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Abstract

In a risk assessment perspective, this work aims to assess the bioaccessibility of PCBs in meat. A standardised *in vitro* static digestion protocol was set up and coupled with extraction, clean-up and GC×GC-ToF/MS multianalyte method to monitor the fate of PCBs in meat during digestion. Starting with spiked meat, PCB bioaccessibility in 11% fat medium-cooked meat varied in adults from 20.6% to 30.5% according to congeners. PCB bioaccessibility increased to 44.2-50.1% in 5% fat meat and decreased to 6.2-9.1% and to 14.6-19.4% in digestion conditions mimicking infants and elderly, respectively. Intense cooking also decreased PCB bioaccessibility to 18.0-26.7%. Bioaccessibility data obtained with spiked meat were validated with measurements carried out in incurred meat samples. Finally, mean uptake distributions are obtained from a modular Bayesian approach. These distributions feature a lower mode when the fat content is higher, the meat is well-done cooked, and the consumers are older.

Highlights

- An *in vitro* digestion protocol was set up to assess PCB bioaccessibility in meat
- The mean PCB bioaccessibility was 26% in 11% fat ground beef
- PCB bioaccessibility and mean uptakes increased when the fat level decreased in meat
- PCB bioaccessibility and mean uptakes decreased when meat is well-done cooked
- PCB bioaccessibility and mean uptakes were lower in elderlies than in adults

Keywords

Bioaccessibility; Polychlorinated biphenyls; Meat; *In vitro* digestion; Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-ToF/MS); Dietary uptake

1. Introduction

Many micropollutants may be found in food, and in particular in products of animal origin (Dervilly-Pinel *et al*., 2017). Among these micropollutants are polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs) massively used as insulators until the 1980s. PCBs accumulate in animal tissues ~~during~~ growth, and may end up in meat products intended for human consumption. The consumption of food of animal origin is considered as one of the main sources of human exposure to PCBs (Tressou *et al.*, 2017). While chemical risk is generally assessed from the total concentration of micropollutants in fresh food, the bioaccessible fraction of the micropollutants present in food (i.e. the fraction liable to cross the intestinal barrier and to induce toxic effects) can be considerably lower than the total amount (Engel *et al.*, 2015). This is due to both the changes occurring during food processing (Planche *et al.*, 2017), and the physiological processes of the digestion modifying the amounts of contaminants truly available for absorption in the systemic circulation. Therefore, the bioaccessibility of micropollutants must be considered to make an accurate assessment of their impact on human health (Tressou *et al.*, 2017).

To study the bioaccessibility of micropollutants such as PCBs, *in vitro* digestion models are largely used because they offer the advantage of being faster, better controlled and less costly than *in vivo* methods, while not being restricted by ethical considerations. However, marked variations are observed among the different *in vitro* digestion models described in the literature (incubation time, pH, constituents and concentrations of digestive and intestinal solutions, etc.), thus yielding widely disparate results. For example, Oomen *et al*. (2003) studied pollutant bioaccessibility in soils with an *in vitro* digestion model and they observed different results according to the type of bile salts used, with for example a bioaccessibility of lead 3–5.5 times greater when chicken bile was used compared to pig and ox biles. More

recently, He *et al.* (2018) observed a near-doubling of triphenyl phosphate (TPP) bioaccessibility when the intestinal digestion time increased from 4h to 8h.

To allow comparing results from different laboratories, a network of scientists proposed a standardised international protocol to mimic as closely as possible the physiological conditions of digestion (Minekus *et al.*, 2014). Minekus *et al.* (2014) have setup a consensus *in vitro* protocol consisting in three steps, namely oral, gastric and intestinal digestion. This protocol has been developed specifically for food matrices, which may explain differences observed in terms of ratios of matrix to digestive fluids or even in terms of sample stirring rate when compared to other protocols such as the protocol developed by Oomen *et al.* (2003) dedicated to soil samples. The consensus protocol developed by Minekus *et al.* (2014) has already been used to assess the bioaccessibility of food contaminants such as polybrominated diphenyl ethers (Cruz *et al*., 2020) or pesticides (Milinčić *et al*., 2020), but to the best of our knowledge, it has never been adapted to the study of PCBs in food.

To assess the bioaccessibility of PCBs in food after digestion, appropriate analytical methods are required for the monitoring and quantification of contaminants. Multianalyte methods are of particular interest because they allow the simultaneous monitoring of many substances in a single run. Several studies have shown the usefulness of comprehensive two-dimensional gas phase chromatography (GC×GC) in tandem with time-of-flight mass spectrometry (ToF-MS) to analyse a great number of different PCB congeners (Planche *et al.*, 2015).

Various factors are liable to influence the bioaccessibility of contaminants in meat, among which the composition of the raw material, and in particular its fat content (Xing *et al.*, 2008; Yu *et al.*, 2010; Shen *et al.*, 2016). Food processing steps such as cooking could also cause variations in bioaccessibility values (He *et al.*, 2010; Shen *et al.*, 2016). Lastly, age-related physiological variations in the human digestion process exist, in particular regarding

99 concentrations of digestive enzymes: the age of consumers could therefore also affect
100 bioaccessibility values (Dupont *et al.*, 2010; Levi *et al.*, 2014).

101 With the ultimate aim of making a better assessment of risks due to chemical
102 contamination of food, we have determined the bioaccessibility of PCBs in meat. For this
103 purpose, the first part of this paper was focused on the set up of an *in vitro* digestion protocol
104 coupled with extraction, clean-up and GC×GC-ToF/MS multianalyte analysis to monitor the
105 fate of the 18 most relevant PCBs in meat during digestion. In a second part, this method was
106 used to assess the influence of fat level, cooking intensity and the age of consumers on the
107 bioaccessibility of PCBs in meat as well as on the uptake to these micropollutants due to beef
108 consumption. The validity of these results obtained with intentionally contaminated (spiked)
109 meat is discussed in the light of measurements carried out on naturally contaminated
110 (incurred) samples.

111

112 ## 2. Materials and Methods

113 ### 2.1. Chemicals and standards

114

115 Hexane, dichloromethane, acetone and toluene were organic trace analysis grade
116 solvents (Sigma-Aldrich, Saint-Quentin Fallavier, France). For *in vitro* digestion, constituents
117 of simulated salivary fluid, simulated gastric fluid and simulated intestinal fluid (KCl,
118 KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$), together with HCl, NaOH, $CaCl_2$,
119 pepsin from porcine gastric mucosa (P6887), pancreatin from porcine pancreas (P7545) and
120 porcine bile extract (B8631) were from Sigma-Aldrich. For PCB extraction, trichloroacetic
121 acid and activated aluminium oxide (acidic, Brockmann I) were from Sigma-Aldrich.
122 Diatomaceous earth was obtained from Thermo Fisher Scientific (Waltham, MA, USA). PCB
123 reference standards including 12 dioxin-like PCBs (3.3'.4.4'-Tetrachlorobiphenyl, 3.4.4'.5-

Tetrachlorobiphenyl, 2.3.3'.4.4'-Pentachlorobiphenyl, 2.3.4.4'.5-Pentachlorobiphenyl, 2.3'.4.4'.5-Pentachlorobiphenyl, 2'.3.4.4'.5-Pentachlorobiphenyl, 3.3'.4.4'.5-Pentachlorobiphenyl, 2.3.3'.4.4'.5-Hexachlorobiphenyl, 2.3.3'.4.4'.5'-Hexachlorobiphenyl, 2.3'.4.4'.5.5'-Hexachlorobiphenyl, 3.3'.4.4'.5.5'-Hexachlorobiphenyl, 2.3.3'.4.4'.5.5'-Heptachlorobiphenyl) and 6 non-dioxin-like PCBs (2.4.4'-Trichlorobiphenyl, 2.2'.5.5'-Tetrachlorobiphenyl, 2.2'.4.5.5'-Pentachlorobiphenyl, 2.2'.3.4.4'.5'-Hexachlorobiphenyl, 2.2'.4.4'.5.5'-Hexachlorobiphenyl, 2.2'.3.4.4'.5.5'-Heptachlorobiphenyl) were from AccuStandard Europe (Niederbipp, Switzerland). Internal standards were used during *in vitro* digestion and PCB extraction for the accurate quantification of target compounds: 3'-F-PCB-28, 3-F-PCB-52, 3'-F-PCB-81, and 5'-F-PCB-156 (Chiron, Trondheim, Norway), ^{13}C-labeled PCB-111 and ^{13}C-labeled PCB-194 (Wellington laboratories, Guelph, ON, Canada).

2.2. Meat samples

Two types of meat samples were used: spiked and incurred samples. Spiked meat was prepared with ground beef samples from the same blend of muscles (<15% fat) purchased from a French supplier. The exact fat level in these samples was measured at 11%. Aliquots weighing 125 g were stored at −80 °C before use. Matrix blanks of these samples were made before spiking. Incurred beef meat samples were obtained under the French project SOMEAT (Contract No. ANR-12-ALID-0004. Safety of Organic Meat, available at www.so-meat.fr), and were ground before use.

2.3. Spiking and cooking

2.3.1. Sample spiking

149 Ground beef was spiked according to Planche *et al*. (2015), combining contaminant
150 addition to ground meat with matrix homogenisation. Briefly, ground beef (120 g) was
151 immersed in dichloromethane (DCM) containing the 18 most relevant PCBs in meat (Sirot *et*
152 *al.*, 2012), the mixture evaporated down under a hood, and the residue homogenised for 2 min
153 in a blender. A spiking concentration of 20 $ng.g^{-1}$ of fresh meat was selected for each
154 congener to give PCB concentrations in ready-to-run samples within the range of linearity of
155 GC×GC-ToF/MS for these compounds.
156
157 *2.3.2. Cooking method*
158
159 To study the effect of cooking on the bioaccessibility of PCBs in meat, circular small
160 ground beef patties weighing 26 g (2.5 cm thick) were then shaped to copy commercial ground
161 beef patties. These ground beef patties were cooked in a stainless-steel frying pan (diameter 17
162 cm) on a temperature-controlled induction hob (Bosch Electroménager, Saint-Ouen, France)
163 according to Planche *et al.* (2017). Briefly, aluminium foil (11 µm thickness) was placed on the
164 bottom of the frying pan to recover juices released during meat cooking. Three different
165 cooking conditions were used to simulate rare (50 °C at the core), medium (70 °C at the core
166 according to WHO recommendations for ground meat) and well-done (85 °C at the core) meat
167 ($n = 3$ for each cooking condition). These cooking conditions were obtained by 7 min heating
168 (turned once) at 160 °C at the bottom of the pan, 14 min heating (turned three times) at 200 °C
169 at the bottom of the pan, and 14 min heating (turned three times) at 250 °C at the bottom of the
170 pan, respectively. Temperatures at the core of the meat and at the bottom of the pan were
171 measured with thermocouples (RS Components, Beauvais, France). Before *in vitro* digestion,
172 the cooked meat was minced in a blender to simulate mastication.
173

174 *2.3.3. Determination of fat content*

175

176 Fat content was determined according to Blanchet-Letrouvé *et al.* (2014) with slight
177 modifications. Raw and cooked meat were first freeze-dried. Aliquots (1 g) of the resulting
178 powder were then extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350
179 extractor (Sunnyvale, CA, USA) with 22 mL stainless-steel extraction cells. Toluene-acetone
180 (70:30) was used as extraction solvent at a temperature of 120 °C and pressure of 1500 psi
181 with three extraction cycles per sample. The extracts obtained were then evaporated down
182 under a hood and weighed to determine fat content.

183

184 **2.4. *In vitro* digestion**

185

186 *In vitro* adult digestions ($n = 3$ for each condition) were performed according to
187 Minekus *et al*. (2014). Firstly, to simulate the oral phase, 5 g of raw or cooked meat was
188 mixed with 3.5 mL of simulated salivary fluid (SSF), 25 µL of 0.3 M $CaCl_2$ and 1.475 mL of
189 ultrapure water for 2 min at 150 rpm in a water bath at 37 °C. A gastric phase was then
190 carried out by adding 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of porcine pepsin
191 solution (25 000 U mL^{-1}) and 5 µL of 0.3 M $CaCl_2$. The pH was adjusted to 3.0 with 1 M
192 HCl, and the mixture was shaken in darkness for 2 h at 150 rpm in a water bath at 37 °C. To
193 simulate the intestinal step, 11 mL of simulated intestinal fluid (SIF), 2.5 mL of 160 mM bile
194 salts, 5 mL of pancreatin solution (800 U mL^{-1}) and 40 µL of 0.3 M $CaCl_2$ were added to
195 gastric chyme. The pH was adjusted to 7.0 with 1 M NaOH, and the mixture was shaken in
196 darkness for 2 h at 150 rpm in a water bath at 37 °C. The digesta obtained were centrifuged
197 for 15 min at 10,000 × *g*. After filtration of the supernatant through a 1.2 µm glass fiber
198 prefilter and a 0.45 µm nylon filter (Phenomenex, Torrance, CA), the filters were rinsed with

199 hexane. The filtrate contained the bioaccessible fraction of PCBs, and the pellet and filter
200 rinse contained the non-bioaccessible fraction of PCBs (Figure 1).

201 Following Dupont *et al.* (2010), *in vitro* infant digestion was simulated by reducing
202 the pepsin concentration by a factor of 8, the bile salt concentration by a factor of 4 and the
203 pancreatin concentration by a factor of 10 compared with *in vitro* adult digestion.

204 Following Levi & Lesmes (2014), the digestion of elderly persons (age 75 years) was
205 simulated *in vitro* by reducing the pepsin concentration by a factor of 1.3, the bile salt
206 concentration by a factor of 1.5 and the pancreatin concentration by a factor of 2.2 compared
207 with *in vitro* adult digestion.

208 The meat digestion protocol was validated by measuring the amount of peptides
209 produced by proteolysis during meat digestion. Absorbance of the digesta at 280 nm was
210 measured after the gastric step according to Gatellier *et al.* (2009) (UVIKON 923, double
211 beam UV/VIS spectrophotometer). Before this measurement, trichloroacetic acid (TCA) (50%
212 by volume) was added to the gastric digesta (TCA/digesta, v/v 1:2) to precipitate undigested
213 proteins at 37 °C for 15 min (Gatellier *et al.*, 2009). The mixture was centrifugated at 4000
214 rpm at 4 °C for 15 minutes. The absorbance at 280 nm ($n = 3$) was then measured on the
215 supernatant, and compared with that of the simulated gastric fluid (SGF), the SGF + raw meat
216 mixture and the SGF + pepsin mixture at the same concentrations as those used in the gastric
217 step of the digestion protocol. The quantity of proteolytic peptides could not be measured for
218 the intestinal digestion step because of the absorption of the pancreatin and bile salts at 280
219 nm.

220

221 **2.5. Multianalyte analysis of PCBs**

222 *2.5.1. Extraction*

223

PCB extraction procedure is shown in Figure 1. To extract bioaccessible PCBs, the filtrate obtained after *in vitro* digestion was first mixed with a trichloroacetic acid (TCA) solution (0.5 $g.mL^{-1}$) in a volume ratio of 2:1 (filtrate: TCA solution) for protein precipitation. After 30 min at 37 °C, the mixture was centrifuged (15 min, 10,000 × *g*) and a liquid-liquid extraction of the resulting supernatant was performed with 70 mL of solvent and repeated three times. For this step, the PCB recovery rates after an extraction by hexane and DCM were compared, these two solvents having already been used for the extraction of organochlorine contaminants in bioaccessible fractions (Xing *et al.*, 2008; Wang *et al.*, 2011). For that, a mixture of PCBs was spiked at 2.5 $\mu g.mL^{-1}$ in 40 mL of ultrapure water (Millipore, Bedford, MA, USA) and a liquid-liquid extraction was performed with 40 mL of hexane (repeated three times) or with 40 mL of DCM (repeated three times) ($n = 3$ for each solvent). The recovered organic solvent was then evaporated to dryness (Rocket, Genevac Ltd.), and 100 µL of hexane was added for PCB analysis by GC×GC-ToF/MS.

For the bioaccessible fraction, after the liquid-liquid extraction with the most efficient solvent, the organic phase containing PCBs was evaporated to ~1 mL. A 34 mL stainless-steel extraction cell was then prepared to extract bioaccessible PCBs by ASE. Briefly, 10 g of acidic alumina was placed at the bottom of the cell. Filter papers were placed underneath and on the top of the alumina layer. The cell was then filled with the pellet obtained after TCA protein precipitation and centrifugation, dispersed in 5 g of diatomaceous earth. The evaporated (~1 mL) solvent phase obtained after liquid-liquid extraction was added at the top of the cell.

Same ASE extraction was performed to extract non-bioaccessible PCBs contained in the pellet obtained at the end of the *in vitro* digestion. The hexane filter rinse was added at the top of the cell.

ASE was then carried out according to Planche *et al.* (2015) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). Hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. ASE extraction included heating (5 min), static time (5 min) and purging (90 s) with two extraction cycles per sample. The extract (approximately 40 mL per extraction cell) was evaporated down (Rocket, Genevac Ltd.) using toluene as a keeper to minimise losses during the evaporation step; 4.5 mL of DCM was then added. For cleaning extracts, gel permeation chromatography (GPC) (Gilson, Middleton, WI, USA) was carried out on an S-X3 Bio-Beads column (Bio-Rad, Philadelphia, USA) using DCM as eluting solvent at a flow rate of 5 mL min^{-1}. The fraction obtained was evaporated to dryness (Rocket, Genevac Ltd.), then 100 µL of hexane were added before analysis. All the samples were spiked with internal standards at 100 ng mL^{-1} at the different steps.

2.5.2. GC×GC-ToF/MS

Samples were analysed with a time-of-flight mass spectrometer (Pegasus 4D, Leco) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped with a dual stage jet cryogenic modulator (licensed from Zoex) according to Planche *et al.* with slight modifications (Planche *et al*., 2015). A Rtx-Dioxin2 1D column (60 m × 0.25 mm × 0.25 µm) (Restek, Bellefonte, PA, USA) was connected by a deactivated ultimate union (Agilent Technologies, Santa Clara, CA) to a BPX-50 2D column (2 m × 0.1 mm × 0.1 µm) (SGE, Austin, TX, USA). A splitless injection of 1 µl of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with a split/splitless inert liner (Restek, Sky® 4.0 mm ID liner) set at 280 °C. Ultra-pure grade helium (purity 99.9995%) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. Purge time was set to 60 s with a flow rate of 50 mL min^{-1}. The primary oven temperature

was initially set at 90 °C for 1 min, ramped to 200 °C at 20 °C min^{-1}, and then to 300 °C at 2 °C min^{-1} for 10 min. The secondary oven temperature was set at 5 °C higher than the primary oven temperature. The modulator temperature was set at 15 °C higher than the primary oven temperature, and the modulation period was 5 s, with 1.20 s and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at 280 °C. The mass spectrometer was operated with an electron ionisation source (ionisation energy of 70 eV), a detector voltage of 1800 V and a data acquisition rate of 100 spectra s^{-1}. The run time for each sample was 66.5 min. Analytical blank samples of pure solvent were run to check the absence of targeted analytes. GC×GC data were processed using the ChromaTOF software (Leco, version 4.50.8.0).

For incurred samples, in which the concentration of contaminants is unknown, PCBs were analyzed by the French National Reference Laboratory (LABERCA, Nantes, France) according to Berge *et al.* (2011), in order not to be restricted by the sensitivity of the GC×GC-ToF/MS method.

2.6. Data processing

Bioaccessibility of PCBs was calculated according to Kang *et al.* (2013):

$$\text{Bioaccessibility (\%)} = \frac{\text{Bioaccessible PCBs}}{\text{Bioaccessible PCBs + Non-bioaccessible PCBs}} \times 100,$$

where bioaccessible PCBs and non-bioaccessible PCBs are the amounts of PCBs found after *in vitro* digestion in the bioaccessible and non-bioaccessible fractions, respectively. Data were processed using the Statistica Software version 12 (Dell Software, Paris, France). Principal component analyses (PCA) were performed on the bioaccessibility data of PCBs in meat after *in vitro* digestion to visualise the structure of the data. To determine whether the cooking process, the meat fat content or the physiological differences due to the age of consumers had

any impact on the bioaccessibility of PCBs in meat, a one-way analysis of variance (ANOVA; $p < 0.05$) was performed on bioaccessibility data from GC×GC-ToF/MS. A Newman-Keuls mean comparison test was then performed on the resulting dataset.

2.7. Determination of the mean uptake distribution according to fat level, cooking mode and consumer age

Using the modular Bayesian approach presented in Tressou *et al.* (2017) and integrating initial contamination data obtained after meat production for both organic and conventional production, data reflecting the effect of cooking, data on the levels and frequency of consumption of conventional and organic meat, and data on the effect of digestion presented in the current paper, we propose to compare the exposure after digestion (uptake) according to the fat level, the cooking mode, and the consumer age. The bioaccessibility step is the last one of our modular approach and the angle used in Tressou *et al.* (2017) was to look at the effect of the cooking mode for adults eating 15% fat beef meat. Due to a lack of information on the percentage of fat of the meat consumed and the inability of the model to deal with consumers eating both 5% and 15% fat beef meat, all meat was considered as 15% fat. In section 3.2, we look at the uptake considering all the beef meat is 5% fat meat, without changing any other assumptions (the study population is adults, the cooking mode is medium as recommended by WHO).

In section 3.3, we show the effect of cooking as in Tressou *et al*. (2017), but using the available data on individuals' body weight. In section 3.4, we consider the effect of age on 3 populations: children less than 6 years old, adults (18-64 years old), and seniors (65 years old and older). For each population, consumption data was extracted from the different databases (SoMeat data, INCA2 data, KANTAR data) described in Tressou *et al*. (2017), to differentiate

323 as much as possible their behaviors. Based on the different data sets, we extract the
324 percentage of meat consumer, the mean quantity consumed and individual body weight, and
325 the proportion of organic meat consumption.

326 For children less than 6 years old, the detailed individual meat consumptions and body
327 weights of 163 children aged 3 to 5 were obtained from the INCA2 data, information about
328 organic meat consumption of 780 households with at least a child less than 6 years old from
329 KANTAR data, and information from 94 respondents belonging to households with children
330 aged 0 to 9 years old from the SOMEAT project survey (http://www.so-meat.fr/). For adults,
331 the detailed individual meat consumptions and body weights of 2276 adults aged 18 to 64
332 were obtained from the INCA2 data, information about organic meat consumption of 3381
333 households from KANTAR data with one the reference adults aged 18 to 64, and information
334 from 360 respondents aged 18 to 64 from the SOMEAT project survey. For seniors, the
335 detailed individual meat consumptions and body weights of 348 adults aged 65 to 79 were
336 obtained from the INCA2 data, information about organic meat consumption of 1474
337 households from KANTAR data with one the reference adults aged 65 or more, and
338 information from 26 respondents aged 65 and more from the SOMEAT project survey.

339

340 ## 3. Results and Discussion

341 ### 3.1. Analysis of PCBs in meat digesta

342

343 To assess the bioaccessibility of the 18 most relevant PCBs in meat (Sirot *et al.*, 2012),
344 the standard *in vitro* static digestion protocol described by Minekus *et al.* (2014) was
345 implemented starting with 5 g of meat spiked with PCBs (Figure 1). The protocol was
346 validated by verifying the increase in the amount of peptides produced by proteolysis after the
347 gastric step of digestion according to Gatellier *et al.* (2009). The mean absorbance of the

348 gastric digesta was 1.56 (OD units) against nil for the simulated gastric fluid (SGF) solution,
349 0.49 for the SGF + meat mixture and 0.48 for the SGF + pepsin mixture. The absorbance of
350 the gastric digesta was thus significantly greater than the other absorbances measured
351 ($p < 0.05$), confirming the proteolysis in the course of the gastric digestion of the meat. After
352 considering dilution steps, the mean absorbance (1.18 OD units) was in the same order of
353 magnitude as that found by Gatellier *et al.* (2009) in their gastric raw meat digesta (0.98 OD
354 units), thus validating our *in vitro* digestion set-up.

355 A protocol for the extraction and analysis of PCBs in the bioaccessible fraction
356 (filtrate) and non-bioaccessible fraction (pellet and filter rinse) was then developed (Figure 1).
357 For the liquid/liquid extraction, a comparison was made between hexane (Xing *et al*., 2008)
358 and dichloromethane (DCM) (Wang *et al*., 2011) as the extraction solvent. Results showed a
359 mean recovery rate for PCBs in the bioaccessible fraction of 98% using hexane, with values
360 ranging from 56 ± 0.7% for PCB 28 to 112 ± 19% for PCB 156 (Table S1. Supplementary
361 material). With DCM, the mean recovery rate was 90%, with values ranging from 71 ± 4.9%
362 for PCB 101 to 118 ± 6.5% for PCB 180 (Table S1. Supplementary material). Except for PCB
363 28 with hexane as the extraction solvent, all the recovery rates lay in the classically accepted
364 range of 70–130% according to the EPA Method 8000C (2003), with RSD ≤ 10% for most of
365 the compounds. Of the 18 PCBs targeted in this study, 14 showed higher recovery rates, and
366 closer to 100%, with hexane than with DCM. Hexane was therefore subsequently used in this
367 study for the liquid/liquid extraction of PCBs in the bioaccessible fraction.

368 After ASE extraction of PCBs and GPC defatting of the extracts obtained respectively
369 for bioaccessible and non bioaccessible fractions (Figure 1), GC×GC-ToF/MS analysis of
370 PCBs was carried out according to Planche *et al.* (2015). The visual separation of the 18
371 PCBs (Figure 2) was validated based on resolution factors (R_s) with $R_s = \Delta r_t / w_b$, where r_t is
372 the retention time and w_b the average peak width at the base (Table S2. Supplementary

material). For the 18 PCBs, as $R_{s,1D} \geq 0.6$ in the first separation dimension or $R_{s,2D} \geq 0.4$ in the second separation dimension, all peaks were considered resolved.

Lastly, to verify the accuracy of our protocol, the recovery rates for PCBs contained in the raw meat were determined after completion of the procedure (*in vitro* digestion, extraction and analysis by GC×GC-ToF/MS). The PCBs contained in the meat before digestion were thus quantified ($n = 3$) and compared with the sum of the PCBs found in the bioaccessible and non-bioaccessible fractions ($n = 3$) after digestion. The mean of the resulting recovery rates was 95 ± 16%, confirming the efficiency and robustness of the protocol.

3.2. Impact of meat fat content on bioaccessibility of PCBs

To determine the impact of meat fat content on the bioaccessibility of PCBs, two different types of meat were purchased: 15% fat ground beef which is the type of beef meat most consumed by the French population (51 g per week) (Mota *et al.*, 2021) and 5% fat ground beef as an example of low fat meat (Cardona *et al*., 2020). After verification, the exact fat level in the first type of meat was 11% which corresponds to a model of high fat meat (Cardona *et al.*, 2020). It is important to note that no significant difference was observed between 11% and 5% fat meat in terms of lipid relative composition (Table S3. Supplementary material). Bioaccessibility results of the 18 most relevant PCBs in meat are reported in Table 1. For meat with 11% fat content, PCB bioaccessibility was between 20.6% and 30.5% ± 1.4-6.7% against 44.2-50.1% ± 3.0-12.5% for meat with 5% fat content. As shown in Figure S1. (Supplementary material), fat content is thus a key determinant in the bioaccessibility of PCBs. Compared to the results previously obtained with meat (Planche *et al*., 2015), the low relative standard deviations (3.0%-12.5% for 5% fat meat; 1.4%-6.7% for 11% fat meat) point out the poor bioacessibility variability between PCB congeners. Note that for PCB 126, which displays the highest toxic equivalent factor (WHO-TEF = 0.1), the

bioaccessibility was 24.0 ± 0.6% for meat with 11% fat content and 44.7 ± 3.4% for meat with 5% fat content, confirming that bioaccessibility is a key factor to be considered in risk analysis.

These values are of the same order of magnitude as those obtained by Shen *et al.* (2016), who reported a mean bioaccessibility of PCBs in beef samples (4.8% fat) of 49.0 ± 3.3% after boiling (5 min at 100 °C). By contrast, they differ with those of Xing *et al*. (2008), who found a mean bioaccessibility of PCBs of 3% in raw fish samples (3–15% fat). Besides the fact that Xing *et al*. worked on raw food matrices whereas we used cooked ones, there were important disparities between the *in vitro* digestion protocols used, in particular regarding the sample incubation times with the digestive enzymes used in the different steps of the protocol (1 h in Xing *et al*. against 2 h in our study for the gastric digestion step, and 6 h against 2 h in the intestinal digestion step). These differences in protocols thus rule out any objective comparison of the data obtained, this is the reason why the standardised international protocol setup by Minekus *et al*. (2014) was implemented in the present study. Xing *et al*. (2008) and Shen *et al*. (2016), like us, found a significantly greater PCB bioaccessibility ($p < 0.05$) when the food fat content is lower. Xing *et al*. (2008) measured a mean PCB bioaccessibility of 3% in fish samples (3–15% fat), whereas bioaccessibility was 25% in spinach and salad samples (0.1% fat on average). In our study, we found the relation between the lipid levels of cooked meat and bioaccessibility of PCBs to be inversely proportional: when the percentage of fat was divided by 1.9, the bioaccessibility was multiplied by 1.8. As PCBs are lipophilic compounds (Log Kow = 4.09–8.18 according to Hawker & Connell (1988)), their digestion starts with their release from the food bolus and their dissolution in the fat phase of the meal, followed by emulsification into lipid droplets of gastric emulsion (Xavier & Mercadante, 2019). Then, they are transferred to mixed micelles, composed by products of lipid hydrolysis, phospho-lipids, cholesterol, and bile salts and only

424 the fraction of PCBs incorporated into mixed micelles is bioaccessible (Xavier & Mercadante,
425 2019). For high fat meat, the solubilization capacity of the mixed micelles may be limited by
426 the concentration of available digestive enzymes and emulsifiers (*e.g.* pancreatin and bile
427 salts) (Tan *et al.*, 2020). Thus, we can suggest that there is a fat level threshold above which
428 lipophilic compounds such as PCBs are not fully released from the lipid phase due to the fact
429 that there were proportionally less mixed micelles available to solubilize it (Tan *et al.*, 2020).
430 Considering that there is no significant difference between 11% and 5% fat meat in terms of
431 lipid relative composition (Table S3. Supplementary material), this may explain why an
432 inversely proportional relation is observed between lipid levels and PCB bioaccessibility. This
433 trend was also observed by Xia *et al*. (2017) for lipophilic micronutrients like the hydrophobic
434 β-carotene, with significantly lower bioaccessibility in a simulated high-fat diet than in a
435 simulated low-fat diet. A significant fraction of the lipid phase was not fully digested and,
436 therefore, a fraction of the β-carotene was not released into the intestinal fluids, remaining in
437 the nondigested lipid phase (Xia *et al*., 2017). Considering that PCB concentrations are
438 positively correlated to the fat content of food (Carlson *et al*., 2005), our results suggest that
439 PCBs in fat-rich food are less bioaccessible, which may therefore mitigate the hazard related
440 to their presence in such food.

441 Figure 3a shows the intake distribution of exposure obtained according to the fat
442 content after digestion. The reduced bioaccessibility obtained with 11% fat meat leads to
443 reduced uptakes compared to 5% fat meat.

444

445 **3.3. Impact of cooking intensity on bioaccessibility of PCBs**

446

447 To determine the impact of cooking intensity on PCB bioaccessibility in meat, *in vitro*
448 digestions were carried out on ground beef containing 11% fat, raw and cooked at three

449 different intensities: rare (50 °C at the core), medium, (70 °C at the core according to WHO
450 recommendation), and well-done (85 °C at the core) ($n = 3$ for each condition). Table S4.
451 (Supplementary material) shows that PCB bioaccessibility was 23.3-35.1% ± 6.0-13.9% for
452 raw meat, 20.5-28.2% ± 1.0-8.9% for rare-cooked meat, 20.6-30.5% ± 1.4-6.7% for medium-
453 cooked meat (WHO recommendations), and 18.0-26.7% ± 2.3-11.6% for well-done meat. As
454 mentioned above, the low coefficients of variation point out the poor bioaccessibility
455 variability between PCB congeners. No significant difference was observed between raw,
456 rare- and WHO-cooked meat, whereas the bioaccessibility measured on well-done meat was
457 significantly lower ($p < 0.05$). For PCB 126, the bioaccessibility decreased from 25.2 ± 10.0%
458 for raw meat to 18.0 ± 8.1% after intense cooking.

459 Although they did not assess the impact of cooking intensity on PCB bioaccessibility,
460 Shen *et al*. (2016) showed that cooking modes need to be considered in bioaccessibility
461 studies: for example, the PCB bioaccessibility in meat was significantly lower ($p < 0.05$) after
462 boiling (5 min at 100 °C) than after frying in cooking oil (5 min at 200-300 °C). Various
463 studies conducted on trace elements (Cu, Cd, Zn, etc.) have also shown that the cooking of
464 fish and other seafood generally lowers bioaccessibility, irrespective of the cooking method
465 used: frying, grilling, boiling or steaming (He *et al*., 2010). The lower bioaccessibility
466 observed by these authors may be explained by the change in meat fat content induced by
467 cooking (Xing *et al*., 2008). In the present study, whereas raw meat contained 11% fat,
468 cooked meat contained respectively 13%, 15% and 16% fat after rare, WHO and intense
469 cooking, respectively. As discussed above, the moderate decrease in bioaccessibility could
470 thus be at least partly linked to the increase in meat fat content. Another possible explanation
471 for the reduced bioaccessibility after intense cooking is the protein denaturing phenomenon
472 that occurs during cooking. This process leads to tissue shrinking, making them harder and
473 more compact, and therefore hindering digestive enzymes activity (Kulp *et al*., 2003; He *et*

474 *al*., 2010). Indeed, pepsin activity on myofibrillary proteins has been shown to be reduced by
475 58% in cooked meat (45 min cooking at 100 °C) compared with raw meat (Santé-Lhoutellier
476 *et al*., 2008). Cooking may also cause the formation of disulphide-bonded proteins, making
477 the proteins less digestible (He *et al*., 2010). All these processes are especially significant
478 under extreme cooking conditions, and may explain the decrease in bioaccessibility only in
479 the case of intense cooking.
480 Figure 3b presents the intake distribution of exposure obtained according to the
481 cooking intensity after digestion. The figure shows that the reduced bioaccessibility obtained
482 with well-done meat compared to rare or WHO-cooked meat leads to reduced uptakes.
483
484 **3.4. Impact of consumer age on bioaccessibility of PCBs**
485
486 To determine the impact of physiological conditions related to the age of the consumer
487 on PCB bioaccessibility in meat (11% fat, WHO cooking), *in vitro* digestions were carried
488 out, simulating the digestion of an infant ($n = 3$) according to Dupont *et al*. (2010), and that of
489 an elderly person ($n = 3$) following Levi & Lesmes (2014). These data were then compared
490 with those previously obtained for adult digestion simulation (Minekus *et al.*, 2014). Table 2
491 shows that the mean PCB bioaccessibility was 7.7 ± 0.8% for the infant model and 17.1 ±
492 1.5% for the elderly model, against 25.9 ± 2.3% for the adult model, with again, a poor
493 variability between PCB congeners. As shown in Figure S1. (Supplementary material),
494 bioaccessibility thus varies significantly ($p < 0.05$) according to the consumer age.
495 These variations are consistent with concentrations of pepsin, bile salts and pancreatin,
496 which were 8, 4 and 10 times lower in the infant model, and 1.3, 1.5 and 2.2 times lower in
497 the elderly model compared to the adult model (Dupont *et al*., 2010; Levi & Lesmes, 2014)
498 confirming that the lower the concentrations of these enzymes, the lower the PCB

499 bioaccessibility values. Accordingly, Jadán-Piedra *et al*. (2016) observed an increase of Hg
500 bioaccessibility in fish with the concentration of pepsin, whose activity facilitated the
501 cleavage of protein-contaminant bonds. Likewise, the bioaccessibility of heterocyclic
502 aromatic amines was found to increase with the concentration of lipase containing pancreatin
503 (Kulp *et al*., 2003). Furthermore, the present study also shows that the concentration of bile
504 salts is a key determinant of bioaccessibility: the concentration of bile salts being 4 times
505 lower in the infant model than in the adult model, PCB bioaccessibility in meat was divided
506 by 3.4. Similarly, in the elderly model, both the concentration of bile salts and the measured
507 bioaccessibility were 1.5 times lower than in the adult model. These results are consistent
508 with those reported by Yu *et al*. (2011), who studied the bioaccessibility of PBDEs in dust.
509 These authors observed that when the concentration of bile salts was multiplied by 2.2, the
510 bioaccessibility of PBDEs rose 2.8-fold. During digestion, bile salts may facilitate the
511 emulsification of fats containing lipophilic substances such as PBDEs or PCBs, thereby
512 inducing higher bioaccessibility values (Yu *et al*., 2011).

513 Figure 3c shows the intake distribution of exposure obtained according to the
514 consumer age after digestion. Comparing the uptakes of the three subpopulations, Figure 3c
515 seems to indicate that the reduced bioaccessibility of children and elderly leads to reduced
516 uptakes for children and elderly. Children uptakes remain more variable because of large
517 differences that exist between children in terms of meat consumption. Moreover, children
518 uptakes can exceed those of adults as their exposure before digestion is higher than adults and
519 senior exposures (Figure S2. Supplementary material). This is mainly due to the fact that the
520 quantity of meat consumed by children (mean 109 g/week) (Figure S3. Supplementary
521 material) is relatively high compared to their body weight (mean 19 kg) while the quantities
522 of meat consumed by adults and elderly (means 191 g/week and 145 g/week, respectively)

(Figure S3. Supplementary material) are smaller compared to their body weights (mean 70 kg).

To conclude, the highest PCB bioaccessibility values may be observed in adults, after a consumption of raw, rare or WHO-cooked meat or with 5% fat meat. In contrast, the lowest PCB bioaccessibility values may be obtained for the elderly, after a consumption of well-done meat or with 11% fat. For children, the exposure after digestion varies widely because, as discussed above, there are large differences between individuals in terms of meat consumption. However, we can note that for children, undercooked ground beef is considered a high-risk product due to the haemolytic uremic syndrome (HUS) associated with Shiga toxin-producing Escherichia coli (STEC)-contaminated beef (Brusa *et al.*, 2020). Following the recommendations issued to limit this risk, bioaccessibility values obtained after medium or intense cooking should therefore cover the most common situations observed for children. In contrast, beef is most frequently eaten rare or medium cooked by adults (Loukiadis *et al*., 2017), which corresponds to the highest bioaccessibility values obtained in this study even if the impact of cooking on PCB bioaccessibility is moderate. These cooking modes are also the most commonly observed with the elderly because individuals with mastication problems may avoid well-done meat (Gil-Montoya *et al.*, 2015). Considering the meat fat level, the results obtained with high fat ground beef (which corresponds to the lowest PCB bioaccessibility values) correspond to the most frequent situation observed for adults (Cardona *et al.*, 2020). For the elderly, it should be recommended to consume low fat meat in order to limit the risk of cardiovascular diseases (Bronzato & Durante, 2017) but it is important to note that this recommendation leads to higher PCB bioaccessibility values.

3.5. Comparison of results with naturally contaminated samples

All the results presented above were achieved with intentionally contaminated (spiked) samples in order to control the matrix composition and its PCB load (181.8 ng/g lipid weight for 11% fat meat and 400 ng/g lipid weight for 5% fat meat). However, PCB bioaccessibility determinations were also carried out on naturally contaminated (incurred) meat samples containing PCBs bioaccumulated at ultra-trace concentration during animal breeding in order to assess the scope of the conclusions drawn from spiked model samples. For this purpose, *in vitro* digestions were carried out, after medium cooking of beef meat samples (n = 4) which concentrations in PCBs have been determined applying sensitive and selective GC-HRMS (BE) ISO17025 accredited method. After mincing and blending, the four raw samples were analysed, showing concentrations ranging from 3.4 to 44.6 ng/g lipid weight for non-dioxin-like PCBs and from 1575 to 4032 pg/g lipid weight for dioxin-like PCBs. As reported in Table 3, mean PCB bioaccessibility was 45.0 ± 8.2% for sample 1 (2.9% fat), 44.0 ± 9.0% for sample 2 (5.0% fat), 49.4 ± 10.9% for sample 3 (5.3% fat) and 31.9 ± 13.6% for sample 4 (9.5% fat) after a medium cooking. The mean values of bioaccessibility obtained for samples 2 and 3, both presenting fat contents close to 5%, were of the same order of magnitude as those obtained, after medium cooking, on spiked 5% fat-meat used in our study (47.5 ± 1.7%). In addition, no significant difference was observed between the bioaccessibility values measured from meat containing 2.9% fat and 5-5.3% fat. This suggests the occurrence of a fat content threshold below which the PCB bioaccessibility remains stable. This trend was already described for polybrominated diphenyl ethers (PBDEs) by Yu *et al*. (2010), who observed that PBDE bioaccessibility was significantly correlated with the fat content of food only above 1.8% of their fresh weight. To confirm this trend and validate this hypothesis for PCB bioaccessibility, measures would have to be carried out on a greater number of low-fat samples.

572 Data presented in Table 3 also indicate that for a higher fat content (e.g. sample 4,
573 9.5% fat), the values of bioaccessibility were significantly lower ($p < 0.05$) than for the other
574 samples, in line with our results with spiked meat, thus confirming the inversely proportional
575 relation between PCB bioaccessibility and meat fat content. Interestingly, unlike the spiked
576 samples, the incurred samples were found to present bioaccessibility values that varied
577 according to the PCB congeners: bioaccessibility was higher for the least chlorinated
578 congeners and lower for the most chlorinated, with bioaccessibility values for example
579 significantly higher ($p < 0.05$) for PCB 28 (trichlorobiphenyl) than for PCBs 180 and 189
580 (heptachlorobiphenyls). This suggests that the relationship that exists in incurred samples
581 between PCBs and matrix components cannot be identically reproduced in spiked samples.
582 Xing *et al*. (2008), Kang *et al*. (2013) and Wang *et al*. (2013) also observed that the
583 bioaccessibility of PCBs in incurred samples was generally higher for the least chlorinated
584 congeners. The most chlorinated PCBs display the highest Log K_{ow} values (Wang *et al*.,
585 2013), so that these congeners may be more readily retained in fat, and as a consequence
586 harder to solubilise in the bioaccessible fraction (Kang *et al*., 2013).
587
588 **4. Conclusion**
589
590 Coupled with GC×GC-ToF/MS analysis, the standardised *in vitro* static digestion protocol
591 implemented in this study enabled to assess the bioaccessibility of PCBs in meat. For ground
592 beef meat with 11% fat, only a quarter of PCBs contained in this matrix is liable to cross the
593 intestinal barrier and induce toxic effects. These data confirm that bioaccessibility is a key
594 factor to be considered in risk analysis. The fat content of food, the consumer age and to a
595 lesser extent, the cooking intensity are factors that cause bioaccessibility to vary. Indeed,
596 PCBs are less bioaccessible when the fat content increases, after an intense cooking or even in

597 the infant and the elderly, thus reducing the exposure to PCBs due to meat consumption in
598 these situations. More extensive investigations will be necessary to elucidate in details the
599 mechanisms responsible for these results. Moreover, in order to study more precisely the main
600 factors influencing contaminant bioaccessibility, *in vitro* dynamic digestion models could be
601 used to simulate digestion as realistically as possible. It would also be interesting to determine
602 how PCBs interact with the gut microbiota and whether these interactions are relevant for
603 human health. Work is underway to address this challenging issue (Defois *et al*., 2018).
604 Finally, our work highlights the great relevance of using a consensus digestion protocol for
605 comparisons between different studies. The wide use of such protocols would allow the
606 creation of a consistent database with the ultimate goal of improving chemical risk assessment
607 procedures considering accurate bioaccessibility data. In this perspective, simplified high
608 throughput digestion protocols were developed, based on the protocol setup in the present
609 study, in order to obtain data for epidemiological studies on the health impact of food
610 (Wedekind *et al*., 2020).

611

612 **Conflict of interest**

613 The authors declare that there is no conflict of interest.

614

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Highlights

- An *in vitro* digestion protocol was set up to assess PCB bioaccessibility in meat
- The mean PCB bioaccessibility was 26% in 11% fat ground beef
- PCB bioaccessibility and mean uptakes increased when the fat level decreased in meat
- PCB bioaccessibility and mean uptakes decreased when meat is well-done cooked
- PCB bioaccessibility and mean uptakes were lower in elderlies than in adults

Table 1

Table 1. Bioaccessibility (BA) (%) and relative standard deviation (RSD) (%) of spiked PCBs in 5% fat meat (*n* = 3) and 11% fat meat (*n* = 3) after medium cooking. WHO-TEF are toxic equivalency factors defined by World Health Organization to assess the toxicity of dioxin-like PCBs. [a,b]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the bioaccessibility obtained for the different fat contents. Two groups with distinct letters can be considered as significantly different ($p < 0.05$).

Compound	Congener	WHO-TEF	5% fat meat		11% fat meat	
			BA (%)	RSD (%)	BA (%)	RSD (%)
2,4,4'-Trichlorobiphenyl	28		49.5[b]	9.9	24.9[a]	1.4
2,2',5,5'-Tetrachlorobiphenyl	52		48.7[b]	12.4	25.9[a]	2.2
3,3',4,4'-Tetrachlorobiphenyl	77	0.0001	44.4[b]	9.8	20.6[a]	6.1
3,4,4',5-Tetrachlorobiphenyl	81	0.0003	45.9[b]	7.8	23.0[a]	4.3
2,2',4,5,5'-Pentachlorobiphenyl	101		48.1[b]	4.7	26.9[a]	3.5
2,3,3',4,4'-Pentachlorobiphenyl	105	0.00003	47.5[b]	12.5	26.4[a]	2.7
2,3,4,4',5-Pentachlorobiphenyl	114	0.00003	47.6[b]	8.6	25.2[a]	5.0
2,3',4,4',5-Pentachlorobiphenyl	118	0.00003	48.4[b]	9.1	24.9[a]	6.1
2',3,4,4',5-Pentachlorobiphenyl	123	0.00003	48.7[b]	8.7	25.8[a]	5.1
3,3',4,4',5-Pentachlorobiphenyl	126	0.1	44.7[b]	7.7	24.0[a]	2.7
2,2',3,4,4',5'-Hexachlorobiphenyl	138		48.4[b]	8.0	27.5[a]	6.7
2,2',4,4',5,5'-Hexachlorobiphenyl	153		50.1[b]	8.3	27.9[a]	6.1
2,3,3',4,4',5-Hexachlorobiphenyl	156	0.00003	46.8[b]	7.6	26.7[a]	6.2
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0.00003	46.5[b]	6.0	23.8[a]	4.7
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0.00003	48.2[b]	9.3	28.0[a]	2.3
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0.03	44.2[b]	3.0	25.9[a]	3.0
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		49.4[b]	4.8	30.5[a]	4.7
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0.00003	47.3[b]	4.8	28.5[a]	3.1

Table 2

Table 2. Bioaccessibility (BA) (%) and relative standard deviation (RSD) (%) of spiked PCBs in medium-cooked meat (11% of fat) after simulating the digestion of an infant (*n*=3), an adult (*n*=3) or an elderly person (*n*=3). [a,b,c]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the bioaccessibility obtained for the different consumer ages. Two groups with distinct letters can be considered as significantly different ($p < 0.05$)

Compound	Congener	WHO-TEF	Infant		Adult		Elderly	
			BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)
2.4.4'-Trichlorobiphenyl	28		7.0 [a]	14.1	24.9 [c]	1.4	15.0 [b]	5.0
2.2'.5.5'-Tetrachlorobiphenyl	52		6.4 [a]	9.6	25.9 [c]	2.2	15.5 [b]	5.0
3.3'.4.4'-Tetrachlorobiphenyl	77	0.0001	7.2 [a]	9.7	20.6 [c]	6.1	14.6 [b]	9.5
3.4.4'.5-Tetrachlorobiphenyl	81	0.0003	6.8 [a]	4.6	23.0 [c]	4.3	15.0 [b]	5.1
2.2'.4.5.5'-Pentachlorobiphenyl	101		8.0 [a]	10.2	26.9 [c]	3.5	17.9 [b]	4.6
2.3.3'.4.4'-Pentachlorobiphenyl	105	0.00003	7.7 [a]	8.3	26.4 [c]	2.7	16.8 [b]	5.8
2.3.4.4'.5-Pentachlorobiphenyl	114	0.00003	7.5 [a]	8.1	25.2 [c]	5.0	16.7 [b]	5.8
2.3'.4.4'.5-Pentachlorobiphenyl	118	0.00003	7.3 [a]	6.5	24.9 [c]	6.1	16.3 [b]	6.3
2'.3.4.4'.5-Pentachlorobiphenyl	123	0.00003	8.1 [a]	7.8	25.8 [c]	5.1	17.7 [b]	4.0
3.3'.4.4'.5-Pentachlorobiphenyl	126	0.1	7.2 [a]	12.9	24.0 [c]	2.7	15.3 [b]	5.5
2.2'.3.4.4'.5'-Hexachlorobiphenyl	138		8.6 [a]	8.7	27.5 [c]	6.7	18.4 [b]	4.2
2.2'.4.4'.5.5'-Hexachlorobiphenyl	153		8.8 [a]	9.5	27.9 [c]	6.1	18.6 [b]	4.8
2.3.3'.4.4'.5-Hexachlorobiphenyl	156	0.00003	7.4 [a]	9.7	26.7 [c]	6.2	18.1 [b]	3.9
2.3.3'.4.4'.5'-Hexachlorobiphenyl	157	0.00003	8.2 [a]	6.7	23.8 [c]	4.7	18.5 [b]	3.2
2.3'.4.4'.5.5'-Hexachlorobiphenyl	167	0.00003	8.4 [a]	6.6	28.0 [c]	2.3	18.1 [b]	5.8
3.3'.4.4'.5.5'-Hexachlorobiphenyl	169	0.03	6.2 [a]	14.3	25.9 [c]	3.0	17.0 [b]	5.3
2.2'.3.4.4'.5.5'-Heptachlorobiphenyl	180		9.1 [a]	7.8	30.5 [c]	4.7	19.4 [b]	3.7
2.3.3'.4.4'.5.5'-Heptachlorobiphenyl	189	0.00003	8.0 [a]	6.3	28.5 [c]	3.1	19.1 [b]	4.5

Table 3. Bioaccessibility (%) of PCBs in four medium-cooked naturally contaminated samples after simulating adult digestion.

PCB congener	**Sample 1** *2.9% of fat* *10.3 ng PCBs/g fat*	**Sample 2** *5.0% of fat* *48.6 ng PCBs/g fat*	**Sample 3** *5.3% of fat* *8.7 ng PCBs/g fat*	**Sample 4** *9.5% of fat* *5.0 ng PCBs/g fat*
PCB 28	61.6	63.9	76.8	70.8
PCB 52	56.4	51.4	65.8	48.7
PCB 77	44.9	46.5	48.8	35.8
PCB 81	46.2	45.1	49.3	38.5
PCB 101	61.2	59.8	65.9	45.7
PCB 105	40.9	39.8	39.7	23.9
PCB 114	44.7	38.3	54.5	37.1
PCB 118	45.0	34.8	47.9	22.3
PCB123	48.9	54.8	57.2	35.3
PCB 126	43.6	47.1	42.5	30.9
PCB 138	31.8	34.5	41.2	20.4
PCB 153	37.9	36.6	39.7	19.9
PCB 156	40.1	38.0	42.9	25.0
PCB 157	39.2	38.3	42.6	22.4
PCB 167	44.8	35.2	45.4	23.0
PCB 169	38.2	48.2	41.3	27.0
PCB 180	39.1	37.0	38.8	16.0
PCB 189	35.9	37.4	41.4	20.5

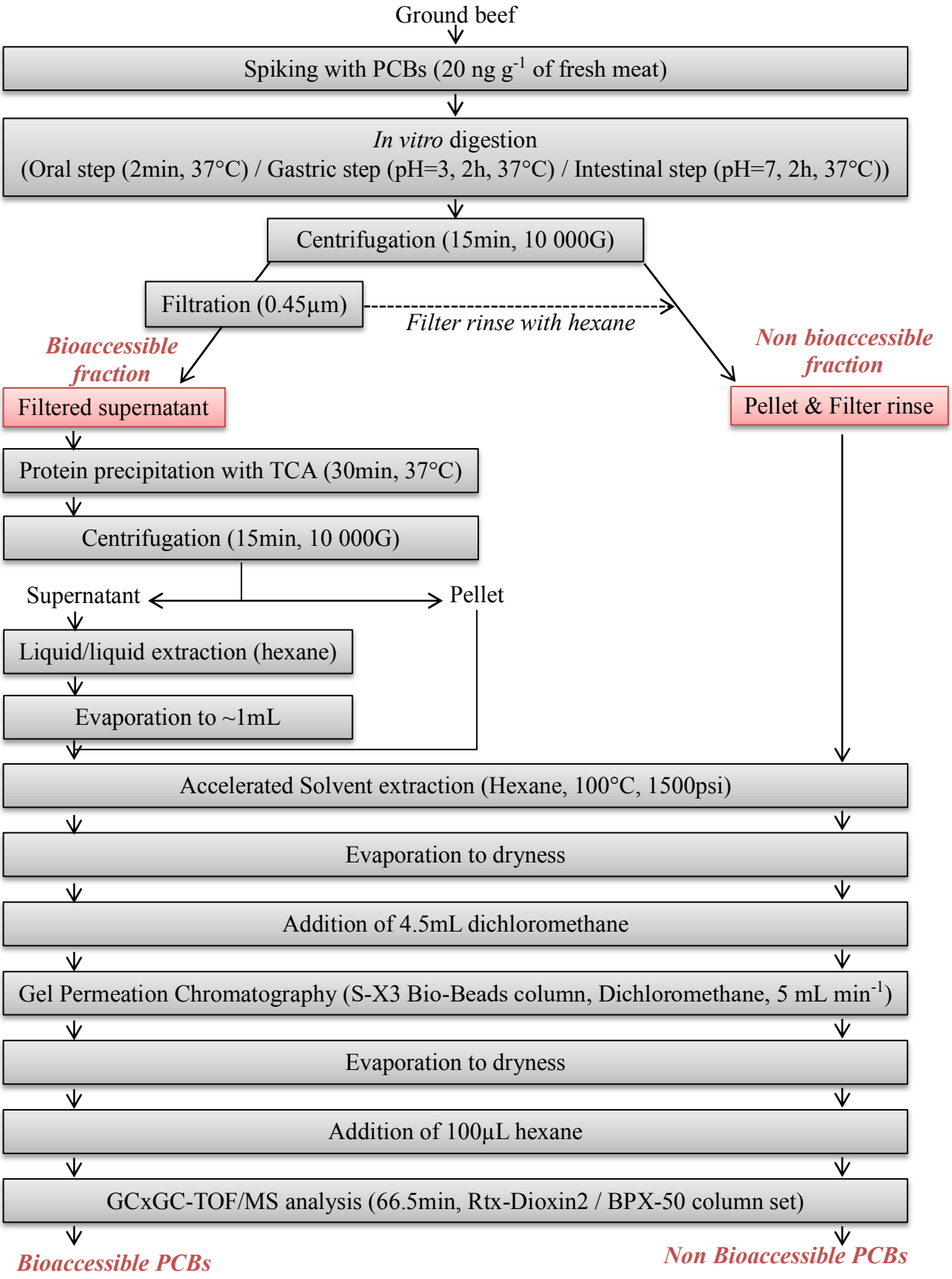

Fig 1: Experimental procedure showing sample preparation, *in vitro* digestion and PCB extraction and analysis.

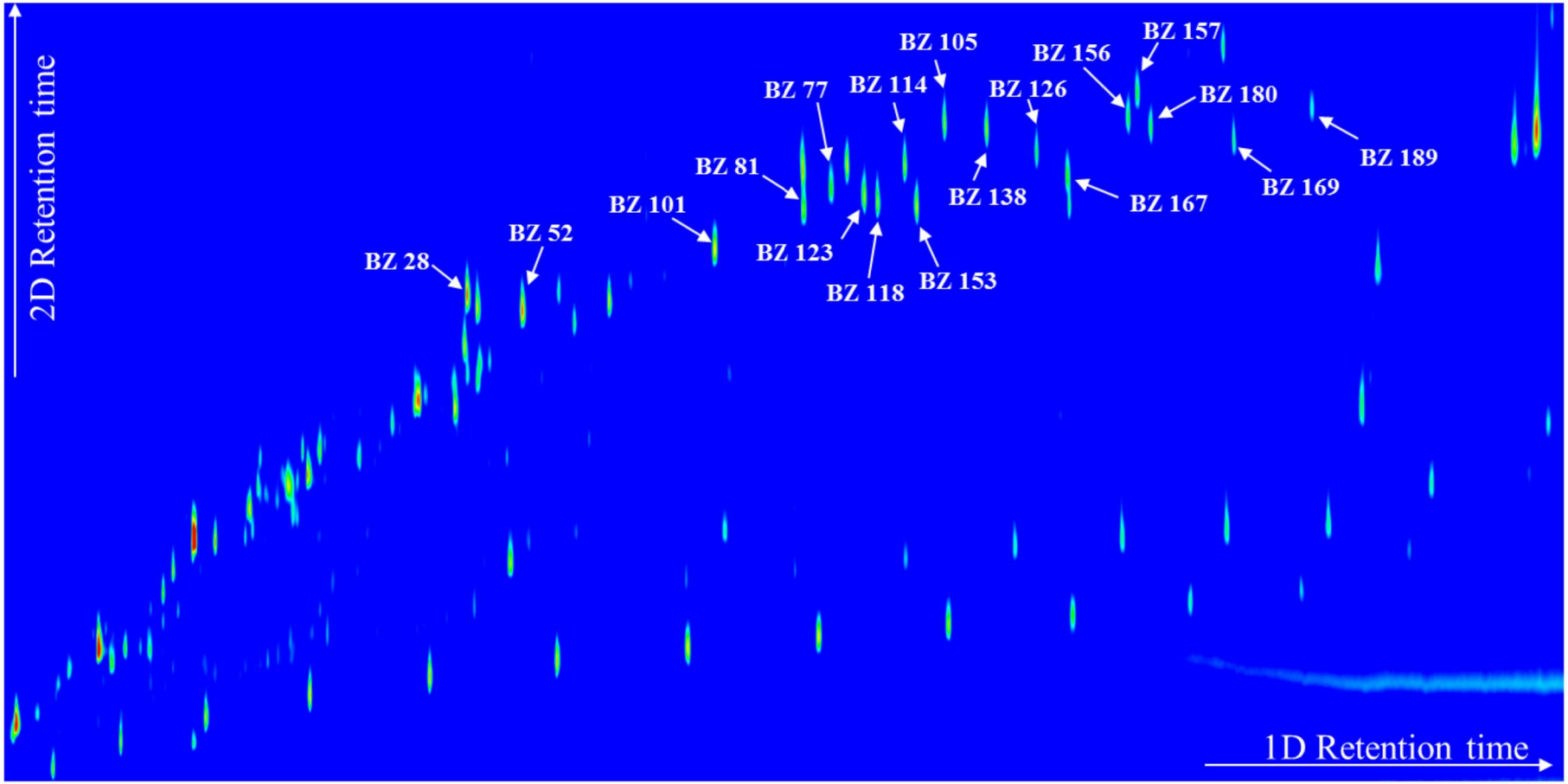

Fig 2: GC×GC-ToF/MS contour plot with the 18 most relevant PCB congeners to monitor in meat. X-axis and y-axis represent the retention of molecules on the first and the second chromatographic column, respectively. The column set consisted of a Rtx-Dioxin2 (60 m × 0.25 mm × 0.25 µm) as the first dimension column and a BPX-50 (2 m × 0.1 mm × 0.1 µm) as the second dimension column according to Planche *et al.* (2015).

Figure 3

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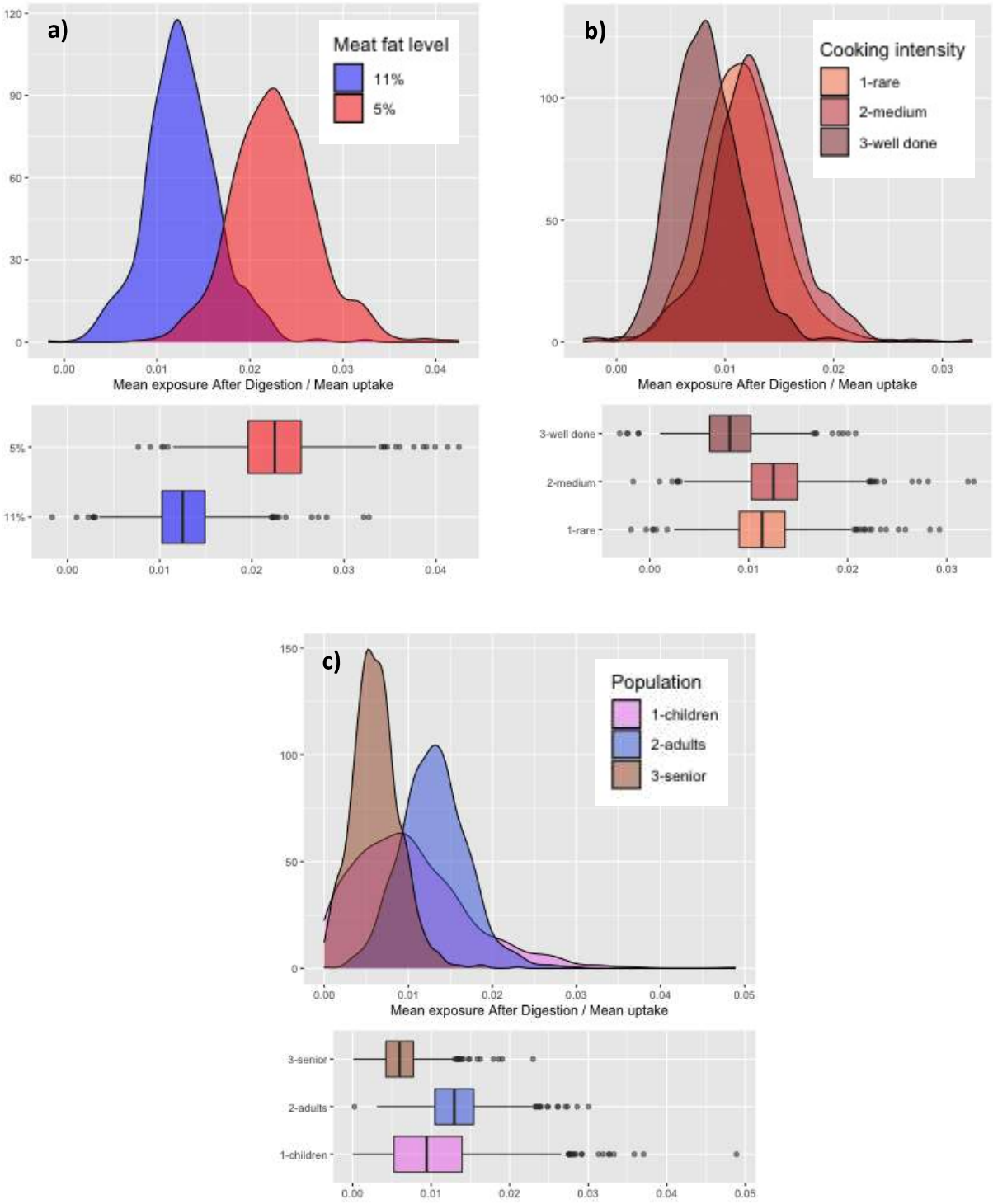

Fig 3: Mean uptakes according to meat fat level (a), cooking intensity (b) or consumer age (c). 3a): red color is 5% fat, blue color is 11% fat; adult population, meat medium cooked. 3b): from the lightest to the darkest: rare, medium, well-done; 11% fat, adult population. 3c): pink color for children, blue color for adults, and brown color for seniors - 11% fat, meat medium cooked.

Table S1. Recovery rate (%) of PCBs in the bioaccessible fraction after a liquid/liquid extraction, with hexane or dichloromethane (DCM) as extraction solvent.

Compound name	PCB congener	WHO-TEF (Toxic equivalency factor)	Recovery rate (%) in the bioaccessible fraction depending on the extraction solvent	
			Hexane	DCM
2,4,4'-Trichlorobiphenyl	28		56 ± 0.7	75 ± 12
2,2',5,5'-Tetrachlorobiphenyl	52		72 ± 3.3	72 ± 8.0
2,2',4,5,5'-Pentachlorobiphenyl	101		93 ± 3.1	71 ± 4.9
3,4,4',5-Tetrachlorobiphenyl	81	0,0003	97 ± 13.0	79 ± 8.6
3,3',4,4'-Tetrachlorobiphenyl	77	0,0001	98 ± 8.7	83 ± 8.9
2',3,4,4',5-Pentachlorobiphenyl	123	0,00003	102 ± 13.0	82 ± 4.0
2,3',4,4',5-Pentachlorobiphenyl	118	0,00003	104 ± 16.0	83 ± 5.2
2,3,4,4',5-Pentachlorobiphenyl	114	0,00003	107 ± 9.7	83 ± 5.6
2,2',4,4',5,5'-Hexachlorobiphenyl	153		93 ± 8.0	89 ± 4.8
2,3,3',4,4'-Pentachlorobiphenyl	105	0,00003	104 ± 4.4	86 ± 3.9
2,2',3,4,4',5'-Hexachlorobiphenyl	138		98 ± 6.5	95 ± 1.3
3,3',4,4',5-Pentachlorobiphenyl	126	0,1	105 ± 13.0	85 ± 2.0
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0,00003	106 ± 15.0	95 ± 5.7
2,3,3',4,4',5-Hexachlorobiphenyl	156	0,00003	112 ± 19.0	103 ± 7.9
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0,00003	107 ± 10.0	101 ± 6.4
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		103 ± 20.0	118 ± 6.5
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0,03	107 ± 8.9	105 ± 6.4
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0,00003	109 ± 8.9	110 ± 6.2

Table S2. Elution order of the 18 PCBs targeted in meat using GC×GC-ToF/MS with a Rtx-Dioxin2/BPX-50 column set. ${}^{1}r_t$ (s) and ${}^{2}r_t$ (s) are the retention time in seconds on respectively the first and the second dimension.

Compound name	PCB congener	WHO-TEF (Toxic equivalency factor)	${}^{1}r_t$ (s)	${}^{2}r_t$ (s)
2,4,4'-Trichlorobiphenyl	28		1580	3,2
2,2',5,5'-Tetrachlorobiphenyl	52		1610	3,4
2,2',4,5,5'-Pentachlorobiphenyl	101		2085	3,6
3,4,4',5-Tetrachlorobiphenyl	81	0,0003	2265	3,7
3,3',4,4'-Tetrachlorobiphenyl	77	0,0001	2320	3,8
2',3,4,4',5-Pentachlorobiphenyl	123	0,00003	2385	3,7
2,3',4,4',5-Pentachlorobiphenyl	118	0,00003	2415	3,7
2,3,4,4',5-Pentachlorobiphenyl	114	0,00003	2465	3,9
2,2',4,4',5,5'-Hexachlorobiphenyl	153		2490	3,7
2,3,3',4,4'-Pentachlorobiphenyl	105	0,00003	2545	4,0
2,2',3,4,4',5'-Hexachlorobiphenyl	138		2630	3,9
3,3',4,4',5-Pentachlorobiphenyl	126	0,1	2730	3,9
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0,00003	2795	3,8
2,3,3',4,4',5-Hexachlorobiphenyl	156	0,00003	2915	4,0
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0,00003	2930	4,1
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		2960	4,0
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0,03	3125	3,9
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0,00003	3280	4,1

Table S3. Lipid relative composition (*n*=3) of 11% and 5% fat meat (g/100 g fatty acids). Total lipids were extracted from skeletal muscle as described by Floch *et al.* (1) and the organic phase was evaporated under nitrogen. Analysis of fatty acid methyl-esters (FAMEs) was performed according to Pinel *et al*. (2) on a gas chromatograph (Thermo Electron Corporation, Waltham, MA, USA) coupled with a flame ionization detector using a select FAME (Agilent Technologies, Les Ulis, France) column (0.25 mm inner diameter, 100 m., 0.25 µm film thickness) and helium as the carrier gas (2.6 bar, constant pressure, inlet temperature of 250 ◦C).

	5% fat meat	11% fat meat
SFA	45 ± 0.25	49.0 ± 0.17
MUFA	44 ± 0.69	45.0 ± 0.35
PUFA	6.5 ± 0.37	3.0 ± 0.03
LA and ALA	4.7 ± 0.19	3.2 ± 0.04

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; ALA: alpha-linolenic acid.

(1) Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem 1957, 226*, 497-509.
(2) Pinel, A., Rigaudière, J. P., Jouve, C., & Capel, F. (2018). Modulation of insulin resistance and the adipocyte-skeletal muscle cell cross-talk by LCn-3PUFA. *International journal of molecular sciences, 19(9)*, 2778.

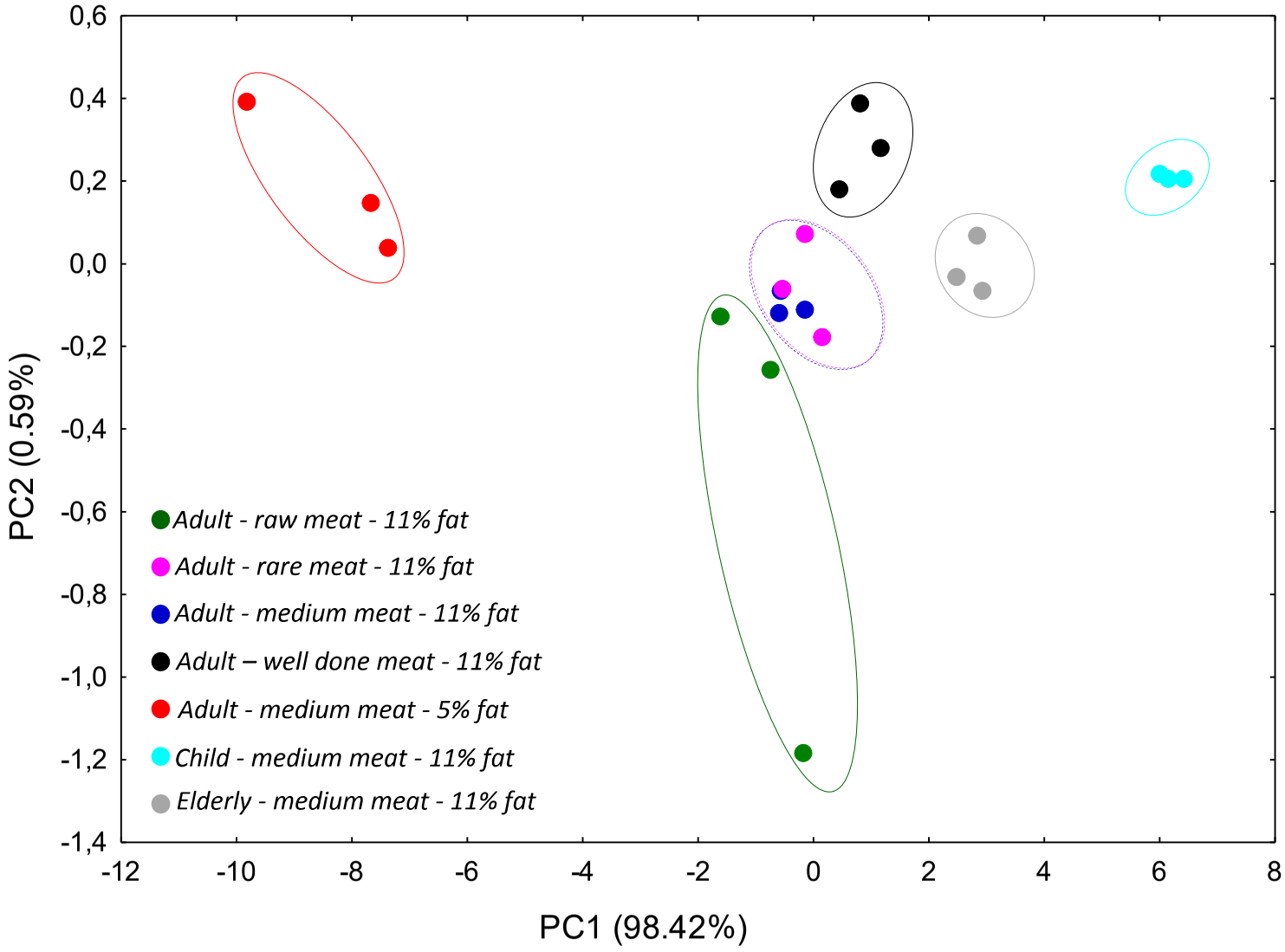

Fig S1. Unsupervised discrimination of meat samples according to the bioaccessibility (%) of 18 targeted PCB. Meat samples differed according to level of meat fat (5 or 11%), intensity of meat cooking (raw, rare, medium and well done) before digestion, and age of consumer (child, adult, elderly)

Table S4. Bioaccessibility (BA) (%) and relative standard deviation (RSD) (%) of spiked PCBs in raw meat ($n = 3$) and in meat cooked at different intensities (rare ($n = 3$), medium ($n = 3$) and well-done ($n = 3$)). [a,b,c]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the bioaccessibility obtained for the different cooking intensities. Two groups with distinct letters can be considered as significantly different ($p < 0.05$)

Compound	Congener	WHO-TEF	Raw meat		Rare cooked meat		Medium cooked meat		Well done cooked meat	
			BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)
2,4,4'-Trichlorobiphenyl	28		26.1[a]	13.5	24.5[a]	7.4	24.9[a]	1.4	22.6[a]	5.1
2,2',5,5'-Tetrachlorobiphenyl	52		27.4[a]	11.8	26.9[a]	8.9	25.9[a]	2.2	24.1[a]	2.3
3,3',4,4'-Tetrachlorobiphenyl	77	0.0001	24.1[b]	12.7	20.5[ab]	4.7	20.6[ab]	6.1	18.3[a]	6.7
3,4,4',5-Tetrachlorobiphenyl	81	0.0003	24.6[c]	8.1	21.4[b]	2.0	23.0[bc]	4.3	19.0[a]	5.1
2,2',4,5,5'-Pentachlorobiphenyl	101		28.9[a]	7.5	27.3[a]	6.8	26.9[a]	3.5	26.7[a]	4.2
2,3,3',4,4'-Pentachlorobiphenyl	105	0.00003	26.1[b]	7.1	24.2[b]	5.4	26.4[b]	2.7	20.5[a]	7.5
2,3,4,4',5-Pentachlorobiphenyl	114	0.00003	25.8[a]	10.1	24.2[a]	4.5	25.2[a]	5.0	22.6[a]	6.5
2,3',4,4',5-Pentachlorobiphenyl	118	0.00003	25.5[a]	9.3	24.3[a]	4.3	24.9[a]	6.1	21.5[a]	5.9
2',3,4,4',5-Pentachlorobiphenyl	123	0.00003	27.0[a]	8.5	26.4[a]	6.8	25.8[a]	5.1	25.8[a]	2.5
3,3',4,4',5-Pentachlorobiphenyl	126	0.1	25.2[b]	10.0	22.1[b]	4.3	24.0[b]	2.7	18.0[a]	8.1
2,2',3,4,4',5'-Hexachlorobiphenyl	138		27.4[a]	8.4	26.7[a]	7.5	27.5[a]	6.7	24.6[a]	5.0
2,2',4,4',5,5'-Hexachlorobiphenyl	153		28.2[a]	8.7	28.2[a]	6.6	27.9[a]	6.1	26.3[a]	7.2
2,3,3',4,4',5-Hexachlorobiphenyl	156	0.00003	27.7[b]	8.7	25.7[b]	1.0	26.7[b]	6.2	21.9[a]	6.2
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0.00003	27.5[b]	10.9	25.4[ab]	2.4	23.8[ab]	4.7	21.9[a]	5.5
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0.00003	28.0[b]	6.0	25.9[ab]	6.0	28.0[b]	2.3	23.3[a]	11.6
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0.03	23.3[ab]	9.1	23.7[ab]	3.8	25.9[b]	3.0	21.2[a]	5.7
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		29.0[ab]	11.1	27.9[ab]	4.3	30.5[b]	4.7	24.4[a]	7.2
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0.00003	35.1[c]	13.9	27.9[b]	4.1	28.5[b]	3.1	21.7[a]	7.5

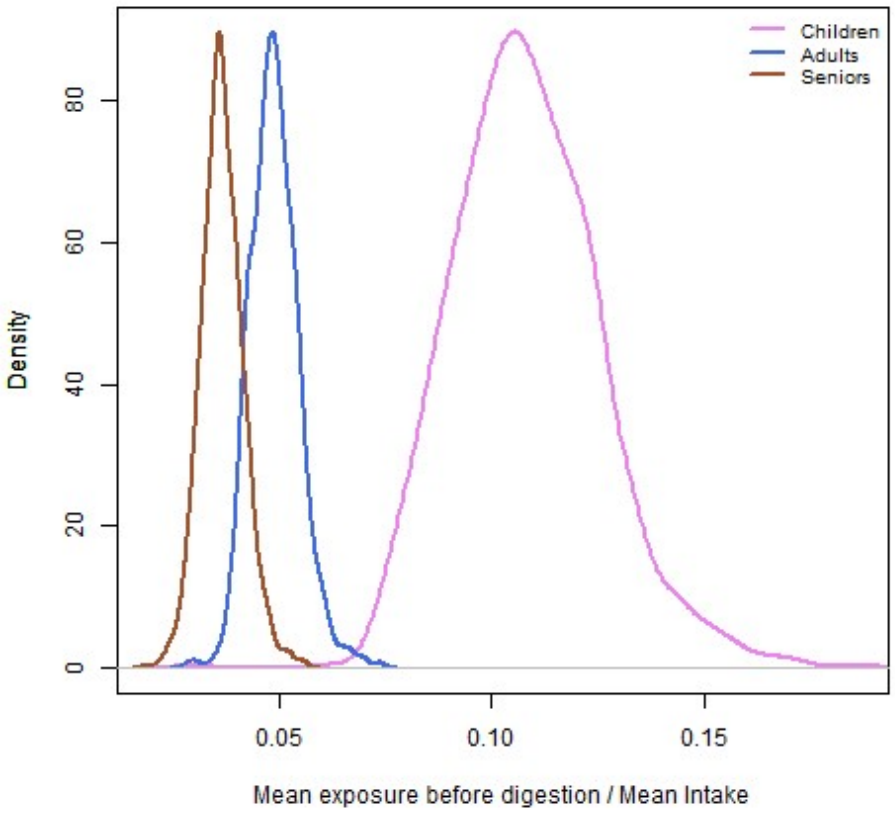

Fig S2. Mean intakes according to consumer age.

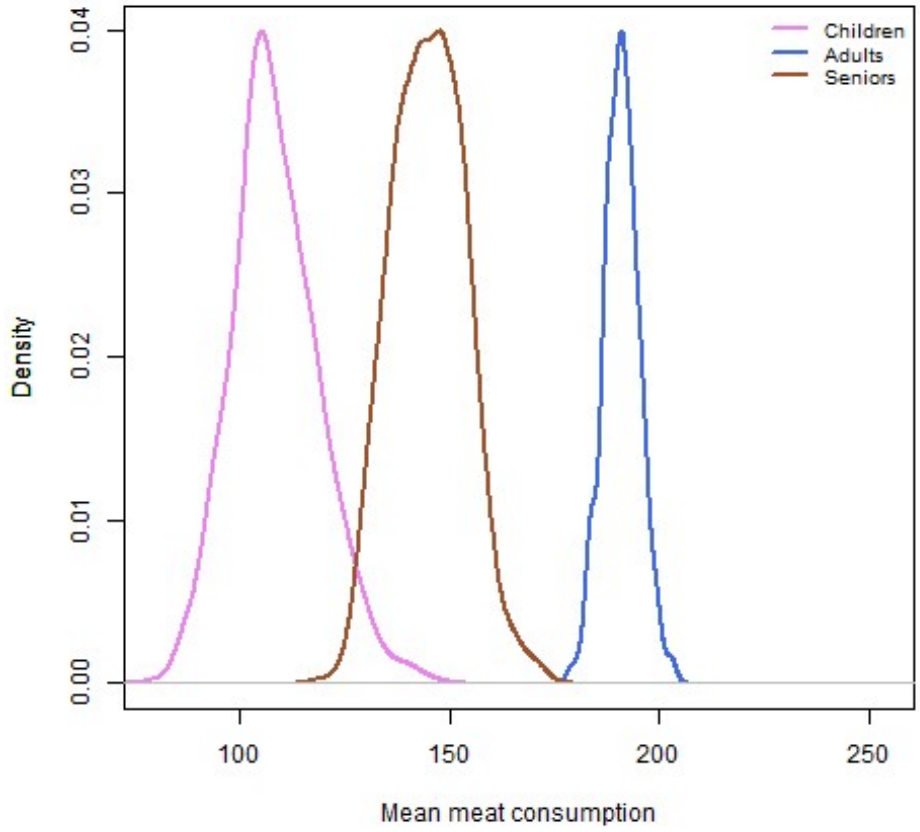

Fig S3. Mean meat consumption (g/week) according to consumer age.