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ENANTIOMER-SPECIFIC ACCUMULATION AND DEPURATION OF α-HEXABROMOCYCLODODECANE (α-HBCDD) IN BROILER CHICKENS (GALLUS DOMESTICUS) AS A TOOL TO IDENTIFY CONTAMINATION SOURCES

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1. Introduction

Hexabromocyclododecane (HBCDD) is mainly used in building sector as an additive for thermal insulation of extruded (XPS) and expanded (EPS) polystyrene foam. It is recognized as a Persistent Organic Pollutants (Stockholm convention). Diet is considered as a major route of exposure for Human, including food from animal origin as terrestrial livestock animals [1]. With regard to rearing environment, identifying exposure sources (e.g. food, insulation materials) to minimise ingestion may help protecting Human health.

Although the γ-isomer predominates the technical mixture, diastereomeric profile in biota displays most frequently domination in α-HBCDD. Indeed, unlike α-HBCDD, β- and γ-HBCDD show faster degradations in vivo. Moreover, isomerisation of γ-HBCDD into α-HBCDD mostly occurs in living organisms but also during extrusion process of polystyrene. Each isomer (α, β, γ) is composed of an enantiomer pair and a non racemic composition indicates that a biological process occurred, in relation with selective uptake, or metabolism and elimination of a preferential enantiomer. Then, chiral signature might be a useful tool to understand if a food from animal origin was contaminated ante- or post-mortem and thereby to clarify the possible contamination sources.

The aim of the present study was to investigate the enantiomer specific accumulation and depuration in broiler chicken and to rule on the contamination source based on chiral signature.

2. Materials and methods

2.1. Feed preparation and broiler chicken experimental design

Details of the feed preparation and National ethic committees approved experimental design were described elsewhere [2]. Briefly, 50 slow growing (SG, JA 657 strain) and 29 fast growing (FG, Ross PM3 strain) broiler chickens were raised in individual cages and given feed contaminated or not with α-HBCDD at ~35 ng/g feed (at 12% H2O) during up to 12 weeks. Composite samples of each feed were prepared all along the experiment. Individuals were regularly killed during accumulation and depuration periods. Abdominal adipose tissue, liver, plasma, pectoralis and thigh muscle were individually collected. Plasma and dropping samples were pooled per treatment group for a given collection time point.

2.2. Sample treatment and analysis

Collected samples were prepared according to an ISO 17025 method described elsewhere [3], quantification being based on isotope dilution method ($^{13}C_{12}$-labelled internal standards). Briefly, after lyophilisation, lipids were extracted by pressurized liquid extraction with a toluene/acetone mixture (70:30, v/v). Purification steps were performed on multi-layer Na_2SO_4, neutral and acidic (H_2SO_4) silica gel flushed with n-hexane and dichloromethane, followed by partitioning between n-hexane and 1 N NaOH. HBCDD diastereoisomers were separated and detected by LC-ESI(-)-MS/MS (6410, Agilent Technologies). Observed α-HBCDD levels reached several hundreds of ng/g lipid weight [2].

2.3. Enantiomer analyses

The sample extract was also analysed for HBCDD enantiomers by LC-ESI(-)-HRMS (Orbitrap Q-Exactive, Thermo Scientific.). HRMS data were acquired in full scan mode over the 600-700 m/z range, at a resolution of 70,000 FWHM (m/z 200). Chromatographic separation was performed on a chiral cellulose tris-(3,5-dimethylphenylcarbamate column (ACQUITY UPC² Trefoil CEL1, 2.1 mm x 150 mm, 2.5 µm) purchased from Waters (Milford, MA, USA) and kept at 35 °C. Mobile phases consisted of 20 mM ammonium acetate in water (A) and acetonitrile (B). The gradient began with A/B 50:50 (v/v) for 1 min, ramped linearly to 30:70 over 7 min to be maintained for 4 min, reached in 1 min 0:100 maintained 6 min before returning to initial conditions. With such parameters, obtained valley between (-) and $(+)_\alpha$-enantiomers was lower than 1%.

2.4. Data analysis and quantification
The enantiomer fraction of α-HBCDD (EF_α) was calculated by the following formula:
$EF_\alpha = [(+)A_\alpha/(+)A_{\alpha(IS)}]/[(+)A_\alpha/(+)A_{\alpha(IS)} + (-)A_\alpha/(-)A_{\alpha(IS)}]$
where $(+)A_\alpha$ and $(-)A_\alpha$ represent the peak areas of the native and internal standard (IS) α-HBCDD enantiomeric pairs.

3. Results and discussion

3.1. Enantiomeric profile of α-HBCDD
Several contaminated feeds (n=11) were analysed and used as control chart for racemic mixture (Figure 1). The average EF_α was found to be 0.505 ± 0.004 (Figure 1).

EF_α calculated in samples collected all along the experiment were similar between SG and FG broilers. Intra-group standard deviations were relatively low (<1.5%, n≥4) revealing a satisfying repeatability. Pectoralis muscle displayed EFs_α included between 0.43 and 0.47, indicating a significant enrichment in $(-)_\alpha$-enantiomer compared to racemic mixture in feed, which was slightly more pronounced during the depuration phase for SG broiler (Figure 1). Abdominal fat, liver tissue, plasma and thigh muscle presented similar profiles to pectoralis muscle, but no significant differences appeared among matrices within kinetic groups. Therefore, most matrices showed EF_α marking a biological transformation revealing ante-mortem contamination. Droppings were the only matrix presenting racemic EF_α values, in the racemic 0.494–0.504 range during the contamination phase (Figure 1). Such racemic elimination suggests that no preferential enantiomer uptake occurs through gastrointestinal barrier. By contrast, during the depuration phase, EF_α of droppings (SG broiler) tended to regularly decrease below 0.4, revealing that, although minor, faeces are a route for a-HBCDD elimination during depuration.

3.2. Application to samples collected in farms
In 2014-2015, we collected pectoralis muscle of broiler chickens directly at rearing farms (n=57 representative of the French production). Three samples showed α-HBCDD levels higher than 1 ng/g lw and were further investigated for EF_α. Samples 1 and 2 showed significant enrichment in $(-)_\alpha$-HBCDD (0.427 and 0.446, Table 1), consistent with the kinetic model (Figure 1). These results suggested a biological transformation and then ante-mortem ingestion. In addition, the diastereomeric profile dominated by α-HBCDD (>97%) was in agreement with a possible biotransformation or a preferential bioaccumulation of the α-diastereoisomer. The third sample showed an EF_α of 0.503, similar to the average value calculated for the feed, indicating a racemic composition and suggesting post-mortem contamination. According to the diastereomeric profile, the contact of muscle with XPS foam was hypothesised [4].

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5. References

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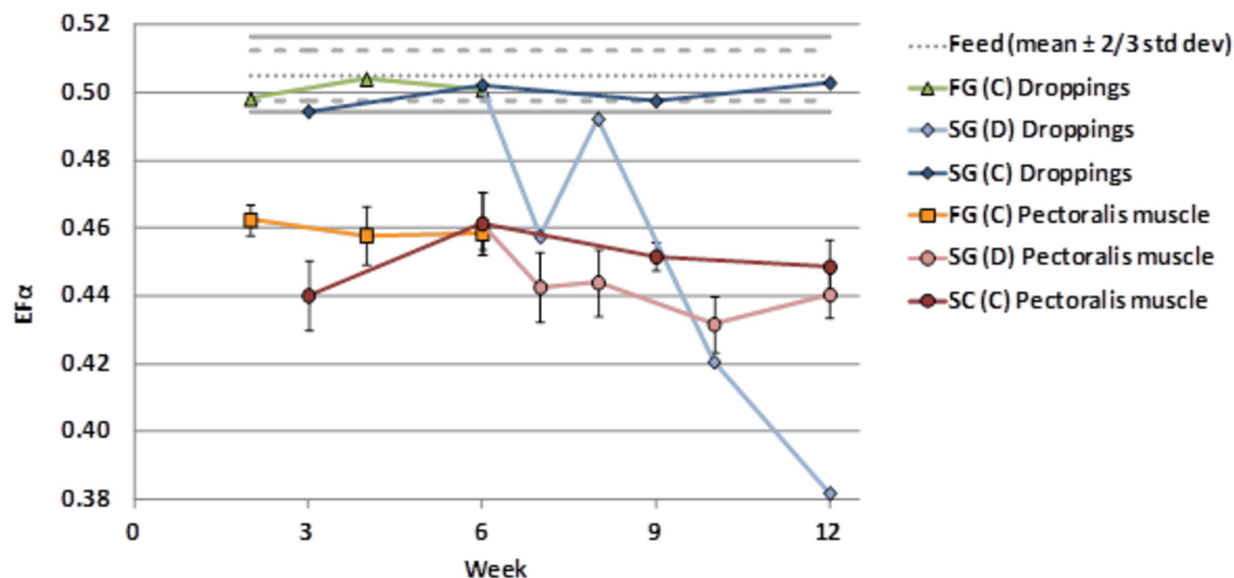

Figure 1: EF_{α} in feed, droppings and pectoralis muscle for SG and FG broilers from the animal experiment during contamination (C) and depuration (D) periods.

Table 1: Total concentration, enantiomer fraction in α-HBCDD and diastereomeric profile for 3 real world broiler pectoralis muscles. nd: not detected.

	Sum HBCDD (ng/g lw)	EF_{α}	Isomer profile (%)		
			α-HBCDD	β-HBCDD	γ-HBCDD
Sample 1	1.96	0.446	>97.5%	nd (<1.4%)	nd (<1.1%)
Sample 2	2.57	0.427	>97.7%	nd (<1.2%)	nd (<1.1%)
Sample 3	2.10	0.503	78.8%	11.9%	9.4%