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

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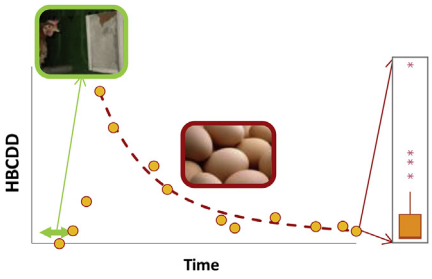
HIGHLIGHTS

- A group of 55 hens ingested a 64-g piece of extruded polystyrene within 3 days.
- Composite samples of eggs contained up to 1037 ng HBCDD g^{-1} fw, mainly as α-HBCDD.
- Egg HBCDD concentration was highly variable between hens.
- Eggs were selectively enriched in (−) α- and (+)β-HBCDD.

GRAPHICAL ABSTRACT



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ABSTRACT

The overall concentration of hexabromocyclododecane (HBCDD) in eggs is low although abnormally high concentrations exceeding 3000 ng g^{-1} lw 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 ± 0.7 and 1.3 ± 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 ± 0.009 and 0.579 ± 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.

Abbreviations: BW, Body weight; EF, Enantiomeric fraction; fw, Fresh weight; HBCDD, Hexabromocyclododecane; lw, Lipid weight; XPS, Extruded polystyrene.

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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 bio-isomerized into β- and mostly α-HBCDD, while bio-isomerization 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. Materials 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 × 126 × 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 × 20 × 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 inter-individual 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 $^{13}C_{12}$-labelled (α-, β-, γ-) and $^{2}H_{18}$-β-HBCDD stable isotopologues were purchased from Wellington Laboratories (Guelph, Ontario, Canada). *n*-Hexane and methanol (Picograde®) were provided by LGC Promochem (Wesel, Germany) and dichloromethane by Biosolve (Valkenswaard, The Netherlands). LC-MS grade acetonitrile and