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Keywords

Sequestration ; Bioavailability ; Exposure ; Chlordecone

Abstract

Chlordecone (Kepone) (CLD) is a highly persistent pesticide formerly used in the French West Indies. High levels of this pesticide are still found in soils and represent a subsequent source of contamination for outdoor-reared animals which may ingest involuntary non-negligible amounts of soil. In that context, sequestering matrices like activated carbons (ACs) may be used to efficiently decrease the bioavailability of such organic pollutants. The present study intends to assess the respective efficiency of two sequestering strategies where two different ACs were provided either *via* feed incorporation or *via* soil amendment. This study involved 20 piglets randomly distributed into 5 experimental groups (4 replicates). All groups were exposed to 10 µg of CLD per kg of BW per day during 10 days via a contaminated soil. In both "Soil-ACs" treatment groups, the contaminated soil was amended by 2% (mass basis) of one of the two ACs. The two "Feed-ACs" groups received the contaminated soil and one dough ball containing 0.5% (mass basis) of one of the ACs. The piglets were then euthanized before collection of pericaudal adipose tissue and the whole liver and CLD analysis. A significant decrease of CLD concentrations in liver and adipose tissue was observed only in the "Soil-ACs" groups in comparison with the control group ($P < 0.001$). This decrease was particularly important for the coconut shell activated carbon where relative bioavailability was found lower than 1.8% for both tissues.

1. Introduction

Chlordecone (CLD) biocidal activity against a wide spectrum of insects formerly led to its extensive use in the tropics, and particularly in Martinique and Guadeloupe (French West Indies) in order to fight against the banana black weevil *(Cosmopolites sordidus)* (Cabidoche et al. 2009). As an illustration, almost one-sixth (~ 300 t) of the CLD total world production *was* spread in French West Indies banana fields from 1972 to 1993, when it *was* formally banned in 1990 (C. Babusiaux et al. 1990). Since this pollutant is strongly retained and persistent in soils, superficial layers of large areas of agricultural land still remain highly contaminated, with registered values exceeding 1 mg of CLD kg ^{r} of soil dry matter (DM) (Cabidoche et al. 2009; Le Déaut and Procaccia 2009; Levillain et al. 2012). This soil contamination will persist for several centuries (Cabidoche et al. 2009) due to the CLD recalcitrance to biodegradation under environmental conditions (Cabidoche et aL 2009). Thus, CLD *was* added *as* a Persistent Organic Pollutant (POP) to the Stockolm Convention in 2009 due to its high persistence and

serious risks to human health and the environment (Cabidoche et al. 2009). Indeed, recent Guadeloupean epidemiological studies suggested that CLD impairs both cognitive and motor developments during early life stages (Dallaire et al. 2012; Boucher et al. 2013), and *acts as* an endocrine-disruptor (Cordier et al. 2015; Multigner et al. 2016)

This long-term CLD soil pollution results in transfer and bio-accumulation of CLD in the food chain (Guldner et al. 2010; Cabidoche and Lesueur-Jannoyer 2012; Boucher et al. 2013). Regarding outdoor-reared animals and derived food products, a number of studies showed that involuntary soil ingestion represents a major route for meat contamination (Jondreville et al. 2013; Bouveret et al. 2013; Jurjanz et al. 2014). Those recent investigations dedicated to assess the relative bioavailability of contaminants as "the comparative bioavailabilities of different forms of a chemical or for different exposure media containing the chemical (e.g., bioavailability of a chemical from soil relative to its bioavailability from water) and is expressed as a fractional relative absorption factor" (NEPI 2000). These articles clearly demonstrated that soil-bound CLD is fully bioavailable for piglets, lambs, and laying hens. This is of great concern in terms of potential impact on human health protection of French West Indian population (Multigner et al. 2010).

Within this context, there is *an* urgent and growing need to propose an environmentally and socially acceptable solution aiming to highly reduce the CLD bioavailability of contaminated soils. The approach which was investigated in this study consisted in the use of activated carbons given either via soil or via feed the CLD transfer to animals. These media are indeed efficient to sequester organic pollutants due to their important porous structure as expressed by surface specific areas (SSA) (Hilber and Bucheli 2010). Concerning CLD, some articles already show the effectiveness of using ACs to sequester CLD (Durimel et al. 2013; Gaspard et al. 2014; Woignier et al. 2015; Yehya et al. 2017) Thus, the present study *was* conducted to assess the potential of two contrasted ACs (DARCO® and ORBO™ respectively originating from peat bog and coconut shells) to efficiently retain CLD during digestive processes of piglets when provided either via feed incorporation or via soil amendment.

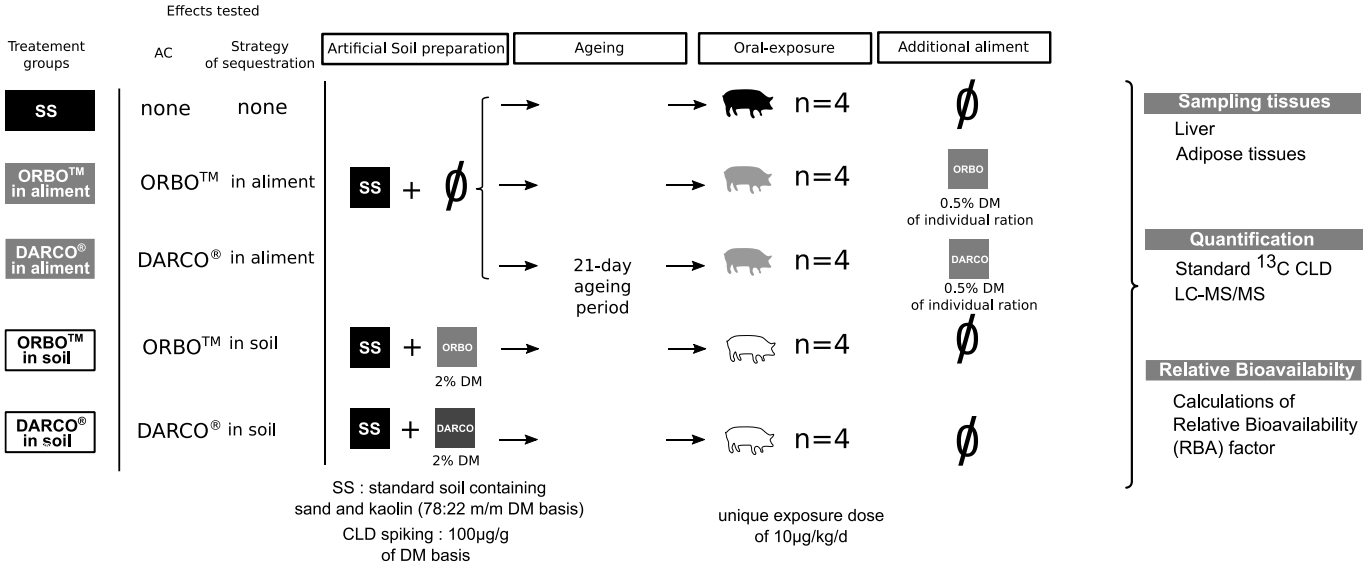

Figure 1: Experimental design of the present study

2. Material and Methods

2.1. Animals, ethics and housing

The overall experimental design is depicted in Figure 1. The experimental protocol was approved by the Ethical Committee of Lorraine and authorized by Ministry of Agriculture for Animal Research (MAAR) (Permit Number: 00270.02). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the French Ministry of Agriculture for Animal Research (MAAR) and European Council Directive (European directive 2010/63/EU). Twenty 35-day-old castrated male piglets (*Sus scrofa domesticus*, EARL des deux chênes, Saint-Maurice-aux-Forges, France) were involved in this experiment. A 7-day acclimation period was realized prior to the start of the exposure period. Each experimental group (n=4) was kept in an individual cage covered with duckboard and individual feeders in the animal facility of Plateforme Biodisponibilité-Bioactivité (Université de Lorraine, Vandœuvre-les-Nancy France). Temperature was kept to 24-25 °C. In order to closely control CLD exposureof animals, all animals were individually weighed three times a week.

Ration was provided daily (SUPER VE, Lorial, Coopérative Agricole Lorraine, France) at 4.5% of body weight (BW). Water was provided ad libitum by nipple waterers throughout the entire study.

2.2. Dosing material and method of exposure to CLD

2.2.1. Contaminated soils

Three artificial soils were prepared as described in Table 1 according to the OECD guideline 207 . First, the artificial standard soil (referred to as SS) contained sand and kaolin only (Sigma-Aldrich, St Louis, USA). This exposure matrix was adapted from the OECD guideline as the composition was modified by not introducing *Sphagnum* peat to avoid sorption competition of CLD. The second soil (referred to as DARCO) and the third one (referred to as ORBO) were based on SS, and they were amended respectively by a 2% mass (dry basis) of DARCO® (Sigma-aldrich, St Louis, USA) and ORBO™ (Sigma-aldrich, St Louis, USA) activated carbons. All the three soils were spiked with 100 µg of CLD (Kepone, Supelco, SigmaAldrich, Saint-Louis) per g of dry matter (DM). 1).

Table 1: Composition of the different artificial soils and treatment of the experiment

Percentages are DM basis of artificial soil.

	Sand See Sand (Carl Roth GmbH, Karlsruhe, Germany)	**Kaolin** (Sigma-aldrich, St Louis, USA)	**Activated carbon** DARCO© ® (Sigma-aldrich, St Louis, USA)	**Activated carbon** ORBO© 32 ® (Sigma-aldrich, St Louis, USA)	**Kepone** Concentration (µg.g^{-1} of DM) (Sigma-aldrich, Supelco)	**Time of maturation** in days
Standard soil (SS)	77.8%	22.2%	-	-	100	21
Soil with DARCO® (AC)	76.2%	21.8%	2%	-	100	21
Soil with ORBO™ (AC)	76.2%	21.8%	-	2%	100	21

CLD was spread over soil as a methanol:water solution (20:80; vol:vol; methanol:water). After spiking solvent traces were evaporated under an extractor hood overnight. Then, artificial soils were moistened with milliQ water to reach 17.5% mass of humidity (wet mass basis). All soils were stored at 20 °C in amber glass vials during three weeks of maturation prior to the first day of CLD-ingestion (day

2.2.2. AC feed supplementation preparation

Two of the three piglet groups exposed to CLD via SS were additionally fed with dough balls containing one of the two ACs: ORBO™ or DARCO®. A mass of AC corresponding to 0.5% of the ration of piglets was introduced in additional dough balls. This mass was chosen according to daily ingested quantities of such materials when feeding animal with commercial AC-enriched feed when applied to prevent gastro-intestinal disorders of animals (Chu et al. 2013a, b). Those AC-enriched dough balls were given to piglets at the same time as CLD contaminated ones (which did not contain any initial AC amendment).

2.2.3. Activated carbons characterization

Specific surface areas (SSA) of both commercialized ACs, ORBO™ and DARCO® were measured at the LIEC laboratory (LIEC, Vandœuvre-lès-Nancy, France). The measurements were performed on the basis of nitrogen adsorption-desorption volumetry at liquid N_2 temperature of 77 K (- 196 °C). Nitrogen adsorption-desorption isotherms were recorded on a Belsorp-mini II set-up (BEL Japan, Inc). The device is equipped with pressure sensors in the range 0-133 kPa. After outgassing the samples at 30 °C during 12 h under a residual vacuum of 0.01 Pa, nitrogen adsorption-desorption isotherms (i.e., volume of nitrogen adsorbed at 77 K vs. relative pressure P/P_0, where P is the equilibrium pressure of the adsorbing gas at that temperature and P_0 is the vapour saturation pressure) were performed using a step-by-step method in the interval of relative pressures, P/P°, extending from 10^{-5} to 0.98. All experiments were carried out with ultra-pure nitrogen (> 99.9995%). The Brunauer–Emmet – Teller (BET) method was used to estimate the SSA, using a 16.3 Å cross-sectional area of nitrogen molecules (Brunauer et al. 1938). The De Boer method (or t –plot, De Boer et al 1965) was carried out to determine microporous volume and external surface area. Micropore filling happens at low and very low relative pressure values, and then includes the domain of the monolayer adsorption on external surface. To be able to distinguish adsorption onto external surface from adsorption into the micropores (pore size<20Á), the experimental isotherm is compared with a reference curve obtained for a non-porous solid, with chemical features and energetic constant as close as possible to the studied matrix.

2.3. Piglet sample collection and chemical analysis of biological matrices

After 10 days of exposure, piglets were anaesthetized by electronarcosis followed by immediate exsanguination. Pericaudal adipose tissue and liver were collected, stored at -20 °C, and freeze-dried. Then, liver samples were analyzed for dry matter (DM) by desiccation (103 °C, 48 h).

The CLD Quantification was performed on both of these biological matrices using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by following the analytical method "ANSES PBM Pest LSA-INS-0164 v5" which was initially developed by the French Agency for

Food, Environmental and Occupational Health and Safety (ANSES, national reference laboratory, Maisons-Alfort Laboratory for food safety). This methodology is the current regulatory analytical reference used in France (French Ministry of Agriculture 2015) to control CLD in foodstuffs in line with the maximum residue levels established in European regulation (Commission of the European Communities 2008). This methodology was described in Yehya et al. (2017) and was performed by the Departmental Analytical Laboratory of Morbihan (LDA 56, Saint-Ave, France). The limit of quantification (LOQ) was 2.0 µg CLD kg ^{1} in these matrices.

Briefly, prior to the extraction step, a ^{13}C ($^{13}C_8C_2Cl_{10}O$, Azur-isotope, Marseille, France, 98% of purity) internal standard of CLD was added to subsamples. Briefly, biological matrices were extracted sequentially. (i) For adipose tissue, 0.5 g of sample were added to 3 ml of a mix of acetonitrile and dichloromethane 75:25 (v:v). After centrifugation (1 200 x *g*, 20 min at -20 °C) the supernatant was extracted. This extraction was performed 2 times. Then, the solvent was evaporated at 40 °C under a nitrogen flux until dry. A mix of 15ml of hexane/acetone 85:15 (v:v) was then added. (ii) For liver, a sample of 2 g was used. Ten mL of hexane:acetone 85:15 (v:v) was added to the sample, which was then grinded using Ultraturrax® (10 000 rpm, 1 min, S25N-10G). After mixing (Vortex® apparatus) and centrifugation (750 x *g*, 3 min) the supernatant was collected.

An alkalinisation step followed by an acidification step were performed to obtain CLD hydrate and to reform CLD, as described elsewhere (Bordet et al. 2007). Analytical grade sodium hydroxide solution (addition of 5 mL of 0.5 M NaOH aqueous solution to supernatant). After a mixing procedure (Vortex® apparatus) and centrifugation (750 x g, 3 min), the supernatant was collected. This step was repeated two times.

The resulting aqueous phase was washed with 5 mL hexane to eliminate fat. The supernatant was collected after centrifugation (750 x *g*, 3 min). CLD was reformed through acidification of the solution by means of sulfuric acid (5mL of 60% solution). A second extraction phase was carried out with hexane:acetone (5mL of 85:15 v/v), followed by mixing (Vortex® apparatus) and centrifugation (750 x *g*, 3 min). This extraction step was repeated 3 times. The organic phase was then rinsed with 2mL of water, before being subsequently evaporated until dry and 1mL of methanol was then added.

Separation of CLD was achieved using a Phenomenex Aqua C18 column (150x2.0 mm 3 µm) and a precolumn Phenomenex Aqua C18 (4x3.0 mm). Two phases were used to perform the separation step: water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Five µL of sample was injected per run and the flow was set to 200 µl/min. After separation, a rinse sequence using acetonitrile was performed. Quantitation by isotope-dilution was performed on API 5500. Parent ions (PI) and child ones (CI) were used to respectively quantify (507/427 Da; PI/CI) and qualify (509/ 429 Da; PI/CI) ^{12}C CLD and quantify (517/436 Da; PI/CI)^{13}C CLD.

2.4. Data analyses

2.4.1. Quality control

In order to optimize the precision of measurements, Belsorp-mini II device is regularly controlled against TiO2 reference material (Community Bureau of reference, SSA=8.23±0.21 $m^2.g^{-1}$). Experimental error is estimated to 0.50 $m^2.g^{-1}$.

CLD quantification in animal tissues was carried out in strict accordance with the COFRAC (French Quality Accreditation Committee) quality accreditation of LDA 56. Values below LOQ (2.0 µg.kg $^{-1}$ of dry matter) were replaced by LOQ value in the data set.

2.4.2. Tissues concentrations of CLD

In order to assess the impact of ACs on CLD bioavailability, an analysis of variances was performed. The experimental unit was the piglet Concentrations of CLD in adipose tissue and liver were compared between the five treatment groups SS versus ORBO or DARCO brought either via amended soil or supplemented feed using the ANOVA procedure and the Tukey–Kramer post -hoc test of R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Differences were considered significant at $P < 0.05$.

2.4.3. Relative bioavailability (RBA) factor calculation

The relative bioavailability (RBA) was used as « the comparative bioavailabilities of different forms of a chemical or for different exposure media containing the chemical (e.g., bioavailability .,of a chemical from soil relative to its bioavailability from water) and is expressed as a fractional relative absorption factor" (NEPI 2000). Statistical analyses were carried out using R (version 3.2.3, R Foundation for Statistical Computing) on CLD concentration using the package agricolae (de Mendiburu 2019). Each piglet was considered as an experimental unit. A 95th confidence interval of concentrations was calculated as described elsewhere (Shafer and Zhang 2013, p. 318).

The RBA was calculated by dividing "CLD -concentrations in one tissue (liver or adipose tissue) after one treatment (DARCO® or ORBO ™) either in soil or in feed" by "CLD concentrations obtained in the same tissue after SS treatment (100% reference)", adapted from a method previously described (Wittsiepe et al. 2007; Delannoy et al. 2014a; Yehya et al. 2017). Linearity between the CLD-dose of exposure and CLD concentrations in the organ was a prerequisite of this method (Littell et al. 1997). This linearity was demonstrated previously for CLD in piglets (Bouveret et al. 2013).

The corresponding equation to calculate RBA of CLD after one AC -treatment in the liver is provided below .

$$RBA_{AC\ treatment;liver} = \frac{C°_{AC\ treatment\ ;\ liver}}{C°_{SS\ treatment\ ;\ iver}}$$

$RBA_{AC\ treatment;liver}$: Relative bioavailability of CLD after one AC – treatment in the liver

$C°_{AC\ treatment;liver}$: CLD concentration in liver after one AC treatment

$C°_{SS\ treatment;liver}$: CLD concentration in liver after SS treatment.

As each part of the division comprises uncertainties, a 95th confidence interval of the RBA factor was calculated via the propagation of uncertainty rule . The corresponding equation is provided above:

$$\frac{\Delta_{RBA}}{RBA} = \frac{\Delta_{C°_{AC\ treatment}}}{C°_{AC\ treatment}} + \frac{\Delta_{C°_{SS\ treatment}}}{C°_{SS\ treatment}}$$

Δ_{RBA}: Relative uncertainty of relative bioavailability after one AC − treatment

RBA: Relative bioavailability

$\Delta_{C°_{AC\ treatment}}$: Relative uncertainty of concentration found in one tissue after one AC treatment

$C°_{AC\ treatment}$: CLD concentration after one AC treatment

$\Delta_{C°_{SS\ treatment}}$: Relative uncertainty of CLD concentration found in one tissue after SS treatment

$C°_{SS\ treatment}$: CLD concentration found in one tissue after SS treatment

2.4.4. Calculation of the transfer reduction factor

A factor of CLD transfer reduction by AC was determined as the ratio between concentrations obtained after SS treatment and concentrations obtained using ORBO™ and DARCO® bought either via soil amendment of in feed, in the same biological matrix. In order to be conservative, this factor was minimized using the lowest and highest values of the 95th confidence interval of concentrations of CLD. Details of the calculations are provided below using concentrations of CLD in liver:

$$R_{AC\ treatement\ ;liver} = \frac{Min_{95}\ C°_{liver}\ AC\ treatment}{Max_{95} C°_{liver}\ SS}$$

$R_{AC\ treatement\ ;\ liver}$: factor of CLD retention by AC DARCO® using concentrations of CLD in liver

$Min_{95}\ C°_{liver}\ AC\ Treatment$: lowest value of 95th confidence inter val of CLD concentration in liver (statistical model explained in 1.4.3)for SS with DARCO© treat − ment

$Max_{95}\ C°_{liver}\ SS$: highest value of 95th confidence interval of CLD concentration in liver (statistical model explained in 1.4.3) $for\ SS\ treatment$

3. Results

The impact of these two microporous ACs to limit CLD contamination of piglets' organs through two contrasted strategies (to provide AC via incorporation in feed or via soil amendment) was assessed. The, the identification of the two ACs surface microstructure through inert gas adsorption such as N_2 was a prerequisite to determine their SSA .(Sing 1982).

3.1. CLD concentration determination in biological matrices and CLD relative bioavailability (RBA) calculation

CLD concentrations in biological matrices showed important differences between the treatment groups as presented in Figure 3. As expected, one of the highest CLD concentrations was obtained in the SS group (control) for both matrices: 90 ± 5 ng.g^{-1} of DM (adipose tissue; mean ± SD) and 1010 ± 103 ng.g^{-1} of DM (liver; mean ± SD). Interestingly, similar levels as SS group were found in both groups where ACs were introduced into the feed, regardless the type of AC used (for ORBO™: 86 ± 4.4 and 1040 ± 250 ng.g^{-1} of DM for adipose tissue and liver respectively; for DARCO®: 98 ± 0.8 and 1030 ± 180 ng.g^{-1} of DM for adipose tissue and liver respectively; Figure 3). In contrast, significantly lower CLD concentrations were obtained in organs when animals were exposed to DARCO® soil: 18.9 ± 4.3 ng .g $^{-1}$g of DM

Table 2: Relative bioavailability factors

1- Relative bioavailability factors of CLD in biological matrices (% and 95th confidence interval)

	Adipose Tissue	Liver
ORBO™ in feed	100%#	100%#
DARCO® in feed	100%#	100%#
ORBO™ in soil	0.7%¤ [NA]	1.4% [1.0% -1.8%]
DARCO® in soil	21.0% [13.5% - 28.34%]	27.4% [14.7% - 40.1%]

2- Reduction factor (%)

	Adipose Tissue	Liver
ORBO™ in feed	0%#	0%#
DARCO® in feed	0%#	0%#
ORBO™ in soil	85%	86%
DARCO® in soil	64%	63%

Values in brackets indicates 95% confidence interval (3.18 x SE). SE were calculated *via* propagation of errors formula. (n=5)
Reduction factor are calculated as described in material and method section.

(adipose tissue; mean ± SD); 276 ± 35 ng.g^{-1} of DM (liver; mean ± SD), and the lowest CLD concentrations were found when piglets were exposed to ORBO™ aged soil resulting in non-quantifiable levels in adipose tissue (< 2 ng/g DM) and 13.7 ± 1 ng.g^{-1} DM in liver (mean ± SD) (Figure 3). Further investigation using a post -hoc Tukey test demonstrated that treatment groups from strategy of sequestration by amendment of soil were distinct from the other ones ($p<0.001$). The ANOVA analyses revealed a significant strategy effect (*cf* Figure 3) for all biological matrices ($p<0.0001$). The activated carbon effect was significant in adipose tissue demonstrating a significant difference between DARCO® and ORBO™; ($p=0.006$). This significance was not achieved in liver ($p=0.11$).

Secondly, the RBA factors were calculated in order to estimate the CLD bioavailable fraction depending on the following treatments: ORBO™ or DARCO®, incorporated either in piglet feed or directly in a contaminated soil. Statistical analyses were performed to obtain 95th confidence interval as presented in Table 3. Concerning "FeedACs" groups, RBA was not different from 100% (Table 3) as concentrations found in organs for these both treatments did not statistically differ from SS group (Figure 3a, b). For "soil ACs" groups compared to SS, the DARCO® treatment showed RBAs of 21.0% and 27.4% (respectively from adipose tissue and liver), whereas the ORBO™ treatment revealed RBAs of 0.7% (maximum value from adipose tissue) and 1.4% (liver) (Table 2). Overall, similar RBA factors were obtained for adipose tissue and liver within each treatment group (Table 2).

At last, in order to assess the CLD sequestration efficiency by ACs during the digestive processes, a sequestration factor was calculated representing a conservative proportion of the CLD retained by the ACs during digestive processes (Table 2). DARCO® exhibited a reduction of 64% (adipose tissue) and 63% (liver tissue) (Table 2) displayed a greater reduction of 85% (adipose tissue; based on LOQ) and 86% (liver) (Table 2).

3.2. Characterization of activated carbons

The isotherms obtained from N_2 adsorption-desorption on ORBO™ and DARCO® are shown on Fig. 3. As depicted in Fig. 3a, the isotherm obtained from gaseous sorption on ORBO™ was characterized by a rapid increase at low

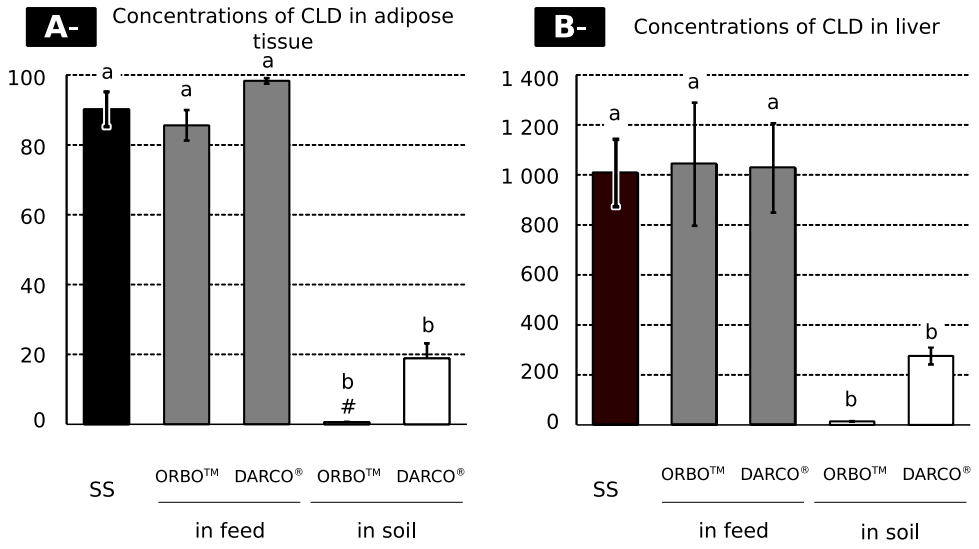

Figure 2: Concentrations of CLD in biological matrices (ng of CLD per g of DM)
Concentrations of CLD are expressed in ng.g^{-1} of DM. Values correspond to the mean ± SD (n=4). Mean values with different superscript letters (a, b) are statistically different ($P<0.05$). Statistical analysis was performed using the two-way ANOVA procedure of R software and Tukey post-hoc test. Two effects were used in the model: AC (none, ORBO and DARCO) and strategy of sequestration (none, in feed, in soil). Both effects were significant ($p<0.0001$) and RMSE =6.97 for adipose tissue) wand RMSE = 530. (n=4). Interaction effect was not significant ($p>0.10$)
RMSE: Root means square error.
#: values are below limit of quantification.

relative pressure values (in the domain of $P/P_0 < 0.1$), indicating an instantaneously monolayer coverage of micropores (Sing 1982). By contrast, the latter observations suggest that the isotherm reaches a plateau with only little adsorption occurring after micropore filling. Therefore, according to the IUPAC classification, the isotherm from ORBO™ exhibited a typical Type I profile (Sing 1982). This is characteristic of microporous adsorbents with a monomodal pore size distribution which is dominated by an average pore diameter below 2 nm (Sing 1982). The isotherm obtained from DARCO® also exhibited type I characteristics (Fig. 3), indicating a micropore-dominant microstructure (Sing 1982). However, the larger quantity of adsorbed N_2 at higher relative pressure values (i.e. in the domain of $P/P_0 > 0.8$, Figure 3) suggests a transition from a monolayer to a multilayer gaseous adsorption phenomenon (and probably N_2 capillary condensation into wide pores (Branton and Bradley 2010).

Hence, the total S_{BET} and pore volume of ORBO™ reach 1130 m^2.g^{-1} and 260 cm^3.g^{-1} respectively (Table 3). These values were about 1.4 times higher than those of

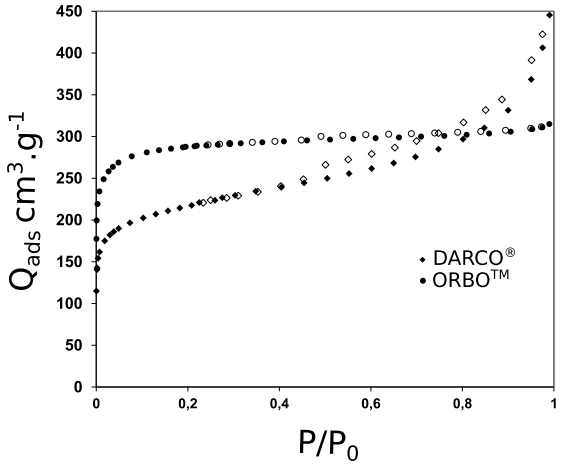

Figure 3: Adsorption isotherms of nitrogen gas on ORBO™ and DARCO® activated carbons at 77 K.
Adsorption of N_2 was performed at 77K on a Belsorp-mini II device (BELJAPAN, Inc Chemicals, JAPAN). Both isotherms exhibit a Type IV profile according to the IUPAC classification.
Q_{ads} : quantity of N_2 adsorbed on activated carbon ($cm^3 .g^{-1}$)

DARCO®, which presented a total S_{BET} of 790 $m^2.g^{-1}$ and a pore volume of 180 $cm^3.g^{-1}$ (Table 3). As DARCO® and ORBO™ presented typical characteristics of microporous media as defined in IUPAC classification (Sing 1982), the t-plot method was secondly used to distinguish the relative part of the total surface due to micropores in these both matrices (de Boer et al. 1966). ORBO™ presented a higher surface of microporosity (970 $m^2.g^{-1}$, 85% of the total surface) than DARCO® (490 $m^2.g^{-1}$, 58% of the total surface) (Table 3). These differences in surface porosity could be explained by a greater N_2 adsorption onto the external surface of the DARCO® AC in comparison with ORBO™

Table 3: Adsorption characteristics of the ACs

	BET surface area	Total pore volume	Surface total	Microporosity surface	Non Microporous Surface
	$m^2.g^{-1}$	$cm^3.g^{-1}$	$m^2.g^{-1}$	volume $cm^3.g^{-1}$	volume $cm^3.g^{-1}$
DARCO® (AC)	793.8±14.5	181.6	829.9	486.8	382.1
ORBO™ (AC)	1126.3±11.9	257.7	1136.3	971.8	242.4

4. Discussion

4.1. Ineffectiveness of the AC feed supplementation strategy

As expected, the highest concentrations of CLD in tissues were found in the SS group. This result is in accordance with previous data obtained with the same soil and for different organic pollutants (Delannoy et al. 2014a, b) and CLD (Yehya et al. 2017). Compared with SS, the feed supplementation strategy (Feed-ACs) was not effective to reduce CLD transfer from soil to piglets. Two main assumptions could explain this absence of sequestration:

The administration of ACs via feed was performed right after the CLD exposure of piglets to favor the contact between ACs and CLD. As no reduction of CLD transfer to piglet was observed, the contact time between the CLD and ACs in the intestinal lumen was probably too short to allow the CLD adsorption on ACs. As recently shown by Wang et al. (2016) with Poly-Chlorinated Biphenyls in bi-solute and chemical mixture systems, competition may occur for sorption sites on porous carbonaceous matrix, as well as pore filling mechanisms by non-target organic compounds. As feed components and digestive enzymes are abundant in the gastrointestinal chime, such competition process could limit the CLD adsorption effectiveness of ACs in the digestive tract.

These both parameters, contact time, and competition, may have synergistically alleviated adsorption (Knettig et al. 1986) of CLD in digestive compartments.

4.2. Efficiency of the AC soil amendment strategy to limit CLD transfer to piglets

When using the soil amendment strategy, CLD remained mainly adsorbed on ACs during the digestive processes. It has to be recalled that the duration of contact between ACs and CLD in soil was of 21 days (i.e., a much longer time than the digestive process in piglets which is not longer than 24 h). Adsorption of CLD onto ACs appears to be of particular efficiency even if differences between

both ACs could be showed. Indeed, the characteristics of ACs used are of particular importance. In the present study, physical characteristics of ACs clearly show that both ACs exhibit a microporosity which could be characterized by the narrowness of their pores (< 2 nm). This particular narrow pore range matches the size of CLD (i.e. 0.652 nm) (Durimel et al. 2013). It should be expected a highly effective entrapment of this hydrophobic organic contaminant, potentially resulting in greater CLD sequestration with ORBO™ compared to the use of micro- to mesoporous DARCO®. This element is in line with RBA results, as the most important CLD RBA limitation was obtained with the highly microporous coconut shell-derived ORBO™ AC (Fig. 2a, b), whereas a less efficient retention was obtained from DARCO® AC. This latter AC comprises a substantial part of meso- to macro- are less effective in limiting contact with the chyme.

Mechanistically, this sequestration of CLD by ACs resulted from adsorption of this POP onto ACs surface. This adsorption relies on physico-chemical weak bindings between chemical functions of CLD and the surface of AC. Such a mechanism is also depicted when a POP is adsorbed onto different soil organic matters (Woignier et al. 2012; Ahmad et al. 2014; Delannoy et al. 2014b). It has been theorized that physico-chemical interactions between sorbent media and the pollutant could likely be disrupted along the digestive tract course due to the peculiar variation of conditions in the distinct gastro-intestinal compartments (such as pH, chemical and enzyme activities, and micellar formation). This ultimately leads to a release of POPs and their solubilisation of organic contaminants in the chime (Delannoy et al. 2014b). Due to the particular binding of POPs on ACs and its structure, such a release should be very limited.

When AC was incorporated in feed, there was no reduction of CLD transfer to piglets. In contrast, when AC were amended to soil major reductions in CLD, concentrations were found in piglet adipose tissue or in livers. Thus, relative bioavailability reduction factors varied from 63 to 86% in liver and 64 to 85% in adipose tissue for DARCO® and ORBO™ respectively.

The coconut shell AC efficiency (i.e. ORBO™) to immobilize CLD was of particular interest since coconut shell is a low-cost and renewable AC source especially abundant in the French West Indies (Arsène et al. 2013), by contrast with conventional raw material of degraded plant matter like peat (i.e., DARCO® precursor). Then, the implementation of a CLD sequestration strategy based on local biomasses may be a real remediation alternative.

It has to be noticed that the interesting results obtained in artificial soils mays not be representative to the soils found in French West Indies (volcanic and tropical). Indeed, the contamination in antillean soils is ancient: 30 years. So, the residual chlordecone retained in tropical soils may not be easily available for adsorption onto ACS surface like in your experience. The pore features (specific surface area and pore size) of the volcanic clay could limit the chlordecone adsorption to the ACs surface. Therefore, this experiment should also be performed in in situ conditions.

5. Conclusion

Overall, this study gives interesting insight in the area of the CDL sequestration by ACs. In the study it can be noticed that a significant decrease of CLD concentrations in liver and adipose tissue was observed when the soil was amended by ACs. This decrease was particularly important for the coconut shell activated carbon where relative bioavailability was found lower than 1.8% for both tissues. In contrast, no reduction of relative bioavailability was noticed when ACs were introduced directly in the feed.

6. References

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