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# Impact of soil characteristics on relative bioavailability of NDL-PCBs in piglets

Matthieu Delannoy<sup>a\*</sup>, Agnès Fournier<sup>a</sup>, Abdourahamane Tankari Dan-Badjo<sup>b</sup>, Jessica Schwarz<sup>a</sup>, Sylvain Lerch<sup>a</sup>, Guido Rychen<sup>a</sup>, Cyril Feidt<sup>a</sup>.

**a** : URAFPA, University of Lorraine-INRAe, 54500 Vandoeuvre, France

**b** : Université Abdou Moumouni de Niamey, Département Sciences du sol, Faculté d'agronomie, BP 10960 Niamey, Niger

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## Highlights

- Relative bioavailability of NDL-PCBs in soil was assessed in vivo.
- Piglet was used as a model of child to assess its exposure through soil ingestion.
- Focus on content and quality of organic matter was realized
- Organic matter content is of major impact on relative bioavailability
- Overall NDL-PCBs relative bioavailability is high for all tested soil (>45%).

## Keywords

Polychlorobiphenils; Children; Soil; Ingestion; Bioavailability; Pollutant

## Abstract

Children may be orally exposed to organic pollutants through involuntary soil ingestion. This study was aimed at determining the impact of the characteristics (organic carbon (OC), black carbon (BC), clay contents and pH) of ten contaminated soils on the bioavailability of non-dioxin-like polychlorobiphenyls (NDL-PCBs). Five juvenile male piglets were exposed to increasing amounts of each of the soils. These soil-fed groups were compared by a relative bioavailability approach (RBA) to a reference group fed with corn oil spiked with increasing doses of Aroclor 1254. After 10 days of oral exposure, the animals were sacrificed and NDL-PCB concentrations were determined by GC-MS in the adipose tissue. The relative bioavailability (RBA) factors were calculated for PCB 101, 138, 153 and 180. Despite high variations in the amount of black carbon (0.50 g kg<sup>-1</sup>–6.0 g kg<sup>-1</sup> d.w.) and organic matter (12 g kg<sup>-1</sup>–180 g kg<sup>-1</sup> d.w.), only 3 soils exhibited a significantly lower RBA for all NDL-PCBs, compared to the oil-group. High levels of OC (>100 g kg<sup>-1</sup>) and BC content (3.0 g kg<sup>-1</sup>) were related to a significant reduction in RBA. Overall, RBA was higher than 45% independently of the soil and the PCB congener.

## 1. Introduction

As children may exhibit hand-to-mouth activities, incidental ingestion of contaminated soils is a growing concern. In risk assessment approaches, U.S. EPA recommends to use an individual ingestion rate of 200 mg per day (U.S. EPA, 2011). Persistent organic pollutants (POPs) like non-dioxin-like-polychlorobiphenyls (NDL-PCBs) are ubiquitous in the environment (Meijer et al., 2003). Soil appears as a principal sink for those pollutants. Furthermore, several studies have linked exposure to polychlorinated biphenyls (PCBs) to neuropsychological impairments like a possible deficit of memory (Elnar et al., 2012, Faroon et al., 2000, Grandjean and Landrigan, 2006). Thus, assessing exposure to NDL-PCBs from contaminated soils appears as an important issue in terms of safety management.

*In vivo* assessment of oral-exposure to NDL-PCB contaminated soils by young children requires the choice and use of an adequate animal model to mimic their digestive processes. Numerous exposure assessment

studies on POPs were performed in rodents (Fouchécourt et al., 1998, Fries et al., 1989), even in the specific field of contaminated soil exposure (Pu et al., 2006). However the rather high ratio soil ingestion over body weight (BW) of rodents could be a concern. In addition, differences in efficiencies of absorption between species were reported (Budinsky et al., 2008, Kelly et al., 2004). These limitations highlight the need to perform further exposure investigation using a more adequate species. Juvenile swine appears to be a valuable model in response to these concerns. Indeed, since piglets are very close in terms of growth and absorptive mechanisms to humans, this animal species is increasingly used to evaluate oral exposure to soil pollutants in soil (Budinsky et al., 2008, Casteel et al., 2006, U.S. EPA, 2007).

Since soil constituents, biological and physico-chemical processes as well as the contaminants characteristics are known to impact the bioavailability of contaminants in the

intestinal lumen (Delannoy et al., 2014a, Pignatello, 1999, Pu et al., 2006, Saghir et al., 2007), the uptake and the absorption of sequestered organic compounds appear to be a complex phenomenon. Some studies based on artificial soils reported the major role of the soil-constituents on the bioavailability of NDL-PCBs during digestion (Delannoy et al., 2014a, Pu et al., 2006). However, few *in vivo* assays described the impact of the characteristics of natural, old-contaminated soils on bioavailability.

The present study was aimed to assess the retention of NDL-PCBs by soil during the digestive processes depending on the physical characteristics of organic matter (OM). More precisely, in order to further investigate the importance of OM, it was designed to assess relative bioavailability of NDL-PCBs in soils sampled in 5 different areas known for their industrial contamination with NDL-PCBs. Common soil characteristics were used as descriptors of those soils in order to distinguish their relative importance on bioavailability of NDL-PCBs in piglets.

## 2. Material and Methods

### 2.1. Experimental design

The experimental design to assess the relative bioavailability (RBA) of NDL-PCBs (i.e. 28, 52, 101, 128 and 153) in 10 sampled soils is shown in Fig. 1. Eleven groups of piglets were fed either one of the 9 sampled-soils, the reference soil or NDL-PCBs spiked corn oil. RBA was estimated using concentrations of NDL-PCBs in adipose tissue of soil-fed and corn oil-fed groups (Delannoy et al., 2014a, Fournier et al., 2012). For each group, one or two additional piglets were fed a non-contaminated standard soil as a negative control. Piglets were randomly allocated to each treatment group. Animals were sacrificed at the end of the contamination period (day 10).

### 2.2. Preparation of exposure matrices

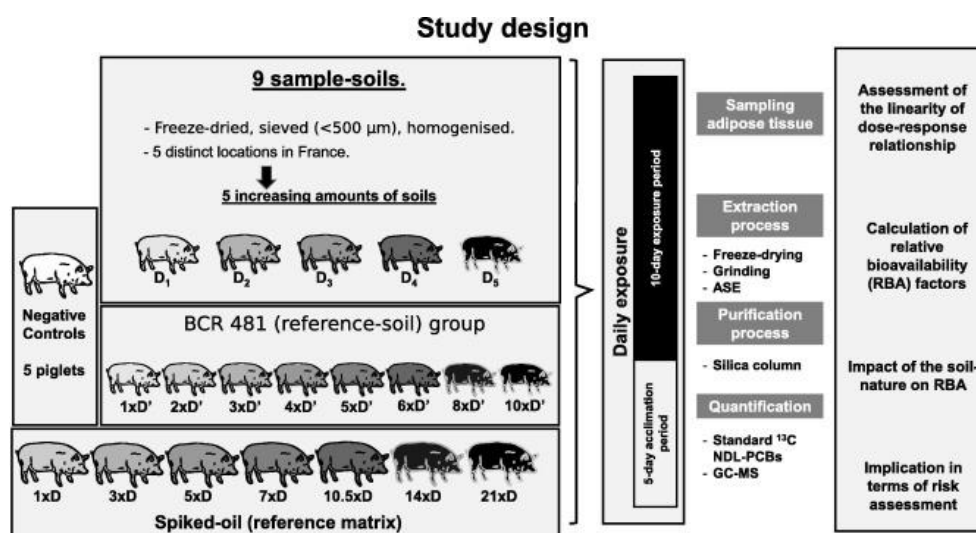
#### 2.2.1. Spiking technique of oil

Spiked oil with NDL-PCBs (SO) was prepared as a reference matrix using corn oil (Sigma Aldrich, St Louis, USA). Seven doses were prepared with increasing amounts of Aroclor 1254 in hexane (Sigma Aldrich,

Supelco) per g of corn oil (Table 1). Resulting concentrations corresponded to multiplications of a reference dose (1D corresponding 3000 ng of Aroclor 1254 per g of oil corresponding to 1000 ng of NDL-PCBs per g of oil). In brief, one hexane solution of NDL-PCBs was prepared and appropriate amounts were sprayed over corn oil (Sigma–Aldrich, St Louis, USA) in order to obtain the seven doses. The resulting seven doses are the following: 1D, 3D, 5D, 7D, 10.5D, 14D, 21D ranging from 3000 ng to 63,000 ng of Aroclor 1254 per g of spiked oil. After spiking, solvent traces were rotary-evaporated (Rotavapor® RE121, Büchi, Flawil, Switzerland) during 2 h at 0.1 mbar and samples were let under an extractor hood overnight. All oil preparations were then stored at 4 °C in amber glass vials until further use.

#### 2.2.1. Spiking technique of oil

BCR 481 (Community Bureau of Reference) supplied from Sigma–Aldrich (BCR 481, Sigma–Aldrich, Saint-Louis, USA) was used as a reference soil. Moreover, nine soils were sampled during a collection campaign which took place in France in 2013. First 5 cm of soils originating from



**Figure 1. Experimental design of the present study**

Amounts of soil (\mu\text{g} of Aroclor 1254 per g of corn oil.

five distinct areas known as contaminated accordingly to the French public database inventorying contaminated soil, BASOL (MEDDE, n.d.), were sampled (March to September 2013). During sampling, the largest vegetative debris and litters were removed. Each soil sample was constituted from a mix of three collection points spaced out by more than 1 m and collected in aluminium containers. As contaminations were reported as high, samples were freeze-dried until stabilization of mass instead of air dried in order to limit volatilization of NDL-PCBs and possible cross-contamination. Then samples were sieved to 500  $\mu\text{m}$ , homogenised by 5 splits in a close-bin riffle splitter and subsampled to aliquots of 200 g prior to experimental use. At last, the tenth soil-sample was BCR 481.

### 2.3. Exposure of piglets to soil and oil: hand-feeding procedure, dosing and repetitions

Moistened dough balls were prepared as exposure vehicle using the usual feed of piglets (Porcelet Super VE 25 kg, Lorial, Laxou, France) and water (1:1, wt/wt). Four dough balls originating from one subsampling of wet pellets ( $28 \pm 2$  g) were used daily for each piglet either in acclimatation or exposure periods. During acclimatation a non-contaminated artificial wet soil (17.5% of mass) containing kaoline (Kaolinite, Sigma–Aldrich, Saint-Louis) and silica (Fontainebleau sand, VWR) (78:22, wt/wt, dry matter basis) were administered to piglets. Increasing amounts of this soil were distributed, over the 5 d of the pre-experimental period, in order to gently acclimate piglets to a limited-extent ingestion of soil (less than 0.1% of the individual daily intake). During the exposure period, piglets were administered daily (day 1 to day 10) a moistened dough ball with the corresponding amount or dose of either spiked oil or one of the soil-samples at 8:00 am (Table 1) according to their treatment group. Soil or oil intake (g of dry matter) for each piglet was calculated on the basis of their individual body weight and their assigned dose. In short, every two days, individual body weight of piglets were measured. Minimum and maximum amount per 10 kg of BW for each soil was calculated to set the ranges of exposure doses for PCB 101, PCB 138 and PCB 153 inside those tested with the spiked oil. Delivered amounts of soil matrix are presented in Table 1. Two g of spiked oil per 10 kg of piglet BW were introduced for all seven doses of oil-fed group. In order to limit possible transfers of NDL-PCBs from soil to the feed matrix, soils were introduced in the centre of the dough ball. Each of the nine soil-sample groups comprised five increasing amounts of ingested soil whereas the oil-fed group included seven increasing doses (Table 1). The reference (R) group was exposed to BCR 481 and comprised 8 doses and piglets (Table 1). The five lowest doses (1D, 3D, 5D, 7D, 10.5D) were provided to 2 piglets whereas the two highest ones (14D, 21D) were delivered to 3 piglets. Spacing between doses of Aroclor 1254 and number of piglets were chosen in order to accurately check the linearity of dose–response relationship. Maximum amount of soil-ingestion was rather

low regardless the soil and dose (maximum of  $203 \text{ mg kg}^{-1} \text{ d}^{-1}$ ). This ingestion amount is lower or of the same order of those used in previous relative bioavailability assay of organic (Bouveret et al., 2013, Budinsky et al., 2008) and inorganic contaminants in soil (Denys et al., 2012) using piglets. At the end of the 10 d of exposure period piglets were subjected to electronarcosis followed by immediate exsanguination after a 12 h-starvation period. Then, pericaudal adipose tissue was collected.

### 2.4. Animals, ethics and housing

In total, 74 Crossbred (Pietrain  $\times$  Youna<sup>®</sup>, EARL des deux chênes, Saint-Maurice-aux-Forges, France) castrated male swine, weaned, of  $35 \pm 3$  days of age were used. A 5-day acclimation period was realized prior to the start of the contamination. In short, animals were housed individually using stainless-steel cages in the animal facility of URAFFPA (Université de Lorraine, Vandœuvre-lès-Nancy, France). Temperature was kept to 26–27 °C. Every 2 days, BW of piglets was checked. Feed was provided daily at 4.5% of BW at 10:00 am. *Ad libitum* access to water was provided throughout the entire study. 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). Furthermore, the protocol was approved by the Ethical Committee of Lorraine (Permit Number: 00344.01, from MAAR). Particular attention was paid to refining and reduction part of the principle of 3R's. Thus, the required number of piglets per group was assessed using available data on bioavailability of NDL-PCBs in artificial soils (Delannoy et al., 2014b). As noticed in a previous publication (Delannoy et al., 2014b), 5 piglets for each soil sample were considered sufficient to allow an adequate calculation of RBA values and suitable 95% fiducial limits (corresponding to  $\pm 20\%$  of RBA). Although, in order to properly establish the linearity of the dose–response relationship it was necessary to use 16 animals for the reference group. Furthermore, instead of using stressful multiple blood sampling to determine bioavailability of ingested NDL-PCBs, we used organs sampling after sacrificing of animals.

### 2.5. Analyses of exposure matrices

Three aliquots of each nine soils and the 7D oil samples were sent to an external laboratory for NDL-PCBs analyses (CARSO – LSEHL, Lyon, France). NDL-PCBs were quantified by GC–MS analysis after an Accelerated Solvent Extraction (ASE) adapted from NF EN 16 167. Moreover prepared samples were sent in duplicate to INRA US10 LAS (Laboratoire d'Analyses des Sols d'Arras, Arras, France) to characterize soil-samples in terms of physical properties (5 fractions size grading, NF X 31-107), pH (using KCl 0.1 N according to NF ISO 10 390), organic carbon content (following decarbonation, NF ISO 10 694) and black carbon content was measured using chemo-

**Table 1. Formulation, description and contamination doses of exposure matrices in experimental dough balls.**

| Soil sample             | Site-name   | Location in France   | Historic   | Age of contamination   | Amount of soil fed (mg DW of soil per 10 kg of BW, $n = 1$ ) |                     |                   |                   |       |       |        |
|-------------------------|---|--|--|--|--|---------------------|-------------------|-------------------|-------|-------|--------|
|                         |   |  |  |  | D1   | D2                  | D3                | D4                | D5    |       |        |
| <i>Soil-samples</i>     |   |  |  |  |  |                     |                   |                   |       |       |        |
| A1                      | St Cyprien <sup>a</sup>   | Saint-Cyprien, Loire (42 160)  | Historical fluid leak from transformer distributed around by a recent fire               | 2008 date of fire event <sup>c</sup>                         | 700  | 1033                | 1366              | 1700              | 2033  |       |        |
| A2                      |   |  |  |  | 15   | 30                  | 45                | 60                | 75    |       |        |
| B1                      | Korsec <sup>a</sup>   | Basse-Ham, Moselle, (57 970)   | Buried industrial waste from a former mechanical workshop. Fluid leaked from transformer | Before 2000 <sup>c</sup>                                     | 50   | 125                 | 200               | 275               | 350   |       |        |
| B2                      |   |  |  |  | 40   | 80                  | 120               | 160               | 200   |       |        |
| B3                      |   |  |  |  | 50   | 110                 | 170               | 230               | 290   |       |        |
| C                       | Lyon <sup>b</sup>   | NK   | NK   | NK   | 50   | 110                 | 170               | 230               | 290   |       |        |
| D                       | Ploufragan <sup>a</sup>   | Ploufragan, Côtes-d'Armor (22 440)   | Heavy oil processing facility  | Before 1993 <sup>c</sup>                                     | 700  | 1033                | 1366              | 1700              | 2033  |       |        |
| E1                      | Reverdy <sup>a</sup>  | Cognière, Haute-Saône (70 230)   | Historical fluid leak from transformer. Former agri-food facility                        | Before 1998 <sup>c</sup>                                     | 50   | 112.5               | 175               | 232.5             | 300   |       |        |
| E2                      |   |  |  |  | 100  | 200                 | 300               | 400               | 500   |       |        |
| <i>Reference soil</i>   |   |  |  |  |  |                     |                   |                   |       |       |        |
| Soil sample             | Site-name   | Description  | Age of contamination   | Amount of soil fed (mg DW of soil per 10 kg of BW, $n = 1$ ) |  |                     |                   |                   |       |       |        |
|                         |   |  |  | 1 × D  | 2 × D  | 3 × D               | 4 × D             | 5 × D             | 6 × D | 8 × D | 10 × D |
| R                       | NK  | BCR 481 (Community Bureau of Reference) supply from Sigmal-Aldrich (Saint-Louis, USA). Former german industrial site                 | NK   | 17.5   | 30   | 42.5 <sup>d</sup>   | 55                | 67.5              | 80    | 105   | 130    |
| <i>Reference matrix</i> |   |  |  |  |  |                     |                   |                   |       |       |        |
| Sample                  | Description   | Concentration of spiked corn oil (µg of Aroclor 1254 per g of corn oil). Number into bracket correspond to the number of repetitions |  |  |  |                     |                   |                   |       |       |        |
|                         |   | 1 × D  | 3 × D  | 5 × D  | 7 × D  | 10.5 × D            | 14 × D            | 21 × D            |       |       |        |
| Oil                     | Each piglet was fed 2 g of spiked cor oil per 10 kg. Concentration of Aroclor 1254 in oil differ along the dose | 3.0<br>( $n = 2$ )   | 9.0<br>( $n = 2$ )   | 15<br>( $n = 2$ )  | 21<br>( $n = 2$ )  | 25.5<br>( $n = 2$ ) | 42<br>( $n = 3$ ) | 63<br>( $n = 3$ ) |       |       |        |

NK: Not known.

<sup>a</sup> Site-name as provided in BASOL database (MEDDE, n.d.).

<sup>b</sup> Location of waste-treatment company which provided this soil..

<sup>c</sup> According to BASOL database (MEDDE, n.d.).

<sup>d</sup> Dose suppressed of the data set.

thermal oxidation methodology, as described previously (Caria et al., 2011)

## 2.6. Analysis of biological samples

Pericaudal adipose tissue was collected. All analytical steps for preparation of adipose tissue samples (freeze-drying, grinding) and chemical analyses are described in details elsewhere (Delannoy et al., 2014a). In brief, these analyses involved three successive steps (Fig. 1). ASE from freeze-dried and milled samples of adipose tissue was first performed (toluene/Acetone, 70:30 vol/vol; 120 °C, ASE 350, Dionex, Sunnyvale, USA) using <sup>13</sup>C-labelled NDL-PCBs from LGC Standard (Molsheim, France) as internal standards. Finally, quantification was achieved by using a GC-MS device (7 890A, Agilent Technologies, Santa Clara, USA). All chemicals used in this study were pesticide residue analytical grade.

## 2.7. Data analyses

### 2.7.1. Quality control of the data set and data treatment

Concentrations in soil were expressed in µg per g of dry soil and those in adipose tissue in ng per g of fat. Externally analysed soil-samples were re-identified to allow randomized, operator-blind analyses. All parameters measured were realized according to COFRAC quality standard criteria, except black carbon because of the lack of standardisation of this methodology. For adipose tissue, regardless the treatment group, PCB 28 levels were below the limit of quantification. PCB 52 exhibited mostly quantifiable but low levels leading to highly variable results. Thus, presented results are focused on the most abundant congeners: PCB 101, PCB 138, PCB 153 and PCB 180. NDL-PCBs concentrations observed from one piglet (R, 1.5D) were removed from the data set because of unexpected low levels of NDL-PCBs.

### 2.7.2. Relative bioavailability (RBA) estimates calculation

The present study aims to determine if some characteristics of soil could impair bioavailability of sequestered PCB. As numerous studies leading to assessment of maximum risk limits for human are based on alimentary exposure (ATSDR, 2000), a comparison of two exposure contexts was realized: contaminated soil (tested matrix) exposure was compared to a spiked corn oil (reference matrix). To perform this comparison, relations between exposure dose and concentrations in target organ have to be clearly assessed to determine the 95% fiducial limits. To cope with this issue, statistical analyses were performed as frequently used in nutrition and exposure assessment fields (Littell et al., 1997). In short, a one-way analysis of covariance using

general linear model procedure of statistical analysis software SAS/STAT (SAS v9.1, SAS Institute, Cary, USA) was performed for each NDL-PCB congener using the treatment group as effect. The statistical process comprises a step-by-step checking of three assumptions: 1/assessing if dose-response relationship is whether linear or quadratic (plateau effect) regardless the exposure matrix, 2/if intercepts are equal independently of the exposure media and 3/if negative controls are not different from the common intercept (refers as  $X_0$  hypothesis in the original work (Littell et al., 1997)).

As the linear model was the most efficient to fit experimental data for the range of doses used in each group, dose-responses were proven linear. Accordingly to this linearity 1/concentrations in adipose tissue of PCB 101, PCB 138, PCB 153 and PCB 180 2/and exposure doses were normalised by dividing them by the maximum daily dose given for one treatment (soil or oil). After this process, slope of the linear regression linking normalised concentrations in adipose tissue and normalised exposure dose was calculated for each treatment group. Relative bioavailability estimates were provided using the following equation:

$$RBA_{soil;A} = \frac{slope_{linear\ soil\ A}}{slope_{linear\ oil}}$$

and 95% fiducial limits were calculated as a previously described statistical methodology (Denys et al., 2012, Littell et al., 1997).

Each piglet was exposed to a unique dose of exposure within its treatment group. This methodology of exposure is in accordance with the original statistical methodology of calculation of RBA (Littell et al., 1997).

Differences were considered significant for  $p < 0.05$  and not statistically significant for  $p > 0.10$ .

### 2.7.3. Principal component analysis (PCA)

To determine the relation between physic-chemical parameters of soils and RBA, a principal component analysis (PCA) has been performed using the statistical software SPAD (SPAD 7.0, Coheris SPAD, France). During this data analysis, Pearson correlation coefficient were retrieved. RBA obtained for PCB 153, organic carbon content (OC), black carbon content (BC), clay content, pH and NDL-PCB concentrations (sum of PCB 101, 138, 153 and 180 concentrations) were used as principal variables. RBA for the other congeners (i.e. PCB 101, 138 and 180) were set as illustrative variable. Each sampled soils take part of the analysis.

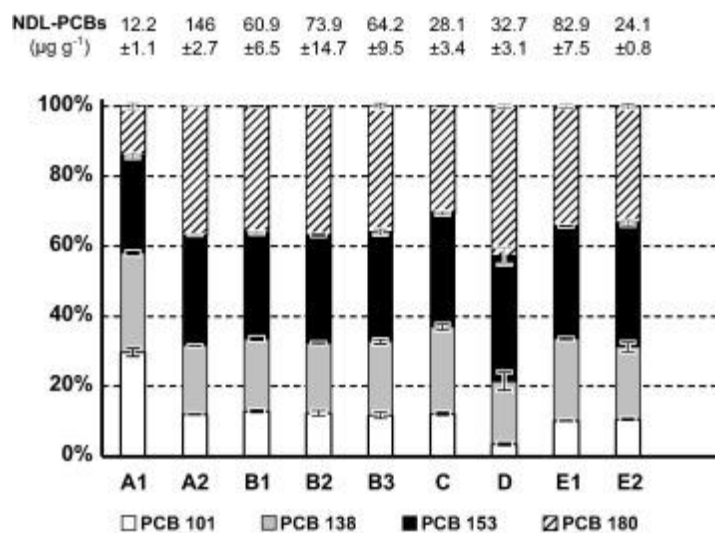
### 3. Results and Discussion

#### 3.1. Experimental matrices

The 9 sampled soils and the reference one covered a consistently wide range of OC as well as BC levels. OC content ranged from  $12.1 \pm 0.4 \text{ g kg}^{-1}$  to  $180.5 \pm 2.5 \text{ g kg}^{-1}$  (mean  $\pm$  SD) whereas BC ranged from  $1.1 \pm 0.4 \text{ g kg}^{-1}$  to  $6.2 \pm 0.2 \text{ g kg}^{-1}$  (mean  $\pm$  SD). Levels of BC are in line with previous data showing levels ranging from 0.2 to  $14.2 \text{ g kg}^{-1}$  for 300 tested soils (Caria et al., 2011).

Concentrations of total soil NDL-PCBs in the present study ranged from  $12.2 \pm 1.1 \mu\text{g g}^{-1}$  to  $146.5 \pm 2.7 \mu\text{g g}^{-1}$  and largely exceeded ubiquitous levels of NDL-PCBs present in the environment (Meijer et al., 2003) ( $19.5 \text{ ng g}^{-1}$  of soil DM) (Fig. 2). However, the observed levels are similar to those found in industrially contaminated sites in France and are of the same order of magnitude than those used in bioavailability assays (Fouchécourt et al., 1998, Fries et al., 1989, Pu et al., 2006).

Seven soils (A2, B1, B2, B3, C, E1 and E2) exhibited a rather similar congener profile characterized by  $11.7 \pm 1.0\%$  PCB 101,  $21.5 \pm 1.9\%$  PCB 138,  $32.0 \pm 1.6\%$  PCB 153 and  $34.8 \pm 2.2\%$  PCB 180 (mean  $\pm$  SD), except A1 and D samples. The D sample displayed a higher chlorinated congener profile:  $3.5 \pm 0.4\%$  PCB 101,  $18.0 \pm 2.6\%$  PCB 138,  $35.6 \pm 2.5\%$  PCB 153 and  $42.9 \pm 0.4\%$  PCB 180 (mean  $\pm$  SD). In contrast, the A1 sample had a less chlorinated congener profile with  $29.7 \pm 1.2\%$  PCB 101,  $28.4 \pm 0.5\%$  PCB 138,  $27.7 \pm 0.6\%$  PCB 153 and  $14.2 \pm 0.9\%$  PCB 180 (mean  $\pm$  SD) (Fig. 2). Since highly chlorinated congeners are more stable in the environment than less chlorinated and more volatile ones, they are more susceptible to be retained by soil. In addition, the similarity of profile also highlights that each soil has been contaminated by a similar source (mostly dielectrical fluid from transformers) and relatively old contamination events were reported (Table 1).



**Figure 2. Profile of NDL-PCBs congener (PCB 101, 138, 153 and 180) and total NDL-PCBs concentrations ( $\mu\text{g g}^{-1}$  dry matter) in 9 contaminated soils collected in 5 different locations in France**

Details on soils origins and physic-chemical characteristics are provided in Table 1. Histograms represent the mass distribution of each congener in the total concentrations of these 4 NDL-PCBs congeners.

#### 3.2. Growing performances of piglets

No effect of the exposure doses was observed on piglets performance regardless the soil and amount of soil ingestion. Measured BW, daily BW gain and weight of liver were unaffected by both the treatment group and the amount of ingested soil ( $p > 0.10$ , data not shown). Over the 10 d-exposure period, initial BW was  $10.6 \pm 1.22 \text{ kg}$  and final one  $13.5 \pm 1.56 \text{ kg}$  (mean  $\pm$  SD). Average daily BW gain was  $0.33 \pm 0.09 \text{ kg}$  (mean  $\pm$  SD). These results also indicate that the daily oral administration of soil does not impair the development of the animals. Indeed, Fries et al. (1982) demonstrated that outdoor reared piglets may ingest up to 5.7% soil of the total daily diet.

#### 3.3. Linearity check of the dose–response relationship

The three necessary assumptions to assess an adequate dose–response relationships were checked (Littell et al., 1997) (Table 2): 1/For each experimental matrix, the response was not quadratic (Ingested PCB  $\times$  Ingested PCB (matrix) interaction,  $p > 0.07$ ). 2/The intercepts adjusted for the treatment groups were not different from each other ( $p > 0.87$ ) leading to consider that, even at low exposure doses, the model describes efficiently the experimental data. 3/The common intercept did not differ from levels found in control piglets ( $p > 0.25$ ). Thus, dose–response curves fitted a linear pattern for all exposed

**Table 2. Assessment of dose–response relationship, linear parameters and relative bioavailability (RBA) factors.**

| <i>Assessment of dose–response relationship</i>                   |                               |                          |             |                                |             |            |             |             |            |             |
|---|-------------------------------|--------------------------|-------------|--------------------------------|-------------|------------|-------------|-------------|------------|-------------|
|   | <i>p-value of model terms</i> |                          |             | <i>Statistics of the model</i> |             |            |             |             |            |             |
|   | <i>Quadratic</i>              | <i>Common in-tercept</i> | <i>X0</i>   | <i>RMSE</i>                    |             |            | <i>RSD</i>  |             |            |             |
| PCB 101   | 0.31                          | 0.97                     | 0.87        | 0.0024                         |             |            | 0.94        |             |            |             |
| PCB 138   | 0.48                          | 0.88                     | 0.60        | 0.0042                         |             |            | 0.97        |             |            |             |
| PCB 153   | 0.35                          | 0.93                     | 0.28        | 0.0041                         |             |            | 0.96        |             |            |             |
| PCB 180   | 0.29                          | 0.92                     | 0.16        | 0.0030                         |             |            | 0.96        |             |            |             |
| <i>Linear parameters of dose–response relationship</i>            |                               |                          |             |                                |             |            |             |             |            |             |
| <i>Slope of the model</i>   |                               |                          |             |                                |             |            |             |             |            |             |
|   | A1                            | A2                       | B1          | B2                             | B3          | C          | D           | E1          | E2         | R           |
| PCB 101   | 0.034                         | 0.019                    | 0.029       | 0.034                          | 0.023       | 0.051      | 0.030       | 0.034       | 0.044      | 0.033       |
| PCB 138   | 0.064                         | 0.048                    | 0.076       | 0.060                          | 0.045       | 0.069      | 0.035       | 0.060       | 0.079      | 0.046       |
| PCB 153   | 0.055                         | 0.035                    | 0.061       | 0.056                          | 0.046       | 0.063      | 0.030       | 0.060       | 0.062      | 0.044       |
| PCB 180   | 0.034                         | 0.032                    | 0.043       | 0.036                          | 0.027       | 0.044      | 0.021       | 0.041       | 0.040      | 0.039       |
| <i>Intercept of the model</i>                                     |                               |                          |             |                                |             |            |             |             |            |             |
| PCB 101   | 0.0025 ± 0.0010               |                          |             |                                |             |            |             |             |            |             |
| PCB 138   | 0.0020 ± 0.0013               |                          |             |                                |             |            |             |             |            |             |
| PCB 153   | 0.0028 ± 0.0018               |                          |             |                                |             |            |             |             |            |             |
| PCB 180   | 0.0016 ± 0.0009               |                          |             |                                |             |            |             |             |            |             |
| <i>Relative bioavailability estimates and 95% fiducial limits</i> |                               |                          |             |                                |             |            |             |             |            |             |
| <i>Adipose tissue</i>   |                               |                          |             |                                |             |            |             |             |            |             |
|   | A1                            | A2                       | B1          | B2                             | B3          | C          | D           | E1          | E2         | R           |
| <i>PCB 101</i>  |                               |                          |             |                                |             |            |             |             |            |             |
| RBA estimates   | 82.6%                         | 45.6%                    | 68.6%       | 81.5%                          | 56.4%       | 123%       | 71.2%       | 81.3%       | 106%       | 80.0%       |
| 95% fiducial limits   | [64.3–101]                    | [30.1–61.1]              | [51.6–85.6] | [63.8–99.1]                    | [40.2–72.6] | [102–143]  | [54.2–88.2] | [63.5–99.1] | [85.8–126] | [63.0–97.0] |
| <i>PCB 138</i>  |                               |                          |             |                                |             |            |             |             |            |             |
| RBA estimates   | 91.7%                         | 68.4%                    | 109%        | 86.3%                          | 64.4%       | 98.3%      | 50.9%       | 86.5%       | 114%       | 75.3%       |
| 95% fiducial limits   | [78.5–105]                    | [56.2–80.5]              | [94.6–123]  | [73.3–99.2]                    | [52.4–76.4] | [84.7–112] | [39.5–62.3] | [73.5–99.6] | [98.9–128] | [63.7–86.9] |
| <i>PCB 153</i>  |                               |                          |             |                                |             |            |             |             |            |             |
| RBA estimates   | 93.7%                         | 59.7%                    | 104%        | 95.4%                          | 78.2%       | 108%       | 50.6%       | 103%        | 105%       | 75.1%       |
| 95% fiducial limits   | [78.7–109]                    | [46.4–73.1]              | [88.0–120]  | [80.2–111]                     | [63.8–92.5] | [92.3–124] | [37.7–63.5] | [87.1–118]  | [88.7–121] | [61.5–88.8] |
| <i>PCB 180</i>  |                               |                          |             |                                |             |            |             |             |            |             |
| RBA estimates   | 83.1%                         | 78.7%                    | 107%        | 88.6%                          | 67.0%       | 110%       | 51.4%       | 101%        | 100%       | 97.2%       |
| 95% fiducial limits   | [67.0–99.2]                   | [62.9–94.6]              | [89.0–124]  | [72.2–105]                     | [51.7–82.2] | [91.8–128] | [37.1–65.8] | [84.1–119]  | [82.3–118] | [80.8–114]  |

Parameters of the linear response of normalised NDL-PCBs concentration in adipose tissue to the amount of normalised ingested indicator PCBs originating from contaminated soils or from contaminated oil..



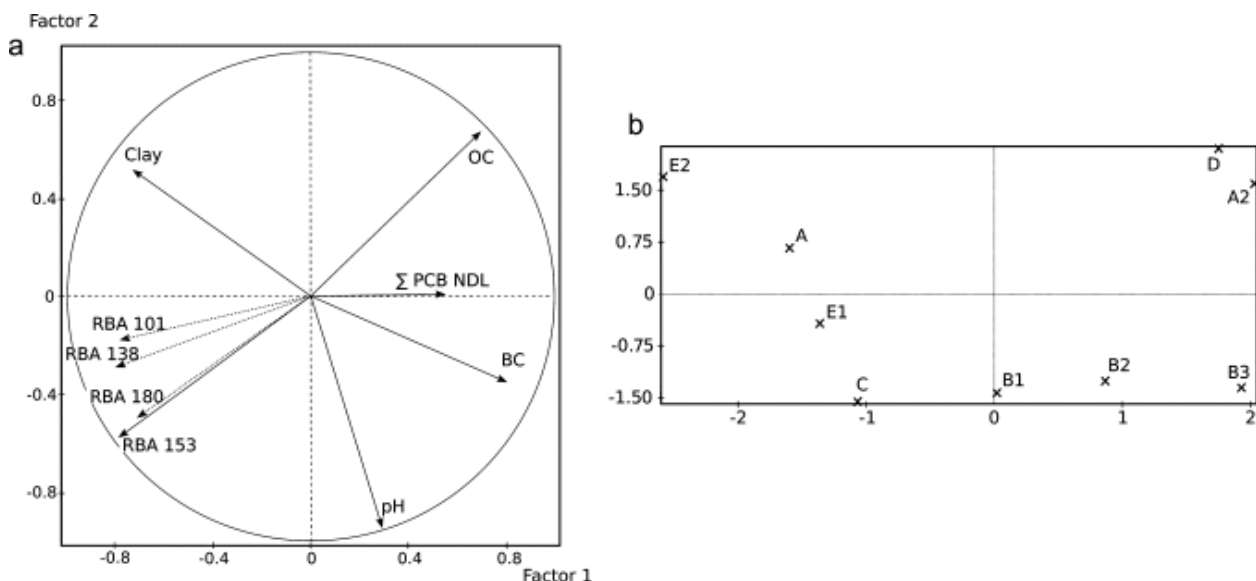
### 3.4. Factors affecting the retention of NDL-PCBs by the soil matrix

No significant correlation was found between the OM characteristics, and the BC or OC content ( $p > 0.10$ ). Those characteristics as well as NDL-PCBs concentrations are highly distributed (supporting info) and no correlations were found ( $R^2 < 0.22$ ,  $p > 0.10$ ) between them. This absence of 2-by-2 variables correlation allowed to perform a principal component analysis PCA, in order to qualitatively determine the effect of each soil-characteristics (content of OC, BC and clay, concentrations of NDL-PCBs and pH). The explained variance was rather high (78.2%). The first component (X-axis, Fig. 3) explained 44.2% of the variance whereas the second one (Y-axis, Fig. 3) explained 34.0% (PCB 153).

The first component comprised mainly RBA, OC and clay content (above the mean contribution of 0.64). Except pH, the other parameters could not be neglected, as they were above the square of the mean contribution (Supporting information). This first component discriminated a soil presenting large amounts of OC and BC, and a reduced

RBA compared to those soils presenting low OC content as well as high proportion of clay and high levels of RBA. The second component (Y-axis), comprised mainly pH, OC, RBA and clay content. This second axis discriminated soils presenting high amount of OC with relatively small amount of BC to those presented high amount of BC in regards of their OC content.

When analysing the repartition of variables (Fig. 3) RBA appeared to be negatively correlated to OC content. As the rate of agreement between variables is expressed by strength (length to origin) and direction of variables (Fig. 3) further observations could be realised. RBA was negatively correlated to OC and NDL-PCBs concentrations in soils. As strength exhibited by NDL-PCBs concentrations is the lowest one, NDL-PCBs seems to be the less informative variable of the exhibited variance. BC content appeared also weakly-correlated to RBA as directions of these variables are almost perpendicular. The same consideration could be raised for clay and pH which had a rather limited impact on RBA. For other congeners, it appeared that these observations are in line with those from PCB 153.



**Figure 3. Principal component analysis between RBA and each soil-characteristic. Figure illustrates correlations between soil-characteristic (pH, clay and OC content, concentration of the four studied NDL-PCBs (101, 138, 153 and 153;  $\Sigma$ PCB NDL) and RBA estimates**

PCB 153 was set as reference (RBA 153). PCB 101, 138 and 180 were added as illustrating variables (respectively RBA 101, RBA 138 and RBA 180). (a) Represent the space of variables whereas the (b) represent the space of the individuals.

Two main soil groups could be distinguished. On the one hand, soils with high amounts of OC ( $>100 \text{ g kg}^{-1}$ ) and BC ( $>3 \text{ g kg}^{-1}$ ) and resulting in significantly reduced RBA ( $<80\%$ ) (A2, B2, B3 and D) and on the other hand soils presenting low OC content ( $<0.5 \text{ g kg}^{-1}$ ) and high RBA (A1, B1, C, E1 and E2).

OC in soil is a well-known characteristic that plays a major role in NDL-PCBs retention (Cornelissen et al., 2005, Pignatello, 1998). In contrast, artificial soil devoid of organic matter did not present any significant reduction of

RBA (Delannoy et al., 2014a). Such results were also obtained in the present study for soil presenting high amounts of clay and low content of OC. This could be explained by the specific apolarity of NDL-PCBs leading to low retention by clay. In addition, clay structure could be intensively degraded along variations of electrochemical conditions (Chenu, 1993) during the digestive process. This impact may lead to a lower retention of apolar compounds by clay than OC. Conversely, since NDL-PCBs are apolar molecules, pH seems to have only a slight incidence on the RBA.

BC is known to be a particular reservoir for environmental contaminants. However, this parameter seems to present rather limited negative-correlation to RBA found in the present study. Despite soil-samples could exhibit higher levels of BC ( $6.2 \text{ g kg}^{-1}$  of DW), RBA found in the present study are far higher than those observed from artificial soils with activated carbon ( $10 \text{ g kg}^{-1}$  of DW) (Delannoy et al., 2014a). Quality of OM has been highlighted to have a major impact on RBA (Delannoy et al., 2014a, Saghir et al., 2007). Condensed organic matter is already known to sequester ND-L-PCBs during digestive processes of piglets (Delannoy et al., 2014a). This observation could be explained by 1/the particular physical properties of activated carbon which is far more nanoporous than BC and 2/the difference in terms of quantity of BC ( $<7 \text{ g kg}^{-1}$  in the present study and  $10 \text{ g kg}^{-1}$  in the previous one (Delannoy et al., 2014a)).

Overall, OC appears to be the main factor affecting soil bound ND-L-PCBs retention through the digestive process.

## 4. Conclusion

Soil characteristics have been shown to impact the retention of ND-L-PCBs during the digestive processes in piglets. OC appears to be the main factor affecting soil bound ND-L-PCBs retention as a high level of OC ( $>100 \text{ g kg}^{-1}$ ) and BC ( $>3.0 \text{ g kg}^{-1}$ ) could be related to a

## 3.5. Processes of sequestration of ND-L-PCBs

Retention of POPs by soil is known to be caused by adsorption on specific components like OM. During the digestive processes, a desorption of those sequestered contaminants should firstly occur before the absorption step. A solubilisation state has to be achieved before to be absorbed by the organism. As ND-L-PCBs are lipophilic molecules ( $\log K_{ow}$  of 6.33–7.22 (Li et al., 2003)), this aqueous solubilisation should be very limited. In contrast, the present results show that even soils presenting high amounts of OC ( $>100 \text{ g kg}^{-1}$ ), bioavailability is high ( $>45\%$ ). Indeed, digestion is a dynamic process involving specific mechanisms such as 1/micellar solubilisation (Oomen et al., 2004) and 2/concentrations gradient (Kelly et al., 2004). These elements explain the particular digestive efficiency despite the high lipophilicity of such molecules by improving the apparent solubilisation and partitioning of these molecules in aqueous phase. They also highlight that, for common soils, a limited reduction of RBA ND-L-PCBs is expected.

significant decrease of RBA ( $<80\%$ ) compared to oil. However, RBAs were never found below 46% for all soil samples despite wide variations of OC ( $7.2\text{--}180 \text{ g kg}^{-1}$ ). This result reveals that a notable fraction of soil bound ND-L-PCBs was bioavailable regardless the soil considered.

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