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# Effects of condensed organic matter on PCBs bioavailability in juvenile swine, an animal model for young children

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## Highlights

- We performed an exposure assessment of NDL-PCBs from contaminated soils to piglets.
- We analyzed the impact of soil organic matter on NDL-PCBs bioavailability.
- Two organic matters with opposite degrees of condensation were tested.
- The condensed one strongly reduced bioavailability of NDL-PCBs.
- No retention of NDL-PCBs was seen with the less condensed one.

## Keywords

Soil; Bioavailability; NDL-PCBs; Children; Piglets; Organic matter

## Abstract

The exposure assessment of polychlorinated biphenyls (PCBs) contaminated soils is a critical issue in terms of human health, especially since little reliable information on transfer of PCBs to humans via involuntary soil ingestion is available. Indeed, young children with their hand-to-mouth activity may be exposed to contaminated soils. The current study addresses the impact of soil organic matter (OM) condensation on bioavailability of sequestered NDL-PCBs. Three artificial soils (ASs) were prepared according to OECD guideline 207. One standard soil (SS), devoid of OM, and two amended versions of this SS with fulvic acid (FA) or activated carbon (AC) were prepared to obtain 1% organic mass. This study involved fourteen juvenile male swine as a digestive physiology model of young children. Animals were randomly distributed into 4 contaminated groups (3 replicates) and a control one (2 replicates). During 10 d, the piglets were fed AS or a corn oil spiked with 19 200 ng of Aroclor 1254 per g of dry matter (6000 ng g<sup>-1</sup> of NDL-PCBs) to achieve an exposure dose of 1200 ng NDL-PCBs kg<sup>-1</sup> of body weight per day. After 10 d of oral exposure, NDL-PCBs in adipose tissue, liver and muscles were analyzed by GC-MS, after extraction and purification. Two distinct groups of treatments were found: on the one hand oil, SS and FA, on the other hand C and AC. This study highlights that condensed OM (AC) strongly reduces bioavailability whereas the less condensed one (FA) does not seem to have a significant effect. This result has to be considered as a first major step for further relative bioavailability studies involving mixture of different humic substances.

## 1. Introduction

The exposure assessment to pollutants from contaminated soils is a critical issue in terms of safety management. Little reliable information is available on the pollutant transfer to target population through involuntary soil ingestion. Children may exhibit hand-to-mouth activities and according to their development they are particularly susceptible to hazardous chemicals. Indeed, the infant has an immature blood-brain barrier (Grandjean and Landrigan, 2006) and at various developmental stages, his metabolism pathways may differ from those of adults (Vieira et al., 1996, NRC, 2003). Concerning PCBs, cohorts studies (on 7 months of age, and at 4 years of age) reported a possible memory deficit following exposure (Jacobson et al., 1992, Jacobson and Jacobson, 1996, Faroon et al., 2000, Grandjean and Landrigan, 2006). Similar results were found following a perinatal exposure in rats (Lilienthal and Winneke, 1991, Corey et al., 1996, ATSDR, 2000), even at environmentally low

doses (Elnar et al., 2012) and in monkeys (Levin et al., 1988, Schantz et al., 1989). These neurodevelopmental and behavioral impacts were linked to non-dioxin like congeners (Boix et al., 2011, Boix et al., 2010). Six of these non-dioxin like PCBs (NDL-PCBs) constitute the most abundant part of PCBs found in environmental matrices like soils, sediments or foodstuff (Meijer et al., 2003, ANSES, 2007, EFSA, 2010) and are laid down in European regulation No 1259/2011 (European Commission, 2011). In this context and in order to perform a quantitative assessment of health risks, it appears of real importance to focus on exposure to NDL-PCBs via involuntary soil ingestion. The relative bioavailability (RBA) approach which relies on the ratio of the bioavailability of a compound in one exposure context to that in another exposure context appears to be a valuable way to perform this exposure assessment (NRC, 2003, USEPA, 2007a, USEPA, 2007b).



However, when considering the RBA of lipophilic organic soil bound compounds, highly variable results were obtained between mammals highlighting the major importance of the animal model (Fries and Marrow, 1992, Saghir et al., 2007, Ounnas et al., 2009, Fournier et al., 2012, Jondreville et al., 2013, Feidt et al., in press). Considering the assessment of children's exposure after ingestion it appears that three animals are particularly used and pertinent to model children: rats, monkeys and swine. Concerning juvenile swine and rats contaminated soil uptakes have been also previously reported to be different (Budinsky et al., 2008). Indeed, gastrointestinal absorption differs between rats and swine regarding specific mechanisms driving absorption, as noticed for metallic ions (Oomen et al., 2004, Casteel et al., 2006, USEPA, 2007a, Denys et al., 2012). The juvenile swine appears to be a better model than rodent one. Indeed, the relative similarity of physiology, growth and mechanisms of absorption between young humans and swine led to an increasing use of this animal as a model of oral bioavailability and its application in risk assessment studies (Casteel et al., 2006, USEPA, 2007a, Budinsky et al., 2008). Such an approach consisting a combination of both RBA methodology and swine model has already been performed for trace metals (Denys et al., 2012).

Overall assessment of the risk linked to contaminated sites is mostly carried out in response to toxicity to humans and in estimating this, the oral bioavailability of the

## 2. Material and Methods

### 2.1. Experimental design

In order to assess the impact of soil OM condensation on RBA of NDL-PCBs the following study was designed (Fig. 1). The first step of the study consisted in assessing the linearity of the NDL-PCBs dose-response pattern. (Fig. 1). The following step was aimed at evaluating the impact of OM condensation on the concentration of NDL-PCBs in tissues. Two well-characterized sources of OM were selected to illustrate opposite degrees of condensation: fulvic acid and activated carbon (Fig. 1). RBA was estimated by comparing the levels obtained in target tissues (liver, adipose tissue, muscle) of spiked soil-feed or corn oil feed (Wittsiepe et al., 2007).

### 2.2. Dosing material

#### 2.2.1. Spiked oil

Spiked corn oil containing graded levels of Aroclor 1254 (Sigma-Aldrich, Supelco, Saint-Louis, USA) were used in the first step of the study. Four doses of Aroclor 1254 were prepared in order to achieve these four exposure doses: 3000 ng Aroclor 1254 per g of spiked oil (1X), 9000 ng g<sup>-1</sup> (3x), 15 000 ng g<sup>-1</sup> (5x), 21 000 ng g<sup>-1</sup> (7x) (Fig. 1). These exposure doses corresponded respectively to 950 ng of NDL-PCBs per g of spiked oil (1x), 2800 ng g<sup>-1</sup> (3x), 4700 ng g<sup>-1</sup> (5x) and 6550 ng g<sup>-1</sup> (7x). The reference spiked oil was contaminated with 19 200 ng

contaminants is a key parameter. Soil particles that are ingested by mammals are exposed to gastro-intestinal fluids, enzymes and salts (Oomen et al., 2004). Bile acids, pancreatic enzymes, the dietary environment may play major roles in the extraction of organic compounds from soil in the gastro-intestinal lumen (Oomen et al., 2004). It is also known that physical/chemical properties of the soil, like porosity, pH, clay, and organic matter (OM) content, along with aging, may impact both sequestration and/or release of contaminants (Saghir et al., 2007). The characteristics of the soil OM is thought to be one of the most important factors that determines the strength of interactions between the soil fractions and the organic lipophilic pollutants (Pignatello and Xing, 1996). Thus, the sorption-desorption process would be driven by OM characteristics like condensation (Pignatello, 1998). Furthermore, ample evidence has been gathered *in vitro* that adsorption of lipophilic chemicals to soil particles is positively correlated to OM content (Ping et al., 2006).

The present study was conducted to assess the effects of different OM characteristics on the oral RBA of NDL-PCBs sequestered in two artificial soils amended with 1% of organic carbon in the juvenile swine. Assessment of the relevance of the target tissues and the range of linearity of the response to oral exposure were tested first.

Aroclor 1254 per g of spiked oil corn corresponding to 6000 ng of NDL-PCBs per g of spiked oil. Corn oil (Sigma Aldrich) was spiked with an adequate volume of a hexane solution of Aroclor 1254 (Sigma Aldrich, Supelco). Secondly, hexane was rotary-evaporated over 2 h and let evaporating under an extractor hood overnight. The difference of weigh of spiked corn oil before and after spiking and evaporation was assessed to determine persistent hexane was less than 1:10 000.

#### 2.2.2. Artificial soils and spiking

Three artificial soils were prepared. They were based on a standard soil (SS) which was prepared according to Organization for Economic Cooperation and Development (OECD) guideline 207 (OECD, 1984). This soil fulfilled the composition and pH of an OECD artificial soil but the sphagnum peat was not introduced to avoid sorption competition between the different OM phases. One of the studied soils was SS. To identify the incidence of OM on NDL-PCBs bioavailability, two well-characterized OMs were used. fulvic acid was purchased from the International Humic Substances Society (Suwannee River II, IHSS, St. Paul) and introduced in a part of SS to form a fulvic acid amended artificial soil (FA). Secondly activated carbon (Activated carbon, Sigma) was introduced in a part of SS to form an activated carbon amended artificial soil (AC). Fulvic acid and activated carbon were introduced in FA and AC soils respectively in order to achieve a mass concentration



of 1% of the organic carbon regarding the whole dry matter (DM) of each soil. The soils were mixed manually thoroughly for 5 min until a homogeneous color soil was reached.

An adequate amount of water and PCBs were introduced at the same time in the three artificial soils which were stored in amber glass container. The spiking solution was prepared using milliQ water and the adequate volume of Aroclor 1254 dissolved in methanol to achieve 19 200 ng g<sup>-1</sup> of DM and a moisture content of 17.5%. Expected exposure concentrations in NDL-PCBs are: 7.3 ng kg BW<sup>-1</sup>

(PCB 28), 206 ng kg BW<sup>-1</sup> (PCB 52), 308 ng kg BW<sup>-1</sup> (PCB 101), 223 ng kg BW<sup>-1</sup> (PCB 138), 145 ng kg BW<sup>-1</sup> (PCB 153) and 26 ng kg BW<sup>-1</sup> (PCB180). The same amount of spiking solution was introduced in 85 g of DM of each artificial soil. The soils were mixed manually thoroughly for 10 min until a homogeneous color was reached and methanol was evaporated under an extractor hood overnight. Then, additional water was added to achieve 17.5% of moisture and soils were thoroughly mixed during 10 additional minutes. The containers were mixed on a rolling mill for 24 h and then placed in a 10 °C room in static position during 4 weeks to allow correct impregnation aging of NDL-PCBs in soil matrices.

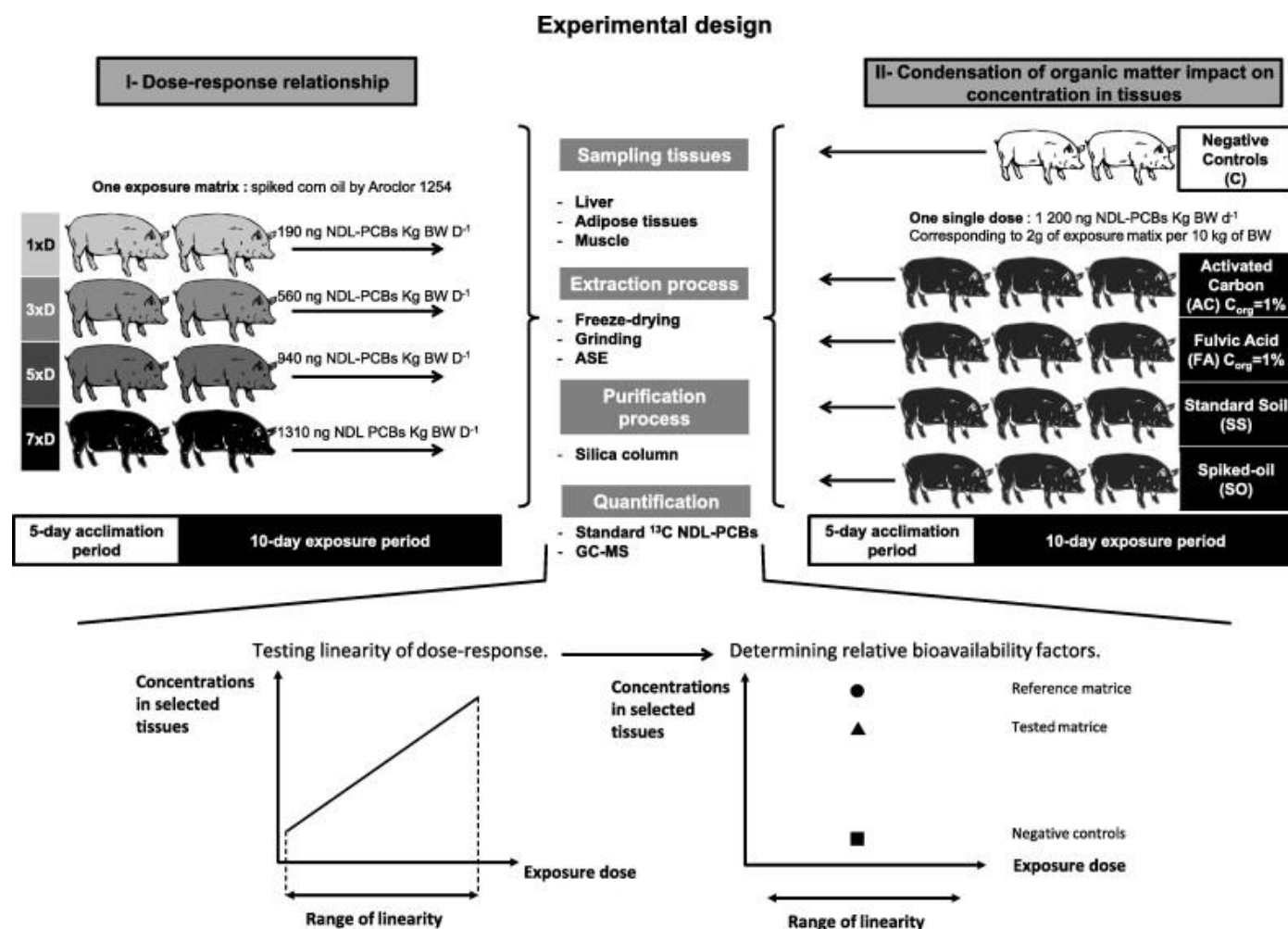


Figure 1. Animal management and experimental design of the study

### 2.2.3. Dough ball manufacturing

Animals were exposed to Aroclor 1254 for 10 d. The daily doses and ration were based on the individual body weight (BW). The oral delivery doses were achieved by transferring a calculated weight of contaminated soil-matrices (200 mg of DM per kg of BW  $\pm$  5%) in the depression of one dough ball. Dough balls were manufactured by squeezing repeatedly 8 g of commercial pellets (Porcelet Super VE 25 kg, Lorial, Laxou, France) moistened with an adequate amount of milliQ water to obtain dough-like consistency. For the dough ball containing spiked oil, 4 g of commercial pellets were pulverized using Analytical mill A 11 (IKA®, Staufen, Germany), mixed with spiked oil and then placed in the depression of 4 g of commercial pellets moistened by distilled water.

### 2.3. Animals

Each step of the experiment was performed with a distinct group of juvenile swine. Crossbred (Pietrain  $\times$  Youna®) castrated male swine, weaned at 28 d were kept in individual stainless-steel metabolism cages with wire floors and nipple waterers which delivered fresh tap water *ad libitum*. The animals were fed an age-appropriate commercial pelleted swine diet once daily at a level of 5% of body weight (Porcelet Super VE 25 kg). All animals were acclimated to the facility during 5 d. One dough ball containing the appropriate dose for acclimation or exposure was hand fed 3 h prior to ration distribution. An ambient temperature of 26–27 °C and a nycthemeral cycle (10 h light) were maintained in the animal room. Human care of swine and administration of spiked artificial soils and oils were carried out in compliance with the guidelines of the French Ministry of Agriculture for Animal Research..

#### 2.3.1. Assessment of the dose–response linearity

Eight swine were acclimated to the facilities and adapted to progressive oil supplementation in hand fed dough balls during 5 d (BW 8.0  $\pm$  0.5 kg). Two swine ( $n = 2$ ) were assigned to each treatment groups 1x, 3x, 5x and 7x. The exposure period of 10 d consisted of a daily administration of one moistened dough ball containing the appropriate quantity of one of the four spiked oils to achieve the expected exposure doses.

#### 2.3.2. Assessment of the oral bioavailability

Fourteen swine were acclimated to the facilities and adapted progressively to oil supplementation and geophagia during 5 d (body weight 8  $\pm$  0.5 kg). Three swine ( $n = 3$ ) were assigned to each treatment group exposed to one of the four exposure matrices: SS, AC, FA or spiked oil

(SO). Two control swine (C) were exposed to a clean standard soil ( $n = 2$ ) through clean dough balls. The exposure period consisted in a daily administration of one moistened dough ball containing the appropriate quantity of one of the four matrices in order to achieve the daily exposure dose (1200 ng of NDL-PCBs per kg BW).

### 2.4. Sampling and analysis of biological samples

After exposure period piglets were slaughtered by electronarcosis immediately followed by exsanguination. After laparotomy, the whole liver was collected after exeresis of the gallbladder, as well as pericaudal adipose tissue and rib-eye muscle (Longissimus dorsi). All samples were kept in individual boxes and chopped. Then, samples were freeze-dried and pulverized thoroughly using Analytical mill (A 11, IKA®, Staufen, Germany). NDL-PCBs were analyzed by gas chromatography coupled with a mass spectrometer (GC–MS). These analyzes involved three successive steps: extraction, purification and quantification. Chemicals used in this study were pesticide residue analytical grade.

#### 2.4.1. Extraction

Before extraction, 6 <sup>13</sup>C-labeled NDL-PCBs from LGC Standard (Molsheim, France) were added to the various samples studied. After spiking, the solid samples were transferred into accelerated solvent extraction (ASE) cells. Pressure and temperature were set at 100 bar and 120 °C respectively. The extraction solvent was a mixture composed of toluene (Sigma–Aldrich, Fluka) and acetone (Sigma–Aldrich, Fluka) at 70:30 (v/v), and three successive extraction cycles (5 min each) were performed by ASE device (ASE 350, Dionex, Sunnyvale, USA). The extracts were redissolved in cyclohexane (Sigma–Aldrich, Fluka) for sample clean up after rotary evaporation.

#### 2.4.2. Purification

The purification process included a glass column (length 50 cm; ID 2.5 cm) which was composed of successive layers including 6.0 g ( $\pm$ 0.10 g) of Florisil (Promochem, Fluka) added with 20 mL of cyclohexane, 5.0 g ( $\pm$ 0.10 g) of anhydrous sodium sulfate (Sigma–Aldrich, Wesel, Germany), 4 g ( $\pm$ 0.1 g) of pure silica gel (Sigma–Aldrich, Fluka) added with 20 mL of cyclohexane (Sigma–Aldrich, Fluka), 8.0 g ( $\pm$ 0.20 g) of silica gel acidified with sulfuric acid (VWR International, Analar, Poole, England) (22%) added with 20 mL of cyclohexane, 10.0 g ( $\pm$ 0.25 g) of silica gel acidified with sulfuric acid (44%) added with 20 mL of cyclohexane and 5.0 g ( $\pm$ 0.10 g) of anhydrous sodium sulfate.

#### 2.4.3. Quantification



PCBs analyzes were performed by GC–MS (7890A, Agilent Technologies, Santa Clara, USA). The mass spectrometer was set at a resolution of 10 000, in electron ionization mode (70 eV electron energy) (5975C, Agilent Technologies, Santa Clara, USA). Single Ion Monitoring (SIM) was used to record the two most abundant signals. A DB5MS (30 m × 0.25 mm × 0.251 m) capillary column from Agilent J&W (Agilent Technologies) was used in splitless mode. The GC temperature program for PCBs analysis was the following: 110 °C (1 min), 20 °C min<sup>-1</sup> to 200 °C (0 min) and 2 °C min<sup>-1</sup> to 230 °C and 5 °C min<sup>-1</sup> to 270 °C and 10 °C min<sup>-1</sup> to 300 °C (5 min). Signals were integrated using Chemstation (chemstation E.02.00, Agilent Technologies, Santa Clara, USA). To avoid any over estimation the control samples were injected at the beginning of the experiment.

## 2.5. Calculations and statistical analyses

### 2.5.1. Assessment of linearity

Linearity of the dose response of NDL-PCBs concentration to ingested NDL-PCBs was assessed by regression analysis performed by means of the REG procedure of SAS (9.1, SAS Institute, Cary, NC, USA). The linear and the quadratic effects of PCBs ingested (ng g<sup>-1</sup> BW d<sup>-1</sup>) were tested. Where not significant ( $P > 0.10$ ) the quadratic term was removed from the model and the response was declared linear.

### 2.5.2. Incidence of condensation of OM on bioavailability

In order to evaluate the incidence of contaminated matrices on bioavailability a variance analysis were performed. For each congener, values below the limit of quantification (LOQ) were replaced by LOQ/2 to avoid missing data. Statistical analyzes were carried out using SAS (9.1, SAS Institute). Each piglet was considered as an experimental unit. Concentrations of each NDL-PCB congener in liver, muscle, and adipose tissue were analyzed using the GLM procedure. The model included the exposure media (AC,

FA, SS, SO,  $n = 3$  and C,  $n = 2$ ). A Tukey–Kramer test was used for comparison of means. Differences were considered significant for  $P < 0.05$ .

### 2.5.3. Relative bioavailability factors (RBA) calculation

In order to compare the bioavailability from the two exposure media (soil and oil), the relative bioavailability in a selected tissue was calculated as the ratio of the bioavailability in soil and the bioavailability in oil, adapted from a method preliminary described (Wittsiepe et al., 2007). Estimation of bioavailability in one matrix of exposure  $m$  in a selected tissue  $t$  was calculated as the ratio of the mass of a PCB-NDL-congener  $p$  in the tissue to the administered mass of the same congener from soil or oil multiplied by 100%. Because no significant differences were found considering ratio of masses of sampled tissues on BW each treatment group, it was assumed that masses of each tissue were similar. Calculation of RBA was performed using the ratio of the adjusted means of concentrations of NDL-PCBs congener in tissues after a soil and an oil exposure after subtracting concentration founded in controls.

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:

$$RBA_{m,t,p} = \frac{C_{m,t,p}^{soil} - C_{controls,t,p}}{C_{m,t,p}^{oil} - C_{controls,t,p}}$$

## 3. Results

The animals remained healthy throughout the experiment. During the exposure period, daily ingestion and growth performance were unaffected by treatments. Thus the average daily gain was  $0.24 \pm 0.03$  kg of BW d<sup>-1</sup> (mean ± SD,  $P > 0.10$ ) in the first step and  $0.27 \pm 0.04$  kg of BW d<sup>-1</sup> for the second one (mean ± SD,  $P > 0.10$ ). At the end of first experiment piglets weighed  $9.04 \pm 1.22$  kg of BW (mean ± SD,  $P > 0.10$ ), and after the follow up one  $9.98 \pm 0.72$  kg of BW (mean ± SD,  $P > 0.10$ ).

### 3.1. Assessment of the dose–response linearity

Limits of quantification are presented in Table 1. Since levels of PCB 28 were found to be mainly below LOQ, this congener was not further considered. Data concerning one animal (dose 5×) was not considered in the statistical analysis because of abnormally high concentrations. Regression analysis for PCBs 52, 101, 138 and 153 demonstrated significant linear relationships ( $P < 0.01$ ) between the administrated doses and the levels of NDL-PCBs analyzed in adipose, hepatic and muscular tissues



**Table 1. Parameters of the linear dose–response relationships between NDL-PCBs doses (ng kg BW<sup>-1</sup> d<sup>-1</sup>) and retrieved concentration in adipose, muscular and hepatic tissues (ng g<sup>-1</sup> of fat).**

		Estimated slope ± SE	Estimated intercept ± SE	LOQ	Calculated R <sup>2</sup>	RMSE
PCB 52	Adipose tissue	0.123 ± 0.010***	NS	0.278	0.82	1.37
	Muscle	0.117 ± 0.001***	NS	1.17	0.77	1.28
	Liver	0.195 ± 0.001***	NS	1.25	0.96	1.16
PCB 101	Adipose tissue	0.082 ± 0.013***	2.45 ± 0.960***	0.292	0.87	1.15
	Muscle	0.119 ± 0.001***	NS	0.813	0.81	1.75
	Liver	0.145 ± 0.001***	NS	0.360	0.92	1.55
PCB 138	Adipose tissue	0.107 ± 0.011***	NS	0.934	0.71	1.72
	Muscle	0.183 ± 0.013***	NS	3.52	0.83	2.23
	Liver	0.229 ± 0.024***	NS	1.08	0.78	3.89
PCB 153	Adipose tissue	0.105 ± 0.012***	1.55 ± 0.430***	0.220	0.92	1.55
	Muscle	0.161 ± 0.019***	NS	0.824	0.57	1.92
	Liver	0.184 ± 0.018***	NS	0.394	0.80	2.16
PCB 180	Adipose tissue	0.084 ± 0.019**	0.490 ± 0.116**	0.309	0.77	0.14
	Muscle	0.176 ± 0.040*	NS	1.64	0.43	0.36
	Liver	0.315 ± 0.031***	NS	0.316	0.86	0.29

A sequential fitting was performed in order to suppress insignificant parameters. P-value and resulting model originated of parameters values are presented. RMSE: root means square error.

P-value of parameters are presented in front of each value: NS > 0.10.

\* : P < 0.05.

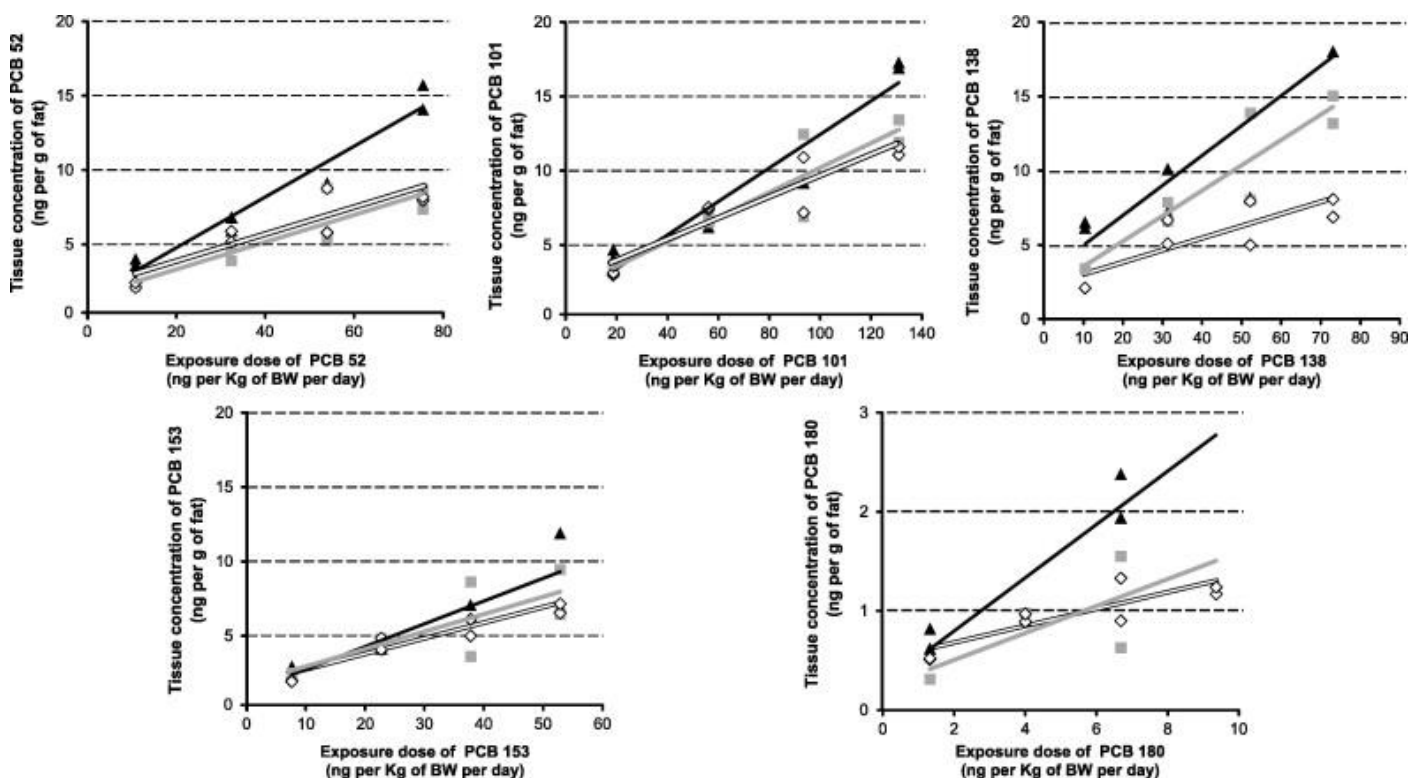
\*\* : P < 0.01.

\*\*\* : P < 0.001.



whereas the quadratic terms were not significant ( $P > 0.10$ , Table 1). No conclusions could be reached considering PCB 180 results because of integration issues in some chromatograms. Considering the coefficient of determination ( $R^2$ ) equal or over than 0.77 for subcutaneous fat, 0.77 for hepatic tissue and 0.71 for muscular one, linear equations provided reliable

predictions of dose–response relationships in these tissues in the tested range of dose (3000–21 000 ng of Aroclor 1254 per kg of BW) (Table 1, Fig. 2). The intercepts did not differ from zero ( $P > 0.10$ ) except for 3 ND-L-PCBs in adipose tissue. Higher slopes of dose–responses were found in hepatic tissues when compared to muscles or adipose tissue (Table 1, Fig. 2)



**Figure 1. Dose–response relationships of ND-L-PCBs in liver, muscle and adipose tissue**

(values are presented respectively by full triangles ▲, grey squares ■, empty diamonds ◇;  $n = 2$ ) to the amount ingested originating from spiked oil. Parameters of the linear models are presented in Table 1 (respective curves are fitted by full black lines, grey and white ones).

### 3.2.3.2. Incidence of condensed OM on RBA

Regardless of the contamination media, PCB 28 levels were below LOQs and were not included in the data set. ND-L-PCBs concentrations in all analyzed tissues (hepatic, muscular and subcutaneous fat) were above LOQs in C for all other congeners. The highest levels of ND-L-PCBs were recovered in tissue samples from piglets contaminated by SO, SS and FA spiked with the same amount of Aroclor 1254 (6000 ng  $g^{-1}$  of soil DM corresponding to a similar exposure dose of 1200 ng  $kg^{-1}$  of BW). Thus, the lowest levels of ND-L-PCBs were observed in the AC and in the C groups.

## 4. Discussion

Although the historic of contamination of the experimental animals were known, most control tissues presented

The ratio of means by treatment indicated that ND-L-PCBs levels in tissues after AC treatment were higher than after C exposure (1.3–5.2 times higher) and much lower than the SO reference tissues (5.2–9.8 times lower). Two distinct groups of treatments composed by “SO, SS, FA” on the one hand and “AC, CS” on the other one ( $P < 0.05$ ) could be differentiated (Table 2). The exposure media effect was significant ( $P < 0.02$ ) except in liver for PCB 52. Higher levels were found in liver than in muscles (1.4–1.7 times higher considering ratios between sum of 5 ND-L-PCBs) and in adipose tissue (1.2–1.4 times) (Table 2). Calculated RBA factors were estimated for the ND-L-PCBs sum (5 ND-L-PCBs) (Table 2).

quantifiable levels of ND-L-PCBs (above LOQs, Table 1). These basal levels were always far below the levels set in regulation (40 ng per g of fat) (European Commission,





2011). Despite formal banning of PCBs use in France since 1989, PCBs are known to be persistent and ubiquitous contaminants of the environment (Meijer et al., 2003). Thus, noticeable levels of PCBs were found in several environmental matrices (soil and sediments) without a known history of contamination (Meijer et al., 2003).

The experimental design of this RBA study required a validation step. Indeed, the need to determine the dose–response pattern in order to evaluate the representativeness of final concentrations of NDL-PCBs in tissues related to an ingested dose of contaminants appeared as a major point. In fact, it is known that ample variability exists between individuals among applied doses (USEPA, 2007a). This variability tends to increase as the dose increases, resulting to a rise of variance. Among factors that could lead to the increase of the variance, metabolism induction which is depending on individual susceptibility and the applied dose, is known to be one of the most important (Devito et al., 1993, Tang et al., 2006). The linearity of the dose–response in the tested range of doses validates the correspondence between the ingested doses and the final concentrations of NDL-PCBs in the studied organs.

No significant different behaviors could be noticed between PCBs from SS, FA, SO whereas far lower concentrations were found after AC treatment. Since OC content were equal for FA and AC treatments, this study reveals that the quality of the OM pilots the retention of PCBs during the digestive process in piglets. It demonstrates therefore that carbon organic content should be analyzed in regards with characteristics of this OM to determine potentialities of bioavailability reduction of this media. This study also highlights that poorly condensed OM, like fulvic acids, does not significantly reduce bioavailability compared to the reference matrix, SO. Some hindsight concerning bioavailability of pharmaceutical apolar compounds like furosemide tends to show that this sort of OM should even increase bioavailability of such compounds (Agarwal et al., 2008, Agarwal et al., 2007). However no statistical evidence was achieved to confirm or infirm this hypothesis comparing SS and FA treatments. Activated carbon is a manufactured and clean type of black carbon and is often used to model this carbonaceous, ubiquitous and highly condensed organic material (Zimmerman et al., 2004, Cornelissen et al., 2006, Saghir et al., 2007). Black carbon is already known to provide strong binding with apolar contaminants like PAHs resulting in a physical trapping and reducing desorption processes and

bioavailability to limbic and mammals (Pikaar et al., 2006). Activated carbon is also suggested as an efficient way to remediate contaminated areas by geosorbing apolar compounds (Zimmerman et al., 2004, Cornelissen et al., 2006, Jakob et al., 2012). Nanoporosity of black carbon has been previously demonstrated as responsible for sorption of pollutants (Pignatello, 1998). An industrial and highly nanoporous activated carbon introduced in a simple artificial soil, was employed during the current experiment to evaluate potentialities of reduction offered by this kind of media during the digestive process. It appeared that activated carbon dramatically reduced RBA of NDL-PCBs resulting to less considerably exposition to these pollutants through direct ingestion of contaminated soil. However this apparently heavy sequestration correspond to an activated carbon particularly well-known for its notable surface specific area, far higher than commonly observed in black carbon (Koelmans et al., 2006) and simple artificial soils. The total organic carbon chosen (1%) ranges in the limit low concentrations found in soils whatever land use (Agarwal and Bucheli, 2011). Median black carbon as a fraction of total organic carbon content (BC/TOC) has been previously reported to be 4% in soils (Cornelissen et al., 2005). However much higher levels were found in fire-impacted soils where black carbon reached 30–45% of total organic carbon (Cornelissen et al., 2005). These considerations let us considerate as possible such levels of organic carbon and black carbon in natural soils. PCBs concentrations employed correspond to 1000 folds medium known-levels concentrations found in soils without historic of contamination (19 500 pg g<sup>-1</sup>) (Meijer et al., 2003). These concentrations correspond also to those found in some industrial areas (Schuhmacher et al., 2004). Therefore this huge reduction of bioavailability may be overestimated comparing to what may occur in naturally contaminated soils.

Thus, the main interest of this study was to simulate extreme conditions with a same amount of organic carbon (OC) either in the form of AC or in the form of FA. No other OM was used during processing of the artificial soils in order to ensure no competition effect between different phases of OM (Burgess et al., 2005). Indeed, sorption of activated carbon in environmental matrices could be weaker than those reported using activated carbon alone. Blockage of sorption of lipophilic apolar contaminants sites in complex matrices by several compounds like natural organic matter (Pignatello et al., 2006) or lipids components (Kwon and Pignatello, 2005) are known to decrease sorption in environmental matrices.

## 5. Conclusion

Characteristic of the OM pilots the retention of PCBs during the digestive process in piglets. Indeed, it appears that condensed OM (AC) strongly reduces bioavailability whereas the less condensed one (FA) does not seem to

have a significant effect. These elements are of great interest for further investigations in terms of risk assessment in contaminated areas



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

		<b>C</b>	<b>AC</b>	<b>FA</b>	<b>SS</b>	<b>SO</b>	<b>Exposure media</b>	<b>RMSE</b>
PCB 52	Adipose tissue	0.934 <sup>a</sup>	1.37 <sup>a</sup>	8.70 <sup>b</sup>	11.4 <sup>bc</sup>	15.0 <sup>c</sup>	0.001	2.1
	Muscle	0.583 <sup>a(2)</sup>	1.83 <sup>a</sup>	6.21 <sup>ab</sup>	7.88 <sup>b</sup>	9.11 <sup>b</sup>	0.003	2.0
	Liver	2.29	3.10 <sup>A</sup>	12.9	12.1 <sup>A</sup>	20.4	0.351	10.3
PCB 101	Adipose tissue	0.505 <sup>a</sup>	1.83 <sup>a</sup>	13.8 <sup>b</sup>	13.6 <sup>b</sup>	16.8 <sup>b</sup>	<0.001	1.7
	Muscle	0.407 <sup>a(2)</sup>	2.24 <sup>a</sup>	9.73 <sup>b</sup>	13.2 <sup>b</sup>	12.2 <sup>b</sup>	<0.001	1.5
	Liver	1.27 <sup>a</sup>	2.37 <sup>a</sup>	10.1 <sup>ab</sup>	17.4 <sup>bc</sup>	23.5 <sup>c</sup>	<0.001	4.1
PCB 138	Adipose tissue	0.467 <sup>a(2)</sup>	1.75 <sup>a</sup>	14.6 <sup>b</sup>	11.8 <sup>b</sup>	14.4 <sup>b</sup>	0.003	3.6
	Muscle	1.76 <sup>ab(2)</sup>	1.76 <sup>ab(3)</sup>	11.7 <sup>bc</sup>	15.5 <sup>c</sup>	14.8 <sup>c</sup>	0.001	3.3
	Liver	1.56 <sup>ab</sup>	2.57 <sup>a</sup>	24.9 <sup>abc</sup>	27.9 <sup>bc</sup>	34.9 <sup>c</sup>	0.005	8.9
PCB 153	Adipose tissue	1.19 <sup>a</sup>	1.97 <sup>a</sup>	12.5 <sup>b</sup>	10.8 <sup>b</sup>	15.0 <sup>b</sup>	<0.001	2.3
	Muscle	0.626 <sup>a(1)</sup>	2.59 <sup>a</sup>	9.06 <sup>ab</sup>	13.4 <sup>b</sup>	12.9 <sup>b</sup>	0.004	3.3
	Liver	4.35 <sup>a</sup>	3.19 <sup>a</sup>	23.3 <sup>b</sup>	23.3 <sup>b</sup>	27.0 <sup>b</sup>	0.002	5.7
PCB 180	Adipose tissue	0.154 <sup>a(2)</sup>	0.493 <sup>a</sup>	2.37 <sup>b</sup>	2.45 <sup>b</sup>	2.45 <sup>b</sup>	<0.001	0.5
	Muscle	0.821 <sup>ab(2)</sup>	0.821 <sup>a(3)</sup>	1.78 <sup>b</sup>	2.59 <sup>c</sup>	2.45 <sup>bc</sup>	0.010	0.6
	Liver	0.530 <sup>abA</sup>	0.618 <sup>a</sup>	4.31 <sup>b</sup>	4.21 <sup>b</sup>	4.56 <sup>b</sup>	0.010	1.2
ΣPCB	Adipose tissue	3.38 <sup>a</sup>	8.96 <sup>a</sup>	72.6 <sup>b</sup>	68.3 <sup>b</sup>	89.9 <sup>b</sup>	<0.001	12.5
	Muscle	4.54 <sup>a</sup>	10.6 <sup>a</sup>	51.5 <sup>b</sup>	66.7 <sup>b</sup>	65.6 <sup>b</sup>	0.001	10.7
	Liver	11.1 <sup>aA</sup>	14.5 <sup>a</sup>	87.6 <sup>b</sup>	95.5 <sup>b</sup>	124 <sup>b</sup>	<0.001	20.6
RBA factors	Adipose tissue		6.4% <sup>B</sup>	80.0% <sup>C</sup>	75.0% <sup>C</sup>			
ΣPCB	Muscle		9.9% <sup>B</sup>	76.9% <sup>C</sup>	101.8% <sup>C</sup>			
	Liver		3.0% <sup>B</sup>	67.8% <sup>C</sup>	74.8% <sup>C</sup>			

Values are adjusted means (n = 3 for AC, FA, SS and SO groups; n = 2 for C).

Letters (a, b, c) indicates values that do not statistically differ from other values within column presenting a common letter (P < 0.05).

Numbers into brackets indicate values below LOQ replaced by LOQ/2.

RMSE: root means square error.



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