Relevance of volatile metabolome in animal-derived products to reveal a food chain contamination by hexabromocyclododecane

Jérémy Ratel, Frederic Mercier, Saïd Abou El Karam, Ronan Cariou, Elena Dominguez-Romero, Angelique Travel, Catherine Jondreville, Erwan Engel

RELEVANCE OF VOLATILE METABOLOME IN ANIMAL-DERIVED PRODUCTS TO REVEAL A FOOD CHAIN CONTAMINATION BY HEXABROMOCYCLODODECANE

J. Ratel[1], F. Mercier[1], S. Abou El Karam[1], R. Cariou[2], E. Dominguez-Romero[3,4,5], A. Travel[4], C. Jondreville[5], E. Engel[1]

[1] INRA, QuaPA UR370, MASS group, 63122 Saint-Genès-Champanelle, France

[2] LUNAM Université, Oniris, LABERCA, USC INRA 1329, 44307 Nantes, France

[3] INRA, URA83, 37380 Nouzilly, France

[4] ITAVI, UMT Bird, UR83 Recherches Avicoles, BP1, 37380 Nouzilly, France

[5] Université de Lorraine, INRA, URAFPA, USC 340, 54500 Vandoeuvre-lès-Nancy, France

Abstract – The flame retardant Hexabromocyclododecane (HBCD) is part of the critical emerging contaminants. The diet is the major route of human exposure to HBCD and consumers may be exposed through a single food intake at very high doses. The only way to guarantee their food safety given the unpredictable isolated cases of high contamination is to propose frequent and large-scale controls, yet the current reference analytical methods are economically inconceivable. Alternative approaches based on targeted quantification of livestock's exposure markers to pollutants are proposed. Among the compounds assumed as potential markers, the volatile organic compounds (VOCs) are particularly promising. Based on animal test involving laying hens contaminated or not by HBCD through their feed, the present study demonstrates the relevance of using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) for profiling VOCs in animal-derived products and for determining the markers of HBCD exposure. The volatile fingerprints of egg yolk and liver allowed the different animal groups to be clearly discriminated and volatile markers of HBCD exposure to be pointed out. The analysis of these markers could enable to reveal systematically suspect samples on market and to guide further analyses to confirm and possibly explain the contamination.

Key Words – Chemical contamination, Food safety, Volatile biomarkers.

I. INTRODUCTION

Human activities cause the emission of pollutants which may accumulate in environmental compartments and subsequently in the food chain. Some brominated flame retardants were part of the critical emerging contaminants, as hexabromocyclododecanes (HBCDs) most notably because of its known endocrine effects [1]. One of the most important route of human exposure to HBCDs is the exposure through the diet [2]. Literature data and monitoring plans carried out on animal-derived food showed that some poultry meat and eggs display abnormally high HBCD concentrations. In the case of the ingestion of a strongly contaminated egg, the consumer can ingest more than 50 mg HBCD at once, which is almost 4000 times the maximum daily exposure in France for a 70 kg man [3].

Today, the only way to guarantee food security to consumers given the unpredictable isolated cases of strong contamination to HBCD is to propose frequent and large-scale controls, and routine controls based on the current reference methods are economically inconceivable. To overcome these limitations, alternative analytical approaches based on the targeted quantification of livestock's exposure markers to pollutants can be proposed. Among all candidate markers, the volatile organic compounds (VOC) are particularly promising. In a recent critical review, Hakim *et al.* [4] proposed that metabolic disorders can produce new VOCs or change the ratio between the VOCs normally produced by the body. Berge *et al.* [5] demonstrated that the composition of volatile metabolome of the liver of animals exposed via their feed to environmental pollutants was changed compared to unexposed animals.

The present study aims to assess the relevance of a SPME-GC-MS analysis of volatile metabolome in animal-derived products to reveal markers of livestock exposure to HBCD.

II. MATERIALS AND METHODS

2.1. Feed for animal experiment.

Contaminated diets were prepared from a commercial basal diet. α-HBCD (1,2,5,6,9,10-hexabromocyclododecane) was chosen for feed contamination step because this isomer prevails in the environment and constitutes the main contamination source of animals. Soy oil was mixed with commercial basal feed according to Fournier *et al.* [6], at two targeted contamination levels (L1 at 50 and L2 at 5 µg/kg feed). The clean feed was obtained by mixing the same amount of blank soy oil in the commercial feed. Feeds were administered as pellets.

2.2. Poultry and exposure to α-HBCD.

Laying hens aged 30-weeks were reared indoors in individual coops under controlled conditions at the AFPA Research Unit (Nancy, France). Two groups of three laying hens received during the 18 weeks of breeding the "L1" contaminated feed and the "L2" contaminated feed, respectively. Three other laying hens received the L1 contaminated feed during the 11 first weeks then the control feed during the last 7 weeks ("L1_D"). Two laying hens were fed with the control feed throughout the breeding ("C"). Hens were sacrificed at the end of the 18 weeks, after 12 h of fasting.

2.3. Preparation of samples.

Eggs and livers were immersed in liquid nitrogen, wrapped in aluminum foil, vacuum packed and stored at −80 °C. In a second step, each egg yolk and liver was ground in liquid nitrogen into a fine homogeneous powder using a home-made stainless steel ball mill. For the targeted quantification of HBCD, an aliquot of each ground tissue was placed in a glass bottle and stored at −80 °C. For the determination of the volatile compound metabolic signature of the exposure to HBCD, a 1.2 g aliquot of each ground tissue was placed in a glass vial sealed under nitrogen flow and stored at −80 °C.

2.4. Quantification of HBCD.

After lyophilisation, lipids were extracted by Pressurised Liquid Extraction (Büchi) using a toluene/acetone mixture (70:30, v/v). Purification steps were achieved on successive columns, manually packed with acidic silica gel and Florisil®. HBCD was further purified by partitioning between hexane and NaOH 1 N. HBCD isomers were separated and detected by LC-ESI(-)-MS/MS (6410, Agilent Technologies).

2.5. Volatile compound signatures.

The volatile compounds of liver samples were extracted by SPME according to Berge *et al.* [5]. An automated sampler was used to carry out the following successive steps: (i) sample preheating in the agitator (500 rpm) for 10 min at 40 and 60 °C for liver and egg yolk samples, respectively, (ii) trapping by SPME (75 µm carboxen-polydimethylsiloxane) of the volatile compounds for 30 min at 40 °C or 60 °C, and (iii) thermal desorption at 250 °C for 2 min in the GC inlet. Further volatile compound analysis was performed according to Berge *et al.* [5] by GC-full scan MS (GC2010; QP2010+, Shimadzu). The SPME-GC-MS chromatograms were first converted into virtual SPME-MS fingerprints to estimate quickly the informative potential of the volatile fraction [5], then the abundances of volatile compounds were determined from a specific mass fragment for each molecule to avoid co-elution problems (GC-MS solution software, Shimadzu).

2.6. Data processing.

Data were processed using the Statistica Software version 10 and the R software version 2.1.4. The variables used in data treatments were the mass fragments for virtual SPME-MS fingerprints and the volatile compounds for SPME-GC-MS signals. To improve both sample set discrimination and biomarker identification, the variables were normalized according to the systematic ratio normalization [7]. This method consists in calculating for each sample all the log-ratios between abundances of variable abundances, then selecting the log-ratios that best maximize the discrimination between sample-sets. Principal component analyses (PCA) were performed on the discriminant variables selected by ANOVA in the signals in order to visualize the structure of the data. For the discriminant analyses (DA), the two

most relevant variables were selected according to the best subset algorithm.

III. RESULTS AND DISCUSSION

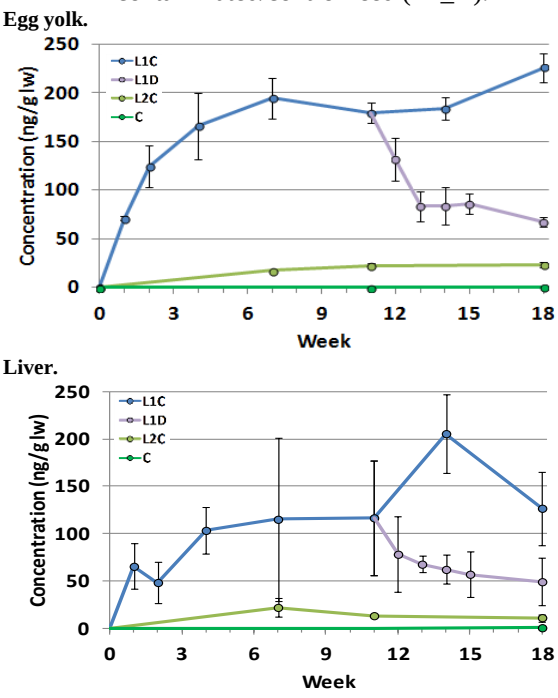
3.1. HBCD in spiked feed.

For control feed, no HBCD has been detected at the limit of reporting (0.040 ng/g at 12% moisture). HBCD levels measured in the contaminated feed L1 and L2 were 36.8 and 3.3 ng/g at 12% moisture, respectively. The actual HBCD levels in contaminated feeds were lower than the targeted levels (50 and 5 ng/g), but the targeted ratio of 10 between the two contamination levels was reached.

3.2. HBCD in egg and liver.

α-HBCD was measured in both egg yolk and liver of laying hens (Figure 1). Results are consistent with the experimental design confirming the animal contamination, with a higher variability observed for the HBCD measured in liver.

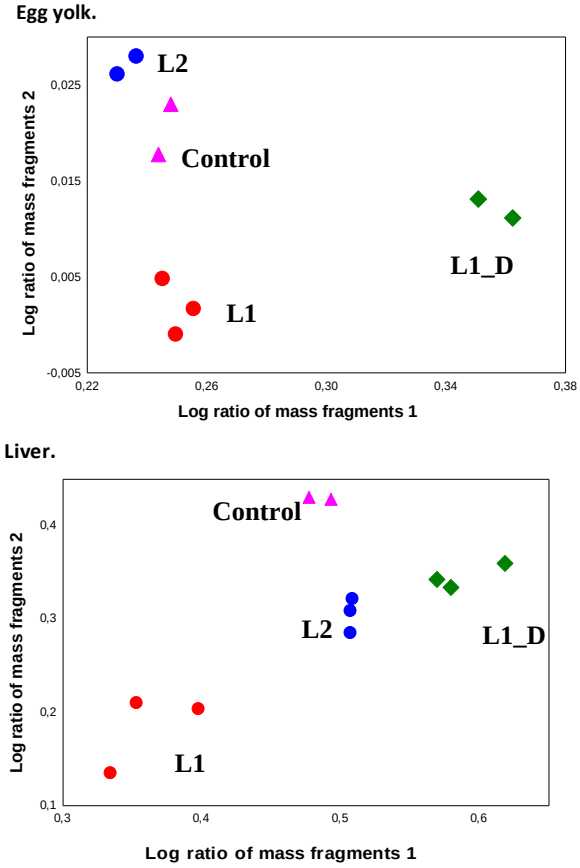
Figure 1. HBCD levels determined by LC-MS/MS in egg yolk and liver of laying hens fed the control feed (C), the contaminated feed (L1, L2) and the contaminated/control feed (L1_D).



3.3. Response of the volatile metabolome in egg and liver to HBCD exposure.

Fingerprints of the volatile metabolome in egg yolk and liver. Laying hens fed control or contaminated feed can be clearly differentiated according to the fingerprints of the volatile metabolome both in egg yolks and in livers. Figure 2 shows the sample discrimination in the plane formed by the DA-selected pair of log ratios of mass fragment in egg yolk and liver, respectively, after 18 weeks of rearing. We can observe a clear distinction of the four hen groups which indicates that changes in metabolism occurred in response to dietary contamination by HBCD and that these changes were revealed by the volatile compound fingerprint. This result is consistent with the study of Berge *et al.* [5] who demonstrated that virtual SPME-MS fingerprints of volatile compounds in poultry liver were relevant for differentiating animals according to the occurrence of pollutants in feed.

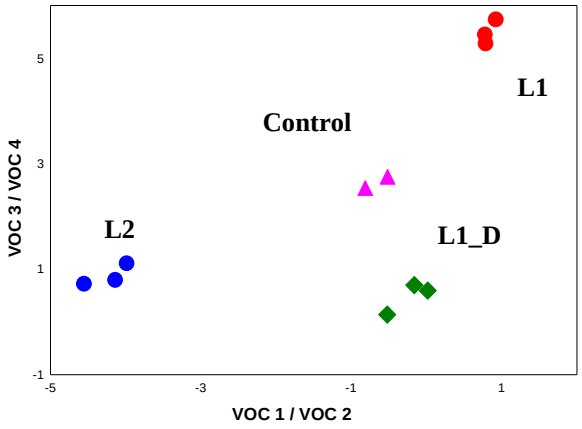
Figure 2. Virtual SPME-MS fingerprints in egg yolk and liver of laying hens fed the control feed (C), the contaminated feed (L1, L2) and the contaminated/control feed (L1_D). Representation of the 2 most discriminative log ratios of mass fragments.



According to Figure 2, animals weakly exposed to HBCD (L2) were closer to control animals than those which were strongly exposed (L1), suggesting a dose dependent metabolic response. The results for egg and liver related to decontaminated animals (L1_D) were clearly separated from the group of control animals. The volatile metabolome could enable to trace-back an acute HBCD contamination event.

Volatile biomarkers in liver for revealing HBCD exposure. More than 100 volatile compounds were monitored in contaminated or control animals. Fifty log ratios of volatile compounds were determined as significantly discriminating the four animal groups. Figure 3 shows that just 2 log ratios of volatile compounds, selected by DA, allowed the livers of contaminated or control laying hens to be discriminated. The identification of the discriminative volatile compounds and the assessment of the relevance of markers of HBCD exposure are in progress.

Figure 3. Discrimination of livers of animals contaminated or not by HBCD on the basis of the 2 best log ratios of volatile compounds.



IV. CONCLUSION

The present study confirms the interest of volatile metabolome of animal derived-food products for revealing animal exposure to HBCD. The use of the SPME-GC-MS could constitute a promising resolutive and fast monitoring method for guaranteeing the chemical safety of food. The further investigation should focus on the identification of the volatile markers and the validation of their robustness.

ACKNOWLEDGEMENTS

This study was supported by the French special purposes account for the agricultural and rural development (CASDAR), in the frame of the project BRAVIPORC.

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