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### ► To cite this version:

François Le Cor, Sylvain Slaby, Vincent Dufour, Alain Iuretig, Cyril Feidt, et al.. Occurrence of pesticides and their transformation products in headwater streams: Contamination status and effect of ponds on contaminant concentrations. *Science of the Total Environment*, 2021, 788, pp.147715. 10.1016/j.scitotenv.2021.147715 . anses-03464225

**HAL Id: anses-03464225**

**<https://anses.hal.science/anses-03464225>**

Submitted on 24 May 2023

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## Title page

Occurrence of pesticides and their transformation products in headwater streams:  
Contamination status and **effect** of ponds on contaminant concentrations

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## 1 **1. Introduction**

2 Diffuse and acute pollution of water by pesticides brings together global health  
3 (Carvalho, 2017; Kim et al., 2017), ecological (Kaur and Garg, 2014; Osborne, 2012;  
4 van Lexmond et al., 2015), and economic concerns (Burgess and Pimentel, 2014;  
5 Pimentel, 2005). With the expansion of their use over the last 60 years,  
6 environmental issues have emerged on a large scale (Carson, 1962; Özkara et al.,  
7 2016). In Europe, the total quantity of pesticides used annually increased from  
8 440,000 tons to more than 475,000 tons between 2000 and 2017 according to the  
9 Food and Agriculture Organization of the United Nations (FAO, 2020), questioning  
10 the overall state of ecosystems (Sharma et al., 2019). According to the same source,  
11 France (16% of all agricultural land in the European Union in 2016) has been ranked  
12 6<sup>th</sup> in the world, over the last 10 years, among countries having the highest pesticide  
13 consumption, with more than 69,600 tons of active substances used for agriculture.

14 It has been estimated that depending on substances, agricultural equipment, and  
15 weather conditions before, during, and after treatment, various amounts of pesticides  
16 are likely to be transported to adjacent ecosystems (Dhananjayan et al., 2020;  
17 Mantzos et al., 2016a; Rousis et al., 2017; Sharma et al., 2019). Pesticides will  
18 spread through water resources in runoff induced by rainfall, resulting in rapid and  
19 widespread contamination events (Lefrancq et al., 2017). Headwater streams are  
20 particularly subject to this contamination, owing to their low dilution capacities and  
21 their proximity to agricultural land (Lorenz et al., 2017; Szöcs et al., 2017). Because  
22 of their position at the top of hydrographic networks, they are key to preserving the  
23 quality of downstream ecosystems (Dodds and Oakes, 2008; Rasmussen et al.,  
24 2013) as well as their rich biodiversity (Meyer et al., 2007). In this regard, several in  
25 situ structures can be implemented to limit their contamination by agricultural  
26 activities, such as constructed wetlands (Gauillier et al., 2019; Tournebize et al.,  
27 2013, 2012; Vallée et al., 2015; Vymazal and Březinová, 2015), vegetated ditches  
28 (Stehle et al., 2011), or riparian buffer strips (Bunzel et al., 2014; Chow et al., 2020).

29 Small waterbodies (i.e., 0.1 up to 100 ha) also seem to be relevant in the  
30 preservation of headwater streams. Ponds appear to have significant, but  
31 underestimated, pesticide mitigation capacities (Gaillard et al., 2016, 2015; Gregoire  
32 et al., 2009). Although small in size, these waterbodies, when added up together,

33 cover an area three times larger than that covered by large natural and artificial lakes  
34 in France. On a European scale they cover nearly 270,000 km<sup>2</sup> (Bartout and  
35 Touchart, 2018). Usually, a high density of small waterbodies occurs at the head of  
36 agricultural watersheds (Drożdżyński, 2008; Lazartigues et al., 2012); they are,  
37 therefore, from the beginning of hydrographic networks, on the path of pesticide  
38 contamination. Moreover, they may also be particularly subject to transformation  
39 product (TP) transfers (Ulrich et al., 2018), making them even more relevant in the  
40 understanding of both levels of contamination (i.e., with parent compounds and TP).  
41 Field data regarding contamination of ponds with pesticides are scarce and, even  
42 though some do exist (Gaillard et al., 2016; Lazartigues et al., 2013; Ulrich et al.,  
43 2018), they rarely take into account TP. The collection of environmental data on  
44 pesticides and TPs seems, therefore, necessary to predict ecotoxicological effects  
45 that may occur.

46 Currently, waterbody status is mainly assessed against environmental quality  
47 standards (EQS) set by the Water Framework Directive (WFD, 2000/60/EC) for  
48 certain priority substances (e.g., EQS for metazachlor = 0.02 µg/L, EQS for MCPA =  
49 0.5 µg/L). Ecotoxicological risks related to the occurrence and concentration of  
50 pesticides can also be assessed by comparing concentrations in waterbodies to  
51 toxicity thresholds (EC, LC50, or PNEC) established for various contaminants and  
52 model species (e.g., algae, invertebrates, fish). However, the diversity of active  
53 substances (466 potentially used in the European Union; Nagy et al., 2020) leads to  
54 gaps in the toxicity of certain substances and does not consider the effect of mixtures  
55 found in ecosystems. Consideration of this combination effect is necessary to assess  
56 the state of natural environments in which multiple pesticides and TP are potentially  
57 present (Özkara et al., 2016). The current approach, based on the toxicity of  
58 individual compounds and not combinations of compounds, leads to an  
59 underestimation of the risk (Pereira et al., 2017).

60 Although ecotoxicological data on active substances are largely incomplete, 60% of  
61 the pesticides monitored in this work had at least one guidance value or toxicity  
62 threshold). As for TP, data are extremely scarce (i.e. 1%; Chemical Substances  
63 Portal, 2009). While issues relating to the latter have received increasing attention  
64 since the late 1990s (Belfroid et al., 1998; Melin et al., 2020), field data are still  
65 scarce (Fonseca et al., 2019; Rousis et al., 2017; Reemtsma et al., 2013). Given that

66 TP, resulting from the transformation of parent compounds, are often more stable  
67 and therefore potentially more present in the environment (Phillips et al., 1999;  
68 Schuhmann et al., 2019), it seems relevant to accurately determine the extent of their  
69 environmental contamination (Gruchlik et al., 2018) especially in headwater streams.  
70 However, there is no official list of relevant TP to be monitored in the environment  
71 (Hernández et al., 2008; Melin et al., 2020). In addition, the scientific community  
72 knows little about the extent of their toxic potential, especially with long-term  
73 exposure. Therefore, in order to assess the extent of ecosystem contamination, it is  
74 necessary to gain such knowledge by analyzing as many substances (pesticides and  
75 TP) as possible (Fonseca et al., 2019) and over several growing seasons (Chow et  
76 al., 2020).

77 In this context, the present work had two main objectives. The first was to provide  
78 innovative data by assessing the numbers and concentrations of pesticides and  
79 several of their TP in small streams located at the head of a hydrographic web. The  
80 second was, on the one hand, to verify the reduction in pesticide concentrations  
81 downstream from a pond reported by Gaillard et al. (2016a). On the other hand, we  
82 wanted to determine whether this reduction was counterbalanced by the production  
83 of TP and their release, downstream. Our work is a case study focusing on the high-  
84 resolution dynamics of a single pond over the long term, as an essential preliminary  
85 step before studying a wider range of cases at a larger scale.

86

## 87 **2. Materials and methods**

### 88 **2.1. Study area, headwater stream, and pond characteristics**

89 The study was carried out in North-Eastern France between February 8<sup>th</sup> and  
90 December 3<sup>rd</sup>, 2019 (Fig. 1). During this period, the average monthly rainfall was 70  
91 mm with a maximum of 116.4 mm in October. Over the whole period, a total of 766  
92 mm of rainfall was recorded. Twenty-three rainfall events (24-hours periods) greater  
93 than 8 mm were observed and the average temperature was approximately  $12.3^{\circ}\text{C} \pm$   
94  $1.8^{\circ}\text{C}$  (Infoclimat, 2020).

95 The study streams were small first-order streams according to the Strahler  
96 classification (Strahler, 1952) receiving water from agricultural catchments. The study

97 area was characterized by hydromorphic clay soils (Keuper marl). Streams were  
98 impeded by a dam pond built in the 15<sup>th</sup> century (Fig. 1). The study pond had a water  
99 surface area of 4.5 ha and supported extensive fish production (without any  
100 additional nutrient inputs). The average pond depth was 0.9 m, its maximum depth  
101 (near the dike) was 2.2 m, and its water residence time when the pond is at its fullest  
102 was 96 days (Gaillard et al., 2015). Regarding physicochemical characteristics, the  
103 pond water had a neutral to slightly alkaline pH. The study began with the pond dike  
104 gate closure and the pond being emptied, a few months before pesticides application  
105 in the agricultural catchment. The pond was then filled up with water from two first-  
106 order streams (i.e., tributaries) over the first two months of the study period (i.e.,  
107 February and March). Next, it was stocked with selected fish species (i.e., *Cyprinus*  
108 *carpio*, *Tinca tinca*, *Rutilus rutilus*, and *Scardinius erythrophthalmus*) in late March.  
109 Finally, it was entirely drained in November in order to collect fish with fishnets. The  
110 whole water volume was discharged to a downstream stream.

111 The pond was supplied with water for the most part from two first-order streams (i.e.,  
112 UP1 and UP2 representing 80% and 20% of its major water inputs, respectively).  
113 Pond water was discharged via a single outlet stream (i.e., DO) located 15 m  
114 downstream and mainly supplied with water from the pond. Even though dam ponds  
115 are no longer recommended by water agencies and the WFD because of the  
116 ecological discontinuity they cause, such a configuration is highly frequent among  
117 fish farming ponds that still represent historical production tools in Europe.

118 The pond watershed had a surface area of 86.2 ha. Since 2016, a large part (33.8  
119 ha) had been cultivated according to a conventional 3-year rotation of rapeseed,  
120 wheat, barley, and potentially peas, whereas 42.5 ha had been used as permanent  
121 pastures (data retrieved by means of a farmer survey). Based on the information  
122 provided by the farmer, all data regarding treatment periods and crops grown on  
123 treated plots are given in Table 1.

## 124 **2.2. Sampling strategy**

125 The aforementioned three streams that were monitored in the study (i.e., UP1, UP 2,  
126 and DO) were each equipped with a sampling station to carry out continuous flow-  
127 based water sampling. Mean sampled water volumes were  $277 \pm 51 \text{ m}^3$  at UP1,  $104$   
128  $\pm 12 \text{ m}^3$  at UP2, and  $356 \pm 58 \text{ m}^3$  at DO. Each sampling station was composed of an

129 automatic sampler (Hach Sigma SD-900, United States) controlled by an IFQ type  
130 logger (Flow-Tronic S.A., Belgium). Each automatic sampler allowed the storage of  
131 24 polyethylene bottles of 1 L samples. Samples were retrieved once a week. The  
132 IFQ logger triggered water sampling by the automatic sampler following  
133 measurements of two sensors. The first was the Beluga flow sensor (Flow-Tronic  
134 S.A., Belgium). It emits in the water column a continuous 1 MHz frequency acoustic  
135 signal, and the receiver samples multiple frequency signals to calculate velocity with  
136 high accuracy ( $\pm 0.01$  m/s) and resolution (0.001 m/s). This probe allows the  
137 calculation of negative flow velocities (-2 to 6 m/s). Given the moderate slope (2.2%)  
138 that characterized the water inlet tributaries, this specification allowed us to take into  
139 account pond backflow events and obtain accurate water flow measurements. For  
140 level calculation, a second sensor was used simultaneously (ULS-02 ultrasonic level  
141 sensor, Flow-Tronic S.A., Belgium). It allows continuous level calculation by  
142 processing continuous echoed ultrasonic waves that are emitted toward the water  
143 flow, with high precision ( $\pm 0.3$  mm) and resolution (1 mm). Water flows were  
144 measured (based on measurements from the two probes) every minute at all three  
145 stations. The sampling frequency was thus automatically adjusted in order for the  
146 number of samples to be proportional to the volume of water flowing. Thus, samples  
147 were mainly collected during flood events and varied with seasonal precipitation.

148 During the study period, UP1, UP2, and DO sampling stations collected a total of  
149 121, 52, and 64 water samples, respectively. Between July 3<sup>rd</sup> and October 15<sup>th</sup>, no  
150 samples were collected as streams were dry. During this period, DO station only  
151 sampled water coming from the main drain channel through dike leaks.

152 Samples were kept in the dark at -18°C and analyzed within 15 days to avoid  
153 degradation and sorption to sampling bottles. The stability of analyzed compounds in  
154 polyethylene bottles was tested prior to sampling and was found to be good for at  
155 least the first 15 days at 20°C and 17 days at 10°C (Table A.4 in the Supplementary  
156 appendix). Nevertheless, a few compounds showed low stability (i.e., less than 15  
157 days at 10°C) in polyethylene bottles and were, therefore, removed from analysis  
158 (e.g., chlorpyrifos, CPO, chlorthiamide, fenthion). High temperatures in the summer  
159 season (37.6°C at their highest) were not an issue for sample conservation because  
160 no sampling was done at that time owing to lack of precipitation.

### 161 **2.3. Pesticide and transformation product quantification in water**

162 The list of pesticides and TP investigated in this study was established upon analysis  
163 of the treatments applied to the watershed since 2000 and historic contaminants now  
164 banned from use. It included 67 substances in total, with 18 herbicides and 35 of  
165 their TP, 2 fungicides, and 8 insecticides and 4 of their TP (Table 2). Each water  
166 sample was analyzed by high-performance liquid chromatography-electrospray  
167 ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) using HPLC-LC20AD  
168 (Shimadzu, Marne-la-Vallée, France) coupled with QTRAP® 5500 system (Sciex,  
169 Villebon-sur-Yvette, France). Limits of detection (LOD) and quantification (LOQ) are  
170 given in Table 2. Details regarding the method of analysis and hardware setup are  
171 given in Tables A.1 to A.3 in the Supplementary appendix.

172 Briefly, a 1 mL aliquot was taken from each initial sample and supplemented with 10  
173 µL of an 8 ng/mL internal standard solution (HPC standards GmbH, Neochema,  
174 Bodenheim, Germany; Fluka, Toronto Research Chemicals, North York, Canada;  
175 Cambridge Isotope Laboratories, Tewksbury, United States) (purity > 96%; the full list  
176 of internal standards is provided in Tables A.1 and A.2 in the Supplementary  
177 appendix). Then, samples were centrifuged at 20,800 g for 15 min at 18°C  
178 (Eppendorf 5810R, Montesson, France), allowing suspended particles to separate.  
179 The supernatant was transferred into 2 mL glass vials and 100 µL of preparation was  
180 injected and analyzed. In between each sample, the automatic sampler was rinsed  
181 with a mixed solution of ultrapure water, methanol, acetonitrile, and isopropanol (in  
182 equal proportions, 25% of final volume), and then with ultrapure water. Ultrapure  
183 water, formic acid, and acetonitrile were of ULC-MS quality (Biosolve, Dieuze,  
184 France). One sample per injection series was spiked with analytical standards to  
185 control accuracy (recovery range, 75%-125%). Blank samples were added at the  
186 beginning and end of each series, and quantification was performed with internal  
187 calibration. Samples with high concentrations (above the highest calibration point, ±  
188 10%) were diluted to meet the calibration range. Injection results were then  
189 interpreted using MultiQuant software (version 3.0.1, Sciex, Villebon-sur-Yvette,  
190 France).

191 Concerning glyphosate and AMPA (aminomethanesulfonic acid), the analysis was  
192 based on online solid-phase extraction coupled with HPLC-MS/MS. As they are too  
193 polar to be analyzed with classical systems, a derivation step with the FMOC  
194 (fluorenylmethoxycarbonyl) protection group was necessary before injection. Briefly,



195 1540  $\mu\text{L}$  of water sample was spiked with 7.5  $\mu\text{L}$  of a 40  $\mu\text{g/L}$  solution of  $^{13}\text{C}^{15}\text{N}$   
196 AMPA-FMOC, 100  $\mu\text{L}$  of 250 mM disodium EDTA, 200  $\mu\text{L}$  of 50 mM sodium  
197 tetraborate buffer, and 160  $\mu\text{L}$  of FMOC mix (0.5 g/L in acetonitrile). Derivation was  
198 performed with a thermomixer at 50°C and for 20 min. The reaction was then  
199 stopped with 20  $\mu\text{L}$  of  $\text{H}_3\text{PO}_4$  (2% in ultrapure water) and the mixture was transferred  
200 into a 1.5 mL polypropylene injection vial for analysis (details on the analytical  
201 methods are available in Tables S.4 and S.5 in the Supplementary appendix). One  
202 sample per injection series was spiked with analytical standards to control recovery  
203 accuracy. Blank samples were added at the beginning and end of each series, and  
204 quantification was performed with internal calibration for AMPA, while glyphosate was  
205 quantified with external calibration based on comparison with ultrapure water, spiked  
206 at 10, 20, 50, 100, 150, 200, 300, and 400 ng/L, and extracted as field samples.  
207 Samples with high concentrations (above the highest calibration point,  $\pm 10\%$ ) were  
208 diluted to meet the calibration range. Injection results were then interpreted using  
209 TraceFinder (4.1) and Xcalibur (2.2 SP1.48) software.

210 All solvents and reagents used were of LC-MS purity grade.

#### 211 **2.4. Data analysis**

212 Pesticide and TP concentrations allowed the calculation of mean concentrations  $\pm$   
213 standard error of the mean (SEM) upstream and downstream from the pond as well  
214 as maximum concentrations of all analyzed compounds. Only substances having a  
215 frequency of quantification (FQ) of at least 80% were averaged, in order to avoid  
216 estimating concentrations for pesticides and TP poorly represented in the dataset.  
217 Concentrations below the LOD were set to 0, those between the LOD and the LOQ  
218 were set to  $\text{LOQ}/2$ .

219 Based on the LOD and LOQ, we calculated the frequency of detection (FD) and  
220 quantification for each compound at upstream and downstream locations. The  
221 numbers of samples with values above the LOD or LOQ were divided by the total  
222 number of samples ( $N = 121$  at UP1, 52 at UP2, and 64 at DO) and set in percentage  
223 form. Maximum and mean reduction rates (%) were calculated based on the  
224 decrease in pesticide and TP concentrations between upstream (UP1 and UP2) and  
225 downstream (DO) locations. For compounds with a FQ  $> 80\%$ , a reduction rate of the  
226 mean concentration was calculated (i.e., downstream mean concentration / upstream

227 mean concentration). In the same way, a reduction rate of the maximum  
228 concentration was obtained for the highest quantified concentrations. When both  
229 tributaries showed different mean and/or maximum concentrations, data from UP1  
230 were used to calculate the reduction rates in order to take into account the major  
231 water source, which represented at least 80% of water inputs every time.

232 For each substance (and for the sum of all quantified substances) and at each  
233 sampling location (UP1, UP2, and DO), based on pesticide and TP concentrations,  
234 the events exceeding environmental or ecotoxicological standards were accounted  
235 for. Environmental and ecotoxicological standards were retrieved from either the  
236 INERIS database on chemical substances (Portail Substances Chimiques, 2009) or  
237 the IUPAC (International Union of Pure and Applied Chemistry) database on  
238 pesticide properties (Lewis et al., 2016).

239 Comparisons of contaminant concentrations between upstream and downstream  
240 stations were assessed using Welch's two-sample t-tests. Graphical representations  
241 and statistical analyses were done with R software (version 4.0.2, © The R  
242 Foundation).

243

## 244 **3. Results**

### 245 **3.1. Occurrence of pesticides and TP in headwater streams**

246 Over the 10-month study period (i.e., an entire fish breeding cycle from pond filling to  
247 pond emptying), 173 samples were retrieved from the upstream stations. Out of the  
248 67 investigated substances, 38 were at least detected in one sample (Table 2). Three  
249 TP (i.e., flufenacet-ESA, metazachlor-ESA, and OXA) were present in all samples. At  
250 UP1, the number of pesticides detected in one sample ranged from 5 to 12. The  
251 number of detected TP was even higher and ranged from 10 to 17 compounds. At  
252 UP2, this number ranged from 1 to 9 for pesticides and from 4 to 12 for TPs in any  
253 sample (Fig. 2). Upstream from the pond, TPs represented on average  $65.8\% \pm 0.6\%$   
254 of the detected substances. Whatever the water sample (UP and DO stations alike),  
255 a mixture of at least 5 compounds was detected, reaching 29 different contaminants  
256 in one sample at its highest (at UP1 station, Fig. 2). The average numbers of  
257 compounds (i.e., pesticides and TP) detected per sample in UP1 and UP2 stations

258 were  $22.5 \pm 0.51$  and  $11.3 \pm 0.78$ , respectively. Among the 38 detected substances,  
259 31 showed concentrations higher than the LOQ at UP1 and 20 at UP2. In both cases,  
260 TP accounted for more than 50% of the substances.

261 At both upstream locations, several groups showing a high FQ were formed by  
262 pesticides and their respective TP. Indeed, the most quantified herbicides were  
263 flufenacet, metazachlor, and metolachlor (with a FQ as high as 100% for metolachlor  
264 at UP1) and TP of metazachlor were present in all samples at the upstream stations.  
265 In addition, the most quantified herbicide TP upstream were AMPA, atrazine-2-  
266 hydroxy, dimethachlor-ESA, dimethenamid-ESA, flufenacet-ESA and OXA, and  
267 metolachlor-ESA and OXA. Regarding fungicides, boscalid was highly quantified at  
268 both UP1 and UP2 stations, and tebuconazole mainly at UP1. Insecticides were  
269 rarely quantified or detected (Table 2).

270 Downstream from the pond, 64 water samples were collected and 27 different  
271 compounds were at least detected in one sample (Table 2), 59% of them being TP.  
272 The average number of compounds detected at DO station was  $14.6 \pm 0.8$ . The  
273 number of pesticides and TP in one sample ranged from 0 to 7 and from 7 to 13,  
274 respectively (Fig. 2).

275 Out of the 27 detected compounds, 24 showed concentrations above the LOQ.  
276 Dimethenamid, flufenacet, and metolachlor were the most quantified herbicides with  
277 a FQ between 23.4% and 39.1%. The fungicide tebuconazole was quantified in  
278 78.1% of the samples (Table 3). More than half (54%) of these 24 substances were  
279 TP.

280 At DO station, just like at UP1/UP2 stations, groups of compounds showing a high  
281 FQ were formed by pesticides and their respective TP (Table 2). Flufenacet,  
282 metazachlor, and metolachlor were quantified in many samples, as were their TP.  
283 For instance, metolachlor was quantified in 39.1% of the samples, and metolachlor-  
284 ESA and OXA in 100% and 82.8%, respectively.

### 285 **3.2. Pesticides and TP concentrations in agricultural headwater streams and** 286 **pond-induced reduction rates**

287 According to the analytical method used (FQ > 80%), 16 substances allowed  
288 calculation of an average concentration  $\pm$  SEM upstream from the pond (Table 3).

289 Considering all 38 compounds per sample (those with a concentration > LOD), the  
290 mean concentration recorded over the study period was  $3.5 \pm 0.18$   $\mu\text{g/L}$  at UP1 and  
291  $5.3 \pm 0.9$  at UP2 (all pesticide and TP kinetics during the study period are shown in  
292 the Supplementary appendix). The maximum concentrations of pesticides were 6.4  
293  $\mu\text{g/L}$  and 23.9  $\mu\text{g/L}$ , and those of TP were 7.7  $\mu\text{g/L}$  and 13.4  $\mu\text{g/L}$  at UP1 and UP2,  
294 respectively (Fig. 2).

295 During the study period, flufenacet had a mean concentration of  $0.529 \pm 0.09$   $\mu\text{g/L}$  at  
296 UP1 (Table 3) and was quantified at high concentration in November and December  
297 at UP1 and UP2 (5.4 and 3.1  $\mu\text{g/L}$ , respectively). In contrast, its TP (i.e., flufenacet-  
298 ESA and OXA) were found to be at respective concentrations as high as 6.2 and 5.6  
299  $\mu\text{g/L}$  at UP2 throughout the whole study period (Fig. 3). Metazachlor was also highly  
300 quantified throughout the same period, with a mean concentration of  $0.0473 \pm 0.007$   
301  $\mu\text{g/L}$  at UP1 (Table 3). Both its monitored TP showed high concentrations during  
302 sampling (Fig. 3). Higher mean and maximum concentrations were observed at UP2,  
303 with a mean concentration of  $0.871 \pm 0.09$   $\mu\text{g/L}$  for metazachlor-ESA (maximum of  
304 2.8  $\mu\text{g/L}$ ) and  $0.816 \pm 0.1$   $\mu\text{g/L}$  for metazachlor-OXA (maximum of 4.8  $\mu\text{g/L}$ ).  
305 Metolachlor had a mean concentration of  $0.0752 \pm 0.008$   $\mu\text{g/L}$  at UP1 (Table 3) and  
306 was mostly present at the beginning of the study period (Fig. 3). Throughout the  
307 course of the study, its TP metolachlor-ESA and OXA were observed to have mean  
308 concentrations of  $0.156 \pm 0.008$  and  $0.169 \pm 0.01$   $\mu\text{g/L}$  (Table 3), respectively, with  
309 the highest concentrations in November and December (Fig. 3).

310 At DO location, the mean total concentration (i.e., all substances per sample  
311 considered) was  $1.08 \pm 0.07$   $\mu\text{g/L}$  during the study. The maximum concentration of  
312 pesticides was 0.5  $\mu\text{g/L}$  and that of their TP was 1.9  $\mu\text{g/L}$  (Fig. 2).

313 Flufenacet-ESA and OXA were present throughout the whole study period (Fig. 3)  
314 with mean concentrations of  $0.244 \pm 0.02$   $\mu\text{g/L}$  and  $0.0719 \pm 0.006$   $\mu\text{g/L}$ , respectively.  
315 Metazachlor-ESA and OXA were always present at higher concentrations at DO than  
316 their parent compound (Fig. 3) and had mean concentrations of  $0.179 \pm 0.008$   $\mu\text{g/L}$   
317 and  $0.263 \pm 0.01$   $\mu\text{g/L}$ , respectively (Table 3) during the same period. Both  
318 metolachlor-ESA and OXA were present in 100% and 82.8% of the water samples  
319 (Table 2), with mean concentrations of  $0.0394 \pm 0.002$   $\mu\text{g/L}$  and  $0.0422 \pm 0.003$   $\mu\text{g/L}$ ,  
320 respectively, throughout the study (Table 3 & Fig. 3).

321 Reduction rates were calculated for both mean and maximum concentrations (Table  
322 3). When UP1 and UP2 had different values, UP1 was selected for the calculation of  
323 the reduction rate, in order to consider the main water input to the pond. For all  
324 substances having a calculated mean concentration (FQ > 80%) except atrazine-2-  
325 hydroxy, reduction rates were positive, meaning that mean concentrations at DO  
326 were lower than those at UP1 and/or UP2. Regarding atrazine-2-hydroxy, its mean  
327 concentration at DO was 16% higher than that at UP2, which was not significantly  
328 different. Regarding dimethachlor-ESA, flufenacet-ESA, and metazachlor-ESA and  
329 OXA, their mean concentrations at DO were significantly lower than those at UP2.  
330 The reduction rates of the mean concentrations ranged from 34% for flufenacet-ESA  
331 up to 75% for metolachlor-ESA and OXA. The low FQs of DIA-OH and several other  
332 compounds including benzamide and dimethachlor did not allow their mean  
333 concentrations to be expressed at UP1/UP2, but since they were never quantified at  
334 DO, positive reduction rates were expected.

335 Based on the maximum concentrations (i.e., peak concentrations), reduction rates  
336 were mainly positive, meaning that maximum concentrations at DO were lower than  
337 those at UP1/UP2. Considering highly quantified compounds, maximum reduction  
338 rates ranged from 20% for atrazine-2-hydroxy up to 100% for DIA-OH, benzamide,  
339 and eight other compounds (Table 3).

### 340 **3.3. Suppression of threshold exceedances events downstream from the pond**

341 In upstream locations and during late 2019, 37 flufenacet exceedance events were  
342 detected regarding regulatory acceptable concentration (RAC) 15 exceedance  
343 events of EC<sub>50</sub> for the bioindicator *Raphidocelis subcapitata* (13 at UP1 and 2 at  
344 UP2) were detected regarding flufenacet concentrations. In addition, 90 exceedances  
345 of the EQS value for metazachlor, set by the WFD (Order of July 27, 2015), were  
346 detected at UP1 between February and May, and again in November (Table 4 and  
347 Fig. 3).

348 Mid-May, 8 exceedances of the regulatory acceptable concentration (RAC) values  
349 were highlighted for metsulfuron-methyl at UP2 (Table 4). Also, the EQS threshold  
350 was exceeded for MCPA mainly at UP1 in May. At DO location, 5 exceedances of  
351 RAC values were observed for flufenacet (Table 4).

352

## 353 **4. Discussion**

### 354 **4.1. Occurrence of pesticides and TP in headwater streams**

355 At the very beginning of the headwater streams, mixtures of pesticides and TP were  
356 present in surface water (22.5 and 11.3 substances on average at UP1 and UP2,  
357 respectively). At DO station, the number of detected substances was found to be  
358 14.6 on average, therefore smaller than at UP1 but greater than at UP2, the reason  
359 for the latter observation being a greater number of TP (Fig. 2) coming from UP1.  
360 Moreover, TP represented more than half of the substances quantified upstream and  
361 downstream from the pond. This result seems to be in agreement with studies carried  
362 out in lentic systems, whose characteristics are similar to those of ponds, and which  
363 reported the occurrence of metazachlor and flufenacet TP in 39% to 94% of their  
364 water samples (Ulrich et al., 2018). In the same way, Fonseca et al. (2019) noted the  
365 occurrence of TP in (Spanish) streams, which accounted for 22% of the detected  
366 pesticide-related substances. These results call attention to the occurrence of TP in  
367 the environment. They show the importance of considering compound mixtures when  
368 studying ecotoxicological effects. In a study carried out across several European  
369 countries (France, Germany, and the Netherlands) and the United States, Schreiner  
370 et al. (2016) revealed, based on the analysis of routine monitoring data (292  
371 compounds for France), the presence of 2 to 5 substances per sample, the majority  
372 of which being herbicides. Our results suggest higher levels of contamination of  
373 headwater streams with pesticide mixtures (as high as 29 different compounds per  
374 sample), especially in areas of intensive agricultural activities. Furthermore, other  
375 studies showed an occurrence of TP in surface waters greater than that of the  
376 present study. Indeed, Moschet et al. (2014) reported water samples containing  
377 between 15 and 25 different TP (as high as 17 in our samples), in rivers with larger  
378 agricultural/urbanized catchments (between 38 km<sup>2</sup> and 105 km<sup>2</sup>) than that studied  
379 here, potentially representing an even greater source of contamination.

380 Our analysis suggests a positive effect of the pond on the number of detected  
381 substances: by decreasing the number of pesticides and TP, ponds may induce a  
382 reduction in ecotoxicity risks (Cedergreen, 2014; Relyea, 2009; Rizzati et al., 2016).

383 The same trend was observed in Ohio (Midwestern United States), where TP are  
384 present at the beginning of channelized agricultural watersheds and mixtures

385 (pesticides and TP) are generally more frequent than single substances.  
386 Concentrations depend on treatments types and climatic conditions, and can vary  
387 annually and seasonally (Smiley Jr et al., 2014). Therefore, our approach, based on  
388 frequent water sampling, appears to be relevant in highlighting the occurrence of TP  
389 in headwater streams. The same results were obtained in southwestern Germany,  
390 where more than 80% of the water samples coming from studied agricultural  
391 headwater streams contained more than 5 substances (pesticides and TP), and half  
392 of them showed high concentrations of TP, such as metazachlor-OXA (0.89  $\mu\text{g/L}$ )  
393 (Weber et al., 2018). Our results showed higher concentrations for some compounds,  
394 with metazachlor-OXA and metolachlor-OXA concentrations exceeding those of  
395 Weber et al. (2018) by a factor of 5.3 and 2.75, respectively.

396 Concerning herbicide parent compounds, flufenacet, metazachlor, and isoproturon  
397 were present in our samples, which is consistent with the type of crops grown in the  
398 watershed (Table 1) and the importance of herbicides in the crop protection market  
399 (Bonanno et al., 2017). Flufenacet was particularly often quantified upstream from the  
400 pond, having been applied in the watershed in 2019. Although this herbicide has a  
401 relatively low organic carbon sorption coefficient ( $K_{oc}$ ) of 401 (Lewis et al., 2016),  
402 which characterizes it as being moderately mobile, it may be highly mobile in  
403 agricultural catchment areas (Willkommen et al., 2019). The latter authors showed  
404 significant and continuous leaching of flufenacet soon after application under both dry  
405 and wet conditions. Our result tends to confirm this mobility, as flufenacet was  
406 applied at the beginning of October, and quantified as early as mid-October (Fig. 3).  
407 Metazachlor, which was used in 2017, was also often quantified at UP1 (FQ =  
408 99.2%). It has a relatively low 50% dissipation time ( $DT_{50}$ ) in soil (6.8 days under field  
409 conditions) characterizing a non-persistent compound but it is stable in the water  
410 phase ( $DT_{50}$  of 216 days) (Lewis et al., 2016). However, field studies have shown that  
411 metazachlor could be detectable in soil up to 170 days after application (LOD of 0.57  
412 ng/g in soil), depending on soil characteristics (Mantzos et al., 2016b). According to  
413 Mantzos et al. (2016b), metazachlor is a potential risk for adjacent surface water  
414 within the first month after application. However, our results suggest a more lasting  
415 presence, even two years after its application on treated lands. Rising aquifers could  
416 explain its occurrence in the study streams, but its low leaching potential ( $GUS =$   
417 1.75 according to Lewis et al., 2016) makes it unlikely to reach groundwater bodies

418 during treatment and then be transferred to surface water, like polar molecules, as  
419 TP could do (Hintze et al., 2020). Thus, it can be assumed that quantification of  
420 metazachlor over the whole study period resulted from accidental spill or undeclared  
421 treatments, but no evidence could confirm it.

422 Moreover, isoproturon, which had no longer been approved as an agricultural  
423 treatment since 2016 (EU 2016/872), was still quantified in water samples but at a  
424 level far below the EQS limit value of 1 µg/L. It was typically used in cereal crops to  
425 control annual grasses and weeds, and considered as a priority hazardous  
426 substance by the WFD because of its high environmental accumulation (Muhammad  
427 et al., 2019). This molecule is still frequently detected in surface water samples  
428 (Casado et al., 2019; Durak et al., 2020), notably because of its historic use and  
429 variable DT<sub>50</sub> in soil ranging from 3 to 200 days (Alletto et al., 2006), thus potentially  
430 explaining its presence in our samples even though it was not actually used during  
431 the sampling period, nor had it been since 2016.

432 The two fungicides used in the study catchment (i.e., boscalid and tebuconazole)  
433 were detected in all UP1 samples and in 100% and 61.5% of UP2 samples,  
434 respectively. Boscalid is one of the most widely used succinate dehydrogenase  
435 inhibitor (SDHI) fungicides in the world. A recent study (Bénit et al., 2019) has  
436 established that human, honeybee, earthworm, and fungal succinate  
437 dehydrogenases (SDHs) are all affected by SDHIs. Furthermore, these authors have  
438 shown that cell death due to SDHI exposure was significantly accelerated in  
439 fibroblasts derived from patients with neurodegenerative diseases. For these  
440 reasons, even though this effect has not been highlighted in vivo yet, SDHIs have  
441 come under the close scrutiny of phytopharmacovigilance committees.  
442 Contamination of surface water by tebuconazole and boscalid has recently been  
443 established in an agricultural catchment in Germany (Tauchnitz et al., 2020). Our  
444 results confirm Tauchnitz et al.'s findings but with maximum concentrations that were  
445 higher by a factor of 10 (0.01 vs. 0.1 µg/L at UP2) and 50 (0.014 vs. 0.5 µg/L at the  
446 highest at UP1) for boscalid and tebuconazole, respectively.

447 Regarding groups of substances formed by pesticides and their respective TP, an  
448 overall decrease in FQ was observed for pesticides between the upstream and  
449 downstream stations (FQ of 91.7% and 67.3% at UP1 and UP2, respectively, vs.  
450 31.3% at DO for flufenacet). TP tended to remain highly present in the samples (FQ



451 of 100% at UP1, UP2, and DO for both TP of metazachlor). Greater persistence of  
452 TP (compared to pesticides) has been established for metazachlor and flufenacet in  
453 waterbodies (Ulrich et al., 2018), which thus can explain their detection at high  
454 concentration in the water sampled in the present study, even downstream from the  
455 pond.

#### 456 **4.2. Pesticides and TP concentrations in agricultural headwater streams and** 457 **pond-induced reduction rates**

458 A significant decrease in mean concentrations ( $p$ -value < 0.001) was observed  
459 between UP1/UP2 and DO stations ( $3.5 \pm 0.2 \mu\text{g/L}$  and  $5.3 \pm 0.9$  at UP1 and UP2,  
460 respectively, vs.  $1.09 \pm 0.07 \mu\text{g/L}$  at DO). Such a decrease was observed for 7  
461 different substances. A clear buffering of contaminant peaks was also observed, with  
462 a maximum of  $27.2 \mu\text{g/L}$  at UP2 compared to  $2.16 \mu\text{g/L}$  at DO. Reduction in pesticide  
463 concentrations between upstream and downstream rivers has already been  
464 demonstrated for ponds, with reduction rates of up to 99% (Gaillard et al., 2016,  
465 2015), as well as for constructed wetlands, which showed variable reduction rates  
466 (Budd et al., 2009; Gaullier et al., 2019; Tournebize et al., 2017; Vymazal and  
467 Březinová, 2015). Our results indicate a similar trend not only for pesticides, with a  
468 100% reduction rate for several substances such as dimethachlor, isoproturon, and  
469 metsulfuron-methyl, but also for TP such as benzamide, dimethachlor-OXA, and DIA-  
470 OH. Such retention effect was never highlighted in such an extent, at sampling as  
471 well as molecular diversity levels. Considering pesticides and TP, the mean reduction  
472 rates of their mean concentrations were 100% and  $72.4\% \pm 8.9\%$ , respectively.  
473 Better reduction rates were observed for parent compounds, reflecting potentially  
474 greater stability of TP, which may be less affected by abiotic and biotic processes  
475 occurring in the pond (Picó and Barceló, 2015). Atrazine-2-hydroxy had the lowest  
476 reduction rate of maximum concentration as compared to the other TP, which may be  
477 explained by its great persistence (with a  $\text{DT}_{50}$  potentially as high as 164 days; Lewis  
478 et al., 2016) or low volatility (with a Henry's law constant at  $25^\circ\text{C}$  of  $6.36\text{e-}8$   
479  $\text{Pa/m}^3/\text{mol}$ ). Atrazine and its TP have been reported to be highly persistent in water  
480 and soil (Jablonowski et al., 2011). These authors showed that, in Germany, atrazine  
481 was the most abundant pesticide in groundwater samples even after having been  
482 banned from use for 15 years. Schreiner et al. (2016) have also reported high  
483 atrazine and atrazine TP contamination in France, Germany, the Netherlands, and

484 the USA, and identified them as being among the most detected pesticides and TP.  
485 The persistence of atrazine-2-hydroxy, combined with the continuous release of  
486 atrazine TPs from surrounding agricultural lands being then transported by the main  
487 tributaries of the pond (FQs of 60.3% and 94.2% at UP1 and UP2, respectively) and  
488 subsurface flows, may thus explain their occurrence at DO station.

489 It was surprising to observe, on the one hand, the presence of TPs upstream from  
490 the pond and, on the other hand, their diversity ( $10 \leq \text{TPs} \leq 17$  at UP1) (Fig. 2).  
491 Indeed, the pond can be seen as a degrading environment, thus leading to increased  
492 TP presence downstream from the pond. Their presence upstream reflected high  
493 contamination at the beginning of the study headwater streams. Such contamination  
494 is related to the soil transformation that takes place between the time of treatment  
495 application (Raina et al., 2011), the first rain events post application, and rain events  
496 heavy enough to lead to the transportation of these substances (Ulrich et al., 2018).

497 Nevertheless, by showing the occurrence of TP at a stable high concentration (6.2  
498  $\mu\text{g/L}$  for flufenacet-ESA at UP2) over a long period of time, we highlighted that they  
499 should be more considered in monitoring programs. Maximum concentrations of  
500 metazachlor and flufenacet TP were particularly high in our samples compared to  
501 those of Ulrich et al. Flufenacet-ESA and OXA were quantified at a maximum  
502 concentration of 6.2  $\mu\text{g/L}$  (vs. 1.9 in Ulrich et al.) and 5.6  $\mu\text{g/L}$  (vs. less than 0.75  $\mu\text{g/L}$   
503 in Ulrich et al.), respectively, and metazachlor-OXA was quantified at 4.8  $\mu\text{g/L}$  at its  
504 highest compared to less than 4  $\mu\text{g/L}$  in Ulrich et al. TP were found to be at higher  
505 concentration than their parent substances, which could potentially be correlated to  
506 the time of treatment of arable lands, as well as to molecule characteristics. A high  
507 FQ of flufenacet in UP1 and UP2 samples may have been caused by direct transfer  
508 from arable lands, after pre-emergent treatment in October. Having both pesticides  
509 and TP at UP1 and UP2 stations is explained by winter treatments (of 2019 and  
510 potentially of previous years) and specific flufenacet characteristics. Longer  $\text{DT}_{50}$   
511 have been estimated under laboratory conditions, correlated with soil clay content  
512 (Gupta and Gajbhiye, 2002), and even higher  $\text{DT}_{50}$  have been determined in field  
513 studies, ranging from 117 up to 145 days (Marín-Benito et al., 2020). Flufenacet will  
514 be slowly degraded in treated soils, leading to the formation of TP. However, the  
515 parent molecule will also be present when heavy rainfall occurs, explaining the  
516 simultaneous presence of pesticides and TP in water (Willkommen et al., 2019).

517 Moreover, it raises the question of monitoring pesticide occurrences and  
518 concentrations without taking into account related TP, which may be, on average,  
519 more concentrated ( $0.459 \pm 0.02 \mu\text{g/L}$  and  $0.728 \pm 0.04 \mu\text{g/L}$  for metazachlor-ESA  
520 and OXA, respectively, vs.  $0.0473 \pm 0.007 \mu\text{g/L}$  for metazachlor at UP1) and  
521 persistent (Fig. 3). Studies that question the relevance of TP in control and  
522 monitoring programs agree and highlight the need to include these substances on  
523 official lists. However, given the range of potential substances to be monitored,  
524 prioritizing pertinent TP is necessary (Melin et al., 2020). Ecotoxicological effects  
525 should be considered, which is the approach adopted by the French Agency for  
526 Food, Environmental and Occupational Health, and Safety (ANSES). Furthermore,  
527 the selection of the TP to be analyzed should be based on extensive environmental  
528 monitoring data (regarding the number of substances searched for and study times),  
529 as has been done in the present study, to determine TP actually occurring in  
530 anthropized natural environments (Fig. 2).

531 Contrary to Gaillard et al. (2016), no isoproturon increase was observed in DO  
532 samples. This may be due to a decrease in overall pollution, since the substance was  
533 banned from agricultural use, or to a difference in sampling strategy. The strategy  
534 used in the present study is potentially more precise because based on stream flows.  
535 Thus, we were less likely to miss isoproturon peaks at upstream stations during  
536 heavy precipitation events, which represent crucial leaching events (Beulke et al.,  
537 2002; Filipović et al., 2016).

538 Counter-intuitively, the pond did not release TP having formed because of hydraulic  
539 conditions through biotic (Vidali, 2001; Zablotowicz et al., 2004) and abiotic (Sarmah  
540 and Sabadie, 2002) transformation processes. Indeed, they were already present  
541 upstream from the pond (Fig. 3), which, however, may have increased their  
542 dissipation via these biotic and abiotic processes, allowing concentration reductions  
543 downstream. The year 2019 was marked by sparse rainfall, which resulted in  
544 incomplete filling of the pond and thus water not reaching the spillway. As a result,  
545 hydraulic residence time, normally close to 3 months, was increased to 7.7 months; it  
546 is thus likely that TP reaching and forming in the pond were degraded over a longer  
547 period of time, explaining such effective reduction between UP1/UP2 and DO  
548 stations. The importance of hydraulic residence time has indeed already been  
549 highlighted for parent molecules (Stehle et al., 2011).

550 It is also relevant to consider the storage/release capacities of the pond between the  
551 sediment compartment and the water column (Vallée et al., 2014). Indeed, according  
552 to their characteristics, pesticides and TP are likely to have a high affinity for  
553 particulate matter, thus being added to the sediment compartment. It is generally  
554 accepted that the sorption coefficient ( $K_{oc}$ ) and the octanol/water partition coefficient  
555 (LogP) of pesticides are good indicators of their fate in waterbodies, and of their  
556 binding to suspended matter (Boithias et al., 2014; Vryzas, 2018). The importance of  
557 the sediment compartment in the retention of contaminants, whether pesticides or  
558 TP, should therefore also be considered and explored in order to explain the  
559 observed upstream/downstream difference.

560 Finally, it is also important to take into account the dilution effect induced by the  
561 pond. Studies focusing on large rivers have shown that pesticide loads are strongly  
562 correlated with hydrological parameters, showing a decrease in concentrations in  
563 downstream locations where water volumes are large (Climent et al., 2019). For  
564 compounds occasionally detected or quantified at UP1 and UP2 but not at DO, such  
565 as DIA-OH, benzamide, DPC, Me-DPC, dimethachlor, metsulfuron-methyl, and  
566 imidacloprid, and thus having a maximum reduction rate of 100%, dilution may  
567 explain the upstream/downstream reduction. All these substances had FQ lower than  
568 20%, and for some of them less than 1% (DIA-OH and benzamide) at UP1 or UP2  
569 location, and were never quantified at DO. They were likely to have remained  
570 undetected in the pond and at DO location (regardless of low LOD) because of large  
571 water volumes and possibly intra pond transformation processes.

572

#### 573 **4.3. Suppression of threshold exceedances events downstream from the pond**

574 A total of 159 exceedances of environmental/ecotoxicological standards were  
575 observed over the whole sampling period at UP1 and UP2 stations, as against 5 at  
576 DO station (Table 4).

577 MCPA and metsulfuron-methyl are compounds used in cereal crops, and their  
578 occurrence at UP2 could be explained by cultivation upstream from the sampling  
579 station (see Section 2.1). The presence of tebuconazole could be linked to the type  
580 of crops grown in the watershed in previous years (see Section 2.1). It has been  
581 reported to have moderate to high persistence depending on soil properties,

582 explaining possible leaching events even though not applied during the study period  
583 (Lewis et al., 2016).

584 In Europe, studies have shown that pesticide concentration thresholds are exceeded  
585 in water streams during site-sampling events (Casado et al., 2019; Schreiner et al.,  
586 2016; Szöcs et al., 2017; Weber et al., 2018). These events are strongly correlated  
587 with pesticide treatments (Zivan et al., 2016) but highly variable because they depend  
588 on environmental conditions and substance intrinsic properties such as solubility or  
589 soil sorption (Kellogg et al., 2002). Flufenacet was used as winter treatment in late  
590 2019 when precipitation levels were high, thus leaving little time for compound  
591 transformation to occur and inducing direct runoff from arable lands. Low stable  
592 concentrations were observed at the beginning of the study period (Fig. 3),  
593 suggesting that most of the applied doses had already been washed off during  
594 rainfall events of late 2018. Flufenacet is well documented, and it is admitted that first  
595 runoff events will induce major transport after application (Milan et al., 2013), and  
596 then potential toxic effects on aquatic organisms like the bioindicator *R.subcapitata*.  
597 Our high frequency sampling indicated peaks of flufenacet (33 events at UP1 and 4  
598 at UP2) from October until the end of December, suggesting significant chronic  
599 toxicity risks for aquatic organisms. Regarding metazachlor, concentrations were less  
600 variable along the study period (reaching 0.6 µg/L at UP1), but higher concentrations  
601 were observed at the beginning, in accordance with rapeseed treatment (Fig. 3).  
602 Major runoff is expected after rainfall events in treatment periods (Mantzios et al.,  
603 2016a). Both of these compounds are characterized by low to moderate  $K_{oc}$  and are  
604 thus likely to be transported by surface and subsurface runoff during precipitation  
605 events.

606 Regarding total pesticide and TP concentrations and the 44 exceedance events  
607 induced (Table 4 & Fig. 3), it is important to note that they take into account all 67  
608 investigated substances (pesticides and TP). If we consider only parent molecules,  
609 then only 7 samples showed concentrations above the established threshold (5  
610 events at UP2 and 2 at UP1). In both cases, exceeding events were only detected at  
611 UP locations, bringing to the fore the positive effect of the pond on pesticide and TP  
612 mixture contamination in agricultural headwater streams. TP were highly present in  
613 our water samples; their inclusion in regulations, such as EQS, but also in  
614 ecotoxicological risk assessments in the future therefore seems relevant.

615 Nevertheless, many ecotoxicological standards and environmental limits are missing  
616 from the literature and pesticide databases. For instance, INERIS provided  
617 environmental and toxicity thresholds for 68% of the 28 pesticides monitored in the  
618 present study, but only 3% of the 39 TP. Therefore, there is an urgent need to obtain  
619 toxicity data on these compounds whose occurrence has been evidenced in the  
620 natural environment, such as in headwater streams.

621

## 622 **4. Conclusion**

623 Headwater streams are of prime importance when considering the chemical status of  
624 surface water. Our study emphasizes their contamination by pesticides and TP, when  
625 located in an agricultural watershed. We detected a minimum of 5-compounds  
626 mixtures in each sample and highlighted that regardless of the sampling location, TP  
627 represented more than half of the detected and quantified substances. Considering  
628 that their concentrations were, in some samples, higher than those of their parent  
629 molecules, it seems essential to collect data on them in anthropized environments.  
630 Our results showed large pesticide and TP mixtures in water, and suggested several  
631 relevant compounds, such as flufenacet TP, to be included in monitoring lists in the  
632 future.

633 The buffer effect of the pond was brought to the fore by the decrease in the diversity  
634 of substances occurring in downstream water. Indeed, by reducing mixture diversity it  
635 may be able to protect downstream ecosystems from toxicity and the occurrence of  
636 synergic and/or additive effects. Finally, exceedances of guidance and threshold  
637 values were observed during sampling for several pesticide concentrations upstream  
638 from the pond. No exceedances were detected downstream from the pond, but these  
639 results should be interpreted with caution as many guidance values were missing  
640 concerning TP.

641 The question raised, then, relates to underlying processes inducing such an  
642 upstream/downstream difference. A dilution effect should not be ruled out, and  
643 reconciling concentration data with waterflows should make it possible to overcome  
644 the biases induced by this approach. Moreover, intra-pond sampling of the  
645 sedimentary matrix should make it possible in future work to highlight its role in

646 pesticide and TP dynamics, the storage of a substantial part of the contaminants  
647 being a relevant hypothesis.

648 The demonstration of the prolonged presence and high concentration of TP  
649 highlights the importance of their inclusion in ecotoxicological studies, especially  
650 when several substances are combined, which is likely to occur repeatedly and on a  
651 scale as small as the watershed. Regarding the understanding of the fate of  
652 pesticides in the environment, as well as their dynamics, TP analysis is required to  
653 avoid missing this insidious contamination.

### 654 **Funding**

655 This research was carried out within the REPOSE project, funded by the Rhin-  
656 Meuse Water Agency and the French Agency for Food, Environmental and  
657 Occupational Health & Safety (ANSES).

### 658 **Declaration of interest**

659 The authors declare that they have no conflict of interest.

### 660 **Acknowledgements**

661 The authors are sincerely grateful to A. and H. L'huillier for their significant help  
662 during sampling, and for providing access to the study pond. They also thank Mr.  
663 Nicolai Miguel of the Rhine-Meuse Water Agency for his help and advice in the  
664 selection of relevant pesticides and transformation products to be monitored. Finally,  
665 they would like to thank L. Pasquini, C. Hollard, and M-C. Clavos for their work during  
666 sample analysis. Finally, the authors sincerely thank ABC translation for the  
667 proofreading work done.

668

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## 934 **Figure captions**

935 **Graphical abstract** *Color should be used for this figure*

936 **Fig. 1.** a) The study pond is located in the Lorraine Region, upstream from the Seille  
937 River. b) Two sampling stations were located upstream from the pond (i.e., UP1 and  
938 UP2) and a single station was located downstream from the pond (i.e., DO).

939 *Color should be used for this figure.*

940 **Fig. 2.** Summary of maximum and minimum characteristics of upstream (UP1 and  
941 UP2) and downstream (DO) water samples (N = 121, 52, and 64, respectively).  
942 Maximum pesticide and TP concentrations (sum of all pesticides or TP in one  
943 sample) as well as maximum and minimum numbers of pesticides and TP (for  
944 molecules with concentrations > LOD) are shown.

945 *Color should be used for this figure.*

946 **Fig. 3.** Seasonal variations in pesticide and associated transformation product (TP)  
947 concentrations at UP1, UP2, and DO stations over the study period (February to  
948 December 2019). Flufenacet, metazachlor, metolachlor, and sum of pesticide and TP  
949 concentrations per sample are represented as a function of time. Dotted red lines  
950 stand for algae (*R. subcapitata*) acute 72-hour EC<sub>50</sub> (2.04 µg/L) for flufenacet,  
951 environmental quality standard (0.02 µg/L) for metazachlor. Concentrations below the  
952 LOD were set to 0 and those between the LOD and LOQ were set to LOQ/2.

953 *Color should be used for this figure.*

954 **Table 1:** Treatments applied to the watershed between 2017 and 2019 and crops  
955 grown on treated plots.

956

957 **Table 2:** Detection (FD) and quantification (FQ) frequencies of pesticides and TP and  
958 associated detection (LOD) and quantification (LOQ) limits in the two upstream  
959 rivers, at UP1 (N=121), UP2 (N=52), and DO (N=64) stations.

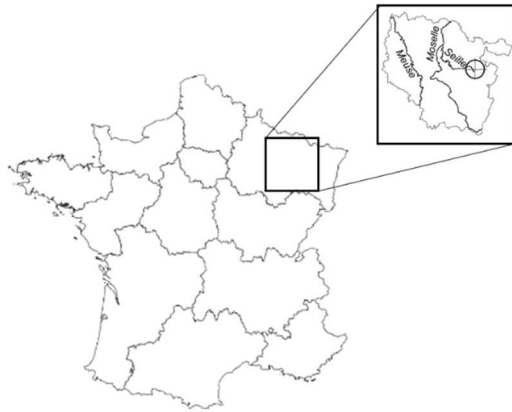
960



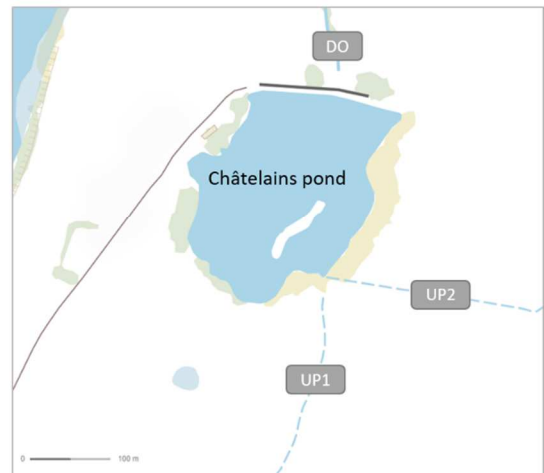
961 **Table 3:** Pesticide and TP mean and maximum concentrations ( $\mu\text{g/L}$ ) at UP1, UP2,  
962 and DO stations and associated reduction rates.

963 **Table 4:** Number of events exceeding environmental/ecotoxicological standards at  
964 UP1, UP2, and DO stations.

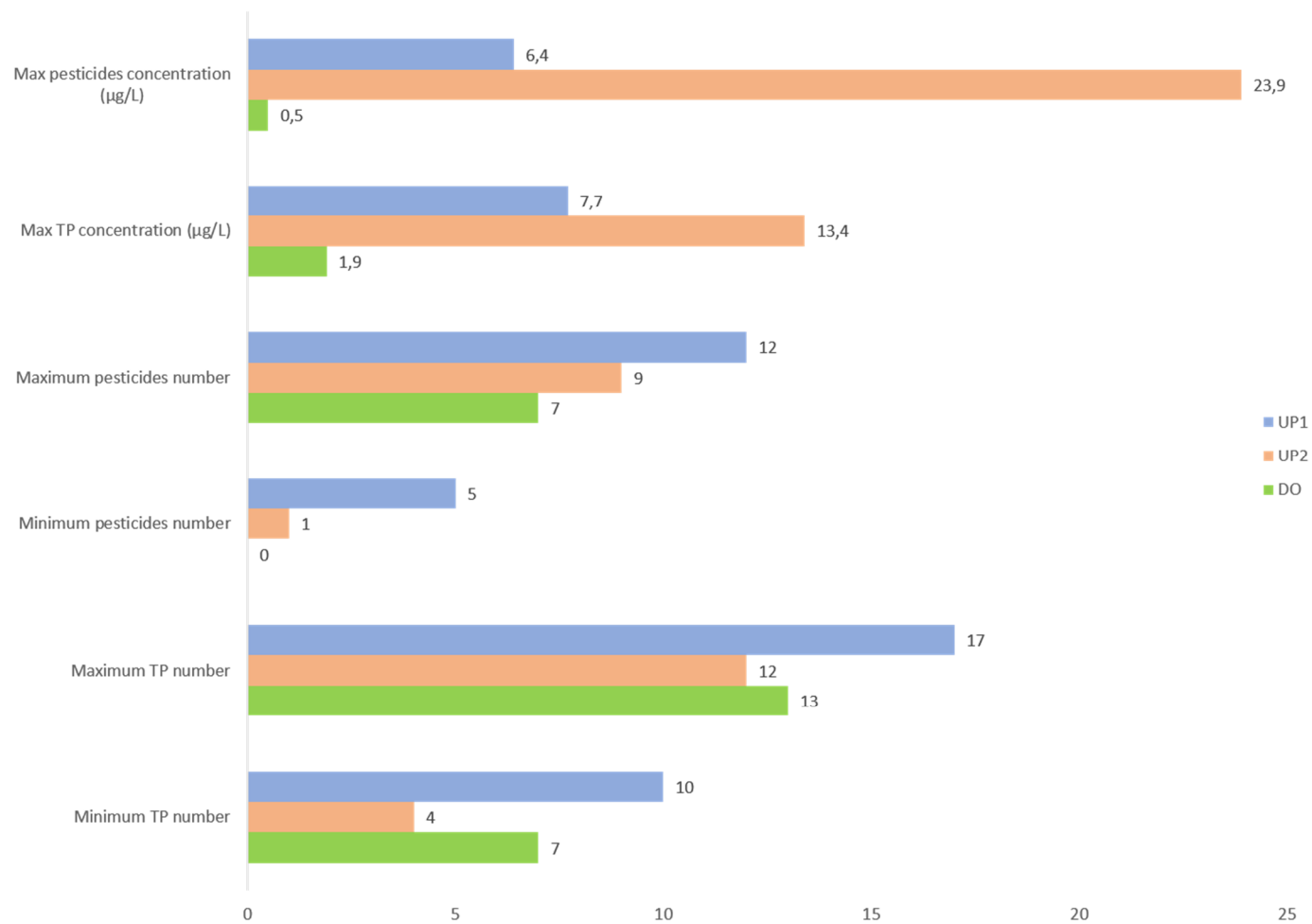
a)



b)

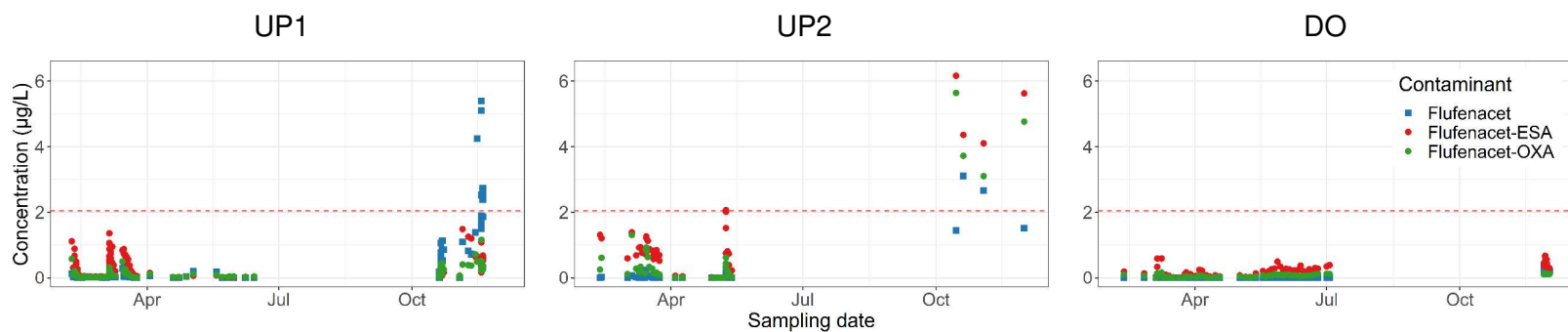


**Fig. 1.** a) The study pond is located in the Lorraine Region, upstream from the Seille River. b) Two sampling stations were located upstream from the pond (i.e., UP1 and UP2) and a single station was located downstream from the pond (i.e., DO).

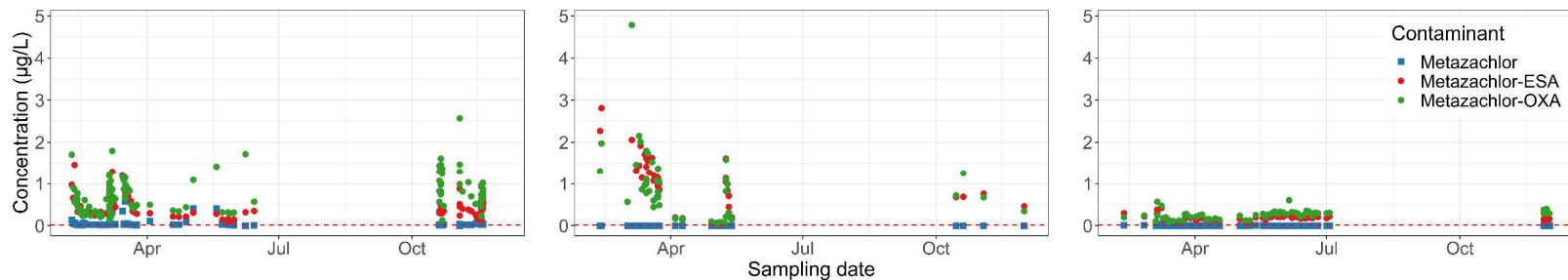


**Fig. 2.** Summary of maximum and minimum characteristics of upstream (UP1 and UP2) and downstream (DO) water samples (N = 121, 52, and 64, respectively). Maximum pesticide and TP concentrations (sum of all pesticides or TP in one sample) as well as maximum and minimum numbers of pesticides and TP (for molecules with concentrations > LOD) are shown.

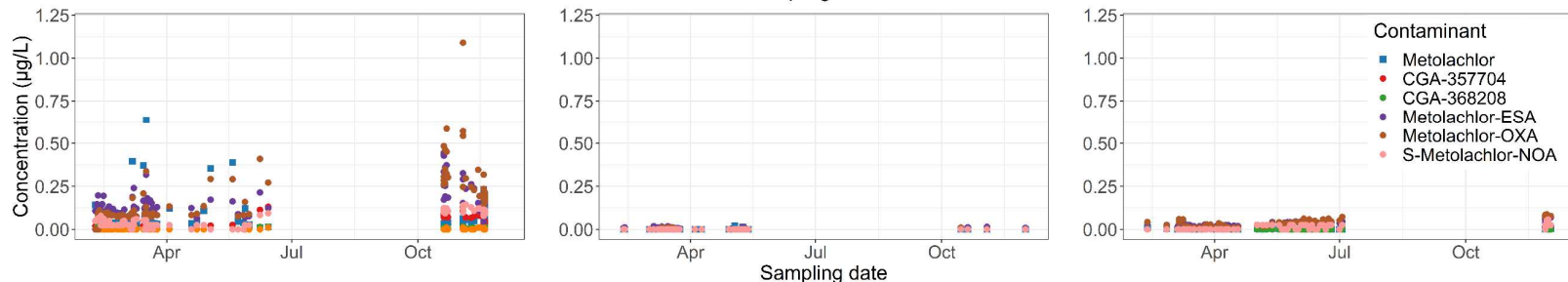
Seasonal variations of flufenacet and associated TP



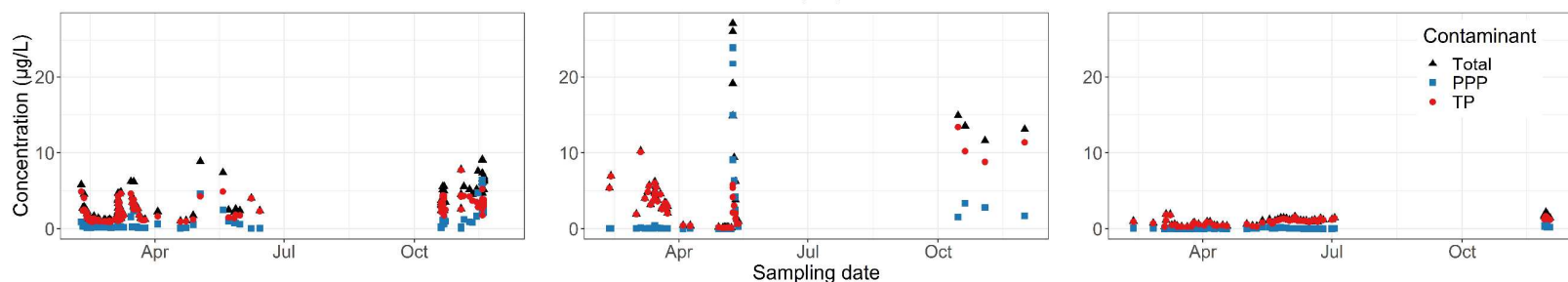
Seasonal variations of metazachlor and associated TP



Seasonal variations of metolachlor and associated TP



Sum of pesticide and TP concentrations per sample over time



**Fig. 3.** Seasonal variations in pesticide and associated transformation product (TP) concentrations at UP1, UP2, and DO stations over the study period (February to December 2019). Flufenacet, metazachlor, metolachlor, and sum of pesticide and TP concentrations per sample are represented as a function of time. Dotted red lines stand for algae (*R. subcapitata*) acute 72-hour  $EC_{50}$  (2.04  $\mu\text{g/L}$ ) for flufenacet, environmental quality standard (0.02  $\mu\text{g/L}$ ) for metazachlor. Concentrations below the LOD were set to 0 and those between the LOD and LOQ were set to LOQ/2.

**Table 1:** Treatments applied to the watershed between 2017 and 2019 and crops grown on treated plots.

Year and crops	Month	Pesticide	Amounts (g)
2017			
Barley and peas			
	April	Napropamide	8100
	August	Glyphosate	10800
	September	Metazachlor	8100
	October	Metazachlor	3360
	October	Prosulfocarb	36000
	October	Clopyralid	600
	October	Prosulfocarb	28800
	October	Esfenvalerate	NA
2018			
Rapeseed and wheat			
	April	Pyroxulam	225
		Florasulam	75
	April	Mesosulfuron-methyl	180
		Iodosulfuron-methyl-sodium	36
	April	Lambda-cyhalothrin	45
	April	Metsulfuron-methyl	36
2019			
Sunflower and wheat			
	April	Pendimethalin	11250
	April	Dimethenamid-P	9547
	May	Nicosulfuron	450
	October	Flufenacet	2880
	October	Flufenacet	2880

**Table 2:** Detection (FD) and quantification (FQ) frequencies of pesticides and TP and associated detection (LOD) and quantification (LOQ) limits in the two upstream rivers, at UP1 (N=121), UP2 (N=52), and DO (N=64) stations.

	LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )	FD UP1 (%)	FD UP2 (%)	FD DO (%)	FQ UP1 (%)	FQ UP2 (%)	FQ DO (%)
<b>Herbicides and TP</b>								
<b>Acetochlor</b>	0.01	0.02	0	0	0	0	0	0
Acetochlor-OXA	0.005	0.01	0	0	0	0	0	0
<b>Alachlor</b>	0.01	0.02	0	0	0	0	0	0
Alachlor-OXA	0.005	0.01	0	0	0	0	0	0
Alachlor-Acetochlor-ESA	0.005	0.01	0	0	0	0	0	0
<b>Atrazine</b>	0.0025	0.005	0	0	0	0	0	0
Atrazine-2-hydroxy	0.005	0.01	87.6	100	100	60.3	94.2	85.9
Desethylatrazine (DEA)	0.005	0.01	0	0	0	0	0	0
DEA-OH	0.005	0.01	0	0	0	0	0	0
Deisopropylatrazine (DIA)	0.005	0.01	0	0	0	0	0	0
DIA-OH	0.01	0.02	0.8	0	0	0.8	0	0
Desethyldeisopropylatrazine (DEDIA)	0.01	0.02	0	0	0	0	0	0
<b>Bentazone</b>	0.0025	0.005	99.2	0	3.13	85.1	0	0
<b>Folpet</b>	NA	NA	NA	NA	NA	NA	NA	NA
Benzamide	0.025	0.05	0.8	0	0	0	0	0
<b>Chloridazon</b>	0.0025	0.005	0	0	0	0	0	0
DPC (desphenyl chloridazon)	0.1	0.2	10	5.8	0	0	1.9	0
Me DPC	0.005	0.01	9.99	0	0	1.7	0	0
<b>Chlorotoluron</b>	0.0025	0.005	51.2	0	39.06	41.3	0	9.4
<b>Dimethachlor</b>	0.0025	0.005	16.5	0	0	13.2	0	0
Dimethachlor-ESA	0.0025	0.005	100	96.2	100	100	82.7	100
Dimethachlor-OXA	0.025	0.05	69.4	61.5	0	53.7	42.3	0
<b>Dimethenamid</b>	0.005	0.01	62	13.5	51.6	47.1	5.8	23.4
Dimethenamid-ESA	0.0025	0.005	99.2	0	85.9	99.2	0	70.3
Dimethenamid-OXA	0.01	0.02	97.5	9.6	57.8	66.1	0	57.8
<b>Flufenacet</b>	0.0025	0.005	92.6	69.2	37.5	91.7	67.3	31.3
Flufenacet-ESA	0.0025	0.005	100	100	100	100	100	100
Flufenacet-OXA	0.005	0.01	100	80.8	93.75	100	78.8	89.1

Table 1 (continued)

	LOD (µg/L)	LOQ (µg/L)	FD UP1 (%)	FD UP2 (%)	FD DO (%)	FQ UP1 (%)	FQ UP2 (%)	FQ DO (%)
<b>Glyphosate</b>	0.033	0.1	69.4	13.5	9.4	28.9	5.8	1.6
AMPA	0.0083	0.025	96.7	40.4	21.9	81.8	5.8	0
<b>Isoproturon</b>	0.0025	0.005	23.1	0	0	18.2	0	0
Desmethyl-Isoproturon	0.0025	0.005	29.8	1.9	12.5	0	0	0
Didesmethyl-Isoproturon	0.0025	0.005	0	0	0	0	0	0
<b>MCPA</b>	0.005	0.01	4.1	21.2	4.7	2.5	17.3	3.1
<b>Metazachlor</b>	0.0025	0.005	99.2	0	21.9	98.3	0	14.1
Metazachlor-ESA	0.01	0.02	100	100	100	100	100	100
Metazachlor-OXA	0.01	0.02	100	100	100	100	100	100
<b>Metsulfuron-Methyl</b>	0.0025	0.005	3.3	25	0	1.7	25	0
Saccharin	0.01	0.02	0.8	17.3	3.1	0	11.5	3.1
<b>Metolachlor</b>	0.0025	0.005	100	15.4	62.5	100	11.5	39.1
Metolachlor-ESA	0.0025	0.005	100	75	100	100	69.2	100
Metolachlor-OXA	0.01	0.02	97.5	17.3	98.4	97.5	0	82.8
CGA-357704	0.01	0.02	71.1	0	21.9	47.1	0	1.6
CGA-368208	0.005	0.01	65.3	0	0	28.9	0	0
CGA-37735	0.0025	0.005	0	0	0	0	0	0
CGA-50267	0.0025	0.005	0	0	0	0	0	0
CGA-50720	0.01	0.02	5.8	0	0	0	0	0
S-Metolachlor-NOA	0.025	0.05	81	0	45.3	52.1	0	3.1
<b>Terbumeton</b>	0.0025	0.005	0	0	0	0	0	0
Terbumeton-Desethyl	0.0025	0.005	0	0	0	0	0	0
<b>Terbuthylazine</b>	0.0025	0.005	17.4	0	12.5	13.2	0	1.6
Terbuth-Desethyl-OH	0.0025	0.005	0	0	0	0	0	0
Terbuthylazine-Desethyl	0.0025	0.005	1.7	0	17.2	0	0	1.6
Terbuthylazine-Hydroxy	0.0025	0.005	0	0	0	0	0	0
<b>Fungicides and TP</b>								
<b>Boscalid</b>	0.0025	0.005	100	100	65.6	99.2	100	9.4
<b>Tebuconazole</b>	0.0025	0.005	100	61.5	96.9	100	25	78.1

**Table 1** (continued)

	LOD (µg/L)	LOQ (µg/L)	FD UP1 (%)	FD UP2 (%)	FD DO (%)	FQ UP1 (%)	FQ UP2 (%)	FQ DO (%)
<b>Insecticides and TP</b>								
<b>Acetamiprid</b>	0.005	0.01	0	0	0	0	0	0
IM-1-4	0.005	0.01	0	0	0	0	0	0
<b>Carbofuran</b>	0.0025	0.005	0	0	0	0	0	0
<b>Clothianidin</b>	0.005	0.01	0	0	0	0	0	0
<b>TZMU</b> (Thiazolyl methylurea)	0.025	0.05	0	0	0	0	0	0
<b>Dimethoate</b>	0.0025	0.005	0	0	0	0	0	0
<b>Imidacloprid</b>	0.005	0.01	1.7	9.6	0	0	5.8	0
Imidacloprid-Urea	0.0025	0.005	0	0	0	0	0	0
Imida-Olefin	0.05	0.1	0	0	0	0	0	0
6-Chloronicotinic-Acid	0.01	0.02	0	0	0	0	0	0
<b>Omethoate</b>	0.0025	0.005	0	0	0	0	0	0
<b>Thiamethoxam</b>	0.005	0.01	0	0	0	0	0	0

NA: Not available, OXA: oxoacetic acid, ESA: ethanesulfonic acid, and NOA: anilino propanoic acid.



**Table 3:** Pesticide and TP mean and maximum concentrations ( $\mu\text{g/L}$ ) at UP1, UP2, and DO stations and associated reduction rates.

	Mean <sup>a</sup> UP1 $\pm$ SEM	Mean UP2 $\pm$ SEM	Max UP1 <sup>a</sup>	Max UP2	Mean DO $\pm$ SEM	Max DO	Reduction rates <sup>b</sup> (mean/max)
<b>Herbicides and TP</b>							
Atrazine-2-hydroxy	-	0.021 $\pm$ 0.002	0.05	0.05	0.0243 $\pm$ 0.002	0.04	-16/20
DIA-OH	-	-	0.09	0	0	0	100/100
Bentazone	0.0086 $\pm$ 0.0006	-	0.05	0	-	0.003	NA/94
Folpet	NA	NA	NA	NA	NA	NA	NA/NA
Benzamide	-	-	0.003	0	0	0	100/100
Chloridazon	-	-	0	0	-	0	NA/NA
DPC	-	-	0	0.2	0	0	100/100
Me-DPC	-	-	0.02	0	0	0	100/100
Chlorotoluron	-	-	0.06	0	-	0.009	NA/85
Dimethachlor	-	-	0.01	0	0	0	100/100
Dimethachlor-ESA	0.0522 $\pm$ 0.003***	0.0707 $\pm$ 0.008***	0.2	0.2	0.0196 $\pm$ 0.0008	0.04	63/80
Dimethachlor-OXA	-	-	0.1	0.2	0	0	100/100
Dimethenamid	-	-	0.7	0.03	-	0.2	NA/71
Dimethenamid-ESA	0.114 $\pm$ 0.01	-	1	0	-	0.05	NA/95
Dimethenamid-OXA	-	-	1.5	0.01	-	0.1	NA/93
Flufenacet	0.529 $\pm$ 0.09	-	5.4	3.1	-	0.5	NA/91
Flufenacet-ESA	0.371 $\pm$ 0.03**	0.992 $\pm$ 0.2***	1.5	6.2	0.244 $\pm$ 0.02	0.7	34/53
Flufenacet-OXA	0.169 $\pm$ 0.02***	-	1.2	5.6	0.0719 $\pm$ 0.006	0.2	58/83
Glyphosate	-	-	1.7	0.2	-	0.1	NA/94
AMPA	0.0739 $\pm$ 0.007	-	0.6	0.05	-	0.01	NA/98
Isoproturon	-	-	0.03	0	0	0	100/100
Desmethyl-isoproturon	-	-	0.003	0.003	-	0.003	NA
MCPA	-	-	1.4	23.7	-	0.02	NA/99
Metazachlor	0.0473 $\pm$ 0.007	-	0.6	0	-	0.02	NA/97
Metazachlor-ESA	0.459 $\pm$ 0.02***	0.871 $\pm$ 0.09***	1.5	2.8	0.179 $\pm$ 0.008	0.4	61/73
Metazachlor-OXA	0.728 $\pm$ 0.04***	0.816 $\pm$ 0.1***	2.6	4.8	0.263 $\pm$ 0.01	0.6	64/77
Metsulfuron-Methyl	-	-	0.02	0.5	0	0	100/100
Saccharin	-	-	0.01	0.06	-	0.06	NA/-500

**Table 2 (continued)**

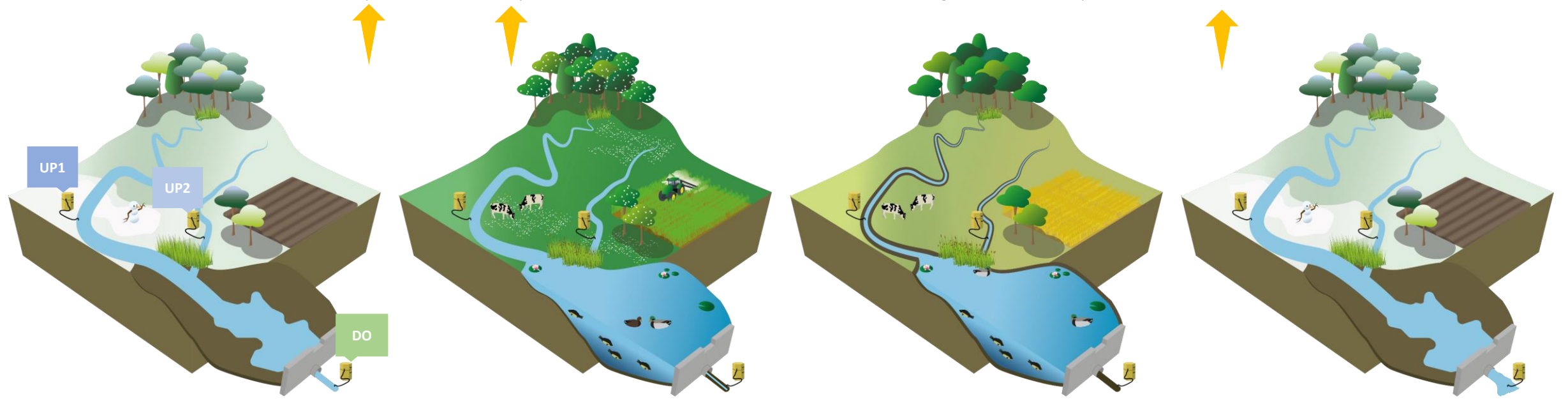
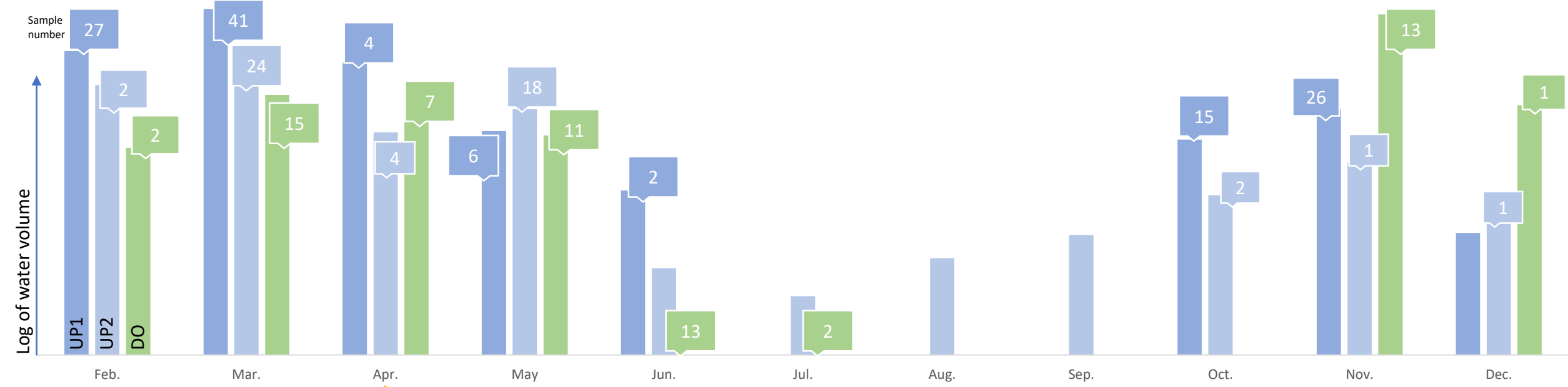
	Mean <sup>a</sup> UP1 ± SEM	Mean UP2 ± SEM	Max UP1 <sup>a</sup>	Max UP2	Mean DO ± SEM	Max DO	Reduction rates <sup>b</sup> (mean/max)
Metolachlor	0.0752 ± 0.008	-	0.6	0.02	-	0.03	NA/95
Metolachlor-ESA	0.156 ± 0.008***	-	0.6	0.02	0.0394 ± 0.002	0.08	75/87
Metolachlor-OXA	0.169 ± 0.01***	-	1.1	0.001	0.0422 ± 0.003	0.009	75/99
CGA-357704	-	-	0.1	0	-	0.02	NA /80
CGA-368208	-	-	0.02	0	0	0	100/100
CGA-50720	-	-	0.01	0	0	0	100/100
S-Metolachlor-NOA	-	-	0.1	0	-	0.06	NA/40
Terbutylazine	-	-	0.01	0	-	0.006	NA/40
Terbutylazine-desethyl	-	-	0.003	0	-	0.01	NA/-233
<b>Fungicides and TP</b>							
Boscalid	0.0128 ± 0.0007	0.0436 ± 0.004	0.05	0.1	-	0.006	NA/88
Tebuconazole	0.052 ± 0.006	-	0.5	0.01	-	0.02	NA/96
<b>Insecticides and TP</b>							
Imidacloprid	-	-	0.005	0.02	0	0	100/100


<sup>a</sup> For the calculation of mean and maximum concentrations at UP1, UP2, and DO, a non-detected molecule was set to 0 and an unquantified molecule (above the LOD) was set to half its LOQ, <sup>b</sup> Reduction rates were calculated regarding mean/max pesticide and TP concentrations (%). UP1 concentrations were used when different from UP2 concentrations, in order to take into account the major water input, \* Molecules having mean concentrations significantly different between UP1 and DO and/or UP2 and DO (\**p*-value < 0.05, \*\* *p*-value < 0.01, \*\*\* *p*-value < 0.001).

**Table 4:** Number of events exceeding environmental/ecotoxicological standards at UP1, UP2, and DO stations.

	Flufenacet	MCPA	Metazachlor	Metsulfuron-methyl	
<b>Sampling station</b>					
UP1	33	13	1	90	0
UP2	4	2	8	0	8
DO	5	0	0	0	0
<b>Standard limit (µg/L)</b>	0.357 <sup>a</sup>	2.04 <sup>b</sup>	0.5 <sup>c</sup>	0.019 <sup>d</sup>	0.036 <sup>e</sup>

<sup>a, e</sup> Regulatory acceptable concentration (RAC), <sup>b</sup> Algae (*Raphidocelis subcapitata*) acute 72-hour EC<sub>50</sub> (µg/L), <sup>c, d</sup> Environmental quality standard (WFD, 2000/60/CE)



 Pesticide treatments