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ACCUMULATION AND TISSUE DISTRIBUTION OF INGESTED α-HEXABROMOCYCLODODECANE (α-HBCDD) IN BROILER CHICKEN (GALLUS DOMESTICUS)

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1. Introduction

Although their content in food is not regulated, some Brominated Flame Retardants (BFRs) are suspected or considered as endocrine disruptors. These lipophilic compounds are prone to bioaccumulation in animal tissues and, according to EFSA[1,2], consumption of animal products represents a major source of consumer's exposure. The BrAviPorc project (2013-2016), co-funded by the French Ministry of Agriculture, Agrifood and Forestry and involving public laboratories as well as organisations of animal production sectors, aims at investigating several aspects related to BFRs at farm scale, more precisely in laying hen, broiler and pig productions, including (i) identifying possible BFRs sources, (ii) evaluating BFRs levels of animal products from representative French farms and (iii) modelling pharmacokinetic behaviour of a selected BFR, α-hexabromocyclododecane (α-HBCDD). The final objective is to provide competent authorities and farmers with new data for an efficient management. The present proceeding describes the levels of α-HBCDD accumulated in tissues of broiler chicken, resulting from a chronic oral exposure during growth.

2. Materials and methods

2.1. Feed preparation

Preparation of contaminated feed was described elsewhere3. Briefly, technical HBCDD was enriched in α-HBCDD by heating (172 °C, 6 h). After purification on Florisil® and preferential precipitation, crystals containing 99.3% α-HBCDD were dissolved in acetone for spiking soy oil at 10 mg/kg. The oil was mixed at 50 µg/kg feed, chosen in order to reach a few hundred of ng/g lipid weight (lw) in animal products, as already observed in a few atypical samples from French monitoring plans in 2009-2010. Feeds (contaminated and control) were formulated according to animal requirements.

2.2. Animal experiments

Contamination experiment, conducted to study pharmacokinetic data of broiler chicken, was approved by National ethic committees. Animals were raised under conventional conditions of temperature and lighting. Fifty slow growing (SG, JA 657 strain) and 29 fast growing (FG, Ross PM3 strain) broiler chicks weighing 42±1 and 49±1 g, respectively, were housed in individual cages after 1 week of adaptation with the control feed. Starter (weeks W1–W3) and grower-finisher (W4–W12) feeds were distributed to SG individuals and a grower feed (W1–W6) to FG individuals according to breeder recommendations. Droppings were individually collected during 72 h before sacrifice. Animals were killed by electrical stunning then exsanguinations at W3 (n=4), W6 (n=4), W9 (n=4) and W12 (n=5) for exposed SG broilers, W6 (n=2) and W12 (n=4) for control SG broilers, W2 (n=4), W4 (n=4) and W6 (n=7) for exposed FG broilers and W6 (n=4) for control FG broilers. Some SG individuals also fed with contaminated feed (W1–W6) followed by control feed were killed at W7 (n=4), W8 (n=4), W10 (n=4) and W12 (n=5). Abdominal adipose tissue, liver, plasma, pectoralis and thigh muscles were individually collected. Plasma and dropping samples were pooled per treatment group. Additionally, 10 SG and 10 FG chicks were killed at W0 and samples pooled per matrix. A composite sample of each feed was prepared all along the experiment.

2.3. HBCDD quantification in feed and animal products

The ISO 17025 method was described elsewhere[3]. Briefly, reference solutions of native and $^{13}C_{12}$-labeled HBCDD isomers were provided by Wellington Laboratories. After lyophilisation, lipids were extracted by Pressurised Liquid Extraction (Büchi) using a toluene/acetone mixture (70:30, v/v). Purification steps were performed on a SPE column manually packed with Na_2SO_4, neutral and acidic (H_2SO_4) silica gel using hexane and dichloromethane, followed by partitioning between n-hexane and 1 N NaOH. HBCDD isomers were separated and detected by LC-ESI(-)-MS/MS (6410, Agilent Technologies).

3. Results and discussion

Figure 1 shows accumulation curves for α-HBCDD in all selected matrices.

3.1. Feed

All composite feed samples were devoid of HBCDD isomers, as well as control feeds in α-HBCDD. α-HBCDD concentrations ranged between 33.6 and 36.8 ng/g of feed normalised at 12% moisture. SG and FG broiler chickens ingested on average 6555 and 4609 g of feed/animal after 12 and 6 weeks, corresponding to 2471 and 2762 g of gained weight and α-HBCDD ingestion of 99±1 and 66±1 ng/g of gained weight, respectively.

3.2. Adipose tissue

The concentration of α-HBCDD in adipose tissue of SG broilers rapidly increased to ~600 ng/g lw before stabilising at ~400 ng/g lw after 9 weeks. It was decreased to ~60 ng/g lw for individuals given control feed from W6 to W12. Dilution from body lipid deposition contributed for 61% to this decrease. For FG broiler, α-HBCDD reached ~300 ng/g lw after 6 weeks.

3.3. Muscle

Regarding pectoralis muscle, α-HBCDD reached ~80 ng/g lw at the end of experiments for both SG and FG broiler, even though it was not stabilised for FG broiler à W6. Regarding thigh muscle, this concentration was higher, at ~280 and ~180 ng/g lw for SG and FG broiler, respectively, while the plateau also seemed to be reached for SG broiler around W9. This higher concentration in thigh compared to pectoralis is all the more striking because lipid contents were lower in pectoralis (1.8%) than in thigh (5.4% and 7.2% for SG and FG broilers, respectively). For SG broilers given control feed from W6 to W12, α-HBCDD concentration in thigh muscle decreased with a similar rate compared to adipose tissue. This rate was higher in pectoralis. In both tissues, lipid deposition contributed for ~50% to this decrease.

3.4. Liver

Regarding liver, α-HBCDD reached ~100 and ~50 ng/g lw at the end of experiments for SG and FG broiler, respectively, with higher intra-group standard deviations. Indeed, this matrix is known to be prone to a higher variability in many species as regard to contaminant levels. Nevertheless, a rapid decrease was also observed for label individuals given control feed from W6 to W12.

3.5. Plasma

Regarding plasma pools, levels appeared relatively stable in the 30–44 ng/g lw range both for SG and FG broilers when exposed. For SG individuals given control feed from W6 to W12, circulating levels shifted to the 7–12 ng/g lw range.

3.6. Droppings

Regarding dropping pools, levels were in the 20–55 ng/g dried weight range both for SG and FG broilers when exposed, but immediately decreased below 3 ng/g dried weight for FG individuals given control feed from W6 to W12, showing limited excretion to the digestive tract after absorption.

4. Conclusion

These results confirm the potential for bioaccumulation of α-HBCDD and will be used for a pharmacokinetic model useful in the context of food chemical safety. Abdominal adipose tissue appeared as the most relevant matrix for monitoring contamination of broiler production, followed by consumed thigh muscle.

5. Acknowledgements
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6. References
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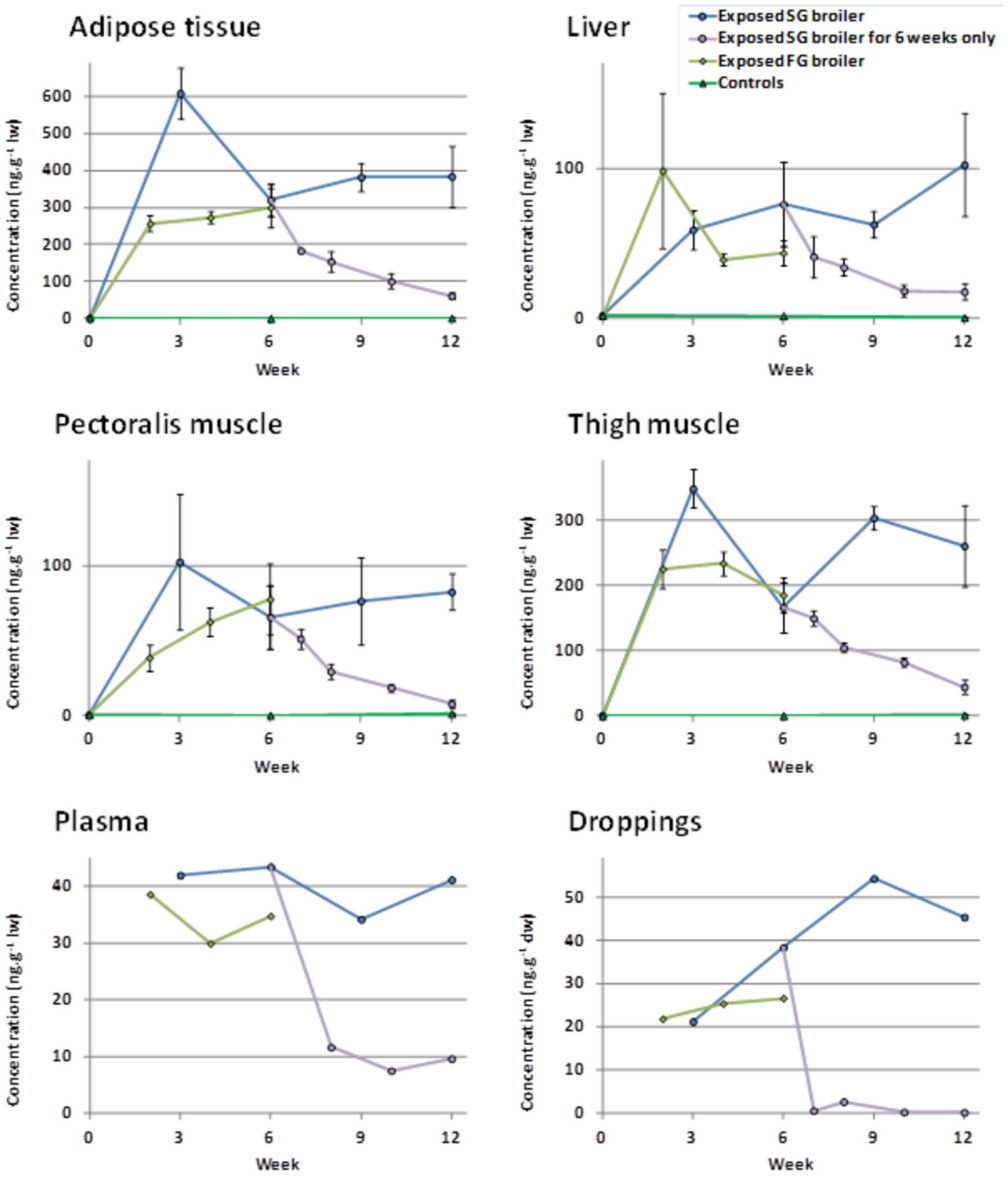

Figure 1: Time-dependent concentration of α-HBCDD in selected matrices of slow and fast growing broiler chicken.