuals except those from the 10 control chickens sacrificed at the beginning of experiment and the excreta and plasma, which were pooled per date of sacrifice. Collected samples were stored at -20 °C (-80 °C for liver) until analysis.

## 2.3. Field survey

Fifty-seven chicken farms from France, including 27 conventional, 22 organic and 8 free-range, were randomly selected among volunteer members of different professional federations and visited during the last week before slaughter (September 2014 to April 2015). Farms were stratified according to production mode (50% conventional, 50% alternative) and geographical distribution (5 regions, proportion according to respective production volumes) (Fig. S1). For each farm, the two breast muscles (*Pectoralis major*) and abdominal adipose tissue from 5 on-site sacrificed chickens were pooled. Feed was collected. Two surface deposited dust samples per farm were also collected from feeders and walls, using wipes to rub a one hundred-square

centimetre area. Collected samples were stored at -20 °C until analysis. Abdominal adipose tissue, feed and wipes were considered as complementary samples to be investigated in case of relevant levels would be observed in muscle.

Limits of reporting (LoR) were defined for each matrix type and each isomer, as the highest value between (i) limits of quantification calculated for each sample at signal-to-noise ratio of 3 and (ii) mean plus 3 times standards deviation of procedural blanks.

## 2.4. Sample preparation

Sample extraction and purification procedure was performed as previously 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). Briefly, abdominal adipose tissue, leg and breast muscles, liver, plasma, excreta as well as feed were prepared according to ISO/IEC 17025:2005 accredited methods. Dried feed (80 °C), wipes and other solid matrices previously lyophilised were extracted by Pressurized Liquid Extraction (PLE) over three successive static cycles (100 bar, 120 °C) (SpeedExtractor, Büchi, Switzerland) with a toluene/acetone mixture (70:30, v/v). Extracted lipids were determined gravimetrically. Plasma (4 mL) 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. Purification steps were conducted on a SPE column manually packed with Na<sub>2</sub>SO<sub>4</sub>, neutral and acidic (H<sub>2</sub>SO<sub>4</sub>) silica gel layers using n-hexane and dichloromethane, followed by partitioning between n-hexane and 1 N NaOH. Final extracts were reconstituted into a mixture of meth-anol/water 80:20 (v/v). Drastic measures were implemented to control contamination by using procedural blanks in each analytical batch.

## 2.5. Instrumental acquisition

Extracts from the experimental assay were first analysed for HBCDD diastereoisomers by LC-ESI(-)-MS/MS (6410, Agilent Technologies) with a Hypersil Gold column (100 mm x 2.1 mm, 1.9 mm) (Thermo Scientific, San Jose, CA, USA), as extensively described and discussed by Jondreville et al. (2017). The same method was also used for the field survey muscle samples.

Enantiomer-specific analysis was performed using an Ulti-Mate 3000 UHPLC pumping system coupled to an Orbitrap Q-Exactive mass spectrometer fitted with a heated electrospray ionisation (HESI) source (Thermo Scientific). High resolution mass spectrometry data were acquired in negative mode in full scan mode over the m/z range [550-750] at a resolving power of 70,000 full width at half-maximum (FWHM) at m/z 200, resulting in about 39,000 FWHM for [M - H]<sup>-</sup> monitored ions. The automatic gain control (AGC Target) was set at high dynamic range (5 x 10<sup>5</sup>) and the maximum injection time was set at 500 ms. HESI parameters were as follows: sheath gas flow at 50 arbitrary units (AU), auxiliary gas flow at 5 AU, capillary temperature at 300 °C; source heater temperature at 150 °C, spray voltage at 2.5 kV and S-lens radio frequency at 50 AU. Chromatographic separation was performed on a

cellulose tris-(3,5-dimethylphenylcarbamate) chiral column (ACQUITY UPC2 Trefoil CEL1, 2.1 mm x 150 mm, 2.5 mm) purchased from Waters (Milford, MA, USA). The injection volume was set at 10 mL. The mobile phase was comprised of 20 mM ammonium acetate (A) and acetonitrile or methanol (B). Ammonium acetate was originally selected to minimise adduct formation and enhance [M - H]<sup>-</sup> signal. The optimised gradient started at A/B 45:55 (v/v) for 1 min, ramped to 33:67 at 2 min, then to 31.5:68.5 at 9 min and to 100% B at 11 min for 6 min prior to a return to initial conditions at 18 min and equilibration for 4 min. The column oven temperature was controlled.

Individual enantiomers were separated and collected using a permethylated b-cyclodextrin chiral column (Nucleodex b-PM, 4 mm x 200 mm, 5 mm) obtained from Macherey-Nagel GmbH & Co. (Düren, Germany) and identified according to Heeb et al. (2005) and Köppen et al. (2007) prior to establishing elution order on the Trefoil CEL1 column.

## 2.6.. Post-acquisition data analysis

## 3. Results and Discussion

### 3.1. Optimisation of enantiomers separation

Preliminary results showed that separation was better achieved using acetonitrile compared to methanol. Additionally, enantiomers were eluted earlier and column pressure was lower. Thus, acetonitrile was selected for further optimisation of parameters known to influence chromatographic separation, such as mobile phase flow, temperature and mobile phase gradient (Snyder et al., 2011).

According to Heeb et al. (2005), the elution order of enantiomers using Nucleodex b-PM column in the described conditions is (-)α (-)β, (+)α, (+)β, (+)γ and (-)γ. Using the Trefoil CEL1 column with acetonitrile or methanol as organic solvent, we determined the elution order as follows: (-)α, (+)α, (-)β, (+)β, (-)γ and (+)γ.

Influence of the mobile phase flow was investigated at 0.3, 0.4 and 0.5 mL min<sup>-1</sup>. In these assays, the column temperature was set at 15°C and the gradient started at A/B 50:50 (v/v) for 1 min, ramped to 25:75 at 15 min and to 100% B at 16 min (Fig. S2). Enantiomers eluted between 11.1 and 17.1 min with decreasing retention times (RT) observed while increasing the flow. While satisfactory resolution factors (>2) were obtained for the separation of most pairs of consecutively eluted enantiomers, they were close to 1.2 and 1.6 for (-)β/(+)β and (+)β/(-)γ, respectively, representing the most challenging part of this development. Peak valleys increased from 8.4% to 13.6% and from 3.9% to 5.8% with increasing flow between (-)β/(+)β and (+)β/(-)γ, respectively. Thus, we selected 0.4 mL min<sup>-1</sup> as a compromise between separation, analysis time and instrument pump capacity.

After various assays, mobile phase gradient was optimised up to the conditions described in Materials and Methods section. Finally, the influence of column temperature (between 5 and 40°C) on the separation was investigated, (Figs. S3 and S4). It appeared that all retention times were highly influenced and

Quantification was achieved according to the isotopic dilution principle, using <sup>13</sup>C<sub>12</sub>-labelled isotopologues as internal standards and <sup>2</sup>H<sub>18</sub>-β-HBCDD as recovery standard. Standard calibration curves were carried out for each enantiomer in the range [0.4e240 pg μL<sup>-1</sup> n = 7 points]. Concentrations were determined based on average Relative Response Factors (RRFs). Enantiomeric fraction of α-HBCDD (EF<sub>α</sub>) was determined according to the following equation:

$$EF_{\alpha} = \frac{\frac{(+A_{\alpha})}{(+A_{IS\alpha})}}{\frac{(+A_{\alpha})}{(+A_{IS\alpha})} + \frac{(-A_{\alpha})}{(-A_{IS\alpha})}}$$

Where (+)A<sub>α</sub> and (-)α<sub>α</sub> represent the peak areas of the α-enantiomeric pair for the native α-HBCDD, (+)A<sub>ISα</sub> and (-)α<sub>ISα</sub> the areas from the corresponding internal standards, assuming that racemic mixtures are used for internal standards and that RRFs are equal for the two enantiomers, as described in the literature (Marvin et al., 2007; Gao et al., 2011) and confirmed by standard calibration curves.

decreased when temperature increased. Additionally, b-enantiomers shifted closer to aenantiomers compared to g-enantiomers when temperature increased, thus significantly impacting selectivity. Consequently, resolution factor for (+)β/(-)γ enantiomers (increasing with temperature) became higher than for (-)β/(+)β enantiomers (decreasing with temperature) when temperature increased from 10 to 15°C (Fig. S5). Moreover, the decrease of temperature resulted in a slight broadening of a-enantiomers chromatographic peaks. A

temperature of 15°C was finally selected as a compromise. The optimised chromatographic conditions enabled resolving the six target HBCDD enantiomers over 11 min (Fig. 1).

### 3.2. Feed as racemic reference

The same α-HBCDD spiked soy oil was used to prepare contaminated livestock feeds (n = 6) for three animal experiments, including the present one. The contaminated feed samples were analysed (each one in triplicate). The EF<sub>α</sub> average value was found to be 0.504 ± 0.004 and was assumed to be a fully racemic mixture having undergone no biologic transformation. Consequently, three standard deviations were considered for the range outside which a statistical enantiomeric enrichment could be observed at the 99% confidence level.

### 3.3. Enantiomer-specific profiles in chicken

Accumulation of α-HBCDD in tissues of FG and SG chickens of the experimental study was previously described by Jondreville et al. (2017), along with growth and tissue characteristics. Fig. S6 shows time-dependent concentrations of α-HBCDD in collected matrices of FG and SG chickens. The accumulation ratios of α-HBCDD in FG and SG chickens calculated as the ratio of the concentration of α-HBCDD in the tissue (ng g<sup>-1</sup> lw) at market age to its concentration in feed (ng g<sup>-1</sup> fw) were 8.4 ± 0.6 and 9.9 ± 0.9 in abdominal adipose

tissue,  $5.2 \pm 0.3$  and  $6.7 \pm 0.7$  in leg muscles,  $2.2 \pm 0.3$  and  $2.1 \pm 0.1$  in breast muscle,  $1.2 \pm 0.1$  and  $2.6 \pm 0.4$  in liver, respectively. It reveals the important influence of tissue on HBCDD levels as well as a slight difference between FG and SG chickens, raising the question of the impact of growth rate on EF $\alpha$ .

Fig. 2 shows calculated EF $\alpha$  kinetics for each matrix from the chicken experiment. Regarding abdominal adipose tissue, muscle and liver for which intra-group variability was assessed, all measured values were found to be significantly lower than the reference racemic feed value. Indeed, average EF $\alpha$  for each continuously exposed group ranged between 0.434 and 0.467, with standard deviations below 0.014, attesting for enantiomeric enrichment as the result of biological process. The observed enrichment in (□)a enantiomer was even accentuated by about 0.020 for slow growing individuals during the 6-week depuration after a 6-week exposure period compared to a 12-week exposure period. This phenomenon stands for reason considering that a continual racemic exposure source would tend to drive EF $\alpha$  closer to 0.5 while, at the opposite, biological enrichment processes would tend to make it deviate. The enrichment process was observed as significant from the first kinetic point (week 2) and quite stable. Furthermore, EF appeared similar between the different matrices investigated at similar kinetic points, suggesting that there was no enantioselectivity in the transportation process between tissues as already pointed out by Zhang et al. (2014) in mirror carp. This is also consistent with another study where enrichment in (□)a-HBCDD was reported for sole muscle and liver (Janak et al., 2005).

Regarding pooled plasma, similar trends were observed. The higher value observed at week 12 for the depuration group was similar to the continuously exposed group, which could be explained by increased uncertainty at such low contamination levels. Indeed, the a-enantiomer sum concentrations for FG and SG at week 12 were 0.037 and 0.123 ng mL<sup>-1</sup>, respectively (4 mL sample size). Then, the EF $\alpha$  of circulating plasma  $\alpha$ -HBCDD appeared to closely reflect EF $\alpha$  in storage tissues (abdominal adipose tissue, breast and leg muscles) and liver, suggesting some equilibrium (Fig. 2).

Excreta were the only matrix presenting EF $\alpha$  values within the racemic range (0.498-0.504) during the contamination phase. The racemic elimination indicates that no preferential enantiomer uptake takes place during gastrointestinal absorption. These results are in line with those of Luo et al. (2013) where no enantioselective uptake was observed in fish gut for all 3 diastereoisomers. By contrast, EF $\alpha$  of slow growing broiler considerably decreased down to 0.382 after 6 weeks depuration phase, more importantly than observed in storage tissues. Peculiarly, EF $\alpha$  seemed to increase temporarily during depuration at week 8. However, values appeared more consistent while relating EF $\alpha$  to a-enantiomer sum concentrations on a dried weight basis (Fig. S7) and considering that analytical confidence was satisfying at any point, with a limit of reporting at only 0.01 ng g<sup>-1</sup> dw for each enantiomer. Such phenomenon suggests that excretion of  $\alpha$ -HBCDD from the circulating compartment to the intestinal lumen occurred (Jondreville et al., 2017) as excreta were accompanied by (-)a enantiomer enrichment as well. If tissues and plasma enrichment in (-)a enantiomer cannot be explained by preferential (+)a enantiomer excretion, an interesting question would be to elucidate the fate of (+)a enantiomer

according to mass balance rule. A first explanation would be that (+)a enantiomer could preferentially accumulate in an organ/ tissue not investigated in the present study. Another plausible explanation may involve a preferential biotransformation. As reported by Jondreville et al. (2017), a OH-HBCDD metabolite was tentatively identified in leg muscles and liver at week 12. Of interest would be to investigate chirality of this metabolite, which at present is unknown.

Thus, the present study demonstrated that ante-mortem exposure to racemic  $\alpha$ -HBCDD resulted in a-isomer accumulation as well as (-)a enantiomer enrichment. This could be advantageously used to investigate the time-line of animal products contamination in the context of monitoring plans or incidents.

### 3.4. Investigation of contamination sources in selected French farms

The present study was part of a larger field investigation aiming at establishing a representative picture of brominated flame retardant contamination levels in edible portions of chicken produced in France, including HBCDD, performed in 2014-2015. The sampling plan was representative of geographical and production mode distributions. Among the 57 analysed muscle samples, 51% showed no HBCDD isomers above established LoR which were 0.1, 0.1 and 0.3 ng g<sup>-1</sup> lw for a-, b- and g-isomers, respectively. As shown in Fig. 3,  $\alpha$ -HBCDD was reported in 25 samples from LoR to 2.51 ng g<sup>-1</sup> lw.  $\beta$ -HBCDD was reported in only one sample at only 0.25 ng g<sup>-1</sup>lw (farm C, see below) while  $\gamma$ -HBCDD was reported in 4 samples all at levels below 0.60 ng g<sup>-1</sup> lw. The presence of  $\gamma$ -HBCDD was attributed to slight uncontrolled procedural contamination, which is also responsible for the higher observed LoR in this analytical series. Observed levels remain relatively low and in accordance with previous monitoring plans organised in France although no atypical value was observed.

The previous French Total Diet Study (ANSES, 2011) also highlighted the predominance of the a-isomer in a range of composite poultry and game food category (0.0510 ng g fw, middlebound, MB). These levels led to an estimate of 6.7% of exposure in the adult population contributed by this food category, i.e. with 0.014 ng kg<sup>-1</sup> bw d<sup>-1</sup> (UB). In the present study, the a-isomer also dominated the contamination profile but at a much lower concentration of 0.0027 ng g fw (MB). This difference of almost a factor of 20 could be explained by (i) differences in poultry and game species, (ii) sampling type (cooked versus raw meat), (iii) sampling dates (2007-2008 versus 2014-2015) if a decrease is expected while regulatory pressure increases and/or (iv) improved analytical performances. The 3 farms (A, B and C), out of the 57, exhibiting the highest  $\alpha$ -HBCDD concentrations (above 1 ng g<sup>-1</sup> lw) in muscle samples were further investigated by evaluating their abdominal adipose tissue, feed and wipes EF $\alpha$  values (Table 1). It was expected that combined information on isomer profile and enantiomer fraction would provide a consistent forensic tool for contamination origin investigations. The technical HBCDD isomeric profile is characterised by around 1-12% for a-, 5-13% for b- and 75-89% for  $\gamma$ -HBCDD (Köppen et al., 2007; Heeb et al., 2007). Expanded polystyrene (EPS) flame retarded with HBCDD and most abiotic environmental contamination exhibit such a profile.

However, due to thermal processes, extruded polystyrene flame retarded with HBCDD presents an isomeric profile dominated by the  $\alpha$ -isomer, typically in the 64-83% range (Jeannerat et al., 2016). Post-mortem contamination of meat by contact with EPS or XPS-type sources would not exhibit any enantiomer enrichment. Conversely, the present data and the research of others shows that any ante-mortem contamination is expected to lead to  $\alpha$ -isomer preferential accumulation as well as (-)- $\alpha$  enantiomer enrichment. In the case of a XPS-type contamination profile, the EF $\alpha$  would allow one to conclude whether ante- or post-mortem contamination of the meat has occurred.

Regarding farms A and B, the isomer profile in muscle was dominated by the  $\alpha$ -isomer (>97%), strongly suggesting ante-mortem contamination. This was confirmed by EF $\alpha$  values showing similar (-)- $\alpha$  enrichment to those observed in the animal experiment (Table 1, Fig. 2). Corresponding abdominal adipose tissues exhibited similar profiles. Feed was first hypothesised as contamination source but no HBCDD isomer was detected above 0.003 ng g<sup>-1</sup> fw. Another considered possible contamination source was the rearing environment. Wipes were rubbed on a wall and the feeders and revealed a few ng of HBCDD dominated by  $\alpha$ -isomer at 75-79% with racemic values. Further investigations would be useful to determine whether buildings are insulated with flame retarded XPS and if animals can ingest XPS particles.

#### 4. Conclusions

Accumulation and depuration kinetics in the different tissues of two *Gallus gallus domesticus* chicken strains pending chronic dietary exposure to racemic  $\alpha$ -HBCDD was previously described (Jondreville et al., 2017). The present study added valuable data regarding specific enrichment in (-)- $\alpha$  enantiomer. Indeed, enriched HBCDD enantiomer profiles in chickens can be considered as a marker of ante-mortem exposure to racemic mixtures, while a racemic profile would rather be

Regarding farm C, isomer profile in muscle was also dominated by  $\alpha$ -isomer but at a lower confidence range of 79-90% (Table 1) which may be compatible with both post-mortem contact with XPS or ante-mortem exposure to a technical mixture. EF $\alpha$  was then useful to guide the suspicion of post-mortem contamination since a racemic value was observed. Conversely, abdominal adipose tissue showed a significant (-)- $\alpha$  enrichment (EF $\alpha$  1/4 0.433) but with slightly lower concentrations. Wipes did not reveal any adsorption of HBCDD isomers on the feeders or the wall greater than 0.15 ng per wipe, suggesting no more than minor environmental contamination. However, feed contained HBCDD at 0.130 ng g<sup>-1</sup> fw (54%  $\alpha$ -isomer), possibly explaining contamination levels found in abdominal adipose tissue but not in breast muscle the lipids of which display a lower affinity to HBCDD (Jondreville et al., 2017). Then, two contamination sources could be hypothesised for this farm, (i) feed explaining the levels in the abdominal adipose tissue and a minor fraction of levels in breast muscle and (ii) an unidentified XPS-like post-mortem contamination source for breast muscle.

derived from post-mortem contamination from non-biological process (e.g. industrial materials). Together with the diastereomeric pattern, the enantiomeric fractions provided powerful information to investigate the contamination timeline of chicken (ante- or post-mortem) and contamination source pattern, which can lead to relevant management options. Similar research on various species is now required in order to expand the applicability of this tool to a larger range of animal productions.

**Table 1. Measurements for three samples sets from three chicken farms. Concentrations in ng.g<sup>-1</sup> lw for muscle and abdominal adipose tissue, ng.g<sup>-1</sup> fw for feed and ng per wipe.**

Matrix	Farm	Dried weight (%)	Lipids (%)	Concentration			α (%)	Enantiomeric fraction		
				α	β	γ		EF <sub>α</sub>	EF <sub>β</sub>	EF <sub>γ</sub>
Muscle	A	27.8	1.14	2.51	<0.03	<0.03	>97	0.427	-	-
	B	27.6	1.29	1.91	<0.03	<0.03	>97	0.446	-	-
	C	28.1	1.16	1.65	0.25	<0.20	79 to 90	0.503	n.d.	-
Abdominal adipose tissue	A	-	86.1	23.10	<0.03	0.07	>99	0.431	-	0.799
	B	-	83.3	4.03	<0.03	0.05	>98	0.437	-	0.712
	C	-	33.3	1.20	<0.03	<0.03	>95	0.434	-	-
Feed	A	-	3.08	<0.003	<0.003	<0.003	-	-	-	-
	B	-	4.49	<0.003	<0.003	<0.003	-	-	-	-
	C	-	6.29	0.070	0.028	0.032	54	0.509	0.521	0.497
Wall wipe	A	-	-	2.87	0.47	0.31	79	0.503	0.494	0.505
	B	-	-	4.40	0.88	0.59	75	0.500	0.498	0.488
	C	-	-	0.14	<0.04	<0.04	>64	0.501	-	-
Feeder wipe	A	-	-	0.33	0.06	<0.04	78 to 91	0.496	0.477	-
	B	-	-	3.17	0.64	0.45	75	0.498	0.499	0.490
	C	-	-	<0.04	<0.04	<0.04	-	-	-	-



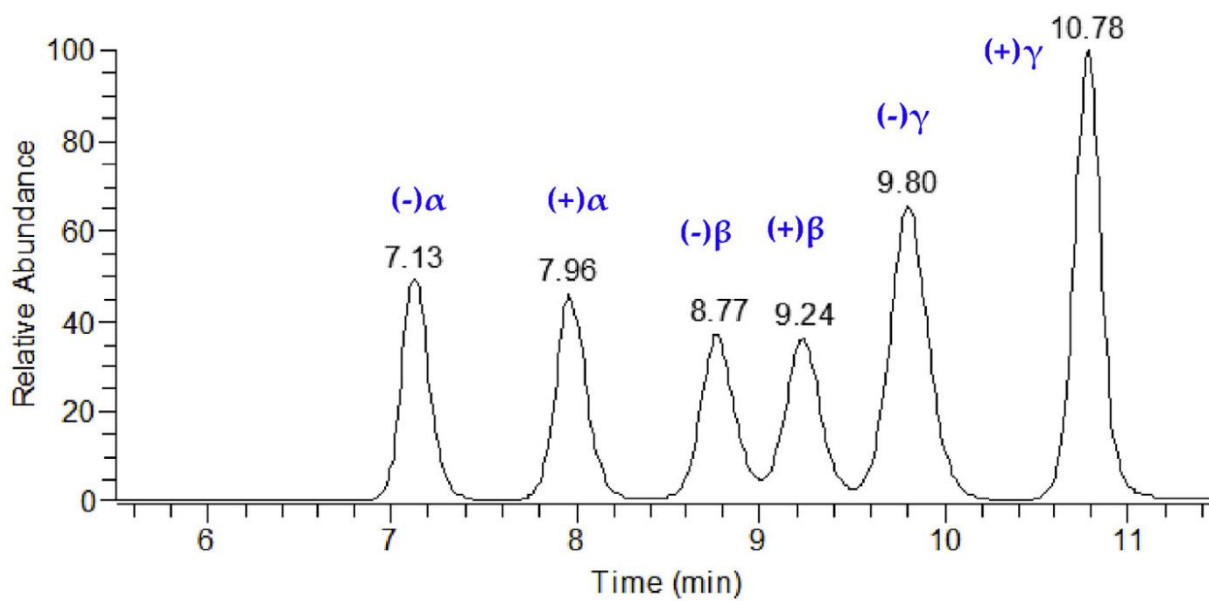
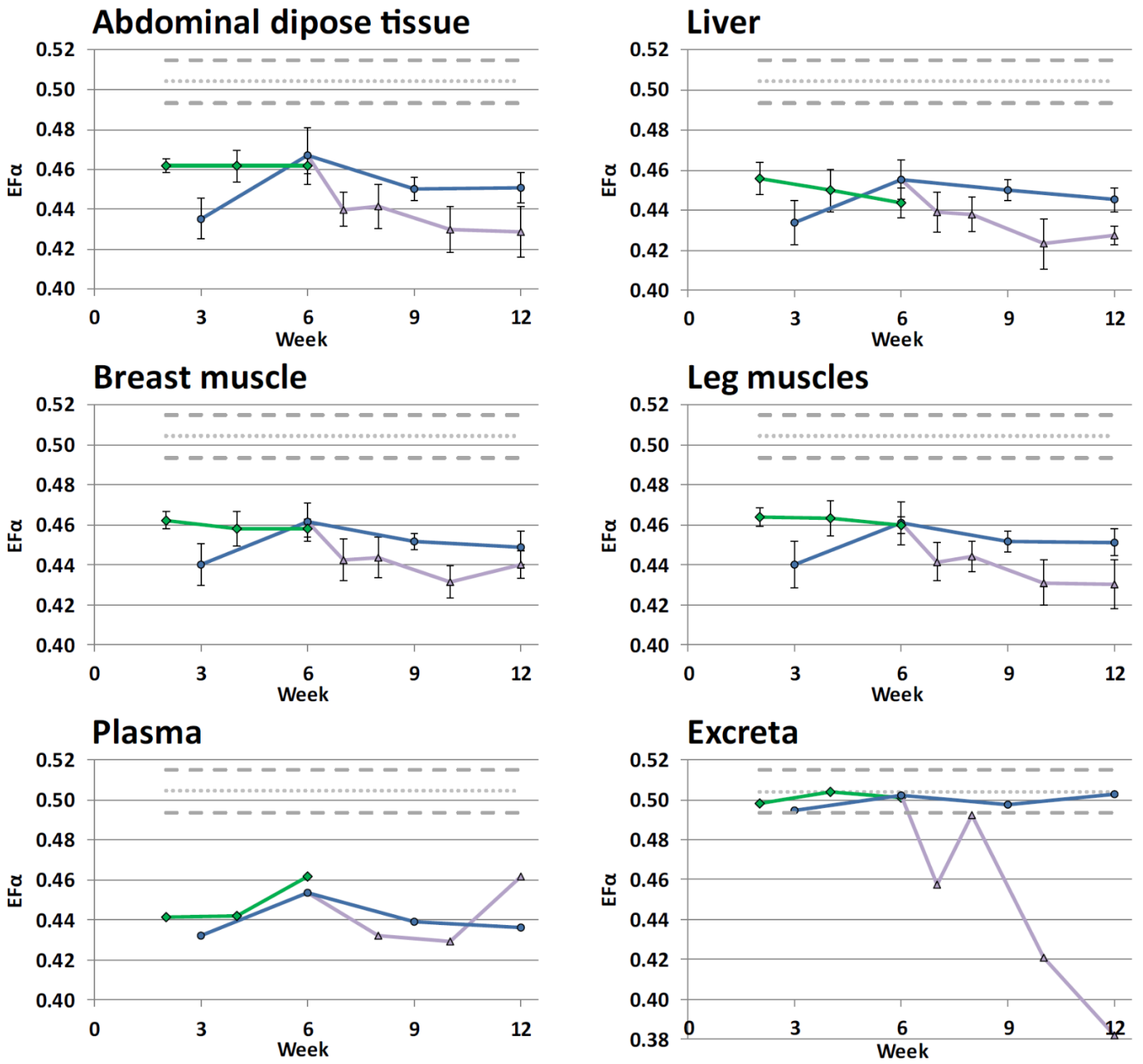
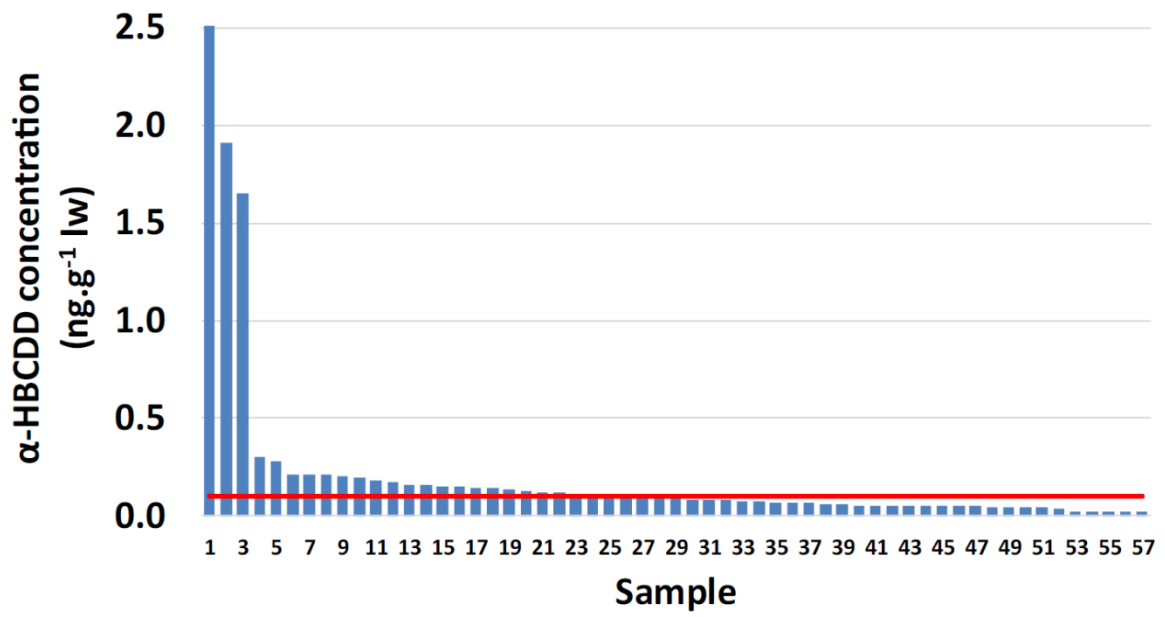


Figure 1. Extracted ion chromatogram obtained at  $m/z$  640.63751 for HBCDD enantiomers pending optimised separation parameters, including column temperature (15°C).



**Figure 1. Calculated EFa for six matrices from the chicken experiment at different time points. Grey dotted (average) and dashed ( $\pm 3$  standard deviation) lines from feed**

Green diamonds: fast growing chicken; Dark blue circles: slow growing chicken; Light blue triangles: slow growing chicken during depuration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Figure 1. Measured  $\alpha$ -HBCDD concentrations in chicken muscle samples from the survey (n = 57). Red line: limit of reporting at  $0.1 \text{ ng g}^{-1} \text{ lw}$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**

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## 5. References

- Abdallah, M.A., Harrad, S., 2011. Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: relationship to external exposure. *Environ. Int.* 37, 443-448.
- ANSES, 2011. Etude de l'alimentation totale française 2 (EAT 2): Contaminants inorganiques, minéraux, polluants organiques persistants, mycotoxines, phytoestrogènes (Tome 1). Agence nationale de sécurité sanitaire, alimentation, environnement et travail, ISBN 978-2-11-128112-7. June 2011.
- Barghi, M., Shin, E.S., Son, M.H., Choi, S.D., Pyo, H., Chang, Y.S., 2016. Hexabromocyclododecane (HBCD) in the Korean food basket and estimation of dietary exposure. *Environ. Pollut.* 213, 268-277.
- Bichon, E., Guiffard, I., Venisseau, A., Marchand, P., Antignac, J.P., Le Bizec, B., 2015. Ultra-trace quantification method for chlordecone in human fluids and tissues. *J. Chromatogr. A* 1408, 169-177.
- Dominguez-Romero, E., Cariou, R., Omer, E., Marchand, P., Dervilly-Pinel, G., Le Bizec, B., Travel, A., Jondreville, C., 2016. Tissue distribution and transfer to eggs of ingested alpha-hexabromocyclododecane (alpha-HBCDD) in laying hens (*Gallus domesticus*). *J. Agric. Food Chem.* 64, 2112-2119.
- ECHA, 2009. Background Document for Hexabromocyclododecane and All Major Diastereomers Identified (HBCDD). European Chemicals Agency. URL: [http://echa.europa.eu/documents/10162/13640/hbccd\\_en.pdf](http://echa.europa.eu/documents/10162/13640/hbccd_en.pdf) (Accessed December 2016).
- EFSA, 2011. Scientific opinion on hexabromocyclododecanes (HBCDDs) in food. *EFSA J.* 9 (7), 2296-2414.
- Fernandes, A.R., Mortimer, D., Rose, M., Smith, F., Panton, S., Garcia-Lopez, M., 2016. Bromine content and brominated flame retardants in food and animal feed from the UK. *Chemosphere* 150, 472-478.
- Fournier, A., Feidt, C., Marchand, P., Venisseau, A., Le Bizec, B., Sellier, N., Engel, E., Rattel, J., Travel, A., Jondreville, C., 2012. Kinetic study of gamma-hexabromocyclododecane orally given to laying hens (*Gallus domesticus*). *Environ. Sci. Pollut. Res.* 19, 440-447.
- Gao, S., Wang, J., Yu, Z., Guo, Q., Sheng, G., Fu, J., 2011. Hexabromocyclododecanes in surface soils from E-waste recycling areas and industrial areas in South China: concentrations, diastereoisomer- and enantiomer-specific profiles, and inventory. *Environ. Sci. Technol.* 45, 2093-2099.
- Guerra, P., Eljarrat, E., Barcelo, D., 2008. Enantiomeric specific determination of hexabromocyclododecane by liquid chromatography-quadrupole linear ion trap mass spectrometry in sediment samples. *J. Chromatogr. A* 1203, 81-87.
- Hakk, H., Szabo, D.T., Huwe, J., Diliberto, J., Birnbaum, L.S., 2012. Novel and distinct metabolites identified following a single oral dose of alpha- or gamma-hexabromocyclododecane in mice. *Environ. Sci. Technol.* 46, 13494-13503.
- He, M.J., Luo, X.J., Yu, L.H., Liu, J., Zhang, X.L., Chen, S.J., Chen, D., Mai, B.X., 2010. Tetrabromobisphenol-A and hexabromocyclododecane in birds from an e-waste region in South China: influence of diet on diastereoisomer- and enantiomer-specific distribution and trophodynamics. *Environ. Sci. Technol.* 44, 5748-5754.
- Heeb, N.V., Schweizer, W.B., Kohler, M., Gerecke, A.C., 2005. Structure elucidation of hexabromocyclododecanes - a class of compounds with a complex stereochemistry. *Chemosphere* 61, 65-73.
- Heeb, N.V., Schweizer, W.B., Mattrel, P., Haag, R., Gerecke, A.C., Kohler, M., Schmid, P., Zennegg, M., Wolfensberger, M., 2007. Solid-state conformations and absolute configurations of (+) and (-) alpha-, beta-, and gamma-hexabromocyclododecanes (HBCDs). *Chemosphere* 68, 940-950.
- Janák K., Covaci, A., Voorspoels, S., Becher, G., 2005. Hexabromocyclododecane in marine species from the Western Scheldt Estuary: diastereoisomer- and enantiomer-specific accumulation. *Environ. Sci. Technol.* 39, 1987-1994.
- Jeannerat, D., Pupier, M., Schweizer, S., Mitrev, Y.N., Favreau, P., Kohler, M., 2016. Discrimination of hexabromocyclododecane from new polymeric brominated flame retardant in polystyrene foam by nuclear magnetic resonance. *Chemo-sphere* 144, 1391-1397.
- Jondreville, C., Travel, A., Meda, B., Dominguez-Romero, E., Omer, E., Cariou, R., Dervilly-Pinel, G., Le Bizec, B., Baeza, E., 2017. Accumulation of alpha-hexabromocyclododecane ( $\alpha$ -HBCDD) in tissues of fast- and slow-growing broilers (*Gallus domesticus*). *Chemosphere* 178, 424-431.
- Köppen, R., Becker, R., Emmerling, F., Jung, C., Nehls, I., 2007. Enantioselective preparative HPLC separation of the HBCD-Stereoisomers from the technical product and their absolute structure elucidation using X-ray crystallography. *Chirality* 19, 214-222.
- Köppen, R., Becker, R., Esslinger, S., Nehls, I., 2010. Enantiomer-specific analysis of hexabromocyclododecane in fish from Etnefjorden (Norway). *Chemosphere* 80, 1241-1245.
- Luo, X.J., Ruan, W., Zeng, Y.H., Liu, H.Y., Chen, S.J., Wu, J.P., Mai, B.X., 2013. Trophic dynamics of hexabromocyclododecane diastereoisomers and enantiomers in fish in a laboratory feeding study. *Environ. Toxicol. Chem.* 32, 2565-2570.
- Marvin, C.H., MacInnis, G., Alae, M., Arsenault, G., Tomy, G.T., 2007. Factors influencing enantiomeric fractions of hexabromocyclododecane measured using liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 21, 1925-1930.
- Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J., Boon, J.P., Belpaire, C., Leonards, P.E., Van Leeuwen, S.P., De Boer, J., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environ. Sci. Technol.* 38, 5497-5504.
- Müller, T.A., Kohler, H.P., 2004. Chirality of pollutants-effects on metabolism and fate. *Appl. Microbiol. Biotechnol.* 64, 300-316.
- Ortiz, X., Guerra, P., Diaz-Ferrero, J., Eljarrat, E., Barcelo, D., 2011. Diastereoisomer- and enantiomer-specific determination of hexabromocyclododecane in fish oil for food and feed. *Chemosphere* 82, 739-744.
- Rani, M., Shim, W.J., Han, G.M., Jang, M., Song, Y.K., Hong, S.H., 2014. Hexabromocyclododecane in polystyrene based consumer products: an evidence of unregulated use. *Chemosphere* 110, 111-119.
- Riddell, N., Mullin, L.G., van Bavel, B., Ericson Jogsten, I., McAlees, A., Brazeau, A., Synnott, S., Lough, A., McCrindle, R., Chittim, B., 2016. Enantioselective analytical- and preparative-scale separation of hexabromocyclododecane stereoisomers using packed column supercritical fluid chromatography. *Molecules* 21, 1509-1520.
- Snyder, L.R., Kirkland, J.J., Dolan, J.W., 2011. Introduction to Modern Liquid Chromatography, third ed., ISBN 978-1-118-21039-0.
- Szabo, D.T., Diliberto, J.J., Huwe, J.K., Birnbaum, L.S., 2011. Differences in tissue distribution of HBCD alpha and gamma between adult and developing mice. *Toxicol. Sci.* 123, 256-263.
- Tomy, G.T., Budakowski, W., Halldorson, T., Whittle, D.M., Keir, M.J., Marvin, C., MacInnis, G., Alae, M., 2004. Biomagnification of alpha- and gamma-hexabromocyclododecane isomers in a Lake Ontario food web. *Environ. Sci. Technol.* 38, 2298-2303.
- Zacs, D., Rjabova, J., Bartkevics, V., 2014. New perspectives on diastereoselective determination of hexabromocyclododecane traces in fish by ultra high performance liquid chromatography-high resolution orbitrap mass spectrometry. *J. Chromatogr. A* 1330, 30-39.
- Zhang, X., Yang, F., Xu, C., Liu, W., Wen, S., Xu, Y., 2008. Cytotoxicity evaluation of three pairs of hexabromocyclododecane (HBCD) enantiomers on Hep G2 cell. *Toxicol In Vitro* 22, 1520-1527.
- Zhang, Y., Sun, H., Ruan, Y., 2014. Enantiomer-specific accumulation, depuration, metabolization and isomerization of hexabromocyclododecane (HBCD) diastereoisomers in mirror carp from water. *J. Hazard. Mater.* 264, 8-15.

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