JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Relative Bioavailability of Tropical Volcanic Soil-Bound Chlordecone in Piglets

Cécile Bouveret, Guido Rychen,* Sylvain Lerch, Catherine Jondreville, and Cyril Feidt

Université de Lorraine, UR Animal et Fonctionnalités des Produits Animaux, EA 3998, USC INRA 340, ENSAIA, 2 avenue de la Forêt de Haye TSA 40402, F-54518 Vandoeuvre-lès-Nancy Cedex, France

ABSTRACT: The application of chlordecone (CLD), a chlorinated polycyclic ketone pesticide, until 1993 in the French West Indies has resulted in long-term pollution of agricultural soils (10% of them exceed 1 mg kg^{-1}). The aim of this study was to assess the impact of two tropical volcanic soils, an andosol and a nitisol, on CLD availability in piglets, using the relative bioavailability (RBA) approach. For both soils and relative to an oil matrix, RBA was close to 100%, indicating that CLD was not retained in the soil matrices during the piglet digestive process. Additionally, after a 14 day exposure period, liver and subcutaneous fat CLD concentrations exceeded the maximum residue limit (10 μg kg^{-1} of fresh matter and 100 μg kg^{-1} of fat for liver and subcutaneous fat, respectively) beyond a CLD ingestion of 2.1 and 6.8 μg CLD kg^{-1} of body weight per day, respectively. Thus, rearing practices in CLD-contaminated areas should avoid involuntary soil ingestion by farm animals.

KEYWORDS: *relative bioavailability, chlordecone, andosol, nitisol, piglet*

INTRODUCTION

Chlordecone (CLD) is a chlorinated polycyclic ketone pesticide, which was used from 1971 until 1993 in Martinique and in Guadeloupe (French West Indies) to fight the banana black weevil (*Cosmopolites sordidus*). The application of this organochlorine insecticide for more than 20 years has resulted in long-term pollution of soils[1] (10% of agricultural soils in Martinique and in Guadeloupe exceed 1 mg CLD kg^{-1} of dry matter (DM)). Such soil pollution has resulted in a subsequent contamination of water and food resources, including fish, root vegetables, and terrestrial animal products (meat, eggs).[2−4] Thus, maximal values were about 144 and 1093 μg CLD kg^{-1} of fresh matter (FM) in meat of poultry and eggs, respectively,[5] and 48 μg CLD kg^{-1} of FM in other meat types.[6] Furthermore, earlier studies showed that the main CLD exposure source for local inhabitants is thought to be the ingestion of contaminated foodstuffs.[2,7] Such context is of great concern regarding human health; CLD is suspected to negatively affect fetal and postnatal development[8,9] and to increase the risk of prostate cancer.[10]

In French West Indies, the main soil types on which CLD was spread are volcanic andosol, ferralsol, and nitisol.[1] CLD affinity to soil particles depends on clay level and structure as well as organic matter content.[1,11,12] In fact, nitisol contains a high content of halloysite, which is an 1:1 aluminosilicate clay mineral, and is structured as a superposition of clay layers displaying a low porosity.[13] In contrast, andosol has a high content of allophane, which is an amorphous clay, and presents a fractal microstructure displaying a high microporosity (i.e., large pore volume and high surface area).[13] When combining chemical retention and physical trapping, Woignier et al.[12] showed that in terms of CLD transfer from soil to plants, CLD is less retained in nitisol than in andosol. Moreover, ferralsol properties appear intermediate between nitisol and andosol. However, in terms of CLD transfer from soil to farm animal tissues and products, Jondreville et al.[14] demonstrated in laying hens (*Gallus gallus domesticus*) that CLD was retained neither in andosol nor in nitisol and was efficiently extracted by the digestive tract of this poultry species. Such a result is of great concern regarding the food safety of animal products. Thus, there is a need to acquire new data on the transfer rate of CLD from soil to animal products in other farm animal species reared directly in contact with soil in French West Indies.

In the present study, the piglet was selected as a complementary monogastric animal model with digestive functions different from those of laying hens. Indeed, there are mechanical processes and enzymatic and microflora functions that strongly differ between the two species.[15] In particular, the poultry digestion process is characterized by retrograde movements of the chyme. Such digestive characteristics may have improved the CLD extraction process in laying hens as described by Jondreville et al.[14] These mechanical processes do not exist in pigs, where the only digestive movement is the oral−aboral one. Moreover, after weaning, the digestive flora of piglets remains unstable and is considered a key factor in causing potential enteric disease (Pelenc[16]). Montagne et al.[17] observed a spatiotemporal sequence of events concerning the morphology, physiology, and ecology of the gut of piglets during the 2 weeks following weaning, suggesting gut postweaning changes. Aumaître et al.[18] also clearly demonstrated that weaned piglets do not possess a full digestive capacity in terms of enzymatic and bacterial functions between weaning and the age of 8 weeks. Thus, the hypothesis of this study was that the soil-bound CLD would be less extracted by the digestive process of weaned piglets than by adult laying hens.[14] In practice, this animal model is also relevant because pig products are widely consumed by Martinican and Guadeloupean populations (around 25% of the meat

Received: February 13, 2013
Revised: August 29, 2013
Accepted: August 30, 2013
Published: August 30, 2013

Table 1. Composition and Analytical Characteristics of the Experimental Diets (per Kilogram As Fed)

	composition (g)						analytical characteristics		
	first age weaned piglet feed[a]	sand	blank oil	andosol[b]	nitisol[c]	spiked oil[d]	dry matter (g)	chlordecone (μg)	expected concn, chlordecone (μg)
adaptation diet	945	50	5				894	<10	<10
exposure diets									
andosol	915	70	5	10			901	45	46
	915	60	5	20			905	87	92
	915	50	5	30			904	129	138
	915	40	5	40			905	192	184
nitisol	915	60	5		20		904	38	48
	915	40	5		40		902	104	96
	915	20	5		60		900	125	144
	915	0	5		80		904	172	192
oil	915	80	3.80			1.20	906	30	46
	915	80	2.53			2.47	904	57	95
	915	80	1.27			3.73	907	105	143
	915	80	0.00			5.00	907	119	192

[a]Commercial feed (Spiggy, Lorial, Laxou, France). [b]Dry matter and chlordecone concentrations in andosol were 860 g kg^{-1} and 4.6 mg kg^{-1}, respectively, fresh matter basis. [c]Dry matter and chlordecone concentrations in nitisol were 835 g kg^{-1} and 2.4 mg kg^{-1}, respectively, fresh matter basis. [d]Rapeseed oil was spiked so that 5.0 g of oil delivers the same dose of chlordecone as 80 g of nitisol or 40 g of andosol, that is, 38.4 mg kg^{-1} of oil.

consumption[19]). To our knowledge, the few available data on CLD measurements in pig products of the French West Indies have been performed on farms where animals were reared inside without access to soil.[2,6] However, in 2000, the French agricultural inventory revealed that half of the total number of sows from Guadeloupe was bred by small farmers owning three sows or fewer and rearing their animals outside, directly in contact with soil.[20] Therefore, it appears of great importance to evaluate the bioavailability of soil-bound CLD in pig, because pigs reared outside may potentially ingest CLD-contaminated soil.[21] The aim of this study was to assess the impact of two tropical volcanic soils, an andosol and a nitisol, on soil-bound CLD availability in the piglet, a young mammal species potentially exposed to CLD, using the RBA approach. Given the contrasting properties of these two soils, the hypothesis was that CLD in andosol would be less available to piglets than CLD in nitisol.

MATERIALS AND METHODS

Chemicals. Chemicals were CLD (Kepone 49046, 99.9% purity determined by GC flame ionization detector, Sigma-Aldrich, St. Louis, MO, USA), and phenyl- and dimethyl-polyxyloxane (origin DB-5MS, Agilent Technologies, Wilmington, DE, USA). The remaining standard chemicals were used inside the LDA26 laboratory, which performed the CLD analysis.

Contaminated Soils and Spiked Oil. The two soil types used in the present study originated from batches already used and described in a previous experiment.[14] They correspond to two surface (A horizon) tropical volcanic soils, one andosol and one nitisol, that were collected in former banana fields in Martinique, where CLD was commonly spread before 1993.

Rapeseed oil (Lesieur, France) was spiked with 38.4 mg kg^{-1} of CLD to provide the same dose of CLD with 5 g of spiked oil rather than with 80 g of nitisol or 40 g of andosol (i.e., 192 μg). CLD was dissolved in rapeseed oil at room temperature by magnetic agitation (90 min) followed by sonication (40 min).

Animals and Diets. The study was carried out at the experimental facilities of the Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (Vandoeuvre-lès-Nancy, France), under the guidelines of the French Ministry of Agriculture for Animal Research.[22] Three successive series of 13 piglets (issued from a terminal crossbreeding Piétrain × (LargeWhite × Landrace), September 15 and October 6 and 27, 2011) from the GAEC des Terres-Noires (Haroué, France) were used (total of 39 piglets, 28 days old and 7.1 ± 1.8 kg of body weight (BW); mean ± standard deviation (SD)). For each animal series, the experiment began with a 5 day adaptation period, which was followed by a 14 day exposure period. During the adaptation period, all piglets received a diet free from CLD and containing 94.5:5.0:0.5 (w/w) of first age weaned piglet commercial feed (Spiggy, Lorial, Laxou, France), Fontainebleau sand (Prolabo, France), and pure rapeseed oil (Lesieur), respectively. The feed used for the adaptation period has been formulated to be intermediate between the feed given at the farm level (no soil) and the experimental with 8% soil.

At the end of the adaptation period, one piglet (used to check the absence of CLD at the farm level) was slaughtered by electronarcosis followed by exsanguination. During the exposure period, the 12 remaining piglets received one of the 12 experimental CLD-contaminated diets. All contaminated diets were based on 91.5:8.0:0.5 (w/w) corresponding to first age weaned piglet commercial feed, Fontainebleau sand, or contaminated soil and unspiked or spiked rapeseed oil, respectively. The 12 CLD-contaminated diets consisted of 4 andosol, 4 nitisol, and 4 spiked oil diets, containing increasing amounts of andosol, nitisol, and spiked oil, respectively. Composition and CLD concentration of all diets are provided in Table 1. Individual ingredients of each diet were mixed and pelleted at INRA UE1295 PEAT (Nouzilly, France) into pellets of 2 mm diameter. The daily allowance of feed was adjusted individually, every 3 days, to 4% of BW, and offered in one meal at 8:30 a.m. Piglets were housed in individual cages in a room at constant temperature (24 °C) and naturally illuminated and were allowed free access to water. At the end of the exposure period, the morning before feeding, each piglet was slaughtered by electronarcosis followed by exsanguination.

Measurements, Sampling, and Chemical Analyses. Feeds offered individually and feed refusals were weighed daily. Piglet BW was measured the morning before feeding at the beginning and at the end of the adaptation period, every 3 days during the exposure period, and the day of slaughter. Subsamples of each of the two soils and of each of the 13 experimental diets (1 adaptation and 12 experimental diets) were collected (amount of 30 g at each ration preparation for each pig and pooled), sieved through a 1 mm screen, and stored at −20 °C before DM and CLD analyses. After piglet slaughter, liver and subcutaneous fat were collected, weighed, and then stored at −18 °C before DM and CLD analyses. Additionally, liver subsamples were lyophilized (Alpha 2-4 LSC, Christ, Osterode am Harz, Germany) and

sieved through a 1 mm screen (A11 basic, IKA, Staufen, Germany), before CLD analysis.

Soils were analyzed by INRA US10 LAS (Arras, France) for physical properties and organic carbon (OC) content according to NF X 31-107[23] and NF ISO 10694,[24] respectively. Soils, diets, and liver DM contents were measured by drying at 103 °C until constant weight. CLD analyses were performed by the Laboratoire Départemental d'Analyses de la Drôme (LDA26, Valence, France), which works under the French accreditation committee COFRAC. CLD concentration was determined according to the method of Cabidoche et al.[1] in soils and according to the method fo Bordet et al.[25] in diets and in animal tissues (liver and subcutaneous fat). Briefly, for soils, CLD was extracted using an accelerated solvent extraction system with an extraction solvent composed of dichloromethane and acetone (50:50, v/v). For experimental diets (2 g, fresh matter (FM) basis), liver (2 g, DM basis), and subcutaneous fats (0.5 g, FM basis), CLD was extracted using a "cold centrifugation extraction" technique with 15 mL of an extraction solvent composed of hexane and acetone (85:15, v/v). ^{13}C-CLD was used as an internal standard. A 5 mL sodium hydroxide solution (0.5 M) was added twice to the extract to transform CLD into CLD hydrate. This compound is soluble in aqueous phase, which was washed with 5 mL of hexane to eliminate fat. Then, CLD was re-formed through acidification of the solution by means of sulfuric acid (5 mL, 60%, v/v). Finally, CLD was extracted using 15 mL of a solvent made of hexane and acetone (85:15, v/v). CLD was quantified by gas chromatography tandem mass spectrometry (GC-MS/MS) using a gas chromatograph (model 3800, Varian, Palo Alto, CA, USA) equipped with a triple-quadrupole mass detector (model MS1200, Varian) and a 30 m fused silica capillary column (i.d. = 0.25 mm) with a 0.25 μm film of 5% phenyl- and 95% dimethylpolyxyloxane. Helium was used as the carrier at a flow rate of 2 mL min^{-1}. The temperature increased from 50 to 250 °C at a rate of 150 °C min^{-1} for the on-column injection. The column was programmed from 80 °C, held for 1 min, and increased by 20 °C min^{-1} to a final temperature of 280 °C that was maintained for 10 min. The triple-quadrupole mass detector operated at 250 °C in electron impact ionization mode. Selected ion monitoring mode was used, where two transitions were systematically applied, 272 → 237 (−20 eV) for quantification and 274 → 239 (−15 eV) for confirmation. Limits of quantification were of 10, 10, 10, and 20 μg kg^{-1} in soil, diet, liver, and subcutaneous fat, respectively (DM basis for liver and in FM basis for soil, diet, and subcutaneous fat). Values below the limit of quantification and above the limit of detection were considered equal to the limit of detection (5, 5, 5, and 10 μg kg^{-1} in liver (DM basis), and in soil, diet and subcutaneous fat (FM basis), respectively), and values below the limit of detection were considered equal to zero in the following calculations. Concentration of CLD in liver were analyzed in micrograms per kilogram of DM and then converted into FM through the determination of DM in liver samples.

Calculations and Statistical Analyses. The individual amount of CLD ingested daily relative to the piglet BW during the exposure period was calculated from (1) the measured CLD concentration in experimental diet, (2) the average amount of feed ingested by day, and (3) the average piglet BW during the exposure period (mean of the individual BW recorded at the beginning and at the end of the exposure period).

All data were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS software (version 9.1, SAS, Cary, NC, USA). The statistical model included treatment (12 experimental diets) and series (3 successive series) as main effects, using piglet as experimental unit. Additionally, the BW at the end of the exposure period was included in the model as a covariate term, to take into account differences in growth rate between piglets. Treatment differences were determined on the bsis of Student tests and declared significant at $P \leq 0.05$.

The bioavailability of CLD present in each soil relative to CLD present in oil was estimated from CLD concentration in liver and in subcutaneous fat by means of the slope ratio method. This method involves a one-way analysis of covariance using the GLM procedure of SAS. First, two assumptions were sequentially tested for validity of the model: (i) linearity of the responses of CLD concentration in target tissues to ingested CLD per kilogram of BW for each matrix (andosol, nitisol, and oil); and (ii) equality of the intercepts for the three lines (common intercept). After these assumptions were checked, the regression of CLD concentration in liver and in subcutaneous fat to the amount of ingested CLD per kilogram of BW was fitted for each of the three matrices. Treatment differences were determined on the basis of Student tests and declared significant at $P \leq 0.05$. RBA of CLD present in each soil was calculated from liver and subcutaneous fat data as the ratio of the slope of the response fitted with each soil to the slope of the response fitted with oil. The standard error of RBA and its 95% confidence limits were calculated according to the method of Littell et al.[26]

RESULTS AND DISCUSSION

Soil and Diet Compositions and Piglet Performances. As already described in a previous study,[14] andosol contained almost 2 times more OC and CLD than the nitisol but >10 times less clay, although in this case the quality of the clay (allophane versus halloysite) seems more important than its quantity. Despite the differences of OC and CLD content, the CLD/OC ratio was almost the same for both soils.

As expected, CLD could not be quantified in the adaptation diet (<10 μg kg^{-1}, Table 1). CLD concentrations measured in the experimental andosol and nitisol diets were close to the target concentrations calculated from the expected CLD level in the diet after inclusion of andosol or nitisol. However, CLD concentrations recorded in oil diets were lower than the expected concentrations, with a ratio CLD analyzed/CLD expected of 0.65 (n = 4). CLD concentrations ranged between 45 and 192 μg kg^{-1}, between 38 and 172 μg kg^{-1}, and between 30 and 119 μg kg^{-1} (FM basis) for andosol diets, nitisol diets, and oil diets, respectively (Table 1).

During the exposure period, the daily ingestion of feed was unaffected by treatment (357 ± 93 g day^{-1} and 38 ± 1 g kg^{-1} of BW day^{-1}, mean ± SD, $P > 0.10$). Daily ingestion of CLD ranged between 1.5 and 7.3 μg kg^{-1} day^{-1}, between 1.4 and 6.7 μg kg^{-1} day^{-1}, and between 1.1 and 4.5 μg kg^{-1} of BW day^{-1} for andosol diets, nitisol diets, and oil diets, respectively. Additionally, the different treatments had no effect on piglet BW at the end of the exposure period (10.7 ± 2.8 kg, mean ± SD, $P > 0.10$) and on the average daily gain during the exposure period (196 ± 63 g day^{-1}, mean ± SD, $P > 0.10$). Faroon et al.[27] also reported no adverse effects on rat BW after 2 years of exposure to a CLD ingestion level of 50 μg kg^{-1} of BW day^{-1}, a dose 7 times higher than the highest dose (7.3 μg kg^{-1} of BW day^{-1}) used in the present experiment.

Chlordecone Concentrations in Liver and Abdominal Subcutaneous Fat. CLD was not detectable in either liver or subcutaneous fat (lower than the limit of detection, i.e., <5 and <10 μg kg^{-1} of DM or FM, respectively) of the three piglets slaughtered after the adaptation period. This result was expected, as piglets were issued in an environment free from CLD (Haroué, Lorraine region, northeastern France) and received a diet free from CLD during the adaptation period. The CLD concentrations in liver and in subcutaneous fat of all the other piglets, which received CLD-contaminated diet, are given in Figures 1 and 2, respectively. CLD was detected in all liver and subcutaneous fat samples (i.e., higher than the limit of detection). However, CLD concentrations were below the limit of quantification of 20 μg kg^{-1} of FM in seven subcutaneous fat samples (two samples with andosol or nitisol and three samples with oil treatments containing the lowest CLD concentrations).

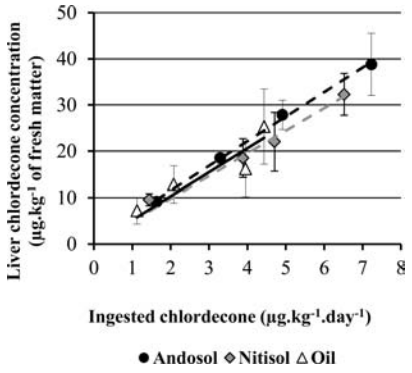

Figure 1. Response of chlordecone (CLD) concentration in liver (values are means ± standard error; $n = 3$) to the amount ingested originating from andosol, nitisol, or rapeseed oil. Linear models are calculated from the parameters presented in Table 2 for andosol in black dashed line, for nitisol in gray dashed line, and for oil in black continuous line.

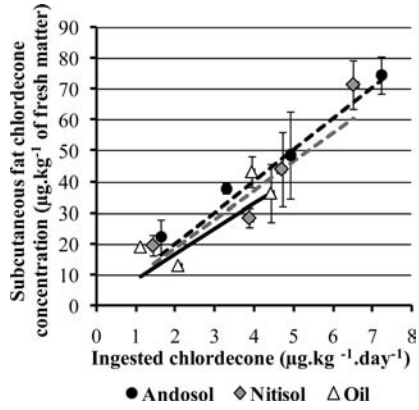

Figure 2. Response of chlordecone (CLD) concentration in subcutaneous fat (values are adjusted means ± standard error; $n = 3$ for a 10.7 kg piglet) to the amount ingested originating from andosol, nitisol, or rapeseed oil. Linear models are calculated from the parameters presented in Table 2 for andosol in black dashed line, for nitisol in gray dashed line, and for oil in black continuous line.

Whatever the type of diet (andosol, nitisol, or spiked oil), CLD concentrations in both liver and subcutaneous fat clearly increased with the CLD concentration of the diet, reaching maximum values of 52 μg kg^{-1} of FM in liver and 75 μg kg^{-1} of FM in subcutaneous fat (Figures 1 and 2). To compare liver and fat levels of CLD, a mean of 5% of fat in liver FM[28] and a mean of 65% of fat in subcutaneous fat FM[29] were used to express CLD level in both liver and subcutaneous fat per kilogram of fat. Thus, liver contents of CLD were found to range from 78 to 1040 μg kg^{-1} of fat, whereas in subcutaneous fat the values ranged from 15 to 115 μg kg^{-1} of fat. As expected, concentrations of CLD were much higher in liver than in subcutaneous fat, expressed on fat basis. This result is in line with previous observations in rat and mouse[30,31] or in humans.[32]

Chlordecone Relative Bioavailability. Before establishing the RBA of CLD, we checked that whatever the tissue (liver and subcutaneous fat), the response to ingested CLD within each of the three matrices (andosol, nitisol, and oil) was not quadratic (ingested CLD × ingested CLD (matrix), $P > 0.10$), and that the intercepts adjusted for the three lines were equal (Table 2; matrix, $P > 0.10$). In the absence of any significant quadratic component in the models, the response to graded levels of ingested CLD from any of the three matrices was proven to be linear (Table 2 and Figures 1 and 2; ingested CLD, $P < 0.001$). Thus, coefficients of determination (R^2) of the fitted models relative to liver and subcutaneous fat were 0.82 and 0.74, respectively (Table 2). Whatever the tissue (liver or subcutaneous fat), the slopes of the three lines could not be differentiated (Table 2 and Figures 1 and 2, ingested CLD × matrix, $P > 0.10$). Similarly, the slope fitted within each of the two soils could not be differentiated from the slope fitted with oil (andosol vs nitisol and nitisol vs oil, $P > 0.10$). The estimates of RBA derived from liver and subcutaneous fat responses were 1.10 and 1.22, respectively, in andosol and 0.97 and 1.13, respectively, in nitisol (Table 2). None of these slope ratios could be differentiated from 1 (Figures 1 and 2, $P > 0.10$).

Contrary to our hypothesis regarding RBA, there was no significant difference in CLD bioavailability from andosol and from nitisol. Thus, even for andosol, known for its high microporosity resulting in a potentially high retention of CLD by a physical trapping,[12,13] the piglet digestive functions appeared efficient enough to extract CLD. Therefore, our results demonstrate that whatever the physical nature of contaminated French West Indies soils, CLD may be extracted, absorbed, and transferred to animal products. Because this study was conducted in the piglet, a young mammal with an immature digestive system, the obtained results may easily be extrapolated to growing pigs, fattening pigs, or sows because of their efficient digestive function. Using the same contaminated soil matrices, Jondreville et al.[14] also reported the absence of impact of andosol and nitisol in terms of CLD retention during the laying hens' digestive process. Thus, these results in piglets, which complete previous results in laying hens, clearly demonstrate that CLD is not retained in soil particles independently of the species (poultry or pigs). Therefore, CLD from soil has to be considered as available for monogastric farm animals (poultry or mammals) reared outside, and this result indicate a clear concern in terms of food safety for local food consumption.

Liver and Subcutaneous Fat Chlordecone Concentrations Regarding Maximum Residue Limits. Regulation EU no. 600/2010[33] has set maximum residue limits (MRLs) for CLD in animal products. In swine the MRLs are the following: 100 μg kg^{-1} of fat in products with a fat content >10% of FM and 10 μg kg^{-1} of FM in products with fat content <10% (ex. liver). In this study, only four subcutaneous fat samples (11%) showed concentrations of CLD above the MRL (maximum of 115 μg kg^{-1} of fat); however, 28 liver samples (78%) showed concentrations of CLD higher than the MRL (maximum of 52 μg kg^{-1} of FM).

Using the parameter of the linear model (Table 2 and Figures 1 and 2), it appears that concentrations of CLD in liver and in subcutaneous fat would exceed the MRL (100 μg kg^{-1} of fat and 10 μg kg^{-1} of FM, for subcutaneous fat and liver, respectively[33]) above an ingestion level of CLD from soil (mean of the slopes for andosol and nitisol) corresponding to 6.8 and 2.1 μg CLD kg^{-1} of BW day^{-1}, respectively.

Thus, CLD-contaminated soils could represent a real risk for the safety of food issued from pigs reared outside and potentially exposed to involuntary soil ingestion. To further assess this risk, knowledge on soil ingestion level of pigs reared outside directly in contact with soil in the French West Indies specific context[20] is needed. Indeed, the available information

Table 2. Parameters of the Linear Response of Chlordecone (CLD) Concentration in Liver and in Subcutaneous Fat to the Amount of Ingested CLD Originating from Andosol, Nitisol, or Contaminated Rapeseed Oil[a]

	liver (μg kg^{-1} of fresh matter)		subcutaneous fat (μg kg^{-1} of fresh matter)	
	parameter	P value	parameter	P value
intercept		NS		NS
ingested CLD (μg kg^{-1} of BW day^{-1})				
andosol	5.17	<0.001	10.0	<0.001
nitisol	4.55	<0.001	9.25	<0.001
oil	4.70	<0.001	8.21	<0.001
ingested CLD × matrix	NS		NS	
andosol vs oil[b]	NS		NS	
nitisol vs oil[b]	NS		NS	
rsd	5.37		16.9	
R^2	0.82		0.74	
RBA[c]				
andosol	1.10	(0.81−1.39)	1.22	(0.64−1.81)
nitisol	0.97	(0.71−1.23)	1.13	(0.60−1.66)

[a]The equation is CLD concentration in liver or in subcutaneous fat (μg kg^{-1} of fresh matter) = a ingested CLD from andosol + b ingested CLD from nitisol + c ingested CLD from oil, where a, b, and c are the estimates of the parameters attributed to the amount of ingested CLD (μg kg^{-1} of body weight day^{-1}) from andosol, nitisol, and oil, respectively. NS , not significant; rsd, residual standard deviation; R^2, coefficient of determination. [b]If $P < 0.05$, the two slopes are significantly different and the RBA significantly differs from 1. [c]Relative bioavailability (RBA) of CLD present in soil: calculated as the ratio of the slopes of the response fitted with soil to the slope of the response fitted with oil; 95% confidence limits calculated as RBA ± 2 standard error are enclosed in parentheses.

on soil ingestion by pigs has been obtained in temperate regions (Fries et al.[21]), and no information is available for tropical areas.

The results of this study are in line with previous studies showing that organochlorine pesticides may be transferred and accumulated in animal products.[34−36]

In conclusion, our results show that agricultural volcanic soils from French West Indies do not retain CLD during the digestive process of piglets. Therefore, CLD-contaminated soil has to be considered as a risk matrix for monogastric animals, as it appears that the ingestion over 14 days of 6.8 and 2.1 μg kg^{-1} of BW day^{-1} of CLD from contaminated andosol or nitisol leads to subcutaneous fat and liver above the MRL. Thus, all rearing practices in CLD-contaminated areas should avoid outdoor rearing.

AUTHOR INFORMATION

Corresponding Author

*(G.R.) Phone: + 33 383 59 58 88. Fax: + 33 383 59 58 89. E-mail: guido.rychen@univ-lorraine.fr.

Funding

This work was funded by the Université de Lorraine and the Département Physiologie Animale et Systèmes d'Elevage of the Institut National de la Recherche Agronomique (INRA, PHASE). The Ministère de l'Enseignement Supérieur et de la Recherche (MERS) funded the Ph.D. fellowship of C.B.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank C. Grandclaudon, P. Hartmeyer, and H. Toussaint (Université de Lorraine, EA 3998) for technical support, N. Besnes and M. Gibelin (INRA, UE 1295 PEAT, Nouzilly, France) for the experimental feed manufacturing, and F. Massat and B. Planel (LDA 26, Valence, France) for the CLD analysis.

ABBREVIATIONS USED

BW, body weight; CLD, chlordecone; DM, dry matter; FM, fresh matter; GC-MS/MS, gas chromatography−tandem mass spectrometry; GLM, General Linear Model; MRL, maximum residue limit; OC, organic carbon; RBA, relative bioavailability; SD, standard deviation

REFERENCES

(1) Cabidoche, Y. M.; Achard, R.; Cattan, P.; Clermont-Dauphin, C.; Massat, F.; Sansoulet, J. Long-term pollution by chlordecone of tropical volcanic soils in the French West Indies: a simple leaching model accounts for current residue. *Environ. Pollut.* **2009**, *157*, 1697−1705.

(2) Dubuisson, C.; Heraud, F.; Leblanc, J. C.; Gallotti, S.; Flamand, C.; Blateau, A.; Quenel, P.; Volatier, J. L. Impact of subsistence production on the management options to reduce the food exposure of the Martinican population to Chlordecone. *Regul. Toxicol. Pharmacol.* **2007**, *49*, 5−16.

(3) Bocquene, G.; Franco, A. Pesticide contamination of the coastline of Martinique. *Mar. Pollut. Bull.* **2005**, *51*, 612−619.

(4) Coat, S.; Monti, D.; Legendre, P.; Bouchon, C.; Massat, F.; Lepoint, G. Organochlorine pollution in tropical rivers (Guadeloupe): Role of ecological factors in food web bioaccumulation. *Environ. Pollut.* **2011**, *159*, 1692−1701.

(5) Clostre, F.; Lesueur-Jannoyer M. Chlordecone bioavailability for laying hens with two soil types. Biodisponibilité de la chlordécone pour les poules pondeuses pour deux types de sol. Document de synthèse *Cirad-INPL*, 2011; 25 pp.

(6) Chlordecone food exposure of the French West Indies population. Evaluation of the impact of measures to control risk (data on habits of consumption extracted from ESCAL and CALBAS studies. Data of contamination extracted from the RESO Guadeloupe and Martinique). AFSSA, Actualisation de l'exposition alimentaire de la population au chlordécone. Evaluation de l'impact de mesures de maîtrise des risques (Données de consommation issues des enquêtes ESCAL et CALBAS. Données de contamination issues des enquêtes RESO Guadeloupe et Martinique). *Document technique AQR/FH/2007*; 2007; 79 pp, http://www.observatoire-pesticides.fr/upload/bibliotheque/31475040681134901497779 16 87356/01_actualisation_exposition_alimentaire_chlordecone.pdf.

(7) Guldner, L.; Multigner, L.; Heraud, F.; Monfort, C.; Thome, J. P.; Giusti, A.; Kadhel, P.; Cordier, S. Pesticide exposure of pregnant women in Guadeloupe: ability of a food frequency questionnaire to estimate blood concentration of chlordecone. *Environ. Res.* **2010**, *110*, 146−151.
(8) Dallaire, R.; Muckle, G.; Rouget, F.; Kadhel, P.; Bataille, H.; Guldner, L.; Seurin, S.; Chajès, V.; Monfort, C.; Boucher, O.; Thomé, J. P.; Jacobson, S. W.; Multigner, L.; Cordier, S. Cognitive, visual, and motor development of 7-month-old Guadeloupean infants exposed to chlordecone. *Environ. Res.* **2012**, *118*, 79−85.
(9) Boucher, O.; Simard, M. N.; Muckle, G.; Rouget, F.; Kadhel, P.; Bataille, H.; Chajès, V.; Dallaire, R.; Monfort, C.; Thomé, J. P.; Multigner, L.; Cordier, S. Exposure to an organochlorine pesticide (chlordecone) and development of 18-month-old infants. *Neurotox* **2013**, *35*, 162−168.
(10) Multigner, L.; Ndong, J. R.; Giusti, A.; Romana, M.; Delacroix-Maillard, H.; Cordier, S.; Jegou, B.; Thome, J. P.; Blanchet, P. Chlordecone exposure and risk of prostate cancer. *J. Clin. Oncol.* **2010**, *28*, 3457−3462.
(11) Li, H.; Sheng, G. Y.; Teppen, B. J.; Johnston, C. T.; Boyd, S. A. Sorption and desorption of pesticides by clay minerals and humic acid-clay complexes. *Soil Sci. Soc. Am. J.* **2003**, *67*, 122−131.
(12) Woignier, T.; Clostre, F.; Macarie, H.; Jannoyer, M. Chlordecone retention in the fractal structure of volcanic clay. *J. Hazard. Mat.* **2012**, *241/242*, 224−230.
(13) Woignier, T.; Pochet, G.; Doumenc, H.; Dieudonne, P.; Duffours, L. Allophane: a natural gel in volcanic soils with interesting environmental properties. *J. Sol−Gel Sci. Technol.* **2007**, *41*, 25−30.
(14) Jondreville, C.; Bouveret, C.; Lesueur-Jannoyer, M.; Rychen, G.; Feidt, C. Relative bioavailability of tropical volcanic soil-bound chlordecone in laying hens (*Gallus domesticus*). *Environ. Sci. Pollut. Res.* **2013**, *20*, 292−299.
(15) Longland, A. C. Digestive enzyme activities in pigs and poultry. In *In Vitro Digestion for Pigs and Poultry*; Fuller, M. F., Ed.; CAB International: Wallingford, UK, 1991; pp 3−18.
(16) Pelenc, F. Le sevrage du porcelet: une phase critique et determinante. Piglet weaning: critical and determining phase. *Bull. G.T.V.* **2010**, *7*, 63−72.
(17) Montagne, L.; Boudry, G.; Favier, C.; Le Huërou-Luron, I.; Lallès, J. P.; Sève, B. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *Br. J. Nutr.* **2007**, *97*, 45−57.
(18) Aumaitre, A.; Peiniau, J.; Madec, F. Digestive adaptation after weaning and nutritional consequences in the piglet. *Pig News Inf.* **1995**, *16*, 73N−79N.
(19) Galan, F.; Traineau, I.; Julien, L.; Duflot, B. Panorama of the animal product sector and types of animal breeding systems in Martinique. "Networks references" program POSEI France. Panorama des filières animales et typologie des systèmes d'exploitation avec élevage de Martinique. Programme "Réseaux de références" POSEI France, 2008; pp 1−60, http://www.odeadom.fr/wp-content/uploads/2012/11/Panorama_filieres_animales_typologie_systemes_exploitation_elevage_Martinique_2008.pdf.
(20) Zébus, M. F.; Alexandre, G.; Diman, J. L.; Paul, J. L.; Despois, E.; Phaëton, E. Diversity of pig rearing systems in Guadeloupe, 1rst technico-economical evaluation. Diversité des élevages porcins en Guadeloupe, 1ère évaluation technico-économique. *J. Recherche Porcine* **2005**, 407−412; http://www.journees-recherche-porcine.com/texte/index.html.
(21) Fries, G. F.; Marrow, G. S.; Snow, P. A. Soil ingestion by swine as a route of contaminant exposure. *Environ. Toxicol. Chem.* **1982**, *1*, 201−204.
(22) http://agriculture.gouv.fr/Animaux-utilises-a-des-fins-scientifiques.
(23) NF X 31-107, Soil quality. Determination of the granulometric distribution of the soil particles - Pipette method. Qualité du sol - Détermination de la distribution granulométrique des particules du sol - Méthode à la pipette , **2003**,.
(24) NF ISO 10694, Soil quality. Dosage of total and organic cabon after dry combustion (elementary analysis) Qualité du sol - Dosage du carbone organique et du carbone total après combustion sèche (analyse élémentaire) , **1995**,.
(25) Bordet, F.; Thieffinne, A.; Mallet, J.; Heraud, F.; Blateau, A.; Inthavong, D. In-house validation for analytical methods and quality control for risk evaluation of chlordecone in food. *Int. J. Environ. Anal. Chem.* **2007**, *87*, 985−998.
(26) Littell, R. C.; Henry, P. R.; Lewis, A. J.; Ammerman, C. B. Estimation of relative bioavailability of nutrients using SAS procedures. *J. Anim. Sci.* **1997**, *75*, 2672−2683.
(27) Faroon, O.; Kueberuwa, S.; Smith, L.; DeRosa, C. ATSDR evaluation of health effects of chemicals. 2. Mirex and chlordecone: Health effects, toxicokinetics, human exposure, and environmental fate. *Toxicol. Ind. Health* **1995**, *11*, 1−195.
(28) Covaci, A.; Gheorghe, A.; Schepens, P. Distribution of organochlorine pesticides, polychlorinated biphenyls and α-HCH enantiomers in pork tissues. *Chemosphere* **2004**, *56*, 757−766.
(29) Monziois, M.; Bonneau, M.; Davenel, A.; Kouba, M. Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Sci.* **2007**, *76*, 54−60.
(30) Egle, J. L.; Fernandez, J. B.; Guzelian, P. S.; Borzelleca, J. F. Distribution and excretion of chlordecone (Kepone) in the rat. *Drug Met. Disp.* **1978**, *6*, 91−5.
(31) Plaa, G. L.; Caille, G.; Vezina, M.; Iijima, M.; Cote, M. G. Chloroform interaction with chlordecone and mirex. Correlation between biochemical and histological indexes of toxicity and quantitative tissue levels. *Fundam. Appl. Toxicol.* **1987**, *9*, 198−207.
(32) Cohn, W. J.; Boylan, J. J.; Blanke, R. V.; Fariss, M. W.; Howell, J. R.; Guzelian, P. S. Treatment of chlordecone (Kepone) toxicity with cholestyramine. Results of a controlled clinical trial. *N. Engl. J. Med.* **1978**, *298*, 243−248.
(33) Regulation (EC) no. 600/2010 of the European Parliament amending Annex I to Regulation (EC) No. 396/2005 of the European Parliament and of the Council as regards additions and modification of the examples of related varieties or other products to which the same MRL applies. *OJ* **2010**, *L174/18*, 1−22, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:174:0018:0039:EN:PDF.
(34) Kan, C. A.; Tuinstra, L. G. M. T. Accumulation and excretion of certain organochlorine insecticides in broiler breeder hens. *J. Agric. Food Chem.* **1976**, *24*, 775−778.
(35) Voorhees, K. J.; Gharaibeh, A. A.; Murugaverl, B. Integrated SFE/SFC/MS system for the analysis of pesticides in animal tissues. *J. Agric. Food Chem.* **1998**, *46*, 2353−2359.
(36) Zhang, Y. D.; Muench, S. B.; Schulze, H.; Perz, R.; Yang, B. L.; Schmid, R. D.; Bachmann, T. T. Disposable biosensor test for organophosphate and carbamate insecticides in milk. *J. Agric. Food Chem.* **2005**, *53*, 5110−5115.