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Hens can ingest extruded polystyrene in rearing buildings and lay eggs contaminated with hexabromocyclododecane

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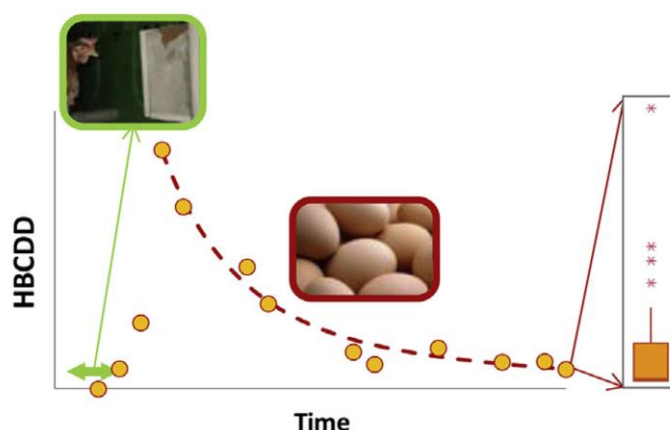
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Keywords

Animal-derived food, brominated flame retardant, building material, enantiomeric fraction, contamination, depuration.

Abstract

The overall concentration of hexabromocyclododecane (HBCDD) in eggs is low although abnormally high concentrations exceeding 3000 ng g⁻¹ fw have been reported. In order to test whether these contaminations may originate from the ingestion of insulating materials in rearing buildings, a group of 55 hens raised in a collective cage was provided with a 64-g piece of extruded polystyrene (XPS, 2.59% HBCDD of which 75, 15 and 10% as α -, β - and γ -HBCDD, respectively). Hens entirely consumed the piece within 3 days, leading to a mean daily exposure of 4.7 mg HBCDD per kg body weight. Whole egg HBCDD concentration reached a maximum of 1037 ng HBCDD g⁻¹ fresh weight (fw), recorded 2 days after the piece had disappeared, and decreased down to 86 ng g⁻¹ fw within the 19 following days. In all these samples, HBCDD was made of 98.7 \pm 0.7 and 1.3 \pm 0.6% α - and β -HBCDD, respectively, and 0.1% γ -HBCDD when quantified; it was enriched in (-) α - and (+) β -HBCDD with enantiomeric fractions of 0.438 \pm 0.009 and 0.579 \pm 0.030, respectively. HBCDD was quantified in all the individual eggs collected the last day of experiment at concentrations ranging between 0.47 and 1361 ng g⁻¹ fw, according to a lognormal distribution. The ingestion of XPS in degraded rearing buildings is thus a plausible cause of on-farm egg contamination by HBCDD which should be strictly avoided.



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 polystyrene, which represented 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.

Food and especially fatty food of animal origin is the main route of human exposure to HBCDD (Marvin et al., 2011; Koch et al., 2015). Overall levels of HBCDD concentrations in animal-derived foodstuffs, generally below 1 ng g⁻¹ lipid weight (lw), may be considered acceptable. Indeed, the median chronic dietary exposure of 0.43 ng kg⁻¹ d⁻¹ in European adults is deemed to be of no public health concern (EFSA, 2011). However, the literature reports some samples of animal-derived foodstuffs exhibiting abnormally high concentrations. In eggs, concentrations reaching 62 ng HBCDD g⁻¹ lw in Belgium (Covaci et al., 2009), 72 ng HBCDD g⁻¹ lw in Canada (Rawn et al., 2011) and 350 ng HBCDD g⁻¹ lw in China (Zheng et al., 2012) were reported. National monitoring plans conducted in Germany (Hiebl and Vetter, 2007) and in France (DGAL, 2009) revealed concentrations in eggs even reaching 2000 to 3400 ng HBCDD g⁻¹ lw. The ingestion of one single egg containing 3000 ng HBCDD g⁻¹ lw by an adult weighing 70 kg would correspond to around 600 times the median European dietary exposure. Thus, the assessment of the risk related to the presence of HBCDD in food is still on-going and the European Commission has recommended the monitoring of animal-derived foodstuffs for HBCDD since 2014 (EU, 2014).

Although the γ -isomer is the major one in technical mixtures of HBCDD, the α -isomer predominates in animal tissues and foodstuffs (Marvin et al., 2011; Koch et al., 2015) including hen

eggs (Rawn et al., 2011; Zheng et al., 2012). This shift is generally ascribed to the rapid degradation of β - and γ -HBCDD compared to α -HBCDD; in addition, γ -HBCDD is bioisomerized into β - and mostly α -HBCDD, while bioisomerization from α -HBCDD to any other isomer has never been reported (Marvin et al., 2011; Koch et al., 2015). However, in animals exposed to γ -HBCDD, α -HBCDD in tissues or eggs does not exceed 10-15% of total HBCDD (Szabo et al., 2010; Fournier et al., 2012). Thus, it is unlikely that hens laying eggs contaminated with mainly α -HBCDD are exposed to a commercial mixture. Rather, they are probably exposed to a mixture in which the α -isomer dominates. In this respect, Cariou et al. (2014) found that XPS used for insulating hen houses contained up to 3.8% HBCDD, of which 75-80% was the α -isomer. This shift in profile compared to the technical mixture results from the thermotransformation of the γ -to the α -isomer during the manufacture of XPS (Heeb et al., 2010). The affinity of hens for polystyrenes was previously observed. Indeed, blocks of polystyrene provided to hens reared in collective cages were readily ingested and were even considered as adequate surrogate stimuli to prevent feather pecking (Huber-Eicher and Wechsler, 1998). Thus, as hypothesized by Hiebl and Vetter (2007), the ingestion of fragments of insulating material, namely XPS, may be a source of on-farm exposure of hens to α -HBCDD. Particularly, hens raised in floor pens may accidentally have access to insulation material if maintenance is lacking in buildings.

The aim of the current study was to test the hypothesis that the ingestion of XPS may be a source of on-farm eggs contamination by HBCDD. For this purpose, we placed a piece of XPS in a cage where a group of laying hens was reared to mimic degraded walls and measured the concentration of the six enantiomers of HBCDD in eggs. In addition to the time-course concentration in daily composite whole egg samples, the variability in HBCDD concentration between eggs was investigated.

2. Material and Methods

2.1. Study design and egg sampling

The experiment was conducted following the Directive 2010/63/ EU on the protection of animals used for scientific purposes in an adequate facility, according to an approved protocol (number 2016022516486530).

Fifty-five ISA Brown laying hens aged 76 weeks and weighing 2.14 ± 0.23 kg were reared according to commercial conditions in a furnished wire cage for 60 hens (336 x 126 x 45.5 cm) equipped with feed troughs, a pecking and scratching area, nests and perches, as described by Huneau-Salaün et al. (2011) (Figure S1). Such cages prevent contact of hens with droppings. Hens had free access to water and to commercial feed; lighting was set to a 16 h light/8 h dark cycle. The parallelepiped piece of XPS (64 g, 20 x 20 x 3.9 cm) provided to hens was collected in a rearing building and analysed by Cariou et al. (2014). Its assayed HBCDD concentration,

confirmed in the present study, was 25.9 g HBCDD kg⁻¹ of which 75, 15 and 10% as α -, β - and γ -HBCDD, respectively (i.e. 1242, 243 and 173 mg, respectively). Racemic proportions of enantiomer pairs were checked and found to be 0.499 ± 0.002 (n = 6).

The XPS piece was attached to the wall of the cage at 2 p.m. on the first day of the experiment, near the pecking and scratching area; the droppings belt below the cage made it possible to collect any debris of XPS that could be present in excreta or fall from the XPS piece. From the moment XPS was positioned, hens were regularly observed to detect any abnormal clinical sign or mortality; the evolution of the XPS piece and the presence of debris on the dropping belt were also carefully monitored. The time the hens would require to consume the entire XPS piece was unknown a priori. Therefore, the duration of the experiment was adjusted a posteriori so that it ended 21 days after the XPS piece had completely disappeared, to allow depuration to start.

Daily, starting 3 days before the XPS plate was positioned, the number and weight of eggs produced by the group of 55 hens was recorded, and a single composite sample was prepared. For this purpose, all the collected eggs were first beaten in omelettes (yolk + albumen) of 5 or 6 eggs. Depending on the number of eggs produced, 5 to 10 omelettes were prepared daily. Then, 10 mL subsamples of each of these omelettes of 5 or 6 eggs were pooled into a daily single composite sample. The last day of experiment, each egg was analysed individually, in addition to the composite sample. In this aim, each whole egg was first individually beaten and a 10-mL separate aliquot was taken before the composite sample was prepared as described above. The whole egg samples were stored at -18 °C before solvent extraction and analysis. In order to depict HBCDD kinetics in the composite whole egg samples, we analysed 50% of them, distributed over the experiment. Nevertheless, we selected in priority the eggs laid the first days after the piece had disappeared. In order to assess the interindividual variability, each whole egg collected on the last day of the experiment was analysed.

2.2. Chemical analysis

Racemic reference solutions of native (α -, β -, γ -) as well as $^{13}\text{C}_{12}$ -labelled (α -, β -, γ -) and $^2\text{H}_{18}$ - β -HBCDD stable isotopologues were purchased from Wellington Laboratories (Guelph, Ontario, Canada). n-Hexane and methanol (Picograde®) were provided by LGC Pro-mochem (Wesel, Germany) and dichloromethane by Biosolve (Valkenswaard, The Netherlands). LC-MS grade acetonitrile and water were obtained from Sigma-Aldrich (ChromaSolv, St. Louis, MO, USA). Sulfuric acid (98%) was purchased from Panreac (Barcelona, Spain), 1 N sodium hydroxide from Reagecon (Clare, Ireland) and ammonium acetate from Merck (Darmstadt, Germany).

As regard to expected high levels, sample size was minimized to 100 mg of whole egg. After addition of 5 ng of each $^{13}\text{C}_{12}$ -labelled isomer (internal standards), samples were vortex mixed with 2 mL H_2SO_4 and a mixture of dichloromethane/n-hexane 2:8 (v/v), then left for one night. After centrifugation, supernatant was vortex mixed with 2 mL NaOH 1 N. After another centrifugation, supernatant was evaporated under a gentle stream of pure nitrogen and reconstituted in 50 mL of a mixture of methanol/ H_2O 4:1 (v/v) with 20 ng of $^2\text{H}_{18}$ - β -HBCDD (recovery standard).

Purified extracts were analysed in a single sequence by liquid chromatography coupled to high resolution mass spectrometry according to the method optimised by Omer et al. (2017), pending slight modifications. Briefly, an UltiMate 3000 UHPLC pumping system coupled to an Orbitrap Q-Exactive mass

spectrometer fitted with a heated electrospray ionisation (HESI) source (Thermo Scientific, San Jose, CA, USA) was used. Chromatographic separation was performed on an ACQUITY UPC2 Trefoil CEL1 chiral column (2.1 mm \times 150 mm, 2.5 mm) purchased from Waters (Milford, MA, USA) and maintained at 10 °C. The mobile phase was constituted of acetonitrile (B) gradient against 20 mM ammonium acetate (A). The 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 set at 13 °C. High resolution mass spectrometry data were acquired in negative mode in full scan mode over the m/z range [620-680] in the full scan mode at a resolving power of 140,000 full width at half-maximum (FWHM) at m/z 200, resulting in about 80,000 FWHM for [M - H]⁻ monitored ions.

All along sampling and analysis, particular attention was paid to procedural contamination management. Quantification was achieved according to the isotopic dilution principle, using $^{13}\text{C}_{12}$ -labelled isotopologues as internal standards and $^2\text{H}_{18}$ - β -HBCDD as recovery standard. Seven-point standard calibration curves (n = 2) carried out for each enantiomer in the range [0.4-240 pg mL⁻¹], procedural blanks (n = 4), fish oil quality controls routinely used for HBCDD isomers analysis by an accredited method (1 g sample size, n = 4), uncontaminated (n = 4) and α -HBCDD containing (~60 ng g⁻¹ fw, n = 3) egg samples supported satisfying analytical performances, including linear range, recoveries, repeatability and trueness (report to supporting information for details). A unique and conservative limit of reporting was set at 0.2 ng g⁻¹ fw for each enantiomer, according to detection performances and procedural contamination. The concentration of each isomer was calculated as the sum of the (+) and (-) enantiomers, and the concentration of HBCDD as the sum of the six enantiomers. Enantiomeric fraction (EF) of each isomer of HBCDD was determined according to the following equation:

$$EF = \frac{\frac{(+)\text{A}}{(+)\text{AIS}}}{\frac{(+)\text{A}}{(+)\text{AIS}} + \frac{(-)\text{A}}{(-)\text{AIS}}}$$

where (+)A and (-)A represent the peak areas of the enantiomeric pair for the native HBCDD isomer, (+)AIS and (-)AIS 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) and confirmed by standard calibration curves.

3. Results and Discussion

The 64-g piece had completely disappeared within 3 days. Consequently, the duration of the experiment was adjusted to 24 days. We observed no debris of XPS on the dropping belt under the cage. This indicates that hens readily ingested XPS and that no visible debris of XPS was present in the excreta. Such an active foraging behaviour of chickens towards polystyrene blocks was previously reported (Huber-Eicher and Wechsler, 1998). The absence of XPS debris in the excreta

was expected because of the grinding capacity of the gizzard (Singh et al., 2014). Over this 3-day period, the mean daily exposure of the group of 55 hens to HBCDD was thus 550 mg distributed in 412, 83 and 55 mg α -, β - and γ -HBCDD, respectively. For a body weight (BW) of 2.14 kg per hen, the daily exposure was thus of 4.7 mg kg⁻¹ BW as 3.5, 0.7 and 0.5 mg α -, β - and γ -HBCDD, respectively. Despite this short term but quite high level of exposure, the group of 55 hens produced daily similar number and weight of eggs before and during the

experiment (41.3 ± 3.8 eggs weighing 66.9 ± 0.1 g each, $n = 3$ days vs. 43.0 ± 3.4 eggs weighing 65.9 ± 3.2 g each, $n = 24$ days). Besides, the hens behaved normally and no loss of appetite or BW was observed. In American kestrels submitted to a 9 fold lower level of exposure (0.51 mg technical HBCDD kg^{-1} BW d^{-1}) but for a 25 fold longer duration (75 days), body characteristics were not affected, but reproductive performance of birds was disturbed with more numerous but smaller eggs laid and an increased eggshell porosity (Letcher et al., 2015).

3.1. HBCDD in composite whole egg samples

The number of eggs laid daily and the concentration of each enantiomer of HBCDD in the composite samples is presented in Table S1. Each daily composite sample was made from all the eggs i.e. 35 to 50 eggs, depending on the day. No HBCDD was detected in the control sample collected prior to the experiment. The two enantiomers of α -HBCDD were quantified in all samples and those of β -HBCDD in all samples except in the first collected one. In contrast, (+) and (-) γ -HBCDD were quantified only in the 5 and 2 most concentrated samples, respectively. The α - and β -isomers made 98.7 ± 0.7 and $1.3 \pm 0.6\%$ of total HBCDD, respectively. The γ -isomer, when quantified, made approximately 0.1% of total HBCDD.

Letcher et al. (2015) previously observed this enrichment in α -HBCDD in American kestrels exposed to technical HBCDD. It was ascribed to the rapid metabolism of β - and γ - compared to α -HBCDD and to the bio-isomerization of γ - and β - to α -HBCDD, while α -HBCDD is recalcitrant to such bio-isomerization. These phenomena were previously reported in mammals (Szabo et al., 2010, 2011; Sanders et al., 2013), in fish (Du et al., 2012; Zhang et al., 2014) and in birds including broilers (Jondreville et al., 2017) and laying hens (Fournier et al., 2012; Dominguez-Romero et al., 2016). In addition, Zheng et al. (2017) observed that egg was more enriched in α -HBCDD than most body tissues of laying hens exposed to a mixture of the 3 isomers and suggested that α -HBCDD was more readily transferred to eggs than β - and γ -HBCDD.

The maximum total HBCDD concentration was 1037 ng g^{-1} fw. Considering that whole egg lipid concentration ranges between 9.2 and 12.2% (Nys and Guyot, 2011), this maximum concentration would be around 8.5-11.3 mg HBCDD g^{-1} lw. These values are 3-6 fold the extreme 2.0 and 3.4 mg g^{-1} lw reported in monitoring plans in Germany and in France (Hiebl and Vetter, 2007; DGAL, 2009). Overall, concentrations increased during 5 days, then decreased rapidly during 10 days and then more slowly (Fig. 1). The maximum concentration of 1037 ng HBCDD g^{-1} fw was thus reached 2 days after the XPS piece was completely consumed and the depuration was biphasic. Hoogenboom et al. (2006) reported a similar pattern of egg contamination with dioxins and dioxin-like poly-chlorobiphenyls. This peculiar profile in egg contamination by lipophilic contaminants is related to the formation of egg yolk into daily concentric layers of unequal sizes deposited during 10-12 days before the egg is laid. Consequently, the pattern of contaminant deposition into egg yolk is proportional to the amount of yolk material layered each day during yolk development (Fournier et al., 2010). Thus, eggs laid up to 10-12 days after the exposure has ceased are contaminated during the exposure. Conversely, the redistribution of contaminants from storage tissues (body fat)

to blood and then to developing egg yolk explains the slower depuration at the end of the experiment.

Except for the γ -isomer, second order kinetics models were adjusted to the concentration data between day 5 and day 24 (Table 1, Fig. 1). The initial half-life, which mainly reflects egg formation, was estimated at 2.4 ± 0.3 , 2.0 ± 0.2 and 2.4 ± 0.3 d for α -, β - and total HBCDD, respectively. The terminal half-life was estimated at 10.6 ± 5.3 , 4.9 ± 2.3 and 10.6 ± 5.3 d, respectively. Although fitted with one single group of hens and with fairly broad confidence limits, these half-lives are in accordance with the well documented rapid elimination of the β -isomer compared to the α -isomer (Sanders et al., 2013; Du et al., 2012; Zhang et al., 2014). These authors also reported similar depuration rates for β - and γ -HBCDD. Thus, although the depuration rate of γ -HBCDD could not be estimated in the current study, our data fit with the depuration half-life of 2.9 d reported by Fournier et al. (2012) in laying hens. For α -HBCDD, the half-life (10.6 ± 5.3 d) is below the value of 17.4 ± 2.3 d HBCDD concentration in eggs down to the average value of 0.54 ng HBCDD g^{-1} fw (i.e. 0.050 - 0.066 ng HBCDD g^{-1} fw) reported in the EU (EFSA, 2011). This duration represents one third of a practical 13-month laying period.

3.2. HBCDD in individual whole egg samples

The concentration of each enantiomer of HBCDD in each of the 42 eggs collected the last day of experiment is presented in Table S2. The two enantiomers of α -HBCDD were quantified in all samples and those of β -HBCDD in the 13 most concentrated ones, while (+) γ -HBCDD was quantified in 7 out of the 10 most concentrated samples and (-) γ -HBCDD was never quantified. The α -isomer made $99.8 \pm 0.3\%$ of total HBCDD.

Total HBCDD concentrations ranged from 0.47 to 1361 ng g^{-1} fw with a clearly lognormal distribution (Table 2, Fig. 2). The mean and the median were 138 ± 261 and 13 ng g^{-1} fw, respectively, and 25% of the individual concentrations were below 3.2 ng g^{-1} fw or above 170 ng g^{-1} fw. Four eggs were outliers with concentrations reaching 1361 , 672 , 603 and 492 total HBCDD g^{-1} fw. Assuming all the eggs were of similar weight, these 4 eggs contributed for 54% to HBCDD present in the 42 eggs collected on day 24. It was noticeable that the mean of the individual concentrations was 61% higher than the concentration measured in the composite sample on the same day (86 ng g^{-1} fw). The variable size of eggs may contribute to this difference. Nevertheless, this bias does not affect the distribution. The quantification of HBCDD in each whole egg indicates that each of the 42 hens was exposed to HBCDD through XPS ingestion. In addition, the great variability between eggs suggests that the hens ingested different amounts of XPS. This would be in accordance with the observations of Shimmura et al. (2008) who reported that hens reared in furnished cages unequally use resources such as nest boxes and the pecking and scratching area, depending on their social hierarchy.

3.3. Enantiomeric fraction

In composite samples, the results show a selective enrichment in (-) α - and (+) β -enantiomers (Table S1, Fig. 1). Based on the few results, an enrichment in (+) γ -enantiomer seemed to occur too. These enrichments appear to be progressive since EF α decreased from 0.445 ± 0.004 (n = 7 time points up to day 10) to 0.429 ± 0.004 (n = 6 time points after day 10). In the meantime, EF β increased from 0.558 ± 0.014 (n = 6) to 0.599 ± 0.029 (n = 6), although less clearly. The results obtained with the 42 individual samples are consistent (0.424 ± 0.019 and 0.585 ± 0.026 for EF α and EF β , respectively) (Table S2). The time-dependent shifts could be due to the significant incorporation of HBCDD of dietary origin, therefore racemic, in the eggs formed up to 10-12 days after the beginning of the experiment and then to the progressive enrichment in the least metabolized enantiomer. Following the experiment reported by Dominguez-Romero et al. (2016), Cariou (unpublished) also observed that EF α in eggs of laying hens orally exposed to racemic α -HBCDD tended to decrease from 0.440 ± 0.013 to

0.404 ± 0.025 along the 7-week depuration. Similarly, EF α in leg muscles of slow-growing broilers decreased from 0.461 ± 0.011 to 0.430 ± 0.012 after 6 weeks of depuration (Omer et al., 2017). Enantioselectivity for α -HBCDD was also reported in eggs of domestic laying hens ($0.44 < EF\alpha < 0.48$) (Zheng et al., 2012), as well as in those of wild birds such as herring gulls ($0.34 < EF\alpha < 0.48$) (Esslinger et al., 2011), common falcons (EF α = 0.14) and peregrine falcons (EF α = 0.21) (Janak et al., 2008) and in muscles of terrestrial passerines ($0.09 < EF\alpha < 0.44$) (Sun et al., 2012). On the contrary, eggs of white-tailed eagles (EF α = 0.77) and of guillemots (EF α = 0.53) were reported to be enriched in (+) α -HBCDD (Janak et al., 2008). As far as we know, whether the accumulation of β -HBCDD in birds is enantioselective is not documented in literature, while the selective accumulation of (+) γ -HBCDD in muscles of passerines ($0.59 < EF\gamma < 0.83$) (Sun et al., 2012) fits with the current observations.

4. Conclusion

Although conducted with one single group of hens, the current study confirms that laying hens reared in collective cages are prone to ingest XPS and that this material is a potential source of on-farm contamination of eggs with HBCDD, especially α -HBCDD. Thus, any access of hens raised in floor pens to insulation material, even of short duration, should be strictly

avoided by preventing any degradation of walls. The experiment also shows that all the eggs were contaminated. However, it highlights the great interindividual variability within the group of hens, possibly as a result of variable amounts of XPS ingested. At the end, we confirm the preferential accumulation of (-) α -HBCDD in poultry and report for the first time the one of the (+) β -HBCDD.

Table 1. Parameters of the two order kinetics models of α -, β -and total HBCDD concentration (ng g^{-1} fw) in composite whole egg samples.

	α -HBCDD	β -HBCDD	Total HBCDD
k1 (d^{-1})	0.293 ± 0.040	0.351 ± 0.031	0.294 ± 0.039
k2 (d^{-1})	0.065 ± 0.033	0.141 ± 0.067	0.066 ± 0.033
R ²	0.99	1.00	0.99
RMSE (ng g^{-1} fw)	52	0.6	52
t _{1/2} phase 1 (d)	2.4 ± 0.3	2.0 ± 0.2	2.4 ± 0.3
t _{1/2} phase 2 (d)	10.6 ± 5.3	4.9 ± 2.3	10.6 ± 5.3

^a k1 and k2 are the first and second phase depuration rate constants, respectively. Half-lives for the first and second phases of depuration are calculated as $\ln(2)/k1$ and $\ln(2)/k2$, respectively. R² is the coefficient of determination between predicted and observed values; RMSE is the root mean square error of the model

Table 2. Distribution of α -, β -, γ - and total HBCDD in the 42 whole eggs collected the last day of experiment.

	α -HBCDD	β -HBCDD	γ -HBCDD	Total HBCDD
^{na}	42	13	7	42
Mean	137	2.3	0.41	138
Standard deviation	260	1.5	0.22	261
Minimum	0.47	0.86	0.20	0.47
First quartile	3.2	1.4	0.23	3.2
Median	13	1.7	0.31	13
Third quartile	169	2.9	0.57	170
Maximum	1355	5.1	0.73	1361

^a Number of eggs in which at least one of the two enantiomers was quantified above the limit of reporting.

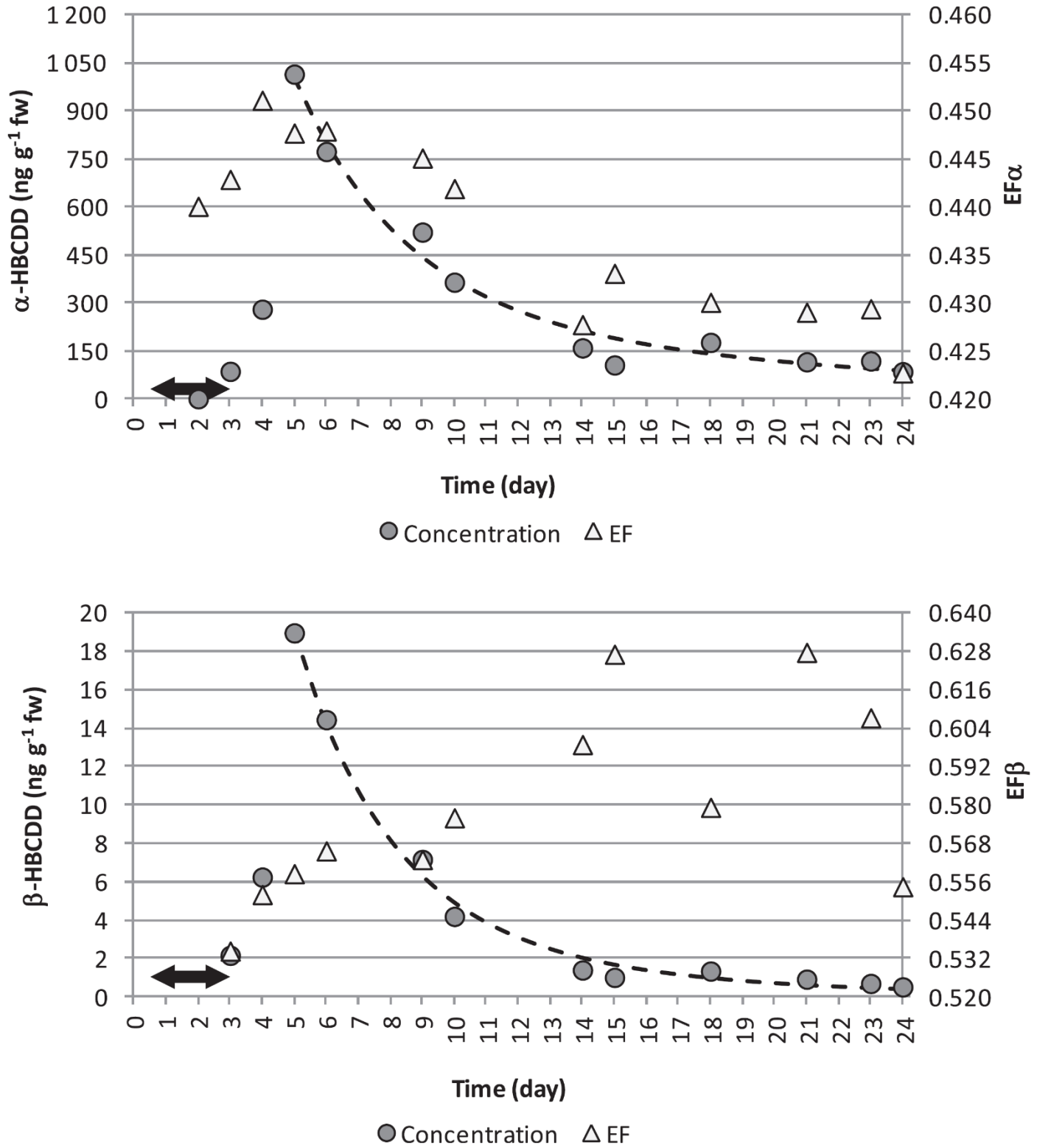


Figure 1. Time-dependent concentration (ng g⁻¹ fresh weight) and enantiomeric fraction (EF) of α - and β -HBCDD in the composite whole egg samples. The double arrow corresponds to the presence of the XPS piece in the cage and the dashed line to the kinetics model presented in Table 1.

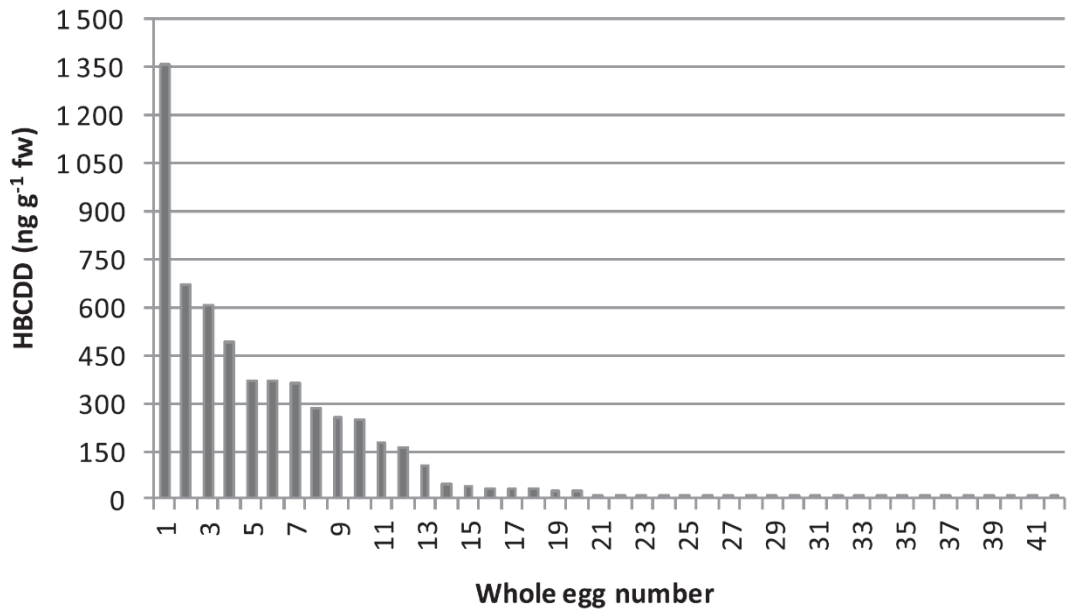


Figure 2. HBCDD concentration in the 42 whole eggs collected the last day of experiment.

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