



**HAL**  
open science

## Correlation between endemic chlordecone concentrations in three bovine tissues determined by isotopic dilution liquid chromatography–tandem mass spectrometry

Gwenaëlle Lavison-Bompard, Julien Parinet, Karelle Huby, Thierry Guérin, Chanthadary Inthavong, Marine Lambert

### ► To cite this version:

Gwenaëlle Lavison-Bompard, Julien Parinet, Karelle Huby, Thierry Guérin, Chanthadary Inthavong, et al.. Correlation between endemic chlordecone concentrations in three bovine tissues determined by isotopic dilution liquid chromatography–tandem mass spectrometry. *Science of the Total Environment*, 2021, 788, pp.147833. 10.1016/j.scitotenv.2021.147833 . anses-03722009

**HAL Id: anses-03722009**

**<https://anses.hal.science/anses-03722009>**

Submitted on 13 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Correlation between endemic chlordecone concentrations in three bovine tissues**  
2 **determined by isotopic dilution liquid chromatography–tandem mass spectrometry**

3

4

5 Gwenaëlle Lavison-Bompard<sup>1a\*</sup>, Julien Parinet<sup>a</sup>, Karelle Huby<sup>a</sup>, Thierry Guérin<sup>b</sup>, Chanthadary  
6 Inthavong<sup>a</sup>, Marine Lambert<sup>a</sup>

7

8 a: ANSES, Laboratory for Food Safety, F-94701 Maisons-Alfort, France

9 b: ANSES, Strategy and Programs Department, Maisons-Alfort, France

10

11

12 \*Corresponding author. Tel.: +33 149 774 677; Email address: [gwenaelle.lavison-](mailto:gwenaelle.lavison-bompard@anses.fr)  
13 [bompard@anses.fr](mailto:gwenaelle.lavison-bompard@anses.fr); ORCID ID <https://orcid.org/0000-0001-6793-6290>

14

## 15 **Highlights**

- 16 • Occurrence and concentrations of chlordecone in fat, muscle and liver in 200 bovines.
- 17 • Chlordecone concentrations are correlated in fat, muscle and liver.
- 18 • Correlation factors: 0.54 muscle/fat, 3.75 liver/fat, and 0.14 muscle/liver.

19

## 20 **Abstract**

21 Chlordecone (CLD) is an organochlorine pesticide widely used from the 1970s to the 1990s in  
22 the French West Indies that induced long-term pollution of the ecosystem. Due to involuntary  
23 soil ingestion, some species bred in open-air areas can be contaminated. As CLD is distributed  
24 in various tissues depending on the breeding species, this study focuses on the distribution of  
25 CLD in bovines. For this purpose, three tissues, i.e. fat, muscle, and liver, from 200 bovines  
26 originating from Martinique and Guadeloupe were sampled in 2016 to determine their endemic  
27 contamination levels. Analyses were performed with the official method for veterinary controls,  
28 isotopic dilution liquid chromatography–tandem mass spectrometry, which has been fully  
29 validated and which reaches a limit of quantification of 3  $\mu\text{g}\cdot\text{kg}^{-1}$  fresh weight (fw).  
30 Irrespective of the matrices, CLD was detected in 68% of samples (404 samples above the  
31 LOD) and quantified in 59% of samples (332 samples above the LOQ). Regarding  
32 contamination levels, the liver had a broader range of concentrations (LOQ up to 420.6  $\mu\text{g}\cdot\text{kg}^{-1}$   
33  $\text{fw}$ ) than fat (LOQ up to 124.6  $\mu\text{g}\cdot\text{kg}^{-1}$   $\text{fw}$ ) and muscle (LOQ up to 67.6  $\mu\text{g}\cdot\text{kg}^{-1}$   $\text{fw}$ ). This  
34 confirms the atypical behaviour of CLD compared to other persistent organochlorine pollutants.  
35 Statistical processing demonstrated a correlation between CLD concentrations among the three  
36 studied tissues. The CLD concentration ratios were 0.54 for muscle/fat, 3.75 for liver/fat, and  
37 0.14 for muscle/liver.

38

39 **Keywords:** Chlordecone, ID-LC-MS/MS, bovine tissues, correlation factors

## 40 1. Introduction

41 Chlordecone (CLD) is an organochlorine pesticide that was widely used in the French West  
42 Indies (Martinique and Guadeloupe) to protect banana plantations from black weevil  
43 (*cosmopolites sordidus*) between 1972 and 1993. CLD is a bi-cyclic chlorinated molecule. As  
44 its octanol/water coefficient exceeds 3, CLD is classified as a lipophilic pollutant. CLD also  
45 exhibits a ketone function (C=O) and the presence of a non-saturated carbon-carbon bond that  
46 can lead to keto-enol equilibrium.

47 CLD was classified in 2009 by the Stockholm Convention as a persistent pollutant. It is known  
48 to be carcinogenic, mutagenic and reprotoxic, and is suspected of being an endocrine disrupter  
49 (Multigner et al., 2010). In a cohort of a study carried out in Guadeloupe (Timoun cohort), an  
50 effect of CLD exposure was found on the duration of pregnancy (Hervé et al., 2016), and on  
51 the thyroid hormone system (Cordier et al., 2015), as well as impairment of fine motor function  
52 in boys (Boucher et al., 2013; Dallaire et al., 2012), and visual contrast sensitivity (Saint-  
53 Amour et al., 2020). For male adults, an increase in the risk of prostate cancer has also been  
54 documented (Multigner et al., 2010).

55 Due to the former use of this pesticide, certain Martinican and Guadeloupean environmental  
56 compartments are widely contaminated with CLD. Soil contamination, linked to former use of  
57 CLD, depends on soil composition (Cabidoche et al., 2009; Woignier et al., 2013). Studies on  
58 the fate of CLD have demonstrated low and poor degradation in soils with decontamination  
59 projected to take decades to centuries (Cabidoche et al., 2009). More recent studies have also  
60 indicated that transformation of CLD occurs in soils through highly complex pathways (Benoit  
61 et al., 2017; Chevallier et al., 2019; Clostre et al., 2015). Food crops (including vegetables)  
62 through root uptake (Clostre et al., 2015; Florence et al., 2015; Létondor et al., 2015), and  
63 ruminants, through involuntary soil ingestion, can also be contaminated by CLD (Clostre et al.,  
64 2014; Collas et al., 2019; Fournier et al., 2017).

65 Regarding food safety, in 2007, the French Food Safety Agency re-examined toxicological  
66 reference values based on previous studies in rats dating back to 2003. No changes were made:  
67 the chronic health-based guidance value was established at 0.5 µg/kg body weight/day, and the  
68 acute value at 10 µg/kg body weight/day. The impact of food consumption, including local food  
69 supply habits, was also examined based on consumption data (AFSSA, 2007): it was  
70 determined that setting maximum concentration limits in foodstuffs would be of limited  
71 effectiveness considering subsistence production (Dubuisson et al., 2007). Moreover, it was  
72 concluded that the maximum residue levels (MRLs) established by the European Regulation on  
73 MRLs of pesticides in and on food and feed of plant and animal origin (Commission Regulation  
74 (EU) No 212/2013, 212AD ; Commission Regulation (EC) No 839/2008) , provided sufficient  
75 protection of the population regarding CLD exposure via food of animal origin (ANSES, 2017).  
76 Concerning livestock, the target tissue for official controls is perirenal fat, since it is the tissue  
77 in which non-polar organochlorine pesticides are supposed to be mostly distributed. However,  
78 some studies have shown that CLD has atypical behaviour regarding its tissue distribution, and  
79 found that CLD is not only distributed in fat but also in muscle in certain species, such as poultry  
80 (Jondreville et al., 2014a), ducks (Jondreville et al., 2014b), growing male Alpine kids (Lastel  
81 et al., 2018), ewes Saint-Hilaire et al., 2018) and pigs (Fourcot et al., 2020). This raises the  
82 question of distribution in other species such as bovines, which are largely consumed and are  
83 one of the most targeted species for official food controls.

84 CLD concentrations can be determined either by gas or liquid chromatography; methods are  
85 mostly adapted from Bordet et al. (Bordet et al., 2007). For gas chromatography, detection can  
86 be performed with an electron capture detector (GC/ECD) or mass spectrometry, with low or  
87 high resolution (Clostre et al., 2014; Florence et al., 2015; Fournier et al., 2017; Lastel et al.,  
88 2018; Méndez-Fernandez et al., 2018). This second type of detection is recommended in the  
89 case of positive results (i.e. > the limit of quantification - LOQ) for confirmation purposes.

90 Although gas chromatography is still used, most of the current methods, including the official  
91 method for controls in France, are based on isotopic dilution followed by reversed-phase liquid  
92 chromatography analysis and tandem mass spectrometry detection with negative electrospray  
93 ionization (Clostre et al., 2015; Lastel et al., 2018; Saint-Hilaire et al., 2018). Isotopic dilution  
94 is recommended to ensure accurate identification and quantification. Recent publications also  
95 report the use of high-resolution mass spectrometry with LC separation for the identification of  
96 CLD transformation products in the environment through biodegradation or food processing  
97 (Martin et al., 2020; Ollivier et al., 2020).

98 The present study aims at determining CLD levels in fat, muscle and liver tissues of 200  
99 endemically contaminated bovines originating primarily from the most contaminated areas of  
100 Martinique and Guadeloupe. The analytical method used was the official method for monitoring  
101 controls in France, and is based on isotopic dilution liquid chromatography hyphenated with  
102 mass spectrometry (ID-LC-MS/MS). These data were then used to assess the correlations  
103 between CLD concentrations in fat, muscle and liver.

104

## 105 **2. Material and methods**

### 106 2.1. Chemicals and reagents

107 All solutions were prepared with analytical reagent grade chemicals and ultrapure water (18.2  
108 M $\Omega$ .cm) obtained by purifying distilled water with a Milli-Q system (Merck Millipore, Saint-  
109 Quentin-en-Yvelines, France). All solvents (acetone, acetonitrile, dichloromethane, hexane,  
110 and methanol) were HPLC grade and were purchased from Fisher Scientific (Illkirch, France).  
111 Sodium hydroxide and formic acid (98%) were also purchased from Fisher Scientific (Illkirch,  
112 France). CLD (98%) and CLD <sup>13</sup>C<sub>10</sub> (98%) standards were purchased from Cluzeau Info Labo  
113 (Sainte-Foy-La-Grande, France). Working solutions were prepared by dilution of the  
114 commercial standards in methanol before use, to prepare calibration standards and fortify the  
115 samples for quality controls and method validation. All solutions were stored at 5 ± 3°C.

116

## 117 2.2. Equipment

118 LC-MS/MS measurements were performed using a 1200 series HPLC binary pump system  
119 (Agilent Technologies, Courtaboeuf, France) coupled with an API 5500 QTRAP hybrid tandem  
120 mass spectrometer (AB Sciex, Les Ulis, France). An Aqua C18 column (150 mm x 2.0 mm i.d.,  
121 3 µm particle size) equipped with an Aqua C18 guard column (4.0 mm x 3.0 mm i.d., 3 µm  
122 particle size) was used for chromatographic separation. An Eppendorf centrifuge 5810 (Sigma  
123 Aldrich, Saint-Quentin-Fallavier, France), a refrigerated Hettich 32 R centrifuge (Sigma  
124 Aldrich, Saint-Quentin-Fallavier, France), and a Genie 2 vortex (Scientific Industries, Bohemia,  
125 NY, United States) were used for the extraction. A Grindomix GM 200 (Retsch Industries,  
126 Haan, Germany) and a Polytron PT 3100 (Fisher Scientific, Illkirch, France) were used for  
127 sample preparation. An IKA Ultraturrax T25 digital Disperser (Retsch Industries) was used for  
128 both sample preparation and extraction. An XS 204 balance (Mettler-Toledo, Viroflay, France)  
129 was used to weigh the samples. A water bath heater from Fisher (Fisher Scientific, Illkirch,  
130 France) was used for fat melting. A Reacti-Therm Pierce heating module (Thermofisher, Les  
131 Ulis France) was used for solvent evaporation.

132

## 133 2.3. Sampling

134 From January to October 2016, an oriented exploratory survey plan was implemented by the  
135 French authorities in charge of food control (Directorate for food, agriculture and forestry of  
136 Guadeloupe and Martinique) toward the most probable contaminated bovines in the French  
137 West Indies. In all, 200 bovines were selected, mostly in Martinique (120 bovines). In  
138 Guadeloupe, open-air farms located in the most contaminated areas were chosen, whereas in  
139 Martinique, animals were selected as a priority when the livestock was found to have positive  
140 results in previous official control plans. Livestock number, age, and sex of the animals were

141 reported, as well as slaughterhouse locations (18 locations for Martinique and a single location  
142 for Guadeloupe). For each bovine, the three tissues – perirenal fat, skirt for muscle, and liver –  
143 were sampled and clearly identified for traceability.

144 For quality controls and method validation, liver and muscle samples were purchased from a  
145 local supermarket (Choisy-le-Roi, France) and perirenal fat was collected from a local butcher  
146 (Saint-Maur-des-Fossés, France). These matrices were determined to be CLD-free.

147

## 148 2.4. Sample preparation and extraction

149 The analytical procedure employed in this study was adapted from (Bordet et al., 2007), with  
150 minor changes regarding sample preparation and major changes regarding separation and  
151 detection: GC-ECD was switched to LC-MS/MS<sup>2</sup>. This is the official method to be  
152 implemented in France for monitoring and control plans for food of animal origin by approved  
153 laboratories.

### 154 2.4.1. Preparation and extraction procedure for fat

155 Frozen fat samples were cut into pieces, blended with a Grindomix apparatus, heated to a  
156 maximum of 60 °C and filtered through a strainer to clean samples of residues. A total of 0.5 g  
157 was precisely weighed in a test tube. Then, 75 µL of internal standard CLD-<sup>13</sup>C<sub>10</sub> at a  
158 concentration of 0.5 ng·µL<sup>-1</sup> was added to melted fat. After 1 min vortex-stirring, the internal  
159 standard was kept in contact for 1 h with the matrix. CLD was extracted from the melted fat  
160 sample 3 times with 3 mL of a mix of acetonitrile/dichloromethane 75/25 (v/v). For each  
161 extraction, the test tube was briefly shaken with a vortex homogenizer and then centrifuged for  
162 20 min at 1,200 g at a temperature of -20 °C. The supernatants were collected into another test  
163 tube and solvent was evaporated to dryness at a temperature not exceeding 40 °C under a gentle  
164 stream of nitrogen. The dry extract was dissolved in 15 mL of a mixture of hexane/acetone  
165 85/15 (v/v) and then transferred to a 50 mL centrifuge tube. 5 mL of 0.5 M aqueous sodium

166 hydroxide solution was added, and the tube was vortexed for 15 s. The lower aqueous phase  
167 was collected in another centrifuge tube. This extraction step was repeated twice, and the three  
168 aqueous phases were combined in the same tube, and then washed with 5 mL of hexane by  
169 gentle manual shaking. The hexane phase was discarded after a 3 min centrifugation step at  
170 750 g. Then, 5 mL of aqueous sulfuric acid solution at 60% were added to the aqueous extract,  
171 and the tube was shaken for 15 s with a vortex homogenizer and gently left to cool at room  
172 temperature. CLD was extracted 3 times with 5 mL of a mixture of hexane/acetone 85/15 (v/v)  
173 by shaking for 15 s with a vortex homogenizer, then centrifuged for 3 min at 750 g, and the  
174 upper organic phases were collected in a 15 mL centrifuge tube and washed with 2 mL of  
175 ultrapure water. After final centrifugation for 3 min at 750 g, the organic phase was evaporated  
176 to dryness at a temperature not exceeding 40 °C under a gentle stream of nitrogen. The dry  
177 extract was dissolved in 1 mL of methanol and transferred to a vial for chromatographic  
178 analysis.

#### 179 *2.4.2. Preparation and extraction procedure for muscle and liver*

180 Due to the lower fat content of liver and muscle compared to perirenal fat, a different sample  
181 preparation method was used. Samples were cut into pieces and homogenized first with a  
182 Grindomix apparatus, then a Polytron and a T25 Ultra-Turrax apparatus. Then, 2 g of  
183 homogenized sample were precisely weighed in a 50 mL centrifuge tube before the addition of  
184 50 µL of internal standard CLD-<sup>13</sup>C<sub>10</sub> at a concentration of 0.6 ng.µL<sup>-1</sup>. After 1 min vortex-  
185 stirring, the internal standard was kept in contact for 1 h with the matrix. CLD was extracted  
186 by 10 mL of a mixture of hexane/acetone 85/15 (v/v) by using a T25 Ultra-Turrax for 1 min at  
187 10,000 rpm. The organic phase was collected in another 50 mL centrifuge tube. The extracts  
188 then followed the same basic and acidic transformations as for the fat extracts.

189

#### 190 2.5. LC-MS/MS analysis

191 A total amount of 5  $\mu\text{L}$  of the individual final extract was injected into the chromatographic  
192 system at room temperature (Phenomenex, Le Pecq, France). Ultrapure water containing 0.1%  
193 (v/v) formic acid (mobile phase A) and methanol containing 0.1% (v/v) formic acid (mobile  
194 phase B) were used for the gradient elution at a constant flow rate ( $200 \mu\text{L min}^{-1}$ ). The gradient  
195 started with 40% of phase B and increased to 100% within 6 min. Phase B was kept constant  
196 for the next 8 min and was then set back to 60% in 2 min before a 5 min equilibrium time,  
197 before the next injection.

198 Electrospray ionization was run in negative mode. The spray voltage was set at -4,500 V. The  
199 curtain gas was set at 25 psi of nitrogen. Spray gas and auxiliary gas were set at 40 and 60 psi  
200 of air, respectively. The collision gas was set at “Medium” value. The source temperature was  
201 set at  $600 \text{ }^\circ\text{C}$ . The instrument was operated in multiple reaction-monitoring mode (MRM). CLD  
202 and its isotopic standard were detected under the deprotonated hydrated form (Bichon et al.,  
203 2015). The quantifying and qualifying transitions for CLD were  $506.7 > 426.7$  and  $506.7 >$   
204  $428.7$ , respectively. For these two transitions, the decluttering potential was -160 V, entrance  
205 potential -10 V, collision cell exit potential -15 V, and collision energy -25 eV. The transition  
206 for CLD- $^{13}\text{C}$  was  $516.7 > 435.7$  with the same parameters, except the collision energy of -28  
207 eV. The transition for CLD- $^{13}\text{C}$  was  $516.7 > 435.7$  with the same parameters, except the  
208 collision cell exit potential -10 V, and the collision energy -30 eV. The dwell time for each  
209 transition was set at 100 ms. Analyst 1.51 Software (AB Sciex) was used for system control  
210 and data acquisition and processing.

211

## 212 2.6. Method validation and quality assurance/quality control

213 The method was validated as per standard NF V03-110: 2010 (AFNOR, 2010), based on  
214 tolerance intervals and overall determination of the performance of the method through the  
215 accuracy profile (Mermet and Granier, 2012).

216 The accuracy profile provides a graphical representation of the risk of error for each  
217 concentration level. The validated range is defined as the domain where  $\beta$ -expectation limits lie  
218 within the acceptance limits ( $\lambda$ ). This method also evaluates the performance of the calibration  
219 model, the accuracy, and the uncertainty. The acceptance limits ( $\lambda$ ) were set to 70-120% in  
220 accordance with European Union guidelines (SANTE/11945/ 2015, 2015). Probability  $\beta$  was  
221 set to 80%, meaning that the risk of results lying outside the limits is below 20% on average.  
222 Initially, the method was validated and implemented to cover two commodity groups mentioned  
223 in the SANTE document: “Fat from food of animal origin” and “Meat (muscle) and seafood”.  
224 For the second commodity group, as fishes were targeted for official controls, it was decided to  
225 select barracuda, a fish consumed in the French West Indies. Since (i) sample preparation is  
226 identical for fish and meat samples, and (ii) the analysis is based on isotopic dilution, the method  
227 was considered to be validated for the whole commodity group. This was confirmed by the  
228 conformity of routine quality control, implemented for the present study, including recoveries  
229 on meat as detailed below. Later, the method was extended to liver tissues. Therefore, three  
230 separate profiles were built: one for the “meat and seafood” commodity, one for liver, and one  
231 covering fat regarding its specific preparation. Five series spiked at 4 levels (3 levels for liver)  
232 from 3 to 500  $\mu\text{g.kg}^{-1}$  fw were analyzed, each on different days in duplicate. The performances  
233 of the method are presented in [Table 1](#).  
234 An overall limit of quantification (LOQ) (3  $\mu\text{g.kg}^{-1}$  fw) was experimentally tested and the range  
235 of application was tested up to 500  $\mu\text{g.kg}^{-1}$  fw, with success. Compliance of the test  
236 concentrations with performance requirements laid down in the SANTE document  
237 ([SANTE/11945/ 2015, 2015](#)) was checked, and the accuracy profiles were validated. The LOQ  
238 is consistent with previous studies (Lastel et al., 2018; Saint-Hilaire et al., 2018) and the  
239 European regulation on MRLs of pesticides ([Commission Regulation \(EU\) No 212/2013](#),

240 [212AD ; Commission Regulation \(EC\) No 839/2008](#)), which sets the MRL at 100  $\mu\text{g.kg}^{-1}$  fw  
241 for fat, muscle and liver.

242 The limit of detection (LOD) was set according to the calibration model used for quantification  
243 with standard solutions and to the enrichment factor between the matrix and the extracts: it was  
244 set at 2  $\mu\text{g.kg}^{-1}$  fw for fat, and 0.5 for  $\mu\text{g.kg}^{-1}$  fw for muscle. For liver, the LOD was set at one  
245 third of the LOQ, i.e. 1  $\mu\text{g.kg}^{-1}$  fw.

246 The repeatability coefficients of variation for all matrices were within the range 1.2–8.3%, all  
247 concentration levels considered. The intermediate precision coefficients of variation ranged  
248 from 2.5 to 9.9%. Both criteria met the requirements of the SANTE Guidelines ([SANTE/11945/](#)  
249 [2015, 2015](#)), which recommend a maximum value of 20%.

250 The expanded uncertainty of the method was estimated taking into account the standard  
251 deviation of the tolerance interval and an estimation of the bias. It was set at 20 %,   
252 corresponding to the maximum estimated uncertainty for each matrix and tested concentrations.  
253 For routine analysis, quality controls were included in each sample batch, with criteria  
254 recommended by SANTE guidelines ([SANTE/11945/ 2015, 2015](#)). Reagent blanks were  
255 injected after each sample to check the absence of cross-contamination, with a tolerance  
256 corresponding to 30% of the LOQ. Bracketing calibration (6 levels) was performed, and  
257 linearity was controlled by residual deviation below 20% from the calibration curve. In every  
258 sample batch, recovery at a level corresponding to 1.5 x LOQ was checked to be in the 70–  
259 120% range. Retention times ( $\pm 0.1$  min between CLD and CLD  $^{13}\text{C}$  retention times) and ion  
260 ratio deviations ( $\pm 30\%$ ) were systematically examined to ensure correct analyte identification  
261 in case of a result above the LOQ. Results were validated only if all criteria were within the  
262 acceptable limits.

263 Some of the analyses (73 out of 200 whole triplets) were subcontracted to the laboratory  
264 Inovalys, Le Mans, France. This laboratory, belonging to the network approved for French

265 official controls, applied the same official method described above after in-house validation  
266 and demonstration of equivalent performance. In both laboratories, analyses were performed  
267 under the international standard ISO/IEC 17025:2005 (ISO/IEC 17025, 2005) by qualified  
268 operators.

269

## 270 2.7. Statistical analysis

271 The correlation study of the CLD concentrations in the three tissues was performed in multiple  
272 steps. In order not to skew the determination of the correlation factors, results below the LOQ  
273 were not included in the statistical analysis. For dataset description, in the lower bound scenario,  
274 zero and the LOD values replaced results below LOD and LOQ, respectively whereas in the  
275 upper bound scenario, these results were replaced by LOD and LOQ, respectively. A Shapiro–  
276 Wilk test was used to determine the normality of the concentration distributions for each of the  
277 three studied tissues. Pearson tests (SPSS software, v 23.0 (IBM, New York, NY, United States)  
278 were used to assess the correlations of CLD concentrations in the triplets (fat, muscle and liver).  
279 The statistical significance was set to  $p < 0.05$ . Linear regressions, prediction models, and  
280 graphical representations of the correlations were performed with Studio software, v 1.2 (R  
281 Development Core Team, 2016).

282

## 283 3. Results and discussion

### 284 3.1. Occurrence of CLD content in bovine perirenal fat, muscle, and liver

285 The main statistical descriptors are presented in [Table 2](#), and [Figure 1](#) illustrates the  
286 distribution of the determined concentrations. The whole dataset is available in the  
287 Supplementary Information. The most contaminated triplets exhibit atypically high values  
288 compared to the whole dataset: CLD concentrations in liver and muscle ( $812 \mu\text{g}\cdot\text{kg}^{-1}$  fw and  
289  $152 \mu\text{g}\cdot\text{kg}^{-1}$  fw) are almost two fold higher than for the penultimate triplet ( $420.6 \mu\text{g}\cdot\text{kg}^{-1}$  fw and

290 67.6  $\mu\text{g}\cdot\text{kg}^{-1}$  fw). This triplet was excluded from the dataset for description and statistical  
291 analysis. Irrespective of the type of matrix, CLD was detected in 68% of samples (404 samples  
292 above LOD) and quantified in 59% of samples (332 samples above LOQ). Considering the  
293 three tissues, CLD was mostly quantified in liver (65%, 130 out of 199 samples), followed by  
294 fat (53%, 106 out of 199 samples), and muscle (48%, 96 out of 199 samples). Regarding  
295 contamination levels, liver covers a broader range of concentrations (LOQ up to 420.6  $\mu\text{g}\cdot\text{kg}^{-1}$   
296 fw) than fat (LOQ up to 124.6  $\mu\text{g}\cdot\text{kg}^{-1}$  fw), and muscle (LOQ up to 67.6  $\mu\text{g}\cdot\text{kg}^{-1}$  fw), as  
297 mentioned in Table 2.

298 For muscle and liver, a previous study regarding food exposure of Martinicans and  
299 Guadeloupeans reported that CLD was detected in 19% of beef meat samples ( $n=38$ )  
300 ([Dubuisson et al., 2007](#)). The samples were collected during a sampling plan that was not only  
301 focussed on contaminated areas (without precision) and was organised between 2002 and 2004.  
302 The type of tissue collected was not detailed. The observed concentration range started at 0.83  
303  $\mu\text{g}\cdot\text{kg}^{-1}$  fw for the average lower-bound residue level, and at 1.16  $\mu\text{g}\cdot\text{kg}^{-1}$  fw for the average  
304 upper-bound, and reached up to 2.88  $\mu\text{g}\cdot\text{kg}^{-1}$  fw for the highest values. The higher detection  
305 occurrence (59% for muscle and 85% for liver) and contamination ranges in the present study  
306 are difficult to compare since they might be linked to a bias in sample collection, since it is  
307 oriented toward most-probable contaminated bovines in the present study.

308  
309 Considering muscle tissue, the most consumed part, the results can be compared with those  
310 reported in the Kannari study ([ANSES, 2017](#)), where meat samples were collected between  
311 2009 and 2015 from different supply chains, including slaughterhouses, local markets,  
312 subsistence production, and medium and large wholesalers. The Kannari study differentiates  
313 contaminated and non-contaminated areas of Martinique and Guadeloupe. In slaughterhouses,  
314 64% ( $n=293$ ) of the samples were above the LOQ of 1–10  $\mu\text{g}\cdot\text{kg}^{-1}$  fw in contaminated areas.

315 These figures are consistent with those found in the present study oriented toward contaminated  
316 areas: 48%, 96 samples out of 199 above the LOQ for muscle, and 65%, 130 samples out of  
317 199 for liver. The median level calculated in the Kannari study ( $11 \mu\text{g.kg}^{-1}$  fw in contaminated  
318 areas) was higher than the median level of  $4.1 \mu\text{g.kg}^{-1}$  fw found in the present study. Moreover,  
319 considering the lower and upper bound hypotheses, the same trend is observed (mean  
320 concentrations of  $5.8$  and  $6.2 \mu\text{g.kg}^{-1}$  fw versus  $0.6$  and  $4 \mu\text{g.kg}^{-1}$  fw in the Kannari study). The  
321 differences regarding these figures may be due to the type of animals sampled (not detailed in  
322 the Kannari study) and to the sampling strategy that included imported commodities for the  
323 purpose of the Kannari plan, whereas only local production was considered in the present work.  
324 Regarding other species, our results in endemically contaminated bovines are similar to those  
325 reported in endemically contaminated pigs originating from Guadeloupe ([ANSES 2019](#)). In pig  
326 skirt sample ( $n=77$ ), CLD concentrations ranged from  $10$  to  $56 \mu\text{g.kg}^{-1}$  fw with a median at  $23$   
327  $\mu\text{g.kg}^{-1}$  fw. In three other muscles (longissimus dorsal muscle, semi-membranous muscle and  
328 flank) collected on another set of  $15$  pigs, the concentration range was also similar ( $11$ – $34$   
329  $\mu\text{g.kg}^{-1}$  fw and median within  $18$ – $27 \mu\text{g.kg}^{-1}$  fw). These figures document the potential endemic  
330 background contamination range of non-artificially fed bovines and pigs bred in Martinique  
331 and Guadeloupe. Another study measured CLD concentrations in fat, legs and liver of free-  
332 range ducks bred in endemically contaminated areas of Martinique ([Jondreville et al., 2014b](#)).  
333 Again, concentration ranges are consistent with those reported in the present study:  $47$ – $1,215$   
334  $\mu\text{g.kg}^{-1}$  fw for liver,  $9$ – $278 \mu\text{g.kg}^{-1}$  fw for fat, and  $6$ – $145 \mu\text{g.kg}^{-1}$  fw for legs (without skin). In  
335 duck tissues, livers were also more highly contaminated than fat and muscle.

336 The distribution of CLD in fat, muscle and liver has also been reported to document the  
337 contamination and/or decontamination kinetics for male Alpine kids ([Lastel et al., 2018](#)),  
338 Muscovy ducks ([Jondreville et al., 2014b](#)) and pigs ([ANSES 2019](#)). It was also documented for  
339 poultry, where liver and fat were investigated ([Jondreville et al., 2014a](#)). The same structure of

340 contamination was observed: liver followed by fat and muscle. Since the above-mentioned  
341 studies concerned artificially contaminated animals, the comparison of both CLD levels and  
342 occurrence is not relevant.

343 The contamination of all studied tissues, both occurrence and concentration levels, documents  
344 the atypical behaviour of CLD in different species, including bovines. It is distributed in  
345 different tissues and does not accumulate mainly in fat. This raises the question of a potential  
346 correlation factor between CLD concentrations in tissues.

347

### 348 3.2. Statistical correlation between tissues

349 For the purposes of the correlation study, only quantified results in the three tissues were taken  
350 into account, thus limiting the dataset to 96 animals. As mentioned above, the most  
351 contaminated triplet (concentrations in fat/muscle/liver: 172/152/812  $\mu\text{g.kg}^{-1}$  fw) was excluded  
352 from data processing.

353 As mentioned in [Table 3](#), Pearson correlation coefficients were above 0.884, indicating a strong  
354 correlation. Moreover, a significance test was performed: values were below 0.01, which  
355 supports the strong correlation.

356 A linear relationship of the observed CLD concentrations was found for muscle/fat,  
357 muscle/liver, and liver/fat. The intercept was forced to zero to avoid negative values at low  
358 concentrations. The slope of the curves represents the CLD correlation factors between the  
359 tissues ([Figure 2](#)). The CLD concentration ratios were estimated: 0.54 for muscle/fat, 3.75 for  
360 liver/fat, and 0.14 for muscle/liver. The coefficients of correlation ( $R^2$ ) of the model were 0.973,  
361 0.901, and 0.906, respectively. To go further, prediction intervals were also determined, based  
362 on the dispersion of the measurements ([ANSES 2018.](#)). Depending on a two standard deviations  
363 confidence level, it was possible to determine a prediction range of CLD concentration in fat  
364 (tissue considered for controls) from a target concentration in skirt or liver (as consumed parts).

365 The value targeted in skirt was set at  $20 \mu\text{g.kg}^{-1}$  fw on the basis of national authorities'  
366 recommendations. For the two standard deviation interval, the CLD concentration in fat ranged  
367 from 27 to  $47 \mu\text{g.kg}^{-1}$  fw: a CLD concentration of  $27 \mu\text{g.kg}^{-1}$  fw in fat was associated at 97.5%  
368 to a CLD concentration below the target value of  $20 \mu\text{g.kg}^{-1}$  fw in skirt. For the three standard  
369 deviation interval, the range was 32 to  $42 \mu\text{g.kg}^{-1}$  fw: a CLD concentration of  $32 \mu\text{g.kg}^{-1}$  fw in  
370 fat was associated at 84% to a concentration of CLD below the target value of  $20 \mu\text{g.kg}^{-1}$  fw in  
371 skirt. For liver, it was not possible to determine a range of concentrations of CLD in fat  
372 corresponding to  $20 \mu\text{g.kg}^{-1}$  fw in liver for any of the two confidence levels (95 nor 68%). It  
373 was only possible to conclude that a concentration of  $20 \mu\text{g.kg}^{-1}$  fw in liver, corresponded to a  
374 value of  $5 \mu\text{g.kg}^{-1}$  fw in fat in average. These prediction interval were used to define a CLD  
375 mitigation concentration of  $27 \mu\text{g.kg}^{-1}$  fw in fat for official control plans ([Ministerial decree of](#)  
376 [Mai the 23<sup>rd</sup>, 2019](#)), corresponding to a MRL of  $20 \mu\text{g.kg}^{-1}$  fw in muscle and liver.

377 As no data on CLD distribution in fat/muscle/liver are reported in the literature for endemically  
378 contaminated bovines, these results were compared to those obtained for other species, i.e.  
379 ducks and pigs. The observed ratios of CLD concentrations in liver/fat and muscle/liver for  
380 bovines match those reported by Jondreville et al. ([Jondreville et al., 2014b](#)) on fresh matter for  
381 Muscovy ducks: 3.9 for liver/abdominal fat, 0.6 for muscle with skin/fat, and 0.5 for muscle  
382 without skin/fat. The muscle/fat ratio was also modelled with an intercept not forced to zero for  
383 endemically contaminated pigs from Guadeloupe (n=77 animals), where skirt muscle was  
384 investigated, and another set of pigs (n=15 animals) where skirt muscle but also three other  
385 types of muscles were considered (longissimus dorsi muscle, semi-membranous muscle, and  
386 flank) ([ANSES 2019](#)). A muscle/fat ratio of 0.64 was determined, considering the two groups  
387 of animals, and thus including muscle type variability. This ratio is similar to that modelled in  
388 the present study, even though animals and muscle types are different.

389 Fourcot et al ([Fourcot et al., 2020](#)) modelled the CLD distribution in two breeds of growing  
390 pigs (Large White and Creole pigs) contaminated with intravenous administration of CLD (n=7  
391 for each breed). To allow comparison with our data, we calculated a muscle/fat ratio from the  
392 published figures in Fourcot *et al.*: total amount of CLD estimated in muscle or fat (Muscle:  
393 8.9 mg for Creole pigs and 12.5 mg for Large White pigs- Fat: 13.2 mg for Creole pigs and 10.0  
394 for Large White pigs) is divided by muscle or fat percentage of the animals respectively  
395 (Muscle: 43.3 % for Creole pigs and 54.8% for Large White pigs. Fat: 34.7% for Creole pigs  
396 and 22.4% for Large White pigs). Muscle/fat ratios are similar between bovine (0.54), Creole  
397 pigs (0.54) and Large White pigs (0.51).

398 The CLD tissue distribution in artificially fed growing kids was also assessed ([Lastel et al.,](#)  
399 [2018](#)). The observed ratios (liver/fat: 31 during the contamination period, 28 during the  
400 decontamination period – muscle (skirt) /fat: 0.5 during the contamination period and 0.6 during  
401 the decontamination period ) are very different from those estimated in the present bovine study:  
402 the liver/fat ratio is 55 times higher and the skirt muscle/fat ratio is 7 times lower in goats. These  
403 differences could be linked to the general tissue composition of the different studied species,  
404 including the differences in lipid classes, the overall fat content of the whole animal, as  
405 mentioned by Lastel *et.al.*, and the metabolic pathways of CLD, as mentioned above.

406

#### 407 **4. Conclusion**

408 An in-house validated ID-LC-MS/MS method was used to analyze CLD in 200 samples of  
409 bovine fat, liver and muscle (skirt) originating from contaminated areas of Martinique and  
410 Guadeloupe. CLD was mostly quantified in liver (65%), followed by fat (53%), and muscle  
411 (48%). These results confirmed that CLD can be distributed in the studied bovine tissues, as  
412 previously reported for goats, poultry and ducks. Strong CLD concentration correlations were  
413 found between these three tissues, with concentration ratios estimated at 0.54 for muscle/fat,

414 3.75 for liver/fat, and 0.14 for muscle/liver. These correlations were used to define a CLD  
415 mitigation concentration of 27  $\mu\text{g}\cdot\text{kg}^{-1}$  fw in fat for official control plans ([Ministerial decree of](#)  
416 [Mai the 23<sup>rd</sup>, 2019](#)), corresponding to an MRL of 20  $\mu\text{g}\cdot\text{kg}^{-1}$  fw in muscle and offal. Moreover,  
417 those data might be the support for MRL revision at European level.

418 It would be interesting to include other types of muscle tissue in a similar study, to take into  
419 account their different composition, including fat content. This could reinforce the correlation  
420 factors to further support consumer protection through dietary exposure.

421

422

### 423 **Acknowledgments**

424 The authors wish to acknowledge the French Directorate General for Food of the French  
425 Ministry for Food, Agriculture and Fisheries for their financial support and for organizing the  
426 oriented exploratory survey plan. The authors also acknowledge the ANSES Risk Assessment  
427 Department- Methodology and Survey Study Unit for its support for the statistical analysis. The  
428 authors are also grateful to Inovalys (Le Mans, France) for its participation in the analyses

429

### 430 **List of figures and tables**

431

432 **Figure 1:** CLD concentrations ( $\mu\text{g}\cdot\text{kg}^{-1}$  fw) of positive results ( $\geq$  LOQ) in muscle (n=96), fat  
433 (n=106) (a), and liver (n=130) (b) in the studied bovines.

434 Boxes represent the concentrations between percentiles 25 and 75. The line dividing the box  
435 represents the median concentration.

436 The whiskers below and above the box represent percentiles 10 and 90.

437 **Figure 2:** Linear model and confidence intervals on means and observances for muscle/fat and  
438 liver/fat correlations of CLD concentrations. Concentrations are expressed on fresh weight.

439

440 **Table 1:** Analytical performances of the method

441 **Table 2:** Main descriptors of the dataset (n=199 animals)

442 **Table 3:** Pearson correlation coefficients

443

444

#### 445 **Funding sources**

446 This work is funded by the French Directorate for food of the French Agriculture ministry

447 (DGAL)

448

#### 449 **Conflicts of interest**

450 The authors declare no conflict of interest.

451

452

#### 453 **References**

454 AFNOR, 2010. NF V03-110 . Analyse des produits agricoles et alimentaires - Protocole de  
455 caractérisation en vue de la validation d'une méthode d'analyse quantitative  
456 construction du profil d'exactitude. Saint Denis.

457 AFSSA, 2007. Actualisation de l'exposition alimentaire au chlordécone de la population  
458 antillaise, évaluation de l'impact de mesures de maîtrises des risques. Document  
459 technique AQR/FH/2007-219. 79 pages.

460 ANSES, 2017. Exposition des consommateurs des Antilles au chlordécone, résultats de l'étude  
461 Kannari.

462 ANSES 2018. NOTE d'appui scientifique et technique de l'Agence nationale de sécurité  
463 sanitaire de l'alimentation, de l'environnement et du travail relative à la fixation d'une  
464 limite maximale de résidus de chlordécone dans la graisse pour les denrées carnées.

465 ANSES 2019. Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de  
466 l'environnement et du travail relatif à la fixation d'une limite maximale de résidus de  
467 chlordécone dans les muscles et dans la graisse pour les denrées carnées. Saisine n°2018-SA-  
468 0265

469 Arrêté du 23 mai 2019, 2019. Arrêté du 23 mai 2019 modifiant l'arrêté du 25 janvier 2019

470 relatif aux limites maximales applicables aux résidus de chlordécone que ne doivent pas  
471 dépasser certaines denrées alimentaires d'origine végétale et animale pour être  
472 reconnues propres à la consommation humaine NOR : AGRG1913466A.

473 Benoit, P., Mamy, L., Servien, R., Li, Z., Latrille, E., Rossard, V., Bessac, F., Patureau, D.,  
474 Martin-Laurent, F., 2017. Categorizing chlordecone potential degradation products to  
475 explore their environmental fate. *Sci. Total Environ.* 574, 781–795.  
476 <https://doi.org/10.1016/j.scitotenv.2016.09.094>

477 Bichon, E., Guiffard, I., Vénisseau, A., Marchand, P., Antignac, J.-P., Le Bizec, B., 2015. Ultra-  
478 trace quantification method for chlordecone in human fluids and tissues. *J. Chromatogr.*  
479 *A* 1408, 169–177. <https://doi.org/10.1016/j.chroma.2015.07.013>

480 Bordet, F., Thieffine, A., Mallet, J., Heraud, F., Bateau, A., Inthavong, D., 2007. In-house  
481 validation for analytical methods and quality control for risk evaluation of chlordecone  
482 in food. *Int. J. Environ. Anal. Chem.* 87, 985–998.  
483 <https://doi.org/10.1080/03067310701461490>

484 Boucher, O., Simard, M.N., Muckle, G., Rouget, F., Kadhel, P., Bataille, H., Chajes, V.,  
485 Dallaire, R., Monfort, C., Thome, J.P., Multigner, L., Cordier, S., 2013. Exposure to an  
486 organochlorine pesticide (chlordecone) and development of 18-month-old infants.  
487 *Neurotoxicology* 35, 162–8. <https://doi.org/10.1016/j.neuro.2013.01.007>

488 Cabidoche, Y.M., Achard, R., Cattan, P., Clermont-Dauphin, C., Massat, F., Sansoulet, J.,  
489 2009. Long-term pollution by chlordecone of tropical volcanic soils in the French West  
490 Indies: A simple leaching model accounts for current residue. *Environ. Pollut.* 157,  
491 1697–1705. <https://doi.org/10.1016/j.envpol.2008.12.015>

492 Chevallier, M.L., Della-Negra, O., Chaussonnerie, S., Barbance, A., Muselet, D., Lagarde, F.,  
493 Darii, E., Ugarte, E., Lescop, E., Fonknechten, N., Weissenbach, J., Woignier, T.,  
494 Gallard, J.-F., Vuilleumier, S., Imfeld, G., Le Paslier, D., Saaidi, P.-L., 2019. Natural  
495 Chlordecone Degradation Revealed by Numerous Transformation Products  
496 Characterized in Key French West Indies Environmental Compartments. *Environ. Sci.*  
497 *Technol.* 53, 6133–6143. <https://doi.org/10.1021/acs.est.8b06305>

498 Clostre, F., Cattan, P., Gaude, J.-M., Carles, C., Letourmy, P., Lesueur-Jannoyer, M., 2015.  
499 Comparative fate of an organochlorine, chlordecone, and a related compound,  
500 chlordecone-5b-hydro, in soils and plants. *Sci. Total Environ.* 532, 292–300.  
501 <https://doi.org/10.1016/j.scitotenv.2015.06.026>

502 Clostre, F., Letourmy, P., Thuries, L., Lesueur-Jannoyer, M., 2014. Effect of home food  
503 processing on chlordecone (organochlorine) content in vegetables. *Sci Total Env.* 490,  
504 1044–50. <https://doi.org/10.1016/j.scitotenv.2014.05.082>

505 Collas, C., Mahieu, M., Tricheur, A., Crini, N., Badot, P.-M., Archimède, H., Rychen, G., Feidt,  
506 C., Jurjanz, S., 2019. Cattle exposure to chlordecone through soil intake. The case-study  
507 of tropical grazing practices in the French West Indies. *Sci. Total Environ.* 668, 161–  
508 170. <https://doi.org/10.1016/j.scitotenv.2019.02.384>

509 Commission Regulation (EU) No 212/2013, 212AD. Commission Regulation (EU) No  
510 212/2013 of 11 March 2013 replacing Annex I to Regulation (EC) No 396/2005 of the European  
511 Parliament and of the Council as regards additions and modifications with respect to the  
512 products covered by that Annex Text with EEA relevance, No 212/2013.

513 Commission Regulation (EC) No 839/2008 of 31 July 2008, 2008. Commission Regulation  
514 (EC) No 839/2008 of 31 July 2008 amending Regulation (EC) No 396/2005 of the  
515 European Parliament and of the Council as regards Annexes II, III and IV on maximum  
516 residue levels of pesticides in or on certain products (No. 839/2008).

517

518 Cordier, S., Bouquet, E., Warembourg, C., Massart, C., Rouget, F., Kadhel, P., Bataille, H.,  
519 Monfort, C., Boucher, O., Muckle, G., Multigner, L., 2015. Perinatal exposure to

520 chlordecone, thyroid hormone status and neurodevelopment in infants: The Timoun  
521 cohort study in Guadeloupe (French West Indies). *Environ. Res.* 138, 271–278.  
522 <https://doi.org/10.1016/j.envres.2015.02.021>

523 Dallaire, R., Muckle, G., Rouget, F., Kadhel, P., Bataille, H., Guldner, L., Seurin, S., Chajès,  
524 V., Monfort, C., Boucher, O., Pierre Thomé, J., Jacobson, S.W., Multigner, L., Cordier,  
525 S., 2012. Cognitive, visual, and motor development of 7-month-old Guadeloupean  
526 infants exposed to chlordecone. *Environ. Res.* 118, 79–85.  
527 <https://doi.org/10.1016/j.envres.2012.07.006>

528 Dubuisson, C., Héraud, F., Leblanc, J.-C., Gallotti, S., Flamand, C., Bateau, A., Quenel, P.,  
529 Volatier, J.-L., 2007. Impact of subsistence production on the management options to  
530 reduce the food exposure of the Martinican population to Chlordecone. *Regul. Toxicol.*  
531 *Pharmacol.* 49, 5–16. <https://doi.org/10.1016/j.yrtph.2007.04.008>

532 Florence, C., Philippe, L., Magalie, L.-J., 2015. Organochlorine (chlordecone) uptake by root  
533 vegetables. *Chemosphere* 118, 96–102.  
534 <https://doi.org/10.1016/j.chemosphere.2014.06.076>

535 Fourcot, A., Feidt, C., Bousquet-Mélou, A., Ferran, A.A., Gourdine, J.L., Bructer, M., Joaquim-  
536 Justo, C., Rychen, G., Fournier, A., 2020. Modeling chlordecone toxicokinetics data in  
537 growing pigs using a nonlinear mixed-effects approach. *Chemosphere* 250, 126151.  
538 <https://doi.org/10.1016/j.chemosphere.2020.126151>

539 Fournier, A., Feidt, C., Lastel, M.-L., Archimède, H., Thome, J.-P., Mahieu, M., Rychen, G.,  
540 2017. Toxicokinetics of chlordecone in goats: Implications for risk management in  
541 French West Indies. *Chemosphere* 171, 564–570.  
542 <https://doi.org/10.1016/j.chemosphere.2016.12.054>

543 Hervé, D., Costet, N., Kadhel, P., Rouget, F., Monfort, C., Thomé, J.-P., Multigner, L., Cordier,  
544 S., 2016. Prenatal exposure to chlordecone, gestational weight gain, and birth weight in  
545 a Guadeloupean birth cohort. *Environ. Res.* 151, 436–444.  
546 <https://doi.org/10.1016/j.envres.2016.08.004>

547 ISO/IEC 17025, 2005. General requirements for the competence of testing and calibration  
548 laboratories.

549 Jondreville, C., Fournier, A., Mahieu, M., Feidt, C., Archimède, H., Rychen, G., 2014a. Kinetic  
550 study of chlordecone orally given to laying hens (*Gallus domesticus*). *Chemosphere*  
551 114, 275–281. <https://doi.org/10.1016/j.chemosphere.2014.05.008>

552 Jondreville, C., Lavigne, A., Jurjanz, S., Dalibard, C., Liabeuf, J.-M., Clostre, F., Lesueur-  
553 Jannoyer, M., 2014b. Contamination of free-range ducks by chlordecone in Martinique  
554 (French West Indies): A field study. *Sci. Total Environ.* 493, 336–341.  
555 <https://doi.org/10.1016/j.scitotenv.2014.05.083>

556 Lastel, M.-L., Fournier, A., Jurjanz, S., Thomé, J.-P., Joaquim-Justo, C., Archimède, H.,  
557 Mahieu, M., Feidt, C., Rychen, G., 2018. Comparison of chlordecone and NDL-PCB  
558 decontamination dynamics in growing male kids after cessation of oral exposure: Is  
559 there a potential to decrease the body levels of these pollutants by dietary  
560 supplementation of activated carbon or paraffin oil? *Chemosphere* 193, 100–107.  
561 <https://doi.org/10.1016/j.chemosphere.2017.10.120>

562 Létondor, C., Pascal-Lorber, S., Laurent, F., 2015. Uptake and distribution of chlordecone in  
563 radish: Different contamination routes in edible roots. *Chemosphere* 118, 20–28.  
564 <https://doi.org/10.1016/j.chemosphere.2014.03.102>

565 Martin, D., Lobo, F., Lavison-Bompard, G., Guérin, T., Parinet, J., 2020. Effect of home  
566 cooking processes on chlordecone content in beef and investigation of its by-products  
567 and metabolites by HPLC-HRMS/MS. *Environ. Int.* 144, 106077.  
568 <https://doi.org/10.1016/j.envint.2020.106077>

569 Ministerial decree of Mai the 23<sup>rd</sup>, 2019. Arrêté du 23 mai 2019 modifiant l'arrêté du 25 janvier  
570 2019 relatif aux limites maximales applicables aux résidus de chlordécone que ne  
571 doivent pas dépasser certaines denrées alimentaires d'origine végétale et animale pour  
572 être reconnues propres à la consommation humaine NOR : AGRG1913466A.  
573

574 Méndez-Fernandez, P., Kiszka, J.J., Heithaus, M.R., Beal, A., Vandersarren, G., Caurant, F.,  
575 Spitz, J., Taniguchi, S., Montone, R.C., 2018. From banana fields to the deep blue:  
576 Assessment of chlordecone contamination of oceanic cetaceans in the eastern  
577 Caribbean. *Mar. Pollut. Bull.* 137, 56–60.  
578 <https://doi.org/10.1016/j.marpolbul.2018.10.012>

579 Mermet, J.M., Granier, G., 2012. Potential of accuracy profile for method validation in  
580 inductively coupled plasma spectrochemistry. *Spectrochim. Acta Part B At. Spectrosc.*  
581 76, 214–220. <https://doi.org/10.1016/j.sab.2012.06.003>

582 Multigner, L., Ndong, J.R., Giusti, A., Romana, M., Delacroix-Maillard, H., Cordier, S., Jégou,  
583 B., Thome, J.P., Blanchet, P., 2010. Chlordecone exposure and risk of prostate cancer.  
584 *J. Clin. Oncol.* 28, 3457–3462.

585 Ollivier, P., Touzelet, S., Bristeau, S., Mouvet, C., 2020. Transport of chlordecone and two of  
586 its derivatives through a saturated nitisol column (Martinique, France). *Sci. Total*  
587 *Environ.* 704, 135348. <https://doi.org/10.1016/j.scitotenv.2019.135348>

588 Saint-Amour, D., Muckle, G., Gagnon-Chauvin, A., Rouget, F., Monfort, C., Michineau, L.,  
589 Thomé, J.-P., Kadhel, P., Multigner, L., Cordier, S., 2020. Visual contrast sensitivity in  
590 school-age Guadeloupean children exposed to chlordecone. *NeuroToxicology* 78, 195–  
591 201. <https://doi.org/10.1016/j.neuro.2020.02.012>

592 Saint-Hilaire, M., Inthavong, C., Bertin, T., Lavison-Bompard, G., Guérin, T., Fournier, A.,  
593 Feidt, C., Rychen, G., Parinet, J., 2018. Development and validation of an HPLC-  
594 MS/MS method with QuEChERS extraction using isotopic dilution to simultaneously  
595 analyze chlordecone and chlordecol in animal livers. *Food Chem.* 252, 147–153.  
596 <https://doi.org/10.1016/j.foodchem.2018.01.092>

597 Saisine n°2018-SA-0265, 2018. AVIS de l'Agence nationale de sécurité sanitaire de  
598 l'alimentation, de l'environnement et du travail relatif à la fixation d'une limite  
599 maximale de résidus de chlordécone dans les muscles et dans la graisse pour les denrées  
600 carnées.

601 SANTE/11945/ 2015, 2015. Guidance document on analytical quality control and method  
602 validation procedures for pesticides residues analysis in food and feed. SANTE/11945/  
603 2015, Leg. Depos. 11945 (2015) 1–42.

604 Woignier, T., Fernandes, P., Soler, A., Clostre, F., Carles, C., Rangon, L., Lesueur-Jannoyer,  
605 M., 2013. Soil microstructure and organic matter: Keys for chlordecone sequestration. *J.*  
606 *Hazard. Mater.* 262, 357–364. <https://doi.org/10.1016/j.jhazmat.2013.08.070>

**Table 1:** Analytical performances of the method

Matrix	CLD concentration levels ( $\mu\text{g}\cdot\text{kg}^{-1}$ fw)	Accuracy				
		Mean recoveries (%)	Trueness (relative bias %)	Precision		
				Repeatability (%)	Intermediate precision (%)	
Perirenal fat	3	109	8.5	2.1	2.5	
	10	108	7.5	1.2	2.8	
	100	104	4.1	2.6	5.8	
	500	99	-1.4	2.5	8.1	
Meat and seafood	Liver	3	99	-1.1	4.6	6.3
		20	102	2.5	4.3	5.1
		500	106	5.9	3.5	4.2
Other commodities	3	100	0.4	4.3	9.1	
	10	101	1.1	1.7	8.3	
	20	99	-0.7	3.3	9.9	
	500	92	-7.9	3.6	7.7	

**Table 2:** Main descriptors of the dataset (n=199 animals)

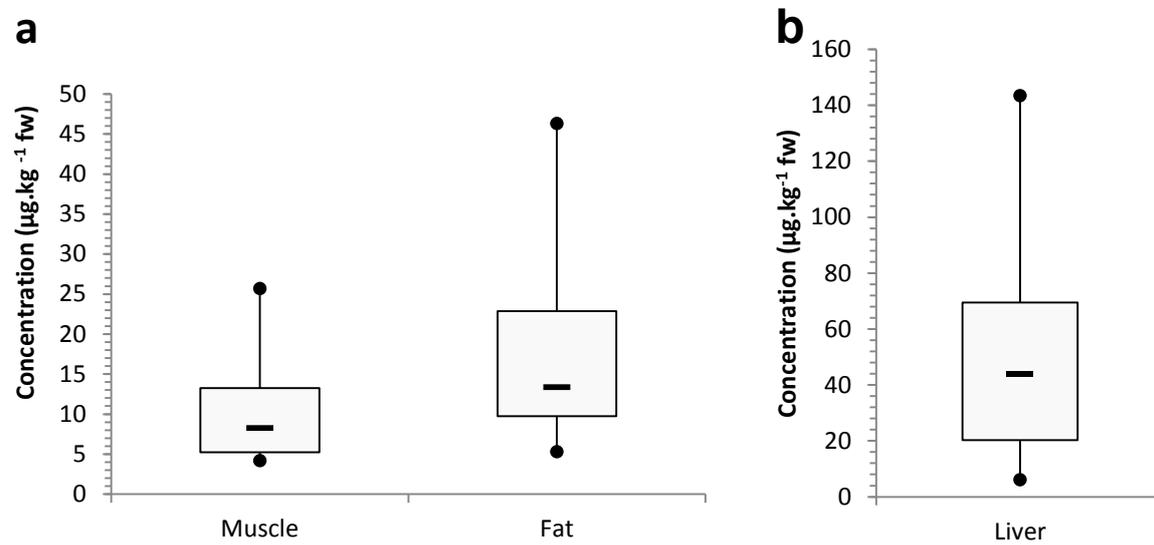
	Number of detections <sup>a</sup>	Number of quantifications <sup>b</sup>	Concentration ( $\mu\text{g}\cdot\text{kg}^{-1}$ fw)				
			Maximum	Lower bound hypothesis		Upper bound hypothesis	
				Mean	Median	Mean	Median
Fat	117	106	124.6	11.4	4.2	12.2	4.2
Muscle	117	96	67.6	5.8	0.5	6.2	3.0
Liver	170	130	420.6	40.0	19.0	40.5	19.0

a:>LOD

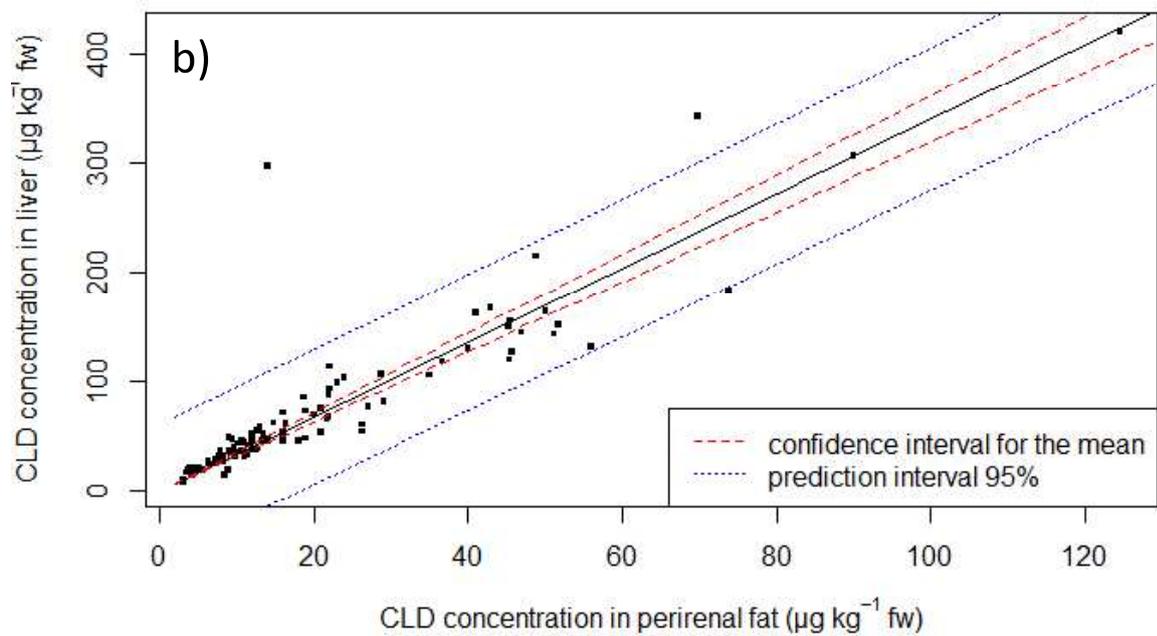
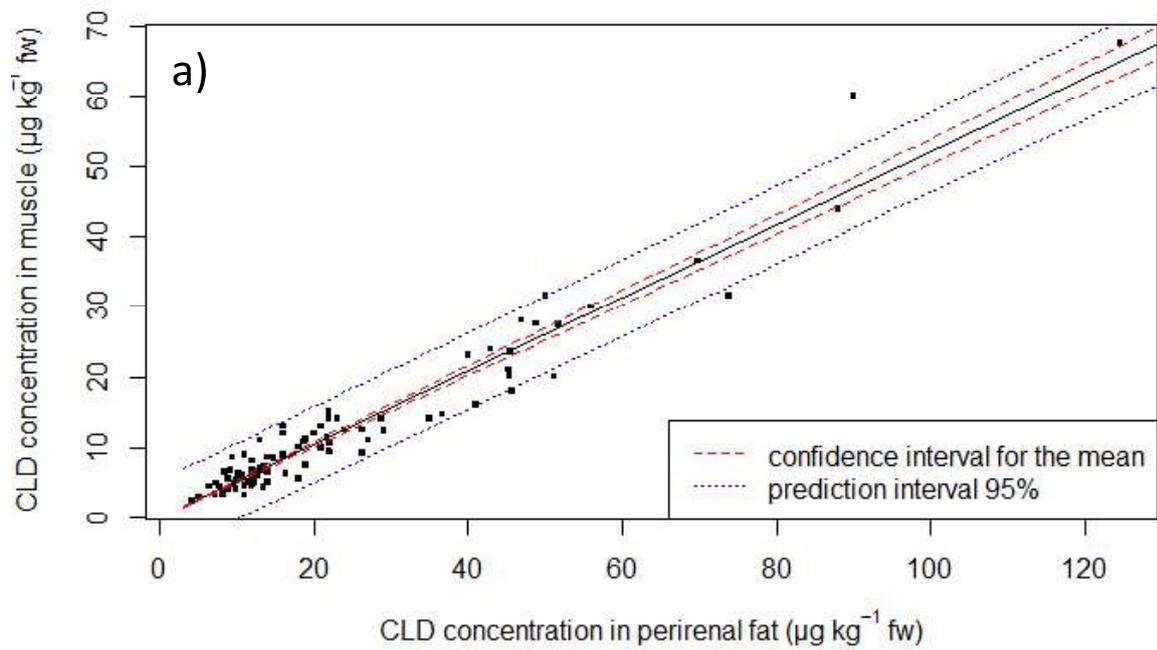
b: >LOQ

**Table 3:** Pearson correlation coefficients

	Fat	Liver
Fat	-	0.884
Muscle	0.974	0.871



**Figure 1:** CLD concentrations ( $\mu\text{g.kg}^{-1}\text{fw}$ ) of positive results ( $\geq \text{LOQ}$ ) in (a) muscle ( $n=96$ ) and fat ( $n=106$ ), and (b) in liver ( $n=130$ ) for the studied bovines. Boxes represent the concentrations between percentiles 25 and 75. The line dividing the box represents the median concentration. The whiskers below and above the box represent percentiles 10 and 90.

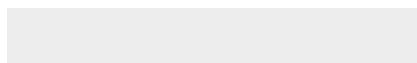
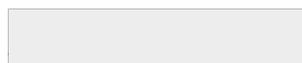


**Figure 2:** Linear model and confidence intervals on means and observances for (a) muscle/fat and (b) liver/fat correlations of CLD concentrations. Concentrations are expressed on fresh weight (fw).



[Click here to access/download](#)

**Supplementary material for on-line publication only**  
**SI.docx**



**Author Contributions:**

Conceptualization : C.I, M.L, .T.G.;

Data curation : G.L-B, M.L, K.H, J.P, T.G.; C.I.;

Formal analysis : G.L-B., M.L, J.P, C.I.

Investigation : M.L, C..I, K.H

Methodology : M.L, C..I, K.H, G. L-B

Project administration, : M.L,C.I,G.L-B.

Supervision : M.L,C.I, G.L-B

Validation : M.L, C..I, G.L-B

Visualization : M.L, G.L-B, J.P

Writing—original draft preparation: G.L-B,

Writing—review and editing : G.L-B ,M.L., T.G, J.P, K.H., C.I.

All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding (Internal public funding: French Agency for Food, Environmental and Occupational Health & Safety (ANSES), grant number CRD-2018-174).

**Acknowledgments:** We would like to express our gratitude to the Directorate General for Food (DGAI - French Ministry of Agriculture and Food) for its facilitating role and without whom this work would have not been possible, and especially to Pauline Favre and Mathilde Palussière. The authors sincerely acknowledge all the Resources and Environment Laboratories (LER) at IFREMER and the coordination of the French programme for monitoring emerging toxins (EMERGTOX scheme) for the collection of samples and for organizing the sampling campaigns. We also would to express our gratitude to the REPHY-REPHYTOX scheme, and in particular, Maud Lemoine and Nadine Neaud Masson, for the information on hydrological and phytoplankton data discussed in this report. We extend special thanks to the Metal and Mineral Trace Elements (ET2M) unit at ANSES for providing gastropods and for preparing them. Finally, the authors would like to thank all the staff at the Marine Biotoxins and Pesticides unit (PBM) at ANSES for their contribution to this work, and especially for preparing the bivalve mollusc samples.