Identification by volatolomics of hydrocarbon, oxygenated, sulfur and aromatic markers of livestock exposure to α-hexabromocyclododecane

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To cite this version:

jérémy ratel, Frédéric Mercier, Magaly Angénieux, Nathalie Kondjoyan, Saïd Abou El Karam, et al.. Identification by volatolomics of hydrocarbon, oxygenated, sulfur and aromatic markers of livestock exposure to α-hexabromocyclododecane. Food Chemistry, 2022, pp.131504. 10.1016/j.foodchem.2021.131504 . hal-03472011

HAL Id: hal-03472011
https://hal.inrae.fr/hal-03472011

Submitted on 8 Jan 2024

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Version of Record: https://www.sciencedirect.com/science/article/pii/S0308814621025103
Manuscript_62c46974d41f0a0ce1c6dd2f16454e35

1 # Identification by volatolomics of hydrocarbon, oxygenated, sulfur and
2 aromatic markers of livestock exposure to α-hexabromocyclododecane

3

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15

16 ## HIGHLIGHTS

17 • An experimental animal exposure to α-hexabromocyclododecane (HBCDD) was designed
18 • Hens, broilers and pigs were exposed to realistic α-HBCDD doses in their diet
19 • Liver volatolomics evidences exposure of the three farm animals to α-HBCDD
20 • Most candidate markers are hydrocarbon, oxygenated, sulfur and aromatic compounds
21 • Volatolomics, as an option for surveillance of chemical contamination in livestock

22

23 ## ABSTRACT

24 Volatile organic Compounds (VOC)-based metabolomics, or volatolomics, was investigated
25 for revealing livestock exposure to chemical contamination. Three farm animals, namely

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laying hens, broilers, and pigs, were experimentally exposed to 5 or 50 ng α-HBCDD g^{-1} feed. Liver and egg yolk for hens were analysed by headspace-SPME-GC-MS to reveal candidate markers of the livestock exposure to α-HBCDD. For hens, 2-butanol was found as marker in egg. In liver, twelve VOCs were highlighted as markers, with three aromatic VOCs – styrene, *o*-xylene, α-methylstyrene – highlighted for the two α-HBCDD doses. For broilers, six markers were revealed, with interestingly, styrene and phenol which were also found as markers in hen liver. For pigs, ten markers were revealed and the seven tentatively identified markers were oxygenated and sulfur VOCs. The candidate markers tentatively identified were discussed in light of previous volatolomics data, in particular from a γ-HBCDD exposure of laying hens.

KEYWORDS: Volatolomics, α-HBCDD, Headspace-SPME, GC-MS, Livestock, Food safety.

1. INTRODUCTION

Hexabromocyclododecane (HBCDD) is a brominated flame retardant that has been a targeted substance in the Stockholm Convention since May 2013. HBCDD is classified as a substance of very high concern (SVHC) on the REACH candidate list due to its persistent bioaccumulative and toxic (PBT) properties. For non-occupationally exposed persons, dietary exposure is a major route of total HBCDD intake (Covaci et al., 2006). Food contaminations by HBCDD are especially problematic because of sometimes high levels, so that the ingestion of a single contaminated animal-derived food can significantly increase consumer exposure to it (Ratel et al., 2017). Based on direct HBCDD detection in food, the current analytical methods for the surveillance of HBCDD contamination are efficient and sensitive but are

51 hindered by cumbersome implementation related in particular to the ubiquitous occurrence of
52 HBCDD. These routine monitoring techniques thus do not allow rapid cost-effective large-
53 scale methods, which seem essential for strengthening food safety with respect to this
54 contaminant (Meurillon et al., 2018).

55 To overcome these limitations, alternative approaches based on omics have emerged to detect
56 contaminations. They are inspired by research showing the usefulness of the rapid cost-
57 effective analysis of expired volatile organic compound (VOC) markers in clinical diagnosis
58 (Hakim et al., 2012). Berge et al. (2011) proved the concept that the signature of the liver
59 metabolome in small compounds, the volatolome, was modified for chickens in response to
60 exposure in their diet to different xenobiotics including brominated flame retardants such as
61 polybrominated diphenyl ethers. Liver is a key target for the determination of markers of
62 exposure to contaminants because of its major function in protecting the organism from
63 potentially toxic chemical insults. Bouhlel et al. (2018) took this concept and identified some
64 VOCs that were impacted in the liver volatolomes of chickens exposed to three types of
65 micropollutants − a pesticide, an environmental contaminant and a coccidiostatic agent. As
66 HBCDD impacts the metabolism of exposed organisms with clear indications of toxicological
67 effects in the liver (Cantón et al., 2008; Germer et al., 2006), liver volatolomics could be
68 useful for identifying markers of HBCDD exposure and back-tracing HBCDD food
69 contamination.

70 Ratel et al. used volatolomics to reveal exposure of laying hens to γ-HBCDD (Ratel et al.,
71 2017) and reported a list of VOCs in livers impacted by γ-HBCDD exposure. The study of
72 Ratel et al. (2017) showed that at least four additional issues needed to be addressed: (i) this
73 first study performed on HBCDD focused on the γ-isomer, whereas the α-HBCDD isomer is
74 probably more relevant because it predominates in the environment, in animal tissues and in
75 animal-derived food (Marvin et al., 2011; Rivière et al., 2014; Koch et al., 2015; Dominguez-

76 Romero et al., 2016), (ii) this first list of VOC candidate markers needed filtering to screen for
77 the most robust one, (iii) the proof of concept had to be confirmed on more realistic HBCDD
78 levels of feed contamination, and (iv) the scope had to be extended to other farm animals.
79 Based on control/test experiments, the present study aimed first to confirm the utility
80 demonstrated by Ratel et al. (2017) of liver volatolomics in demonstrating the exposure of
81 laying hens to HBCDD, by considering the α-HBCDD isomer. In addition, realistic exposure
82 levels of laying hens were implemented and the potential of egg volatolomics was explored.
83 Egg is widely consumed and the literature has reported high HBCDD levels in chicken eggs,
84 sometimes above the mg kg^{-1} LW level (Hiebl and Vetter, 2017; Dominguez-Romero et al.,
85 2016). According to its lipid rate, egg yolk may contain many volatile compounds and it is
86 therefore very relevant for considering the implementation of non-invasive volatolomics
87 methods for food safety surveillance. Liver volatolomes were also investigated for two other
88 major monogastric farm animals, namely broilers and pigs. The potential markers discovered
89 are discussed especially in the light of the candidates found in the two main studies carried
90 out from liver volatolomics of livestock exposed or not to micropollutants: the study of Ratel
91 et al. (2017), based on the exposure of laying hens to γ-HBCDD, and that of Bouhlel et al.
92 (2018), based on the exposure of broilers to different micropollutants.
93

94 **2. MATERIALS AND METHODS**

95 **2.1. Animal feeding**

96 Compositions of experimental feeds for the three farm animals were detailed in Table S1.
97 Contaminated diets were obtained by replacing 5 g of clean soy oil in the control diet by 5 g
98 of soy oil spiked at appropriate levels with α-HBCDD. The synthesis of α-HBCDD has been
99 described by Dominguez-Romero et al. (2016). Briefly, technical HBCDD, containing 1, 5,
100 and 93% of α-, β-, and γ-HBCDD, respectively, was enriched in α-isomer by thermal

101 rearrangement (172 ± 0.4 °C, 6 h), according to a method adapted from that described by
102 Szabo et al. (2011). Purification was performed on neutral silica gel and magnesium silicate
103 manually packed solid phase extraction (SPE) columns and also by preferential precipitation
104 at −20 °C, using dichloromethane and n-hexane. The purity of resulting crystals has been
105 estimated at 99.3% α-HBCDD, η-HBCDD being the only identified impurity. Crystals of α-
106 HBCDD were dissolved in acetone used to spike soy oil at the targeted concentration. All
107 spiked feeds were prepared at the same time in the INRAE PEAT feed mixing unit (Nouzilly,
108 France) by using dilutions of a single 50 µg g^{-1} contaminated oil prepared by LABERCA
109 (Nantes, France). However, for pigs, the volume of contaminated feeds needed for all the
110 experiment was too high to be produced in the INRA PEAT facilities. To overcome this issue,
111 the contaminated feeds for pigs have been concentrated 10 times more than the expected
112 level, then contaminated feed was brought each day of the experiment in the pig trough in a
113 1:10 ratio (w/w) mixed with uncontaminated feed.
114 For the three farm animals, a contaminated diet with the target concentration of 50 ng α-
115 HBCDD g^{-1} was prepared. This concentration was expected to enable the animal-derived
116 products to reach several hundreds of ng of HBCDD g^{-1} LW, as previously reported in heavily
117 contaminated samples shown by French monitoring plans (Jondreville et al., 2017). For laying
118 hens and pigs, a second lower dose of 5 ng g^{-1} feed was tested to obtain animal-derived
119 products with more realistic α-HBCDD contamination levels. For broilers, the high
120 occupancy of the INRAE facilities did not allow testing two exposure doses with individual
121 animal cages.
122
123 **2.2. Animal testing**
124 Animal experiments have been designed to study the exposure to α-HBCDD in feed of laying
125 hens, slow-growing broiler chickens and growing pigs. These experiments were conducted in

126 compliance with Directive 2010/63/EU in France and approved by the relevant ethics
127 committee. Animal experiments were conducted in appropriate facilities with cages allowing
128 feed ingestion of individual animals to be monitored. Laying hens, broilers and pigs were fed
129 with non-contaminated feeds (control groups) or with α-HBCDD contaminated feeds
130 (exposed groups) for 18, 12 and 16 weeks, respectively. The main features of the three
131 experiments conducted in laying hens, broilers and pigs are summarized in Table 1, with the
132 detailed conditions of each animal experiment which are given in Table S2. Information about
133 body weight and feed ingested of animals during animal testing is given in Table S3.
134
135 **2.3 Slaughtering and sampling**
136 At the end of the exposure period, all animals were sacrificed and the weight of each warm
137 carcass was recorded at slaughter (Table S3). Poultry and pigs were killed after a 12-hour and
138 a 18-hour fast, respectively, by electronarcosis followed by exsanguination (electric stunner
139 for poultry, Ducatillon, Cysoing, France; Morphee M4 electric stunner for pigs, Lelong &
140 Cie, Savigny, France). "Control samples" and "exposed samples" correspond to the samples
141 collected from control and exposed animal carcasses, respectively. For eggs, the eggs laid
142 before slaughter and on the day of slaughter were collected. The egg laid before slaughter was
143 dedicated to HBCDD analysis. It was weighed and removed from its shell before separating
144 and storing at -20°C the white and yolk. The egg laid on the day of slaughter was dedicated to
145 volatolomics analysis. It was collected, immersed in liquid nitrogen and stored at −80 °C. For
146 fat and muscle samples dedicated to HBCDD quantification, abdominal fat and muscles from
147 one thigh (without skin and bone) for poultry and samples of back fat and semi-membranous
148 muscle for pigs were collected, weighed and stored at -20°C. For liver samples dedicated to
149 HBCDD quantification and volatolomics, the whole liver for poultry and a sample prepared
150 from the left lateral and right medial lobes for pigs were collected, weighed, immersed in

151 liquid nitrogen, wrapped in aluminum foil, vacuum-packed, and stored at −80 °C. No visible
152 sign of pathology was detected during the autopsy carried out during the cutting of the post-
153 slaughter animals. Data about weight and lipid content of collected samples are given in Table
154 S3.
155
156 **2.4 HBCDD quantification**
157 HBCDD isomers (α, β and γ) were analysed in feed and animal samples according to a
158 method covered by the scope of the ISO/IEC 17025:2005 accreditation of the LABERCA
159 laboratory, and described by Dominguez-Romero et al. (2016). Briefly, all glassware and
160 Na2SO4 were baked prior to use at 400 °C for 4 h or at 650 °C for 6 h, respectively. Feed was
161 dried in an oven at 80 °C. Other matrices, animal tissues and egg yolk, were lyophilized. The
162 sample size ranges used for HBCDD quantification was given for each matrix in Table S4.
163 Lipids were extracted by pressurized liquid extraction (PLE) over three successive static
164 cycles (100 bar, 120 °C) (SpeedExtractor, Büchi, Switzerland) with a toluene/acetone mixture
165 (70:30, v/v), evaporated to dryness, and weighed. Purification steps were conducted on a SPE
166 column manually packed with Na2SO4, neutral, and acidic (H2SO4) silica gel using hexane
167 and dichloromethane, followed by partitioning between n-hexane and 1 N NaOH. Final
168 extracts were reconstituted into a mixture of methanol/water 80:20 (v/v). HBCDD isomers
169 were analyzed by LC-ESI(−)-MS/MS (6410, Agilent Technologies). Separation was achieved
170 on a Hypersil Gold column (100 mm × 2.1 mm, 1.9 µm) (Thermo Scientific, San Jose, CA,
171 USA) fitted to a 1260 series HPLC pump. The mobile phase was constituted of 20 mM
172 ammonium acetate (A) and a mixture of acetonitrile/methanol 1:1 (v/v) (B) in isocratic
173 conditions (A/B 30:70, v/v). The transitions monitored through the triple-quadrupole mass
174 filter corresponded to $[M - H]^- \rightarrow [Br]^-$. Each analytical series comprised a procedural blank
175 and a quality control sample. Quantification was achieved according to the isotopic dilution

176 principle (13C-labeled isomers as internal standards and 2H18-β-HCBDD as external
177 standard). Limits of quantification (LOQ) were determined individually for each sample and
178 isomer, based on a signal-to-noise ratio of 3. A limit of reporting (LoR) higher than each LOQ
179 was established by matrix, species and isomer (considering procedural contamination as well),
180 as presented in Table 2.
181
182 **2.5 Volatolome analysis**
183 All of each egg yolk and liver were ground in liquid nitrogen for 1 and 3 min, respectively,
184 into a fine homogeneous powder using a home-made stainless steel ball mill. A 1.2 g aliquot
185 of powder was placed in a 20mL-glass vial (Supelco, Sigma-Aldrich, St. Louis, MO), sealed
186 under a nitrogen flow with magnetic caps with PTFE/silicone septa (Supelco, Sigma-Aldrich),
187 and stored at −80 °C. The volatolome of powdered liver and egg yolk samples was analyzed
188 by headspace-solid-phase microextraction (HS-SPME) coupled to gas chromatography - mass
189 spectrometry (GC-MS) according to Ratel et al. (2017). Briefly, the following steps were
190 carried out with an automated sampler (AOC-5000 Shimadzu, Japan): (i) preheating of the
191 sample for 10 min in the agitator (500 rpm), (ii) SPME trapping (75 µm
192 carboxen/polydimethylsiloxane, 23-gauge needle, Supelco) of the volatile organic compounds
193 (VOCs) for 30 min. For the liver samples, the extraction temperature was set at 40 °C, as
194 recommended by Bouhlel et al. (2017) for better extraction of liver VOCs with a narrower
195 analytical variability and improved sensitivity. For egg yolk samples, this temperature had to
196 be increased to 60 °C to boost extraction rates. After extraction, thermal desorption was
197 performed at 250 °C for 2 min in the GC inlet. Further VOC analysis was performed by
198 GC/MS-full scan (GC2010; QP2010+, Shimadzu). VOCs were injected in splitless mode into
199 a DB-5MS capillary column (60 m × 0.32 mm × 1 µm; Agilent J&W) with Helium as carrier
200 gas at a flow rate of 1 $ml.min^{-1}$. Oven temperature was held at 40 °C for 5 min, ramped to

201 230 °C at 3 °C.min^{-1}, and held at 230 °C for 10 min. The temperature of the transfer line
202 between GC and MS was set at 230 °C. Temperature was fixed at 180 °C in the MS source
203 and 150 °C in the MS quadrupole. Electron impact energy was set at 70 eV, and data was
204 collected in the range *m/z* 33–250 at 10 scans per second.
205
206 **2.6. Data treatment**
207 All calculations and statistical analyses applied to animal testing and HBCDD quantification
208 data have been previously detailed (Dominguez-Romero et al., 2016; Jondreville et al., 2017).
209 Peak areas of the VOCs were integrated from the SPME-GC-MS signals using a mass
210 fragment selected for both being specific to the sought-after molecule and free of any co-
211 elution with a home-made automatic algorithm developed in Boulhel et al. (2017) under
212 Matlab R2017 (The MathWorks, Natick, USA). VOCs were tentatively identified on the basis
213 of both mass spectra, by comparison against the NIST 17 mass spectral library (version 2.3,
214 build May 4 2017), and retention indices (RI), by comparison with published RI values and
215 those of our internal database. For calculation of the experimental RI, an alkane standard
216 solution (Supelco, Sigma-Aldrich) was analyzed by SPME-GC-MS at the end of the
217 analytical campaign. Data were processed using Statistica (version 12, StatSoft) and R
218 (version 2.1.4., http://www.R-project.org) software. Student's *t*-test and one-way ANOVA
219 were performed on the abundances of the VOCs monitored in volatolomes for the
220 comparisons of control *vs*. one or two groups of α-HBCDD contaminated animals.
221
222 **3. RESULTS**
223
224 **3.1. HBCDD in feed**

225 HBCDD concentrations determined in feed and animal tissues are summarized in Table 2.
226 Control feeds used for the three animals tested were HBCDD-free according to limits of
227 reporting. For contaminated feed intended to contain 50 ng α-HBCDD g^{-1} spiked feed,
228 compliant concentrations of 40, 38 and 31.8 ng α-HBCDD g^{-1} feed were measured for laying
229 hens, broilers and pigs, respectively. The concentration of α-HBCDD in contaminated feeds
230 was 20–36% lower than expected. For the second lot of contaminated feed intended to contain
231 5 ng α-HBCDD g^{-1} spiked feed for laying hens and pigs, concentrations of 3.62 ng and 3.42
232 ng α-HBCDD g^{-1} feed were measured, respectively. The intended 1:10 ratios between the two
233 α-HBCDD levels (5 and 50 ng α-HBCDD g^{-1}) in spiked feed were moderately well kept
234 following the preparation of contaminated feed, with a 1:9.0 ratio for laying hens and 1:9.3
235 for pigs. The difference observed in feed between the expected and measured concentrations
236 is probably related to the uncertainty in the weight of the produced crystals of α-HBCDD used
237 for spiking oils. But no degradation product of α-HBCDD has been identified in feed and
238 only α-HBCDD measured values were considered for further calculations. No β-HBCDD was
239 detected in any spiked feed. Some γ-HBCDD was quantified at levels of 0.18 and 0.14 ng g^{-1}
240 fw in the high- and low-level feeds, respectively, representing 0.45 and 3.9% of the total
241 HBCDD.

242

243 **3.2. HBCDD in animal samples**

244 The results of HBCDD quantification in animal samples obtained at the end of the exposure
245 period are presented in Table 2. The results of tissue distribution and transfer to eggs of
246 ingested α-HBCDD have been detailed in laying hens and in broilers by Dominguez-Romero
247 et al. (2016) and Jondreville et al. (2017), respectively. The exposed animals, at any level,
248 performed as well as the control animals in terms of body weight, growth rate, feed

249 efficiency, and laying rate, and the weight and lipid content of their tissues were not
250 significantly different (Dominguez-Romero et al., 2016, Jondreville et al., 2017). While the
251 HBCDD levels in ground liver samples from control laying hens should have been almost
252 zero, traces of α- and γ-HBCDD isomers have been detected, with 2.2 ± 1.8 ng and 5.0 ± 3.3
253 ng g^{-1} lw for α- and γ-isomer, respectively (Table 2). The γ-HBCDD content was very similar
254 to that from exposed hens (2.6 ± 1.7 ng and 5.6 ± 4.1 ng γ-HBCDD g^{-1} lw for the exposure
255 "dose 5" and "dose 50", respectively; Table 2), which suggested a systematic contamination
256 in all ground liver samples. The profile in HBCDD isomers determined in ground control liver
257 samples was fairly constant. Dominated by the γ-isomer (29%, 5%, 66% of α-, β- and γ-
258 HBCDD, respectively), it matched that of a technical mixture. HBCDD is known as a
259 ubiquitous environmental contaminant commonly found in industrial and domestic polymeric
260 materials. The analysis of the polystyrene box used during the liver grinding (Laberca
261 analysis ID 14.1842.3) has revealed a similar profile in HBCDD isomers (26%, 14%, 60% of
262 α-, β- and γ-HBCDD, respectively). HBCDD traces detected in ground control liver samples
263 could thus be ascribed to the polystyrene box. The analyses of HBCDD quantification pointed
264 out that the feeds given to control animals were α/β/γ-HBCDD-free and that the liver grinding
265 step was the source of the contamination revealed in control ground liver samples.
266 Accordingly, the volatolome of the liver from control hens can then be used to reveal α-
267 HBCDD-exposure markers by comparison with the volatolome of liver from exposed animals
268 deliberately fed with α-HBCDD-contaminated feeds. In the experiments conducted on pigs
269 and laying hens, the α-HBCDD concentration measured in tissues was proportional to the
270 level of diet exposure of the pigs and laying hens.

271

272 **3.3. Change in volatolomes in response to α-HBCDD exposure**

273 To confirm the promising interest of liver volatolomics (Ratel et al., 2017) and to explore the
274 potential of egg volatolomics for revealing livestock exposure to HBCDD, changes in
275 volatolomes were investigated in SPME-GC-MS signals obtained from livers and eggs of
276 control and α-HBCDD exposed animals. Analyses of liver volatolomes found 98, 105, and
277 134 VOCs for laying hens, broilers and pigs, respectively, and 51 VOCs were detected in egg
278 yolk volatolomes (Table S5).

279 ***3.3.1. Laying hens***

280 The results of liver volatolomics presented in Table 3 show that 8 VOCs were impacted in the
281 livers of laying hens for the two exposure levels. Figure S1 presents the first map of PCA
282 plotted on these candidate markers. The hepatic disturbance was thus visible in the
283 volatolome even for realistic low doses of α-HBCDD. Levels of HBCDD in feed in our study
284 (5 and 50 ng α-HBCDD g^{-1} feed) were reduced by a factor of 20 and 200 compared to the
285 levels of HBCDD used in Ratel et al. (2017) (0.1 and 10 μg γ-HBCDD g^{-1} feed). Table 3
286 includes mainly hydrocarbon, oxygenated (alcohols, ketones, acids), aromatic and sulfur
287 compounds. These results are consistent with the list of candidate markers published by Ratel
288 et al. (2017) in response to γ-HBCDD exposure, the authors highlighting in the livers of
289 laying hens mainly hydrocarbon compounds (alkanes and branched alkanes), oxygenated
290 compounds (alcohols, aldehydes, ketones) and aromatic compounds. **Hydrocarbons.** Heptane
291 (increased in exposed animals) and 2,2,4-trimethylpentane (decreased in exposed animals)
292 were found as significant markers of α-HBCDD exposure. Several hydrocarbons were
293 previously found as markers in the livers of laying hens after a γ-HBCDD exposure, with
294 levels in these compounds also increased or decreased in exposed animals according to the
295 compound considered (Ratel et al., 2017). The changes in the hydrocarbon content of livers in
296 response to α-HBCDD exposure may result from an imbalance between their production,
297 mainly due to unsaturated fatty acid peroxidation by reactive oxygen species (ROS), and their

298 hydroxylation, by detoxifying enzymes resulting in the production of alcohols (Hakim et al.,
299 2012). **Alcohols.** Table 3 includes 2-butanol. In response to the exposure to toxic xenobiotics,
300 the level of alcohols in livers could result from an equilibrium between anabolism (e.g.
301 hydroxylation of hydrocarbons) and catabolism (e.g. activation of CYP-450) reactions.
302 Several alcohols were included in the list of Ratel et al. (2017), and primary and secondary
303 alcohols have been proposed as candidate markers for VOC-based clinical diagnoses (Hakim
304 et al., 2012). 2-Butanol can be thus considered as particularly useful for revealing exposure of
305 laying hens to α-HBCDD. **Ketones.** Like for alcohols, these compounds are at the crossroads
306 of cell metabolism elicited in response to exposure to toxic xenobiotics. The levels of 2,5-
307 octanedione could be modified because of an impact of the toxic exposure on the lipid
308 metabolism with a high oxidation rate of fatty acids or on the protein metabolism with amino
309 acid metabolism-induced ketone formation (Hakim et al., 2012). **Sulfur compounds**. Two
310 compounds (sulfur dioxide, dimethylsulfone) were identified as markers at the highest
311 HBCDD dose. Among the candidate markers not classified according to their chemical
312 structure ("others" class) by Ratel et al. (2017), we can note two sulfur-containing compounds
313 (thiazole and thiadiazole). Bouhlel et al. (2018) also highlighted dimethylsulfone and carbon
314 disulfide among the most important liver VOC contributors to the separation of control
315 chickens and exposed chickens to micropollutants such as pesticides or polychlorobiphenyls.
316 In the review of Shubert et al. (2004) on the medical diagnostic potential of endogenous
317 VOCs, the authors report a possible relation between impairment of liver function, which
318 could be initiated by exposure to α-HBCCD given its toxicological effects reported in the
319 liver (Cantón et al., 2008; Germer et al., 2006), and level of sulfur-containing compounds.
320 The generation of these compounds may be connected to an incomplete metabolism of
321 methionine in the transamination pathway. **Aromatic compounds.** Table 3 shows 3 aromatic
322 compounds derived from alkylbenzenes among candidate markers: styrene, o-xylene and α-

323 methylstyrene. Alkylbenzenes were already highlighted by Ratel et al. (2017) when animals
324 were exposed to γ-HBCDD. In their study, the levels of all alkylbenzene candidate markers
325 were higher in exposed laying hens, like in our study. *o*-Xylene and α-methylstyrene were
326 also among the VOCs identified by Bouhlel et al. (2017) as the major contributors to the
327 separation of control and exposed chickens based on liver volatolomics. Although it is largely
328 agreed that alkylbenzenes have an exogenous origin (Hakim et al., 2012), their level in the
329 liver could be impacted by cellular and enzymatic defense mechanisms elicited to eliminate
330 hazardous compounds such as α-HBCDD. It is of note that we found styrene, o-xylene, α-
331 methylstyrene and one unknown VOC (RI 1028) as candidate markers in the livers of laying
332 hens for both α-HBCDD doses. However, the abundance of these VOCs and the α-HBCDD
333 concentration in liver were not significantly correlated according to the R-Pearson test (r
334 Pearson indices between -0.13 and -0.46; p values between 0.26 and 0.75).

335 Regarding the egg volatolome, only 2-butanol was affected by the α-HBCDD exposure
336 (Table 3). This VOC was also identified as a marker in the livers of laying hens exposed to
337 the higher dose of α-HBCDD. 2-Butanol is a secondary alcohol in one of the VOC families
338 reported as likely to be impacted by a chemical risk exposure (Ratel et al., 2017). Given the
339 high utility of eggs in any non-invasive volatolomics-based control, it would be interesting to
340 deepen our knowledge of the potential impact of liver disorders on egg composition.
341 Saraswati et al. (2013) showed that liver functions modified yolk precursor synthesis and
342 depositions in the developing follicles. It would also be of interest to improve egg volatolome
343 analysis by implementing recent advances in sample preparation and volatile fraction
344 collection (Majchrzak et al., 2018).

345 ***3.3.2. Broilers***

346 The exposure to α-HBCDD generates a detectable metabolic disturbance in the liver
347 volatolome of broilers. The first map of PCA plotted on the candidate markers allows the
348 separation of case/control groups to be visualized (Figure S1). With levels higher in control
349 animals, the candidate markers listed in Table 4 are hydrocarbon (2-methylbutane) or
350 aromatic (phenol, 2-phenoxyethanol, methoxybenzene and styrene) compounds. These
351 chemical families were already highlighted in the liver volatolome of laying hens exposed to
352 α-HBCDD (Table 3) and to γ-HBCDD (Ratel et al., 2017). Concerning aromatic compounds,
353 styrene was already identified as affected in liver of laying hens for the two exposure doses of
354 α-HBCDD (Table 3). Styrene levels were detected significantly higher in control hens than in
355 exposed hens. These results make it promising candidate marker. Phenol was already
356 highlighted in the livers of laying hens as a candidate marker of γ-HBCDD exposure by Ratel
357 et al. (2017), with levels also higher in the livers of controls. To explain changes in liver
358 phenol content in response to γ-HBCDD exposure, the authors hypothesize an imbalance
359 between production and degradation of phenol related to (i) the double involvement of CYP-
360 450 enzymes in the detoxication process of toxic xenobiotics and in microsomal
361 hydroxylation of phenol in liver, and (ii) the hepatic degradation of tyrosine and tryptophan
362 amino acids responsible for phenol generation, which could be increased in the case of liver
363 function impairment. Our result thus confirms that phenol is a promising candidate marker to
364 reveal differences in liver metabolism after a diet exposure to HBCDD.

365 ***3.3.3. Pigs***

366 Table 5 shows that 1 and 9 VOCs were significantly impacted when animals were exposed to
367 5 and 50 ng α-HBCDD g^{-1} feed, respectively. This result suggests a dose effect and possibly a
368 higher metabolic response threshold in the case of this animal. Figure S1 presents the first
369 map of PCA plotted on the candidate markers revealed for the exposure at 50 ng α-HBCDD
370 g^{-1} feed. The candidate markers, levels of which were all higher in exposed animals, were

371 mainly oxygenated compounds (alcohols, ketones, lactones) and one sulfur-containing
372 compound (carbon disulfide). Among alcohols, we found the primary alcohol 3-methyl-1-
373 butanol. This compound was reported as a hepatic candidate marker for exposure of laying
374 hens to γ-HBCDD (Ratel et al., 2017), with levels also higher in exposed animals. Carbon
375 disulfide was reported in the liver volatolome of chickens by Bouhlel et al. (2017) as a major
376 contributor to the discrimination between control chickens and chickens exposed to pesticide.
377 Further work is needed to identify the unknown VOC (RI 1132), which is a candidate marker
378 for pigs and broilers for the higher α-HBCDD level.

379

380 **4. CONCLUSION**

381 The present paper confirms that liver volatolome is relevant to highlight metabolic
382 disturbances induced by the exposure of animals to a chemical contamination with α-
383 HBCDD. Based on realistic α-HBCDD exposures, our work supports the study of Ratel et al.
384 (2017) by detecting, by liver volatolomics, the α-HBCDD exposure of laying hens. It shows
385 the effectiveness of this approach in the case of two other farm animals, namely broilers and
386 pigs. But given the numbers of animals involved in this proof-of-concept study, further work
387 is needed to assess the robustness of the markers identified before considering to use them for
388 food safety surveillance. The chemical families of most candidate markers are consistent with
389 the two main cellular reactions put forward as affecting the anabolism and catabolism of
390 VOCs studied as marker of pathologies as cancers (Hakim et al., 2012). First, oxidative stress
391 could be induced, with synthesis of reactive oxygen species that leak from the mitochondria
392 or from peroxidated polyunsaturated fatty acids in the cell membranes. Second, detoxifying
393 enzymes like cytochrome P-450 enzymes could be induced with catalysis of the oxidation of
394 organic substances. Additional case/control experiments need to be investigated to go further
395 in assessing the robustness of the candidate markers, understanding of the cellular reaction

396 mechanisms which affect the VOC production and ascertaining the relationship between level
397 of risky exposure and volatolomic response. In this purpose, repeating *in vivo* experiments
398 with higher numbers of animals, especially for volatolomic studies on pig livers and poultry
399 eggs, or implementing *in vitro* experiments with hepatocyte culture cells are two very useful
400 opportunities. The recent advances in sensors for non-invasive and early detection of VOCs
401 (Li et al., 2020) should be followed since they may lead rapidly to the design of routine
402 sensors for easy, rapid detection of volatolomics markers useful for food chemical safety
403 surveillance.
404

405 **ACKNOWLEDGMENTS**

406 The authors thank the French Ministry for Food, Agriculture and Fisheries for financial
407 support from the Compte d'Affectation Spéciale Développement Agricole et Rural (CASDAR
408 project 1256). They are also grateful to the technical staff of PEAT and BOA (INRAE
409 Nouzilly) and of GIE VGS (IFIP Villefranche-de-Rouergue) for diet preparation, animal
410 rearing and sample collection.
411

412 **REFERENCES**

413 Aaslyng, M.D., Elmore, J.S., Mottram, D.S. (1998). Comparison of the aroma characteristics
414 of acid-hydrolyzed and enzyme-hydrolyzed vegetable proteins produced from soy,
415 Journal of Agricultural and Food Chemistry, https://doi.org/10.1021/jf9806816.

416 Amann, A., Mochalski, P., Ruzsanyi, V., Broza, Y. Y., & Haick, H. (2014). Assessment of
417 the exhalation kinetics of volatile cancer biomarkers based on their physicochemical
418 properties. Journal of Breath Research, https://doi.org/10.1088/1752-7155/8/1/016003

419 Beaulieu, J.C., Grimm, C.C. (2001). Identification of volatile compounds in cantaloupe at
420 various developmental stages using solid phase microextraction, Journal of
421 Agricultural and Food Chemistry, https://doi.org/10.1021/jf0005768.
422 Berge, P., Ratel, J., Fournier, A., Jondreville, C., Feidt, C., Roudaut, B., Le Bizec, B., &
423 Engel, E. (2011). Use of Volatile Compound Metabolic Signatures in Poultry Liver to
424 Back-Trace Dietary Exposure to Rapidly Metabolized Xenobiotics. *Environmental*
425 *Science & Technology*, https://doi.org/10.1021/es200747h
426 Bouhlel, J., Ratel, J., Abouelkaram, S., Mercier, F., Travel, A., Baéza, E., Jondreville, C.,
427 Dervilly-Pinel, G., Marchand, P., Le Bizec, B., Dubreil, E., Mompelat, S., Verdon, E.,
428 Inthavong, C., Guérin, T., Rutledge, D. N., & Engel, E. (2017). Solid-phase
429 microextraction set-up for the analysis of liver volatolome to detect livestock exposure
430 to micropollutants. Journal of Chromatography A,
431 https://doi.org/10.1016/j.chroma.2017.03.008
432 Cajka, T., Hajslova, J., Cochran, J., Holadova, K., Klimankova, E. (2007). Solid phase
433 microextraction - comprehensive two dimensional gas chromatography - time-of-flight
434 mass spectrometry for the analysis of honey volatiles, Journal of Separation Science,
435 https://doi.org/10.1002/jssc.200600413.
436 Cantón, R. F., Peijnenburg, A. A. C. M., Hoogenboom, R. L. A. P., Piersma, A. H., van der
437 Ven, L. T. M., van den Berg, M., & Heneweer, M. (2008). Subacute effects of
438 hexabromocyclododecane (HBCD) on hepatic gene expression profiles in rats.
439 Toxicology and Applied Pharmacology, https://doi.org/10.1016/j.taap.2008.04.013
440 Covaci, A., Gerecke, A. C., Law, R. J., Voorspoels, S., Kohler, M., Heeb, N. V., Leslie, H.,
441 Allchin, C. R., & de Boer, J. (2006). Hexabromocyclododecanes (HBCDs) in the
442 Environment and Humans: A Review. Environmental Science & Technology,
443 https://doi.org/10.1021/es0602492

444 de Montellano, P. R. O. (2015). Substrate oxidation by cytochrome P450 enzymes. In
445 *Cytochrome P450* (pp. 111–176). Springer.
446 Dominguez-Romero, E., Cariou, R., Omer, E., Marchand, P., Dervilly-Pinel, G., Le Bizec, B.,
447 Travel, A., & Jondreville, C. (2016). Tissue Distribution and Transfer to Eggs of
448 Ingested α-Hexabromocyclododecane (α-HBCDD) in Laying Hens (*Gallus*
449 *domesticus*), https://doi.org/10.1021/acs.jafc.5b05574.
450 Engel, E., Ratel, J. (2007). Correction of the data generated by mass spectrometry analyses of
451 biological tissues: Application to food authentication. Journal of Chromatography A,
452 https://doi.org/10.1016/j.chroma.2007.02.012.
453 Fournier, A., Feidt, C., Marchand, P., Vénisseau, A., Bizec, B. L., Sellier, N., Engel, E., Ratel,
454 J., Travel, A., & Jondreville, C. (2012). Kinetic study of γ-hexabromocyclododecane
455 orally given to laying hens (*Gallus domesticus*). Environmental Science and Pollution
456 Research, http://doi.org/10.1007/s11356-011-0573-6
457 Germer, S., Piersma, A. H., van der Ven, L., Kamyschnikow, A., Fery, Y., Schmitz, H.-J., &
458 Schrenk, D. (2006). Subacute effects of the brominated flame retardants
459 hexabromocyclododecane and tetrabromobisphenol A on hepatic cytochrome P450
460 levels in rats. Toxicology, http://doi.org/10.1016/j.tox.2005.10.019
461 Haick, H., Broza, Y. Y., Mochalski, P., Ruzsanyi, V., & Amann, A. (2014). Assessment,
462 origin, and implementation of breath volatile cancer markers. Chemical Society
463 Reviews, http://doi.org/10.1039/c3cs60329f
464 Hakim, M., Broza, Y. Y., Barash, O., Peled, N., Phillips, M., Amann, A., & Haick, H. (2012).
465 Volatile organic compounds of lung cancer and possible biochemical pathways.
466 Chemical Reviews, https://doi.org/10.1021/cr300174a

467 Hiebl, J., Vetter, W. (2007). Detection of hexabromocyclododecane and its metabolite
468 pentabromocyclododecene in chicken egg and fish from the official food control.
469 Journal of Agricultural and Food Chemistry, https://doi.org/10.1021/jf063428b.
470 Jondreville, C., Cariou, R., Méda, B., Dominguez-Romero, E., Omer, E., Dervilly-Pinel, G.,
471 Le Bizec, B., Travel, A., & Baéza, E. (2017). Accumulation of α-
472 hexabromocyclododecane (α-HBCDD) in tissues of fast- and slow-growing broilers
473 (*Gallus domesticus*). Chemosphere,
474 https://doi.org/10.1016/j.chemosphere.2017.03.064
475 Koch, C., Schmidt-Kötters, T., Rupp, R., & Sures, B. (2015). Review of
476 hexabromocyclododecane (HBCD) with a focus on legislation and recent publications
477 concerning toxicokinetics and-dynamics. Environmental Pollution,
478 https://doi.org/10.1016/j.envpol.2015.01.011
479 Li, H. Y., Zhao, S. N., Zang, S. Q., & Li, J. (2020). Functional metal–organic frameworks as
480 effective sensors of gases and volatile compounds. Chemical Society Reviews,
481 https://doi.org/10.1039/c9cs00778d
482 Majchrzak, T., Wojnowski, W., Piotrowicz, G., Gębicki, J., & Namieśnik, J. (2018). Sample
483 preparation and recent trends in volatolomics for diagnosing gastrointestinal diseases.
484 TrAC Trends in Analytical Chemistry, https://doi.org/10.1016/j.trac.2018.08.020
485 Marvin, C. H., Tomy, G. T., Armitage, J. M., Arnot, J. A., McCarty, L., Covaci, A., & Palace,
486 V. (2011). Hexabromocyclododecane: Current Understanding of Chemistry,
487 Environmental Fate and Toxicology and Implications for Global Management.
488 Environmental Science & Technology, https://doi.org/10.1021/es201548c
489 Meurillon, M., Ratel, J., & Engel, E. (2018). How to secure the meat chain against toxicants?
490 Innovative Food Science & Emerging Technologies,
491 https://doi.org/10.1016/j.ifset.2017.10.004

492 Radulovic, N., Blagojevic, P., Palic, R. (2010). Comparative study of the leaf volatiles of
493 Arctostaphylos uva-ursi (L.) Spreng. and Vaccinium vitis-idaea L.
494 (Ericaceae), Molecules, https://doi.org/10.3390/molecules15096168.
495 Ratel, J., Planche, C., Mercier, F., Blinet, P., Kondjoyan, N., Marchand, P., Fournier, A.,
496 Travel, A., Jondreville, C., & Engel, E. (2017). Liver volatolomics to reveal poultry
497 exposure to γ-hexabromocyclododecane (HBCD). Chemosphere,
498 https://doi.org/10.1016/j.chemosphere.2017.09.074
499 Rivière, G., Sirot, V., Tard, A., Jean, J., Marchand, P., Veyrand, B., Le Bizec, B., & Leblanc,
500 J. (2014). Food risk assessment for perfluoroalkyl acids and brominated flame
501 retardants in the French population: results from the second French total diet study.
502 Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2014.01.104
503 Saraswati, T. R., Manalu, W., Ekastuti, D. R., & Kusumorini, N. (2013). Increased egg
504 production of Japanese quail (Cortunix japonica) by improving liver function through
505 turmeric powder supplementation. International Journal of Poultry Science,
506 https://doi.org/10.3923/ijps.2013.601.614
507 Schubert, J. K., Miekisch, W., Geiger, K., & Nöldge–Schomburg, G. F. (2004). Breath
508 analysis in critically ill patients: potential and limitations. Expert Review of Molecular
509 Diagnostics, https://doi.org/10.1586/14737159.4.5.619
510 de Simon, B.F., Estruelas, E., Munoz, A.M., Cadahia, E., Sanz, M. (2009). Volatile
511 compounds in acacia, chestnut, cherry, ash, and oak woods, with a view to their use in
512 cooperage, Journal of Agricultural and Food Chemistry, 2009, 57, 8, 3217-3227,
513 https://doi.org/10.1021/jf803463h
514 Szabo, D. T., Diliberto, J. J., Hakk, H., Huwe, J. K., & Birnbaum, L. S. (2010).
515 Toxicokinetics of the Flame Retardant Hexabromocyclododecane Gamma: Effect of

516 Dose, Timing, Route, Repeated Exposure, and Metabolism. Toxicological Sciences,
517 https://doi.org/10.1093/toxsci/kfq183.
518 Szabo, D. T., Diliberto, J. J., Hakk, H., Huwe, J. K., Birnbaum, L. S. (2011). Toxicokinetics
519 of the flame retardant hexabromocyclododecane alpha: effect of dose, timing, route,
520 repeated exposure, and metabolism. Toxicological Sciences,
521 https://doi.org/10.1093/toxsci/kfr059.
522 Vasta, V., Ratel, J., Engel, E. (2007). Mass spectrometry analysis of volatile compounds in
523 raw meat for the authentication of the feeding background of farm animals. Journal of
524 Agricultural and Food Chemistry, https://doi.org/10.1021/jf063432n.
525 Xie, J., Sun, B., Zheng, F., Wang, S. (2008). Volatile flavor constituents in roasted pork of
526 Mini-pig. Food Chemistry, https://doi.org/10.1016/j.foodchem.2007.12.074.
527

Table 1. Summary of the experiments conducted in laying hens, broilers and pigs

Livestock animals	**Laying hens**			**Broilers**		**Pigs**		
Strain	Novo Brown			JA657		(LW x Ld) x Piétrain		
Initial age (day)	210			1		70		
Duration of exposure (week)	18			12		16		
Target dose of α-HBCDD (ng g^{-1} feed)	50	5	0	50	0	50	5	0
Number of animals	3	3	2	5	4	3	3	3

Table 2. HBCDD concentrations determined in feeds and animal tissues. Values are means ± standard err

Samples	Laying hens			Broilers		Pigs		
	dose 50 [a]	*dose 5* [a]	*control*	*dose 50*	*control*	*dose 50*	*dose 5*	*control*
Feed (ng g^{-1} fw)								
LoR [b]		0.04		0.03			0.04	
α-HBCDD	40	3.62	< LoR	38.0	< LoR	31.8	3.42	< LoR
β-HBCDD	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR
γ-HBCDD	0.18	0.14	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR
Liver (ng g^{-1} lw)								
LoR		0.4		0.2			0.08	
number of samples	3	3	2	5	4	3	3	3
α-HBCDD	142±22*	11.0±2.2*	2.2 ± 1.8	100 ± 16	0.42 ± 0.29	15.6 ± 3.2	1.71 ± 0.17	< LoR
β-HBCDD	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR
γ-HBCDD	5.6 ± 4.1	2.6 ± 1.7	5.0 ± 3.3	< LoR	< LoR	< LoR	< LoR	< LoR
Egg (ng g^{-1} lw)								
LoR		0.1						
number of samples	3	3	2	5	4	3	3	3
α-HBCDD	242±8.5	23.3±1.5	< LoR	N/A	N/A	N/A	N/A	N/A
β-HBCDD	< LoR	< LoR	< LoR	N/A	N/A	N/A	N/A	N/A
γ-HBCDD	< LoR	< LoR	< LoR	N/A	N/A	N/A	N/A	N/A
Fat [c] (ng g^{-1} lw)								
LoR		0.1		0.2			0.1	
number of samples	3	3	2	5	4	3	3	3
α-HBCDD	302 ± 6.6	33.1 ± 6.3	0.26 ± 0.10	384 ± 82	0.46 ± 0.18	179 ± 21	14.1 ± 0.3	0.32 ± 0.19
β-HBCDD	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR	< LoR
γ-HBCDD	0.89 ± 0.42	0.46 ± 0.29	0.45 ± 0.29	< LoR	< LoR	< LoR	< LoR	< LoR
Muscle [d] (ng g^{-1} lw)								
LoR		0.2		0.3			0.1	
number of samples	3	3	2	5	4	3	3	3
α-HBCDD	378± 41	N/A	N/A	260 ± 63	0.88 ± 0.64	142 ± 15	13.0 ± 2.8	< LoR
β-HBCDD	< LoR	N/A	N/A	< LoR	< LoR	0.7 ± 0.5	< LoR	< LoR
γ-HBCDD	< LoR	N/A	N/A	< LoR	< LoR	< LoR	< LoR	< LoR

[a] Diet contaminated with the target concentration of 5 or 50 ng α-HBCDD g^{-1}

[b] Limit of Reporting

[c] Abdominal fat for hens and broilers, back fat for pigs

[d] Thigh muscle for hens and broilers, semi-membranous muscle for pigs

* Each α-HBCDD concentration in liver of exposed hens was corrected in removing the part of α-HBCDD brought by the polystyre

:or. Levels of α- and γ-HBCDD measured in liver samples of control laying hens were not zero because of a

:ne box used during liver grinding, according to Dominguez-Romero et al. (2016) and Jondreville et al. (2017).

an accidental contamination during liver sample treatment (see paragraph 3.2. in Results section).

Table 3. Laying hens - Volatile compounds in liver volatolome impacted by the exposure to α-HBCDD**.** Values are the mean of abundances ($\times 10^4$) of each candidate marl

Candidate markers	Tentatively identification[a]	m/z[b]	Exp. RI[c]	Ref. RI[d]	Liver			Egg yolk		
					control	*dose 50* [e]	*dose 5* [e]	*control*	*dose 50* [e]	*dose 5* [e]
Hydrocarbons										
2,2,4-trimethylpentane	MS+RI	57	688	687 [1]	5.7 (9.1%)		1.4** (24.7%)			
heptane	MS+RI	100	700	700	13.7 (26.9%)		21.8* (5.3%)			
Alcohols										
2-butanol	MS+RI	59	602	603 [1]	13.4 (32.8%)	23.7* (3.9%)		42.5 (22.0%)		82.8* (1.1%)
Ketones	MS+RI									
2,5-octanedione	MS+RI	99	985	985 [2]	2.0 (47.1%)		6.6* (26.1%)			
Acids										
acetic acid	MS+RI	60	575	602 [1]	512.8 (29.7%)	775.5* (2.6%)				
Aromatic compounds										
styrene	MS+RI	104	898	897 [1]	4.0 (11.2%)	2.7* (15.7%)	2.6* (16.4%)			
o-xylene	MS+RI	91	898	898 [3]	5.8 (1.6%)	3.7* (15.9%)	3.5* (26.6%)			
α-methylstyrene	MS+RI	117	994	994 [4]	1.1 (0.3%)	2.1* (19.6%)	4.1** (13.7%)			
Sulfur compounds										
sulfur dioxide	MS	64	<500		32.7 (7.2%)	20.9* (18.6%)				
dimethylsulfone	MS+RI	79	906	915 [1]	9.7 (6.1%)	38.3* (23.3%)				
Unknown										
unknown		54	1028		0.4 (31.3%)	1.7** (11.7%)	3.5* (26.2%)			
unknown		100	1141		0.3 (39.4%)		1.5** (4.7%)			

[a] MS + RI, mass spectrum and RI agree with literature data; MS, mass spectrum agrees with literature spectrum

[b] Mass fragment used for area determination

[c,d] Retention indices on a DB5 capillary column from experimental run (c) or bibliographic data (d)

[1] Engel and Ratel, 2007; [2] Xie et al., 2008; [3] Vasta et al., 2007; [4] Cajka et al., 2007.

[e] Diet contaminated with the target concentration of 5 or 50 ng α-HBCDD g^{-1}

***** *p* <0,05

****** *p* <0,01

(shaded) Level found higher in liver from "control" animals compared to "exposed animals"

‹er with its standard deviation (in bracket).

Table 4. Broilers - Volatile compounds in liver volatolome impacted by the exposure to α-HBCDD**.** Values are the mean of abundances ($\times 10^4$) of each candidate m

Candidate markers	Tentatively identification[a]	m/z[b]	Exp. RI[c]	Ref. RI[d]	*control*	*dose 50* [e]
Hydrocarbons						
2-methylbutane	MS	72	508		63.9 (31.2%)	28.2* (45.1%)
Aromatic compounds						
styrene	MS+RI	104	898	897 [1]	13.7 (19.2%)	8.9* (32.2%)
methoxybenzene	MS+RI	108	922	918 [2]	9.3 (29.5%)	3.2** (21.0%)
phenol	MS+RI	94	976	983 [3]	92.4 (23.2%)	38.3** (54.3%)
2-phenoxyethanol	MS+RI	94	1207	1220 [4]	270.7 (26.2%)	38.9** (100.9%)
Unknown						
unknown		77	1132		10 (16.2%)	3.4** (35.2%)

[a] MS + RI, mass spectrum and RI agree with literature data; MS, mass spectrum agrees with literature spectrum

[b] Mass fragment used for area determination

[c,d] Retention indices on a DB5 capillary column from experimental run (c) or bibliographic data (d)

[1] Engel and Ratel, 2007; [2] Leffingwell and Alford, 2011; [3] Vasta et al., 2007; [4] de Simon et al., 2009.

[e] Diet contaminated with the target concentration of 50 ng α-HBCDD g^{-1}

***** p <0,05

****** p <0,01

(shaded) Level found higher in liver from "control" animals compared to "exposed animals"

ıarker with its standard deviation (in bracket).

Table 5. Pigs - Volatile compounds in liver volatolome impacted by the exposure to α-HBCDD**.** Values are the mean of abundances ($\times 10^4$) of each candidate m

Candidate markers	Tentatively identification[a]	m/z[b]	Exp. RI[c]	Ref. Ri[d]	*control*	*dose 50* [e]	*dose 5* [e]
Alcohols							
3-methyl-1-butanol	MS+RI	55	733	734 [1]	5.0 (28.5%)	8.7* (20.5%)	
2,6-dimethylcyclohexanol	MS+RI	95	1119	1114 [2]	832.2 (33.0%)	2116.5* (32.7%)	
Ketones							
2,4,4-trimethylcyclopentanone	MS	83	1010		15.1 (19.3%)	62.2* (44.6%)	
2,2,6-trimethylcyclohexan-1-one	MS+RI	82	1042	1047 [3]	104.2 (32.6%)	229.8** (13.9%)	
isophorone	MS+RI	82	1062	1080 [4]	51.5 (36.7%)	151.0** (18.2%)	
Lactones							
dihydro-5-methyl-2(3H)-furanone	MS+RI	56	953	954 [1]	31.1 (6.3%)	50.5* (22.3%)	
Sulfur compounds							
carbone disulfide	MS+RI	76	570	568 [5]	526.8 (46.3%)	1102.0* (9.5%)	
Unknown							
unknown		69	1087		1.7 (41.3%)		3.4* (12.8%)
unknown		77	1132		1.6 (18.6%)	3.1* (28.1%)	
unknown		55	1162		4.7 (18.5%)	15.1** (12.8%)	

[a] MS + RI, mass spectrum and RI agree with literature data; MS, mass spectrum agrees with literature spectrum

[b] Mass fragment used for area determination

[c,d] Retention indices on a DB5 capillary column from experimental run (c) or bibliographic data (d)

[1] Engel and Ratel, 2007; [2] Radulovic et al., 2010; [3] Cajka et al., 2007; [4] Aaslyng et al., 1998; [5] Beaulieu and Grimm, 2001.

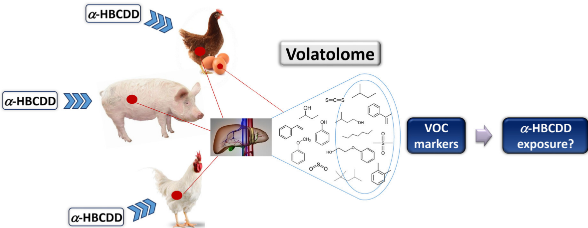
[e] Diet contaminated with the target concentration of 5 or 50 ng α-HBCDD g^{-1}

***** *p* <0,05

****** *p* <0,01

Level found higher in liver from "control" animals compared to "exposed animals"

arker with its standard deviation (in bracket).



α-HBCDD
α-HBCDD
α-HBCDD
Volatolome
VOC markers
α-HBCDD exposure?