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ORIGINAL PAPER

Relative bioavailability of soil-bound chlordecone in growing lambs

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Abstract The pollution of soil with the pesticide chlordecone (CLD) is a problem for the use of agricultural surfaces even years after its use has been forbidden. Therefore, the exposure of free-ranged animals such as ruminants needs to be investigated in order to assess the risk of contamination of the food chain. Indeed, measured concentrations could be integrated in a lowered extent if the soil binding would reduce the bioavailability of the pesticide. This bioavailability of soil-bound CLD in a heavily polluted andosol has been investigated relatively of CLD given via spiked oil. Twenty-four weaned lambs were exposed to graded doses of 2, 4 or 6 μg CLD/kg body weight during 15 days via the contaminated soil in comparison to spiked oil. The concentration of this pesticide has been determined in two target tissues: blood serum and kidney fat. The relative bioavailability (RBA) corresponds to the slope ratio between the test matrix-contaminated soil- in comparison to the reference matrix oil. The RBA of the soil-bound CLD was not found to significantly differ from the reference matrix oil in lambs meaning that the pesticide ingested by grazing ruminants would not be sequestered by soil binding. Therefore, CLD from soil gets bioavailable within the intestinal level and exposure to contaminated soil has to be integrated in risk assessments.

Keywords Availability · Chlordecone · Food safety · Ruminants · Soil

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Introduction

Chlordecone (CLD) is a chlorinated polycyclic ketone pesticide which was used until 1993 in the French West Indies to fight against 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: Le Déaut and Procaccia (2009) reported that about 10 % of agricultural soils contain more than 1 mg CLD.kg^{-1} of dry matter (DM). Such context is of great concern regarding human health, as CLD is suspected to negatively impact fetal and postnatal development (Dallaire et al. 2012; Boucher et al. 2013) and to increase the risk of prostate cancer (Multigner et al. 2010).

In the French West Indies, CLD was spread mainly on tropical volcanic soils. These soils are rich in organic carbon (OC) and may display a variable affinity for organic compounds, depending on the

properties of clay (Cabidoche et al. 2009). In particular, the fractal and tortuous microstructure of allophane contained in andosols would favor CLD retention in soils (Woignier et al. 2012). Therefore, this soil may be heavily polluted with CLD compared with other soils on which this pesticide was sprayed, such as nitisol. This physical trapping of CLD in andosol leads to a time of decontamination by leaching of five to seven centuries (Cabidoche et al. 2009) against 60–100 years in nitisol and a plant contamination capacity 18 times lower than that of the nitisol (Cabidoche and Lesueur-Jannoyer 2012).

The local livestock systems in French West Indies are widely based on ruminants generally reared outside. Moreover, one-third of cattle and more than 80 % of small ruminants reared in Guadeloupe belong to small farmers owning five cattle or less (Galan et al. 2008). These animals are mainly elevated on fallow areas what would enhance huge variations in the quality and the quality of the plant cover, and therefore facilitate the direct contact of the animals with soil. By the way, it has been demonstrated that under unfavorable grazing conditions, ruminants may ingest up to 8 % of soil in the daily ingested DM (Jurjanz et al. 2012). Thus, since ruminants may be reared on such historical contaminated areas, there is a potential risk of transfer of CLD in the food chain and it appears therefore of great importance to evaluate the bioavailability of soil-bound CLD in ruminants.

However, in term of CLD transfer from soil to farm animal tissues and products, Jondreville et al. (2013) observed in laying hens that CLD was probably not retained in andosol nor in nitisol. Bouveret et al. (2013) confirmed the absence of a significant retention of CLD by any of these soils in the digestive tract of weaned piglets.

Thus, the question arising is to characterize the effect of the digestive process of ruminants on soil-bound CLD. Recent publications seem to indicate that the effects of ruminant digestive process may be different than that of monogastric species. Thus, it has, for example, been shown that soil-bound polychlorobiphenyls (PCBs) were as available to laying hens than PCBs ingested through oil (Fournier et al. 2012). In contrast, the relative bioavailability (RBA) of PCBs in the same soil was estimated to be 0.5 in lactating goats (Feidt et al. 2013). Therefore, a different behavior concerning RBA of CLD in monogastric animals and in ruminants cannot be excluded.

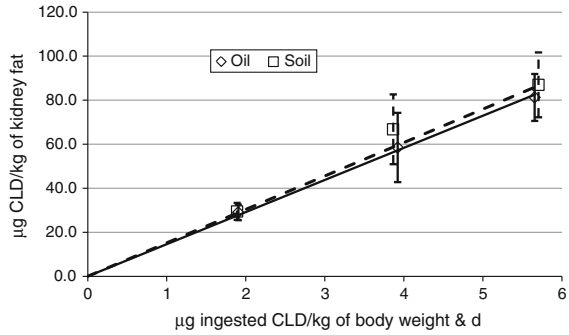

Fig. 1 Concentration of CLD (µg kg^{-1} kidney fat) depending on the amount ingested originating from andosol or from oil (µg kg^{-1} body weight and day). Fitted models for spiked oil and soil are, respectively, in dotted and full lines

Thus, there is need to acquire data on the availability of soil-bound CLD in ruminants that may be reared directly in contact with soil in the French West Indies. Therefore, the aim of this experiment was to determine the bioavailability of soil-bound CLD in young ruminants in comparison to a reference matrix, spiked oil. The hypothesis of this study was that CLD bound in a priori better retaining andosol would be less extracted by the digestive process of ruminants in comparison to oil.

Materials and methods

The experimental design aimed to compare the content of CLD in target tissues after the exposure of the animals to three graded doses of polluted soil in comparison to an exposure to oil spiked at the same doses. Then, the RBA of the pollutant in soil was estimated as the ratio of the concentrations in target tissues obtained with soil to the concentrations in target tissues obtained with oil.

Contaminated soil and spiked oil

A polluted andosol from a former banana field in Guadeloupe was used for the experiment. The surface soil (A horizon) was sampled. Then, plant fragments and stones were manually eliminated. The soil batch was dried at room temperature during 5 days and finally crushed and sieved at 2 mm. The soil was analyzed for physical properties and OC content according to NF X 31-107 and NF ISO 10694,

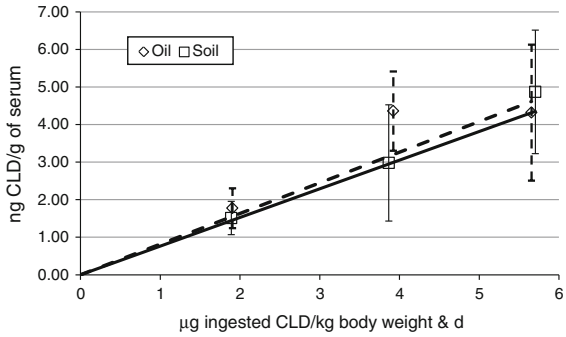

Fig. 2 Concentration of CLD in blood serum (ng g^{-1}) according to the amount ingested originating from andosol or from oil (µg kg^{-1} body weight and day). Fitted models for spiked oil and soil are, respectively, in dotted and full lines. Values are mean ± SE ($n = 4$)

Table 1 Characteristics of andosol

Characteristic	Unit	Value
Humidity	g/kg fresh soil	340
Particle size		
Clay (<2 µm)	g/kg dry soil	331
Silt (2–50 µm)	g/kg dry soil	440
Sand (50 à 2,000 µm)	g/kg dry soil	229
Organic carbon	g/kg dry soil	84.3
Organic matter	g/kg dry soil	146
pH		5.24
Cationic exchange capacity	$cmol^{+}$/kg dry soil	8.15
Chlordecone	mg/kg dry soil	26.2

respectively (Table 1). The concentration of CLD was determined according to the French standard AFSSA/LERQAP/TOP POP04 based on (Blanke et al. 1977) used as routine procedure (internal procedure CMO_MT06) by the Laboratoire Départemental d'Analyses de la Drôme (LDA26, Valence, France).

An alimentary rapeseed oil (Lesieur®, France) was spiked with purchased CLD (Kepone®, 49046 Supelco, Sigma-Aldrich Chimie Sarl). CLD was dissolved in the oil at room temperature by magnetic agitation (90 min) followed by sonication (40 min) in order to get a stock solution of 30 mg CLD/kg. Starting from this solution, the two other doses of 10 and 20 mg CLD/kg of oil were obtained by consecutive dilutions followed by magnetic agitation and sonication. The concentrations of CLD in oils were determined in the same manner as kidney fat.

In order to maintain its initial structure, andosol samples were integrated in a handmade feed pellet of approximately 50 g, daily prepared. Spiked oil also was integrated daily in a handmade feed pellet starting from the corresponding oily solution.

Animals and management

The study was carried out at the experimental facilities of our center (Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires, Vandoeuvre, France), respecting the European regulation for experimentation with animals (Directive 2010/63/EU).

Twenty-eight weaned and 2-month old lambs of the breed INRA (BW of 19.0 ± 2 kg) were purchased from a local farm in the east of France. Four control animals and 24 treated lambs were carried out in the experiment. These animals were adapted during 5 days to the experimental facilities. After this adaption period, the four control lambs were slaughtered and sampled. The 24 exposed lambs were assigned to one of the six treatments: three doses of CLD (2, 4 or 6 µg/kg BW) supplied orally through two matrices (polluted soil or spiked oil). Each treatment was repeated on four animals (two animals for each of the two successive periods) which received the fixed doses orally during 15 days.

The animals were housed by two in boxes of 2 m × 1 m and fed a commercial grower feed (ADAGIO®, Sanders, Einville) distributed at 3 % of the BW. Moreover, hay and water were available ad libitum. The contaminated feed pellet was given individually every morning before feeding.

The exposed lambs were slaughtered at the end of the contamination period (electrical anesthesia followed by bleeding).

Measurements, sampling and chemical analysis

Feeds refusals were recorded daily. Lambs were weighed twice weekly during the exposure period in order to adjust the composition of the pellet. They were also weighed at the day of slaughter. Blood of the jugular vein was sampled from each lamb the day before slaughter. After coagulation (2 h at room temperature followed by 24 h in the refrigerator), the serum was separated by centrifugation (1400*g* during 15 min at room temperature). After slaughter, kidney

fat samples were collected, weighed and stored at −18 °C.

CLD in oil and in kidney fat was analyzed according to the method described by Bordet et al. (2007) by the Laboratoire Départemental d'Analyses du Morbihan (LDA56, Saint-Ave, France), which works under the French Accreditation Committee COFRAC. Determination of CLD in serum was done by the Center for Analytical and Research Technology at Liege University (Belgium) according to Multigner et al. (2010). Limits of quantification were of 0.06 ng g^{-1} of serum, as well as 2 μg kg^{-1} fresh matter in kidney fat, and in oil. Values below these limits were considered equal to zero in the following calculations.

Calculations and statistical analysis

The individual amount of CLD ingested daily relative to the BW of the lamb during the exposure period was calculated from (1) the measured CLD concentration in the experimental matrix (soil or oil), (2) the sum of the ingested matrix incorporated in the feed pellet during the exposure period divided by 15 exposure days and (3) the average BW of the animal during the exposure period (mean of the individual BW determinations recorded during the exposure period).

All data were analyzed by ANOVA using the GLM (general linear model) procedure of SAS software (version 9.3, SAS®, Cary, NC). The statistical model to analyze animal performances included the main effects matrix (soil or oil) and CLD doses (3 levels) as well as the interaction between the matrix and the dose. The lamb was the experimental unit. Least square means were compared based on Student's t tests and declared significant at $P \leq 0.05$.

Bioavailability of CLD present in each soil relative to CLD present in oil was estimated from CLD concentration in serum and in kidney fat, by means of the slope ratio method. This method involved 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.kg^{-1} of BW for each matrix (soil and oil) and (ii)equality of the intercepts for the two lines (common intercept). After these assumptions were checked, the regression of CLD concentration in serum and in kidney fat to the amount of ingested CLD.kg^{-1} of BW was fitted for each of the two matrices. RBA of CLD present in soil was calculated from serum and kidney fat data as the ratio of the slope of the response fitted with soil to the slope of the response fitted with oil. The standard error of RBA and its 95 % confidence limits were calculated according to (Littell et al. 1997).

Results and discussion

The used andosol was characterized by a high concentration of OC and CLD with 84.3 g OC/kg dry soil and 26.2 mg CLD/kg dry soil, respectively (Table 1), falling within the range of OC values obtained in banana fields in the Caribbean, as reported by Feller et al. (2001) and within the highest pollution levels in andosol cited in other studies (Woignier et al. 2012; Cabidoche and Lesueur-Jannoyer 2012). This is in accordance with the distribution of CLD mainly in the first 0–10 cm layer, which reached more than 50 mg kg^{-1} of CLD (Cabidoche et al. 2009).

As such polluted land would be used for grazing of ruminants, the exposure of the lambs to such a soil corresponded to realistic experimental conditions. In order to respect the aimed target doses, only small amounts of soil were incorporated in the daily feed pellets, generally <5 g soil per day.

The lambs ingested the distributed feed without any effort. The animal performances, i.e., average daily gain (ADG; 184 ± 80 g/d), the average and final BW (22.9 ± 2.2 kg) and the weight of the collected kidney fat (31.1 ± 14 g) were not affected neither by the exposure matrix nor by the exposure dose of CLD ($P > 0.1$, Table 2).

As expected by the absence of an environmental background for CLD in east of France, all control animals had no CLD in their blood or kidney fat as concentrations were systematically under the limit of detection.

The necessary hypotheses for a relevant comparison of slopes were checked: the response to ingested CLD within both kidney fat and blood serum was not quadratic [effect ingested CLD * ingested CLD (matrix), $P > 0.10$], and the intercepts adjusted for the two lines were equal (matrix effect $P > 0.10$). In the absence of any significant quadratic component in the models, the response to the graded level of ingested CLD from any of the two matrices was

Table 2 Body weight, growth and yield of kidney fat at slaughter depending on experimental treatments

Variable	Control	Soil			Oil			Effects			Root
Exposure dose[a]	0	2	4	6	2	4	6	Matrix	Dose	m × d[b]	MSE[c]
Average Body Weight (kg)	22,1	22,1	21,6	23,2	21,9	21,3	22,9	NS[d]	NS[d]	NS[d]	2,3
Final Body Weight (kg)	23,2	22,9	22,3	24,1	22,2	21,9	23,7	NS[d]	NS[d]	NS[d]	2,4
Average Daily Gain (g/d)	110	213	184	227	152	133	215	NS[d]	NS[d]	NS[d]	80
Kidney fat (g fresh matter)	41,5	35,4	23,0	30,7	30,5	31,2	26,8	NS[d]	NS[d]	NS[d]	13,4

[a] µg chlordecone/kg BW & d

[b] m × d: interaction matrix × dose

[c] *MSE* mean square error

[d] *NS* not significant ($P > 0.05$)

Table 3 Parameters of the linear response of chlordecone (CLD) concentration in serum (ng mL^{-1}) and in kidney fat (µg kg^{-1} of fresh matter) to the amount of ingested CLD (µg kg^{-1} of body weight.day^{-1}) originating from andosol or contaminated rapeseed oil[a]

	Serum		Kidney fat	
	Parameter	*P* value	Parameter	*P* value
Intercept		NS		NS
Andosol	0.763	<0.001	15.9	<0.001
Oil	0.816	<0.001	14.6	<0.001
Ingested CLD * matrix[b]	NS		NS	
rsd	1.09		10.7	
R^2	0.66		0.85	
RBA[c]	0.94	(0.68–1.20)	1.09	(0.95–1.23)

[a] The equation is CLD concentration in serum or in kidney fat = a ingested CLD from andosol + b ingested CLD from oil, where a and b are the estimates of the parameters attributed to the amount of ingested CLD (µg kg^{-1} of body weight day^{-1}) from andosol and oil, respectively

[b] NS ($P < 0.1$) the two slopes do not differ from each other and RBA does not differ from 1

[c] 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 brackets

NS not significant ($P > 0.10$), *rsd* residual standard deviation, R^2 coefficient of determination

proven to be linear (ingested CLD $P < 001$, Table 3). As pointed out by Budinsky et al. (2008), the linearity of the response to graded levels of ingested pollutant is of primary importance because it avoids bias in RBA calculations.

The parameters of the fitted linear models are shown in Table 3. With respect to kidney fat, the estimate of RBA of andosol (Fig. 1) was 1.09 in comparison to spiked oil and the slope fitted for soil could not be differentiated from the slope fitted with oil (CLD ingested × matrix, $P > 0.10$). The estimate of RBA derived from blood serum (Fig. 2) was 0.94 and could not differentiated from 1 (CLD ingested × matrix, $P > 0.10$) (Table 3). It can be concluded, therefore, that andosol would not modify in notable manner the bioavailability of soil-bound CLD. The interpretation of the serum-based approach has been confirmed, with higher statistical preciseness, by the results obtained in the target tissue kidney fat. Coefficients of determination (R^2) of the models relative to blood serum and to kidney fat were 0.66 and 0.85, respectively. The blood serum appeared a less appropriated target fluid of CLD as the determination coefficient of this model was lower than in kidney fat. The wider confidence interval of the RBA model of blood serum would confirm its lower precision in comparison to the model for kidney fat (Table 3). Blood is easy to sample and would, therefore, be preferred for practical reasons for the monitoring of exposed animals. Nevertheless, the biological role of the blood compartment corresponds to transport

functions what would be less appropriate to show the response, i.e., storage, of a compound after an orally exposure. Moreover, the generally low concentrations of fat in blood serum of sheep (<1 μmol mL^{-1} and <2 μmol mL^{-1} for triglycerides and cholesterol, respectively; Casamassima et al. 2008) would disadvantage this compartment as target fluid in comparison to body fat which showed 15-fold higher CLD concentrations. The low concentrations in blood could be linked to a metabolization of CLD in CLD alcohol as it has been reported in blood of pig and man (Soine et al. 1983). Nevertheless, no data are available concerning this activity in blood of ruminants.

Despite small differences in the statistical preciseness in the fitted models, the response in blood and fat gave the same ascertainment: andosol does not reduce the bioavailability of CLD compared with spiked oil. The exposure of ruminants to soil-bound CLD confirmed the results previously reported in hens and piglets that soil binding would not reduce the bioavailable of CLD. Contrarily to the current results, the RBA of soil-bound PCBs was reduced in lactating goats in comparison to spiked oil (Feidt et al. 2013) in contrast to results obtained in laying hens (Fournier et al. 2012). The soil characteristics differ between these PCB studies and this work on CLD, especially the content of organic matter and the clay structure. Moreover, the stronger lipophilicity of especially higher chlorinated PCBs (log Kow > 6) in comparison to CLD (log Kow 4, 5–6) could also be an element of explanation of this difference of the soil-binding effect between both pollutants in ruminants. Due to the sparse number of such comparisons in the literature, more detailed work is necessary to understanding better the RBA of soil-bound organic pollutants and their variation factors.

Finally, it has been recognized that ruminants would extract CLD from soil as fully as previously studied monogastric animals the soil-bound CLD (Jondreville et al. 2013; Bouveret et al. 2014). The microbial digestion in the rumen of ruminants is not specialized in extraction of lipophilic compounds (Ruckebusch et al. 1995). Nevertheless, the post-ruminal digestion of the fat fraction has generally a quite similar efficiency as in other mammalians, which has been shown in studies using encapsulated fat sources (Doreau and Chilliard 1997).

That would mean that in risk assessment purposes, the soil concentration of CLD should be fully integrated, even in ruminants, as these animals would extract and absorb CLD as strongly from soil as from spiked oil. Indeed, lambs exposed daily to 6 μg of CLD/kg BW did approach the regulation threshold (Regulation UE 396/2005) of 100 μg $CLD.kg^{-1}$ fat (or 20 μg $CLD.kg^{-1}$ fresh matter) after only 15 days even if the daily ingested amount of soil was very low (5 g). Therefore, soil intake should generally be limited. It should also be checked that soil intake estimates, carried out in temperate conditions (Healy 1968; Jurjanz et al. 2012), can be extrapolated to subtropical conditions and could give us management tools for grazing on very lightly contaminated surfaces. In the absence of precise knowledge of soil intake in such rearing systems and given our results, grazing of ruminants on CLD-polluted areas has to be generally avoided. Otherwise a rapid contamination of free-ranged animals has to be feared.

Conclusions

This work on weaned lambs showed that the bioavailability of soil-bound CLD assessed by means of the response of the target tissues blood serum and kidney fat was not reduced by its binding on andosol in comparison to an exposure to spiked oil. Although the structure of andosol would favor CLD binding, it must be concluded that the digestive process in ruminants would extract very strongly this soil-bound pesticide and, therefore, the animals reared on polluted land will be exposed to the fully concentration of CLD contained in the soil potentially ingested. This point is to be taken into account in risk assessment approaches.

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