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1 **Exposure of wild mammals to glyphosate, AMPA, and glufosinate: a case for “emerging organic**  
2 **contaminants”?**

3

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29

30 **Abstract**

31 Glyphosate (GLY) is the most widely used herbicide worldwide and its use is still increasing.  
32 Accumulating evidences show that GLY and its metabolite aminomethylphosphonic acid (AMPA) are  
33 more persistent and toxic than expected, but little is known about risks to wildlife. Glufosinate (GLUF)  
34 was considered as an alternative broad-spectrum herbicide but its field ecotoxicology has rarely been  
35 studied. This study aims at assessing the exposure to GLY, AMPA, and GLUF of free-ranging rodents  
36 and shrews from treated (cereals under conventional farming) and nontreated habitats (cereals under  
37 organic farming and hedgerows) in France through residue analyses in hair. We investigated the  
38 patterns of accumulation according to species, habitats, and treatment intensity at plot, landscape, or  
39 township scale. We showed a generalized exposure of small mammals to GLY, AMPA and GLUF as they  
40 were detected in all species, in 64%, 51% and 44% of hair samples, respectively. The detection and  
41 levels of GLY, AMPA and GLUF were higher in herbivorous and granivorous voles than in insectivorous  
42 shrews and omnivorous wild mice. The three compounds showed comparable ranges of  
43 concentrations but the highest were reached for GLY (0.018-7.74 pg/mg with a maximum of 522  
44 pg/mg). The frequencies of detection and concentrations did not significantly differ according to  
45 farming practices or proxies of pesticide treatment intensity. The concentrations of GLY were higher  
46 in individuals captured in hedgerows than in cereal fields. Based on dose reconstruction approaches  
47 and toxicological thresholds from the literature, GLY and GLUF levels may be associated to risk in small  
48 mammals and endanger local populations. Our findings raise issues about the omnipresence of GLY,  
49 AMPA and GLUF in agricultural landscapes including in animals from habitats considered as refuges,  
50 questioning their ecological safety. This work provides new insights into current broad-spectrum  
51 herbicide wildlife ecotoxicology that may support decision making to protect biodiversity.

52

53 **Keywords:** agro-ecosystem; bioaccumulation; currently used pesticides; landscape ecotoxicology;  
54 wildlife toxicology

55

## 56 **1. Introduction**

57 Since its registration as a broad-spectrum contact herbicide in 1974, the use of glyphosate (GLY)  
58 continuously increased, making GLY-based pesticides the most widely applied herbicides worldwide  
59 nowadays (Gandhi et al. 2021; Richmond 2018). Maggi et al. (2020) showed that GLY and its metabolite  
60 aminomethylphosphonic acid (AMPA) may be persistent contaminants in about 30% and 93% of global  
61 cropland soils, respectively. Based on persistence, accumulation and toxicity criteria, they computed  
62 that 1% of croplands worldwide (i.e. about 385,000 km<sup>2</sup>) undergoes mid-high to high hazard. Outside  
63 these hotspots, the contamination is expected to be low but globally pervasive (Maggi et al. 2020).

64 Initially GLY has been marketed as an “environmentally friendly” herbicide, due to its low expected  
65 environmental persistence (easily bio-degraded), low predicted toxicity to wildlife and human  
66 (absence of the shikimate pathway, that is GLY target, in animals), and lack of potential for  
67 bioaccumulation (hydrophilic, low  $K_{ow}$ , poorly metabolised and rapidly eliminated in mammals) (EFSA  
68 2015a; Gandhi et al. 2021; Kissane and Shephard 2017; Richmond 2018). However, concerns have been  
69 rising within the last decades about environmental safety and risks for health associated to GLY,  
70 especially in a context of continuously increasing use (Gandhi et al. 2021; Székács and Darvas 2018;  
71 Van Bruggen et al. 2018; Vandenberg et al. 2017). GLY and AMPA are more persistent in the  
72 environment than assessed initially, as shown by ubiquitous occurrence of residues in soils, waters,  
73 and air (Bai and Ogbourne 2016; Gandhi et al. 2021; Richmond 2018; Van Bruggen et al. 2018; Xu et al.  
74 2019). They can accumulate in environmental compartments, crops, and human food chain (Bai and  
75 Ogbourne 2016; Richmond 2018). They are associated to risks for chronic low-dose exposure in animals  
76 and human, and can be more harmful to ecosystems than expected (Gandhi et al. 2021; Kissane and  
77 Shephard 2017; Ojelade et al. 2022; Peillex and Pelletier 2020; Richmond 2018). Furthermore, GLY and  
78 AMPA may be more bioaccumulative than predicted from their physico-chemical properties (Pelosi et  
79 al. 2022; Richmond 2018; Ruuskanen et al. 2020; Serra et al. 2021). However, the controversial issues  
80 regarding GLY-based herbicides mostly focused on human health (Székács and Darvas 2018;

81 Vandenberg et al. 2017), while GLY and AMPA exposure and impacts in wildlife have been overlooked  
82 (Kissane and Shephard 2017).

83 In the USA and in EU, the registration reviews concerning GLY use as a plant protection product (PPP)  
84 identified potential risk to terrestrial and aquatic plants and non-target terrestrial arthropods, and risks  
85 to mammals and birds (EFSA 2015b, 2015c, 2015a; US EPA 2020). In the EU assessment for vertebrates,  
86 the taxa identified the most at risk were mammals, in particular herbivorous mammals (EFSA 2015b,  
87 2015a). Glufosinate (GLUF) is, as GLY, an organophosphorus compound used as a broad-spectrum non-  
88 selective contact herbicide with a systemic action and was considered as “*one of the very few*  
89 *alternatives to glyphosate*” (European Commission 2017). Risks for herbivorous and insectivorous  
90 mammals to the herbicidal use of GLUF have however been reported in the environmental risk  
91 assessment (ERA) processes (EFSA 2005, 2012). GLUF was classified as a candidate for substitution in  
92 EU according to the Commission Regulation EC No 1107/2009 (PPP Regulation) in 2015 and the  
93 regulatory exclusion was scheduled at its end of approval in EU in 2018 (Robin and Marchand, 2023).  
94 However, GLUF is still used in many countries worldwide.

95 The singular physico-chemical properties of GLY (a particularly hydrophilic polar compound, only in  
96 ionic form, making it difficult to extract) and closely related compounds such as AMPA or GLUF render  
97 challenging the measurement of their residues, and prevent these compounds to be included in multi-  
98 residue analyses (Delhomme et al. 2021). Such technical limitations in analytical chemistry may have  
99 hampered the developpement of studies about GLY, AMPA and GLUF exposure and effects in wildlife,  
100 and more generally knowledge building about the field terrestrial ecotoxicology of these compounds.  
101 Ultimately, this lack of knowledge could be an impediment for regulatory decisions with regard to the  
102 objectives of reducing the risks and impacts of pesticide use on human health and the environment  
103 (e.g. Directive 2009/128/EC). Assessing and characterizing exposure of wildlife to GLY, AMPA and GLUF  
104 is therefore a critical issue for environmental protection and biodiversity conservation.

105 The aim of this study was to investigate wildlife exposure in an arable landscape to GLY, AMPA and  
106 GLUF using a lowly invasive sampling method based on residue analyses in hair, and focusing on small  
107 mammal species having various ecological traits (rodents and shrews). The compound residues can be  
108 detected and quantified in hair, due to the effectiveness of this matrix to assess exposure to multiple  
109 pesticides in wildlife (Fritsch et al. 2022; Krief et al. 2017), and as shown for GLY in bats (Hooper et al.  
110 2022) and both GLY and AMPA in humans (Alvarez et al. 2022). Moreover, GLY concentrations were  
111 relative to the intensity of exposure in human samples (i.e. GLY was quantified at lower concentrations  
112 in hair samples from nonoccupationally exposed subjects than in hair from farmers who often used  
113 GLY) (Alvarez et al. 2022). We expect exposure to GLY to be higher than to the other compounds.  
114 Indeed, GLY is more used and is still authorized in France contrarily to GLUF (BNVD 2022). Mammals  
115 can be exposed to herbicides through diet and overspray (European Commission, 2015; Martinez-Haro  
116 et al. 2022; Mayer et al. 2020; Newton et al. 1984), therefore small mammals may be exposed to GLY  
117 through a higher number of pathways than in the case of AMPA. Herbivorous voles were expected to  
118 exhibit a greater exposure than granivorous or insectivorous species according to higher daily dietary  
119 doses estimated during ERA processes (EFSA 2005, 2012; European Commission 2015). Moreover,  
120 herbivorous voles are less mobiles than omnivorous mice and voles or shrews (van den Brink et al.  
121 2011; Wijnhoven et al. 2006), and may not avoid treated areas. We expect omnivores and  
122 carnivores/insectivores/vermivores to be exposed as well because residues of GLY, AMPA and GLUF  
123 have been found in earthworms that are dietary items of these trophic groups (Pelosi et al. 2022), and  
124 GLY residues have been found in small carnivorous and omnivorous mammals in a forest ecosystem  
125 (Newton et al. 1984). Higher levels of exposure were expected in treated areas (i.e. conventional crops)  
126 than in non-targeted habitats (i.e. organic farming (OF) fields and hedgerows). Since small mammals  
127 are mobile species the influence of herbicide use intensity was also considered at landscape scale (i.e.  
128 using the proportion of OF fields within the landscape as a proxy) and at township level (i.e. using sales  
129 of active substances as a proxy of usage intensity).

## 130 2. Material and methods

### 131 2.1. Study sites

132 The study site was the Long-Term Socio-Ecological Research (LTSER) “Zone Atelier Plaine & Val de  
133 Sèvre” (ZAPVS, <https://za-plaineetvaldesevre.com/>) in mid-Western France (see (Bretagnolle et al.  
134 2018) for a general site description). Sampling was conducted in Spring 2018 (May-June). The sampling  
135 design was based on 40 landscape windows of 1 km<sup>2</sup> in which sampling by trapping was conducted in  
136 an arable cereal field (winter cereals), and a woody patch (hedgerow or woodlot edge) as close as  
137 possible to the sampled arable field. Cropped cereals and hedgerows were referred to as “habitats”  
138 hereafter. Cropped fields in which the sampling was carried out were cultivated under conventional  
139 farming (CF) or organic farming (OF). The farming practices in the organic fields respected the rules of  
140 the “AB (Agriculture Biologique) France label” and were under OF for at least three years at the time  
141 of sampling. A few fields were in transition from CF to OF and were referred to as “OF/CF”.

### 142 *Sampling*

143 Small mammals were captured in each habitat in each landscape window using lines of non-lethal traps  
144 (25 traps, 2 meter-spaced) with dormitory (Le Quilliec and Croci 2006) filled with hay and food (i.e.  
145 pieces of carrots and apples, sunflower seeds, cat food and peanut butter). Species were determined  
146 during handling of individuals alive based on morphometrics, and confirmed by molecular analyses in  
147 case of doubt (see below). A sample of 50-100 mg of hair was taken by shaving over the posterior part  
148 of the back, wrapped in aluminium foil and stored in plastic zip-lock bags at room temperature during  
149 the field session and later at -20°C until analysis. Individuals were released after handling. For animals  
150 that did not survive to the capture, bodies were collected and stored at -20°C until they were shaved  
151 for hair sampling. Because *M. arvalis* and *M. agrestis* are morphologically similar species, identification  
152 of *Microtus* voles was confirmed by molecular analyses (Sup Info Annex 1. Text A.1). The suspected  
153 *Mus spp* individuals were as well confirmed genetically. For that, a tiny piece of ear was sampled (using  
154 medical clean and disinfected scissors, followed by disinfection of the ear) and stored by freezing (-  
155 20°C) until DNA extraction.

156 The experimentation was performed under the authorization of the French National Ethical Committee  
157 (Project APAFIS N°5340) by skilled and experienced investigators from Chrono-environnement  
158 research department (EU0592), following directive 2010/63/EU on the protection of animals used for  
159 scientific purposes. All precautions to limit as much as possible stress and deleterious effects on  
160 animals were taken. Sampling and handling of small mammals was conducted under the supervision  
161 and with the participation of people authorized for animal experimentation and experienced in small  
162 mammal capture and handling, and using appropriate and authorized (EU0592) facilities (vehicle  
163 equipped with mobile anaesthesia unit, and all materials and equipment required for animal welfare).  
164 The sample size resulted in 61 individuals captured in 29 traplines distributed over 25 landscape  
165 windows out of the 40 landscape windows initially surveyed (Sup Info Annex 1. Table A.1). Individual  
166 samples of 18 wood mice (*Apodemus sylvaticus*), 14 greater white-toothed shrews (*Crocidura russula*),  
167 16 common voles (*Microtus arvalis*), 11 bank voles (*Myodes glareolus*), and two house mice (*Mus*  
168 *musculus*) were analyzed. A total of 41 individuals were captured in habitats under CF farming, 10 in  
169 the category OF/CF, and 10 in OF. A total of 39 individuals were collected in hedgerows or woodlots  
170 and 22 in cereal fields.

### 171 *Proxies related to the intensity of treatments at various spatial scales*

172 First of all, to investigate the role of agricultural practices in shaping exposure of small mammals,  
173 habitats were classified as cultivated under OF or CF, according to the type of agriculture organic or  
174 conventional practiced in cultivated fields. A few fields were in transition from CF to OF and were  
175 referred to as "OF/CF" and considered as treated to conservatively analyze the data. Hedgerows and  
176 woodlots are not supposed to be targeted by pesticide treatments, but they may be unintentionally  
177 contaminated due to drift or run-off (Gandhi et al. 2021), and the homerange of small mammals can  
178 overlap both the woody patches and the surrounding cropped habitats. In order to classify hedgerows  
179 or woodlots with regards to farming practices, the adjacent plots were considered and each woody



180 patch was classified “OF” if none of the adjacent fields was under CF, “OF/CF” if at least one of the  
181 adjacent fields was under CF, and “CF” when all adjacent fields were under CF.

182 For the proxies of treatment intensity, at the plot scale (arable field or woody patches where trapping  
183 was realized), a general proxy of potential for pesticide treatment was computed. The fields cultivated  
184 under OF and hedgerows/woodlots were considered as not intentionally targeted by treatments (NT)  
185 while the arable fields under CF and transitional fields from CF to OF were considered as possibly  
186 targeted (T). A second proxy to characterize the intensity of potential treatments was computed at the  
187 landscape scale. The location and boundaries of cultivated plots in 2018 were obtained from the official  
188 French “Registre Parcellaire Graphique” (“[https://www.data.gouv.fr/fr/datasets/parcelles-en-  
189 agriculture-biologique-ab-declarees-a-la-pac](https://www.data.gouv.fr/fr/datasets/parcelles-en-agriculture-biologique-ab-declarees-a-la-pac)”). The software Quantum GIS (QGIS 3.16) was used to  
190 calculate the proportion of OF plots in landscape, which was computed in each landscape windows of  
191 1 km<sup>2</sup> as the proportion of the total surface occupied by OF arable fields. The final proxy resulted in a  
192 two-level factor, with a category “OF dominant” with proportion varying from 50 to 100% ([49-79], 6  
193 landscape windows) in which treatment intensity at the landscape scale was expected as lower since  
194 arable fields not targeted by pesticide treatments prevailed within the landscape mosaic. The second  
195 category “CF dominant” gathered windows with landscape OF proportion ranging from 0 to 50% ([0-  
196 49], 19 landscape windows). Finally, to consider the intensity of potential treatments over the range  
197 of several farmlands, a proxy at the township scale was computed. The sales of active substance (a.s.)  
198 were obtained from the “Banque Nationale des ventes de produits phytopharmaceutiques par les  
199 Distributeurs agréés (BNVD)” as released to the public by official agencies at township level (i.e.  
200 according to postal code, "<https://geo.data.gouv.fr/fr/datasets>"). The data were extracted for 2017  
201 and 2018 (the year before and the year when sampling was conducted). The sales of GLY and GLUF  
202 were associated to each trapping location based on township positioning. The sales of GLY were highly  
203 correlated between years at the township level ( $R^2=0,950$ ), rendering impossible to study separately  
204 the influence of potential use intensity in the year before or in the year of sampling. The marketing  
205 authorisation of pesticides containing GLUF has been withdrawn in France by October 2017. The

206 quantities sold in 2017 were very low (i.e. 0.75 kg) and sporadic (i.e. only one level quantity reported  
207 as sold in the townships studied) while in 2018, no sale of GLUF was reported in the townships of the  
208 study area of concern. The quantities sold in 2017 and 2018 were thus summed for each a.s., and  
209 classified in two categories. For GLUF, the categories were “0” and “0.75”. For GLY, the median of the  
210 summed quantities in the dataset was calculated at 7300, and the categories were set at “lower than  
211 7000” ([4138-7000[, 2 townships) and “higher than 7000” ([7000-9366[, 3 townships).

## 212 *2.2. Chemical analyses of glyphosate, AMPA and glufosinate*

### 213 *Chemicals and Reagents*

214 Methanol Pesti-S grade and water ULC-MS grade were purchased from Biosolve (Dieuze, France).  
215 Sodium dodecyl sulfate, FMOCl Chloride, sodium tetraborate decahydrate and glyphosate, glufosinate  
216 ammonium, (Aminomethyl)phosphonic acid and glyphosate-1,2-13C<sub>2</sub>,15N standards were purchased  
217 from Sigma Aldrich (Diegem, Belgium).

### 218 *Glyphosate, glufosinate and AMPA analysis*

219 In order to differentiate compounds biologically incorporated into hair from chemicals externally  
220 deposited on hair surface due to contaminated air or dust, samples were decontaminated with sodium  
221 dodecyl sulfate solution and with methanol (Duca et al. 2014). After decontamination, samples (50mg)  
222 were spiked with 5 µL internal standard solution and 1mL of water were added before overnight  
223 extraction at 40°C with 350 rpm agitation. Supernatants were evaporated to dryness at 50°C under N<sub>2</sub>  
224 flow and 100 µL of borate buffer and FMOCl were added before derivatization and injection into the  
225 LC-MS/MS Waters system Acquity UPLC H-class system coupled to Waters Xevo TQ-S triple quadrupole  
226 mass spectrometer equipped with a Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm particle  
227 size). With each analytical run, quality controls consisting in hair samples supplemented at several  
228 levels of concentration (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 pg/mg) in order to control for possible  
229 sensitivity drift, plus blank (sample without the target chemicals) in order to verify possible cross-  
230 contamination.

231 The concentrations were expressed as pg/mg hair. Limits of quantification (LOQ) and lowest detected  
232 values (LOD) are presented in Table 1. The limit of detection was set at the lowest detected value. All  
233 detected concentration values, involving values between the LOQ and the LOD, were included as  
234 continuous data. The use of concentrations below the LOQ has been shown as a suitable method  
235 preferable to the absence of data, which shows better efficiency in terms of bias and precision than  
236 several other recognized established methods (Keizer et al. 2015).

### 237 *2.3. Dose reconstruction to assess risk for deleterious effects in small mammals*

238 Providing concentrations of GLY, AMPA and GLUF in keratinized tissues of small mammals, our study  
239 allows enlightening exposure of the animals. However, the toxicological meaning of such hair  
240 concentrations is hard to interpret with regards to toxicity benchmarks (which are expressed as daily  
241 dose in mg active substance per kg body weight) because relationships between residues in hair and  
242 exposure doses or between residues in hair and in other organs are not known. To overcome this  
243 gap and assess whether exposure levels could reach toxic levels, approaches of reverse dosimetry have  
244 been applied to estimate levels of exposure in terms of daily dose intake, in order to get data  
245 comparable to toxicological thresholds. Different types of data (i.e. concentrations in mammal body  
246 fluids and hair, residues in viscera and body burden in small mammals, toxicokinetics in small mammal  
247 blood, pharmacokinetics of fast-elimination chemicals) that may be used for such purpose have been  
248 collected from the literature (Sup Info Annex 1. Text A.2 for full description), leading to different  
249 approaches of back-calculations computed under several assumptions in case of lack of available data.  
250 Five different approaches could be computed for GLY and one for GLUF (Table 2).

251 The objective of our risk assessment was to get overall orders of magnitude of daily dose intake relative  
252 to the concentrations measured in hair to evaluate whether wild small mammals may experience  
253 deleterious toxicological effects. The lack of data and assumptions that had to be applied hamper the  
254 calculated dose values to be accurate. The interpretation of the findings was thus developed in order  
255 to consider ranges and global patterns rather than refined (but biased) toxicological assessments. The

256 doses calculated were compared with toxicity data using the usual and regulatory procedure of  
257 Toxicity-Exposure Ratio (TER) calculation with associated triggers of 5 for chronic toxicity data and of  
258 10 for acute toxicity data. The doses were reconstructed for each species, using four levels of residue  
259 concentrations measured in hair: minimum, median, 3<sup>rd</sup> quartile and maximum values (only one value  
260 available in house mice for GLY, and in house mice and shrews for GLUF). The dose of GLY was  
261 compared to five toxicological thresholds: the short-term dietary no observed effect level (short-term  
262 dietary NOEL), the chronic 21 days no observed adverse effect level (chronic NOAEL, which is the same  
263 value than reproductive NOAEL), the long-term NOAEL, the No Mortality Dose to the Oregon vole, and  
264 a “Chronic Low Dose” limit. Details about the values of the thresholds are provided in Sup Info Annex  
265 1. Text A.2. The dose of GLUF was compared to four available toxicological thresholds: the acute oral  
266 median lethal dose, the short-term dietary NOEL, the chronic 21 days NOAEL, and the long-term  
267 NOAEL.

268 The process ended in TER values computed for each approach (i.e. back-calculations based on  
269 concentrations in human urine and hair, body burden in rats, toxicokinetics in rat blood, residues in  
270 viscera and body of wild small mammals, prediction of incorporation in hair from pharmacokinetics),  
271 under different scenarii of assumption, for the different levels of residue concentrations measured in  
272 hair (minimum, median, 3<sup>rd</sup> quartile and maximum values), for each species, for the different  
273 toxicological thresholds (see details in Sup Info Annex 2). Finally, the number of cases when TER  
274 exceeded the triggers, which is supposed to highlight a risk for toxic effects in small mammals, and the  
275 number of individuals concerned were summarized.

#### 276 *2.4. Data analysis and statistics*

277 Analyses were performed using R (3.3.1), with the additional packages “AICcmodavg”, “effects”,  
278 forestmodel”, “ggeffects”, “ggplot2”, “gtsummary”, “multcomp”, “pgirmess”, and “questionr”.

279 As a first step, exploratory analyses were performed on the whole dataset of detection and  
280 concentration, with non-detect set to zero value, using non-parametric tests because statistical

281 distributions were skewed and zero-inflated. Correlations between raw values of the concentrations  
282 of the three compounds were investigated using Spearman's rank correlation tests. The differences in  
283 raw values of the concentrations between species, habitats, farming practices, and the proxies of  
284 treatment intensity were tested using Wilcoxon-Mann-Whitney test or Kruskal-Wallis test adequately  
285 depending on the number of levels of the factors (i.e. two or more). Post-hoc multiple comparison  
286 tests after Kruskal-Wallis tests were used to investigate pairwise-comparisons when relevant.  
287 Correlations between concentrations and landscape OF proportions or sales of active substances used  
288 as raw continuous data were tested using Spearman's rank correlation test. This preliminary set of  
289 analyses on concentrations with no detection set at 0 value showed (1) that significant correlations  
290 were identified between GLY, AMPA, and GLUF, and (2) that concentrations differed significantly  
291 according to species, while no other significant differences were found depending on habitat, farming  
292 practices or proxies of treatment intensity. Owing to such a species influence and because sample size  
293 by species was in several cases unbalanced according to the other factors (unbalanced capture success,  
294 Sup Info Annex 1. Table A.1), further analyses were conducted taking species into account in statistics.  
295 Since more than 2 individuals were obtained from the same trapline in only 8 traplines over the 29  
296 where animals were captured, pseudo-replication was considered not an issue. Moreover, the  
297 traplines extended over 50 meters, which should limit the influence of spatial auto-correlation  
298 between individuals. Thus, generalized linear models (GLMs) were preferred to generalized linear  
299 mixed effect models. The relationships between the concentrations of each compound were further  
300 studied according to species using GLMs on log-transformed data ( $\log(x+1)$ ) including non-detect  
301 values set at zero to meet the assumptions of normality and variance homogeneity. Models were  
302 checked for homogeneity of variance, normality of error and linearity/additivity and leverage was  
303 checked (i.e. standardized residuals vs leverage). An extreme outlier of GLY concentration was  
304 identified (one *Microtus* vole) as having overinfluence on the estimated parameters (i.e. Cook distance  
305 larger than 1). This outlier was removed from the dataset to compute model outputs and graphics in  
306 this part of the analyses.

307 Further statistical analyses were then performed on (1) GLY, AMPA or GLUF frequency of detection  
308 and (2) GLY, AMPA or GLUF concentrations quantified above the LOD (i.e. in samples where the  
309 compound was detected, without non-detect). GLMs with the factors “species”, “habitat”, and “type  
310 of farming” with interactions were used as explanatory factors in a first set of models and then  
311 “species” and the “proxies of treatment intensity” with interactions were used as explanatory factors  
312 in a second set of models. The analyses were separated in the two sets described above because of  
313 lack of indepeny between the two sets of factors. First, full models with all first-order interactions  
314 were run. Since none of the interaction was statistically significant, models without interactions better  
315 fitted the data according to corrected Akaike Information Criterion (AICc) comparisons, and due to  
316 numerous NA’s the final models used to compute statistics were run without interactions.

317 The influence of species, habitat, farming practices and proxies for treatment intensity on the  
318 detection frequency of GLY, AMPA or GLUF was investigated using binomial GLMs (link = logit). The  
319 detection was coded as “0” for non-detect and “1” when detected. The models were built using species  
320 alone and species with each one of the other variables as explanatory factors. The significance of  
321 factors was checked by *chi-squared* test. Odds ratios with confidence intervals were computed to  
322 characterize differences between levels. The influence of species, habitat, farming practices and  
323 proxies for treatment intensity on the concentrations of GLY, AMPA or GLUF in samples showing value  
324 above the LOD was investigated using GLMs on log-transformed data to meet the assumptions of  
325 normality and variance homogeneity. The models were built using species alone and species with each  
326 one of the other variables as explanatory factors. The significance of explanatory factors was checked  
327 by *F*-test and statistical differences between levels were checked based on *t*-test.

### 328 **3. Results & Discussion**

#### 329 *3.1. Detection and quantification of GLY, AMPA and GLUF*

330 Glyphosate, AMPA and glufosinate were detected in 64%, 51%, and 44% of the individuals, respectively  
331 (Table 1). Comparing the quantities sold in the townships studied, GLUF quantities were 2484 to 4844

332 times lower than for GLY in 2018, and 1653 to 4548 times lower in 2017. Indeed, after its ban in 2017,  
333 GLUF could only have been used until stock depletion or usage derogation. Focusing on the part of the  
334 region where the study was carried out (i.e. “Département des Deux-Sèvres”), the purchases of GLUF  
335 rapidly dropped after 2015, and reached 0 in 2019 (“[https://ventes-produits-  
336 phytopharmaceutiques.eaufrance.fr](https://ventes-produits-phytopharmaceutiques.eaufrance.fr)”). Despite such a low intensity of application compared to GLY,  
337 GLUF was detected in about half of small mammals. A study conducted over the same year over the  
338 same sampling sites showed the detection of GLY in 88% of the soil samples and 74% of the earthworm  
339 samples, of AMPA in 58% of soil samples and 38% of earthworms, and of GLUF in 35% of soil samples  
340 and 12% of earthworms (Pelosi et al. 2022). These detections in soils, earthworms and small mammals  
341 collected in arable fields and hedgerows, both in conventional and organic farming fields, showed a  
342 generalized occurrence of GLY, AMPA and GLUF in agricultural environment and fauna over this French  
343 typical agricultural area. In comparison, GLY was detected in all the three analysed county-level hair  
344 pools of carcasses of big brown bats (*Eptesicus fuscus*; insectivore species, weighing 15 to 26 g) in  
345 Missouri (USA) (Hooper et al. 2022). The frequency of exposure to GLY in a farmland herbivorous  
346 mammal, the Iberian hare (*Lepus granatensis*) via analyses of residues in samples of gastric content,  
347 reached 45% of individuals found dead due to roadkill or unknown causes (Martinez-Haro et al. 2022).  
348 In hunted hares from pesticide-treated areas, the frequency of detection in gastric content ranged  
349 between 9 and 22%. These values look lower than the frequencies found in our study in rodents, maybe  
350 because residues in gastric content represent a snapshot of recent exposure while measurements in  
351 hair represent a temporally integrative exposure assessment during the time of hair growth. Issues  
352 related to local contamination and/or different analytical sensitivity may further explain the lower  
353 detection frequency in hares.

354

355 **Table 1. Frequency of detection and concentrations of glyphosate, AMPA and glufosinate in hair of small mammals.**

356 *Descriptive statistics were computed on samples where the given compound was detected, i.e. excluding samples where concentrations were below the lowest detected value*  
 357 *(LOD). Abbreviations: LOQ, limit of quantification; LOD, Lowest detected value. GWT shrew stands for Greater white-toothed shrew.*

	GLYPHOSATE						AMPA						GLUFOSINATE					
	All species	Wood mouse <i>Apodemus sylvaticus</i>	GWT shrew <i>Crocidura russula</i>	Common vole <i>Microtus arvalis</i>	House mouse <i>Mus musculus</i>	Bank vole <i>Myodes glareolus</i>	All species	Wood mouse <i>Apodemus sylvaticus</i>	GWT shrew <i>Crocidura russula</i>	Common vole <i>Microtus arvalis</i>	House mouse <i>Mus musculus</i>	Bank vole <i>Myodes glareolus</i>	All species	All species	Wood mouse <i>Apodemus sylvaticus</i>	GWT shrew <i>Crocidura russula</i>	Common vole <i>Microtus arvalis</i>	House mouse <i>Mus musculus</i>
<b>Number of individuals</b>	<b>61</b>	18	14	16	2	11	<b>61</b>	18	14	16	2	11	<b>61</b>	18	14	16	2	11
<b>Detections</b>																		
Number of detection	<b>39</b>	6	7	15	1	10	<b>31</b>	4	2	14	1	10	<b>27</b>	2	1	13	1	10
Frequency (%)	<b>64</b>	33	50	94	50	91	<b>51</b>	22	14	88	50	91	<b>44</b>	11	7	81	50	91
<b>Concentrations (pg/mg)</b>																		
LOQ	<b>5</b>						<b>1</b>						<b>1</b>					
Minimum (LOD)	<b>0.018</b>	0.600	0.600	0.018	NA	1.43	<b>0.240</b>	0.400	0.400	0.240	NA	0.515	<b>1.16</b>	2.04	NA	1.29	NA	1.16
Q1	<b>1.040</b>	0.795	0.600	2.23	NA	2.60	<b>1.025</b>	0.519	0.617	1.08	NA	1.19	<b>2.01</b>	7.88	NA	2.39	NA	1.59
Median	<b>2.65</b>	1.77	0.800	4.42	NA	3.65	<b>1.39</b>	1.29	0.833	2.51	NA	1.61	<b>3.51</b>	13.8	NA	4.05	NA	3.01
Mean	<b>16.9</b>	2.73	0.817	39.4	NA	4.14	<b>5.00</b>	1.26	0.833	5.84	NA	6.54	<b>5.74</b>	13.7	NA	5.64	NA	4.29
Q3	<b>5.77</b>	4.83	0.950	8.13	NA	5.63	<b>6.45</b>	2.03	1.05	7.65	NA	6.51	<b>7.25</b>	19.7	NA	5.80	NA	4.87
Maximum	<b>522</b>	5.92	1.22	522	3.79	7.74	<b>33.6</b>	2.07	1.27	19.4	1.37	33.6	<b>25.5</b>	25.5	1.48	21.8	9.75	12.6
SD	<b>83.0</b>	2.45	0.239	133	NA	2.07	<b>7.37</b>	0.906	0.612	6.55	NA	10.2	<b>6.04</b>	16.6	NA	5.49	NA	3.75

358

359



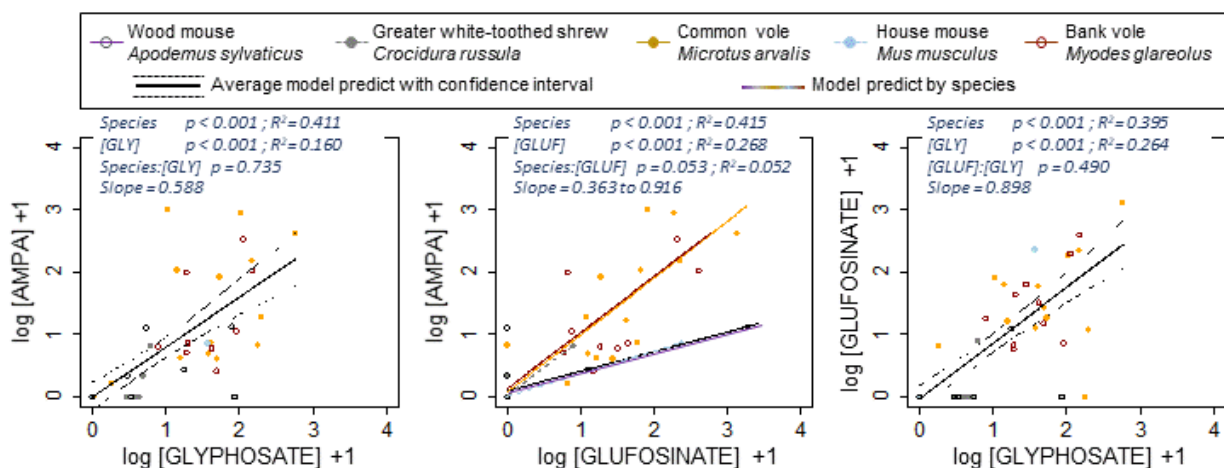
360 Regarding residue concentrations (when quantified), we found that the three compounds showed  
361 overall similar ranges of concentrations, but the highest concentrations were reached for GLY, with  
362 values varying from 0.018 to 522 pg/mg (median = 2.65) while its metabolite AMPA ranged from 0.240  
363 to 33.6 pg/mg (median = 1.39) and GLUF from 1.16 to 25.5 pg/mg (median = 3.51) (Table 1). In the  
364 county-level hair pools from big brown bats in Missouri, Hooper et al. reported concentrations varying  
365 from 5.7 to 4505.2 pg/mg. These maximum concentrations are much higher than those measured here  
366 in shrews (median = 0.8 pg/mg) and almost ten times higher than the maximum concentration  
367 measured in our study in a vole (i.e. 522 pg/mg). Several non-exclusive hypotheses may be proposed  
368 to explain such high levels found in bats: analytical issues (i.e. recovery of the extraction method, lack  
369 of internal standard), physiological and ecological traits of the species enhancing exposure or  
370 bioaccumulation, and issues related to the amount of GLY used and the timing of exposure of the  
371 animals after GLY application. Newton et al. (1984) studied the fate and behaviour of GLY and AMPA  
372 in forest brush field ecosystems in Oregon Coast Range (USA) following aerial application of GLY at  
373 doses comparable the recommended GLY application rate in EU (i.e. 3.3 kg a.s./ha there, maximum  
374 field application 4.32 kg a.s./ha in EU (European Commission 2015)). They measured the residues of  
375 GLY and AMPA in viscera and body of free-ranging small mammals captured over the sites before  
376 treatment and during the 55 days post-treatment. As in the present work, GLY (LD of 0.10 mg/kg = 100  
377 pg/mg) was found in all trophic groups while AMPA was detected in two voles only at 130 and <160  
378 pg/mg (LD of 100 pg/mg) (Newton et al. 1984), which is far lower than here but may be due to the fact  
379 that the LOD in our work was much lower (i.e. 0.240 pg/mg).

380 Interestingly, considering the previous findings from Newton et al. (1984) in forest small mammal  
381 tissues, the concentrations varied over the same order of magnitude as the maximum concentrations  
382 measured in our hair samples from agricultural landscapes. They reported concentrations in viscera  
383 ranging from < 100 pg/mg (LD) to 1690 pg/mg in shrews, 5080 pg/mg in deermice and 1700 pg/mg in  
384 voles, and concentrations in body tissues ranging from < 100 to 410 pg/mg in shrews, 400 pg/mg in  
385 deermice and 250 pg/mg in voles. The concentrations were the highest during the first days following

386 herbicide treatment both in viscera and body tissues. Body concentrations dropped under LD by 2  
 387 weeks after treatment while they were still detectable in viscera, and concentrations in viscera  
 388 decreased to nondetectable or near the LD by day 55 after treatment (Newton et al. 1984). Together  
 389 with differences in the matrices analyzed, this (rapid) concentration decrease over time following a  
 390 single GLY application may explain overall lower GLY levels in our study, and suggest that the highest  
 391 concentrations found here may correspond to animals exposed to GLY soon after its application. Our  
 392 samples were collected during springtime, in May-June, while glyphosate is usually applied in winter  
 393 cereals during late summer or fall (often in October). The seasonal moult of small mammal was  
 394 expected to be mostly completed by May-June when they were captured, and the sampled fur might  
 395 have partly or even totally grown within the period February-May (Abad 1991; López-Fuster et al. 1986;  
 396 Sealander 1972). However, the post-winter moult may be incomplete, depending on the age of  
 397 individuals and the species of concern, allowing the possibility for hair grown during autumn to be  
 398 included in the analysed fur sample (Beltran et al. 2018).

399 **Figure 1. Correlations between the concentrations of glyphosate, AMPA and glufosinate in hair of small**  
 400 **mammals.**

401 *The concentrations are expressed in (pg/mg). The statistical outputs of general linear models are provided on*  
 402 *graphs. Predicts are plotted according to species when the interaction between the factors is significant, average*  
 403 *predicts are provided when the interaction is not significant.*



404  
 405 The concentrations of the three compounds in hair of small mammals exhibited significant  
 406 relationships (Fig. 1, Annex Fig. 1). Positive correlations were observed between AMPA and GLY  
 407 (correlation coefficients Spearman's  $\rho = 0.84$ ), between GLUF and GLY ( $\rho = 0.79$ ), and between

408 AMPA and GLUF ( $\rho=0.89$ ) (Annex Fig. A1). The variance explained (*cf* determination coefficient  $R^2$ )  
409 was greater for the factor species (0.41 to 0.42) than for compounds (0.16 to 0.27) (Fig. 1). The  
410 correlation between AMPA and GLUF only differed according to species, with greater slopes for shrews  
411 and voles than for the two species of wild mice (Fig. 1). The correlation between AMPA and GLY and  
412 between GLUF and GLY were found similar between species (Fig. 1). The strength of the association  
413 between AMPA and GLUF was higher than between AMPA and GLY (i.e. greater  $\rho$  values, greater  $R^2$   
414 values, and greater slope coefficients, Fig. 1, Sup Info Annex 1. Fig. A.1). This may be seen as  
415 unexpected since since AMPA is a GLY transformation product. Such patterns of correlation between  
416 the compounds may reflect different exposure pathway contributions between GLY *versus* AMPA and  
417 GLUF. As GLY is poorly metabolized in mammals (EFSA 2015a), the origin of AMPA should be external  
418 (environmental) rather than internal (degradation of GLY in body). Studying GLY and AMPA residues in  
419 human hair, Alvarez et al. (2022) concluded in different origin of GLY and AMPA because AMPA was  
420 rarely detected in comparison to GLY both in farmers and nonoccupationally exposed subjects, and  
421 the ratio GLY to AMPA greatly differed between the subjects. The correlation between GLY and AMPA  
422 or between GLY and GLUF likely originates from spatial relationship related to the geographic  
423 occurrence of the herbicidal treatments, which enhance the probability of co-occurrence of parent and  
424 transformation products in herbicide-treated areas. As hypothesized, small mammals may be exposed  
425 to GLY via dietary route but also via direct spraying if present in the plots during the treatments, and  
426 orally after spraying treatments through grooming activities. Conversely, dietary exposure, related to  
427 accumulation of the compounds in soil, biota and food webs, may be the predominant exposure  
428 pathway to AMPA and to GLUF (which PPP use is no longer authorized) in small mammals.

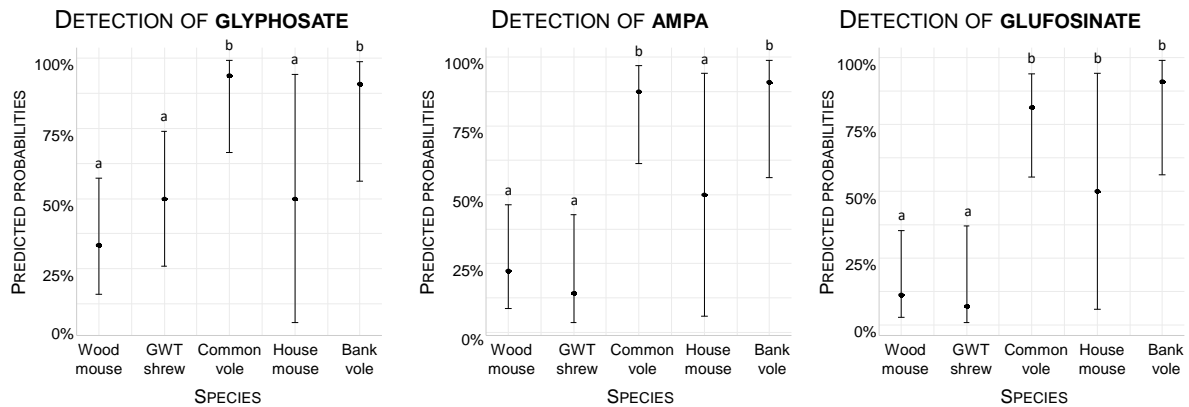
### 429 *3.2. Differences between species*

430 The frequency of detection differed between species for the three compounds (Fig. 2, Sup Info Annex  
431 1. Table A.2). In all cases, odds ratios showed significant differences between voles and the reference  
432 (i.e. wood mice) (Sup Info Annex 1. Table A.2). The probabilities of detection of GLY, AMPA and GLUF

433 were significantly higher in common and bank voles than in shrews and wood mice (Fig. 2). The results  
 434 were contrasted for the house mouse, likely because of low sample size (Fig. 2).

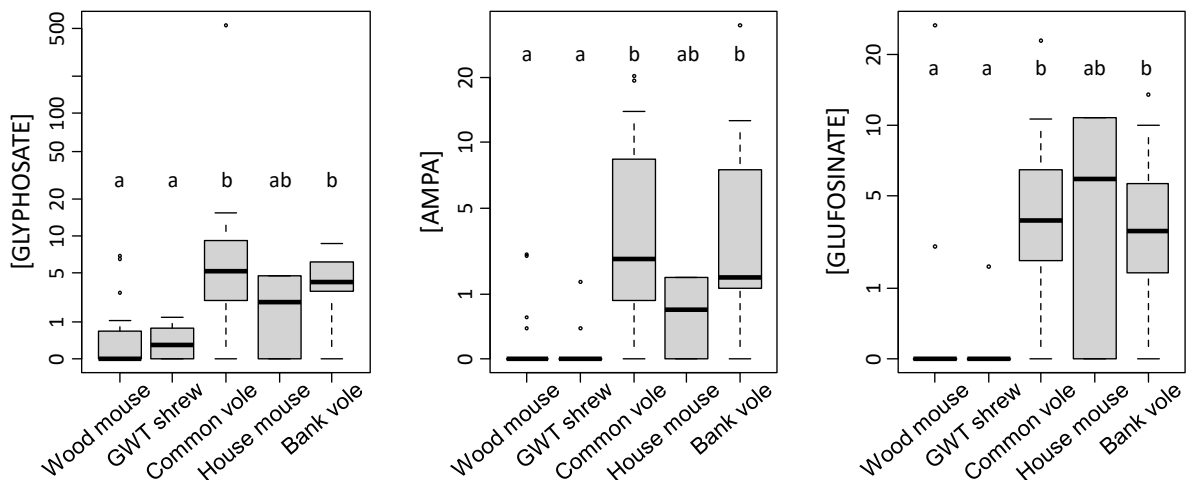
435 **Figure 2. Detection probabilities of glyphosate, AMPA and glufosinate in hair of small mammals according to**  
 436 **species.**

437 *Predicted probabilities of detection were obtained from the statistical binomial models "Detection ~ Species".*  
 438 *Statistical outputs of comparisons between levels are provided, the species sharing similar letters did not differ*  
 439 *statistically. Abbreviations: GWT shrew stands for Greater white-toothed shrew. The numbers of individuals for*  
 440 *each category are provided in Table 1.*



441  
 442 **Figure 3. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals according to species**  
 443 **with data under detection limits included and set at 0.**

444 *Concentrations are expressed in pg/mg. Not detected are set to 0 value. Statistical significance is indicated with*  
 445 *letters, different letters between levels indicate significant differences. Statistics were performed using Kruskal-*  
 446 *Wallis test followed by post-hoc multiple comparison tests to investigate pairwise differences. Abbreviations: GWT*  
 447 *shrew stands for Greater white-toothed shrew. The numbers of individuals for each category are provided in Table*  
 448 *1.*



449  
 450 When running analyses on the subset of data with quantified concentrations in the mammals (i.e.  
 451 without data under LOD), the concentrations of GLY, AMPA and GLUF did not significantly differ  
 452 between species (Sup Info Annex 1. Table A.3). However, GLY and AMPA trends were overall

453 comparable, since estimates for concentrations were found as the lowest in shrews and wood mice  
454 for GLY, and as the highest in voles for AMPA (Sup Info Annex 1. Table A.3). For GLUF, the highest  
455 estimates were observed for wood mice and house mice (Sup Info Annex 1. Table A.3). When the tests  
456 are run including non-detection set as 0 values, the results showed higher levels of GLY, AMPA, and  
457 GLUF in common voles and bank voles than in wood mice and shrews (Fig. 3). Concentrations in house  
458 mice did not significantly differ from other species, showing intermediate values (note that the sample  
459 size is very low) (Fig. 3).

460 In their forest study where residues of GLY and AMPA were measured in several free-ranging  
461 mammalian herbivores, carnivores, and omnivores, Newton et al. (1984) found GLY in all trophic  
462 groups but AMPA only in a few herbivorous voles. Our results are in line with these findings, with  
463 confirmed exposure to GLY (and AMPA) in both herbivorous/granivorous or omnivorous rodents and  
464 insectivorous/carnivorous shrews, and greater levels of AMPA in herbivorous mammals. Newton et al.  
465 (1984) emphasized that exposure to and accumulation of GLY in mammals varied according to diet  
466 preference, since omnivores (deermice) had the highest concentrations of GLY in viscera during the 2  
467 weeks post-treatment, showing a higher initial intake of GLY in omnivores, but such visceral levels  
468 decreased the most fastly in comparion to carnivores (shrews and weasel) and herbivores (squirrel,  
469 vole, chipmunk). The detailed data showed the highest body concentrations in shrews and deermice  
470 (0.35 to 0.41 mg/kg) in comparison to squirrels and voles (0.13 to 0.25 mg/kg) within the first days  
471 post-treatment. Our results similarly evidenced differences in accumulation/uptake of GLY and AMPA  
472 between trophic groups or species, but various trends were identified according to the trophic  
473 preferences. Overall, we here found lower detection frequency and concentration in insectivores while  
474 the highest detection frequency and concentration were observed in herbivores and  
475 granivores/omnivores voles. Both detection probability and concentrations were here greater for the  
476 two species of voles than for the omnivorous wood mice and house mice. The differences in the  
477 matrices analysed may be a reason explaining the discrepancies between the findings of Newton et al.  
478 and ours regarding the influence of diet on accumulation, in addition to differences in identity of the

479 species (i.e. European vs American species) and ecosystems (i.e. agrosystem vs forest). Physiological  
480 and metabolic traits affecting uptake and accumulation in body tissues/fluids and ecological traits  
481 affecting exposure may shape the differences found here between species or trophic groups but their  
482 role is difficult to disentangle. The species exhibiting the lowest detection frequency differed for  
483 phylogeny, since both *Apodemus* and *Mus* mice of the subfamily Murinae as well as Crocidurinae  
484 *Crocidura* shrews were represented (Sup Info Annex 1. Fig. A4). They also differ for their ecological  
485 traits since they are omnivorous/granivorous and insectivorous species. The most herbivorous and, to  
486 a lesser extent, the less mobile species which are the two species of voles studied here showed the  
487 highest detection frequencies and greatest concentrations. *Microtus* and *Myodes* voles are classified  
488 in two different genera but both belong to the same sub-family Arvicolinae (Sup Info Annex 1. Fig. A4).  
489 The correlation between levels of GLUF and AMPA varied under species-specific patterns as detailed  
490 above. The wood mouse and the house mouse which are both granivorous/omnivorous Murinae  
491 species showed a different slope than the herbivorous common vole, the  
492 herbivorous/granivorous/omnivorous bank vole and the insectivorous greater white-toothed shrew  
493 which all the three exhibited a steeper slope. Usually, the bank vole is classified as showing  
494 intermediate features of traits between the wood mouse and the common vole in terms of mobility  
495 and homerange size, habitat selectivity, and diet breadth (van den Brink et al. 2011). According to  
496 ecotoxicological studies where dietary route is the predominant exposure pathway, its responses are  
497 expected to be intermediate or closer to the trends exhibited by the wood mouse than by the common  
498 vole (Baudrot et al. 2018; van den Brink et al. 2011). Although the importance of toxicokinetics on  
499 accumulation in hair cannot be ignored (Faÿs et al. 2023), our results may again suggest the  
500 involvement of different origins of the compounds and of different exposure route contribution in  
501 shaping inter-species accumulation dissimilarities. The differences found here between species and  
502 between animals captured in different habitats within landscape advocate for the need for including  
503 both ecological and physiological features in wildlife risk assessment process and post-registration  
504 survey of unintentional effects of PPP (Morrissey et al. 2023). Importantly, these results highlight the

505 need for additional research to gain knowledge about the traits that shape the exposure and uptake  
506 of PPP in wildlife and about all interplaying mechanisms.

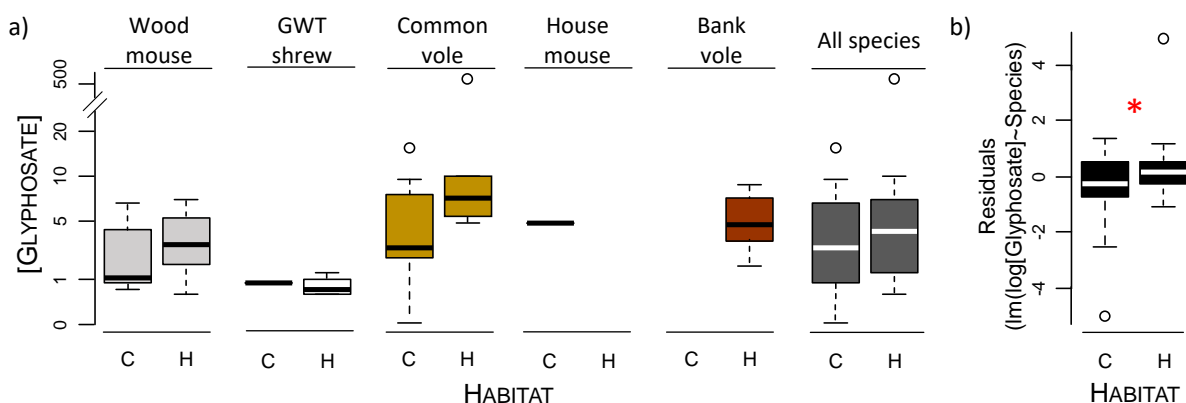
### 507 3.3. Influence of environmental factors

508 The frequency of detection did not differ according to habitat or farming practices (Sup Info Annex 1.  
509 Table A.2). Accordingly, the study of Pelosi et al. (2022) which is based on the same sampling scheme  
510 (same location of sampled plots in 2018 as well) did not show differences in GLY detection frequency  
511 in soils sampled from cereal fields and from hedgerows. But in earthworms, GLY and AMPA were  
512 detected more frequently in cereal fields than in hedgerows.

513 Concerning the concentrations of the compounds when quantified, GLY concentrations differed  
514 between small mammals captured in different habitats, being higher in individuals from hedgerows  
515 than from cereal fields (Fig. 4, Sup Info Annex 1. Table A.3, Fig. A2). Such a pattern was not observed  
516 in the case of AMPA and GLUF (Sup Info Annex 1. Table A.3, Fig. A2).

517 **Figure 4. Concentrations of glyphosate in hair of small mammals without data under detection limits (a)**  
518 **according to species and habitat and (b) according to habitats taking species into account statistically in**  
519 **modelling.**

520 *The concentrations are expressed in pg/mg in hair. The statistical significance is depicted by red asterisk. The*  
521 *habitats are represented as "C" for cereals and "H" for hedgerows. GWT shrew stands for Greater white-toothed*  
522 *shrew. The numbers of individuals for each category are provided in Table A.1.*



523 This may be seen as a confirmation of the different contribution of exposure pathways for GLY in  
524 comparison to AMPA and GLUF. Such a pattern may indeed be related to a contribution of overspray  
525 in addition to dietary exposure, because of direct spraying and/or elevated GLY deposits on hedgerow  
526 vegetation (1) during spraying due to drift and/or (2) due to (wet) deposition (Gandhi et al. 2021). GLY  
527

528 is known as showing a low volatility but is found in air and rainfall in intensively treated areas, at  
529 relatively high frequency and concentrations, and submitted to wet deposition (Chang et al. 2011).  
530 Contaminant deposit, including PPP, on vegetation in hedgerows is known to be enhanced by larger  
531 surfaces of interception and by “edge effect” compared to open habitats (Davis et al. 1994; Fowler et  
532 al. 2004; Lazzaro et al. 2008; Ould-Dada et al. 2002). Parallely, Mayer et al. (2020) computed for hares  
533 (*Lepus europaeus*) that pesticide uptake via foraging could be 7-fold lower than uptake via  
534 overspray/oral grooming. Moreover, GLY has been found to persist in vegetation for up to one year  
535 following treatments in perennial forested areas, representing a potent dietary source of exposure for  
536 wildlife (Edge et al. 2021). In a previous study dealing with the exposure of small mammals to banned  
537 and currently used PPP by residue screening in hair, based on animals sampled over the same study  
538 area and another region in France, we found a higher contamination (number of compounds and  
539 concentrations) in individuals captured in hedgerows than in grasslands, but the contamination did not  
540 differ between animals captured in hedgerows from those sampled in cereal crops (Fritsch et al. 2022).  
541 In Pelosi et al. (2022), GLY and AMPA concentrations in both soils and earthworms were, however,  
542 higher in samples from cereal fields than from grasslands and hedgerows. This may be seen as an  
543 argument in favour for the contribution of processes related to overspray in determining small  
544 mammal exposure to GLY. The role of external exposure to herbicides in small mammals and the  
545 importance of oral exposure via grooming remain to be investigated. The contribution of non-dietary  
546 routes in wildlife is still an overlooked field of research that need to be addressed.

547 The frequency of detection of GLY, AMPA and GLUF were not significantly dependent on the proxies  
548 of treatment intensity at plot, landscape and township scales, except in the case of GLUF sales (Sup  
549 Info Annex 1. Table A.2, Fig. A5). However, such an effect of a.s. sales on GLUF detection probabilities  
550 was negative, i.e. the detection probabilities were lower in townships where GLUF sales were the  
551 highest, and the differences between categories were not significant (Sup Info Annex 1. Table A.2, Fig.  
552 A5). To investigate further this paradoxical result and avoid any interpretation bias, a model with GLUF  
553 sales as the only explanatory factor (i.e. without the factor “species” first in the model formula) was



554 run, showing an absence of significant differences (Sup Info Annex 1. Table A.2, Fig. A5). No differences  
555 in the quantified concentrations of GLY, AMPA or GLUF in hair of small mammals were detected  
556 according to farming practices or proxies of pesticide treatment intensity at the different scales (Sup  
557 Info Annex 1. Table A.3). Running the analyses based on concentrations and including non-detection  
558 as 0 value provided similar results (Sup Info Annex 1. Fig. A3). Previously, we also highlighted an  
559 absence of differences in contamination by current use pesticides between small mammals captured  
560 in conventional or organic farming plots (neither for the number of compounds nor for their  
561 concentrations in individuals) (Fritsch et al. 2022). In agricultural topsoil sampled from conventional  
562 and organic farms across Europe screened for, GLY and AMPA (together with pendimethalin) showed  
563 the greatest frequency of detection and the highest concentrations, and AMPA was the residue the  
564 most often detected in both conventional and organic fields with 96% of occurrence in conventional  
565 systems and 83% in organic systems (Geissen et al. 2021). AMPA and GLY were also the most detected  
566 pesticides in soils under various land uses sampled over mainland France in which 111 pesticide  
567 residues were screened (Froger et al. 2023). The detection frequency was 83% for AMPA and 70% for  
568 GLY, and the two compounds were found both in treated cultivated fields and non-targeted habitats  
569 as they occurred both in conventional and organic farming plots, and in the different types of land  
570 uses: arable lands, orchards, forests, grasslands, and brownfields (Froger et al. 2023). Such a global  
571 occurrence of these two compounds, including in organic fields and grasslands or forest, echoes the  
572 pervasive exposure found here in small mammals. However, both Geissen et al. (2021) and Froger et  
573 al. (2023) reported that pesticide concentrations (for the full mixture of residues) in conventional soils  
574 were significantly higher than in organic soils (higher by 70-90% in Geissen et al. (2021)), and Froger et  
575 al. (2023) evidenced higher cumulative concentrations in cultivated soils than soils from forests,  
576 brownfields, and permanent grasslands. Martinez-Haro et al. (2022) reported results contrasted with  
577 the present findings about the influence of farming practices on exposure to GLY: the prevalence was  
578 9–22% in gastric content of hunted Iberian hares and 45% in hares found dead in pesticides-treated  
579 areas while no residues were detected in hares from organic crops. Again, such a pattern may be due

580 to the differences in the matrices analysed, with an assessment of recent short-term exposure in the  
581 case of gastric content versus past integrated measure of exposure in the case of hair. Further, the  
582 Iberian hares from conventional and organic crops were collected by hunting from October to January  
583 while our sampling session was conducted during springtime. The authors indicated that frequencies  
584 of detection in hares progressively increased over the hunting season, being of 0% and 11% in October  
585 and November and later 50% both in December and January (Martinez-Haro et al. 2022). The hares  
586 found dead were collected in March-April (45% positive for GLY). Finally, the intensity and extent of  
587 GLY treatments may be higher in our study region and the OF and CF plots may be geographically closer  
588 in our case. Beyond the study of GLY levels in environmental matrices such as soil or biota, more  
589 research is required to characterize the patterns of exposure of wildlife to GLY, AMPA and GLUF and  
590 quantify the underlying processes and spatio-temporal dynamics.

591 In soils and earthworms sampled within the ZAPVS, Pelosi et al. (2022) also found no significant  
592 differences in GLY, AMPA and GLUF levels according to pesticide use (i.e. comparing treated  
593 conventional fields to nontreated habitats such as grasslands, hedgerows and organic fields) or  
594 according to farming system in cropped fields (i.e. field cropped under conventional or organic  
595 farming). The ubiquity of exposure to GLY, AMPA and GLUF in arable landscapes, both in crops and  
596 agroecological infrastructure was therefore showed from recent studies for soils, and for both  
597 terrestrial invertebrates and vertebrates belonging to various taxa and of different trophic levels. The  
598 transfer of GLY, AMPA and GLUF in food webs is thus questioned and require further attention. Such  
599 a widespread occurrence of GLY, AMPA and GLUF in environment and biota whatever the farming  
600 practices and intensity of use at the landscape scale evidenced a pervasive contamination that may  
601 reflect the magnitude of repeated GLY-based (and formerly GLUF-based) herbicidal use over huge  
602 surfaces in French agrosystems. A similar situation has been found for neonicotinoids, in nectar (Henry  
603 et al. 2015), earthworms (Pelosi et al. 2021), small mammals (Fritsch et al. 2022) and birds (Fuentes et  
604 al. 2023).

605 Based on our results and recent literature, the beneficial role of agroecological infrastructures (e.g.  
606 hedgerows) and non-treated crops as refuges or recovery areas towards pesticide treatments in  
607 agroecosystems under the current agricultural model is questioned. Our findings highlight that the  
608 current surfaces of non-treated habitats within the agricultural landscapes studied are insufficient to  
609 mitigate the exposure of wildlife to GLY, AMPA and GLUF. We ended up in similar conclusions while  
610 studying the exposure of small mammals to 73 residues of fungicides, herbicides (excluding GLY, AMPA  
611 and GLUF), and insecticides in Fritsch et al. (2022). With regards to the mitigation of unintentional  
612 effects of PPP on wildlife, these results call for a re-assessment of the relevance and efficiency of the  
613 current procedures and a strengthening of national and regional initiatives for agri-environment  
614 schemes. Further investigation about pathways of wildlife exposure to PPP and responses at individual  
615 and population levels is needed to understand and predict the role of agroecological infrastructures in  
616 shaping direct and indirect effects of PPP. The beneficial role of hedgerows in limiting inputs of PPP on  
617 non-target plots around the treated fields can be leveraged in agro-environment schemes, but this  
618 desirable use of agroecological infrastructures to protect the environment and biodiversity may turn  
619 semi-natural habitats into “ecotoxicological traps”. Such issues deserve attention and should be  
620 addressed both in future research studies and monitoring schemes.

### 621 *3.4. Dose reconstruction to assess risk*

622 Trying to evaluate the risk of unintentional impacts in small mammals by comparing exposure dose values  
623 estimated from back-calculation using the concentrations measured in hair to toxicological data, we  
624 found that toxic thresholds could be exceeded for both GLY and GLUF. In the case of GLY, at least one  
625 of the five considered toxicological thresholds was exceeded according to the trigger of concern for at  
626 least one species in all the approaches (Table 2). Potential for toxicity impairment was thus observed  
627 in all the approaches developed. This suggests a risk for small mammals, especially for rodents and  
628 particularly for herbivorous voles (Table 2). Often, only the maximum value measured in *Microtus* voles  
629 reached toxic levels (10 times out of 16 scenarii). In five scenarii out of the 16 computed within the  
630 different approaches, all the species were found at risk with maximum but also median and minimum

631 concentrations measured being involved. At the population level, in the “best-case” scenario  
632 toxicological effects related to GLY exposure would cause impairment in at least 6% of individuals in  
633 the common voles studied here, which represents risk of effects in 2.1% of the rodents and in 1.6%  
634 within the small mammal community studied including insectivorous species (Table 2). In the worst-  
635 case scenario, toxicological impairment would occur in 87.5% of the common voles, 88.9% of the voles  
636 (bank voles and common voles), 66% of all rodent species, 50% of shrews and 62.3% of individuals  
637 within the small mammal community (Table 2). Up to 93.8% of the common voles and 92.6% of  
638 individual of all vole species may be impacted if individuals close to the trigger value of 5 are included  
639 in the calculation (Table 2).

640 For GLUF, the four toxicological thresholds considered were exceeded in the two scenarii for all rodent  
641 species, including the maximum concentrations measured in our samples but also the third quartile,  
642 median and even minimum values (Table 2). This would translate, based on our captured individuals,  
643 in a possible impairment related to GLUF exposure in 31.3 to 75% of the common voles, 25.9 to 70.4%  
644 of the vole individuals, 19.1 to 46.8% of individuals within the rodent population and 14.8 to 36.1% of  
645 individuals within the small mammal community (Table 2). This result suggests that a large part of the  
646 free-ranging non-target small mammals could be at risk for toxicological effects related to exposure to  
647 GLUF, especially herbivorous/granivorous species, at the time of sampling (i.e. 2018) and although the  
648 use of this compound in France has been largely decreased since 2015.

649 Thus, our findings are consistent with the first conclusions of the risk assessments performed during  
650 the registration processes, which identified potential threats to herbivorous, granivorous and  
651 insectivorous terrestrial vertebrates, especially herbivorous voles for both GLY and GLUF (EFSA 2005,  
652 2012, 2015a). Note that the regulatory assessments were based on dietary exposure, but wildlife may  
653 be exposed to PPPs - and this is especially the case for GLY when used through spraying - via additional  
654 routes such as oral uptake via grooming, inhalation, dermal contact, and/or ocular contact as  
655 mentioned also for human populations (Gandhi et al. 2021; Mayer et al. 2020; Mineau 2011).

656 **Table 2. Summary of risk assessment for glyphosate and glufosiate based on reverse dosimetry approaches.**

657 *Details are provided in Sup Info Annex 1 and Annex 2. Abbreviations: Nb number; Ind individuals; L-T NOAEL Long-term No Observed Adverse Effect Level; S-T NOEL short-term*  
 658 *dietary No Observed Effect Level; Ac NOAEL Acute NOAEL; Ch NOAEL Chronic NOAEL; CLD Chronic Low Dose threshold; Apsy Apodemus sylvaticus (wood mouse); Crru Crocidura*  
 659 *russula (greater white-toothed shrew); Miar Microtus arvalis (common vole); Mumu Mus musculus (house mouse); Mygl Myodes glareolus (bank vole); Max maximum; Q3 3<sup>rd</sup>*  
 660 *quartile; Med median; Min minimum; PK pharmacokinetics.*

Compounds Approach Scenarii	Type of data / Specific data / Assumptions	Cases when Toxicity/exposure ratio exceed trigger value (of 10 for Ac toxicity data or of 5 for Ch toxicity data)											
		Nb cases	Threshold of concern	Species of concern	Level [C] <sub>hair</sub> of concern	Nb ind	Nb rodents	Nb shrews	% All species	% Rodents	% Voles	% Microtus	% Shrews
<b>GLYPHOSATE</b>													
Approach 1	[GLY] in human urine and hair												
Scenario 1.1	Head hair												
Scenario 1.1.a	Urinary excretion 10%	3	L-T NOAEL, S-T NOEL, CLD	Miar	Max	1	1		2	2	4	6	
Scenario 1.1.b	Urinary excretion 20%	2	L-T NOAEL, CLD	Miar	Max	1	1		2	2	4	6	
Scenario 1.1.c	Urinary excretion 1%	16	L-T NOAEL, S-T NOEL, Ac NOAEL, Ch NOAEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med	29 (33)	25 (26)	4 (7)	48 (54)	53 (55)	74 (78)	69 (75)	29 (50)
Scenario 1.2	Pubic hair												
Scenario 1.2.a	Urinary excretion 10%	12	L-T NOAEL, S-T NOEL, Ac NOAEL, Ch NOAEL, CLD	Miar, Apsy, Mygl	Max, Q3, Med	16 (18)	16 (17)	(1)	26 (30)	34 (36)	48 (48)	50 (50)	(7)
Scenario 1.2.b	Urinary excretion 20%	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Scenario 1.2.c	Urinary excretion 1%	31	L-T NOAEL, S-T NOEL, Ac NOAEL, Ch NOAEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med, Min	37	30	7	61	79	85	81	50
Approach 2	Body burden in small mammals 7 days after dose												
Scenario 2.1	Proportions hair /body burden	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Scenario 2.2	[C] <sub>whole body</sub> = [C] <sub>hair</sub>	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	

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667 **Table 2. continued**

Compounds Approach Scenarii	Type of data / Specific data / Assumptions	Cases when Toxicity/exposure ratio exceed trigger value (of 10 for Ac toxicity data or of 5 for Ch toxicity data)											
		Nb cases	Threshold of concern	Species of concern	Level [C] <sup>hair</sup> of concern	Nb ind	Nb rodents	Nb shrews	% All species	% Rodents	% Voles	% Microtus	% Shrews
<b>GLYPHOSATE</b>													
Approach 3	Toxicokinetics in small mammal blood												
Scenario 3.1	Anadon et al. (2009)												
Scenario 3.1.a	GLY residues	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Scenario 3.1.b	AMPA residues	16	L-T NOAEL, S-T NOEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med	24	23	1	39	49	74	63	7
Scenario 3.2	Kim et al. (2023) GLY residues	1	CLD	Miar	Max	1	1		2	2		6	
Approach 4	Residues in viscera and body of small mammals												
Scenario 4.1	Min ratio viscera/body residues	1	CLD	Miar	Max	1	1		2	2		6	
Scenario 4.2	Average ratio viscera/body residues	1	CLD	Miar	Max	1 (2)	1 (2)		2 (3)	2 (4)	7 (7)	6 (13)	
Scenario 4.3	Max ratio viscera/body residues	2	L-T NOAEL, CLD	Miar	Max	1	1		2	2		6	
Approach 5	Prediction of incorporation in hair from PK												
Scenario 5.1	Glyphosate residues	20	L-T NOAEL, S-T NOEL, Ac NOAEL, Ch NOAEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med, Min	38 (39)	31 (32)	7 (7)	62 (64)	66 (68)	89 (93)	88 (94)	50 (50)
Scenario 5.2	AMPA residues	20	L-T NOAEL, S-T NOEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med, Min	30 (31)	28 (29)	2 (2)	49 (51)	60 (62)	85 (89)	81 (88)	14 (14)
<b>GLUFOSINATE</b>													
Approach 1	Toxicokinetics in small mammal blood												
Scenario 1.1	values in males	18	L-T NOAEL, S-T NOEL, Acute LD50, Chronic NOAEL	Miar, Apsy, Mumu, Mygl	Max, Q3, Med, Min	22 (23)	22 (22)		36 (38)	47 (47)	70 (70)	75 (75)	(7)
Scenario 1.2	values in females	10	L-T NOAEL, S-T NOEL, Acute LD50, Chronic NOAEL	Miar, Apsy, Mumu, Mygl	Max, Q3, Med	9 (10)	9 (10)		15 (16)	19 (21)	26 (30)	31 (31)	

\*Values considered close to the trigger : less than 1 of difference, e.g. value of 5.2 for a trigger of 5

670 Importantly, in the study carried out in the same area on small mammals mentioned above (Fritsch et  
671 al. 2022), 112 different residues of banned and currently used PPPs (parent chemicals and metabolites)  
672 were detected in hair samples from wood mice and greater white-toothed shrews, with 32 to 65  
673 residues detected per individual. A widespread exposure of small mammal to fungicides, herbicides  
674 and insecticides was shown, with an overall higher contamination (number of compounds or  
675 concentrations) in shrews than in rodents (Fritsch et al. 2022). This means that “cocktail effects” are  
676 likely to occur, which can ultimately increase the risk of GLY- or GLUF-induced toxicity assessed here  
677 due to mechanisms of additivity and synergy (Martin et al. 2021). Indeed, in their review Martin et al.  
678 (2021) showed that additivity was the interaction most often occurring in chemical mixtures, followed  
679 by synergistic interactions. For instance, Brodeur et al. (2014) evidenced synergic toxic impacts in the  
680 common South American toad (*Rhinella arenarum*) of both equitoxic and nonequitoxic binary mixtures  
681 of GLY-and cypermethrin-based (two compounds that have been detected in more than half of small  
682 mammals’ individuals in our studies) commercial PPP products based on the acute toxicity endpoint  
683 LC50 in tadpoles (median lethal environmental concentration). They showed that the degree of  
684 synergy, computed by comparison to the toxicity induced by concentration addition, ranged from 2 to  
685 9 times depending on the mixture tested (Brodeur et al. 2014).

686 From terrestrial wildlife and biodiversity conservation perspectives, our findings may be qualified as  
687 worrisome. As mentioned earlier, GLY concentrations were found at higher levels than here in bats  
688 (Hooper et al. 2022). The authors emphasized that GLY reached the highest concentrations measured  
689 in their study where 8 compounds including neonicotinoids and several systemic or selective  
690 herbicides were analysed. They suggested that extremely high environmental inputs of GLY may  
691 counterweight the role of its short half-life in mitigating risks to wildlife (Hooper et al. 2022).  
692 Furthermore, some wild taxa may be highly sensitive to GLY, more than expected from laboratory rat  
693 and mice studies and more than terrestrial small mammals. For instance, in amphibians that can  
694 experience elevated probability of exposure to GLY in agrosystems (Berger et al. 2013), acute effects  
695 have been shown. The mortality rate reached 68 to 86% of the post-metamorphosis juveniles of the

696 three species *Rana sylvatica*, *Bufo woodhousii fowleri*, and *Hyla versicolor* exposed by spraying a GLY-  
697 based commercial herbicide at recommended dose (Relyea 2005).

698 Following GLY treatments, altered habitat characteristics (delay in vegetation growth, reduction in  
699 height of vegetation and vegetation cover) and altered plant and invertebrate food resources have  
700 been associated to reduced (breeding) populations and negative effects on reproduction in birds  
701 (Lazaran et al. 2013; Ojelade et al. 2022), reduction in availability of foraging resource and less  
702 frequentation for elk populations (Milner et al. 2013), and reduced abundance of small mammals,  
703 especially insectivores (Soricidae) and herbivores (Microtinae) (Santillo et al. 1989). Such indirect  
704 effects on wildlife via disturbances of natural habitats and dietary resources due to the risks to non-  
705 target plants and arthropods [from off-site spray drift] were indeed pointed out in risk assessment  
706 (EFSA 2015c; US EPA 2020), but considered as acceptable because *“these risks are expected to be*  
707 *limited to the application area or areas near the application area”* and side effects including those via  
708 trophic interactions relied on the intended consequence of herbicide use, i.e. elimination of competing  
709 vegetation (EFSA 2015c; US EPA 2020). Such application zones and nearby areas may, though,  
710 represent huge surfaces since GLY-based PPPs are registered in most countries for use in both  
711 agricultural (including horticulture, viticulture, and silviculture) and non-agricultural sites (e.g.  
712 commercial, industrial, and residential areas) and are among the most applied herbicides. Our results  
713 showing a pervasive exposure, they question the magnitude of positive compensatory effects (i.e.  
714 refuges and connectivity recolonization) expected from untreated areas within the agricultural  
715 landscape mosaic (see for instance Dalkvist et al. (2013) about the role of landscape features on  
716 impacts of pesticides on vole population). Moreover, recurring treatments over seasons and years  
717 likely limit the possibility for recovery. Indirect effects may thus occur in addition to direct toxic effects.  
718 Our findings warrant further research to confirm their interpretation and gather knowledge about the  
719 ecological impacts of GLY and GLUF use as PPP. There is a need to better characterize the toxicokinetics  
720 and the toxicodynamics of the compounds in wild species, and to provide data in order to relate  
721 concentrations in hair with exposure dose, accumulation in other tissues, and toxicological responses.



722 Further, more research is needed to assess whether the potential risks computed here actually  
723 translates in deleterious effects in free-ranging population, which would require field surveys and  
724 monitoring of wildlife exposure and health. The dynamics of GLY, AMPA and GLUF transfer and impacts  
725 in food webs crucially need to be investigated. We provide here lines of evidence to support the  
726 identification of the organophosphate chemicals GLY and GLUF as ‘emerging organic contaminants’ as  
727 emphasized by Kissane and Shephard (2017). Showing a pervasive exposure of wildlife to GLY, AMPA  
728 and GLUF and suggesting a chronic exposure at sub-lethal levels based on non-destructive sampling in  
729 biosentinel species, our study meets their statement about the research needed to fill gaps in  
730 knowledge about early-warning signals of impacts (Kissane and Shephard 2017).

### 731 *5. Conclusion*

732 Our results provide new insights into ecological risks of GLY- and GLUF-based herbicides, showing that  
733 in addition to the evidences related to environmental and human health issues, these PPP are of  
734 concern for wildlife exposure and may be of concern for impacts and further trophic cascading effects.  
735 The exposure of wild small mammals to GLY, AMPA and GLUF was here shown as being widespread in  
736 all trophic groups or taxa and in treated and nontreated habitats within the studied agricultural area,  
737 and unrelated to the intensity of treatments at plot or landscape scale. These results underpin the  
738 most recent findings about the pervasive GLY environmental contamination, occurrence in food webs,  
739 and associated ecological risks. Our results warrant further research about the ecological safety of GLY  
740 and GLUF use as PPP and provide support for assessing their potential as ‘emerging organic  
741 contaminants’.

742 Our study relied on measurements of residues in hair samples, a method that is developing in human  
743 epidemiology, and looks a promising non-destructive approach to study the field ecotoxicology of PPP  
744 and monitor wildlife exposure or detect early-signals in wild species. Such surveys may represent  
745 valuable opportunities for the assessment of regulatory measures and post-registration monitoring,  
746 and provide support to phytopharmacovigilance and toxicovigilance schemes.

747 This study highlights the need for research about the pathways of wildlife exposure to PPP and the  
748 toxicokinetics and toxicodynamics of GLY, AMPA and GLUF and more broadly of PPP initially assessed  
749 as not persistent and not bioaccumulative based on criteria established on legacy compounds. Our  
750 findings strongly suggest that current agro-environment schemes do not allow to achieve the  
751 objectives of ecological risk mitigation. They call for additional research and monitoring about the role  
752 of agroecological infrastructure in shaping PPP impacts on biodiversity, and provide support for an  
753 overall reflexion if not an overhaul of agro-environment policies.

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## 764 **Author’s contribution**

765 Clémentine Fritsch: Conceptualization, Funding acquisition, Project administration, Supervision,  
766 Investigation, Data curation, Formal analysis, Validation, Visualization, Writing - original draft

767 Brice Appenzeller: Project administration, Investigation, Methodology; Supervision, Validation, Writing  
768 - review & editing

769 Bertrand Colette: Conceptualization, Project administration, Investigation, Data curation, Writing -  
770 review & editing

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780 review & editing

## 781 **Additional Information**

782 Competing financial interests: The authors declare no competing financial interests.

## 783 **Supplementary information**

784 List of information in appendix document:

### 785 *Annex 1.*

786 Text A.1: Molecular identification of small mammal species

787 Text A.2: Approaches of reverse dosimetry to assess risk for deleterious effects in small  
788 mammals

789 Figure A.1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in  
790 small mammal hair.

791 Figure A.2. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals with  
792 data under detection limits set at 0 according to species (a), to habitat where captured (b), and to  
793 farming practices where captured (c).

794 Figure A.3. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals  
795 according to proxies of treatment intensity at the plot scale (a), at the landscape scale (b), and at the  
796 township scale (c).

797 Figure A.4. Simplified phylogeny tree of rodents and shrews involved in the study.

798 Figure A.5. Detection probabilities of glufosinate in hair of small mammals according to sales  
799 of the active substance at the township scale.

800 Table A.1. Overview of sample size.

801 Table A.2. Statistical outputs of binomial GLMs to investigate the influence of explanatory  
802 factors on detection frequencies of glyphosate, AMPA, and glufosinate in hair of small mammals.

803 Table A.3. Statistical outputs of GLMs to investigate the influence of explanatory factors on the  
804 concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals.

### 805 *Annex 2.*

806 Computation of reverse dosimetry approaches.

807

808 *The data will be made accessible on a repository after acceptance for publication, with raw data*  
809 *provided as Annex 3.*

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977 **Appendix – Annex 1**

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997 Table A.3. Statistical outputs of GLMs to investigate the influence of explanatory factors on the

998 concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals. .... 53

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1000

1001 *Text A.1: Molecular identification of small mammal species*

1002 Total genomic DNA was extracted from 10-25mg of ear tissue using the DNeasy Blood and  
1003 Tissue kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations (pp 28-30,  
1004 version 07/2006). Each sample was processed independently in an automated manner using the  
1005 QIAcube robot (Qiagen). DNA concentration was then measured using a NanoVue Plus  
1006 spectrophotometer (Biochrom). DNA extracts were stored at -20 °C until DNA amplification.

1007 All DNA extracts were then amplified for an approximately 900 bp-long fragment (excluding  
1008 primers) of the *cytochrome b* gene. We used primers CytB Uni fw 5' – TCATCMTGATGAAAYTTYGG – 3'  
1009 and CytB Uni rev 5' – ACTGGYTGDCBCCRATTCA – 3' published in Schlegel et al. (2011). Amplifications  
1010 were performed in 50µL reactions containing 1× HotStart Taq Master Mix (Qiagen), 0.32µM of each  
1011 primer, 0.5ng/µL of bovine albumin serum, 0.75mM of MgCl<sub>2</sub>, and 5µL of DNA extract (10-70 ng/µL).  
1012 The PCR program consisted in an activation step of 15mn at 95°C, followed by 40 cycles of denaturation  
1013 at 94°C during 30s, annealing at 53°C during 45s, and extension at 72°C during 1mn. A final extension  
1014 was performed at 72°C during 10mn. Amplification reactions were performed using an Eppendorf  
1015 Mastercycler DNA Engine.

1016 The PCR products were separated and visualized using the QIAxcel device and a QIAxcel DNA  
1017 Screening kit (Qiagen). The amplified products were purified using the Illustra GFX PCR DNA and Gel  
1018 Band Purification Kit (GE Healthcare) according to the manufacturer's instructions. Direct sequencing  
1019 of the PCR products was performed with an automated sequencer (Applied Biosystems Seqstudio  
1020 Genetic Analyzer). All the samples were sequenced with primers employed for the PCR reactions. The  
1021 DNA sequences obtained were submitted to GenBank with the Blast algorithm  
1022 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A query sequence was then assigned to a given species when  
1023 identity and coverage matches the database sequence with a threshold of at least 99% for only one  
1024 species.

1025

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1031 *Text A.2: Approaches of reverse dosimetry to assess risk for deleterious effects in small*  
1032 *mammals*

1033 In this part of the work, approaches of reverse dosimetry have been applied to estimate daily dose  
1034 intake of small mammals from the concentrations of glyphosate (GLY), AMPA or glufosinate (GLUF)  
1035 measured in hair and using several types of data that may be useful to apply dose reconstruction  
1036 processes. Concentrations in mammal body fluids and hair, residues in viscera and body burden in  
1037 small mammals, toxicokinetics in small mammal blood have been collected from the literature. Several  
1038 approaches of back-calculations were thus applied depending on the type of input data and the  
1039 different approaches were computed under several assumptions in case of lack of available data.

1040 For GLY, four different approaches have been applied based on data about (1) concentrations in human  
1041 urine and hair (Alvarez et al. 2022), (2) body burden in rats 7 days after dose administration (Brewster,  
1042 1991), (3) toxicokinetics in rat blood (Anadón et al. 2009; Kim et al. 2023), (4) residues of GLY in viscera  
1043 and body of wild small mammals in a field study (Newton et al. 1984), and (5) equations for fast-  
1044 elimination chemicals relating concentration in hair in rats and level of exposure taking  
1045 pharmacokinetics into account (Faÿs et al. 2023). For GLUF, one approach could be proposed based  
1046 on toxicokinetics in rat blood (Joint FAO/WHO Meeting on Pesticide Residues (JMPR) et al. 2012). The  
1047 body weight of small mammals came from actual morphometrics measured in our experiment by  
1048 weighting individuals in which residues of GLY, AMPA and GLUF were measured (Sup Info Annex 2).  
1049 Data about urinary excretion percentage of GLY were obtained in documents released by EFSA and  
1050 recent reviews (Connolly et al. 2020; EFSA 2015a; Peillex and Pelletier 2020). The concentrations of  
1051 GLY in viscera and body reported in Newton et al. (1984) for shrews (carnivores), deer mice (omnivores)  
1052 and voles (herbivores) were used separately according to diet preference to compute reverse  
1053 dosimetry in shrews (carnivores), wood mice, house mice and bank voles (omnivores), and common  
1054 voles (herbivores), respectively. Food intake for the different species have been computed using the  
1055 mean of food intake of different items provided in Crocker et al. (2002). The mean food intake of

1056 arthropods and cereal seeds provided for the wood mouse has been used for the wood mouse and the  
1057 house mouse, the mean food intake of arthropods and earthworms provided for the common shrew  
1058 has been used for the greater white-toothed shrew, and the mean food intake of grasses and forbs  
1059 provided for the field vole has been used for the common vole (Crocker et al. 2002). The assumptions  
1060 that had to be made to allow calculation were that distribution was equivalent between body parts  
1061 and hair, that concentration in hair were equivalent to concentrations in plasma/blood and  
1062 proportional to administered dose, or that concentration in viscera reflects concentrations in diet.  
1063 These assumptions, if not verified, might however be considered as acceptable according to current  
1064 knowledge about GLY pharmacokinetics. It is acknowledged that *“absorbed GLY is poorly metabolised,  
1065 widely distributed in the body, does not undergo enterohepatic circulation and is rapidly eliminated”*  
1066 (EFSA 2015a). Similarly to GLY, GLUF is distributed ubiquitously in the body rapidly after administration  
1067 and mostly excreted in faeces and urine (Joint FAO/WHO Meeting on Pesticide Residues (JMPR) et al.  
1068 2012). It is considered that chemicals are mostly incorporated into hair bulb living cells from blood  
1069 stream, and that residue concentrations in hair are representative of the internal dose during the time  
1070 of hair sample growth (Appenzeller et al. 2017; Faÿs et al. 2023). In a field study where GLY was aerielly  
1071 applied at 3.3 kg a.s./ha in forest, the concentrations measured in remainder of small mammal bodies  
1072 (without viscera) were of the same order of magnitude than maximum concentrations measured here  
1073 in hair owing that in EU the maximum field application of GLY in cropped fields is comparable (4.32 g  
1074 a.s./ha) (Newton et al. 1984). Newton et al. (1984) considered concentrations in viscera as reflecting  
1075 intake, and reported that *“GLY in the viscera of herbivores was roughly equal to or somewhat below  
1076 concentrations found in litter and ground cover”*.

1077 The dose of GLY was compared to five toxicological thresholds. Three of them were regulatory  
1078 thresholds: the short-term dietary NOEL (no observed effect level, 150 mg active substance/kg body  
1079 weight), the chronic 21 days NOAEL which is the same value than reproductive NOAEL (no observed  
1080 adverse effect level, 351 mg a.s./kg bw/d) and the long-term NOAEL (maternal and developmental  
1081 NOAEL which is the same than long-term NOAEL for terrestrial vertebrates, 50 mg a.s./kg bw/d) (EFSA,

1082 2015a; PPDB, <http://sitem.herts.ac.uk/>). The No Mortality Dose to the Oregon vole (NOAEL Acute  
1083 toxicity (Intraperitoneal), 450 mg a.s./kg bw) was also used to consider a wild species (McComb et al.  
1084 2008). Some more recent publications highlighted that GLY harmful effects were detected below  
1085 regulatory limits (e.g. within the spectrum of the acceptable daily intake) (Cai et al. 2017; Clair et al.  
1086 2012; Mesnage et al. 2015). We therefore added a « Chronic Low Dose » limit, with the value of 5 mg  
1087 a.s./kg bw/d being obtained from studies showing effects on reproductive development, endocrine  
1088 disruption, maternal behaviour, neuroplasticity and gut microbiota in rats (Dechartres et al. 2019;  
1089 Romano et al. 2010).

1090 The dose of GLUF was compared to four available toxicological thresholds: the acute oral LD50 (median  
1091 lethal dose; 416 mg/kg bw), the short-term dietary NOEL (64 mg a.s./kg bw), the chronic 21 days NOAEL  
1092 (6.3 mg a.s./kg bw/d) and the long-term NOAEL (2 mg a.s./kg bw/d) (Joint FAO/WHO Meeting on  
1093 Pesticide Residues (JMPR) et al. 2012; PPDB, <http://sitem.herts.ac.uk/>).

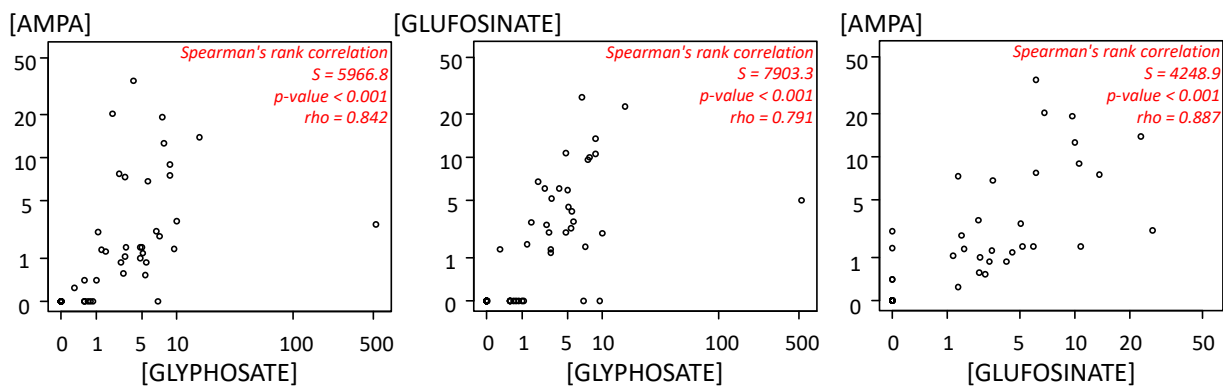
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- 1143
- 1144

1145 **Figure A.1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in small mammal**  
1146 **hair.**

1147 *Concentrations are expressed in pg/mg. Not detected are set to 0 value.*



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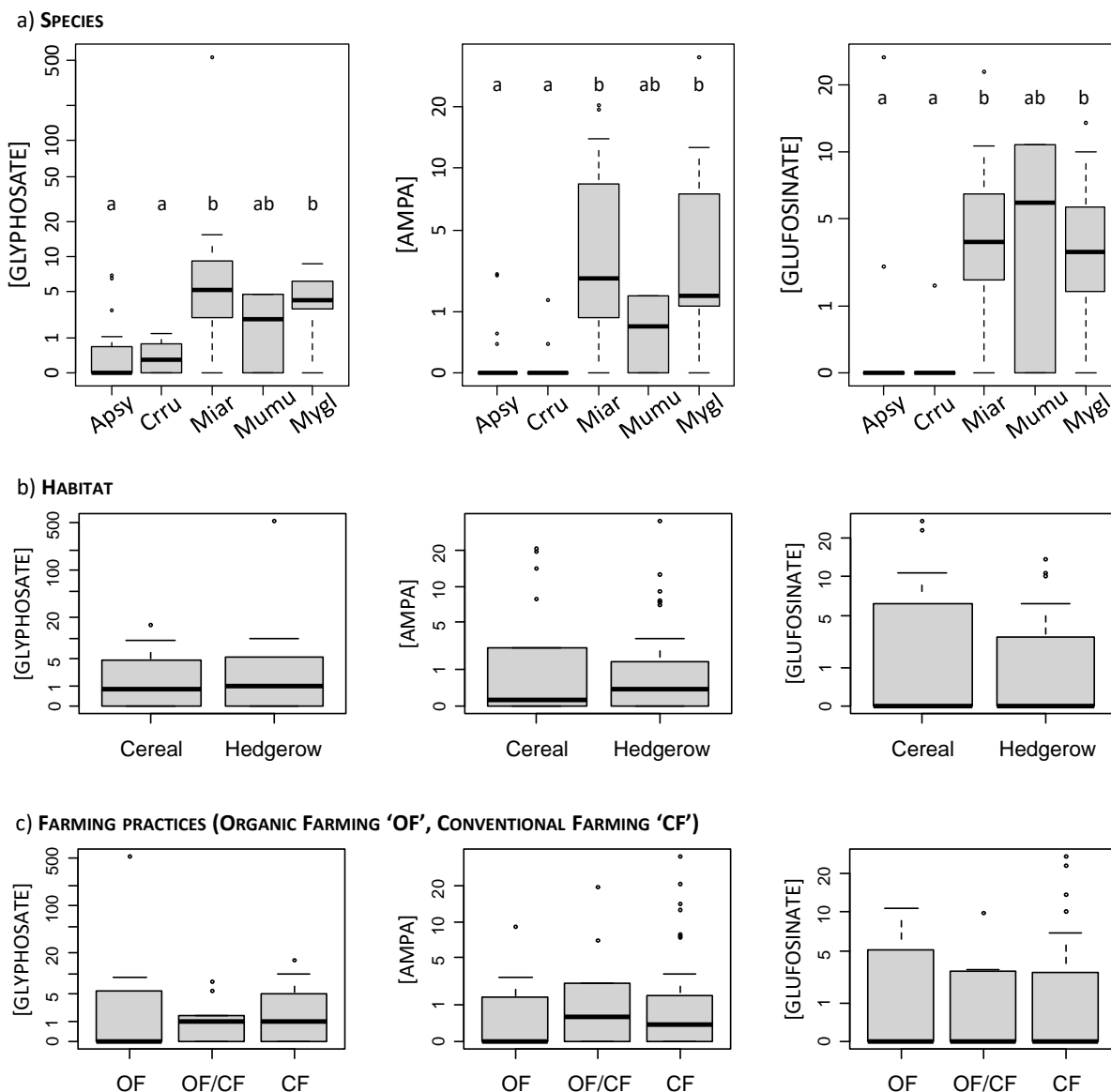


1150 **Figure A.2. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals with data under**  
 1151 **detection limits set at 0 according to species (a), to habitat where captured (b), and to farming practices where**  
 1152 **captured (c).**

1153 Concentrations are expressed in pg/mg. Not detected are set to 0 value. Statical significance is indicated with  
 1154 letters, different letters between levels indicate significant differences. In absence of significant differences, letters  
 1155 are not provided on graphics. Statistics were performed using Wilcoxon-Mann-Whitney test or Kruskal-Wallis test  
 1156 depending on the number of levels of the factor of concern. Post-hoc multiple comparison tests after Kruskal-Wallis  
 1157 tests were used to investigate pair-comparisons when relevant.

1158 Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus Wood mouse,  
 1159 Crru=Crocidura russula Greater white-toothed shrew, Miar=Microtus arvalis Common vole, Mumu=Mus musculus  
 1160 House mouse, Mygl=Myodes glareolus Bank vole.

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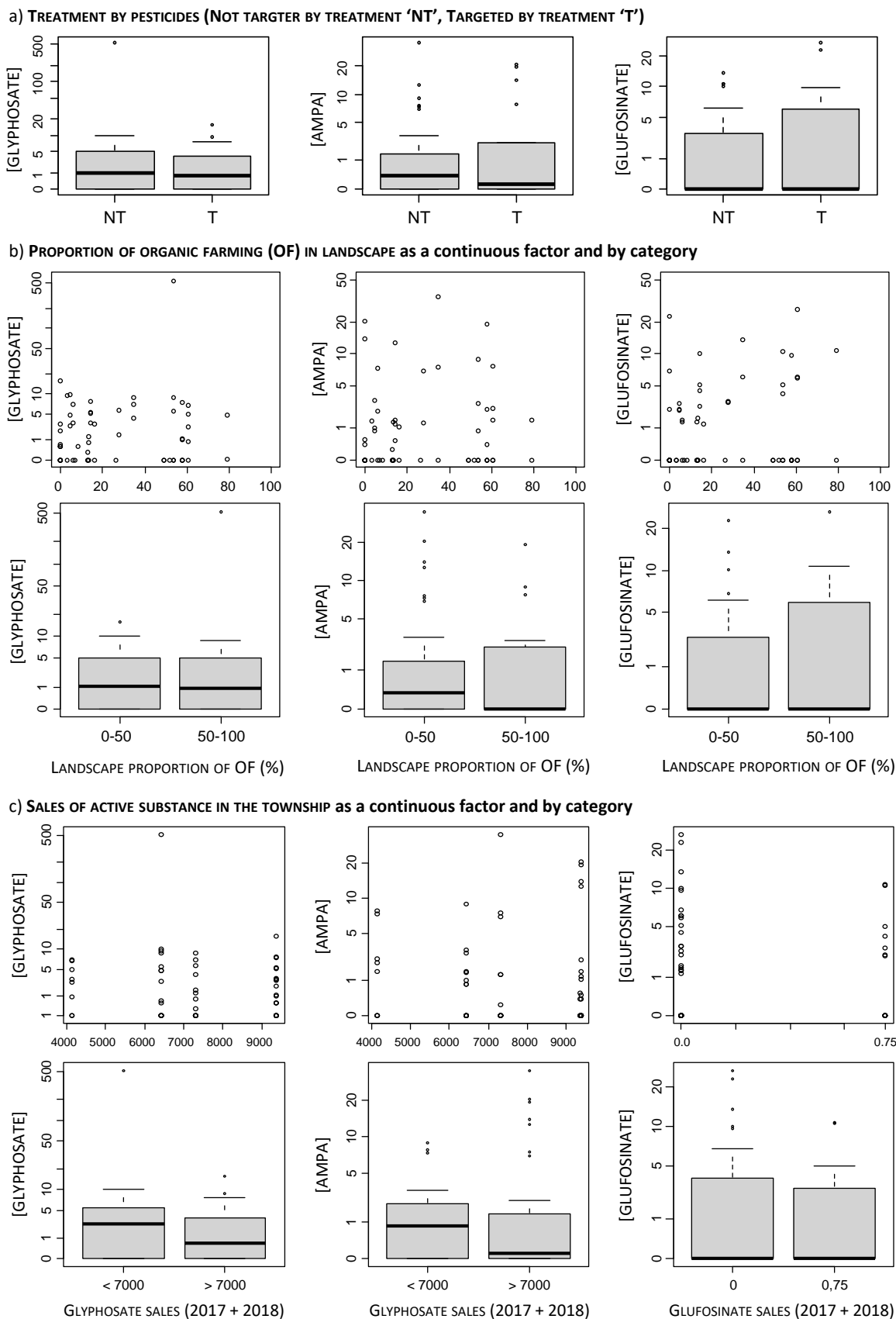


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1164 **Figure A.3. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals according to proxies**  
 1165 **of treatment intensity at the plot scale (a), at the landscape scale (b), and at the township scale (c).**

1166 Concentrations are expressed in pg/mg. Not detected are set to 0 value. Statistics were performed using Wilcoxon-  
 1167 Mann-Whitney test or Kruskal-Wallis test depending on the number of levels of the factor of concern, and  
 1168 Spearman's rank correlation test. No significant differences were detected.

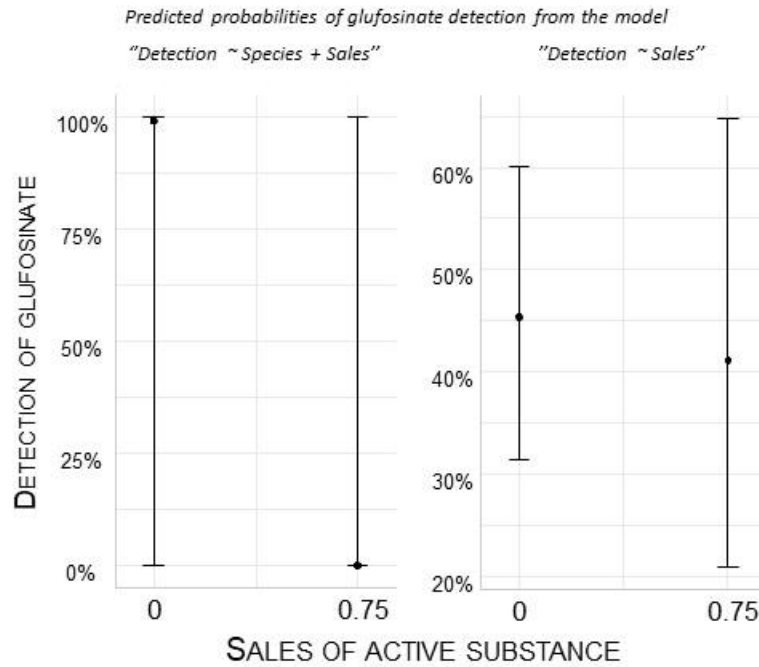




1183 **Figure A.5. Detection probabilities of glufosinate in hair of small mammals according to sales of the active**  
1184 **substance at the township scale.**

1185 *Predicted probabilities of detection were obtained from the statistical binomial models "Detection ~ species +*  
1186 *sales"(left panel) and "Detection ~ sales" (right panel).*

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1211 **Table A.1. Overview of sample size.**

1212 Abbreviations: OF=Organic Farming, CF=Conventional Farming, NT=not targeted by treatments, T=targeted by treatments, AS=Active Substance.

Habitat	Species	Wood mouse <i>Apodemus sylvaticus</i>	Greater white-toothed shrew <i>Crocidura russula</i>	Common vole <i>Microtus arvalis</i>	Bank vole <i>Myodes glareolus</i>	House mouse <i>Mus musculus</i>	Total
<b>Farming</b>							
<b>Cereal</b>							
	CF	5	2	7		1	15
	OF/CF	4		1			5
	OF			1		1	2
	<b>Total</b>	<b>9</b>	<b>2</b>	<b>9</b>		<b>2</b>	<b>22</b>
<b>Hedgerow or woodlot</b>							
	CF	7	7	2	10		26
	OF/CF		3	1	1		5
	OF	2	2	4			8
	<b>Total</b>	<b>9</b>	<b>12</b>	<b>7</b>	<b>11</b>		<b>39</b>
	<b>Total CF</b>	<b>12</b>	<b>9</b>	<b>9</b>	<b>10</b>	<b>1</b>	<b>41</b>
	<b>Total OF/CF</b>	<b>4</b>	<b>3</b>	<b>2</b>	<b>1</b>		<b>10</b>
	<b>Total OF</b>	<b>2</b>	<b>2</b>	<b>5</b>		<b>1</b>	<b>10</b>
	<b>Total « NT »</b>	<b>9</b>	<b>12</b>	<b>8</b>	<b>11</b>	<b>1</b>	<b>41</b>
	<b>Total « T »</b>	<b>9</b>	<b>2</b>	<b>8</b>		<b>1</b>	<b>20</b>
<b>Landscape OF proportion</b>							
	0-50%	11	9	8	11	1	40
	50-100%	7	5	8		1	21
<b>AS sales GLY</b>							
	< 7000	6	4	11	2	2	25
	> 7000	12	10	5	9		36
<b>AS sales GLUF</b>							
	0	15	11	7	11		44
	0.75	3	3	9		2	17
	<b>Total</b>	<b>18</b>	<b>14</b>	<b>16</b>	<b>11</b>	<b>2</b>	<b>61</b>

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1214 **Table A.2. Statistical outputs of binomial GLMs to investigate the influence of explanatory factors on detection frequencies of glyphosate, AMPA, and glufosinate in hair**  
 1215 **of small mammals.**

1216 *The binomial models were computed as “Detection ~ Species” and “Detection ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AS sales”. \**  
 1217 *alternative model with the factor “glufosinate sales” alone without the factor “species”. Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus*  
 1218 *(Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew), Miar=Microtus arvalis (Common vole), Mumu=Mus musculus (House mouse), Mygl=Myodes glareolus (Bank*  
 1219 *vole), NT=Not targeted, T=target, AS=active substance, p=p-value. Significant p-values are highlighted in bold.*

Factor	GLYPHOSATE							AMPA						GLUFOSINATE						
	Levels	n	Deviance	Df	p	Odds Ratio	95% CI (lower, upper)	p	Deviance	Df	p	Odds Ratio	95% CI (lower, upper)	p	Deviance	Df	p	Odds Ratio	95% CI (lower, upper)	p
Species			20.484	56	<b>&lt; 0.001</b>				32.464	56	<b>&lt; 0.001</b>				39.079	56	<b>&lt; 0.001</b>			
Apsy	18					Reference	---	---				Reference	---	---				Reference	---	---
Crru	14					2.00	(0.48, 8.76)	0.344				0.58	(0.07, 3.55)	0.571				0.62	(0.03, 7.14)	0.700
Miar	16					30.0	(4.48, 612)	<b>0.003</b>				24.5	(4.56, 208)	<b>&lt; 0.001</b>				34.7	(6.05, 321)	<b>&lt; 0.001</b>
Mumu	2					2.00	(0.07, 56.9)	0.644				3.50	(0.12, 103)	0.411				8.00	(0.25, 273)	0.200
Mygl	11					20.0	(2.86, 414)	<b>0.010</b>				35.0	(4.73, 753)	<b>0.003</b>				80.0	(9.16, 1999)	<b>&lt; 0.001</b>
Habitat			0.191	55					0.018	55	0.892				0.182	55	0.669			
Cereal	22				0.662	Reference	---	---				Reference	---	---				Reference	---	---
Hedgerows	39					0.71	(0.14, 3.31)	0.663				1.12	(0.22, 6.59)	0.893				1.50	(0.24, 12.3)	0.673
Farming			3.273	54	0.195				3.364	54	0.186				1.496	54	0.473			
OF	10					Reference	---	---				Reference	---	---				Reference	---	---
OF/CF	10					6.98	(0.57, 217)	0.169				12.7	(0.79, 4745)	0.102				5.83	(0.35, 133)	0.200
CF	41					6.96	(0.84, 174)	0.119				6.35	(0.65, 172)	0.161				2.49	(0.30, 27.1)	0.400
Treatment			0.000	55	0.999				0.030	55	0.863				0.016	55	0.900			
NT	41					Reference	---	---				Reference	---	---				Reference	---	---
T	20					1.00	(0.22, 4.47)	0.999				1.15	(0.23, 5.63)	0.863				0.90	(0.14, 5.12)	0.900
Landscape OF			0.331	55	0.565				0.031	55	0.860				0.013	55	0.908			
0-50%	40					Reference	---	---				Reference	---	---				Reference	---	---
50-100%	21					0.67	(0.16, 2.60)	0.568				1.14	(0.24, 5.18)	0.860				0.91	(0.15, 4.88)	0.908
AS sales			0.009	55	0.925				1.257	55	0.262				5.266 (0.091)*	55 (59)*	<b>0.022</b> (0.763)*			
Low category	25					Reference	---	---				Reference	---	---				Reference	---	---
High category	36					1.07	(0.26, 4.58)	0.925				2.66	(0.51, 21.9)	0.289				0.00	NA	> 0.900
																		(0.84)*	(0.26, 2.59)*	(0.800)*

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1222 **Table A.3. Statistical outputs of GLMs to investigate the influence of explanatory factors on the concentrations of glyphosate, AMPA, and glufosinate in hair of small**  
 1223 **mammals.**

1224 *The models were computed as “log(concentration) ~ Species” and “log(concentration) ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AI sales”.*  
 1225 *Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus (Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew), Miar=Microtus*

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*arvalis* (Common vole), *Mumu*=*Mus musculus* (House mouse), *Mygl*=*Myodes glareolus* (Bank vole), *NT*=Not targeted, *T*=target, *AS*=active substance, *p*=*p*-value. Significant *p*-values are highlighted in bold.

Factor	GLYPHOSATE										AMPA						GLUFOSINATE											
	Factor			Mode			Levels				Factor			Model			Levels			Factor			Model			Levels		
	Levels	n	F	Df	p	R <sup>2</sup>	R <sup>2</sup> R <sup>2</sup> Adj	Estimate	SE	p	F	Df	p	R <sup>2</sup>	R <sup>2</sup> R <sup>2</sup> Adj	Estimate	SE	p	F	Df	p	R <sup>2</sup>	R <sup>2</sup> R <sup>2</sup> Adj	Estimate	SE	p		
Species		1.643	4	0.186	0.162	0.162					1.110	4	0.373	0.146	0.146				1.008	4	0.425	0.155	0.155					
						0.063									0.014							0.002						
	Apsy	6					Reference	---	---						Reference	---	---					Reference	---	---				
	Crru	7					-0.840	0.815	0.310						-0.322	1.09	0.770					-1.58	1.06	0.150				
	Miar	15					0.723	0.707	0.314						1.08	0.715	0.142					-0.546	0.657	0.415				
	Mumu	1					0.729	1.58	0.648						0.333	1.41	0.815					0.310	1.06	0.773				
	Mygl	10					0.701	0.756	0.360						1.06	0.746	0.169					-0.819	0.670	0.235				
Habitat		4.37	1	<b>0.044</b>	0.098	0.260					0.750	1	0.395	0.025	0.171				2.759	1	0.112	0.098	0.253					
						0.148									0.005							0.075						
	Cereal	14					Reference	---	---						Reference	---	---					Reference	---	---				
	Hedger	25																										
	ows						1.20	0.572	<b>0.044</b>														-0.716	0.431	0.112			
Farming		0.017	2	0.983	0.001	0.162					0.725	2	0.495	0.049	0.195				0.087	2	0.917	0.007	0.162					
						0.006									0.000							0.000						
	OF	5					Reference	---	---						Reference	---	---					Reference	---	---				
	OF/CF	6					-0.184	1.05	0.861						0.800	0.988	0.426					-0.194	0.750	0.799				
	CF	28					-0.146	0.890	0.871						0.022	0.838	0.979					-0.252	0.606	0.682				
Treatment		0.232	1	0.633	0.006	0.168					0.750	1	0.395	0.025	0.171				2.759	1	0.112	0.098	0.253					
						0.042									0.005							0.075						
	NT	27					Reference	---	---						Reference	---	---					Reference	---	---				
	T	12					-0.289	0.600	0.633						0.521	0.602	0.395					0.716	0.431	0.112				
Landscape OF		0.151	1	0.700	0.004	0.166					0.754	1	0.393	0.025	0.171				3.454	1	0.077	0.119	0.274					
						0.039									0.005							0.101						
	0-50%	27					Reference	---	---						Reference	---	---					Reference	---	---				
	50-100%	12					0.227	0.582	0.700						0.496	0.570	0.393					0.790	0.425	0.077				
AS sales		0.104	1	0.749	0.003	0.165					0.595	1	0.448	0.020	0.166				0.884	1	0.358	0.034	0.189					
						0.038									0.000							0.000						
	Low category	17					Reference	---	---						Reference	---	---					Reference	---	---				
	High category	22					-0.172	0.535	0.749						0.416	0.539	0.448					-0.454	0.483	0.358				

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