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Characterization and quantification of chlordecone elimination in ewes

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Abstract

To reduce the exposure of the French West Indies population to the organochlorine insecticide chlordecone (Kepone; CLD), the contamination of currently consumed foodstuffs must be reduced. Depuration of contaminated animals before slaughter could be a strategy to obtain safe animal products. The aim of this study was to characterize and quantify CLD elimination in contaminated ewes during depuration process. Experiments A and B consisted in a single intravenous (*i.v.*) administration of CLD (n=5) and CLDOH (chlordecol; n=3) followed by a 84-d and 3-d depuration period respectively with collection of blood, faeces and urine samples. After CLD administration, CLD and conjugated-CLDOH (CLDOH-C) were quantified in serum and urine and CLD and CLDOH were quantified in faeces. Based on calculations of faecal, urinary and body clearances of CLD and CLDOH-C, faeces appeared as the major route of CLD excretion with 86% of the CLD administered dose eliminated in faeces, either as CLD (51%) or as CLDOH (35%).

Keywords: Chlordecone, Ruminants, Depuration, Chlordecol, Faeces

Highlights

- After chlordecone (CLD) administration, CLD and conjugated-chlordecol (CLDOH-C) were quantified in serum and urine and CLD and chlordecol (CLDOH) were quantified in faeces.
- 86% of CLD is excreted in faeces as parent form or CLDOH.
- 3.6% of CLD is excreted in urine as parent form or CLDOH-C.

1. Introduction

Persistent organic pollutants (POPs) can lead to toxicologically significant soil pollution and solutions must be found to adapt production systems in order to avoid contamination of human food products. In the French West Indies (FWI), extensive use of chlordecone in banana fields from 1972 to 1993 has resulted in long-term pollution of soils (Le Déaut and Procaccia, 2009). Parts of these agricultural areas are used as pasture for grazing animals. It is known that CLD may transfer from soil to the food chain, thus, production of CLD-free products in contaminated areas may be compromised. However, farming operations located on contaminated areas are important since they provide real cultural, ecosystemic and economic benefits. In the FWI, animal breeding activity represents almost 20% of the total turnover of the agricultural production (IEDOM, 2015). One of the strategies that have been proposed to adapt breeding systems and ensure consumers' health protection is based on depuration process of contaminated animals (Fournier et al., 2017). This strategy involves removing the animals from CLD exposure to ensure their depuration. As the elimination half-life of CLD in ruminants is relatively short (between 20 and 40 days; Mahieu et al., 2014; Fournier et al., 2017; Saint-Hilaire et al., 2019; Fourcot et al., 2021), with respect to the duration of the rearing period (around 1 year for small ruminants and 2-3 years for cattle), it allows the introduction of a depuration step. In such a scenario, the potential pollution impact must be evaluated of the environmental release of contaminated effluents containing CLD and its metabolites.

Some data are already available on CLD elimination by organisms and recent studies estimated the main toxicokinetic parameters of CLD in goats, ewes and pigs (Fournier et al., 2017; Saint-Hilaire et al., 2019; Fourcot et al., 2020). CLD is known to be metabolized in humans, gerbils, pigs and

ruminants (Fariss et al., 1980; Houston et al., 1981; Soine et al., 1983; Saint-Hilaire et al., 2018b). This compound is reduced to chlordecol (CLDOH) by an aldo-keto reductase (AKR1C4); then CLDOH can be conjugated by UDP glucuronosyl - or sulfonyl - transferase forming glucuronidated chlordecol (CLDOH-G) and sulfated chlordecol respectively (CLDOH-S; Fariss et al., 1980). In contaminated ewes, it has been observed that CLD is excreted in faeces either unchanged or in its metabolized form (CLDOH) and also in urine either unchanged or in conjugated CLDOH form (CLDOH-C; Saint-Hilaire et al., 2018a). Overall, the faecal route is also considered as the main route of CLD excretion in rats and gerbils (Egle et al., 1978; Houston et al., 1981). Therefore, in summary, (1) the main CLD metabolites have been identified, (2) the main excretion route of CLD and these metabolites is predicted to be the faeces via the bile. However, no data is available on quantification of CLD excretion.

The aims of this study were to characterize and quantify the main CLD elimination pathways in ewes. This quantification is essential because the transfer of CLD from animal to non-contaminated areas during any depuration process raises concerns in terms of potential CLD or CLD metabolite release into the environment.

2. Material and methods

Two experiments (A and B) based on intravenous (*i.v.*) administration of CLD and CLDOH, respectively, were performed to obtain toxicokinetic parameters of CLD and CLDOH.

Both experiments were performed in the experimental facilities of the Bio-DA platform (URAFPA, Université de Lorraine, Vandoeuvre-lès-Nancy, France) and were approved by the Lorraine Ethics Committee (CELMEA) and the French Ministry of Higher Education and Research under the project number APAFIS#2016062217188805 and APAFIS#14815-2018042409503299 for the experiments A and B, respectively.

2.1. Experimental designs

2.1.1. Ewes and management

Eight non-lactating and non-pregnant adult ewes (*Ovis aries*, from farms located in Pulligny, France) were used. For the experiments A and B, five ewes (63.8 ± 4.9 kg body weight (BW), mean ± SD) and three ewes (76.0 ± 2.2 kg BW, mean ± SD) were respectively placed in individual barns. Water, salt and prairie hay were delivered *ad libitum*. Ewes were weighed at least once a week. No sign of physical stress or side effects on the body weight, physiological status and behavior were reported.

2.1.2. Experiment A

A 14-d adaptation period was applied before the beginning of the experiment, consisting in a single *i.v.* administration of CLD (1 mg CLD kg^{-1} BW). CLD supplied by *Azur Isotopes SAS* (CLD purity > 98%) was dissolved in cremophor (polyethoxylated castor oil, Kolliphor® EL, Sigma-Aldrich). Before the experiment, two external catheters (B. Braun, Germany) were inserted in the jugular veins, one being used for CLD administration and the other one for blood sampling. After CLD administration, an 84-d depuration period was applied. Blood samples (2 x 10 mL) were collected at 0, 5, and 30 min, 1, 3, 8, and 12 h and 3, 7, 36 and 84 d after CLD administration, using one catheter for the first 24 h and then by direct venipuncture on the jugular vein (BD vacutainer®, ref 368815). Faeces were entirely collected thanks to layers regularly changed during the first 48 h and over an 8-h period at 3, 7, 36 and 84 d. Urines were entirely collected with a Foley sonde (Rüsch Gold, France) during periods 0-2, 2-4, 4-7, 7-25, 25-28 and 28-34 h. Then, urines were collected at 3, 7, 36 and 84 d after CLD administration, over an 8-h period for each day.

2.1.3. Experiment B

A 7-d adaptation period was applied before the experiment. The experiment consisted in a single administration of CLDOH (1 mg CLDOH kg^{-1} BW). CLDOH supplied by *Azur Isotopes SAS* (CLDOH purity > 98%) was dissolved in cremophor (polyethoxylated castor oil, Kolliphor® EL, Sigma-Aldrich). Before the experiment, two external catheters (B. Braun, Germany) were inserted in jugular veins to allow CLDOH administration and blood sampling. After CLDOH administration, a 3-d depuration period was applied. Blood samples (2 x 10 mL) were collected at 0, 30 min, 1, 3, 8, 12, 24, and 72 h after CLDOH *i.v.* administration, using one catheter for the first 24 h and then by direct

venipuncture on the jugular vein (BD vacutainer®, ref 368815). Faeces and urines were entirely collected during periods 4-24, 24-45, 45-58 and 58-72 h.

2.2. Sample treatment

Collected blood samples were allowed clotting for 2 h at room temperature and at 4°C during 24 h before centrifugation at 3000 rpm for 15 min to obtain serum samples which were stored at -20°C until analysis. Faeces samples were weighed, lyophilized, crushed and then stored in amber vial at ambient temperature until analysis. Urines samples were weighed, filtrated and stored in amber vials at -20°C until analysis.

2.3. Analytical methods of CLD and metabolites in serum, faeces and urine

2.3.1. Analysis in serum

2.3.1.1. CLD analysis in serum

CLD analysis in serum was conducted by the Center for Analytical and Research Technology at Liege University (CART, Belgium) with a proven method used routinely in the laboratory according to Saint-Hilaire et al. (2019). Briefly, after protein denaturation with trimethylamine and formic acid, CLD was extracted with n-hexane/diethyelether (85/15, v/v) using a solid-phase extraction (SPE) on Supelclean Envi-C18 microcartridges (Supelco, Bellafonte, PA, USA) and subjected to acidic purification using concentrated sulfuric acid 98% w/v. The extracts were analyzed by High Resolution Gas Chromatography coupled to an ion trap mass spectrometer (Trace GC Ultra and ITQ 1100 from ThermoQuest, ThermoScientific, Waltham, MA, USA) in MS/MS mode as detailed in Fournier et al. (2017).

2.3.1.2. Analysis of CLD metabolites in serum

In order to quantify CLD metabolites, a method based on a validated method to extract CLD and its metabolites in urine (Saint-Hilaire et al., 2018a) was developed for the serum matrix with slight modifications. Since serum contains more proteins than urine, it was necessary to adapt the extraction protocol. Two milliliters of serum (2.000 ± 0.001 mL) were placed in a 50 mL centrifuge tube named

"1" for the extraction of CLD and CLDOH. Another 2 mL of serum (2.000 ± 0.001 mL) was added to a second 50 mL centrifuge tube named "2" for the extraction of total CLD (abbreviated CLD-t to stand for extraction of both CLD and conjugated chlordecone CLD-C) and the extraction of total CLDOH (abbreviated CLDOH-t to stand for extraction of both CLDOH and conjugated chlordecol CLDOH-C). Then, the deconjugaison step was realized as indicated in Saint-Hilaire et al. (2018a) for the urine matrix. For the extraction step, ten millimeters of acetonitrile (ACN) was added to each tube and then were stirred for 1 min with the Genie 2 vortex in order to allow the protein precipitation. Instead of directly adding the QuEChERS extraction kit for the original method as made for urine, each tube was then centrifuged at 4000 rpm for 5 min with the Eppendorf 5810 centrifuge. After centrifugation, the supernatant of tubes 1 and 2 were collected and the QuEChERS extraction kit for the original method was then added to each tube. The two mixtures were stirred for 1 min with the Genie 2 vortex and then centrifuged at 4000 rpm for 5 min with the Eppendorf 5810 centrifuge. The supernatant of tubes 1 and 2 (10 mL in each) was evaporated under a nitrogen flow at 37 °C. After evaporation, 1 mL of ACN was added to the residues of each tube and analyzed by LC-MS/MS. As for urine, the conjugated metabolites concentration (CLDOH-C, corresponding to CLDOH-G + CLDOH-S) in serum was calculated by subtracting the result of the analysis without the enzymatic pretreatment (CLDOH) from the result of the analysis with enzymatic pretreatment (CLDOH-t).

2.3.2. Analysis of CLD and metabolites in faeces and urine

CLD, CLDOH and its conjugated metabolites in faeces and urine samples were analyzed as indicated by Saint-Hilaire et al. (2018a). Briefly, free CLD and free CLDOH were extracted from faeces using the Quick, Easy, Cheap, Rugged and Safe (QuEChERS) method followed by the LC-MS/MS analysis. In urines, CLD and CLDOH were extracted with and without an enzymatic pretreatment using a QuEChERS method and the LC-MS/MS analysis. At the end, the conjugated metabolites concentration in urines was calculated subtracting the analysis with the enzymatic pretreatment from the analysis without the enzymatic pretreatment.

2.4. Toxicokinetic analysis

2.4.1. Serum data analysis

2.4.1.1. Experiment A

CLD serum concentration vs. time profiles after *i.v.* administration were analyzed using R software (R version 3.6.2.). A two-compartmental model provided the best fitting with individual curves. The relation followed Eq. (1):

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C(t) (ng mL^{-1}) is the CLD serum concentration at time t; A and B (ng mL^{-1}) are the intercepts of the distribution and elimination phases, respectively; α and β (d^{-1}) are the first-order rate constants of the distribution and elimination phases, respectively.

Individual areas under the serum concentration-time curve ($AUC_{0\rightarrow\infty,CLD}$) and the main toxicokinetic parameters of CLD were calculated with the classical equations of the non-compartmental approach: total body clearance (Cl_{CLD}), mean residence time (MRT_{CLD}), steady-state volume of distribution (V_{ssCLD}) and serum elimination half-life ($t_{1/2CLD}$).

2.4.1.2. Experiment B

CLDOH serum concentration vs. time profiles after CLDOH *i.v.* administration were analyzed using R software (R version 3.6.2.). A three-compartmental model provided the best fitting with individual curves. The relation followed Eq. (2):

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + Pe^{-\gamma t} \quad (2)$$

where C(t) (ng mL^{-1}) is the CLDOH serum concentration at time t; A, B and P (ng mL^{-1}) are the intercepts of the distribution (A, B), and elimination (P) phases, respectively; α, β and γ (d^{-1}) are the first-order rate constants of the distribution (α, β), and elimination (γ) phases, respectively.

Individual areas under the serum concentration-time curve ($AUC_{0\rightarrow\infty,CLDOH}$), total body clearance (Cl_{CLDOH}), mean residence time of CLDOH (MRT_{CLDOH}), steady-state volume of distribution of CLDOH (Vss_{CLDOH}) and serum CLDOH elimination half-life ($t_{1/2CLDOH}$) were calculated with the classical equations of the non-compartmental approach.

CLDOH-C serum concentration vs. time profiles after *i.v.* CLDOH administration were analyzed using R software (R version 3.6.2.). A two-compartmental model provided the best fitting with individual curves. Using the same method as presented in the section 2.4.1.1., individual areas under the serum concentration-time curves ($AUC_{0\rightarrow\infty,CLDOH\text{-}C}$), total body clearance of CLDOH-C ($Cl_{CLDOH\text{-}C}$) were calculated. This calculation requires the assumption that CLDOH is entirely and rapidly biotransformed in CLDOH-C. This assumption is supported by the results obtained in serum (see results).

2.4.2. Urine and faeces data analysis

CLD and CLDOH have very close molar mass. Although the molar mass of CLDOH-C is much higher than CLD and CLDOH ones, the analytes concentrations were not conversed in molar concentrations for the following calculations for two reasons. Firstly, the exact molar mass of the conjugated CLDOH (CLDOH-C) form is unknown (CLDOH-C is composed of CLDOH-G and CLDOH-S and their respective parts were not determined). Secondly, as the urinary route is the minor excretion route of CLD and CLDOH-C is exclusively found in urine (see results), not using the molar mass has little impact on the mass balance results.

2.4.2.1. Estimation of faeces and urine excretion rates

A mean urine excretion rate ($R_{u,mean}$) of 68.2 ± 13.8 mL (mean ± SE) of urine per hour was obtained based on the experiment A. A mean faeces excretion rate ($R_{f,mean}$) of 31.5 ± 7.7 g (mean ± SE) of faeces (DM) per hour was obtained following different experiments carried out on ewes on the Bio-DA platform.

2.4.2.2. Quantification of CLD and metabolites excretion following the *i.v.* administration of CLD

The analysis of experiments A and B data allowed to quantify the excreted CLD in faeces and urine following the *i.v.* administration of CLD. This estimation method is based on clearance calculations.

Calculation of urinary and faecal excretion rates of CLD and its metabolites. Urinary and faecal excretion rates of CLD correspond to the excreted quantity of CLD during the collection period (ΔT) of the sample i and were calculated according to Eqs. (3) and (4):

$$\text{Urinary excretion rate}_{\text{CLD i}} = [\text{CLD}]_{\text{urine, }\Delta\text{Ti}} \times R_{\text{u,mean}} \quad (3)$$

$$\text{Faecal excretion rate}_{\text{CLD i}} = [\text{CLD}]_{\text{faeces, }\Delta\text{Ti}} \times R_{\text{f,mean}} \quad (4)$$

where $[\text{CLD}]_{\text{urine, }\Delta\text{Ti}}$ and $[\text{CLD}]_{\text{faeces, }\Delta\text{Ti}}$ are the individual concentrations of CLD in urine or faeces measured during the ΔTi period of the sample collection i; $R_{\text{u,mean}}$ and $R_{\text{f,mean}}$ are as previously described.

The urinary and faecal excretion rates of CLDOH (Urinary excretion $\text{rate}_{\text{CLDOH i}}$ and Faecal excretion $\text{rate}_{\text{CLDOH i}}$, respectively) were calculated using the same method. For this calculation, CLDOH concentrations were considered under the excreted form (either deconjugated CLDOH or conjugated CLDOH i.e. CLDOH-C; see results).

Calculation of urinary and faecal clearances of CLD. The urinary and faecal excretion rates calculated above were used to calculate urinary ($Cl_{\text{u,CLD}}$) and faecal ($Cl_{\text{f,CLD}}$) clearances of CLD. As clearance is the proportionality factor between excretion rate and circulating (blood/serum) concentrations, and because several collection periods were performed on each animal, the data were analyzed by the linear models described in Eq. (5) and (6):

$$\text{Urinary excretion rate}_{\text{CLD i}} = Cl_{\text{u,CLD}} \times [\text{CLD}]_{\text{serum, }\Delta\text{Ti}} \quad (5)$$

$$\text{Faecal excretion rate}_{\text{CLD i}} = Cl_{\text{f,CLD}} \times [\text{CLD}]_{\text{serum, }\Delta\text{Ti}} \quad (6)$$

where $[\text{CLD}]_{\text{serum, }\Delta\text{Ti}}$ is the CLD concentrations in serum during the collection period ΔTi.

Calculation of urinary and faecal clearances of CLDOH-C. The urinary ($Cl_{\text{u,CLDOH-C}}$) and faecal ($Cl_{\text{f,CLDOH-C}}$) clearances of CLDOH-C were calculated using the same method previously described for $Cl_{\text{u,CLD}}$ and $Cl_{\text{f,CLD}}$, considering the urinary and faecal excretion rates of CLDOH (under the excreted form) and the CLDOH-C concentration in serum during the collection period ΔTi.

Percentages of CLD excreted in urine and faeces. For each ewe, the percentage of CLD excreted in urine as parent form (%excreted$_{u,CLD}$) was defined as the ratio of the urinary clearance of CLD ($Cl_{u,CLD}$) and the body clearance of CLD (Cl_{CLD}) following Eq. (7):

$$\% \text{ excreted}_{u,CLD} = \frac{Cl_{u,CLD}}{Cl_{CLD}} \times 100 \quad (7)$$

In the same way, for each ewe, the percentage of CLD excreted in faeces as parent form (%excreted$_{f,CLD}$) was defined as the ratio of the faecal clearance of CLD ($Cl_{f,CLD}$) and the body clearance of CLD (Cl_{CLD}) following Eq. (8):

$$\% \text{ excreted}_{f,CLD} = \frac{Cl_{f,CLD}}{Cl_{CLD}} \times 100 \quad (8)$$

Percentages of metabolites excreted in urine and faeces. For the following calculations, because no other metabolite was detected (see results), we hypothesized that all CLD not excreted in parent form (in faeces or urine) was metabolized to CLDOH-C. The excreted CLDOH was considered under its excreted form (either deconjugated or conjugated; see results).

For each ewe, the percentage of CLDOH excreted in urine (% excreted$_{u,CLDOH}$) after administration of CLD was calculated from the individual urinary clearance of CLDOH-C ($Cl_{u,CLDOH-C}$), the average total body clearance (n=3) of CLDOH-C ($Cl_{CLDOH-C,mean}$; obtained following the experiment B) and the individual percentage of CLD remaining to be excreted, following Eq. (9):

$$\% \text{ excreted}_{u,CLDOH} = \frac{Cl_{u,CLDOH-C}}{Cl_{CLDOH-C,mean}} \times (100 - [\% \text{ excreted}_{u,CLD} + \% \text{ excreted}_{f,CLD}]) \quad (9)$$

In the same way, for each ewe, the percentage of CLDOH excreted in faeces (% excreted$_{f,CLDOH}$) after administration of CLD was calculated following Eq. (10):

$$\% \text{ excreted}_{f,CLDOH} = \frac{Cl_{f,CLDOH-C}}{Cl_{CLDOH-C,mean}} \times (100 - [\% \text{ excreted}_{u,CLD} + \% \text{ excreted}_{f,CLD}]) \quad (10)$$

For each ewe, percentages of administered CLD which were excreted via urine (%Dose$_u$) and faeces (%Dose$_f$) during the depuration period have been calculated by summing percentages of the dose

excreted as CLD and as CLDOH (under deconjugated or conjugated forms), following Eqs. (11) and (12):

$$\% \text{ Dose}_u = \% \text{ Excreted}_{u,CLD} + \% \text{ Excreted}_{u,CLDOH} \quad (11)$$

$$\% \text{ Dose}_f = \% \text{ Excreted}_{f,CLD} + \% \text{ Excreted}_{f,CLDOH} \quad (12)$$

3. Results and discussion

3.1. CLD metabolic and elimination pathways

The analysis of CLD and its metabolites in different matrices (serum, urine and faeces) allowed the characterization of the elimination pathways of CLD in ewes. Figure 1 shows the kinetics of CLD and its metabolites in serum, urine and faeces after *i.v.* administration of CLD (experiment A). For the first time, CLDOH-C was quantified in serum but the predominant form was CLD. In accordance with results obtained in humans and pigs (Boylan et al., 1979; Soine et al., 1983), CLDOH and CLD-C were not quantified in serum.

In urine samples, CLD and CLDOH-C have been quantified in accordance with the hypothesis made by Houston et al. (1981). The mean ratio of CLD to CLDOH-C concentrations being 0.10 ± 0.02 (mean ± SE), the predominant form in urine was CLDOH-C. The quantification of CLD at low concentrations in urine was also observed in humans and rats (Cohn et al., 1978; Egle et al., 1978). In faecal samples, CLD and CLDOH could be quantified, whereas conjugated metabolites were not detected. These results are similar with previous studies in humans, gerbils and pigs (Cohn et al., 1978; Fariss et al., 1980; Houston et al., 1981; Soine et al., 1983). The mean ratio of CLD to CLDOH concentrations in faeces samples being 1.9 ± 0.1 (mean ± SE), the main form quantified in faeces was CLD. CLD/CLDOH faecal concentrations ratios in gerbils, humans and pigs were respectively of 2.4, 0.25 and between 8 and 10 (Boylan et al., 1979; Houston et al., 1981; Soine et al., 1983). The CLD/CLDOH faecal concentration ratio observed in ewes was then closer to that obtained in gerbils. CLD/CLDOH ratio differences between species could be explained by metabolic variations. Indeed, CLD is known to be metabolized in these species and differences in specific chlordecone reductase activity between humans, gerbils and rabbits have been demonstrated (Molowa et al., 1986).

According to Houston et al. (1981), the differences in CLD/CLDOH ratio between species may be due to CLDOH reconversion to CLD. However, this hypothesis does not seem to be relevant because after the single *i.v.* administration of CLDOH, CLD serum, urine and faeces concentrations were lower or very close to the LOQ. This implies that the reversibility of CLDOH to CLD parent probably exists, but is negligible. The second hypothesis already proposed by Houston et al. (1981) is the existence of differences in intestinal absorption or reabsorption between species.

This study thus enabled the identification of the main metabolic and elimination pathways of CLD in ewes. CLD is eliminated as parent form in urine and faeces and is also metabolized in CLDOH, which is very rapidly and extensively conjugated in CLDOH-C. CLDOH-C is excreted in urine, where it is found unchanged, and in faeces, where it is found as the deconjugated form (CLDOH), probably due to microbiome-mediated deconjugation occurring in the gut.

3.2. CLD toxicokinetics and quantification of elimination pathways

3.2.1. Characterization of global elimination of CLD and CLDOH

Serum concentration vs. time profiles of CLD and CLDOH after *i.v.* administration (experiments A and B, respectively) allowed to calculate toxicokinetic parameters of CLD and CLDOH presented in Table 1. The serum half-life of CLD was 24.8 ± 1.2 d (mean ± SE). Figure 2 shows the concentration vs. time profiles of CLDOH and its conjugated forms in serum after *i.v.* administration of CLDOH (experiment B). Following the *i.v.* administration of CLDOH, CLDOH concentrations in serum decreased quickly while CLDOH-C concentrations increase rapidly and then decrease. These observations suppose a quick and full transformation of CLDOH into CLDOH-C. The body clearance of CLDOH is 18.9 times higher than the body clearance of CLDOH-C and confirms the rapid elimination of CLDOH by biotransformation into CLDOH-C.

Table 1: CLD and CLDOH toxicokinetic parameters obtained in ewes following a single *i.v.* administration of CLD (1 mg CLD kg^{-1} BW) or CLDOH (1 mg CLDOH kg^{-1} BW)

Parameters	experiment A single *i.v.* (n=5) 1 mg CLD kg^{-1} mean ± SE	experiment B single *i.v.* (n=3) 1 mg CLDOH kg^{-1} mean ± SE
CL_{CLD} mL d^{-1} kg^{-1} BW	50.0 ± 6.03	-
Vss_{CLD} mL kg^{-1} BW	1 747 ± 145	-
$t_{1/2CLD}$ d	24.8 ± 1.19	-
MRT_{CLD} d	35.8 ± 1.72	
CL_{CLDOH} mL d^{-1} kg^{-1} BW	-	11 116 ± 2 626
Vss_{CLDOH} mL kg^{-1} BW	-	8 573 ± 2 206
$t_{1/2CLDOH}$ d	-	1.62 ± 0.21
MRT_{CLDOH} d	-	0.76 ± 0.08
$CL_{CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	-	589 ± 87
$Cl_{f,CLD}$ mL d^{-1} kg^{-1} BW	25.1 ± 2.80	-
$Cl_{u,CLD}$ mL d^{-1} kg^{-1} BW	0.12 ± 0.05	-
$Cl_{f,CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	422 ± 65	-
$Cl_{u,CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	39.1 ± 4.81	-
CLD/CLDOH-C_u	0.10 ± 0.02	-
CLD/$CLDOH_f$	1.9 ± 0.1	-

CLD: chlordecone; CLDOH: chlordecol; CLDOH-C: conjugated chlordecol; CL_{CLD}: total body clearance of CLD; Vss_{CLD}: steady-state volume of distribution of CLD; $t_{1/2CLD}$: elimination half-life of CLD; MRT_{CLD}: mean residence time of CLD; CL_{CLDOH} : total body clearance of CLDOH; Vss_{CLDOH}: steady-state volume of distribution of CLDOH; $t_{1/2CLDOH}$: elimination half-life of CLDOH; MRT_{CLDOH}: mean residence time of CLDOH; $CL_{CLDOH\text{-}C}$: total body clearance of CLDOH-C; $Cl_{f,CLD}$: faecal clearance of CLD; $Cl_{u,CLD}$: urinary clearance of CLD; $Cl_{f,CLDOH\text{-}C}$: faecal clearance of CLDOH-C; $Cl_{u,CLDOH\text{-}C}$: urinary clearance of CLDOH-C; CLD/CLDOH-C: CLD/CLDOH-C_u: urine concentrations ratio; CLD/$CLDOH_f$: CLD/CLDOH faecal concentrations ratio; SE: Standard Error; *i.v.*: intravenous administration.

3.2.2. Quantification of elimination pathways

Estimation of urinary and faecal clearances of CLD and CLDOH-C. The estimated urinary and faecal clearances of CLD and CLDOH-C are presented in Table 1. Urinary and faecal clearances of CLD are 0.12 ± 0.05 and 25.1 ± 2.8 mL d^{-1} kg^{-1} BW, respectively. Urinary and faecal clearances of CLDOH-C are 39.2 ± 4.8 and 422 ± 65 mL d^{-1} kg^{-1} BW, respectively. These results highlight the small contribution of the urinary route in CLD and CLDOH-C excretion regarding the faecal route.

Quantification of CLD and metabolites elimination pathways. Serum, urinary and faecal clearances of CLD and its metabolites obtained following the *i.v.* administrations of CLD (experiment A) and CLDOH (experiment B) allowed to quantify the elimination pathways of CLD (Figure 3). 51% and 0.2% of CLD are excreted as parent form in faeces and in urine respectively. To quantify the percentage of biotransformation of CLD into CLDOH-C, we hypothesized that (1) all non-excreted CLD in urine and faeces is metabolized to CLDOH and (2) CLDOH in serum is rapidly and fully transformed into CLDOH-C. 48.6% of CLD in serum is metabolized in CLDOH-C. Then, 71.7% and 6.6% of the CLDOH-C in serum is excreted in faeces and in urine respectively.

Table 2: Estimations of CLD excreted during the depuration period following a single *i.v.* administration of 1 mg CLD kg^{-1} (experiment A)

		Experiment A (n=5)
	Parameters	mean ± SE
Faecal excretion	**%excreted$_{f,CLD}$**	51.2 ± 3.81
	%excreted$_{f,CLDOH}$	34.6 ± 5.44
	%Total$_{f,excreted}$	85.8 ± 5.90
Urinary excretion	**%excreted$_{u,CLD}$**	0.2 ± 0.1
	%excreted$_{u,CLDOH}$	3.3 ± 0.5
	%Total$_{u,excreted}$	3.6 ± 0.6

CLD: chlordecone; CLDOH: excreted CLDOH (under conjugated or deconjugated form); %excreted$_{f,CLD}$: percentage of the CLD administrated dose excreted as CLD form in faeces; %excreted$_{f,CLDOH}$: percentage of the CLD administrated dose excreted as CLDOH in faeces; %excreted$_{u,CLD}$: percentage of the CLD administrated dose excreted as CLD form in urine; %excreted$_{u,CLDOH}$: percentage of the CLD administrated dose excreted as CLDOH in urine; %Total$_{f,excreted}$: percentage of the CLD administrated dose excreted in faeces as CLD and CLDOH forms; %Total$_{u,excreted:}$ percentage of the CLD administrated dose excreted in urine as CLD and CLDOH forms; SE: Standard Error.

These calculations allowed the estimation of the percentages of CLD excreted as parent or metabolites forms (Table 2). The average percentage of CLD excreted in faeces was 86 ± 5 %, with 51 ± 4 % under its parent form CLD and 35 ± 5 % under its CLDOH form. The average percentage of CLD excreted in urine was 3.6 ± 0.6 %, with 0.2 ± 0.1 % under its parent form CLD and 3.3 ± 0.5 % under its CLDOH-C form.

The elimination mass balance of CLD. We have estimated that more than 89% of CLD is excreted in faeces and urine as parent CLD, CLDOH or CLDOH-C. Therefore, about 10% of CLD is absent from the excretion massed balance. This difference does not appear to be the result of an incorrect estimation of faeces production, which influences the estimation of CLD faecal excretion. Indeed, the sampling method of faeces was individual, layers used allowed the collection of all faeces over the sampling period and the number of samples was significant enough to calculate an average faecal excretion. The elimination mass balance of CLD is incomplete but the authors would suggest it is an improvement on previous studies, such as those which employed radioactivity. In rats, the excretion of CLD estimated after a depuration period was estimated using radioactivity at 65.5% of the administered dose (Egle et al., 1978). Even using radioactivity, the excreted balance was far from the CLD administered dose. Another hypothesis which might account for the mass balance issues could be the presence of other metabolic pathways. In fact, according to Fariss et al. (1980), pretreatment of human bile with deconjugating enzymes plus acid hydrolysis released an additional increment of free CLD; indeed, 19% of CLD was present in a form releasable only by acid hydrolysis. In gerbil bile, the same study was carried out (Houston et al., 1981), but no difference was observed between CLD concentrations obtained with or without deconjugating enzymes plus acid hydrolysis. This could indicate a significant metabolic difference between humans and gerbils. It would be interesting to determine the effects of acid hydrolysis on the bile of ewes, as well as the exploration of other potential metabolic pathways for CLD in these animals.

In urine, only 3.6 ± 0.6 % of the administered dose was found to be eliminated. This result is consistent with the excreted percentage in urine of 1.6% observed in rats (Egle et al., 1978). However,

an unidentified metabolite comprising two percent of the administered radioactivity appeared in the gerbil urine during the first two days after injection of the radioisotope (Houston et al., 1981). In the FWI, other transformation products of CLD have been detected in water and soils samples from Martinique (Chevallier et al., 2019). Hence the complete metabolic fate of CLD remains to be determined in most animal species.

3.3. Applications for depuration strategies

Recent studies revealed that 86.5% of the pesticides found in the analyzed watercourses of Guadeloupe (Basse-Terre) were CLD, 5b hydro CLD and CLDOH (Rochette et a. 2017). CLDOH was quantified in Curlone® that was the main formulation used in the FWI. According to Macarie et al. (2016), CLDOH accounted for 13-17 % (weight/weight) of CLD in the Curlone® formulation. Presence of CLDOH in the environment could be then partially explained by this impurity. However, we have demonstrated in our study that a significant quantity of CLDOH released into the environment is produced by animals.

Two conditions are necessary to demonstrate the viability of depuration strategies and to implement them *in situ*. Firstly, the elimination half-life of CLD in ruminants must be short compared to the duration of the rearing periods. Recent studies, have adhered to this guideline successfully (Mahieu et al., 2014; Fournier et al. 2017; Saint-Hilaire et al., 2019). Secondly, CLD and CLDOH release by animals on depuration areas is likely to be negligible compared to existing CLD concentrations of contaminated areas. Future studies will centre on the development of a rapid and reliable method using TK parameters obtained in this study to estimate CLD amounts excreted by animals during depuration. These data will allow a more informed discussion amongst regulators and local stakeholders regarding the acceptability of depuration strategies.

Conclusion

This study has provided novel data on CLD elimination in sheep and has identified the main CLD elimination pathways. It confirmed that the faecal excretion is the major route of elimination of CLD: 51% of CLD is excreted as parent form in faeces vs. 0.2% in urine. In addition to examining the parent

molecule elimination, this study focused on CLDOH elimination, the main metabolite of CLD. The faecal route is also the major route of CLDOH excretion: 72% of CLDOH-C in serum is excreted as CLDOH in faeces vs. 6.6% as CLDOH-C in urine. These valuable results could be used later to quantify CLD transfer from animals to soil during depuration processes. These data are essential for the risk assessment of depuration strategies by local stakeholders.

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Conflict of interest

The authors have no conflicts of interest.

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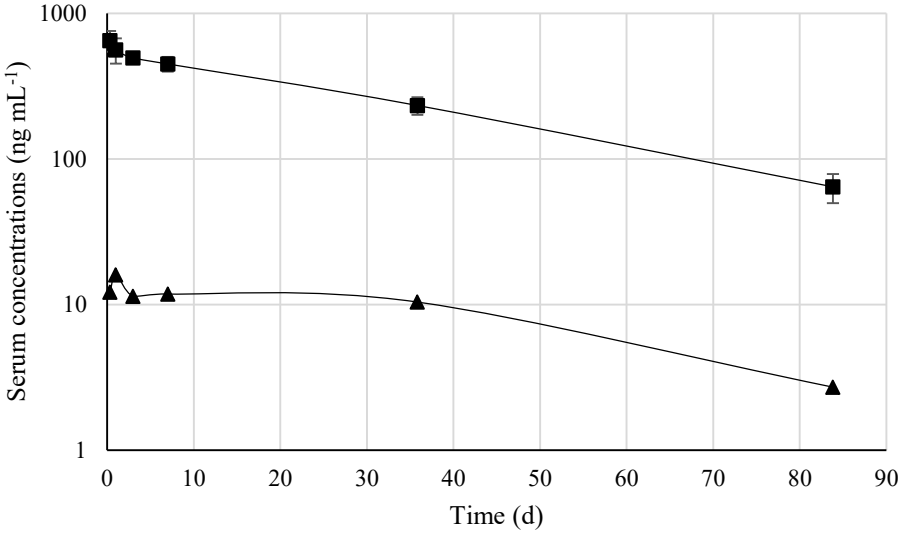
Figure 1: Chlordecone, chlordecol, conjugated-chlordecol concentration-time curves in serum, urine and faeces following an *i.v.* administration of 1 mg CLD kg^{-1} BW (experiment A, n=5)

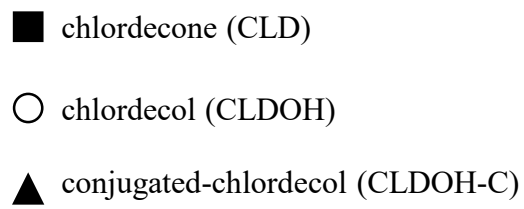
FM: fresh matter. Values are means ± SE.

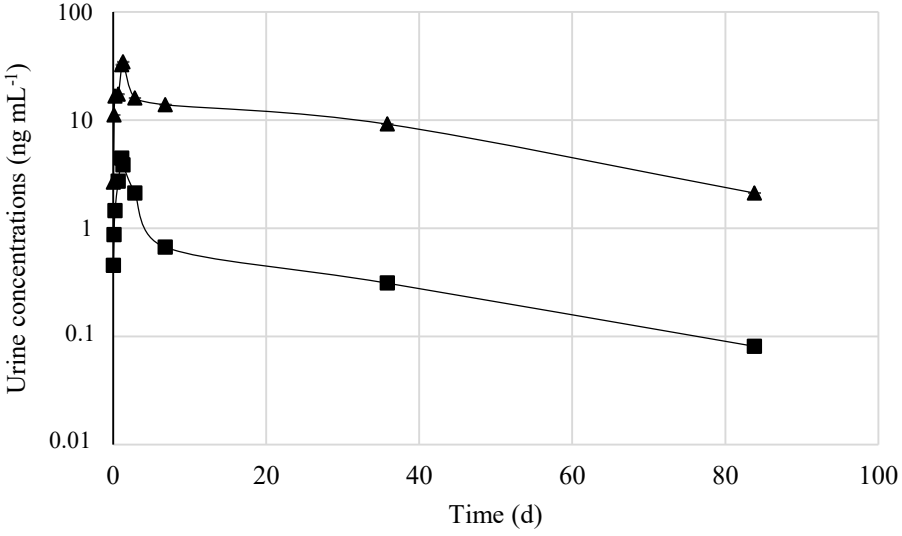
Figure 2: Chlordecol and conjugated-chlordecol concentration-time curves in serum following an *i.v.* administration of 1 mg CLDOH kg^{-1} BW (experiment B, n=3)

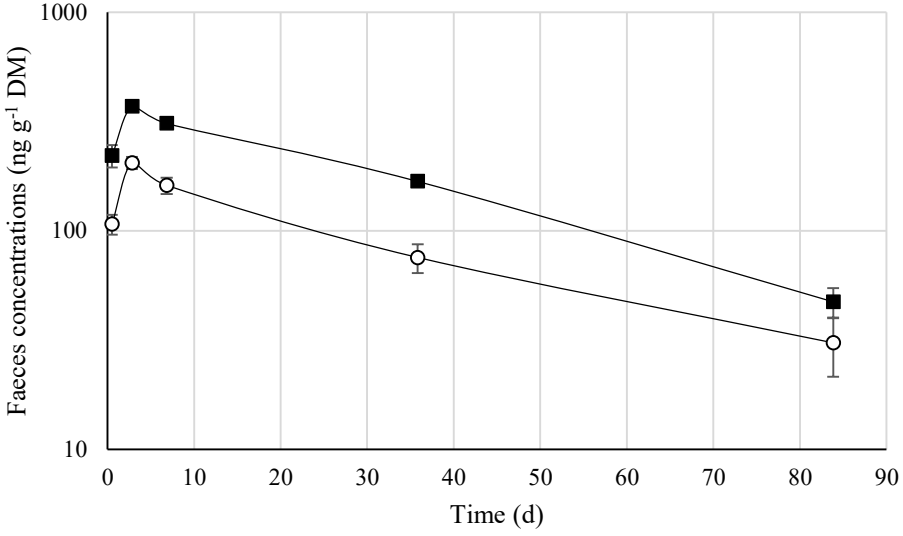
FM: fresh matter. Values are means ± SE.

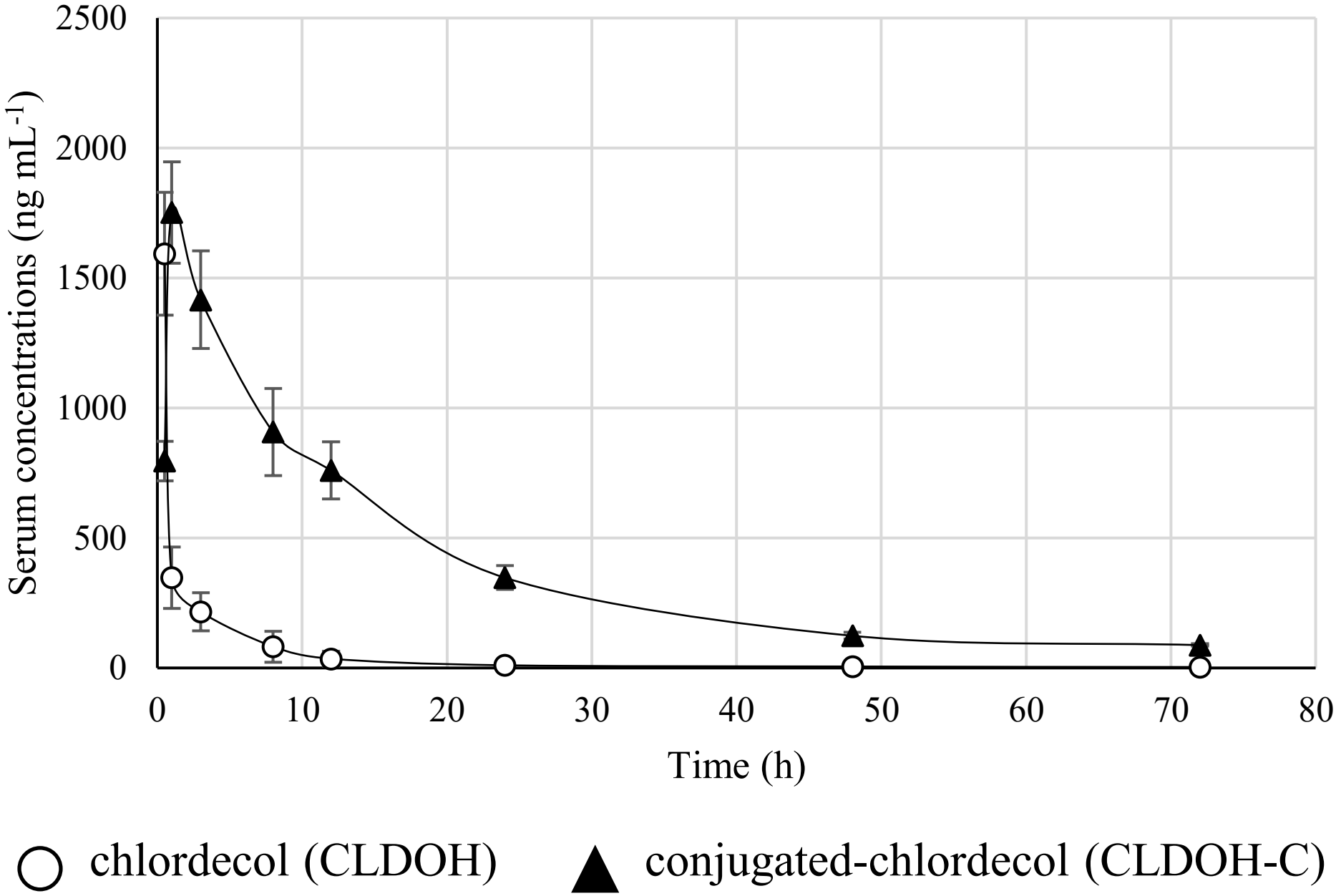
Figure 3: Mass balance of CLD elimination following a single *i.v.* administration of CLD


1000
100
10
1
Serum concentrations (ng mL-1)
0
10
20
30
40
50
60
70
80
90
Time (d)


chlordecone (CLD)
chlordecol (CLDOH)
conjugated-chlordecol (CLDOH-C)


100
10
1
0.1
0.01
Urine concentrations (ng mL-1)
0
20
40
60
80
100
Time (d)


1000
100
10
Faeces concentrations (ng g-1 DM)
0
10
20
30
40
50
60
70
80
90
Time (d)


2500
2000
1500
1000
500
0
Serum concentrations (ng mL-1)
0
10
20
30
40
50
60
70
80
Time (h)
chlordecol (CLDOH)
conjugated-chlordecol (CLDOH-C)

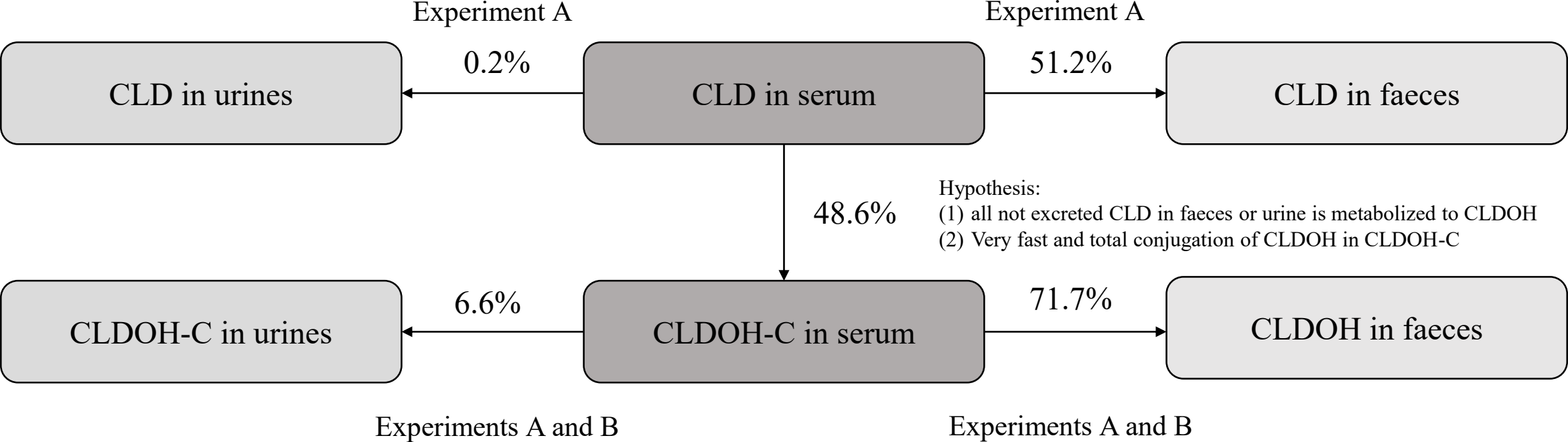

Experiment A
Experiment A
CLD in urines
0.2%
CLD in serum
51.2%
CLD in faeces
48.6%
Hypothesis:
(1) all not excreted CLD in faeces or urine is metabolized to CLDOH
(2) Very fast and total conjugation of CLDOH in CLDOH-C
CLDOH-C in urines
6.6%
CLDOH-C in serum
71.7%
CLDOH in faeces
Experiments A and B
Experiments A and B

Table 1: CLD and CLDOH toxicokinetic parameters obtained in ewes following a single *i.v.* administration of CLD (1 mg CLD kg^{-1} BW) or CLDOH (1 mg CLDOH kg^{-1} BW)

Parameters	experiment A single *i.v.* (n=5) 1 mg CLD kg^{-1} mean ± SE	experiment B single *i.v.* (n=3) 1 mg CLDOH kg^{-1} mean ± SE
CL_{CLD} mL d^{-1} kg^{-1} BW	50.0 ± 6.03	-
Vss_{CLD} mL kg^{-1} BW	1 747 ± 145	-
$t_{1/2CLD}$ d	24.8 ± 1.19	-
MRT_{CLD} d	35.8 ± 1.72	
CL_{CLDOH} mL d^{-1} kg^{-1} BW	-	11 116 ± 2 626
Vss_{CLDOH} mL kg^{-1} BW	-	8 573 ± 2 206
$t_{1/2CLDOH}$ d	-	1.62 ± 0.21
MRT_{CLDOH} d	-	0.76 ± 0.08
$CL_{CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	-	589 ± 87
$Cl_{f,CLD}$ mL d^{-1} kg^{-1} BW	25.1 ± 2.80	-
$Cl_{u,CLD}$ mL d^{-1} kg^{-1} BW	0.12 ± 0.05	-
$Cl_{f,CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	422 ± 65	-
$Cl_{u,CLDOH\text{-}C}$ mL d^{-1} kg^{-1} BW	39.1 ± 4.81	-
CLD/CLDOH-C_u	0.10 ± 0.02	-
CLD/$CLDOH_f$	1.9 ± 0.1	-

CLD: chlordecone; CLDOH: chlordecol; CLDOH-C: conjugated chlordecol; CL_{CLD}: total body clearance of CLD; Vss_{CLD}: steady-state volume of distribution of CLD; $t_{1/2CLD}$: elimination half-life of CLD; MRT_{CLD}: mean residence time of CLD; CL_{CLDOH} : total body clearance of CLDOH; Vss_{CLDOH}: steady-state volume of distribution of CLDOH; $t_{1/2CLDOH}$: elimination half-life of CLDOH; MRT_{CLDOH}: mean residence time of CLDOH; $CL_{CLDOH\text{-}C}$: total body clearance of CLDOH-C; $Cl_{f,CLD}$: faecal clearance of CLD; $Cl_{u,CLD}$: urinary clearance of CLD; $Cl_{f,CLDOH\text{-}C}$: faecal clearance of CLDOH-C; $Cl_{u,CLDOH\text{-}C}$: urinary clearance of CLDOH-C; CLD/CLDOH-C: CLD/CLDOH-C_u: urine concentrations ratio; CLD/$CLDOH_f$: CLD/CLDOH faecal concentrations ratio; SE: Standard Error; *i.v.*: intravenous administration.

Table 2: Estimations of CLD excreted during the depuration period following a single *i.v.* administration of 1 mg CLD kg^{-1} (experiment A)

		Experiment A (n=5)
	Parameters	mean ± SE
Faecal excretion	**%excreted$_{f,CLD}$**	51.2 ± 3.81
	%excreted$_{f,CLDOH}$	34.6 ± 5.44
	%Total$_{f,excreted}$	85.8 ± 5.90
Urinary excretion	**%excreted$_{u,CLD}$**	0.2 ± 0.1
	%excreted$_{u,CLDOH}$	3.3 ± 0.5
	%Total$_{u,excreted}$	3.6 ± 0.6

CLD: chlordecone; CLDOH: excreted CLDOH (under conjugated or deconjugated form); %excreted$_{f,CLD}$: percentage of the CLD administrated dose excreted as CLD form in faeces; %excreted$_{f,CLDOH}$: percentage of the CLD administrated dose excreted as CLDOH in faeces; %excreted$_{u,CLD}$: percentage of the CLD administrated dose excreted as CLD form in urine; %excreted$_{u,CLDOH}$: percentage of the CLD administrated dose excreted as CLDOH in urine; %Total$_{f,excreted}$: percentage of the CLD administrated dose excreted in faeces as CLD and CLDOH forms; %Total$_{u,excreted:}$ percentage of the CLD administrated dose excreted in urine as CLD and CLDOH forms; SE: Standard Error.