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1 Modeling chlordecone toxicokinetics data in growing pigs using a nonlinear mixed-  
2 effects approach

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15

16 Abstract

17 The use of chlordecone (CLD), a chlorinated polycyclic pesticide used in the French Indies  
18 banana fields between 1972 and 1993, resulted in a long-term pollution of agricultural areas.  
19 It has been observed that this persistent organic pollutant (POP) can transfer from  
20 contaminated soils to food chain. Indeed, CLD is considered almost fully absorbed after  
21 involuntary ingestion of contaminated soil by outdoor reared animals. The aim of this study  
22 was to model toxicokinetics (TKs) of CLD in growing pigs using both non-compartmental and  
23 nonlinear mixed-effects approaches (NLME). In this study, CLD dissolved in cremophor was  
24 intravenously administrated to 7 Creole growing pigs and 7 Large White growing pigs (1 mg  
25 kg<sup>-1</sup> body weight). Blood samples were collected from time t=0 to time t=84 days. CLD  
26 concentrations in serum were measured by GCMS/MS. Data obtained were modeled using  
27 Monolix (2019R). Results demonstrated that a bicompartmental model best described CLD  
28 kinetics in serum. The influence of covariates (breed, initial weight and average daily gain) was

29 simultaneously evaluated and showed that average daily gain is the main covariate explaining  
30 inter-individual TKs parameters variability. Body clearance was of 76.7 mL kg<sup>-1</sup> d<sup>-1</sup> and  
31 distribution volume at equilibrium was of 6 L kg<sup>-1</sup>. This modeling approach constitutes the first  
32 application of NLME to study CLD TKs in farm animals and will be further used for rearing  
33 management practices in contaminated areas.

34 Keywords: Chlordecone, Toxicokinetics, Pigs, Modeling, Monolix

## 35 1. Introduction

36 Chlordecone (CLD) is a chlorinated polycyclic ketone pesticide which was extensively used in  
37 the French West Indies (FWI) from 1972 until 1993 to control the banana black weevil  
38 (*Cosmopolites sordidus*) population (Cabidoche et al., 2009). The use of this insecticide has  
39 resulted in long-term soils pollution, this compound transfers from soil to food chain and local  
40 population is thus exposed to CLD through food (Dubuisson et al., 2007). Human exposure to  
41 CLD is therefore a public health concern since CLD is known for its neurotoxic and  
42 carcinogenic properties (Multigner et al., 2010; Costet et al., 2015). To limit human exposure  
43 to CLD related to self-produced or informal sector products (ANSES, 2017), risk of food  
44 contamination has to be reduced. Meat from local outdoor reared pigs can contribute to CLD  
45 human exposure. Thus, CLD distribution and elimination processes need to be characterized  
46 in pigs to further propose rearing strategies aiming at removing CLD from contaminated  
47 animals. Furthermore, a study was conducted in 2013-2014 on 742 FWI inhabitants to assess  
48 CLD levels in serum (KANARRI study). Although CLD was detected in serum of 90% of the  
49 participants, impregnation value could not be used to assess health risk due to lack of an  
50 internal health-based guidance value (Dereumeaux et al., 2019). The currently existing chronic  
51 toxicological reference value (TRV) is an external exposure value fixed at 0.5 µg kg<sup>-1</sup> body  
52 weight (BW) d<sup>-1</sup> (AFSSA, 2007). CLD toxicokinetics (TKs) in pigs could also be used to build a  
53 PBPK model in human because pig is an animal model close to the human model. This model  
54 would then allow to convert the external TRV in an internal reference dose and then to discuss  
55 the impregnation level of population.

56 The main route of animal exposure to CLD is related to soil ingestion. Level of soil ingestion is  
57 known in ruminants (Jurjanz et al., 2017; Collas et al., 2019). In pigs reared outdoor, level of  
58 soil ingestion has been studied in temperate conditions (Jurjanz and Roinsard, 2014) but not  
59 yet in tropical conditions. However, because of their exploratory behavior, risk of pig meat  
60 contamination must be even higher than for ruminant meat. It has been demonstrated that soil  
61 bound CLD is not retained in soil matrices during digestive processes (Bouveret et al., 2013;  
62 Jurjanz et al., 2014) and CLD is considered almost fully absorbed after oral administration  
63 (Fournier et al., 2017). Unlike other POPs, CLD has a peculiar distribution with a preferential  
64 accumulation in liver and a lower one in fat tissues (Soine et al., 1983; Jondreville et al., 2014;  
65 Lastel et al., 2016). Thus, because of different distribution profiles within the organism (Soine  
66 et al., 1982; Soine et al., 1983; Lerch et al., 2016), TK extrapolations from other POPs to CLD  
67 are not appropriate. Limited information is currently available concerning TKs of CLD in human  
68 or in animals. Some data are available in rodents (Egle et al., 1978; Houston et al., 1981),  
69 piglets (Soine et al., 1983), ruminants (Fournier et al., 2017; Saint-Hilaire et al., 2019) and  
70 humans (Adir et al., 1978; Cohn et al., 1978; Fariss et al., 1980). Extrapolations from rodents  
71 or piglets to growing pigs are not appropriate because of metabolic differences between  
72 animals of different species or different physiological status (Houston et al., 1981). Therefore,  
73 it appears as a major priority to characterize CLD behavior in growing pigs. In this study, the  
74 two main breeds of pigs reared in the FWI will be compared.

75 Until now, TKs of CLD in animals have been studied by compartmental approaches that did  
76 not consider parameters like breed or body weight (Fournier et al., 2017; Saint-Hilaire et al.,  
77 2019). The population approach, initially developed for pharmacokinetics applications, was  
78 used to evaluate pharmacokinetics variability between individuals. Nonlinear mixed-effects  
79 (NLME) have not been used yet for the modeling of TKs CLD data. Advantages of such an  
80 approach are that parameters are estimated in the population of individuals, variability within  
81 the population is evaluated and factors that control that variability may be identified.

82 The aim of this study was to model TKs of CLD in growing pigs using both a non-compartmental  
 83 approach and a NLME approach. Such data are essential to better characterize elimination  
 84 mechanisms of CLD in pigs.

## 85 2. Material and methods

### 86 2.1. Animals and management

87 This study was performed at the experimental facilities of INRAE-PTEA in Guadeloupe (FWI;  
 88 16°N, 61°W). The experimental protocol was approved by the Ethics Committee of Guyana  
 89 and West Indies (CEMEA-AG) under the project number APAFIS#6070-2016070721289156.

90 A total of 14 growing pigs born in the herd of the INRAE experimental station of Duclos (Petit-  
 91 Bourg, Guadeloupe) were placed in individual barns. To compare TK parameters between the  
 92 two main pig breeds reared in the FWI, two different breeds were studied: Large White (n=7)  
 93 and Creole (n=7). Large White breed is one of the mainstream pig breed used worldwide that  
 94 has been selected for high growth performance in optimal conditions (ie specialist type of  
 95 breed). On the other hand, Creole breed is a generalist type of breed that has not been  
 96 genetically selected and this local tropical Caribbean breed is mainly characterized by early  
 97 maturity, a higher fat deposition than Large White breed and a better ability to cope with harsh  
 98 tropical conditions (heat, nutritional stress; Gourdine et al., 2018). Physiological and growth  
 99 performance differences between the two groups may affect TKs of CLD.

100 Table 1: Average values of IBW, FBW and ADG for each breed

Parameters	Creole pigs (n=7) mean ± SE	Large White pigs (n=7) mean ± SE	All pigs (n=14) mean ± SE
<b>IBW</b> kg	68.8 ± 1.8 <sup>a</sup>	67.9 ± 3.1 <sup>a</sup>	68.3 ± 1.7
<b>FBW</b> kg	91.2 ± 2.8 <sup>a</sup>	95.6 ± 2.4 <sup>a</sup>	92.8 ± 1.9
<b>ADG</b> g d <sup>-1</sup>	248 ± 25 <sup>a</sup>	327 ± 13 <sup>a</sup>	288 ± 17

IBW: Initial Body Weight; FBW: Final Body Weight; ADG: Average Daily Gain; SE: Standard Error.  
 Values not followed by the same superscript letter significantly differ (P < 0.05; Student test)

101

102 Table 1 gives average values of initial body weight (IBW), final body weight (FBW) and average  
103 daily gain (ADG) for each breed. At the beginning of the experiment, pigs were 26 and 23  
104 weeks old for Creole and Large White, respectively, to obtain comparable animals in term of  
105 IBW. In order to limit ADG of growing pigs and to limit dilution effect, a daily feed ration  
106 composed by a commercial feed was established to obtain an ADG of 250 g d<sup>-1</sup>. Water was  
107 delivered *ad libitum*. All pigs were individually weighted every 7 days during the 84-days  
108 depuration period. ADG was calculated by the equation (1):

$$109 \text{ ADG} = (\text{FBW} - \text{IBW}) / \text{number of days} \quad (1)$$

110 To compare IBW, FBW and ADG between the two breeds, a Student *t* test was used. Before  
111 apply this parametric test, normality of data and equality of variances has been checked with  
112 a Shapiro-test and a F-test, respectively. Statistical analysis were performed using R version  
113 3.5.0. software. The  $\alpha$  level for statistical significance was set at 0.05.

## 114 2.2. Experimental design

115 One week before the experiment, a catheter was inserted in the jugular vein of each pig to  
116 allow CLD administration and blood sampling. After this surgical intervention, pigs were  
117 allowed a 7-days adaptation period in individual barns (metal-slatted pen of 0.85 x 1.50 m).  
118 The experiment consisted in a single administration of CLD by intravenous (*i.v.*) route (1 mg  
119 kg<sup>-1</sup> BW, Kepone 99.9%, Sigma Aldrich). This high dose was chosen to be able to follow the  
120 decrease of CLD concentrations in serum and easily compare to other species. Indeed, this  
121 dose has already be used in goats and ewes (Fournier et al., 2017; Saint-Hilaire et al., 2019).  
122 For these studies, no side effects were observed.

123 CLD was dissolved in cremophor (polyethoxylated castor oil, Kolliphor<sup>®</sup>, Sigma-Aldrich) at  
124 room temperature by sonication (2 hours). Pigs received 0.04 g kg<sup>-1</sup> BW of contaminated oil  
125 concentrated at 24.8 mg CLD g<sup>-1</sup> oil. The catheter was flushed several times after CLD  
126 administration to avoid contamination of following blood samples.

127 At 0, 30, 60 min, 2, 3, 7, 12, 24 hours, 3, 8, 14, 21, 28, 49, 70 and 84 days after administration,  
128 blood samples (4 x 10 mL) were taken using the *i.v.* catheter for the first weeks (in dry tubes)  
129 and then, by a direct venipuncture on the jugular vein (BD vacutainer®, ref 368815), when the  
130 sampling was less intensive. Collected blood samples were allowed clotting for 2 hours at room  
131 temperature and at 4°C during 24 hours before centrifugation at 3000 rpm for 10 min to get  
132 serum samples which were stored at -20°C until analysis. After 84-depuration days, pigs were  
133 slaughtered (stunning followed by bleeding). Liver, peri-renal fat and shaft (diaphragm muscle)  
134 were collected. Tissues were stored at -20°C until analysis.

### 135 2.3. Analytical methods

#### 136 2.3.1. Analysis of CLD in serum

137 CLD analysis was performed by the Center for Analytical Research and Technology (LEAE-  
138 CART, University of Liège, Belgium) according to Fournier et al. (2017) with slight  
139 modifications. Briefly, 10-monohydrochlordecone kindly synthesized by Dr. P.L. Saaidi  
140 (Génomique Métabolique, Evry, France) according to Chevallier et al. (2019) was used as  
141 internal standard (recovery surrogate). This compound was never detected in previous studies  
142 for CLD analysis in goat, lamb and ewe serum (Jurjanz et al., 2014; Lastel et al., 2016; Lerch  
143 et al., 2016; Fournier et al., 2017). This internal standard was added to 2 mL of pig serum  
144 samples. After proteins denaturation with trimethylamine and formic acid, CLD was extracted  
145 with n-hexane/diethylether/acetonitrile/ethanol (80/15/4/1, v/v) using a solid-phase extraction  
146 (SPE) on Supelclean Envi-C<sub>18</sub> microcartridges (Supelco, Bellafonte, PA, USA) and subjected  
147 to acidic purification using concentrated sulfuric acid 98% w/v. The final extract (100 µL of  
148 hexanic solution) was spiked with PCB-209 (100 pg µL<sup>-1</sup> in hexane) used as a volume internal  
149 standard. As described in Fournier et al. (2017), all PCBs and organochlorine pesticides were  
150 eluted in the first hexanic solution from C<sub>18</sub> microcartridge. CLD and related compounds (5b  
151 monohydroCLD and 10-monohydroCLD) were recovered in the second elution  
152 (hexane/diethylether; 85/15; v/v). Then, there was no PCBs in the CLD elution. Furthermore,  
153 PCB-209 was not present in commercial PCB mixtures such as Aroclor used in the past.

154 However, this congener was reported in organic paint pigment (Hu and Hornbuckle, 2010;  
155 Anezaki and Nakano, 2014). Moreover, Koh et al. (2015) reported traces of PCB-209 in human  
156 serum in some areas in USA but at trace levels and concentrations measured (0.00 to 0.01 ng  
157 mL<sup>-1</sup>) were far below the LOQ of the present study (0.06 ng mL<sup>-1</sup>). As a consequence, when  
158 considering all these elements and as PCB-209 was added as internal volume standard just  
159 prior the injection in HRGC MS/MS, this compound cannot come from environmental  
160 contamination. For each series of 21 experimental samples there was a procedural blank, a  
161 matrix blank (porcine serum from Sigma Aldrich chemie Gmbh, Steinheim, Germany) and a  
162 quality control (QC) sample composed of porcine serum (Sigma Aldrich chemie Gmbh,  
163 Steinheim, Germany) spiked with 0.1 or 5 or 10 ng CLD mL<sup>-1</sup>. These different QC were  
164 distributed among the series in a random manner. The CLD limit of detection was 0.02 ng mL<sup>-1</sup>  
165 in the serum with a limit of quantification (LOQ) at 0.06 ng mL<sup>-1</sup>. The mean  $\pm$  standard  
166 deviation CLD recoveries in the spiked porcine serum used as QCs were 87.0%  $\pm$  2.3%, 99.0%  
167  $\pm$  4.2% and 97.4%  $\pm$  4.3% for 0.1 ng mL<sup>-1</sup>, 5 ng mL<sup>-1</sup> and 10 ng mL<sup>-1</sup> QCs respectively.  
168 Recovery rates were always between 60 and 140% according to the requirement of SANCO  
169 (2014). The measurement uncertainty has been assessed from spiked CLD human serum  
170 according to Eppe et al. (2017). The uncertainty was 4.66% for 0.05 ng mL<sup>-1</sup> CLD human  
171 spiked serum, 5.82% for 2.5 ng mL<sup>-1</sup> CLD human spiked serum and 6.34% for 10 ng mL<sup>-1</sup> CLD  
172 human spiked serum. The mean uncertainty was 5.61%  $\pm$  0.7%.

173 The extracts were analyzed by High Resolution Gas Chromatophy coupled to an ion trap mass  
174 spectrometer to confirm the identity and concentrations of CLD (Trace GC Ultra and ITQ 1100  
175 from ThermoQuest, ThermoScientific, Waltham, MA, USA). GC-MS was equipped with a J&W  
176 Scientific DB-XLB capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film) purchased from Agilent  
177 (Santa Clara, CA, USA), using helium as a carrier gas. Chromatographic conditions were  
178 described elsewhere (Fournier et al., 2017). The extracts were injected into the column using  
179 a split/splitless injector. The transfer line temperature was kept at 290°C and the ion trap



180 temperature was set at 250°C. The electron ionisation was performed at 70 eV and the ion  
181 trap was operating in MS/MS mode. Compounds identification was performed with MS/MS  
182 transitions of 272>237, 238>203 and 498>428 for CLD, 10-monohydrochlordecone and PCB-  
183 209 respectively. The collision energy was 2.5 eV, for all the compounds. Concentrations were  
184 measured by quantifying m/z 238 ion for 10\_monohydrochlordecone, m/z 272 ion for  
185 chlordecone and m/z 498 ion for PCB-209.

### 186 2.3.2. Analysis of CLD in tissues

187 CLD concentrations in liver, shaft and peri-renal fat were determined by Liquid  
188 Chromatography coupled with a tandem Mass Spectrometry (LC-MS/MS) as described in  
189 Dromard et al. (2017), using the official method of the French National Reference Laboratory  
190 (ANSES), in LABOCEA laboratory (Quimper, France), labeled by the French Accreditation  
191 Committee (COFRAC). Briefly, CLD C13 was used as internal standard and add to all samples.  
192 CLD was extracted from tissues with a solution of solvents: acetonitrile/dichloromethane 75/25  
193 (v/v) and hexane/acetone 75/25 (v/v) for adipose tissue and hexane/acetone 85/15 (v/v) for  
194 liver and muscle. CLD was transformed into chlordecone hydrate with soda and the aqueous  
195 phase was rinsed with hexane. CLD was then subjected to acidic purification using  
196 concentrated sulfuric acid 60% and extracted with a solution of hexane/acetone 85/15 (v/v).  
197 Concentrations of CLD in tissues were expressed in  $\mu\text{g CLD kg}^{-1}$  fresh weight (FW). The LOQ  
198 was  $1.0 \mu\text{g CLD kg}^{-1}$  in muscle and liver tissues and  $3.0 \mu\text{g CLD kg}^{-1}$  in adipose tissues.

### 199 2.4. Estimations of CLD amounts in tissues

200 CLD concentrations at slaughtering obtained in tissues were used to estimate CLD amounts  
201 in liver, muscle and fat tissues. The liver was weighed after slaughtering. The weight of the  
202 total muscle mass was estimated considering that muscle percentages of BW in Creole and  
203 Large White pigs are 43.3% and 54.8%, respectively (Renaudeau et al., 2005). These  
204 percentages were multiplied by the BW at slaughter to obtain weight of total muscle mass in

205 the organism. Weight of total fat mass was estimated in the same way considering that fat  
206 percentages of BW in Creole and Large White pigs are 34.7% and 22.4%, respectively  
207 (Renaudeau et al., 2005). Total amounts of CLD in matrices were obtained multiplying the total  
208 weight of each matrix by corresponding concentrations at slaughtering.

## 209 2.5. Non-compartmental analysis

210 Non-compartmental analysis describes the evolution of CLD concentrations without any  
211 assumptions. This analysis is mainly used to obtain basic TK parameters. The non-  
212 compartmental analysis was performed using the PKanalix software (Lixoft, version 2019R1).

213 To compare TK parameters between different species, a non-compartmental analysis was  
214 performed on CLD kinetic data in pigs and with data previously obtained on goats and ewes  
215 (Fournier et al., 2017; Saint-Hilaire et al., 2019). Non-lactating and non-pregnant adult ewes  
216 (n=5) and goats (n=4) of  $65.8 \pm 2.2$  and  $33.0 \pm 3.5$  kg (mean  $\pm$  standard error (SE)) BW  
217 respectively were used in these studies.

218 For each species, four TK parameters were calculated with the non-compartmental analysis:  
219 total body clearance (CL), steady-state distribution volume ( $V_{ss}$ ), mean residence time (MRT)  
220 and area under the serum curve (AUC). Area under the serum curve from zero to infinity was  
221 calculated by the linear log up log down method.

222 To compare TK parameters obtained in the three species, an analysis of variance (ANOVA)  
223 test was performed and followed by a Tukey test (R version 3.5.0. software). The  $\alpha$  level for  
224 statistical significance was set at 0.05.

## 225 2.6. Population toxicokinetic model development

226 Population TK analysis aims to provide TK parameters on a set of individuals and to assess  
227 the variability between individuals.

### 228 2.6.1. Methods and software

229 The population TK analysis was performed using a NLME approach with the Monolix software  
230 (Lixoft, version 2019R1). Monolix estimates TK parameters using the stochastic approximation  
231 expectation maximization (SAEM) algorithm.

## 232 2.6.2. Model building

### 233 **Individual modelisation**

234 Experimental data were best described by a linear two-compartmental model with a  
235 proportional error. The two-compartmental model is represented in Figure 1. In this model, the  
236 body is divided into central and peripheral compartments. The central compartment consists  
237 mainly of the plasma and well perfused organs where the distribution of the molecule is almost  
238 instantaneous. The peripheral compartment consists of less perfused organs, where the  
239 molecule is distributed slower.

240 TK parameters were calculated for each pig individually with the two-compartmental model.  
241 Parameters chosen in the structural model were body clearance (CL), distribution volume of  
242 the central compartment (V1), intercompartmental clearance (Q) and distribution volume of the  
243 peripheral compartment (V2). Clearances and volumes of distribution were normalized to BW  
244 with the IBW. TK parameters (CL, V1, Q and V2) were assumed to follow a lognormal  
245 distribution in the population of pigs.

246 Mean residence time (MRT) and elimination half-time life ( $t_{1/2elim}$ ) were calculated with individual  
247 parameters estimates by Eq. (2) and (3):

$$248 \text{ MRT} = V_{ss} / \text{CL} \quad (2)$$

249 With  $V_{ss} = V1 + V2$

$$250 t_{1/2elim} = \ln(2) / \beta \quad (3)$$

$$251 \text{ With } \beta = \frac{1}{2} ((k_{12} + k_{21} + k_{10}) - \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4 \cdot k_{21} \cdot k_{10}})$$

### 252 **Population modelisation: full model and covariate analysis**

253 CLD concentration  $C_{ij}$  in an individual  $i$  at time  $t_{ij}$  can be expressed as a function of the dose,  
 254 the time  $t_{ij}$  and the model individual parameters ( $CL_i$ ,  $V1_i$ ,  $Q_i$  and  $V2_i$ ). The full model can be  
 255 written in the general form:

$$256 \quad C_{ij} = F(\Phi_i, t_{ij}) \times (1 + \varepsilon_{ij}), \quad j = 1, \dots, n_i$$

$$257 \quad \Phi_i = \begin{pmatrix} CL_i \\ V1_i \\ Q_i \\ V2_i \end{pmatrix} = \begin{pmatrix} \mu_{CL} \cdot e^{\eta_{CL,i}} \\ \mu_{V1} \cdot e^{\eta_{V1,i}} \\ \mu_Q \cdot e^{\eta_{Q,i}} \\ \mu_{V2} \cdot e^{\eta_{V2,i}} \end{pmatrix}$$

258 Where  $C_{ij}$  is the observed CLD serum concentration measured on the individual  $i$  at time  $t_{ij}$ ,  $F$   
 259 is the function describing a two compartment model (bi-exponential),  $\Phi_i$  is the vector of  
 260 individual parameters,  $F(\Phi_i, t_{ij})$  is the value of the predicted CLD serum concentration at time  
 261  $t_{ij}$  for an individual with parameters  $\Phi_i$ , and  $\varepsilon_{ij}$  is independent random variable normally  
 262 distributed around zero with a variance of one.  $F(\Phi_i, t_{ij})$  is the structural model and  $\varepsilon_{ij}$  is  
 263 describing the residual error model.  $\varepsilon_{ij}$  measures the difference between the predicted value  
 264 and the observed value of  $C_{ij}$  and was assumed to describe a proportional residual error model.  
 265  $\mu$  is the population median of a model parameter. The variability sources ( $e^\eta$ ) between the  
 266 individual parameters  $\Phi_i$  can be explained by covariates. Each parameter of  $\Phi_i$  is assumed to  
 267 be log-normally distributed.

268 Once the structural model was built, covariates were added. One factor (genotype with two  
 269 categories: Large White vs. Creole) and two continuous variables (IBW and ADG) were tested  
 270 as potential covariates on the TK parameters. First, variables were added one by one on  
 271 individual CL and V2 estimations and were selected if their addition was able to cause a drop  
 272 of the log-likelihood estimate (LL). Bayesian Information Criteria (BIC) was calculated and  
 273 models with lower values of BIC were considered as better fitted to the observed data. P-value  
 274  $< 0.05$  after a Wald test implemented in Monolix was considered statistically significant to  
 275 include the covariate in the final model. Two covariates were eliminated from the model in a  
 276 step-by-step manner, only ADG was statistically significant and added as covariable to obtain  
 277 the final full model.

### 278 2.6.3. Model evaluation

279 Evaluation of goodness-of-fit was based on the following plots: (i) individual (IPRED) and  
280 population predicted (PRED) concentrations versus observed concentrations (DV), (ii)  
281 individual plots (PRED and OBS vs. time), (iii) population weighted (PWRES) and individual  
282 weighted (IWRES) residuals versus time and (iv) normalized prediction distribution errors  
283 (NPDE) versus time, IPRED and PRED (NPDE has to be normally distributed with a mean of  
284 0 and a standard deviation of 1). Normality of residuals were evaluated using density plots of  
285 weighted residuals.

### 286 2.6.4. Comparison of toxicokinetic parameters between the two breeds

287 To compare TK parameters obtained by the NLME model, a Student *t* test was used (R version  
288 3.5.0. software). The  $\alpha$  level for statistical significance was set at 0.05.

## 289 3. Results and discussion

### 290 3.1. Global kinetic analysis

291 Figure 2 shows CLD TKs in pig's serum over the 84-depuration days following the single *i.v.*  
292 administration of 1 mg CLD kg<sup>-1</sup> BW. The first part of the curve (until 24 hours) corresponds  
293 mainly to a distribution phase. During the first day following *i.v.* administration of CLD, serum  
294 concentrations declined rapidly compared to the remaining kinetics, because of the partitioning  
295 of the molecule in the body (Toutain and Bousquet-Melou, 2004). The second part of the curve  
296 corresponds mainly to an elimination phase.

### 297 3.2. Non-compartmental analysis

298 TK parameters obtained with the non-compartmental analysis for CLD in pigs, ewes and goats  
299 are presented in Table 2. The dose of 1 mg CLD kg<sup>-1</sup> BW was already used in goats and ewes  
300 (Fournier et al., 2017; Saint-Hilaire et al., 2019) and was chosen in this study to allow  
301 comparisons of TK parameters between the three species. Body clearance is the total  
302 clearance, it depends on the elimination of the parent compound and on CLD metabolism. In

303 pigs, the body clearance was  $79.6 \pm 4.4 \text{ mL kg}^{-1} \text{ d}^{-1}$  (mean  $\pm$  SE). The body clearance in pigs  
 304 is comparable with the clearance determined in goats but 1.6 higher than in ewes ( $P < 0.05$ ).  
 305 Higher CLD elimination capacities in pigs than ewes could be explained either by higher  
 306 capabilities for CLD elimination in the parent form (via biliary excretion for example), or higher  
 307 metabolic capabilities, or both. Indeed, the enzyme *chlordecone reductase* involved in the  
 308 biotransformation of CLD is known to be present in pigs as in humans, gerbils, ewes, goats  
 309 (Fariss et al., 1980; Houston et al., 1981; Soine et al., 1983; Molowa et al., 1986a; Molowa et  
 310 al., 1986b; Saint-Hilaire, 2018). Further studies could be performed to compare enzymatic  
 311 activity of *chlordecone reductase* between species.

312 Table 2: Comparison of CLD toxicokinetic parameters obtained with non-compartmental  
 313 analysis between pigs, ewes and goats following a single intravenous administration of 1 mg  
 314 CLD  $\text{kg}^{-1}$

315

Parameters	Pigs (n=14)		Ewes (n=5)		Goats (n=4)	
	Mean $\pm$ SE	CV (%)	Mean $\pm$ SE	CV (%)	Mean $\pm$ SE	CV (%)
<b>CL</b> $\text{mL kg}^{-1} \text{ d}^{-1}$	$79.6 \pm 4.4^a$	5.5	$51.0 \pm 6.1^b$	12.0	$66.6 \pm 16.2^{ab}$	24.4
<b>Vss</b> $\text{mL kg}^{-1}$	$5,982 \pm 354^a$	5.9	$1,807 \pm 157^b$	8.7	$1,615 \pm 446^b$	27.6
<b>MRT</b> d	$77 \pm 5^a$	6.6	$36 \pm 2^b$	4.5	$24 \pm 1^b$	3.9
<b>AUC</b> $\text{ng d mL}^{-1}$	$12,505 \pm 636^a$	5.1	$21,202 \pm 3,373^b$	15.9	$18,911 \pm 5,687^{ab}$	30.1

CL: Body clearance; Vss: Volume of distribution at the equilibrium; MRT: Mean Residence Time; AUC: Area Under the Curve;  
 CV: coefficient of variation; SE: Standard Error.

Values not followed by the same superscript letter significantly differ ( $P < 0.05$ ; Tukey test).

Toxicokinetic parameters calculated from data obtained in Saint-Hilaire et al. (2019) and Fournier et al. (2017) for ewes and goats, respectively.

316

317 Despite high body clearance values showing high elimination capacities, the mean residence  
 318 time (MRT) in pigs is 2.1 and 3.2 times higher than those obtained in ewes and goats ( $P <$   
 319  $0.05$ ), respectively. The MRT represents the average time the molecule stays in the body. This  
 320 parameter is a function of the Vss and CL. Because of the high distribution volume, the MRT  
 321 is higher in pigs (MRT =  $78 \pm 3$  days) compared to both other species (MRT = 36 and 24 days  
 322 in ewes and goats, respectively;  $P < 0.05$ ). CLD elimination in pigs is slower than in ewes and  
 323 goats because the Vss is 3.3 and 3.7 times larger in pigs than in ewes and goats, respectively.  
 324 This assumes that pigs may store more CLD into deeper peripheral compartments than ewes

325 and goats. Deeper peripheral compartments represent tissues from which the molecule  
326 depletes slower than other tissues because of CLD bindings to lipid or protein components.

327 This non-compartmental analysis allowed comparing the main TK parameters between pigs,  
328 ewes and goats. Coefficients of variation of estimated mean parameters are ranged from 5.1  
329 to 6.6. TK parameters estimation would probably be improved by considering inter-individual  
330 variability. Thus, a NLME approach in pigs was applied to estimate more precisely TK  
331 parameters.

### 332 3.3. Population toxicokinetic analysis

#### 333 **Model evaluation**

334 The two-compartmental model best described the observed data, as indicated by a relative  
335 standard error < 20% for most of all the model parameters. Good correlations were found  
336 between observed concentrations vs. population predicted (PRED) and individual predicted  
337 (IPRED) concentrations. Plots of population weighted (PWRES) and individual weighted  
338 residuals (IWRES) vs. time and IPRED show that for both types of residuals, points are  
339 randomly distributed around the zero line and most points fall within  $\pm 3$  standard deviations.  
340 Simulation plots of NPDE vs. time and IPRED do not reveal biases because points are  
341 scattered randomly around the zero line. Thus, these data support the model stability.

342 TK parameters obtained with the NLME approach are comparable with results obtained with  
343 the non-compartmental approach. However, TK parameters seem to be better estimated with  
344 the NLME approach because coefficients of variation are lower (Table 2 and Table 3).

#### 345 **CLD elimination**

346 Estimated parameters derived from the population TK model are summarized in Table 3. Body  
347 clearance (CL) is the most important TK parameter; it expresses the overall ability of the blood  
348 compartment to eliminate the molecule. For all pigs, the body clearance is  $76.7 \pm 3.2$  mL kg<sup>-1</sup>.  
349 Average daily gain (ADG) has a very significant effect on CL ( $P < 0.05$ ): the higher the ADG

350 is, the lower the CL is. Despite high CL, mean residence time and elimination half-life in pigs  
 351 are quite long ( $MRT = 78 \pm 3$  days and  $t_{1/2elim} = 54.7 \pm 2.3$  days) when compared to other species  
 352 due to the molecule distribution in organism.

353 Table 3: Population toxicokinetic model estimates for CLD following a single intravenous  
 354 administration of 1 mg CLD  $kg^{-1}$  to 14 pigs  
 355  
 356

Parameters	Model estimate median $\pm$ SE	P <sup>1</sup>	CV (%)
<b>CL</b> mL $kg^{-1}$ d <sup>-1</sup>	76.7 $\pm$ 3.2	0.0009	4.1
<b>V1</b> mL $kg^{-1}$	604 $\pm$ 195	-	32.4
<b>Q</b> mL $kg^{-1}$ d <sup>-1</sup>	16,680 $\pm$ 2,870	-	17.1
<b>V2</b> mL $kg^{-1}$	4,840 $\pm$ 262	0.0142	5.4
<b>Vss</b> mL $kg^{-1}$	6,002 $\pm$ 280	-	4.6
<b>MRT</b> d	78 $\pm$ 3	-	4.3
<b>t<sub>1/2elim</sub></b> d	54.7 $\pm$ 2.3	-	4.2

CL: Body clearance; V1: Central compartment volume of distribution; Q: Intercompartmental clearance; V2: Peripheral compartment volume of distribution; Vss: Steady-state volume of distribution; MRT: Mean residence time;  $t_{1/2elim}$ : Elimination half-life; CV: Coefficient of variation; SE: Standard Error.

<sup>1</sup> P-value obtained after a Wald test, testing significant effect of Average Daily Gain (ADG) on CL and V2 parameters.

357

### 358 **CLD distribution in organism**

359 Intercompartmental clearance (Q) expresses ability of the peripheral compartment to mobilize  
 360 the molecule. The high intercompartmental clearance ( $16.7 \pm 2.9$  L  $kg^{-1}$ ) suggests that  
 361 equilibrium between central compartment and peripheral compartment is quickly reached.  
 362 These results are consistent with the distribution half-life obtained in ewes: in less than one  
 363 hour and a half, the equilibrium between the two compartments is reached (Saint-Hilaire et al.,  
 364 2019).

365 Peripheral volume of distribution (V2) is not a physiologic value but a proportionality factor  
 366 between amount of CLD in the organism at a given time and blood concentration at that time  
 367 (Toutain and Bousquet-Mélou, 2004). The peripheral distribution volume V2 largely exceeds



368 the central distribution volume  $V_1$ . The steady-state volume of distribution ( $V_{ss}$ ) is calculated  
369 by adding up volumes of central compartment ( $V_1$ ) and peripheral compartment ( $V_2$ ). In this  
370 study, the  $V_{ss}$  ( $V_{ss} = 6,002 \pm 280 \text{ mL kg}^{-1}$ ) largely exceeds the total volume of body water  
371 (around  $0.6 \text{ L kg}^{-1}$ ). The ratio between  $V_{ss}$  and the total volume of body water expresses the  
372 overall partition coefficient ( $K_p$ ).  $K_p$  expresses the overall affinity of the body for the molecule  
373 (Toutain and Bousquet-Mélou, 2004).  $K_p$  can also be defined as the ratio between the unbound  
374 fraction in plasma and the unbound fraction outside plasma. In this study, the high  $K_p$  ( $10.0 \pm$   
375  $1.7$ ) supposes that most of CLD is stored in the peripheral compartment and extensively bound  
376 to proteins in tissues or to adipocytes (Toutain and Bousquet-Mélou, 2004).

377 ADG has a very significant effect on peripheral distribution volume  $V_2$  ( $P < 0.05$ ): the higher  
378 the ADG, the lower the  $V_{ss}$ . This may be explained by carcass composition, when the animal  
379 gain weight, lean and fat composition of carcass may change over time.

#### 380 **Breed, average daily gain, composition of carcass: which factor may really affect TKs** 381 **variability in growing pigs?**

382 Covariate analysis showed that ADG is an important cofactor and that inter-individual  
383 differences in parameters are rather related to the ADG than to breed. However, these results  
384 must be put into perspective because breed and growth performance factors are inextricably  
385 interlinked. Moreover, comparing a covariate (ADG) and a factor (breed) induce statistical  
386 biases. These results must be discussed to explore which factor may impact inter-individual  
387 variability.

388 TK parameters obtained with the NLME approach were used to compare the two breeds.  
389 Estimated parameters obtained for Creole and Large White pigs with the NLME approach are  
390 summarized in Table 4. Significant differences between Creole and Large White pigs were  
391 observed for CL,  $V_2$  and  $V_{ss}$ . Differences in CLD distribution ( $V_2$  and  $V_{ss}$ ) and elimination  
392 (CL) may be explained by percentage of lean meat in carcass or by carcass fatness. Indeed,  
393 Large White pigs have a better growth potential, higher ADG, feed efficiency and lean carcass

394 content than Creole pigs and Creole pigs have higher carcass fatness and bigger adipocytes  
395 than Large White pigs (Renaudeau et al., 2005). Recently, Pouillet et al. (2019) have confirmed  
396 the difference in pig metabolism between Creole and Large white pigs, by observing a lower  
397 N utilization efficiency in Creole breed with a higher level of blood urea nitrogen and a lower  
398 level of globulin compared to Large White pigs. Moreover, age differential between the two  
399 breeds may have amplified growth performance differences and fatness of carcass  
400 (Renaudeau et al., 2006).

401 CLD has a different behavior from other POPs such as PCB because CLD is known to be more  
402 concentrated in liver and muscle than in fat tissues (Lastel et al., 2016; Lastel et al., 2018).  
403 High concentrations of CLD in liver and muscles may be explained by its affinity for plasmatic  
404 proteins (Soine et al., 1982). CLD linked to plasma proteins would arrive faster and in greater  
405 quantities in organs and tissues heavily irrigated (liver and muscles) than in those weakly  
406 perfused (fat tissues). Slaughtering data were analyzed to characterize molecule distribution  
407 in the three matrices analyzed (liver, muscle and fat) and to highlight possible differences in  
408 CLD distribution between the two breeds. Estimated amounts of CLD into tissues after 84  
409 depuration-days in Creole and Large White pigs are presented in Table 5. No significant  
410 difference was observed for CLD amounts in liver and muscle between the two breeds.  
411 However, repartition of CLD in fat is significantly different between the two breeds and Creole  
412 pigs have higher carcass adiposity. Although CLD concentrations in fat tissue are lower than  
413 in muscle or liver, fat tissues store an important portion of CLD body burden, as shown in Table  
414 5. Thus, carcass fatness may impact volume of distribution. To explain inter-individual  
415 variability between pigs, animal-specific features (age, breed, sex), growth performance and  
416 carcass fatness seem to be interesting. Breed effects we found suggest that variability between  
417 breeds (especially between specialist and generalist breeds) should be taken into account in  
418 decision support for a healthy farming practice. Further studies are needed to better  
419 understand CLD storage, depuration capacities between tissues and characterize  
420 physiological factors that drive TK parameters.

421 Table 4: Comparison of toxicokinetic parameters obtained with the nonlinear mixed-effects  
 422 approach between Creole and Large White pigs  
 423

Parameters	Creole (n=7) median ± SE	Large White (n=7) median ± SE	P
<b>CL</b> mL kg <sup>-1</sup> d <sup>-1</sup>	82.1 ± 4.6 <sup>a</sup>	68.8 ± 1.6 <sup>b</sup>	<b>0.0081</b>
<b>V1</b> mL kg <sup>-1</sup>	686 ± 420 <sup>a</sup>	1,061 ± 329 <sup>a</sup>	0.5236
<b>Q</b> mL kg <sup>-1</sup> d <sup>-1</sup>	15,896 ± 2,435 <sup>a</sup>	22,841 ± 2,940 <sup>a</sup>	0.9604
<b>V2</b> mL kg <sup>-1</sup>	5,792 ± 405 <sup>a</sup>	4,433 ± 232 <sup>b</sup>	<b>0.0128</b>
<b>Vss</b> mL kg <sup>-1</sup>	6,714 ± 270 <sup>a</sup>	5,316 ± 392 <sup>b</sup>	<b>0.0158</b>
<b>MRT</b> d	79 ± 3 <sup>a</sup>	71 ± 6 <sup>a</sup>	0.4240
<b>t<sub>1/2elim</sub></b> d	55 ± 2 <sup>a</sup>	49 ± 4 <sup>a</sup>	0.4112

CL: Body clearance; V1: Central compartment volume of distribution; Q: Intercompartmental clearance; V2: Peripheral compartment volume of distribution; Vss: Steady-state volume of distribution; MRT: Mean residence time; t<sub>1/2elim</sub>: Elimination half-life; SE: Standard Error. Values not followed by the same superscript letter significantly differ (Student test).

424

425 Table 5: Estimated amounts of CLD into tissues after 84 depuration-days

	Creole (n=7) mean ± SE	Large White (n=7) mean ± SE	P
Liver (mg)	2.2 ± 0.1 <sup>a</sup>	2.2 ± 0.2 <sup>a</sup>	0.5619
Muscle <sup>1</sup> (mg)	8.9 ± 1.2 <sup>a</sup>	12.5 ± 1.8 <sup>a</sup>	0.9352
Fat <sup>2</sup> (mg)	13.2 ± 0.5 <sup>a</sup>	10.0 ± 0.8 <sup>b</sup>	<b>0.0039</b>

<sup>1</sup> Considering CLD concentrations in shaft muscle and that muscle percentages of body weight in Creole and Large White pigs are 43.3% and 54.8%, respectively (Renaudeau et al., 2005)

<sup>2</sup> Considering CLD concentrations in peri-renal fat tissue and that fat percentages of body weight in Creole and Large White pigs are 34.7% and 22.4%, respectively (Renaudeau et al., 2005)

Values not followed by the same superscript letter significantly differ (Student test).

## 426 Conclusion

427 This study permitted to establish for the first time a NLME model of CLD in growing pigs. Major  
 428 TK parameters like body clearance (76.7 mL kg<sup>-1</sup> d<sup>-1</sup>) and distribution volume (6 L kg<sup>-1</sup>) were  
 429 determined. ADG is an important covariate explaining inter-individual TK parameters  
 430 variability. This study provided relevant and original data to describe distribution and  
 431 elimination of CLD in pigs. In the near future, these results may be used by the risk manager  
 432 to assess adequate rearing management practices in CLD contaminated areas. Moreover,

433 since the pig is a valuable model for humans, these results may also be used to build a TK  
434 model of CLD in humans.

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#### 445 References

446 Adir, J., Caplan, Y.H., Thompson, B.C.,1978. Kepone® serum half-life in humans. *Life*  
447 *Sciences*. 22, 699–702. [https://doi.org/10.1016/0024-3205\(78\)90494-0](https://doi.org/10.1016/0024-3205(78)90494-0).

448 AFSSA, 2007. Avis de l'agence française de sécurité sanitaire des aliments relatif à  
449 l'actualisation des données scientifiques sur la toxicité du chlordécone en vue d'une éventuelle  
450 révision des limites tolérables d'exposition proposées par l'AFSSA en 2003.  
451 <https://www.anses.fr/fr/system/files/RCCP2007sa0305.pdf>.

452 Anezaki, K. and Nakano, T., 2014. Concentration levels and congener profiles of  
453 polychlorinated biphenyls, pentachlorobenzene, and hexachlorobenzene in commercial  
454 pigments. *Environmental Science and Pollution Research*. 21, 998-1009.  
455 <https://doi.org/10.1007/s11356-013-1977-2>.

456 ANSES, 2017. Exposition des consommateurs des Antilles au chlordécone, résultats de  
457 l'étude Kannari. <https://www.anses.fr/fr/system/files/ERCA2014SA0029Ra.pdf>.

458 Bouveret, C., Rychen, G., Lerch, S., Jondreville, C., Feidt, C., 2013. Relative bioavailability of  
459 tropical volcanic soil-bound chlordecone in piglets. *Journal of Agricultural and Food Chemistry*.  
460 61, 9269–9274. <https://doi.org/10.1021/jf400697r>.

461 Cabidoche, Y.-M., Achard, R., Cattan, P., Clermont-Dauphin, C., Massat, F., Sansoulet, J.,  
462 2009. Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies:  
463 A simple leaching model accounts for current residue. *Environmental Pollution*. 157, 1697–  
464 1705. <https://doi.org/10.1016/j.envpol.2008.12.015>.

465 Chevallier, M.L., Dela-Negra, O., Chaussonnerie, S., Barbance, A., Muselet, D., Lagarde, F.,  
466 Darii, E., Ugarte, E., Lescaop, E., Fonknechten, N., Weissenbach, J., Woignier, T., Gallard, J.-  
467 F., Vuilleumier, S., Imfeld, G., Le Paslier, D., Saaidi, P.-L., 2019. Natural chlordecone  
468 degradation revealed by numerous transformation products characterized in key French West  
469 Indies environmental compartments. *Environmental Science & Technology*. 53, 6133-6143.  
470 <https://doi.org/10.1021/acs.est.8b06305>.

471 Cohn, W.J., Boylan, J.J., Blanke, R.V., Fariss, M.W., Howell, J.R., Guzelian, P.S., 1978.  
472 Treatment of chlordecone (Kepone) toxicity with cholestyramine – Results of a controlled  
473 clinical trial. *The New England Journal of Medicine*. 298, 243–48.  
474 <https://doi.org/10.1056/NEJM197802022980504>.

475 Collas, C., Mahieu, M., Tricheur, A., Crini, N., Badot, P.-M., Archimède, H., Rychen, G., Feidt,  
476 C., Jurjanz, S., 2019. Cattle exposure to chlordecone through soil intake. The case-study of  
477 tropical grazing practices in the French West Indies. *Science of the Total Environment*. 668,  
478 161-170. <https://doi.org/10.1016/j.scitotenv.2019.02.384>.

479 Costet, N., Pelé, F., Comets, E., Rouget, F., Monfort, C., Bodeau-Livinec, F., Liganiza E.M.,  
480 Bataille, H., Kadhel, P., Multigner, L., Cordier, S., 2015. Perinatal exposure to chlordecone and  
481 infant growth. *Environmental Research*. 142, 123–134.  
482 <https://doi.org/10.1016/j.envres.2015.06.023>.

483 Dereumeaux, C., Saoudi, A., Guldner, L., Pecheux, M., Chesneau, J., Thomé, J.-P., Ledrans,  
484 M., Le Tertre, A., Denys, S., Fillol, C., 2019. Chlordecone and organochlorine compound levels  
485 in the French West Indies population in 2013-2014. *Environmental and Pollution Research*.  
486 <https://doi.org/10.1007/s11356-019-07181-9>.

487 Dromard, C.R., Guéné, M., Bouchon-Navaro, Y., Lemoine, S., Cordonnier, S., Bouchon, C.,  
488 2017. Contamination of marine fauna by chlordecone in Guadeloupe : evidence of a seaward  
489 decreasing gradient. *Environmental Science and Pollution Research*. 25, 14294-14301.  
490 <https://doi.org/10.1007/s11356-017-8924-6>.

491 Dubuisson, C., Héraud, F., Leblanc, J.-C., Gallotti, S., Flamand, C., Bateau, A., Quenel, P.,  
492 Volatier, J.-L., 2007. Impact of subsistence production on the management options to reduce  
493 the food exposure of the Martinican population to chlordecone. *Regulatory Toxicology and*  
494 *Pharmacology*. 49, 5–16. <https://doi.org/10.1016/j.yrtph.2007.04.008>.

495 Eppe, G., Schaechtele, A., Haedrich, J., Fernandes, A., 2017. Guidance document on  
496 measurement uncertainty for laboratories performing PCDD/F and PCB analysis using isotope  
497 dilution mass spectrometry. Working Group for Measurement Uncertainty in PCDD/F and PCB  
498 Analysis. [https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-](https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_document_pcdd-f_pcb_en.pdf)  
499 [guidance\\_document\\_pcdd-f\\_pcb\\_en.pdf](https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_document_pcdd-f_pcb_en.pdf). <https://doi.org/10.13140/RG.2.2.35446.09285>.

500 Egle, J.L., Fernandez, S.B, Guzelian, P.S., Borzelleca, J.F., 1978. Distribution and excretion  
501 of chlordecone (Kepone) in the rat. *Drug Metabolism and Disposition*. 6, 91–95.

502 Fariss, M.W., Blanke, R.V., Saady, J.J., Guzelian, P.S., 1980. Demonstration of major  
503 metabolic pathways for Chlordecone (Kepone) in Humans. *Drug Metabolism and Disposition*.  
504 8, 434–438.

505 Fournier, A., Feidt, C., Lastel, M.-L., Archimede, H., Thomé, J.-P., Mahieu, M., Rychen, G.,  
506 2017. Toxicokinetics of Chlordecone in goats: Implications for risk management in French  
507 West Indies. *Chemosphere*. 171, 564–570.  
508 <https://doi.org/10.1016/j.chemosphere.2016.12.054>.

509 Gourdine, J-L, Bambou, J-C., Giorgi, M., Loranger-Merciris, G., Archimède, H., 2018.  
510 Performance of growing pigs reared indoors or outdoors in sweet-potato fields. Revue  
511 d'élevage et de médecine vétérinaire des pays tropicaux. 71, 41-46.  
512 <https://doi.org/10.19182/remvt.31347>.

513 Houston, T.E., Mutter, L.C., Blanke, R.V., Guzelian, P.S., 1981. Chlordecone alcohol formation  
514 in the Mongolian gerbil (*Meriones Uniguiculatus*): a model for human metabolism of  
515 Chlordecone (Kepone). Fundamental and Applied Toxicology. 1, 293–298.

516 Hu, D. and Hornbuckle, K.C., 2010. Inadvertent polychlorinated biphenyls in commercial paint  
517 pigments. Environmental Science and Technology. 44 (8), 2822-2827.  
518 <https://doi.org/10.1021/es902413k>.

519 Jondreville, C., Fournier, A., Mahieu, M., Feidt, C., Archimède, H., Rychen, G., 2014. Kinetic  
520 study of chlordecone orally given to laying hens (*Gallus Domesticus*). Chemosphere. 114,  
521 275–281. <https://doi.org/10.1016/j.chemosphere.2014.05.008>.

522 Jurjanz, S. and Roinsard, A., 2014. Valorisation de l'herbe par des truies plein-air.  
523 ALTERAGRI. May 2014, 125, p. 25-28.

524 Jurjanz, S., Jondreville, C., Mahieu, M., Fournier, A., Archimède, H., Rychen, G., Feidt, C.,  
525 2014. Relative bioavailability of soil-bound chlordecone in growing lambs. Environmental  
526 Geochemistry and Health. 36, 911–917. <https://doi.org/10.1007/s10653-014-9608-5>.

527 Jurjanz, S., Collas, C., Lastel, M.-L., Godard, X., Archimède, H., Rychen, G., Mahieu, M., Feidt,  
528 C., 2017. Evaluation of soil intake by growing Creole young bulls in common grazing systems  
529 in humid tropical conditions. Animal. 11. <https://doi.org/10.1017/S1751731116002755>.

530 Lastel, M.-L., Lerch, S., Fournier, A., Jurjanz, S., Mahieu, M., Archimède, H., Feidt, C., Rychen,  
531 G., 2016. Chlordecone disappearance in tissues of growing goats after a one month  
532 decontamination period - Effect of body fatness on Chlordecone retention. Environmental  
533 Science and Pollution Research. 23, 3176–3183. <https://doi.org/10.1007/s11356-015-5833-4>.

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

540 Lerch, S., Guidou, C., Thomé, J.-P., Jurjanz, S., 2016. Non-dioxin-like Polychlorinated  
541 Biphenyls (PCBs) and chlordecone release from adipose tissue to blood in response to body  
542 fat mobilization in Ewe (*Ovis aries*). *Journal of Agricultural and Food Chemistry*. 64, 1212–  
543 1220. <https://doi.org/10.1021/acs.jafc.5b05817>.

544 Molowa, D.T., Shayne, A.G., Guzelian, P.S., 1986a. Purification and characterization of  
545 chlordecone reductase from Human liver. *The Journal Of Biological Chemistry*. 261, 12624–  
546 12627.

547 Molowa, D.T., Wrighton, S.A., Blanke, R.V., Guzelian, P.S., 1986b. Characterization of a  
548 unique aldo-keto reductase responsible for the reduction of chlordecone in the liver of the gerbil  
549 and man. *Journal of Veterinary Pharmacology and Therapeutics*. 17, 375–384.  
550 <https://doi.org/10.1080/15287398609530832>.

551 Multigner, L., Ndong, J.-R., Giusti, A., Romana, M., Delacroix-Maillard, H., Cordier, S., Jégou,  
552 B., Thomé, J.-P., Blanchet, P., 2010. Chlordecone exposure and risk of prostate cancer.  
553 *Journal of Clinical Oncology*. 28, 3457–3462. <https://doi.org/10.1200/JCO.2009.27.2153>.

554 Pouillet, N., Bambou, J.-C., Loyau, T., Trefeu, C., Feuillet, D., Beramice, D., Bocage, B.,  
555 Renaudeau, D., Gourdine, J.-L., 2019. Effect of feed restriction and refeeding on performance  
556 and metabolism of European and Caribbean growing pigs in a tropical climate. *Scientific*  
557 *Reports*. 9, 4878. <https://doi.org/10.1038/s41598-019-41145-w>.



558 Renaudeau, D., Hilaire, M., Mourot, J., 2005. A comparison of carcass and meat quality  
559 characteristics of Creole and Large White pigs slaughtered at 150 days of age. *Animal*  
560 *Research*. 54, 43-54. <https://doi.org/10.1051/animres:2004042>.

561 Renaudeau, D., Giorgi, M., Silou, F., Weisbecker, J.-L., 2006. Effect of breed (lean or fat pigs)  
562 and sex on performance and feeding behavior of group housed growing pigs in a tropical  
563 climate. *Asian-Australasian Journal of Animal Sciences*. 19, 593-600.  
564 <https://doi.org/10.5713/ajas.2006.593>.

565 Saint-Hilaire, M., 2018. Toxicocinétique de la chlordécone chez la brebis. Université de  
566 Lorraine. 250 p.

567 Saint-Hilaire, M., Rychen, G., Thomé, J.-P., Joaquim-Justo, C., Le Roux, Y., Feidt, C.,  
568 Fournier, A., 2019. Linear toxicokinetic of chlordecone in ewe's serum. *Environmental Science*  
569 *and Pollution Research*. <https://doi.org/10.1007/s11356-019-05800-z>.

570 SANCO, 2014. Guidance Document on Analytical Quality Control and Validation Procedures  
571 for Pesticide Residues Analysis in Food and Feed. SANCO/12571/2013 Supersedes  
572 SANCO/12495/2011, 48 p.. [http://www.eurl-](http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf)  
573 [pesticides.eu/library/docs/allcrl/AqcGuidance\\_Sanco\\_2013\\_12571.pdf](http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf).

574 Soine, P.J., Blanke, R.V., Guzelian, P.S., Schwartz, C.C., 1982. Preferential binding of  
575 chlordecone to the protein and high density lipoprotein fractions of plasma from humans and  
576 other species. *Journal of Toxicology and Environmental Health*. 9, 107–118.  
577 <https://doi.org/10.1080/15287398209530146>.

578 Soine, P.J., Blanke, R.V. and Schwartz, C.C, 1983. Chlordecone metabolism in the pig.  
579 *Toxicology Letters*. 17, 35–41. [https://doi.org/10.1016/0378-4274\(83\)90032-2](https://doi.org/10.1016/0378-4274(83)90032-2).

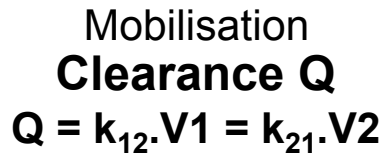
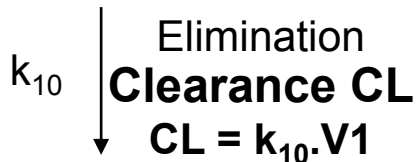
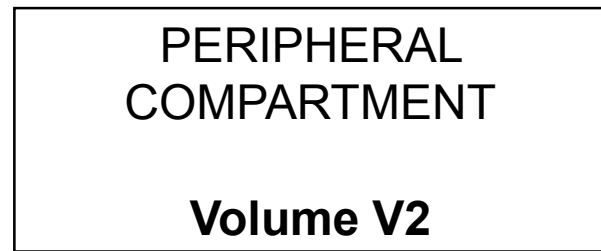
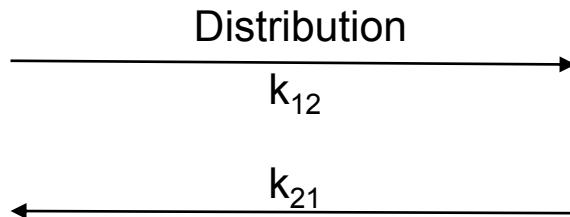
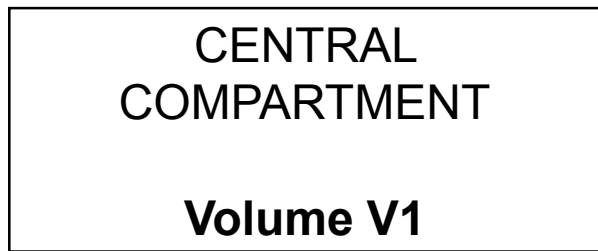
580 Toutain, P. L. and Bousquet-Melou, A. 2004. Volumes of Distribution. *Journal of Veterinary*  
581 *Pharmacology and Therapeutics*. 27, 441–53. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2885.2004.00602.x)  
582 [2885.2004.00602.x](https://doi.org/10.1111/j.1365-2885.2004.00602.x).

583 Koh, W.X., Hornbuckle, K.C., Thorne, P.S., 2015. Human serum from urban and rural  
584 adolescents and their mothers shows exposure to polychlorinated biphenyls not found in  
585 commercial mixtures. *Environmental Science and Technology*. 49, 8105-8112.  
586 <https://doi.org/10.1021/acs.est.5b01854>.

Figure 1: Two-compartmental modelisation of the equilibrium between the central compartment and the peripheral compartment after a single intravenous administration of chlordecone (adapted from Toutain and Bousquet-Melou, 2004)

Figure 2: Serum CLD concentration ( $\text{ng.mL}^{-1}$ ) vs. time in growing pigs ( $n=14$ ) after a single intravenous administration of CLD at  $1 \text{ mg.kg}^{-1} \text{ BW}$ : observed kinetics from  $t=0$  to  $t=84$  days  
Top graph on the right corresponds to an enlargement of the main graph from  $t=0$  to  $t=1.4$  days.

Chlordecone  
*single intravenous administration*



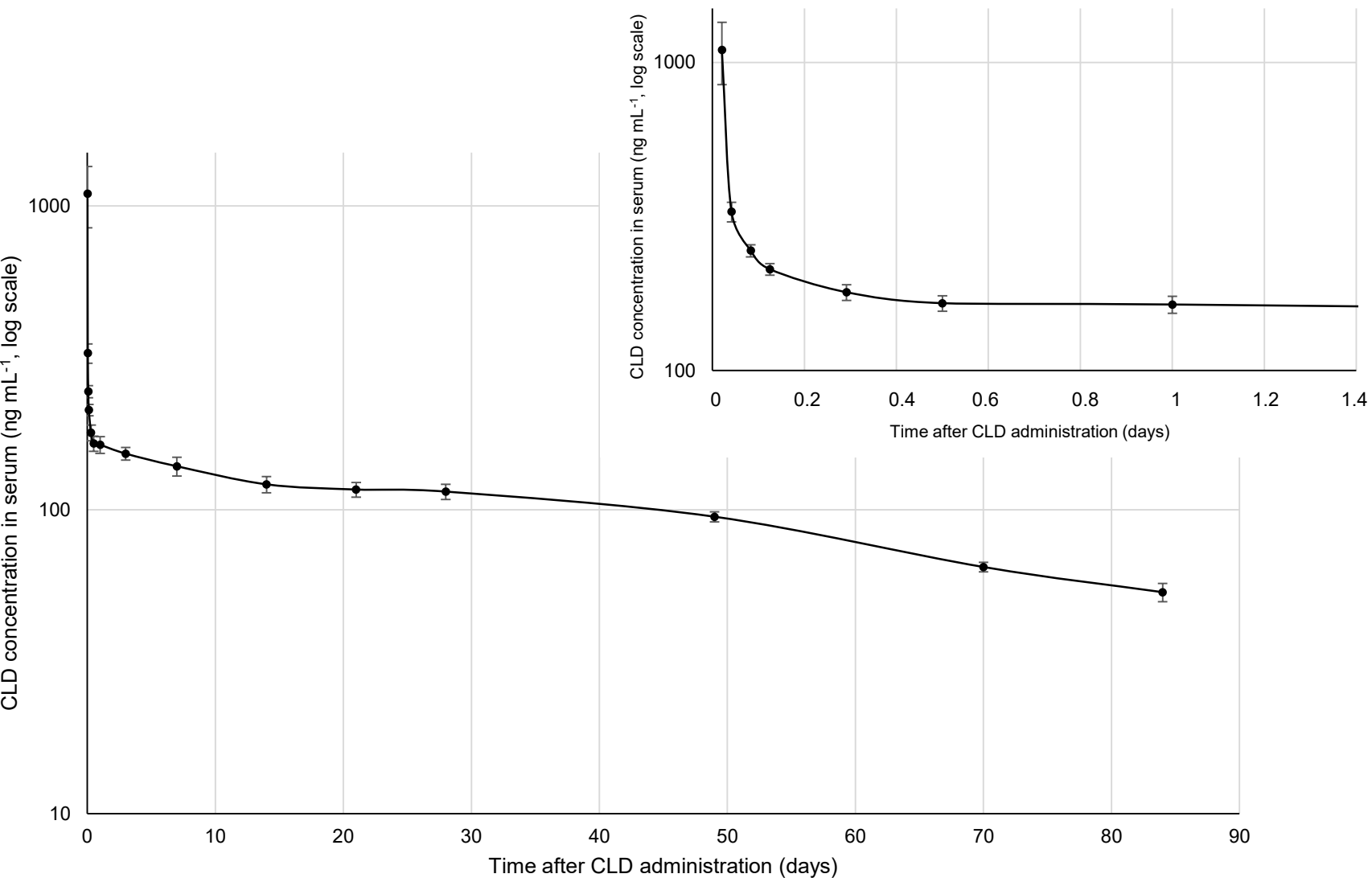


Table 1: Average values of IBW, FBW and ADG for each breed

Parameters	Creole pigs (n=7)	Large White pigs (n=7)	All pigs (n=14)
	mean $\pm$ SE	mean $\pm$ SE	mean $\pm$ SE
<b>IBW</b> kg	68.8 $\pm$ 1.8 <sup>a</sup>	67.9 $\pm$ 3.1 <sup>a</sup>	68.3 $\pm$ 1.7
<b>FBW</b> kg	91.2 $\pm$ 2.8 <sup>a</sup>	95.6 $\pm$ 2.4 <sup>a</sup>	92.8 $\pm$ 1.9
<b>ADG</b> g d <sup>-1</sup>	248 $\pm$ 25 <sup>a</sup>	327 $\pm$ 13 <sup>a</sup>	288 $\pm$ 17

IBW: Initial Body Weight; FBW: Final Body Weight; ADG: Average Daily Gain; SE: Standard Error.  
 Values not followed by the same superscript letter significantly differ (P < 0.05; Student test)

Table 2: Comparison of CLD toxicokinetic parameters obtained with non-compartmental analysis between pigs, ewes and goats following a single intravenous administration of 1 mg CLD kg<sup>-1</sup>

Parameters	Pigs (n=14)		Ewes (n=5)		Goats (n=4)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
<b>CL</b> mL kg <sup>-1</sup> d <sup>-1</sup>	79.6 ± 4.4 <sup>a</sup>	5.5	51.0 ± 6.1 <sup>b</sup>	12.0	66.6 ± 16.2 <sup>ab</sup>	24.4
<b>Vss</b> mL kg <sup>-1</sup>	5,982 ± 354 <sup>a</sup>	5.9	1,807 ± 157 <sup>b</sup>	8.7	1,615 ± 446 <sup>b</sup>	27.6
<b>MRT</b> d	77 ± 5 <sup>a</sup>	6.6	36 ± 2 <sup>b</sup>	4.5	24 ± 1 <sup>b</sup>	3.9
<b>AUC</b> ng d mL <sup>-1</sup>	12,505 ± 636 <sup>a</sup>	5.1	21,202 ± 3,373 <sup>b</sup>	15.9	18,911 ± 5,687 <sup>ab</sup>	30.1

CL: Body clearance; Vss: Volume of distribution at the equilibrium; MRT: Mean Residence Time; AUC: Area Under the Curve; CV: coefficient of variation; SE: Standard Error.

Values not followed by the same superscript letter significantly differ ( $P < 0.05$ ; Tukey test).

Toxicokinetic parameters calculated from data obtained in Saint-Hilaire et al. (2019) and Fournier et al. (2017) for ewes and goats, respectively.

Table 3: Population toxicokinetic model estimates for CLD following a single intravenous administration of 1 mg CLD kg<sup>-1</sup> to 14 pigs

Parameters	Model estimate median ± SE	P <sup>1</sup>	CV (%)
<b>CL</b> mL kg <sup>-1</sup> d <sup>-1</sup>	76.7 ± 3.2	0.0009	4.1
<b>V1</b> mL kg <sup>-1</sup>	604 ± 195	-	32.4
<b>Q</b> mL kg <sup>-1</sup> d <sup>-1</sup>	16,680 ± 2,870	-	17.1
<b>V2</b> mL kg <sup>-1</sup>	4,840 ± 262	0.0142	5.4
<b>Vss</b> mL kg <sup>-1</sup>	6,002 ± 280	-	4.6
<b>MRT</b> d	78 ± 3	-	4.3
<b>t<sub>1/2elim</sub></b> d	54.7 ± 2.3	-	4.2

CL: Body clearance; V1: Central compartment volume of distribution; Q: Intercompartmental clearance; V2: Peripheral compartment volume of distribution; Vss: Steady-state volume of distribution; MRT: Mean residence time; t<sub>1/2elim</sub>: Elimination half-life; CV: Coefficient of variation; SE: Standard Error.

<sup>1</sup>P-value obtained after a Wald test, testing significant effect of Average Daily Gain (ADG) on CL and V2 parameters.



Table 4: Comparison of toxicokinetic parameters obtained with the nonlinear mixed-effects approach between Creole and Large White pigs

Parameters	Creole (n=7) median ± SE	Large White (n=7) median ± SE	P
<b>CL</b> mL kg <sup>-1</sup> d <sup>-1</sup>	82.1 ± 4.6 <sup>a</sup>	68.8 ± 1.6 <sup>b</sup>	<b>0.0081</b>
<b>V1</b> mL kg <sup>-1</sup>	686 ± 420 <sup>a</sup>	1,061 ± 329 <sup>a</sup>	0.5236
<b>Q</b> mL kg <sup>-1</sup> d <sup>-1</sup>	15,896 ± 2,435 <sup>a</sup>	22,841 ± 2,940 <sup>a</sup>	0.9604
<b>V2</b> mL kg <sup>-1</sup>	5,792 ± 405 <sup>a</sup>	4,433 ± 232 <sup>b</sup>	<b>0.0128</b>
<b>Vss</b> mL kg <sup>-1</sup>	6,714 ± 270 <sup>a</sup>	5,316 ± 392 <sup>b</sup>	<b>0.0158</b>
<b>MRT</b> d	79 ± 3 <sup>a</sup>	71 ± 6 <sup>a</sup>	0.4240
<b>t<sub>1/2elim</sub></b> d	55 ± 2 <sup>a</sup>	49 ± 4 <sup>a</sup>	0.4112

CL: Body clearance; V1: Central compartment volume of distribution; Q: Intercompartmental clearance; V2: Peripheral compartment volume of distribution; Vss: Steady-state volume of distribution; MRT: Mean residence time; t<sub>1/2elim</sub>: Elimination half-life; SE: Standard Error. Values not followed by the same superscript letter significantly differ (Student test).

Table 5: Estimated amounts of CLD into tissues after 84 depuration-days

	Creole (n=7) mean ± SE	Large White (n=7) mean ± SE	P
Liver (mg)	2.2 ± 0.1 <sup>a</sup>	2.2 ± 0.2 <sup>a</sup>	0.5619
Muscle <sup>1</sup> (mg)	8.9 ± 1.2 <sup>a</sup>	12.5 ± 1.8 <sup>a</sup>	0.9352
Fat <sup>2</sup> (mg)	13.2 ± 0.5 <sup>a</sup>	10.0 ± 0.8 <sup>b</sup>	<b>0.0039</b>

<sup>1</sup> Considering CLD concentrations in shaft muscle and that muscle percentages of body weight in Creole and Large White pigs are 43.3% and 54.8%, respectively (Renaudeau et al., 2005)

<sup>2</sup> Considering CLD concentrations in peri-renal fat tissue and that fat percentages of body weight in Creole and Large White pigs are 34.7% and 22.4%, respectively (Renaudeau et al., 2005)

Values not followed by the same superscript letter significantly differ (Student test).