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Bioaccumulation of perfluoroalkyl compounds in midge (*Chironomus riparius)* larvae exposed to sediment

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Abstract

Midge larvae (*Chironomus riparius*) were exposed to sediments from a deposition sampled at a site along the Rhône River (France) downstream of an industrial site releasing various perfluorinated chemicals. This sediment is characterized by high concentrations of perfluoroundecanoic acid (PFUnA) and perfluorotridecanoic acid (PFTrDA) and a low perfluorooctane sulfonate (PFOS) concentration. Concentrations of 23 perfluoroalkyl compounds, including C_4-C_{14} carboxylate acids, C_4-C_{10} sulfonates, and seven precursors, were analyzed in overlying and pore water, sediment, and larvae. Midge larvae accumulated carboxylate acids (C_{11}-C_{14}), PFOS, and two precursors (perfluorooctane sulfonamide: FOSA and 6:2 fluorotelomer sulfonic acid, 6:2 FTSA). These substances accumulated mainly during the fourth instar larvae exponential growth phase. Accumulation of 6:2 FTSA, PFUnA, and PFOS occured via trophic and tegumentary routes. Other compounds mainly accumulated from food. Kinetics followed a partition model, from which uptake and elimination constants were derived.

Keywords: Perfluoroalkyl compounds, sediment, *Chironomus riparius*, bioaccumulation.

Capsule: *Chironomus riparius* mainly bioaccumulates long-chain PFASs via trophic and/or tegumentary routes during the fourth instar larvae growth phase.

1. Introduction

Polyfluoroalkyl and perfluoroalkyl substances (PFASs) have been produced since the early 1950s. The production and use of these compounds have resulted in their

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global distribution in the environment (Houde et al., 2011; Houde et al., 2006; Prevedouros et al., 2006). In 2000, the 3M company voluntarily phased -out C_8-based-chemicals (i.e., sulfonamide based polymers), at the base of the formation of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), and replaced them with shorter- chain chemicals (e.g., perfluorobutane sulfonate, PFBS) (Renner, 2006; Lindstrom et al., 2011). In 2009, PFOS was listed under Annex B of the Stockholm convention (United Nations Environmental Program -UNEP) on Persistent Organic Pollutants (POPs) (Lindstrom et al., 2011).

PFASs have been found in all aquatic media (Houde et al., 2011; Houde et al., 2006). On the basis of global modelling, marine sediments have been designated as the ultimate containment for PFASs (Armitage et al., 2006). Several studies have shown the presence of PFASs in sediment with concentrations ranging from 0.5 ± 0.1 to 38.3 ± 16.8 $ng.g^{-1}$ dw (Ahrens et al., 2009; Bao et al., 2009; Bao et al., 2010; Higgins et al., 2005; Labadie and Chevreuil, 2011; Myers et al., 2012; Zushi et al., 2010). Sediment has been suggested as a major source of contamination of aquatic organisms (Martin et al., 2004). However, the distribution of water, sediment, and biota as well as the role of the sediment compartment in biota contamination is still poorly understood. The length of the fluorocarbon chain is an important criterion for PFAS distribution in the environment (Ahrens et al., 2009; Higgins and Luthy, 2006; Myers et al., 2012). PFASs with eight or more carbon atom backbones are known to be bioaccumulative (Kannan et al., 2005; Kelly et al., 2009; Loi et al., 2011; Tomy et al., 2004).

In France, PFASs were found in fish from the Rhône River near Lyon (Miège et al., 2012). Highest levels of PFASs were found in fish from this latter river (mean total PFAS concentration: 241.5 $ng.g^{-1}$ dry weight (dw)), the Rhône River was therefore

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chosen as the study site for this research which aimed to better understand the origin of PFAS contamination in aquatic organisms by evaluating the transfer of these compounds from sediment to invertebrates.

A benthic invertebrate, the non-biting midge *Chironomus riparius*, is found in sediment from the Rhône River and is recommended by the OECD for toxicity testing (OECD, 2004). Midges are holometabolous, that is they go through complete metamorphosis consisting of egg, larva, pupa, and adult stages. *C. riparius* mates in aerial swarms. After mating, the female deposits the eggs on the water surface. Larvae undergo four instars; the first (L1) is planktonic and the second to fourth larval stages (L2, L3, L4) are in direct contact with sediment (Fig. S1). Larvae L2 to L4 are collector-gatherers, feeding mainly on detritus and its associated bacteria and fungi. This species represents an important food source for fish, making it a useful species for documenting the bioaccumulation of perfluoroalkyl compounds (Armitage et al., 1995).

The objectives of the present study were (i) to experimentally determine the role played by sediment in the accumulation of eleven carboxylic acids (from 4 to 14 carbons), five sulfonates (ranging from 4 to 10 carbons), and seven precursors by *C. riparius* larvae and (ii) to describe the kinetics of the bioaccumulation process.

2. Materials and Methods

The study site, Beurre Island (BER), is a fluvial annex of the Rhône River (eastern central France, N45°28'17,0"E4°46'43,4", Fig. 1) and located downstream of a fluoropolymer manufacturing plant that has been found to be an important source of PFAS contamination with a typical profile (Dauchy et al., 2012).

2.1 Sediments

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In 2012, 50 L of natural sediments were collected from the river bed with a Van-Veen grab, kept on ice and brought to the laboratory, where they were sieved at 2 mm, pooled in a polypropylene (PP) jar, and stored at 4°C. Six aquaria (38 × 20 × 24.5 cm in polystyrene) were prepared with 4 L of homogenized sediment and 15 L of groundwater mixed with treated water via an osmosis system in order to reach a conductivity of 300 $\mu S.cm^{-1}$. Each aquarium was allowed to settle for 1 week before introducing the chironomids. Three control aquaria were prepared in the same manner with silica sand (particle size distribution: 90% 50-200µm, 10% <50µm) (Péry et al., 2002).

The sediment was characterized for water content, nitrogen, carbonate using a Bernard calcimeter, and loss on ignition (LOI) (AFNOR, 1994, 2000, 2007; Vatan 1967) and showed a water content of 43.4%, an organic carbon content of 4.3%, a carbonate content of 27.4%, and a nitrogen content of 0.19%. The particle size distribution was determined by laser diffractometry (Cilas 1190, France) and found to be 10% clay -silt (< 5.83µm), 40% silt (5.83 - 52.24 µm), and 50% of silt -very fine sand (>52.24 mm).

2.2 *C. riparius* exposure

Chironomids were obtained from laboratory cultures made according to standard methods (AFNOR, 2010; OECD, 2004). Chironomids were exposed to the BER sediment at standard temperature (21°C). Two experiments were conducted to examine the extent to which developmental stage influences the amount of accumulated chemicals in *C. riparius* (experimental designs shown in Fig. S2). The first experiment (E1) started with L2 larvae until the end of the L4 stage and lasted for 9 days. The second experiment (E2) started directly with L4 larvae.

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In experiment E1, 2400 L2 larvae (2-day-old larvae post-hatching) were added to three study aquaria, and to three control aquaria (400 in each aquarium). In experiment E2, 1200 early L4 larvae (7-day-old larvae post-hatching) were exposed in three aquaria. Overlying water (OW) was continuously renewed (four times a day) to maintain adequate water quality and oxygenated under a 16:8-h light: dark photoperiod during the experiment. Larvae were fed ad libitum each day with 1mg commercial food (Tetramin®) per organism per day. We decided to feed the chironomids during the experiment because previous experiments showed that larvae did not survive without an external supply of food in this sediment. A quantity of 1mg Tetramin® per organism per day was selected because preliminary tests (data not shown) conducted with 0.6mg Tetramin® per organism per day showed low survival and growth. E1 organisms were sacrificed after 11 post-hatching days, i.e., 9 days (Tfinal, Tf) after being introduced in the aquaria. E2 organisms were sacrificed at 2 (T1), 3 (T2), and 4 (T3) days, respectively, after introducing L4 chironomids. The water quality parameters (pH, concentration of dissolved oxygen, conductivity, NO_2^-, and NH_4^+) were monitored once a week and at the beginning and the end of the exposure period (Fig. S2).

2.3 Sample collection and chironomid measurements

Overlying water (OW), pore water (PW), and sediment were sampled at T1, T2, T3, and Tf. E1 organisms were sampled only at Tf. E2 chironomids were sampled at T0 (fourth instar larvae), T1, T2 and T3, and Tf. The overlying water was sampled directly in a 1L polyethylene (PE) bottle. For pore water, we used a Rhizon® system (SDEC, Reignac-sur-Indre, France), which consists of one porous polymer part inside a fiberglass rod. Organisms were collected by sieving the upper layer of sediment at 500µm; sub-samples from deeper sediment were deposited in PE tubes. Organisms were

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split into two different batches for PFAS analysis (about 800 mg ww, representing about 200 larvae) and $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis (about 5 mg dw of larvae). Biota samples were cryopreserved in liquid nitrogen and stored at -21°C and abiotic samples frozen and kept at -21°C (Fig S2).

Chironomid survival, length, and weight were determined for all aquaria. To determine length, four groups of ten larvae were photographed and mean sizes assessed using digital image analysis software (Jmicrovision, freely available via http://www.jmicrovision.com/). The same groups were then weighed (weighing scales: Sartorius CPA225D, France) to obtain mean weights.

2.4 PFAS extraction

2.4.1 Overlying and pore water samples

Water samples were each spiked with 2 ng of IS and processed using Srata X-AW cartridges as described by Labadie and Chevreuil (2011).

Eluates were concentrated to 400 µL under a nitrogen stream at 40°C and transferred into PP vials (final volume: 250-300 µL). Extracts were stored at -20°C until analysis.

2.4.2 Sediment sample

Sediment samples were extracted by sonication, using a method adapted from Sun et al. (2011). Samples (1 g dw) were spiked with ISs (1.8 ng each) and extracted with 5 mL of MeOH for 20 min, prior to centrifugation for 3 min (20°C, 2900 *g*). This procedure was repeated with 2.5 mL of MeOH and the two extracts were combined and concentrated to 800 µL under a nitrogen stream at 40°C. Samples were purified using ENVI-Carb cartridges previously conditioned with 4 mL of MeOH and eluted with 2× 4 mL of MeOH. Eluates were concentrated to 400 µL under a nitrogen stream at 40°C and transferred into injection vials.

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144 2.4.3 *C. riparius* samples and Tetramin® samples

145 Extraction of PFASs in 120 mg dw *C. riparius* (800 mg wet weight (ww)) and in
146 540 mg Tetramin® followed the same protocol used for the sediment samples. Extract
147 clean-up was adapted from Ballesteros-Gomez et al. (2010). Extracts were diluted 20
148 times with ultra-pure water (5% extract in water) and then passed through a Strata X-
149 AW cartridge previously conditioned as described above. After extract loading,
150 cartridges were washed with 5 mL of sodium acetate buffer (pH=4.5, 25 mM). They
151 were vacuum-dried (30-40 min) and connected via a polyethylene adaptor cap to an
152 ENVI-Carb cartridge previously conditioned with 8 mL of MeOH. Neutral PFASs were
153 eluted with 8 mL of MeOH (fraction A). The ENVI-Carb cartridge was then replaced
154 and a second fraction (fraction B) containing acidic analytes was eluted with 8 mL of
155 MeOH containing 0.2% NH_4OH. Fraction A eluates were concentrated to 300 µL and
156 then transferred into PP injection vials. Fraction B eluates were processed almost to
157 dryness before adding 200 µL of acetonitrile and 200 µL ultrapure water. Extracts were
158 then passed through MeOH-rinsed centrifuge tube filters and transferred into PP
159 injection vials.

160 2.5 Isotopic analysis

161 Isotopic analysis (^{13}C and ^{15}N) was performed by the INRA-Nancy laboratory
162 (PTEF-isotopy pole, France) on decarbonated sediment, Tetramin®, and chironomids
163 (i.e., chironomids that have eliminated their gut content following 48h in a beaker
164 containing silicate, overlying water with food, and chironomids with gut content).

165 2.6 LC-MS/MS analysis

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166 PFAS analyses were performed using an Agilent 1200 LC system (Agilent
167 Technologie, Massy, France) interfaced with an Agilent 6460 triple quadrupole mass
168 spectrometer (details in Table S2).

169 2.7 Quality control and performance methods

170 Analyte recoveries were determined using spiked samples for each matrix
171 (surface and pore water, sediment, and chironomids). Native PFAS recoveries ranged
172 from 50 to 110% (except for PFTeDA, MeFOSA and EtFOSA), with a relative standard
173 deviation below 15% (Table S3).

174 Mean IS recoveries, estimated by external calibration, were in the range 70 -
175 135% for the different matrices. Replicate procedural blanks were analyzed for each
176 series of samples. The predominant compounds in surface water blanks were PFHpA
177 (mean level: 166 pg) and PFHxA (115 pg). For solid samples, the prevailing analytes in
178 blanks were PFOA (63 pg) and PFPA (26 pg). PFAS concentrations were therefore
179 blank-corrected. For compounds present in blanks, the limits of detection (LDs) were
180 defined as three times the standard deviation, and the limits of quantification (LQs)
181 were set at ten times the standard deviation of the blank. For analytes not detected in the
182 blanks, LDs and LQs were determined as the concentration with a signal-to-noise ratio
183 of 3 and 9, respectively. This calculation was performed on matrices spiked at 2-5 $ng.g^{-1}$
184 (sediment and *C. riparius*) and 2-3 $pg.L^{-1}$ (Vittel® mineral water samples) (Table S4).

185 2.8 Data processing

186 Data were analyzed using the Student' *t*-test. All data were checked for normality and
187 homogeneity of variance using the Shapiro-Wilk test and the Bartlett test, respectively.
188 For all statistical tests, the significance level (alpha) was set at 0.05 and calculations

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were performed using the software package R (version 2.11.0) (R Development Core Team, 2013). For calculating data below the LQ, 0.5*LQ was used.

When relevant, accumulation data were fit to an exponential rise model (Eq.1) with Sigma Plot 10.0 software (Systat software Inc., Point Richmond, CA, USA-www.systat.com).

$$C_{org}(t) = a.(1-e^{-bt}) \qquad \text{Eq. (1)}$$

Where C_{org} is the concentrations in chironomids (ng. g^{-1} ww), t the time in (h), a and b are defined in the discussion.

This model has been adapted to existing bioaccumulation models, including the model described by Spacie and Hamelink (1985) and Landrum (Landrum, 1989).

3. Results

3.1 PFAS distribution in water, sediment, food, and *C. riparius*

Short- (PFBA to PFHxA) and long-chain PFCAs (PFOA, PFNA, PFDA, PFUnA, and PFTrDA), short- and long-chain PFSAs (PFBS, PFHxS, and PFOS) and one precursor (6:2 FTSA) were detected in OW test aquaria. PFBA, PFPA, PFHxA, PFNA, PFDA, PFBS, PFHxS, and PFOS were also detected in OW control aquaria, at levels comparable to those observed in test aquaria. Concentrations were consistent throughout the replicates (test and control) (Table S5). The same compounds were observed in pore water, except PFDA and PFTrDA; PFHpA was also measured in this compartment. In PW samples from control aquaria, PFBA, PFPA, PFHxA, PFHpA, and PFHxS were detected at levels lower than PW from test aquaria (n =3, p-value<0.001). In sediment, short- (PFBA to PFHpA) and long-chain (PFOA to PFTeDA) PFCAs, PFOS, and two precursors (6:2 FTSA and FOSA) were detected > LQs and EtFOSAA were detected < LQs. Concentration of long-chain PFCAs were higher in sediment:

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PFTrDA (2.54 ± 0.23 ng.g^{-1}dw), PFUnA (1.70 ± 0.22 ng.g^{-1}dw), PFDoA (1.54 ± 0.63 ng.g^{-1}dw), PFTeDA and PFDA (0.99 ± 0.58 ng.g^{-1}dw and 0.91 ± 0.43 ng.g^{-1}dw respectively), whereas PFOS was lower (0.26 ± 0.02 ng.g^{-1}dw). In sediment control samples, only PFPA and PFOA results were between LDs and LQs; the other compounds were <LDs.

The analysis of Tetramin® indicated the presence of 4 PFASs: PFNA (0.20 ± 0.05 ng.g^{-1}dw), PFUnA (0.34 ± 0.32 ng.g^{-1}dw), PFOS (1.43 ± 0.17 ng.g^{-1}dw), and FOSA (0.39 ± 0.03 ng.g^{-1}dw). The range of PFAS concentrations in chironomids at the end of E1 and E2 exposures are presented in Table 1, PFAS concentrations in chironomids control were <LDs.

3.2 Growth and accumulation kinetics

Survival throughout the experiments (E1 and E2) was greater than 80%. Chironomid weight was significantly different ($p < 0.001$) between growth in sediment (two at fourth instar larvae: 7.15 ± 0.60 mg) and in silica (5.84 ± 0.83 mg) at 11 days post-hatching (Fig. S3). However, no adverse effect of contaminated sediment on chironomid growth was observed. In addition, fourth instar larvae weight data (E2) fit an exponential growth curve well ($R^2 = 0.999$, $p < 0.0001$); growth rate (g) 0.0148 (± 0.0001).

The results indicated that *C. riparius* accumulated four long-chain PFCAs (> 10 carbons: PFUnA, PFTrDA, PFDoA, and PFTeDA), PFOS, one precursor (FOSA), and one fluorotelomer (6:2 FTSA); the other compounds were all <LDs (Fig. 2). Fourth instar chironomid larvae accumulated PFASs as early as the second day of exposure and a steady state was observed at the end of exposure for most of the accumulated compounds, except for PFTeDA (LD< PFTeDA concentration < LQ) and perhaps for

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6:2 FTSA. No differences in accumulation were observed between organisms exposed only at the fourth instar and those exposed from the second to the fourth instar (Fig. 3, Table 1).

The chironomids were not depurated before PFAS analyses; therefore the gut content estimation was based on Brooke et al. (1996). According to this calculation there was no difference in PFAS concentrations between chironomids in which gut content was or was not estimated. Therefore, the data without gut content correction were kept for further interpretation.

The biota-to-sediment accumulation factor (BSAF) between organisms was calculated according to (Higgins et al., 2007):

$$BSAF_{ww} = C_{org} / C_{sed,oc} \quad \text{Eq. (2)}$$

where C_{org} (ng.g^{-1} ww) is the PFAS concentration in the organism (at steady state) and $C_{sed,oc}$ (ng.g$_{oc}^{-1}$ dw) is the PFAS concentration in sediment normalized by the organic carbon content (Table 2).

3.3 Isotopic analysis

$\delta^{13}C$ signatures of -27.57 ± 0.005‰ and -23.11‰ and $\delta^{15}N$ signatures of 4.12 ± 0.64‰ and 7.58‰ were found in sediment and chironomid food (Tetramin®) respectively. No differences were observed between depurated or non-depurated chironomids: the $\delta^{13}C$ signature was -20.37 ± 0.42‰ and the $\delta^{15}N$ signature 11.61 ± 0.80‰ (Fig. S4).

4. Discussion

4.1 PFAS distribution in water, sediment, food, and *C. riparius*

The sediment from the study site was characterized by high concentrations of PFNA, PFUnA, and PFTrDA, whereas PFOS concentrations remained low as compared

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261 with the data reported in the literature (Table S6). This pattern of PFAS contamination
262 may be characteristic of the upstream industrial discharge from a fluoropolymer and
263 polyvinylidene fluoride facility (Dauchy et al., 2012). In most published studies from
264 other sites, PFUnA and PFDA were usually below 1 $ng.g^{-1}dw$ (Clara et al., 2009;
265 Higgins et al., 2005; Higgins et al., 2007; Labadie and Chevreuil, 2011), except for the
266 Coosa River in Alabama (3.80 $ng.g^{-1}dw$) and Tennessee, USA (4.66 $ng.g^{-1}dw$) (Lasier et
267 al., 2011). Detection of other long-chain PFCAs was scarce: a maximum value of 1.19
268 $ng.g^{-1}dw$ for PFTrDA was reported in Tokyo Bay, Japan (Zushi et al., 2010), and
269 PFDoA and PFTeDA values were below 1 $ng.g^{-1}dw$ in the Orge River (near Paris,
270 France) (Labadie and Chevreuil, 2011), whereas in the Coosa River, USA,
271 concentrations above 1 $ng.g^{-1}dw$ (1.7 and 4.64 $ng.g^{-1}dw$ respectively) were found
272 (Lasier et al., 2011). For PFOS, BER sediment concentrations (0.247 - 0.294 $ng.g^{-1}$ dw)
273 were in the same range as reported in other studies around the world (Table S6) (Bao et
274 al., 2009; Benskin et al., 2012; Nakata et al., 2006) and lower than PFOS levels in the
275 Orge River (France), Coosa River (USA), Sydney Harbor (Australia), and the North and
276 Baltic Seas (Labadie and Chevreuil, 2011; Lasier et al., 2011; Theobald et al., 2012;
277 Thompson et al., 2011).

278 Comparison of the respective concentration ranges for the PFASs measured in
279 control and test aquaria make it possible to design a conceptual diagram of PFASs
280 transport between compartments (Fig. 4). Since PFBA, PFPA, PFHxA, PFNA, PFDA,
281 PFBS, and PFHxS display the same concentrations on overlying waters in control and
282 test aquaria, we inferred that sediment is not a source for these compounds to water.
283 Conversely, PFUnA, PFTrDA, and 6:2 FTSA, which were present only in OW test
284 aquaria and therefore were clearly released from the sediment. Similarly, PFBA, PFPA,

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PFHxA, PFOA, PFNA, PFUnA, PFOS, and 6:2 FTSA associated with test sediments were in much higher concentrations in PW from test aquaria than in PW from controls. Therefore, their presence in PW cannot be related to OW. PFNA and PFOS were measured in both food (Tetramin®) and OW controls aquaria. As a consequence, it could not be exclude that PFNA and PFOS in Tetramin® were dissolved into OW. However, all the PFASs measured were <LD in control chironomids; therefore Tetramin® is not a source of PFASs. PFUnA, PFOS, and 6:2 FTSA were present in sediment, PW, and chironomids: both PW (respiration) and sediment (food) contribute to the contamination. Conversely, PFDoA, PFTrDA, PFTeDA, and FOSA present only in sediment and chironomids contributed to the contamination by the ingestion of sediment particles.

4.2 PFASs in benthic invertebrates

Chironomids bioaccumulated four PFCAs, one PFSA (PFOS), and two PFASs precursors. Among these, 6:2 FTSA was found to bioaccumulate poorly in fish and rats (DuPont, 2008). Reported concentrations were generally higher for benthic invertebrates than chironomids, especially for PFOS; ranges of PFOS in Capitellidae and Nereidae were 0.82 -12.6 $ng.g^{-1}$ww and 0.26 - 0.67 $ng.g^{-1}$ww (Loi et al., 2011). Mean PFAS concentrations reported for lugworm were 0.41 $ng.g^{-1}$ww (Nakata et al., 2006) and 280 ± 33 $ng.g^{-1}$ww for *Diporeia hoyi* (Martin et al., 2004). The latter study also reported that concentrations of PFUnA, PFDoA, PFTrDA, and PFTeDA were higher in *Diporeia hoyi* than in chironomids (Martin et al., 2004). In Nereidae and Capillidae worms, concentrations of PFUnA were lower than in chironomids (Loi et al., 2011). Compared to chironomids from the present study, lower concentrations were also reported for PFDoA in Capillidae (Loi et al., 2011), for PFOS in clams (Nakata et al.,

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2006; Nania et al., 2009), for FOSA in clams and lugworms (Nakata et al., 2006), and for PFTeDA in Nereidae worms (Loi et al., 2011). However, comparisons between freshwater and marine organisms should be interpreted with caution because salt may affect the chemical activity (Jeon et al., 2010a; Jeon et al., 2010b).

4.3 Accumulation kinetics

The biota-to-sediment accumulation factors (BSAFs; Eq. 2) indicated values for FOSA > PFTrDA > PFOS > PFUnA > 6:2 FTSA > PFTeDA > PFDoA, and no trend was observed between BSAF and PFAS chain length. In contrast, Higgins et al. (2007) observed that BSAF values tended to decrease with increasing chain length and Lasier et al. (2011) reported that BSAF values increased with increasing chain length. BSAF values in the present study were lower than BSAFs reported by Higgins et al. (2007) and Lasier et al. (2011) (e.g., PFUnA $BSAF_{ww}$ chironomid = 0.020; $BSAF_{ww}$ = 0.44 ± 0.11; $BSAF_{ww}$ = 0.29, CV = 40, respectively). This might be explained by the fact to the food added during the present experiment, in contrast to the absence of fedding in previous studies (Higgins et al., 2007 and Lasier et al., 2011). Adding food may decrease the exposure and dilute the contamination because without this addition the chironomids would have fed only on sediment organic matter. Our experiment was conducted in this manner because preliminary trials showed that survival rates were too low when no food was added to the aquaria.

In the Higgins et al. (2007) study, *Lumbriculus variegatus* reached steady state for PFOA and PFDS but not for PFNA, PFOS, PFDA, PFUnA, and PFDoA. In this experiment *C. riparius* reached a steady state both for PFCAs > C_{11} (PFUnA, PFDoA, PFTrDA) and PFOS and FOSA. However, in Lasier et al. (2011), *L. variegatus* accumulated other compounds that were not accumulated by chironomids such as

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PFHpA, PFOA, PFNA, and PFDA, although they were measured in sediment. This could be explained by the difference in feeding habits between the two organisms (Nogaro et al., 2009). In addition, chironomid growth during the accumulation phase is not negligible, unlike for *L. variegatus*, which lost weight (because it went unfed), as growth dilutes contamination. PFAS accumulation in chironomids was as effective when exposure started at 2nd instar as when it started at 4th instar. PFASs concentrations were the same between the end of E1 and E2 exposures (Table 1, Fig. 3). At the latter stage, growth is more rapid than at previous stages (Péry et al., 2002). These results suggest that the sole use of the 4th instar in could be included in the design of future experimental studies with this organism.

For PFTrDA, PFUnA, PFOS, and FOSA, accumulation data obtained in the present study fit an exponential rise model well (Eq. 1), with R^2 values ranging from 0.99 (PFOS) to 0.97 (PFUnA). Equation (1) supports either a classical two-compartment partition model (Higgins et al., 2007; Landrum, 1989) or a more sophisticated adsorption-like accumulation model (Liu et al., 2011). Model parameter *a* is related to the exposure concentration ($C_{sed\text{-}oc}$) and uptake and depuration rates (k_u and k_e respectively).

Model parameter *b* is related to the elimination rate (k_e). As chironomids grow during the experiment, the elimination rate ($k_{e'}$) should be corrected according to Eq. (3) (Spacie and Hamelink, 1985),

$$k_e' = k_e + g \qquad \text{Eq. (3)}$$

The complete two-compartment model includes a λ term in order to account for the chemical concentration decline in sediment. This term is set to 1, as in eq. 1, when sediment concentrations do not vary significantly during the experiment (that is, if the

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357 flux from sediment to organisms is much lower than the overall compound mass in
358 sediment). This was the case in the current experiment (Table S5). In this case, equation
359 (1) can be solved easily, with *b* being the apparent elimination rate (k_e' in h^{-1}), and

360 $a = (k_u/k_e)*C_{sed\text{-}oc}$ eq. (4)

361 with k_u the uptake rate ($g_{oc}.g_{ww}.h^{-1}$) and $C_{sed\text{-}oc}$ the concentration in sediment normalized
362 to the organic carbon content ($ng.g_{oc}^{-1}$).
363 Applying this approach, yields (k_u, k_e, and $k_{e'}$) were obtained for PFTrDA, PFUnA,
364 PFOS, and FOSA) (Table 3). These data are only informative because no depuration
365 data were available.
366 The adsorption-like model (Liu et al., 2011) was developed initially for an uptake
367 experiment from water. We assumed that it could also apply to accumulation from
368 sediment and food as in our study. Nevertheless, it cannot be solved without depuration
369 data because three variables are included in the terms *a* and *b* instead of two as in the
370 two-compartment model:
371 $a = (n*k_u*C_{sed})/(k_u*C_{sed} + k_e)$ Eq. (5)
372 $b = k_u*C_{sed} + k_e$ Eq. (6)
373 where *n* is the number of adsorption sites.

374 4.4 Contamination routes

375 All compounds accumulated by the chironomids were detected in sediment. PFUnA,
376 PFOS, and 6:2 FTSA were also quantified in both pore and overlying water and
377 PFTrDA was detected in overlying water. In addition, some compounds, such as
378 PFUnA, PFOS, and FOSA, were found in food added (Tetramin®) to chironomids,
379 which did not affect accumulation in chironomids (i.e., the balance between

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accumulation and depuration) because these compounds were not detected in control chironomids (PFAS concentrations <LDs).

These results therefore suggest that there are two contamination routes for the PFASs, which differ according to the compounds. The contamination routes for substances found in both pore water and sediment (particles) were via food and tegument. For other compounds, found only in sediment, the contamination route was only trophic.

These observations are supported by the growth data: larvae grew more in test sediments than in the control setting, although they received the same quantity of Tetramin®. These results indicate that larvae fed on organic carbon from the sediment and thus may have absorbed the PFAS associated with particles. On the other hand, the chironomid $\delta^{13}C$ values were almost identical in test and control organisms, suggesting that the proportion of C obtained by the larvae from the sediment was limited. This is consistent with the findings reported by Goedkoop et al. (2006), which showed that about 90% of the carbon intake was from added food. However, both $\delta^{13}C$ and $\delta^{15}N$ values for Tetramin® were mean values because Tetramin® is a mixture of animal (fish, crustaceans, mollusks) and plant (cereals) ingredients, each having its own isotopic composition. Chironomids may feed selectively on certain components of the added food (Goedkoop et al., 2006). Therefore, the observed chironomid $\delta^{13}C$ signature may be influenced by sediment organic carbon.

5. Conclusion

Results from this study indicate that *C. riparius* accumulated four long-chain perfluoroalkyl carboxylates (PFUnA, PFDoA, PFTrDA, and PFTeDA), a perfluoroalkyl sulfonate (PFOS), a precursor (FOSA), and a fluorotelomer (6:2 FTSA) from sediment.

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No short-chain PFAS, detected mostly in water, were accumulated by chironomids. No trends were observed for BSAFs, in contrast to studies by Higgins et al. (2007) and Lasier et al. (2011). The contamination pathways appeared to be trophic (sediment particles) and tegumentary (pore water) for PFUnA, PFOS, and 6:2 FTSA and only via the trophic pathway for the other compounds. Growth data support the trophic contamination route but isotopic data are difficult to interpret because of food added during the experiments. PFUnA, PFTrDA, and PFOS data fit an exponential rise model; apparent uptake and elimination constants can thus be proposed on the basis of the classical partition model (Landrum, 1989; Spacie and Hamelink, 1985). Elimination experiments should be conducted to determine the elimination kinetics experimentally in this species.

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Table 1: PFAS concentrations in chironomids at the end of E1 and E2 exposures ($ng.g^{-1}$ ww). E1 with standard deviation (n = 3) and E2 with analytical error calculation relative to spiked samples (Table S3). PFTeDA "trace" means that concentrations were between LD and LQ.

Table 2: $BSAF_{ww}$ ($g_{oc}.g_{ww}^{-1}$) for chironomids and for *Lumbriculus variegatus* (Higgins et al., 2007; Lasier et al., 2011); n.d, not detected, *PFTeDA, as guidelines only (because LD < PFTeDA concentrations in chironomids < LQ).

Table 3: Model parameters with (k_e') and without growth correction (k_u, k_e)

Figure 1: Study site of the Rhône River, France. BER = Beurre Island, Plant = PFAS production plant. The river runs from north to south.

Figure 2: Kinetics of PFASs accumulation in fourth instar larvae of *C. riparius*, in the E2 experiment. Left, PFSAs; right, PFCAs (PFTeDA concentrations were between LD and LQ). Error bars represent the analytical error estimated from the relative standard deviation of triplicate recovery rate tests performed on spiked samples (Table S3).

Figure 3: PFCA accumulation in E1 (left, LD < PFTeDA concentrations < LQ) and E2 (right) experiments. Error bars represent the analytical error estimated from the relative standard deviation of triplicate recovery rate tests performed on spiked samples (Table S3); on the right graph they represent the standard deviation between three replicates (n = 3).

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596 Figure 4: Partitioning between different compartments: green represents PFASs in food
597 or from a food source, and brown, in the same manner, for sediment. For chironomids,
598 the blue frames represent both sediment (brown) and pore water (blue) sources for
599 PFASs.

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Table 1 : PFAS concentrations in chironomids at the end of E1 and E2 exposures

Compounds	PFASs in chironomids at the end of E1 (ng.g^{-1} ww)	PFASs in chironomids at the end of E2 (ng.g^{-1} ww)
PFUnA	0.79 ± 0.10	0.80 ± 0.08
PFDoA	0.11 ± 0.01	0.14 ± 0.01
PFTrDA	1.71 ± 0.42	2.42 ± 0.17
PFTeDA trace	0.05 ± 0.001	0.08 ± 0.02
PFOS	0.16 ± 0.02	0.13 ± 0.02
FOSA	0.05 ± 0.01	0.05 ± 0.02
6:2 FTSA	0.07 ± 0.03	0.08 ± 0.005

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Table 2: $BSAF_{ww}$ ($g_{oc}.g_{ww}^{-1}$)

Compounds	Fluorinated carbon number	$BSAF_{ww}$ chironomid	$BSAF_{ww}$ Higgins et al., 2007	$BSAF_{ww}$ Lasier et al., 2011
PFUnA	10	0.020	0.44 ± 0.11	0.29, CV= 40
PFDoA	11	0.004	0.45 ± 0.08	0.34, CV=40
PFTrDA	12	0.042	n.d	0.62, CV=44
PFTeDA*	13	0.004	n.d	0.63, CV=47
PFOS	8	0.023	0.83 ± 0.20	0.49, CV=50
6:2 FTSA	6	0.018	n.d	n.d
FOSA	8	0.098	n.d	n.d

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Table 3: Model parameters

Compounds	a	ku	ke' (b)	ke
PFUnA	0.83 ± 0.03	0,0009	0.04 ± 0.01	0,03
PFTrDA	2.56 ± 0.11	0,0015	0.03 ± 0.01	0,02
PFOS	0.14 ± 0.008	0,0009	0.04 ± 0.01	0,03
FOSA	0.06 ± 0.01	0,0022	0.02 ± 0.01	0,01

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Figure 1 : Study site

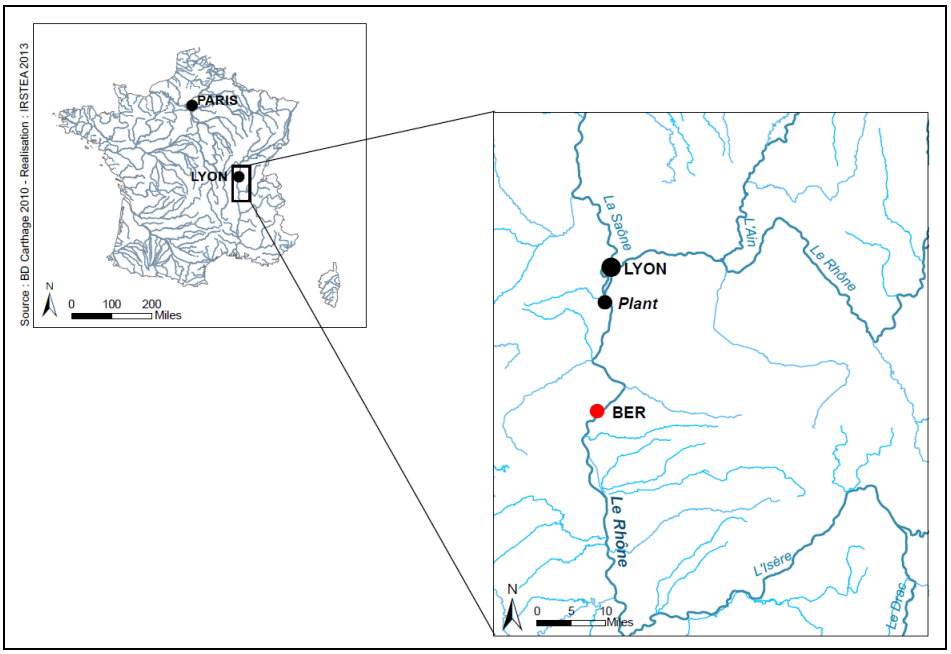

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Figure 2: Kinetics of PFAS accumulation in fourth instar larvae of *Chironomus riparius.*

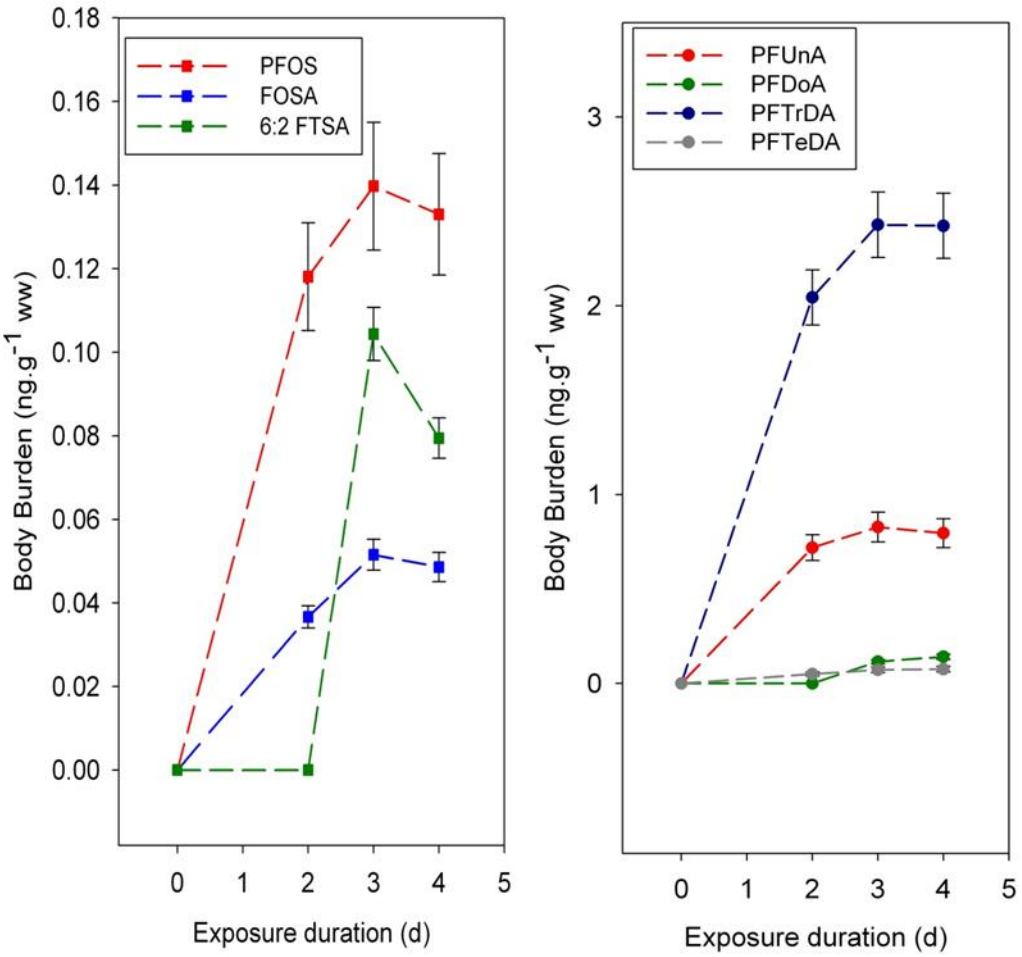

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Figure 3: PFCA accumulation in E1 (left) and E2 (right) experiments.

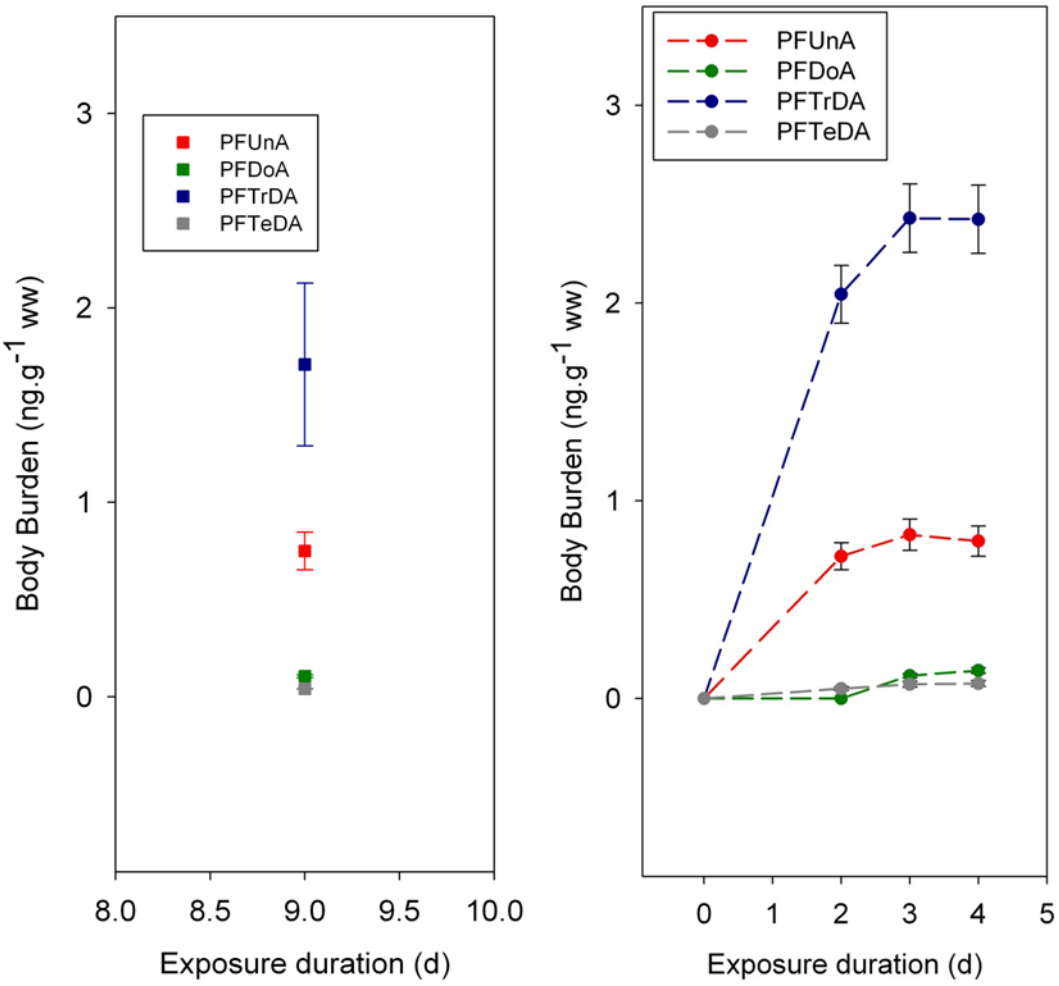

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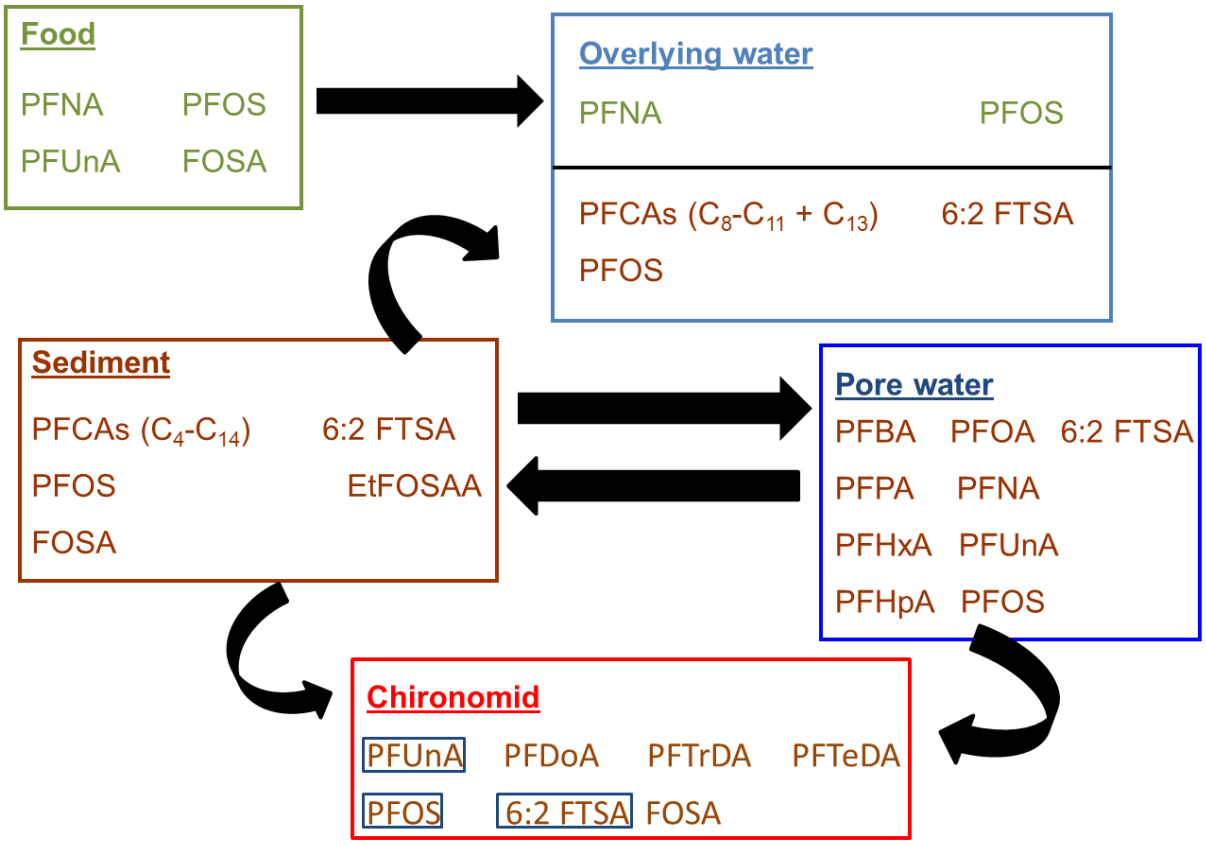
Figure 4: Partition between different compartments



Supplementary Material

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