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Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod:

***Gammarus* spp. (Crustacea)**

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1 **Highlights**

- 2 • Gammarids were exposed to field sediments from downstream of a fluoropolymer
3 plant.
- 4 • The steady state was not achieved after 21 days.
- 5 • Elimination rates were dependent on the perfluorinated carbon chain length.
- 6 • Clearance was high - up to 8-9 perfluorinated carbons.
- 7 • Kinetic BSAFs exceeded 1 for compounds having more than 8 perfluorinated
8 carbons.

9 **Abstract**

10 Gammarids were exposed to sediments from a deposition site located on the Rhône River
11 (France) downstream of a fluoropolymer manufacturing plant. Gammarids accumulated to
12 various extents four long-chain perfluoroalkyl carboxylic acids (PFCAs) from C₉ to C₁₃, one
13 sulfonate, perfluorooctane sulfonate (PFOS) and three of its precursors (the perfluorooctane
14 sulfonamide (FOSA), the N-methyl perfluorooctane sulfonamidoacetic acid (MeFOSAA), the
15 N-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA) and the 6:2 fluorotelomer
16 sulfonic acid (6:2 FTSA). Whatever the compound, the steady state was not achieved after a
17 3-week exposure; elimination was almost complete after a 3-week depuration period for
18 perfluorononanoic acid (PFNA), PFOS, the three precursors and the 6:2FTSA. However, this
19 was not the case for long-chain PFCAs, whose elimination rates decreased with increasing
20 chain length. PFAS accumulation in gammarids occurred via the trophic and respiratory
21 pathways, in proportions varying with the carbon chain length and the terminal moiety.

22 **Keywords**

23 perfluorinated compounds; sediment; *Gammarus* spp.; exposure route; uptake rate; clearance;

24 BSAF

25 **1. Introduction**

26 Poly- and perfluorinated alkyl compounds (PFASs) are present in all media (water, sediment,
27 biota, humans) and are subject to large-scale distribution as far as the polar regions (Giesy and
28 Kannan, 2001; Houde et al., 2006; Houde et al., 2011). Many studies have shown PFASs'
29 bioaccumulation in various species, including top-predators, and a few of them point to
30 sediment as a PFAS source (Martin et al., 2004; Armitage et al., 2006; Loi et al., 2011). In
31 2009, perfluorooctane sulfonate (PFOS) and related compounds were registered under Annex
32 B of the Stockholm Convention on Persistent Organic Pollutants
33 (<http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx>).

34 Although they are located at the basis of many food webs, little is known about PFAS transfer
35 from sediment to benthic organisms. The oligochaete *Lumbriculus variegatus* accumulated
36 seven perfluoroalkyl carboxylic acids (PFCAs; carbon chain length ranging from C₇ to C₁₄)
37 and three perfluoroalkane sulfonates (perfluorobutane sulfonic acid - PFBS, perfluorooctane
38 sulfonic acid - PFOS and perfluorodecane sulfonic acid - PFDS) from sediment in two
39 laboratory experiments (Higgins et al., 2007; Lasier et al., 2011). However, the PFAS
40 accumulation pathways and the factors influencing their bioaccumulation were not identified.
41 Two studies of midge (*Chironomus riparius*) larvae exposed to field sediment showed rapid
42 accumulation and elimination of two PFCAs (C₁₁, perfluoroundecanoic acid [PFUnDA] and
43 C₁₃ acid perfluorotridecanoic [PFTrDA]), PFOS and its perfluorooctane sulfonamide
44 precursor (FOSA) (Bertin et al., 2014; Bertin et al., submitted). Like oligochaetes, chironomid
45 larvae are in direct contact with sediment, and accumulation occurs through food ingestion

46 and diffusion from pore water (PW) through the tegument. Biota to sediment accumulation
47 factors (BSAFs) for chironomids were lower than BSAFs reported previously for
48 *Lumbriculus variegatus* (Higgins et al., 2007; Lasier et al., 2011). This difference could be
49 related to the respective experimental designs (uncontaminated food added to chironomids
50 experiments), to physiology, or to differing life traits, in particular feeding habits (Bertin et
51 al., submitted). We found no study addressing these issues for PFASs.

52 The *Gammarus* sp. is a freshwater invertebrate widely used in ecotoxicology. It is a
53 widespread epi-benthic species, present throughout the Northern hemisphere. Furthermore,
54 this species is an important food source for fish, amphibians and birds (MacNeil et al., 1997).
55 It feeds mainly by shredding the leaf litter (Tachet et al., 2010). This behaviour plays an
56 important role in the nutrient cycle and contributes to redistributing organic matter in the
57 riverine ecosystem. In addition, gammarids graze organic biofilm at the litter surface, and
58 ingest fine organic matter particulates from sediment deposits. They can also adopt a predator
59 position (MacNeil et al., 1997). Gammarids are also known for their capacity to accumulate
60 various organic and inorganic contaminants (Amiard et al., 1987; Ashauer et al., 2010; Lebrun
61 et al., 2011; Tlili et al., 2012).

62 The aim of the present study was to determine the PFAS accumulation pathways and kinetics
63 in a benthic species, *Gammarus* sp. in realistic conditions, because its biology and life traits
64 differ from those previously studied. The objectives were (i) to identify the contamination
65 routes in the accumulation of PFASs by gammarids, (ii) to describe PFAS accumulation and
66 elimination kinetics and (iii) to compare the kinetic constants and BSAFs obtained for
67 gammarids to those available for other benthic species and to discuss the differences
68 according to the respective life traits and physiology.

69 **2. Materials and methods**

70 2.1. Sediments

71 In October 2013, a sample of approximately 60 L of sediment was taken from the river bed of
72 a deposition site in the Rhone River (eastern central France, N45°28'17.0"E4°46'43.4"). This
73 site is located 40 km downstream of a fluoropolymer manufacturing plant, where
74 polyvinylidene fluoride (PVDF) and various fluorinated polymers have been synthesized
75 since the 1980s (Dauchy et al., 2012). The known releases from this site include mainly a
76 range of perfluoroalkyl acids, in particular the perfluorohexanoic (PFHxA) and
77 perfluorononanoic acids (PFNA), used as a carrier (solvent) of fluorinated polymers. Some
78 longer chain perfluoroalkyl acids, such as the perfluoroundecanoic and perfluorotridecanoic
79 acids (PFUnDA, PFTrDA respectively), are believed to be impurities of the technical PFNA.
80 Surface sediment was collected using a Van-Veen grab, sieved at 2 mm, pooled in a
81 polypropylene (PP) jar and stored at 4°C in the laboratory. Twelve aquaria (38 x 20 x 24.5 cm
82 in polystyrene) were prepared with 4 L of homogenized sediment and 15 L of groundwater
83 with 400-500 $\mu\text{S}\cdot\text{cm}^{-1}$ conductivity. Each aquarium was allowed to settle for 1 week before
84 introducing the gammarids. Twelve aquaria were prepared with only groundwater for the
85 controls.

86 2.2. Collection and maintenance of gammarids

87 Three weeks before the start of the experiment, adult gammarids, i.e. a mixture of *Gammarus*
88 *fossarum* and *Gammarus pulex*, (9.5 ± 0.8 mm) were collected with a hand net at a
89 remote/uncontaminated site (La Mouge River, N46°50'97.2"E4°75'63.9"). Gammarids were
90 kept in a bucket on ice, and brought to the laboratory. They were acclimatized for 3 weeks in
91 aquaria with continuously renewed groundwater under constant aeration; a 16:8 h light:dark
92 photoperiod was maintained and the temperature was kept at 12°C. Organisms were fed ad
93 libitum with alder leaves (*Alnus glutinosa*).

94 2.3. *Gammarus sp.* exposure

95 One experiment was conducted in two phases: the first step examined PFAS accumulation
96 kinetics while the second step considered PFAS elimination (Figure S1 in SI). Only male
97 gammarids were selected, in order to eliminate potential biases due to neonate release by
98 females. For the accumulation step, 450 individuals were added to nine sediment aquaria (50
99 per aquarium) and 150 individuals were added to three control aquaria (50 organisms per
100 aquarium). All the aquaria were made in polystyrene (PS) materials; flexible pipes were made
101 of low-density poly-ethylene (PE, Versilic ®). Gammarids were collected at 7 (T1), 15 (T2)
102 and 21 (T3) days, one aquarium being sacrificed at each time. For the elimination step, 450
103 gammarids were added to three sediment aquaria (150/aq.) and exposed for 21 days. After 3
104 weeks of sediment exposure, the organisms were transferred to a clean media (about 450
105 organisms in three flow-through aquaria filled with groundwater completely renewed four
106 times per day). About 50 gammarids were killed at 7 (T4), 15 (T5), and 21 (T6) days after the
107 transfer. The control gammarids were also killed after 42 days (Te, about 50 organisms). For
108 both experiments, overlying water (OW) was continuously renewed (four times a day) under
109 constant aeration. A 16:8 h light:dark photoperiod and a temperature of 12°C were maintained
110 during the experiments. The organisms were fed ad libitum with alder leaves (*Alnus*
111 *glutinosa*), previously conditioned for 3 days in groundwater; freshly pre-conditioned leaves
112 were added once a week. Each week the water quality parameters (pH, temperature,
113 concentration of dissolved oxygen, conductivity, NO₂⁻ and NH₄⁺) were monitored
114 (experimental design shown in Fig. S1).

115 2.4. Sample collection

116 Overlying water (OW) was sampled directly in a 1 L polyethylene (PE) bottle at T1, T2, T3,
117 T4, T5, T6 and in the controls. PW was obtained with Rhizon® systems (SDEC, Reignac-sur-

118 Indre, France) following the method developed in (Seeberg-Elverfeldt et al., 2005) and was
119 sampled at T1, T2 and T3. Sediment was also sampled at T1, T2 and T3. Leaves were
120 sampled at T0, T1, T2, T3, T4, T5, T6 and once in the controls. Before freezing, leaf samples
121 were rinsed with groundwater to remove sediment particles. Like the leaf samples, organisms
122 were sampled at T0, T1, T2, T3, T4, T5, T6 and in the controls. Then these biota samples
123 were cryopreserved in liquid nitrogen and stored at -21°C (Reiner et al., 2012).

124 2.5. PFAS extraction

125 For sediment, gammarid samples, leaf samples and the fish tissues used as reference matrix
126 (NIST SRM 1947, Reiner et al., 2012), PFASs were extracted using sonication following the
127 method described in Bertin et al. (2014). The OW and PW samples were processed using
128 Strata-X-AW cartridges as described by Labadie and Chevreuil (2011). Sediment or tissue
129 samples were extracted by sonication using methanol (MeOH), concentrated under a nitrogen
130 stream, purified on ENVI-Carb cartridges and eluted with MeOH. Eluates were concentrated
131 to 400 µl under a nitrogen stream and transferred into injection vials. Details are provided in
132 SI.

133 2.6. LC-MS/MS analysis and chemicals

134 PFASs (list of compounds and acronyms in SI, table S1) were analysed by LC-MS/MS using
135 an Agilent 1200 LC system (Agilent Technology, Massy, France) interfaced with an Agilent
136 6490 triple quadrupole mass spectrometer (details in SI, Table S2). The purchase of the
137 chemicals and their source are described in the supplementary data; most were purchased
138 from Wellington Laboratories (via BCP Instruments, Irigny, France) and Sigma-Aldrich (St
139 Quentin Fallavier, France).

140 2.7. Quality control and method performance

141 Analyte recovery was determined using spiked samples for each matrix (OW and PW,
142 sediment and gammarids). Native PFAS recovery ranged from 73% to 122% with a relative
143 standard deviation below 33% for fish and spiked sand except for perfluoropentanoic acid
144 (PFPeA) and PFBS. Native PFAS recovery for spiked waters (OW and PW) ranged from 65%
145 to 118% with a relative standard deviation below 12% except PFTTrDA,
146 perfluorotetradecanoic acid (PFTeDA), 6:2 fluorotelomer sulfonic acid (6:2 FTSA), N-ethyl
147 perfluorooctane sulfonamidoacetic acid (EtFOSAA), N-methyl perfluorooctane sulfonamide
148 (MeFOSA) and N-ethyl perfluorooctane sulfonamide (EtFOSA) (Table S3 in SI).

149 Replicate procedural blanks were analysed for each series of samples (details in Table S4 in
150 SI). For the water sample procedure, the predominant compound was PFPeA (mean level: 160
151 \pm 8 pg, $n=4$) and perfluorodecanoic acid (PFDA) (mean level: 34 \pm 5 pg, $n=4$). For tissue,
152 sediment and leaf samples, the prevailing analytes in blanks were PFPeA (123 pg) and PFDA
153 (31 pg). PFAS concentrations were therefore blank-corrected when applicable. For
154 compounds present in blanks, the limits of detection (LDs) were defined as three times the
155 standard deviation, and the limits of quantification (LQs) were set at 10 times the standard
156 deviation of the blank (Muir and Sverko, 2006; Munoz et al., 2015). For analytes not detected
157 in blanks, LDs and LQs were determined as the concentration with a signal-to-noise ratio of 3
158 and 9, respectively. This was calculated on matrices spiked at 0.3-1.8 ng g⁻¹ (sediment and
159 *Gammarus spp.*) and 0.9-17.3 ng L⁻¹ (Vittel® mineral water samples) (Table S6). The PFASs
160 measured in NIST SRM 1947 Lake Michigan Fish Tissue compared well with reference
161 values (SI Table S6).

162 2.8. Data analyses and modelling

163 Data were checked for normality using the Shapiro-Wilk test and were analysed using the
164 Kruskal-Wallis test (a non-parametric test) and the Mann-Whitney test using R language (R
165 Core Team, 2014a, b).

166 Assuming sediment was the main source of PFASs for the gammarids, accumulation data
167 were adjusted to a two-compartment model (Spacie and Hamelink, 1985; Landrum, 1989).
168 Equation (1) was used to estimate the uptake and elimination coefficients (k_u and k_e ,
169 respectively).

$$170 \quad \frac{dC_{org}}{dt} = k_u \times C_{sed} - k_e \times C_{org} \quad \text{Eq. (1)}$$

171 with C_{org} the PFAS concentration in organisms ($\text{ng}\cdot\text{g}^{-1}\text{ww}$), C_{sed} the PFAS concentration in
172 sediment ($\text{ng}\cdot\text{g}^{-1}\text{dw}$), k_u expressed in $\text{g}\cdot\text{g}^{-1}\text{ww}\cdot\text{d}^{-1}$ and k_e in d^{-1} . In the selected size range,
173 gammarids do not grow significantly within the experiment duration, so there is no need to
174 correct for growth.

175 Elimination data can also be adjusted directly to an exponential decrease model (Eq. 2).

$$176 \quad C_{org}(t) = C_0 \cdot e^{-k_e t} \quad \text{Eq. (2)}$$

177 where C_0 is the PFAS concentration at the start of the elimination phase ($\text{ng}\cdot\text{g}^{-1}\text{ww}$).
178 Conceptually, k_e values obtained from Eqs. 1 and 2 are identical: Eq. 2 corresponds to the
179 integrated form of Eq. 1 when C_{sed} is set to 0. Nevertheless k_e values obtained from the two
180 approaches can differ when fitted on independent data sets. Model calculations were
181 performed in R language using the `<<ode>>` function, and k_u and k_e were optimized
182 simultaneously with the least square method using the `<<optim>>` function implemented in
183 the `<<Desolve>>` package (R Core Team, 2014a). When k_u and k_e are processed in this way,
184 the whole data set including both uptake and elimination experimental data can be used,
185 avoiding differences in k_e values derived from separate adjustments.

186 The kinetic biota-to-sediment accumulation factor ($BSAF_{kinetic}$) is derived from the uptake and
187 elimination rate constants (Eq. 3):

$$188 \quad BSAF_{kinetic} = \frac{k_u}{k_e} \quad \text{Eq. (3)}$$

189 The elimination half-life ($T_{1/2}$) is obtained according to Eq. (4):

$$190 \quad T_{1/2} = \frac{\ln 2}{k_e} \quad \text{Eq. (4)}$$

191 3. Results

192 3.1. PFAS distribution in water, sediment and leaves

193 Water in control aquaria displayed low concentrations of PFHpA, PFOA, PFNA, PFDA,
194 PFUnDA, as well as perfluorohexane sulfonate (PFHxS), PFOS and 6:2FTSA (0.01 – 0.07 ng
195 L^{-1}) while PFBA, PFPA and PFHxA reached higher concentrations (1.35, 0.25 and 0.24 ng L^{-1} ,
196 respectively). The contamination profile in sediments (test aquaria, T1 to T3) was
197 dominated by PFUnDA ($1.19 \pm 0.15 \text{ ng g}^{-1}$ dry weight – dw) and PFTTrDA ($2.09 \pm 0.10 \text{ ng g}^{-1}$
198 dw); PFHxA, PFNA, PFDA, perfluorododecanoic acid (PFDoDA), PFOS and 6:2FTSA
199 ranged from 0.1 to 0.5 ng g^{-1} dw, while other compounds, such as PFOA, N-methyl
200 perfluorooctane sulfonamidoacetic acid (MeFOSAA) and FOSA, ranged from <LD (0.003–
201 0.013 ng g^{-1} dw) to 0.1 ng g^{-1} dw. In PW the dominant compounds were PFHxA ($97.95 \pm$
202 17.41 ng L^{-1}), PFNA ($7.10 \pm 1.98 \text{ ng L}^{-1}$) and PFUnDA ($6.05 \pm 0.97 \text{ ng L}^{-1}$); PFPA, PFOA,
203 PFBS and PFOS were within the range 1.0–5.0 ng L^{-1} , with other compounds undetected (e.g.
204 PFDA, PFTTrDA, PFBS, FOSA, 6:2FTSA) to $\leq 1.0 \text{ ng L}^{-1}$ (PFHpA, MeFOSAA). PFHxA (2.59
205 $\pm 0.23 \text{ ng L}^{-1}$) was still the most concentrated compound in OW; PFBA, PFPA, PFHpA,
206 PFOA, PFTTrDA, PFBS, PFHxS and PFOS concentrations were within the range 0.5–1.5 ng L^{-1} .
207 The concentrations of other compounds, including PFNA, PFUnDA and 6:2FTSA were <
208 0.5 ng L^{-1} . PFUnDA ($0.53 \pm 0.34 \text{ ng g}^{-1}$ dw), PFTTrDA ($0.40 \pm 0.23 \text{ ng g}^{-1}$ dw) and PFOS

209 (0.63 ± 0.38 ng g⁻¹ dw) displayed the highest concentrations in alder leaves; PFPA, PFOA,
210 PFNA, and EtFOSAA ranged from 0.05 to 0.5 ng g⁻¹ dw. PFHxA, PFDODA, PFHxS,
211 MeFOSAA and FOSA were measured at levels <0.05 ng g⁻¹ dw; other compounds such as
212 PFDA or 6:2FTSA remained undetected in this matrix. Details are shown in SI (Table S6).

213 3.2. Gammarid survival, PFAS accumulation and elimination

214 Organisms from the reference site (La Mouge) displayed a limited contamination by a range
215 of PFCAs (PFOA, PFNA, PFDA, PFDODA and PFTrDA, all around 0.1–0.3 ng g⁻¹ (ww),
216 and PFOS (0.93 ± 0.2 ng g⁻¹ww). After acclimatization for 3 weeks, all concentrations
217 remained similar, except for PFUnDA, which increased to 0.19 ng g⁻¹ (ww) and PFOS, which
218 fell to 0.09 ± 0.002 ng g⁻¹ww (Table S7 in SI). Gammarid survival was > 75% in control
219 aquaria, and > 80% in test aquaria except at T6 (54%, 66% and 6% respectively). Organisms
220 from this aquarium with a low survival rate were not considered further.

221 PFAS concentrations in OW remained steady during the course of the experiment (Figure S2-
222 A in SI), except PFOA (decrease at day 21), PFTrDA (<LD at day 7, 2.62 ng L⁻¹ at day 15,
223 and 0.14 ng L⁻¹ at day 21) and PFBS (increase at day 21). PFAS concentrations in PW were in
224 the 1-10 ng L⁻¹ range (PFPA, PFHpA, PFOA, PFNA, PFUnDA, PFHxS, PFOS) or below
225 (PFDA, PFBS), and steady (Figure S2-B), with two exceptions: PFHxA, which decreased
226 from 116 ng L⁻¹ at day 7 to 81 ng L⁻¹ at day 21 (Figure S2-B), and PFTrDA which remained
227 undetected. PFAS concentrations were also steady in the sediment (Figure S2-C).

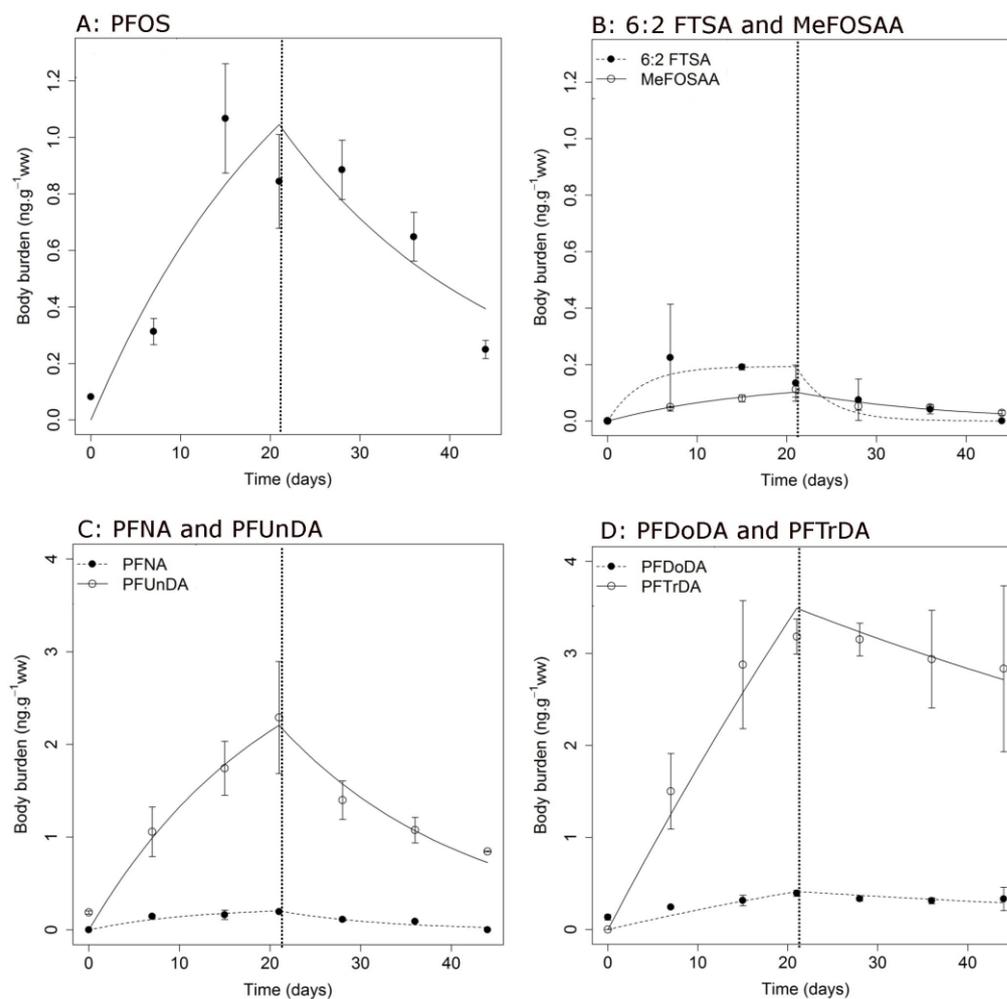
228 Nine of ten compounds (PFCAs: PFNA, PFUnDA, PFDODA, PFTrDA; PFOS and precursors:
229 PFOS, MeFOSAA, EtFOSAA, FOSA; 6:2FTSA) displayed significantly higher
230 concentrations in test gammarids at 21 days (T3) compared with control organisms (Table 1).
231 The most accumulated compounds were PFUnDA and PFTrDA, and PFOS to a lesser extent.

232 **Table 1:** Mean PFAS concentration in gammarids in control aquaria and at T3 (ng g⁻¹wet weight (ww)) (±
 233 standard deviation, *n*=3). (*) significant difference between test and control (*p*-value < 0.05).

Compounds	Concentration in gammarids in control aquaria (ng g ⁻¹ ww)	Concentration in gammarids (ng g ⁻¹ ww) at 21 days
PFOA	0.43 ± 0.14	0.36 ± 0.04
PFNA	<0.09 (LQ)	0.20 ± 0.01 (*)
PFUnDA	0.11 ± 0.02	2.29 ± 0.60 (*)
PFDoDA	0.12 ± 0.01	0.39 ± 0.03 (*)
PFTTrDA	<0.02 (LD)	3.18 ± 0.19 (*)
PFOS	0.17 ± 0.07	0.84 ± 0.17 (*)
6:2 FTSA	<0.01 (LD)	0.13 ± 0.06 (*)
MeFOSAA	<0.04 (LQ)	0.11 ± 0.03 (*)
EtFOSAA	< 0.02 (LD)	0.16 ± 0.05 (*)
FOSA	<0.02 (LQ)	0.14 ± 0.01 (*)

234

235 Concentrations of most PFASs were still increasing at T3 (21 days), so the steady state was
 236 not reached at 3 weeks of exposure (Figure 1).



237

238 **Figure 1** : Accumulation and elimination kinetics of selected PFAS; errors bars represent the standard deviation
239 of measurements ($n = 3$, except for the last depuration point T6) – Curves represent model outputs.

240 MeFOSAA, the fluorotelomer 6:2 FTSA and PFNA were rapidly eliminated after gammarids
241 transfer in uncontaminated water (Figure 1B and C, respectively), with half-lives ranging
242 from 7.5 days for 6:2 FTSA to 10.8 days for MeFOSAA. The elimination was slower for
243 FOSA, PFOS and PFUnDA (Figure 1A and C), with half-lives of 15.0, 19.4 and 14.5 days,
244 respectively). It was not significant at 21 days for PFDoDA and PFTrDA (Figure 1D; Mann-
245 Whitney test, p -value > 0.05).

246 The results from the accumulation and elimination experiments fitted the two-compartment
 247 model well (Eq. 1; Figures 1 and S3 and S4 in SI): the distance between the observed and
 248 simulated data ranged from 0.0001 to 0.039, and R^2 ranged from 0.78 to 0.97 except for
 249 FOSA ($R^2 = 0.54$) and PFDoDA ($R^2 = 0.29$) (Table 2). The uptake rates (k_u) ranged from
 250 0.053 to 0.242 $\text{g}\cdot\text{g}_{\text{ww}}\cdot\text{d}^{-1}$ and the elimination rates (k_e) from 0.042 to 0.276 d^{-1} (Table 2). Given
 251 that elimination was not significant for PFDoDA and PFTrDA, the k_e values provided by the
 252 model calculations for these compounds are indicative, as are the corresponding BSAFs.

253
 254 **Table 2:** Model outcomes. The values in brackets for PFDoDA and PFTrDA are tentative, because elimination
 255 was not significant for these compounds.

Compounds	PFCAs				PFOS, 6:2 FTSA and PFOS precursors				
	PFNA	PFUnDA	PFDoDA	PFTrDA	PFOS	6:2 FTSA	MeFOSAA	EtFOSAA	FOSA
Distance	0.001	0.013	0.004	0.039	0.023	0.002	0.0001	0.001	0.001
R^2	0.899	0.965	0.290	0.968	0.804	0.782	0.947	0.821	0.537
k_u ($\text{g}\cdot\text{g}_{\text{ww}}\cdot\text{d}^{-1}$)	0.094	0.141	0.078	0.089	0.202	0.242	0.129	0.053	0.218
k_e (d^{-1})	0.087	0.048	(0.015)	(0.011)	0.042	0.276	0.060	nd	0.066
BSAF	1.08	2.92	(5.05)	(8.13)	4.76	0.88	2.13	nd	3.32
N perfluorinated C	8	10	11	12	8	6	8	8	8

256
 257 BSAF_{kinetic} values tended to increase as the PFCA chain length increased. In addition, BSAF
 258 is also higher for PFOS than for PFNA, which has the same number of perfluorinated carbons
 259 (Table 2). All compounds with more than 8 perfluorinated carbons display BSAF_{kinetic} values
 260 above 1, with the exception of EtFOSAA. Note that EtFOSAA concentrations varied between
 261 LD and LQ during the elimination phase, so both k_e and BSAF_{kinetic} values could not be
 262 determined.

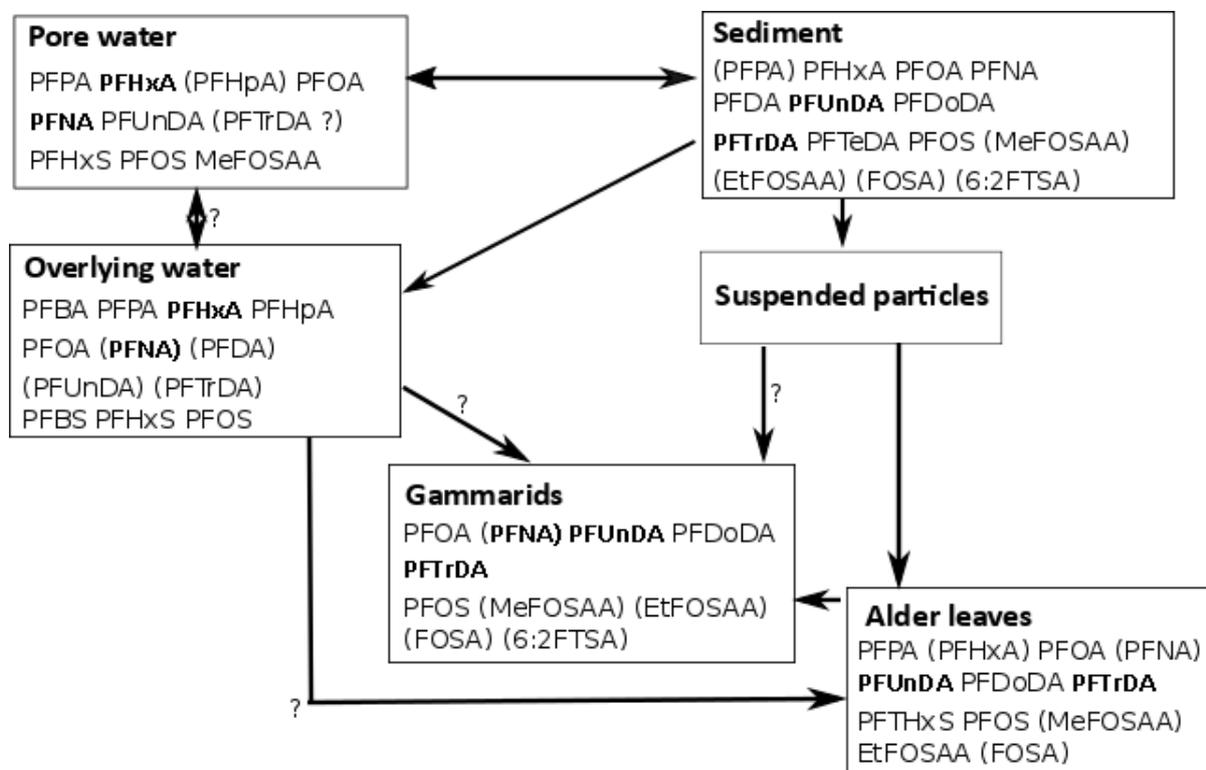
263 4. Discussion

264 4.1. PFAS distribution in water, sediment, food and *Gammarus* sp.

265 The comparison of PFAS concentrations in each compartment (OW, PW, sediment, leaves
266 and gammarids) in control and test aquaria at the beginning and end of the experiment makes
267 it possible to identify the transfers between the compartments and design a conceptual
268 diagram of PFAS transport (Figure 2). A transfer from one compartment to another is
269 assumed when a compound is present in both compartments in test aquaria, but undetected in
270 one of them in controls. PFBA, MeFOSAA and EtFOSAA are present at similar
271 concentrations in both control and test OWs throughout the experiment, while other PFAS
272 concentrations increase by several orders of magnitude, suggesting transfers from PW (e.g.
273 PFHxA, PFHpA, PFHxS) or sediment particles via PW (e.g. PFUnDA). Thus PFUnDA was
274 presumably transferred from sediment to leaves. PFUnDA is both associated to sediment
275 particles and dissolved in PW: therefore gammarids may have taken PFUnDA from both PW
276 and leaves or sediment. PFTrDA concentrations varied widely in OW (range <LD – 2.62 ng
277 L⁻¹) but remained undetected in PW. Since the water was not filtered prior to extraction and
278 analysis, the concentrations measured in OW and PW represented the total concentrations
279 (dissolved + sorbed to suspended particles). On the other hand, PFTrDA concentrations could
280 be underestimated in PW, as a consequence of sorption to Rhizon ® materials.
281 Notwithstanding these uncertainties concerning PFTrDA measurements in water
282 compartments, the higher load on sediment particles as well as its *K_{OC}* value (Munoz et al.,
283 2015) supports the hypothesis that PFTrDA uptake occurred much more through the ingestion
284 of contaminated particles than from water. This hypothesis is consistent with previous
285 conclusions about the PFCA benthic source signature for Lake Ontario *Diporeia* (an
286 amphipod species) (Martin et al., 2004).

287 During the exposure period, gammarid activity mobilized particles, which deposited on the
288 leaves' surface. Two PFAS transfer pathways to the leaves are therefore possible: (i) by
289 diffusion from water, or (ii) from sediment particles and water. These transfers could involve

290 the microbial biofilm on the leaves' surface. Indeed, the leaf conditioning before their
 291 addition to the aquaria led to the microorganism's colonization (Abelho, 2001; Aßmann et al.,
 292 2011) and biofilms are known for accumulating trace elements (Frag et al., 1998; van
 293 Hullebusch et al., 2003) as well as organic compounds (Widenfalk et al., 2008; Writer et al.,
 294 2011). Nevertheless, PFAS transfer to the leaves themselves was probably limited, because
 295 leaves were renewed every week. Like PFUnDA and PFNA, PFOS accumulation by
 296 gammarids could have occurred via water (OW) and leaves concurrently. In summary,
 297 gammarids accumulate PFASs by two potential contamination routes: the trophic route, i.e.
 298 ingestion of leaves and sediment particles, and the respiratory route. The former was
 299 predominant for long-chain compounds such as PFTrDA and PFDoDA, while the latter was
 300 also involved for PFOS, PFNA and PFUnDA (Figure 2). As a consequence, the k_u values are
 301 overestimated for these three compounds, because the derivation process assumed sediment
 302 was the main source. The k_e values are not affected.



303

304 **Figure 2: Conceptual diagram of PFAS distribution between compartments**

305 4.2. PFAS bioaccumulation

306 The concentrations observed in gammarids in the present experiment are within the same
307 range as in the few studies that reported PFAS concentrations in crustaceans from the field
308 (Kannan et al., 2005; Haukås et al., 2007). FOSA concentrations ($<2 \text{ ng g}^{-1}\text{ww}$) were lower in
309 freshwater amphipods from a Great Lake food web (Kannan et al., 2005) than those found in
310 gammarids in our study. PFOS and PFOA concentrations were higher in marine gammarids
311 (*Gammarus wilkitzkii*) from the Barents Sea than in the present study, whereas the opposite
312 was found for 6:2 FTSA ($0.48 \pm 0.24 \text{ ng g}^{-1}\text{ww}$)(Haukås et al., 2007). Long chain compounds
313 were not often measured in monitoring studies, except in two shrimp species (*Peneaus*
314 *monodon* and *Metapenaeus ensis*) (Loi et al., 2011). (Martin et al., 2004) also analysed a
315 range of PFCAs in the Ontario food-web, observing decreasing concentrations with increasing
316 chain length, but they did not report the respective concentrations in sediments. They stated
317 that the contamination profile observed in *Diporeia* and sculpin (a benthic-feeding fish
318 species) reflected a benthic source signature. Such comparisons nevertheless remain difficult,
319 because (i) different species were considered and (ii) the exposure concentrations were
320 different and did not necessarily involve water and sediment in all cases.

321 According to accumulation kinetics, the steady state was not reached at 3 weeks of exposure.
322 For the pesticides chlorpyrifos and pentachlorophenol (Ashauer et al., 2006), the steady state
323 was achieved between 48 and 72h for *Gammarus pulex*: for chlorpyrifos resulted from a
324 decline of the exposure concentration in the test vessel, while for carvedilol and fluoxetine the
325 steady state was not reached in 48 hours (Meredith-Williams et al., 2012). Ashauer et al.,
326 (2006) explained the differences between pentachlorophenol and chlorpyrifos by
327 hydrophobicity. Furthermore, Meredith-Williams et al. (2012) assigned the variations in
328 uptake rates of several pharmaceuticals (5-fluorouracil, carbamazepine, diazepam,
329 moclobemide, carvedilol and fluoxetine) at an estimated steady state to the respective

330 ionization state. Nevertheless, both studies were conducted in water, with neither sediment
331 nor food present, which presumably lead to different kinetics compared to the present study.
332 These observations and the need for longer equilibration times for PFASs suggest that
333 transport mechanisms across membranes are more complex than simple diffusion. The failure
334 to achieve steady state within 3 weeks could also be due to the formation of PFCAs (or
335 PFSA) in sediment or gammarids from unanalysed precursors. We nevertheless discarded
336 this hypothesis, because (i) PFCA and PFSA concentrations remained steady in sediment
337 during the experiment, and (ii) the corresponding kinetics in sediments are deemed to be very
338 low (Liu and Mejia Avendaño, 2013).

339 In spite of the failure to achieve the steady state, the determination of the uptake and
340 elimination constant rates allowed determining kinetic BSAFs. For PFCAs, these BSAFs are
341 positively correlated with the number of perfluorinated carbons (Pearson correlation
342 coefficient, 0.958; p -value 0.042; when all compounds including PFOS, its precursors and 6:2
343 FTSA are accounted for, the Pearson coefficient is still 0.797, and the p -value = 0.01).
344 Moreover, the BSAF for PFOS was higher than for PFNA, which has the same number of
345 fluorinated carbons, as already shown in previous studies (Martin et al., 2003a, b; Lasier et al.,
346 2011).

347 4.3. Comparison between gammarids and chironomids

348 Despite the inherent spatial and temporal variability of the PFAS sediment concentrations in
349 the field, the sediment molecular profile contamination in this study and in studies with the
350 midge *Chironomus riparius* using a similar design (Bertin et al., 2014; Bertin et al.,
351 submitted) are comparable (Table S8). Uptake and elimination rates were estimated with the
352 approach in both studies. The respective kinetics presented several differences between these
353 two species: chironomids did not accumulate PFNA, unlike gammarids. Despite a shorter

354 exposure (4 days) according to its life cycle, chironomids' k_u for PFUnDA (0.70 g g_{ww}.d⁻¹),
355 PFTrDA (0.93 g g_{ww}.d⁻¹), PFOS (1.27 g g_{ww}.d⁻¹) and FOSA (2.02 g g_{ww}.d⁻¹) were higher than for
356 gammarids (Table 2). PFAS elimination by chironomids was faster than for gammarids and
357 complete after 42 h. In addition, PFCAs k_e values for chironomids were negatively correlated
358 with the carbon chain-length for compounds with more than nine perfluorinated carbons in
359 gammarids. BSAFs were also higher for all PFASs for gammarids than for chironomids,
360 consistent with the gammarids' slower elimination. These differences between these two
361 invertebrates could be explained by different exposure routes related to the organisms'
362 lifestyles and physiology. The chironomid is a benthic invertebrate feeding on organic matter
363 associated with sediment particles, whereas the gammarids are shredder epi-benthic
364 organisms, living in the water column with occasional contact with sediment. For
365 chironomids, the trophic pathways were identified as the main PFAS exposure route (Bertin et
366 al., 2014; Bertin et al., in prep.) while for gammarids the trophic route was predominant for
367 longer chain (>11) PFCAs, and both respiratory and trophic routes were involved for C8-C10
368 compounds.

369 **5. Conclusion**

370 The study results reported here show an accumulation by gammarids of four long-chain
371 PFCAs (PFNA, PFUnDA, PFDoDA and PFTrDA), one PFSA (PFOS), three precursors
372 (MeFOSAA, EtFOSAA, FOSA) and one fluorotelomer (6:2 FTSA) from the sediment. The
373 steady state was not reached at 3 weeks of exposure. In addition, the depuration of PFOS,
374 precursors and 6:2 FTSA was almost complete in 21 days but not for the long-chain PFCAs.
375 The PFCA depuration time increases as the chain length increases. Moreover, in agreement
376 with previous studies (Martin et al., 2003a, b; Lasier et al., 2011), the BSAFs of PFCA values
377 also seem to increase as the chain length increases. The PFAS pathway follows digestive and
378 respiratory routes. In comparison with PFAS bioaccumulation by chironomids, where the

379 PFAS accumulation and elimination were fast and complete after 42 h, and the behavioural
380 differences existing between these two organisms, it is clear that lifestyle has an impact on
381 PFAS exposure routes and subsequent bioaccumulation.

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485 **Figure captions**

486 Figure 2 : Accumulation and elimination kinetics of selected PFAS; errors bars represent the standard deviation
487 of measurements ($n = 3$, except for the last depuration point T6) – Curves represent model outputs.

488 Figure 2: Conceptual diagram of PFAS distribution between compartments

489 **Table captions**

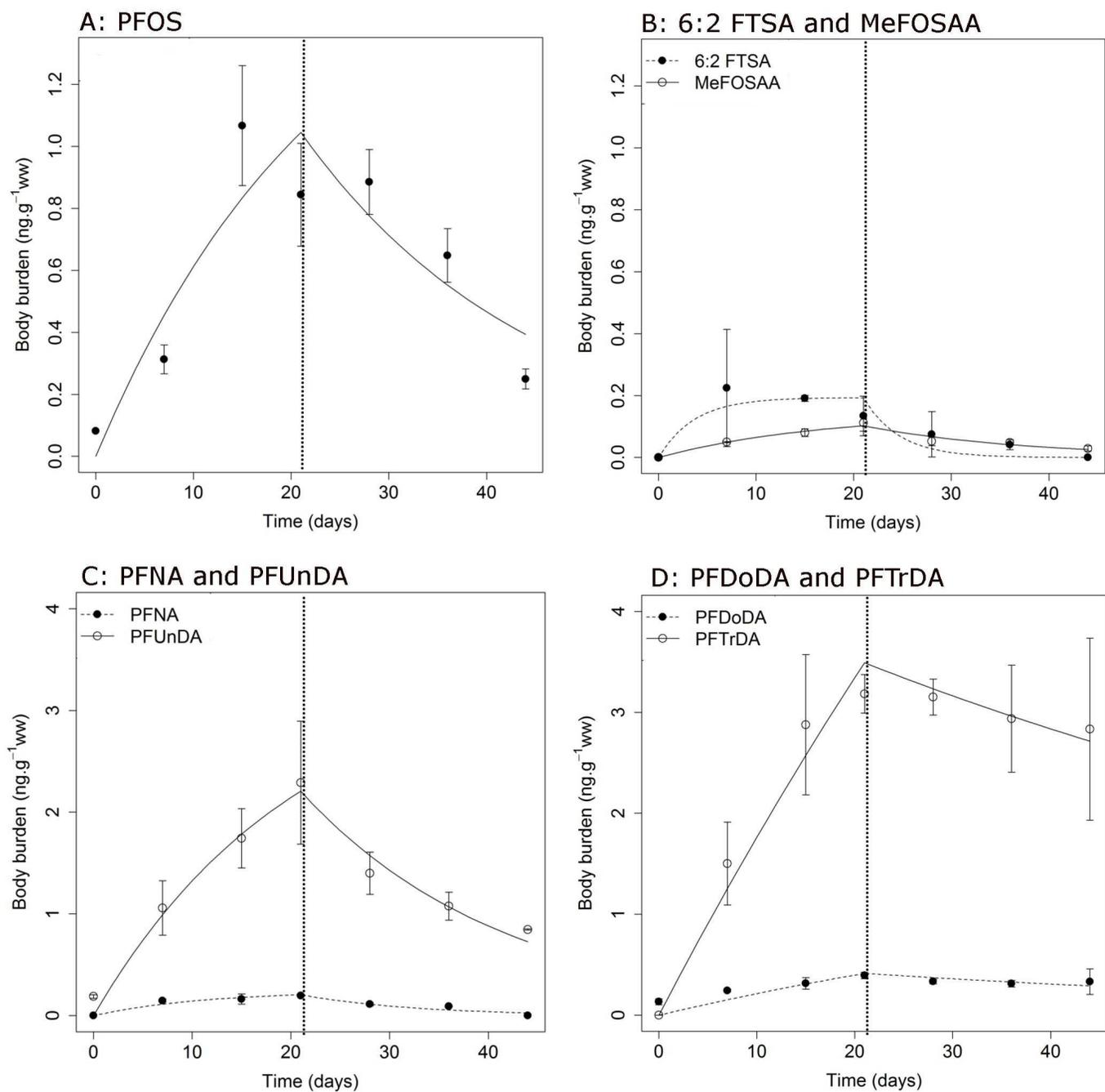
490 Table 3: Mean PFAS concentration in gammarids in control aquaria and at T3 ($\text{ng}\cdot\text{g}^{-1}$ wet weight (ww)) (\pm
491 standard deviation, $n=3$). (*) significant difference between test and control (p -value < 0.05).

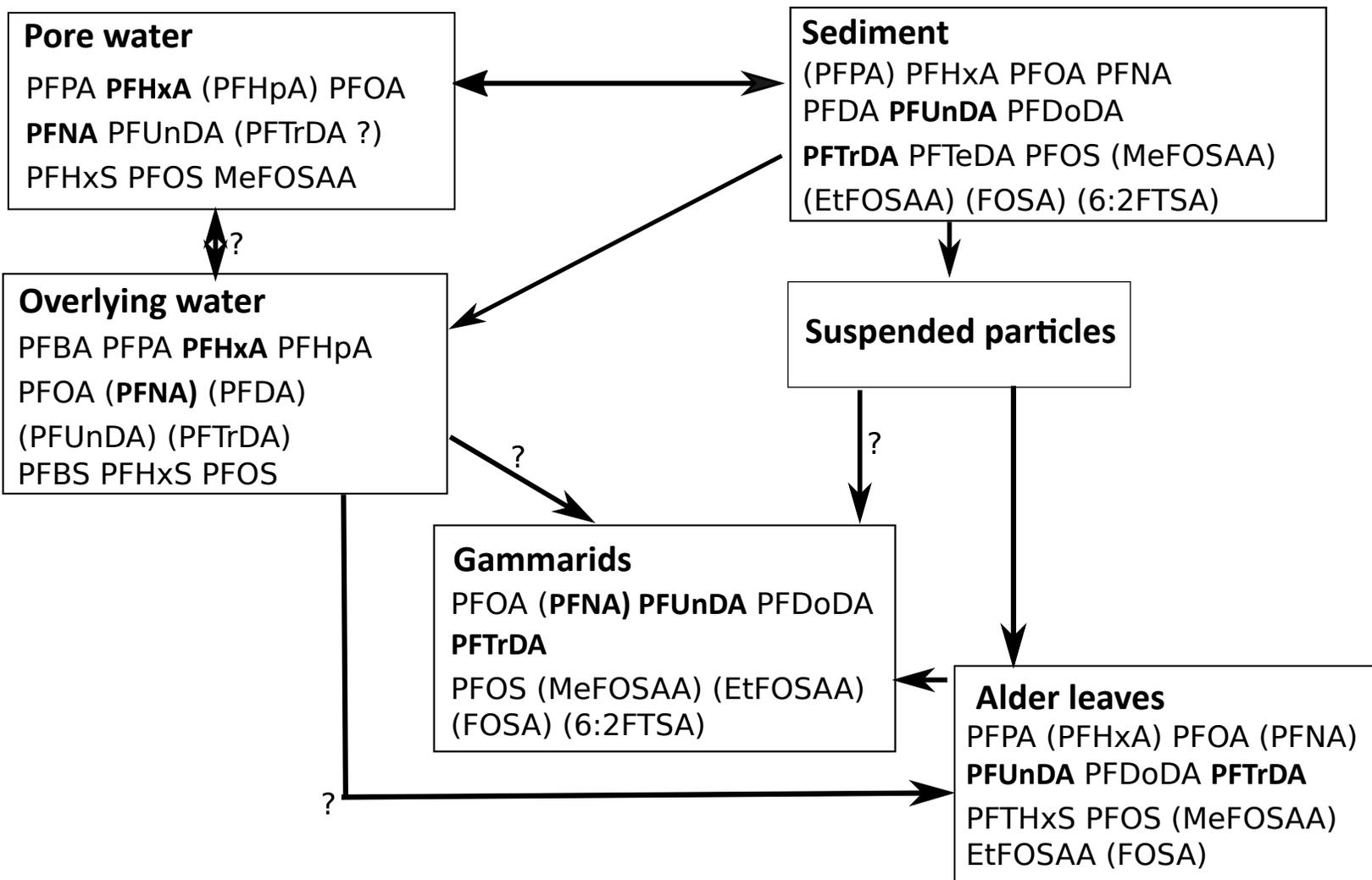
492 Table 4: Model outcomes. The values in brackets for PFDoDA and PFTrDA are tentative, because elimination
493 was not significant for these compounds.

Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: Gammarus spp. (Crustacea)**Figure captions**

Figure 1 : Accumulation and elimination kinetics of selected PFAS; errors bars represent the standard deviation of measurements ($n = 3$, except for the last depuration point T6) – Curves represent model outputs.

Figure 2: Conceptual diagram of PFAS distribution between compartments





Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: *Gammarus* spp. (Crustacea)

Delphine Bertin, Pierre Labadie, Benoît J. D. Ferrari, Alexandre Sapin, Jeanne Garric, Olivier Geffard, H  l  ne Budzinski, Marc Babut

(Table 1)

Compounds	Concentration in gammarids in control aquaria (ng.g ⁻¹ ww)	Concentration in gammarids (ng.g ⁻¹ ww) at 21 days
PFOA	0.43 ± 0.14	0.36 ± 0.04
PFNA	<0.09 (LQ)	0.20 ± 0.01 (*)
PFUnDA	0.11 ± 0.02	2.29 ± 0.60 (*)
PFDoDA	0.12 ± 0.01	0.39 ± 0.03 (*)
PFTTrDA	<0.02 (LD)	3.18 ± 0.19 (*)
PFOS	0.17 ± 0.07	0.84 ± 0.17 (*)
6:2 FTSA	<0.01 (LD)	0.13 ± 0.06 (*)
MeFOSAA	<0.04 (LQ)	0.11 ± 0.03 (*)
EtFOSAA	< 0.02 (LD)	0.16 ± 0.05 (*)
FOSA	<0.02 (LQ)	0.14 ± 0.01 (*)

Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: *Gammarus* spp. (Crustacea)

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(Table 2)

Compounds	PFCAs				PFOS, 6:2 FTSA and PFOS precursors				
	PFNA	PFUnDA	PFDoDA	PFTrDA	PFOS	6:2 FTSA	MeFOSAA	EtFOSAA	FOSA
Distance	0.001	0.013	0.004	0.039	0.023	0.002	0.0001	0.001	0.001
R ²	0.899	0.965	0.290	0.968	0.804	0.782	0.947	0.821	0.537
k_u (g.g _{ww} .d ⁻¹)	0.094	0.141	0.078	0.089	0.202	0.242	0.129	0.053	0.218
k_e (d ⁻¹)	0.087	0.048	(0.015)	(0.011)	0.042	0.276	0.060	nd	0.066
BSAF	1.08	2.92	(5.05)	(8.13)	4.76	0.88	2.13	nd	3.32
N perfluorinated C	8	10	11	12	8	6	8	8	8