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Abstract

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

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

Keywords: Chlordecone, Toxicokinetics, Pigs, Modeling, Monolix

1. Introduction

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

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

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

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

2. Material and methods

2.1. Animals and management

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

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

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
IBW kg	68.8 ± 1.8 [a]	67.9 ± 3.1 [a]	68.3 ± 1.7
FBW kg	91.2 ± 2.8 [a]	95.6 ± 2.4 [a]	92.8 ± 1.9
ADG g d^{-1}	248 ± 25 [a]	327 ± 13 [a]	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)

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

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

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

2.2. Experimental design

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

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

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

2.3. Analytical methods

2.3.1. Analysis of CLD in serum

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

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

The extracts were analyzed by High Resolution Gas Chromatophy coupled to an ion trap mass spectrometer to confirm the identity and concentrations of CLD (Trace GC Ultra and ITQ 1100 from ThermoQuest, ThermoScientific, Waltham, MA, USA). GC-MS was equipped with a J&W Scientific DB-XLB capillary column (30 m × 0.25 mm i.d., 0.25 µm film) purchased from Agilent (Santa Clara, CA, USA), using helium as a carrier gas. Chromatographic conditions were described elsewhere (Fournier et al., 2017). The extracts were injected into the column using 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: acetronitrile/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 µg CLD kg^{-1} fresh weight (FW). The LOQ
198 was 1.0 µg CLD kg^{-1} in muscle and liver tissues and 3.0 µg 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

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

2.5. Non-compartmental analysis

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

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

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

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

2.6. Population toxicokinetic model development

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

2.6.1. Methods and software

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

2.6.2. Model building

Individual modelisation

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

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

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

$$MRT = Vss / CL \quad (2)$$

With $Vss = V1 + V2$

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

With $\beta = \frac{1}{2}\left((k12 + k21 + k10) - \sqrt{(k12 + k21 + k10)^2 - 4.k21.k10}\right.$

Population modelisation: full model and covariate analysis

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

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

$$\Phi_i = \begin{pmatrix} Cl_i \\ V1_i \\ Q_i \\ V2_i \end{pmatrix} = \begin{pmatrix} \mu_{CL}.\, e^{\eta_{CL,i}} \\ \mu_{V1}.\, e^{\eta_{V1,i}} \\ \mu_{Q}.\, e^{\eta_{Q,i}} \\ \mu_{V2}.\, e^{\eta_{V2,i}} \end{pmatrix}$$

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

Once the structural model was built, covariates were added. One factor (genotype with two categories: Large White vs. Creole) and two continuous variables (IBW and ADG) were tested as potential covariates on the TK parameters. First, variables were added one by one on individual CL and V2 estimations and were selected if their addition was able to cause a drop of the log-likelihood estimate (LL). Bayesian Information Criteria (BIC) was calculated and models with lower values of BIC were considered as better fitted to the observed data. P-value < 0.05 after a Wald test implemented in Monolix was considered statistically significant to include the covariate in the final model. Two covariates were eliminated from the model in a step-by-step manner, only ADG was statistically significant and added as covariable to obtain 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 α 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^{-1} 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^{-1} 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

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

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^{-1}

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

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

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

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

3.3. Population toxicokinetic analysis

Model evaluation

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

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

CLD elimination

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

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

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

Parameters	Model estimate median ± SE	P[1]	CV (%)
CL mL kg^{-1} d^{-1}	76.7 ± 3.2	0.0009	4.1
V1 mL kg^{-1}	604 ± 195	-	32.4
Q mL kg^{-1} d^{-1}	16,680 ± 2,870	-	17.1
V2 mL kg^{-1}	4,840 ± 262	0.0142	5.4
Vss mL kg^{-1}	6,002 ± 280	-	4.6
MRT d	78 ± 3	-	4.3
$t_{1/2elim}$ 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; $t_{1/2elim}$: Elimination half-life; CV: Coefficient of variation; SE: Standard Error.
[1] P-value obtained after a Wald test, testing significant effect of Average Daily Gain (ADG) on CL and V2 parameters.

CLD distribution in organism

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

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

368 the central distribution volume V1. The steady-state volume of distribution (Vss) is calculated
369 by adding up volumes of central compartment (V1) and peripheral compartment (V2). In this
370 study, the Vss (Vss = 6,002 ± 280 mL kg^{-1}) largely exceeds the total volume of body water
371 (around 0.6 L kg^{-1}). The ratio between Vss and the total volume of body water expresses the
372 overall partition coefficient (Kp). Kp expresses the overall affinity of the body for the molecule
373 (Toutain and Bousquet-Mélou, 2004). Kp 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 Kp (10.0 ±
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 V2 ($P < 0.05$): the higher
378 the ADG, the lower the Vss. 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, V2 and Vss. Differences in CLD distribution (V2 and Vss) 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

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

CLD has a different behavior from other POPs such as PCB because CLD is known to be more concentrated in liver and muscle than in fat tissues (Lastel et al., 2016; Lastel et al., 2018). High concentrations of CLD in liver and muscles may be explained by its affinity for plasmatic proteins (Soine et al., 1982). CLD linked to plasma proteins would arrive faster and in greater quantities in organs and tissues heavily irrigated (liver and muscles) than in those weakly perfused (fat tissues). Slaughtering data were analyzed to characterize molecule distribution in the three matrices analyzed (liver, muscle and fat) and to highlight possible differences in CLD distribution between the two breeds. Estimated amounts of CLD into tissues after 84 depuration-days in Creole and Large White pigs are presented in Table 5. No significant difference was observed for CLD amounts in liver and muscle between the two breeds. However, repartition of CLD in fat is significantly different between the two breeds and Creole pigs have higher carcass adiposity. Although CLD concentrations in fat tissue are lower than in muscle or liver, fat tissues store an important portion of CLD body burden, as shown in Table 5. Thus, carcass fatness may impact volume of distribution. To explain inter-individual variability between pigs, animal-specific features (age, breed, sex), growth performance and carcass fatness seem to be interesting. Breed effects we found suggest that variability between breeds (especially between specialist and generalist breeds) should be taken into account in decision support for a healthy farming practice. Further studies are needed to better understand CLD storage, depuration capacities between tissues and characterize physiological factors that drive TK 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
CL mL kg^{-1} d^{-1}	82.1 ± 4.6 [a]	68.8 ± 1.6 [b]	**0.0081**
V1 mL kg^{-1}	686 ± 420 [a]	1,061 ± 329 [a]	0.5236
Q mL kg^{-1} d^{-1}	15,896 ± 2,435 [a]	22,841 ± 2,940 [a]	0.9604
V2 mL kg^{-1}	5,792 ± 405 [a]	4,433 ± 232 [b]	**0.0128**
Vss mL kg^{-1}	6,714 ± 270 [a]	5,316 ± 392 [b]	**0.0158**
MRT d	79 ± 3 [a]	71 ± 6 [a]	0.4240
$t_{1/2elim}$ d	55 ± 2 [a]	49 ± 4 [a]	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; $t_{1/2elim}$: 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 [a]	2.2 ± 0.2 [a]	0.5619
Muscle[1] (mg)	8.9 ± 1.2 [a]	12.5 ± 1.8 [a]	0.9352
Fat[2] (mg)	13.2 ± 0.5 [a]	10.0 ± 0.8 [b]	**0.0039**

[1] 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)
[2] 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).

Conclusion

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

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

Acknowledgements

The experiment was funded by ANR through research program INSSICCA (ANR-16-CE21-0008-01). The authors thank Harry Archimède (URZ), David Beramice, Bruno Bocage, Katia Benony, Laurent Dantec, Mario Giorgi, Felix Silou (PTEA), Valeriuse Calif, Yoann Félicité, Dalila Feuillet, Lucien Philibert (URZ) and Pamela Hartmeyer (URAFPA) for their technical support. They are grateful to Alain Chauvin and Francis Le Gouevec (UEPR, Rennes) for surgeries. They warmely thank Catherine Adam (LEAE-CART) for analysis and Pierre-Loic Saaidi (Génomique Métabolique, CEA, CNRS, Univ Evry, Université Paris-Saclay, Evry, France) for the 10-monohydrochlordecone synthesis. They thank Jean-Pierre Thomé (LEAE-CART) for his advices.

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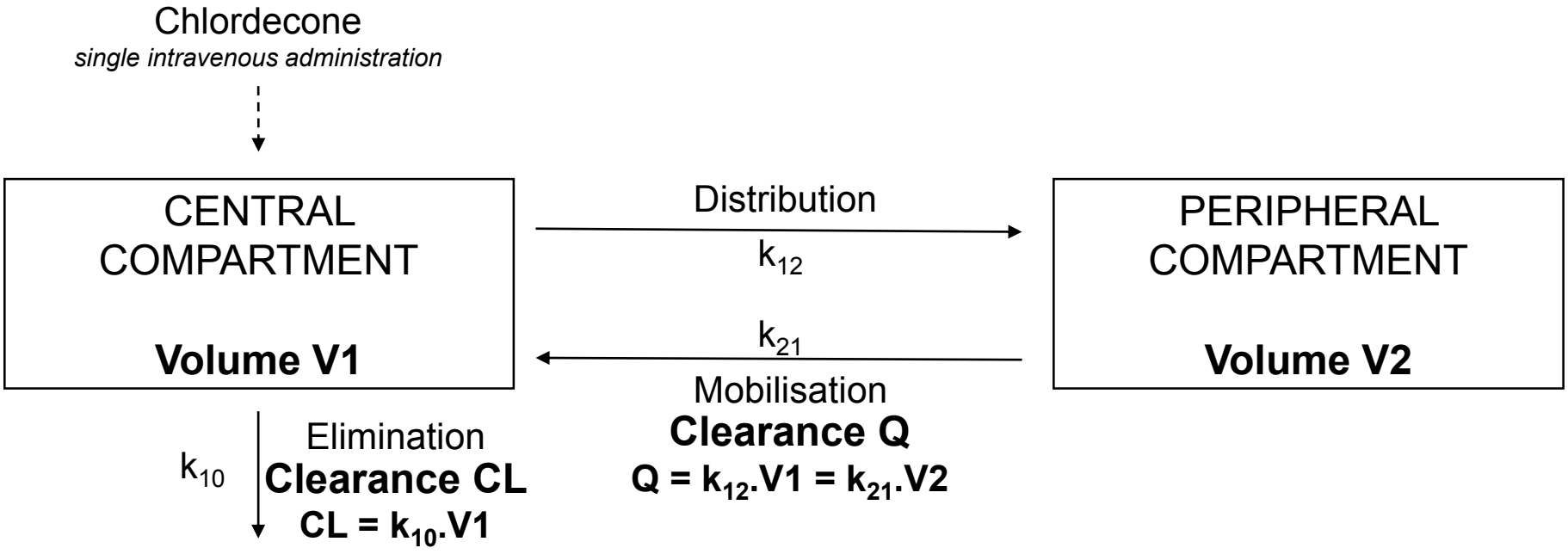
Figure 1: Two-compartmental modelisation of the equilibrium between the central compartment and the peripheral compartment after a single intravenous administration of chlordecone (adapted from Toutain and Bousquet-Melou, 2004)

Figure 2: Serum CLD concentration ($ng.mL^{-1}$) vs. time in growing pigs (n=14) after a single intravenous administration of CLD at 1 $mg.kg^{-1}$ 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
CENTRAL COMPARTMENT
Volume V1
Distribution
k12
k21
Mobilisation
PERIPHERAL COMPARTMENT
Volume V2
k10
Elimination
Clearance CL
CL = k10.V1
Clearance Q
Q = k12.V1 = k21.V2

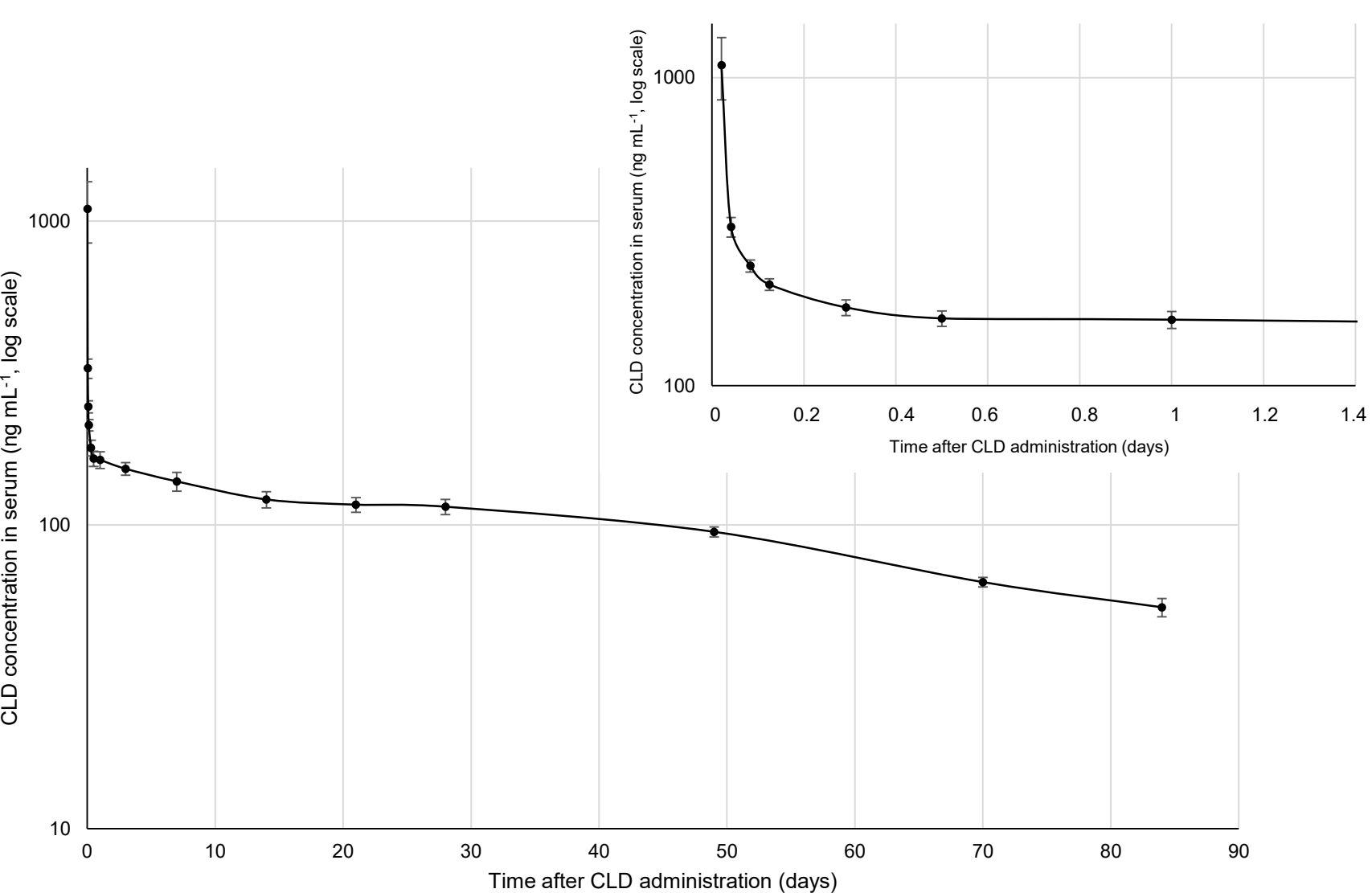

CLD concentration in serum (ng mL-1, log scale)
1000
100
10
0
10
20
30
40
50
60
70
80
90
Time after CLD administration (days)
CLD concentration in serum (ng mL-1, log scale)
1000
100
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Time after CLD administration (days)

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
IBW kg	68.8 ± 1.8 [a]	67.9 ± 3.1 [a]	68.3 ± 1.7
FBW kg	91.2 ± 2.8 [a]	95.6 ± 2.4 [a]	92.8 ± 1.9
ADG g d^{-1}	248 ± 25 [a]	327 ± 13 [a]	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)

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^{-1}

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

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

Parameters	Model estimate median ± SE	P[1]	CV (%)
CL mL kg^{-1} d^{-1}	76.7 ± 3.2	0.0009	4.1
V1 mL kg^{-1}	604 ± 195	-	32.4
Q mL kg^{-1} d^{-1}	16,680 ± 2,870	-	17.1
V2 mL kg^{-1}	4,840 ± 262	0.0142	5.4
Vss mL kg^{-1}	6,002 ± 280	-	4.6
MRT d	78 ± 3	-	4.3
$t_{1/2elim}$ 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; $t_{1/2elim}$: Elimination half-life; CV: Coefficient of variation; SE: Standard Error.

[1] 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
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Q mL kg^{-1} d^{-1}	15,896 ± 2,435 [a]	22,841 ± 2,940 [a]	0.9604
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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; SE: Standard Error.
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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 [a]	2.2 ± 0.2 [a]	0.5619
Muscle[1] (mg)	8.9 ± 1.2 [a]	12.5 ± 1.8 [a]	0.9352
Fat[2] (mg)	13.2 ± 0.5 [a]	10.0 ± 0.8 [b]	**0.0039**

[1] 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)
[2] 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).