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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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31 **Abstract**

32 Glyphosate (GLY) is the most widely used herbicide worldwide, and its use continues to increase.
33 Accumulating evidence shows that GLY and its metabolite aminomethylphosphonic acid (AMPA) are
34 more persistent and toxic than expected, but little is known about the risks to wildlife. Glufosinate
35 (GLUF) was considered an alternative broad-spectrum herbicide, but its field ecotoxicology has rarely
36 been studied. This study aimed to assess the exposure of free-ranging rodents and shrews from
37 treated (cereals under conventional farming) and nontreated habitats (cereals under organic farming
38 and hedgerows) in France to GLY, AMPA, and GLUF through residue analyses in hair. We investigated
39 the patterns of accumulation according to species, habitat, and treatment intensity at the plot,
40 landscape, or township scale. We showed a generalized exposure of small mammals to GLY, AMPA
41 and GLUF, as they were detected in all of the species, and in 64%, 51% and 44% of the hair samples,
42 respectively. The detection and levels of GLY, AMPA and GLUF were greater in herbivorous and
43 omnivorous voles than in insectivorous shrews and omnivorous wild mice. The three compounds
44 showed comparable ranges of concentrations: 0.018-7.74 pg/mg with an outlier of 522 pg/mg for
45 GLY (median = 2.65), 0.240-33.6 pg/mg for AMPA (median = 1.39), and 1.16-25.5 for GLUF (median =
46 3.51). The frequencies of detection and concentrations did not significantly differ according to the
47 farming practices or proxies of pesticide treatment intensity. The concentrations of GLY were greater
48 in individuals captured in hedgerows than in those captured in cereal fields. On the basis of dose
49 reconstruction approaches and toxicological thresholds from the literature, GLY and GLUF levels may
50 be associated with risk in small mammals and endanger local populations. Our findings raise issues
51 about the omnipresence of GLY, AMPA and GLUF in agricultural landscapes, including in animals from
52 habitats considered refuges, questioning their ecological safety. This work provides new insights into
53 current broad-spectrum herbicide wildlife ecotoxicology that may support decision-making to protect
54 biodiversity.

55

56 **Keywords:** agroecosystem; bioaccumulation; currently used pesticides; landscape ecotoxicology;
57 wildlife toxicology
58

59 **1. Introduction**

60 Since its registration as a broad-spectrum contact herbicide in 1974, the use of glyphosate (GLY) has
61 continuously increased, making GLY-based pesticides the most widely applied herbicides worldwide
62 (Gandhi et al. 2021; Richmond 2018). Maggi et al. (2020) reported that GLY and its main degradation
63 product aminomethylphosphonic acid (AMPA) may be persistent contaminants in approximately 30%
64 and 93% of global cropland soils, respectively. On the basis of persistence, accumulation and toxicity
65 criteria, they computed that 1% of croplands worldwide (i.e. approximately 385,000 km²) experience
66 mid-high to high hazard conditions. Outside these hotspots, contamination is expected to be low but
67 globally pervasive (Maggi et al. 2020).

68 Initially, GLY was marketed as an “environmentally friendly” herbicide owing to its low expected
69 environmental persistence (easily biodegraded), low predicted toxicity to wildlife and humans
70 (absence of the shikimate pathway in animals, which is found only in plants and microorganisms and
71 is the target pathway of GLY in plants), and lack of potential for bioaccumulation (hydrophilic, low log
72 octanol–water partition coefficient K_{ow} (– 3.12) which is a measure to assess whether a substance is
73 more or less hydrophobic (and/or lipophilic, fat solubility) or hydrophilic (and/or lipophobic, water
74 solubility) and is used as one of the regulatory criteria to classify a compound as bioaccumulative if
75 $\log K_{ow} > 5$, poorly metabolized and rapidly eliminated in mammals) (EFSA 2015a; Gandhi et al. 2021;
76 Kissane and Shephard 2017; Richmond 2018). However, concerns about the environmental safety
77 and health risks associated with GLY have increased within recent decades, especially in the context
78 of its continuously increasing use (Gandhi et al. 2021; Székács and Darvas 2018; Van Bruggen et al.
79 2018; Vandenberg et al. 2017). GLY and AMPA are more persistent in the environment than initially
80 assessed, as shown by the ubiquitous occurrence of residues in soil, water, and air (Bai and Ogbourne
81 2016; Gandhi et al. 2021; Richmond 2018; Van Bruggen et al. 2018; Xu et al. 2019). They can
82 accumulate in environmental compartments, crops, and the human food chain (Bai and Ogbourne
83 2016; Richmond 2018). They are associated with chronic low-dose exposure risks in animals and
84 humans, and can be more harmful to ecosystems than expected (Gandhi et al. 2021; Kissane and

85 Shephard 2017; Ojelade et al. 2022; Peillex and Pelletier 2020; Richmond 2018). Furthermore, GLY
86 and AMPA may be more bioaccumulative substances than predicted from their physico-chemical
87 properties (Pelosi et al. 2022; Richmond 2018; Ruuskanen et al. 2020; Serra et al. 2021). However,
88 the controversial issues regarding GLY-based herbicides have focused mostly on human health
89 (Székács and Darvas 2018; Vandenberg et al. 2017), whereas GLY and AMPA exposure and their
90 impacts on wildlife have been overlooked (Kissane and Shephard 2017).

91 In the USA and EU, registration reviews concerning GLY use as a plant protection product (PPP)
92 identified potential risks to terrestrial and aquatic plants and nontarget terrestrial arthropods and
93 risks to mammals and birds (EFSA 2015b, 2015c, 2015a; US EPA 2020). In the EU assessment for
94 vertebrates, the taxa identified as the most at risk were mammals, particularly herbivorous mammals
95 (EFSA 2015b, 2015a). Glufosinate (GLUF), like GLY, is an organophosphorus compound used as a
96 broad-spectrum nonselective contact herbicide with a systemic action and was considered “*one of*
97 *the very few alternatives to glyphosate*” (European Commission 2017). However, risks for
98 herbivorous and insectivorous mammals associated with the herbicidal use of GLUF have been
99 reported in environmental risk assessment (ERA) processes (EFSA 2005, 2012). GLUF was classified as
100 a candidate for substitution in the EU according to Commission Regulation EC No 1107/2009 (PPP
101 Regulation) in 2015, and withdrawal was scheduled at its end of approval in the EU in 2018 (Robin
102 and Marchand, 2023). However, GLUF is still used in many countries worldwide.

103 The singular physicochemical properties of GLY (a particularly hydrophilic polar compound, existing in
104 different ionic states depending on pH, making it difficult to extract) and closely related compounds
105 such as AMPA or GLUF make measurement of their residues challenging and prevent these
106 compounds from being included in multiresidue analyses (Delhomme et al. 2021). Such technical
107 limitations in analytical chemistry may have hampered the development of studies about GLY, AMPA
108 and GLUF exposure and their effects in wildlife and, more generally, knowledge building about the
109 terrestrial ecotoxicology of these compounds. Ultimately, this lack of knowledge could be an
110 impediment for regulatory decisions with respect to the objectives of reducing the risks and impacts

111 of pesticide use on human health and the environment (e.g. Directive 2009/128/EC). Assessing and
112 characterizing the exposure of wildlife to GLY, AMPA and GLUF is therefore a critical issue for
113 environmental protection and biodiversity conservation.

114 The main aim of this study was to investigate the exposure of wildlife in an arable landscape to GLY,
115 AMPA and GLUF and evaluate the risk for toxicological impairment, with a focus on small mammals.
116 This was achieved via a minimally invasive sampling method based on residue analyses in hair and on
117 reverse dosimetry approaches. The compound residues can be detected and quantified in hair, owing
118 to the effectiveness of this matrix for assessing exposure to multiple pesticides in wildlife (Fritsch et
119 al. 2022; Krief et al. 2017), as shown for GLY in bats (Hooper et al. 2022) and both GLY and AMPA in
120 humans (Alvarez et al. 2022). Moreover, GLY concentrations were relative to the intensity of
121 exposure in human samples (i.e. GLY was quantified at lower concentrations in hair samples from
122 nonoccupationally exposed subjects than in hair samples from farmers, who often used GLY) (Alvarez
123 et al. 2022). Differences in exposure between the three compounds were investigated, and the
124 influences of farming practices and herbicide use intensity were studied. We expect exposure to GLY
125 to be greater than that to the other compounds. Indeed, GLY is more commonly used and is still
126 authorized in France, in contrast to GLUF (BNV-D Traçabilité), and AMPA is not a pesticide active
127 substance itself but a metabolite of GLY. Mammals can be exposed to herbicides through diet and
128 overspray (European Commission, 2015; Martinez-Haro et al. 2022; Mayer et al. 2020; Newton et al.
129 1984). Therefore, small mammals may be exposed to GLY through both trophic exposure and/or
130 overspray, whereas direct exposure during spraying should be negligible for the other two
131 compounds. Higher concentrations in hair were expected in treated areas (i.e. conventional crops)
132 than in nontargeted habitats (i.e. organic farming (OF) fields and hedgerows). Since small mammals
133 are mobile species, the influence of herbicide use intensity was also considered at the landscape
134 scale (i.e. using the proportion of OF fields within the landscape as a proxy) and at the township level
135 (i.e. using sales of active substances as a proxy of usage intensity). To further document the factors
136 that may shape exposure to GLY, AMPA and GLUF, differences between small mammal species

137 (rodents and shrews) with various ecological traits were investigated. Herbivorous voles are expected
138 to exhibit greater exposure than are granivorous, omnivorous or insectivorous species according to
139 the higher daily dietary doses estimated during ERA processes (EFSA 2005, 2012; European
140 Commission 2015). Moreover, herbivorous voles are less mobile than omnivorous mice and voles or
141 shrews are (van den Brink et al. 2011; Wijnhoven et al. 2005) and may not avoid treated areas. We
142 expect species that feed on animal matter (i.e. omnivores, carnivores, insectivores or vermivores) to
143 also be exposed because residues of GLY, AMPA and GLUF have been found in earthworms that are
144 the prey of these trophic groups (Pelosi et al. 2022), and GLY residues have been found in small
145 carnivorous and omnivorous mammals in a forest ecosystem (Newton et al. 1984).

146 **2. Material and methods**

147 *2.1. Study design*

148 The study site was the Long-Term Socio-Ecological Research (LTSER) “Zone Atelier Plaine & Val de
149 Sèvre” (ZAPVS, <https://za-plaineetvaldesevre.com/>) in mid-western France (see Bretagnolle et al.
150 (2018) for a general site description). The sampling design was based on 40 sampling squares of 1
151 km², initially selected on the basis of landscape features (gradient of seminatural habitats such as
152 hedgerows and a gradient of organic farming surfaces), in which sampling by trapping was conducted
153 in an arable cereal field (winter cereals) and a woody patch (hedgerow or woodlot edge) as close as
154 possible to the sampled arable field (cf. Pelosi et al. 2022), for a total of 78 plots investigated (Fig. 1).
155 Cropped cereals and hedgerows are referred to as “habitats” hereafter. Cropped fields in which the
156 sampling was carried out were cultivated either under conventional farming (CF) or organic farming
157 (OF). The farming practices in the organic fields followed the rules of the “AB (Agriculture Biologique)
158 France label” and were under OF for at least three years at the time of sampling. A few fields were in
159 transition from CF to OF and were referred to as “OF/CF”. Sampling was conducted in spring 2018
160 (May-June).

161 *2.2. Sampling process*

162 Small mammals were captured using traplines, which were set in each sampling plot over the
163 sampling squares using lines of nonlethal traps (25 traps, 2 m apart) with dormitory (Le Quilliec and
164 Croci 2006) filled with hay and food (i.e. pieces of carrots and apples, sunflower seeds, cat food and
165 peanut butter). The hay, vegetables, fruits and seeds originated from organic agriculture. The traps
166 were checked every day, rebaited and refilled as necessary, and replaced by a new trap in case of
167 capture. The traplines were set during one to four nights depending on logistics and capture success
168 (i.e. longer duration, if possible, in case of an absence of capture during the first night(s)). Species
169 were determined during the handling of individuals on the basis of morphometrics and confirmed by
170 molecular analyses in case of doubt (see below). A sample of 50-100 mg of hair was taken by shaving

171 over the posterior part of the back, wrapped in aluminium foil and stored in plastic zip-lock bags at
172 room temperature during the field session and later at -20 °C until analysis. Individuals were released
173 after handling. In case of recapture, the individuals were immediately released without any
174 measurement or sampling. For animals that did not survive capture, the bodies were collected and
175 stored at -20 °C until they were shaved for hair sampling. Because *Microtus arvalis* and *Microtus*
176 *agrestis* are morphologically similar species, the identification of *Microtus* voles was confirmed by
177 molecular analyses (Sup Info Annex 1. Text A1). The suspected *Mus* spp individuals were also
178 confirmed genetically. For that, a tiny piece of ear was sampled (using medical clean and disinfected
179 scissors, followed by disinfection of the ear) and stored by freezing (-20 °C) until DNA extraction.

180 The experiments were performed under the authorization of the French National Ethical Committee
181 (Project APAFIS N°5340) by skilled and experienced investigators from the Chrono-environnement
182 research department (EU0592), following directive 2010/63/EU on the protection of animals used for
183 scientific purposes. All precautions to limit stress and deleterious effects on the animals as much as
184 possible were taken. The sampling and handling of small mammals were conducted under
185 supervision and with the participation of people authorized for animal experimentation and
186 experienced in small mammal capture and handling and using appropriate and authorized (EU0592)
187 facilities (a vehicle equipped with a mobile anaesthesia unit and all of the materials and equipment
188 required for animal welfare).

189 *2.3. Proxies related to the intensity of treatments at various spatial scales*

190 First, to investigate the role of agricultural practices in shaping the exposure of small mammals,
191 habitats were classified as cultivated under OF or CF according to the type of agricultural organic or
192 conventional practices in the cultivated fields. To classify hedgerows or woodlots with respect to
193 farming practices, the adjacent plots were considered, and each woody patch was classified as “OF”
194 if adjacent fields were under OF and none of the adjacent fields was under CF, “OF/CF” if at least one
195 of the adjacent fields was under CF, or “CF” when all adjacent fields were under CF.

196 For the proxies of treatment intensity, at the plot scale (arable field or woody patches where
197 trapping was realized), a general proxy of potential for pesticide treatment was computed. The fields
198 cultivated under OF and hedgerows or woodlots were considered not intentionally targeted by
199 treatments (NT), whereas the arable fields cultivated under CF and transitional fields cultivated from
200 CF to OF were considered possibly targeted (T). A second proxy to characterize the intensity of
201 potential treatments was computed at the landscape scale. The locations and boundaries of
202 cultivated plots in 2018 were obtained from the official French “Registre Parcellaire Graphique”
203 (<https://www.data.gouv.fr/fr/datasets/parcelles-en-agriculture-biologique-ab-declarees-a-la-pac>).
204 The software Quantum GIS (QGIS 3.16) was used to calculate the proportion of OF plots in the
205 landscape, which was computed in each sampling square of 1 km² as the proportion of the total
206 surface occupied by OF arable fields. The final proxy resulted in a two-level factor, with a category
207 “OF dominant” with proportions varying from 50 to 100% ([49-79], 6 sampling squares), in which
208 treatment intensity at the landscape scale was expected to be lower since arable fields not targeted
209 by pesticide treatments prevailed within the landscape mosaic. The second category, “CF dominant”,
210 gathered squares with landscape OF proportions ranging from 0 to 50% ([0-49], 19 sampling
211 squares). Finally, to consider the intensity of potential treatments over the range of several
212 farmlands, a proxy at the township scale was computed. The sales of active substance (a.s., data
213 provided in Kg) were obtained from the “Banque Nationale des ventes de produits
214 phytopharmaceutiques par les Distributeurs agréés (BNV-D Traçabilité)” as released to the public by
215 official agencies at the township level (i.e. according to postal code, <https://www.data.gouv.fr/fr>).
216 These data were extracted for 2017 and 2018 (the year before and the year when sampling was
217 conducted). The sales of GLY and GLUF were associated with each trapping location on the basis of
218 township positioning. The sales of GLY were highly correlated between years at the township level
219 ($R^2=0.950$), rendering it impossible to study the influence of potential use intensity separately in the
220 year before or in the year of sampling. The marketing authorisation of pesticides containing GLUF
221 was withdrawn in France by October 2017. The quantities sold in 2017 were very low (i.e. 0.75 kg)

222 and sporadic (i.e. only one quantity reported as sold in the townships studied) whereas in 2018, no
223 sales of GLUF were reported in the townships of the study area of concern. The quantities sold in
224 2017 and 2018 were thus summed for each a.s. and classified into two categories. For GLUF, the
225 categories were “0” and “0.75”. For GLY, the median of the summed quantities in the dataset was
226 calculated at 7300, and the categories were set at “lower than 7000” ([4138-7000[, 2 townships) and
227 “higher than 7000” ([7000-9366[, 3 townships).

228 *2.4. Chemical analyses of glyphosate, AMPA and glufosinate*

229 Full detailed information about chemicals and reagents, sample preparation, methodological
230 approaches used for the chemical analyses and issues related to metrology are provided in Sup Info
231 Annex 1. Text A2.

232 Briefly, the surface of hair samples were decontaminated with sodium dodecyl sulfate solution and
233 methanol (Duca et al. 2014) After decontamination, the samples were spiked with internal standard
234 solution, and water was added before overnight extraction. The supernatants were evaporated to
235 dryness, and borate buffer and FMOC-Chloride were added before derivatization and injection into
236 the LC-MS/MS Waters system Acquity UPLC H-class system equipped with a Waters Acquity BEH C18
237 column (100 × 2.1 mm, 1.7 µm particle size) coupled to a Waters Xevo TQ-S triple quadrupole mass
238 spectrometer. With each analytical run, quality controls consisting in hair samples supplemented at
239 several concentrations (0.1 to 100 pg/mg) and a blank sample were included.

240 The concentrations are expressed as pg/mg hair. The limits of quantification (LOQ) and lowest
241 detected values (Minimum) are presented in Table 1. We did not perform specific experiments and
242 calculations to compute limits of detection (see Sup Info Annex 1. Text A2). Importantly, only one
243 concentration value was reported below the lowest concentration in quality controls (the minimum
244 GLY concentration, see Table 1). All values above the lowest analytical detected value were included
245 as “detects” contrary to “nondetects”. All reported concentration values, including values between
246 the LOQ and the lowest detected value, were included as continuous data. The use of concentrations

247 below the LOQ has been shown to be a suitable method than may be preferred to the absence of
248 data or other established approaches such as imputation-based methods (i.e. efficiency in terms of
249 bias and precision)(Keizer et al. 2015).

250 *2.5. Dose reconstruction to assess the risk of deleterious effects in small mammals*

251 Given the concentrations of GLY, AMPA and GLUF in the keratinized tissues of small mammals, our
252 study highlights the exposure of these animals. However, the toxicological meaning of such hair
253 concentrations is hard to interpret with respect to toxicity benchmarks (which are expressed as daily
254 doses in mg a. s. per kg body weight) because relationships between residues in hair and exposure
255 doses or between residues in hair and in those other organs are not known. To overcome this gap
256 and assess whether exposure levels could reach toxic levels, approaches of reverse dosimetry have
257 been applied to estimate levels of exposure in terms of daily dose intake, to obtain data comparable
258 to toxicological thresholds. Different types of data (i.e. concentrations in mammal body fluids and
259 hair, residues in viscera and body burden in small mammals, toxicokinetics in small mammal blood,
260 pharmacokinetics of fast-elimination chemicals) that may be used for such purposes have been
261 collected from the literature (Sup Info Annex 1. Text A3 for full description), leading to different
262 approaches of back-calculations computed under several assumptions when there was a lack of
263 available data. Five different approaches could be computed for GLY and one for GLUF (Table 2).

264 The objective of our risk assessment was to determine the overall orders of magnitude of daily dose
265 intake relative to the concentrations measured in hair to evaluate whether wild small mammals may
266 experience deleterious toxicological effects. The lack of data and assumptions that had to be applied
267 hamper the accuracy of the calculated dose values. The interpretation of the findings was thus
268 developed to consider ranges and global patterns rather than refined (but biased) toxicological
269 assessments. The doses calculated were compared with toxicity data using the usual and regulatory
270 procedure of toxicity-exposure ratio (TER) calculation, with associated triggers of 5 for chronic
271 toxicity data and of 10 for acute toxicity data. The doses were reconstructed for each species, using

272 four levels of residue concentrations measured in hair: minimum, median, 3rd quartile and maximum
273 values (only one value available in house mice for GLY and in house mice and shrews for GLUF). The
274 dose of GLY was compared to five toxicological thresholds: the short-term dietary no observed effect
275 level (short-term dietary NOEL), the chronic 21 day no observed adverse effect level (chronic NOAEL,
276 which is the same value as the reproductive NOAEL), the long-term NOAEL, the no mortality dose to
277 the Oregon vole, and a “chronic low dose” limit. Details about the values of the thresholds are
278 provided in the Sup Info Annex 1. Text A3. The dose of GLUF was compared to four available
279 toxicological thresholds: the acute oral median lethal dose, the short-term dietary NOEL, the chronic
280 21 day NOAEL, and the long-term NOAEL.

281 The process ended with the TER values computed for each approach (i.e. back-calculations on the
282 basis of concentrations in human urine and hair, body burden in rats, toxicokinetics in rat blood,
283 residues in viscera and body of wild small mammals, prediction of incorporation in hair from
284 pharmacokinetics), under different scenarios of assumption, for the different levels of residue
285 concentrations measured in hair (minimum, median, 3rd quartile and maximum values), for each
286 species, for the different toxicological thresholds (see details in Sup Info Annex 2). Finally, the
287 number of cases where TER exceeded the triggers, which highlights the risk for toxic effects in small
288 mammals, and the number of individuals involved were summarized.

289 *2.6. Data analysis and statistics*

290 Analyses were performed using R (3.3.1), with the additional packages “AICcmodavg”, “effects”,
291 “EnvStats”, “forestmodel”, “ggeffects”, “ggplot2”, “gtsummary”, “multcomp”, “pgirmess”, and
292 “questionr”. Since more than 2 individuals were obtained from the same trapline in only 8 out of the
293 29 where the animals were captured, pseudoreplication was not considered an issue. Moreover, the
294 traplines extended over 50 metres, which should limit the influence of spatial autocorrelation
295 between individuals. The statistical approaches were designed to handle left-censored data within a
296 situation where the smallest observations of data are nondetects, with high proportions of

297 nondetects, skewed distributions, and small sample sizes, which hampered the use of methods
298 considered “gold standards” to manage left-censored data (ITRC 2013; Shoari and Dubé 2018).
299 Following recommendations to analyse datasets including nondetected and left-censored
300 observations, we used tests dedicated to censored data when possible, computed descriptive
301 statistics separately on detection frequencies and on detected concentrations (for which median,
302 interquartile range, and outer percentiles were reported), performed group comparisons separately
303 on detection frequencies and on detected concentrations, and preferred nonparametric tests to
304 analyse the combined dataset, including uncensored data and nondetects with the latter being
305 substituted by 0 (ITRC 2013; Shoari and Dubé 2018). Compared with other methods (i.e. imputation-
306 and nonimputation-based approaches), the traditional substitution approach has been shown to be
307 appropriate for handling nondetects in a study that investigated a dataset of pesticide
308 concentrations in pollen and nectar to assess the exposure of bumble bees (Adjei and Stevens 2022).
309 Substitution with a half limit of detection outperformed the other approaches in the study of Adjei
310 and Stevens (2022), which used parametric tests (nested ANOVA), but we preferred to use 0 because
311 (1) we used all reported values, including those between the LOQ and lowest detected values,
312 instead of data left-censored by the limit of detection (Keizer et al. 2015), (2) the lowest detected
313 values reached low values that would have brought the value of half of the LOD close to 0, (3) this
314 approach allows easy identifying nondetects in the databases and graphics, and (4) nonparametric
315 tests were used to compute statistics on both nondetects and quantified values. Comparisons
316 between the distributions of the compounds were performed using Tarone-Ware two-sample test
317 for censored data (i.e. GLY vs. AMPA, GLY vs. GLUF, and AMPA vs. GLUF), with nondetects set to the
318 corresponding lowest detected value of the corresponding compound as needed in such tests, for all
319 species together (the sample sizes were too small to run the tests by species).

320 Correlations between raw values with nondetects set to 0 of the concentrations of the three
321 compounds were investigated using Spearman’s rank correlation tests. The relationships between
322 the concentrations of each compound were further studied according to species via generalized

323 linear models (Gaussian family) on log-transformed data ($\log(x+1)$) to meet the assumptions of
324 normality and variance homogeneity. The models were checked for homogeneity of variance,
325 normality of error and linearity/additivity and leverage was checked (i.e. standardized residuals vs.
326 leverage). An extreme outlier of GLY concentration was identified (one *Microtus* vole) as having
327 overinfluence on the estimated parameters (i.e. Cook distance greater than 1). This outlier was
328 removed from the dataset to compute model outputs and graphics in this part of the analyses.

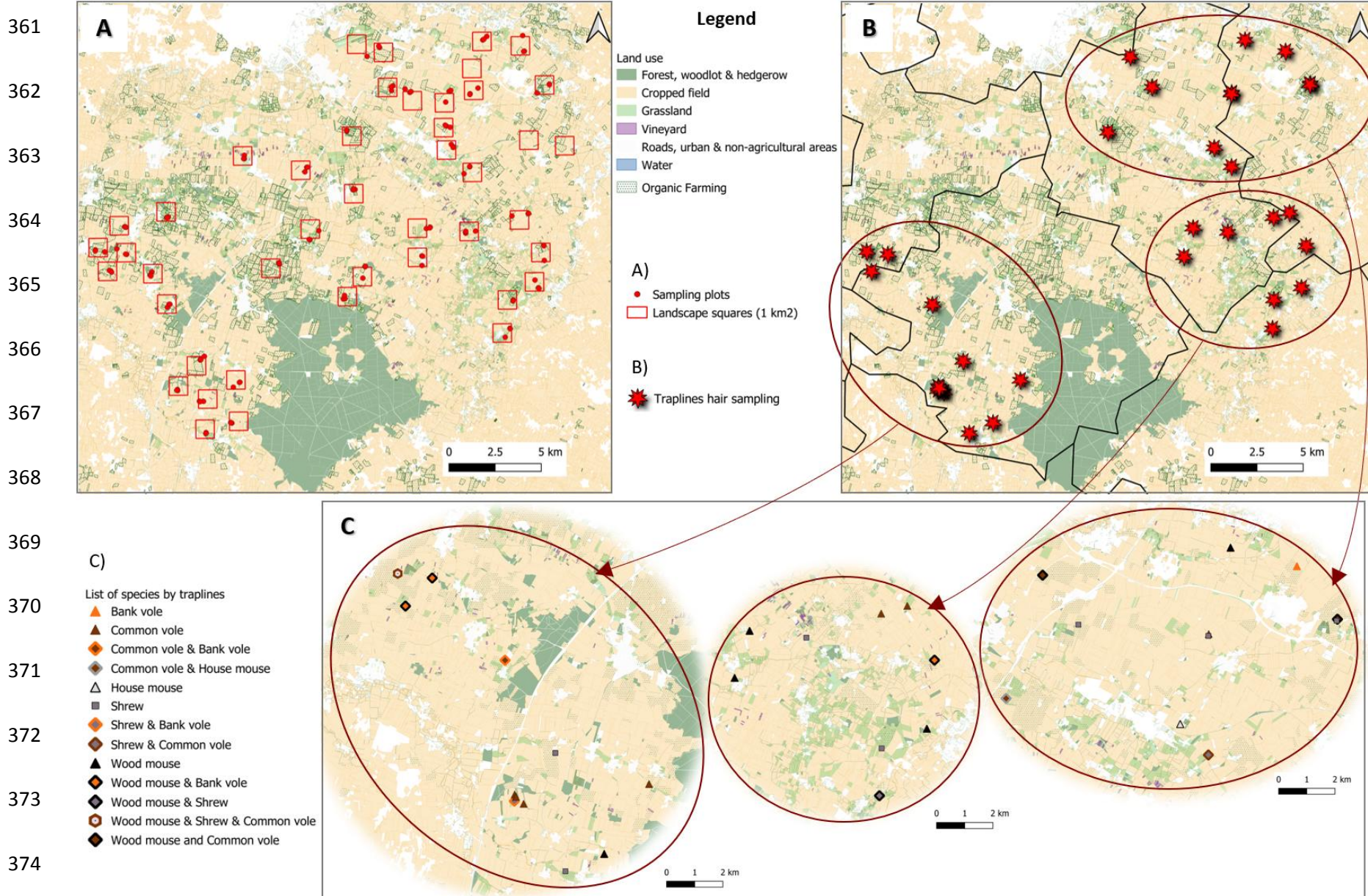
329 Then, statistical analyses were performed on (1) GLY, AMPA or GLUF frequencies of detection and (2)
330 GLY, AMPA or GLUF concentrations quantified above the lowest detected value (i.e. in samples
331 where the compound was detected, without nondetects). According to the preliminary analyses
332 showing differences between species, further analyses were conducted taking species into account in
333 statistics using the factor “species” first in the models with the other factors. Full models including all
334 explanatory factors and first-order interactions could not be used due the large number of NAs.
335 Preliminary tests performed on combinations of factors, including interactions, revealed that none of
336 the interactions were statistically significant and that models without interactions better fit the data
337 (according to corrected Akaike information criterion (AICc) comparisons). The final models used to
338 compute the statistics were therefore run without interactions. We tested whether the detection of
339 GLY, AMPA or GLUF in hair of small mammals was dependent on species, habitat, farming practices
340 and proxies for treatment intensity using binomial GLMs (link = logit). The detection was coded as “0”
341 for nondetected and “1” when detected. The models were built using species alone and species with
342 each of the other variables as explanatory factors. The significance of the factors was checked by the
343 *chi-squared* test. Odds ratios with confidence intervals were computed to characterize differences
344 between levels. We tested whether the concentrations of GLY, AMPA or GLUF in the hair of small
345 mammals were dependent on species, habitat, farming practices and proxies for treatment intensity
346 using GLMs on log-transformed values above the lowest detected value (i.e. without nondetects) to
347 meet the assumptions of normality and variance homogeneity. The models were built using species
348 alone and species with each of the other variables as explanatory factors. The significance of the

349 explanatory factors was checked via an *F* test, and statistical differences between levels were via a *t*
350 test.

351 To support the results and interpretation, both the detection and concentration data were analyzed
352 via nonparametric tests on all of the data, with nondetects included and set to 0. The differences
353 between species, habitats, farming practices, and the proxies of treatment intensity were tested
354 adequately via the Wilcoxon-Mann-Whitney test or the Kruskal-Wallis test adequately depending on
355 the number of levels of the factors (i.e. two or more). Post hoc multiple comparison tests after
356 Kruskal-Wallis tests were used to investigate pairwise comparisons when relevant. Correlations
357 between concentrations and landscape OF proportions or sales of a.s. used as raw continuous data
358 were tested via Spearman's rank correlation test.

359

360 **Figure 1. Maps of the study design (A) and location of the traplines where hair samples were collected (B and C) across the study area.**



375 **3. Results & Discussion**

376 *3.1. Captures of small mammals*

377 The sample size resulted in hair samples from 61 individuals captured in 29 traplines distributed over
378 25 squares of 1 km² out of the sampling plots initially surveyed (Fig. 1, Table 1; further details about
379 the sample sizes are provided in Sup Info Annex 1. Table A1). Six individuals were recaptured. The
380 number of recaptures was quite low since most of the traplines were set for only one night (1 night:
381 $n = 63$, 2 nights: $n = 12$, 4 nights: $n=3$). Individual samples of 18 wood mice (*Apodemus sylvaticus*), 14
382 greater white-toothed shrews (*Crocidura russula*), 16 common voles (*Microtus arvalis*), 11 bank voles
383 (*Myodes glareolus*), and two house mice (*Mus musculus*) were analysed. A total of 41 samples were
384 collected from individuals captured in habitats under CF farming, 10 from OF/CF category, and 10
385 from the OF category. A total of 39 samples were collected from hedgerows or woodlots, and 22
386 were collected from cereal fields.

387 *3.2. Detection frequency of GLY, AMPA and GLUF in the hair of small mammals*

388 Considering all of the species together, GLY, AMPA and GLUF were detected in 64%, 51%, and 44% of
389 the individuals, respectively (Table 1). Compared with the quantities sold in the townships studied,
390 the quantities of GLUF were 2484 to 4844 times lower than those of GLY in 2018 and 1653 to 4548
391 times lower in 2017. Indeed, after its ban in 2017, GLUF could only have been used until stock
392 depletion or usage derogation. Focusing on the part of the region where the study was carried out
393 (i.e. “Département des Deux-Sèvres”), the purchases of GLUF rapidly decreased after 2015 and
394 reached 0 in 2019 (BNV-D”). Despite its low intensity of application compared with that of GLY, GLUF
395 was detected in approximately half of the small mammal individuals. Within the “PING” research
396 programme, the part of the study conducted over the same year over the same sampling sites as the
397 present one detected GLY in 88% of the soil samples and 74% of the earthworm samples, AMPA in
398 58% of the soil samples and 38% of the earthworms, and GLUF in 35% of the soil samples and 12% of
399 the earthworms (Pelosi et al. 2022). These detections in soil, earthworms and small mammals

400 collected from arable fields and hedgerows, both in conventional and organic farming fields, revealed
401 a generalized occurrence of GLY, AMPA and GLUF in the agricultural environment and fauna across
402 this typical French agricultural area. In comparison, in three county-level hair pools of carcasses of
403 big brown bats (*Eptesicus fuscus*; an insectivorous species weighing 15 to 26 g) in Missouri (USA), GLY
404 was detected in all three analysed sample pools (100% detection frequency) (Hooper et al. 2022).
405 The frequency of exposure to GLY in a farmland herbivorous mammal, the Iberian hare (*Lepus*
406 *granatensis*) via analyses of residues in samples of gastric content, reached 45% of individuals found
407 dead due to roadkill or unknown causes (Martinez-Haro et al. 2022). In hunted hares from pesticide-
408 treated areas the frequency of detection in gastric content ranged between 9% and 22%. These
409 values look lower than the frequencies found in rodents in our study, possibly because residues in
410 gastric content represent a snapshot of recent exposure, whereas measurements in hair represent a
411 temporally integrative exposure assessment during the time of hair growth. Issues related to local
412 contamination and/or different analytical sensitivities may further explain the lower detection
413 frequency in hares.

414 **Table 1. Sample size and frequency of detection and concentrations of glyphosate, AMPA and glufosinate in the hair**
 415 **of small mammals.**

416 *Descriptive statistics on concentrations were computed for samples where the given compound was detected, i.e. nondetects (samples whose*
 417 *concentrations were below the lowest detected value (Minimum)) were excluded. . Home range size were obtained from Chambers et al. (2000) and*
 418 *Wijnhoven et al. (2005). The bank vole is often considered a mostly granivorous species but recent studies have shown omnivory in this taxon.*
 419 *Abbreviations: OF=Organic farming, CF=Conventional farming, NT=Not targeted by treatments, T=Targeted by treatments, LOQ= Limit of*
 420 *quantification.*

Diet	All species	Wood mouse	Greater white-toothed shrew	Common vole	House mouse	Bank vole
		<i>Apodemus sylvaticus</i>	<i>Crocidura russula</i>	<i>Microtus arvalis</i>	<i>Mus musculus</i>	<i>Myodes glareolus</i>
Mean home-range size (m ²)		Omnivore 230–12200	Insectivore 50–200	Herbivore 161–1350	Omnivore 2710	Omnivore 260–4100
NUMBER OF INDIVIDUALS						
Habitat						
Cereal	22	9	2	9	2	
Hedgerow or woodlot	39	9	12	7		11
Farming						
CF	41	12	9	9	1	10
OF/CF	10	4	3	2		1
OF	10	2	2	5	1	
Not treated or treated						
« NT »	41	9	12	8	1	11
« T »	20	9	2	8	1	
Landscape OF proportion						
0-50%	40	11	9	8	1	11
50-100%	21	7	5	8	1	
Sales GLY						
< 7000	25	6	4	11	2	2
> 7000	36	12	10	5		9
Sales GLUF						
0	44	15	11	7		11
0.75	17	3	3	9	2	
Total	61	18	14	16	2	11
GLYPHOSATE						
Detections						
Number of detection	39	6	7	15	1	10
Frequency (%)	64	33	50	94	50	91
Concentrations (pg/mg)						
LOQ	5					
Minimum	0.018	0.600	0.600	0.018		1.43
Q1	1.040	0.795	0.600	2.23		2.60
Median	2.65	1.77	0.800	4.42		3.65
Q3	5.77	4.83	0.950	8.13		5.63
Maximum	522	5.92	1.22	522	3.79	7.74
AMPA						
Detections						
Number of detection	31	4	2	14	1	10
Frequency (%)	51	22	14	88	50	91
Concentrations (pg/mg)						
LOQ	1					
Minimum	0.240	0.400	0.400	0.240		0.515
Q1	1.025	0.519	0.617	1.08		1.19
Median	1.39	1.29	0.833	2.51		1.61
Q3	6.45	2.03	1.05	7.65		6.51
Maximum	33.6	2.07	1.27	19.4	1.37	33.6
GLUFOSINATE						
Detections						
Number of detection	27	2	1	13	1	10
Frequency (%)	44	11	7	81	50	91
Concentrations (pg/mg)						
LOQ	1					
Minimum	1.16	2.04		1.29		1.16
Q1	2.01	7.88		2.39		1.59
Median	3.51	13.8		4.05		3.01
Q3	7.25	19.7		5.80		4.87
Maximum	25.5	25.5	1.48	21.8	9.75	12.6

422 3.3. Concentrations of GLY, AMPA and GLUF in the hair of small mammals

423 With respect to residue concentrations above the lowest detected value, we found that the three
424 compounds presented overall similar ranges of concentrations, and the highest concentrations were
425 reached for GLY, with values varying from 0.018 to 522 pg/mg (median = 2.65), whereas the
426 concentrations of AMPA ranged from 0.240 to 33.6 pg/mg (median = 1.39), and those of GLUF
427 ranged from 1.16 to 25.5 pg/mg (median = 3.51) (Table 1). The concentrations of GLY did not differ
428 significantly from those of AMPA or GLUF, and the concentrations of AMPA did not differ from those
429 of GLUF (Tarone-Ware tests, p-values > 0.05, Sup Info Annex 1. Text A4).

430 In county-level hair pools from big brown bats in Missouri, Hooper et al. (2022) reported
431 concentrations of GLY varying from 5.7 to 4505.2 pg/mg. These maximum concentrations are much
432 higher than those measured here in shrews (median = 0.8 pg/mg) and almost ten times higher than
433 the maximum concentration measured in our study in a vole (i.e. 522 pg/mg). Several nonexclusive
434 hypotheses may be proposed to explain such high levels found in bats: analytical issues, physiological
435 and ecological traits of the species enhancing exposure and/or bioaccumulation (Fritsch et al. 2024),
436 and issues related to the amount of GLY used and the timing of exposure of the animals after GLY
437 application. Newton et al. (1984) studied the fate and behaviour of GLY and AMPA in forest brush
438 field ecosystems in the Oregon Coast Range (USA) following aerial application of GLY at doses
439 comparable to the recommended GLY application rate in the EU (i.e. 3.3 kg a.s./ha there, maximum
440 field application of 4.32 kg a.s./ha in the EU (European Commission 2015)). They measured the
441 residues of GLY and AMPA in the viscera and body of free-ranging small mammals captured at the
442 sites before treatment and over the 55 days posttreatment. As in the present work, GLY was
443 detected in all of the trophic groups (LOD of 0.10 mg/kg = 100 pg/mg). However, AMPA was detected
444 in two voles only at 130 and <160 pg/mg (LOD of 100 pg/mg) (Newton et al. 1984), which is far lower
445 than in our study. This may be because the lowest detected value in our work was much lower (i.e.
446 0.240 pg/mg) and because different tissues were used to measure residues.

447 Interestingly, considering these previous findings of Newton et al. (1984) in forest small mammal
448 tissues, the concentrations varied over the same order of magnitude as the maximum concentrations
449 measured in our hair samples from agricultural landscapes. They reported concentrations in viscera
450 ranging from < 100 pg/mg (LOD) to 1690 pg/mg in shrews, 5080 pg/mg in deermice and 1700 pg/mg
451 in voles, and concentrations in body tissues ranging from < 100 to 410 pg/mg in shrews, 400 pg/mg in
452 deermice and 250 pg/mg in voles. The concentrations were the highest during the first days following
453 herbicide treatment both in the viscera and body tissues. Body concentrations decreased under the
454 LOD by 2 weeks after treatment while they were still detectable in the viscera, and concentrations in
455 the viscera decreased to nondetectable concentrations or near the LOD by day 55 after treatment
456 (Newton et al. 1984). Together with differences in the matrices analysed, this (rapid) concentration
457 decrease over time following a single GLY application may explain the overall lower GLY levels in our
458 study and suggests that the highest concentrations found here may correspond to animals exposed
459 to GLY soon after its application. Our samples were collected during springtime, from May-June,
460 whereas glyphosate is usually applied to winter cereals during late summer or fall (often in October).
461 The seasonal moult of small mammals is expected to be completed mostly by May-June, when they
462 were captured, and the sampled fur might have partly or even totally grown within the period of
463 February-May (Abad 1991; López-Fuster et al. 1986; Sealander 1972). However, the postwinter moult
464 may be incomplete, depending on the age of individuals and the species of concern, allowing the
465 possibility for hair grown during autumn to be included in the analysed fur sample (Beltran et al.
466 2018).

467 **Figure 2. Correlations between the concentrations of glyphosate, AMPA and glufosinate in the hair of small mammals.**

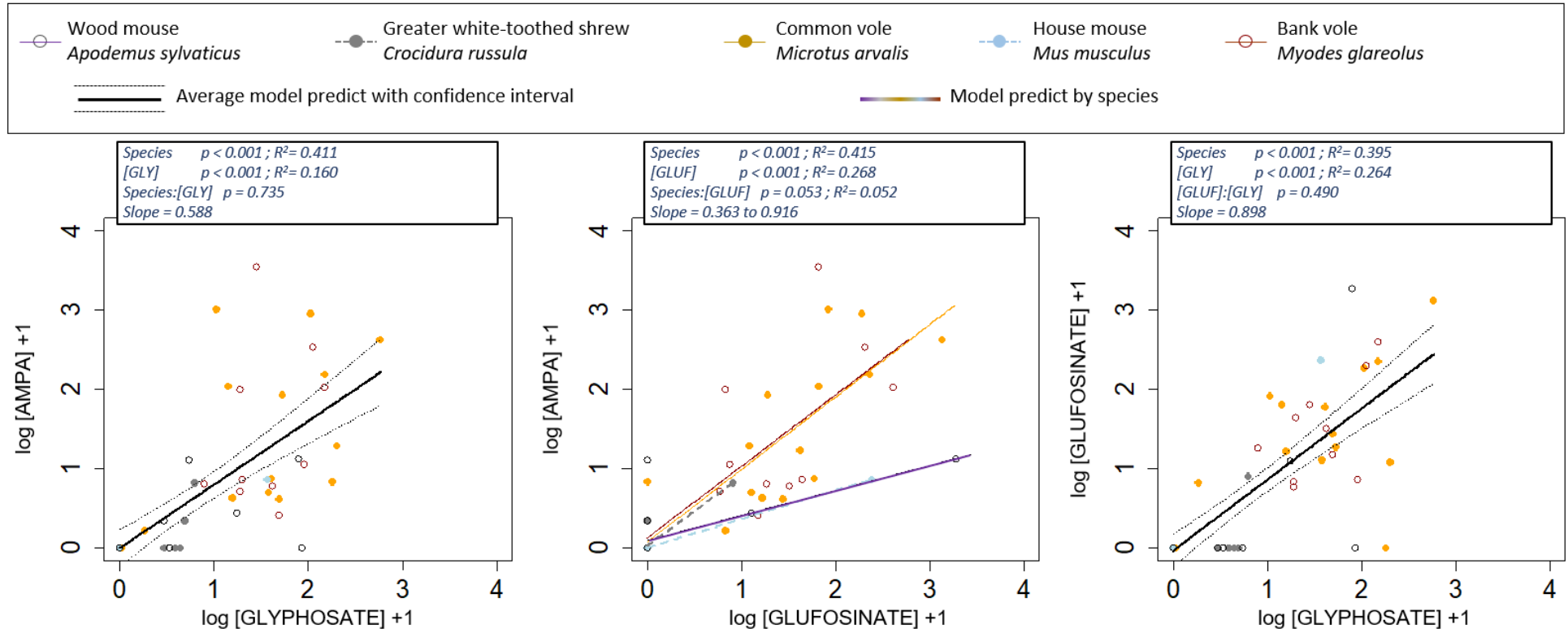
468 *The concentrations are expressed in pg/mg. The statistical outputs of general linear models are provided on graphs. Predictions are plotted according to species when the interaction*
 469 *between the factors is significant, and average predictions are provided when the interaction is not significant.*

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474 The concentrations of the three compounds in the hair of small mammals were significantly related
475 (Fig. 2, Annex Fig. A1). Positive correlations were observed between AMPA and GLY (correlation
476 coefficient Spearman's $\rho=0.84$), between GLUF and GLY ($\rho=0.79$), and between AMPA and GLUF
477 ($\rho=0.89$) (Annex Fig. A1). The variance explained (*cf.* determination coefficient R^2) was greater for
478 the factor species (0.41 to 0.42) than for compounds (0.16 to 0.27) (Fig. 2). The correlation between
479 AMPA and GLUF differed only according to species, with greater slopes for shrews and voles than for
480 the two species of wild mice (Fig. 2). The correlations between AMPA and GLY and between GLUF
481 and GLY were similar between species (Fig. 2). The strength of the association between AMPA and
482 GLUF was greater than that between AMPA and GLY (i.e. greater ρ values, greater R^2 values; Fig. 2,
483 Sup Info Annex 1. Fig. A1), and the relationship presented greater slope coefficients. This may be
484 seen as unexpected since AMPA is a GLY transformation product. Such patterns of correlation
485 between the compounds may reflect different exposure pathway contributions between GLY *versus*
486 AMPA and GLUF. As GLY is poorly metabolized in mammals (EFSA 2015a), the origin of AMPA should
487 be external (environmental) rather than internal (degradation of GLY in the body), as suggested in
488 humans (Alvarez et al.,2022). Studying GLY and AMPA residues in human hair, Alvarez et al. (2022)
489 reported different origins of GLY and AMPA because AMPA was rarely detected in comparison with
490 GLY in both farmers and nonoccupationally exposed subjects, and the ratio of GLY to AMPA greatly
491 differed between the subjects. The correlation between GLY and AMPA or between GLY and GLUF
492 likely originates from a spatial relationship related to the geographic occurrence of the herbicidal
493 treatments, which increases the probability of co-occurrence of parent and transformation products
494 in herbicide-treated areas. As hypothesized, small mammals may be exposed to GLY via the dietary
495 route but also via direct spraying if they are present in the plots during the treatments and orally
496 after spraying treatments through grooming activities. Conversely, dietary exposure, related to the
497 accumulation of the compounds in the soil, biota and food webs, may be the predominant exposure
498 pathway to AMPA and to GLUF (whose use as a PPP is no longer approved) in small mammals.

499 *3.4. Differences between species in detection frequency and concentration of the*
500 *compounds*

501 The frequency of detection differed among species for the three compounds (Fig. 3, Sup Info Annex
502 1. Table A2). In all cases, the odds ratios were significantly different between the voles and the
503 reference (i.e. wood mice) (Sup Info Annex 1. Table A2). The probabilities of detection of GLY, AMPA
504 and GLUF were significantly greater in common and bank voles than in shrews and wood mice (Fig.
505 3). The results were contrasted for the house mouse, likely because of the small sample size (Fig. 3).

506 When analyses were run on the subset of data with quantified concentrations in small mammals (i.e.
507 without nondetects), the concentrations of GLY, AMPA and GLUF did not significantly differ among
508 species (Sup Info Annex 1. Table A3). However, the trends considering GLY and AMPA were
509 comparable overall, since estimates for concentrations were found to be the lowest in shrews and
510 wood mice for GLY and the highest in voles for AMPA (Sup Info Annex 1. Table A3). Considering
511 GLUF, the highest estimates were observed for wood mice and house mice (Sup Info Annex 1. Table
512 A3). When the tests included nondetects set to 0, the results revealed higher levels of GLY, AMPA,
513 and GLUF in common voles and bank voles than in wood mice and shrews (Fig. 4). The concentrations
514 in house mice did not significantly differ from those in other species, with intermediate values (note
515 that the sample size is small) (Fig. 4).

516 **Figure 3. Probabilities of the detection of glyphosate, AMPA and glufosinate in the hair of small mammals according to species.**
 517

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

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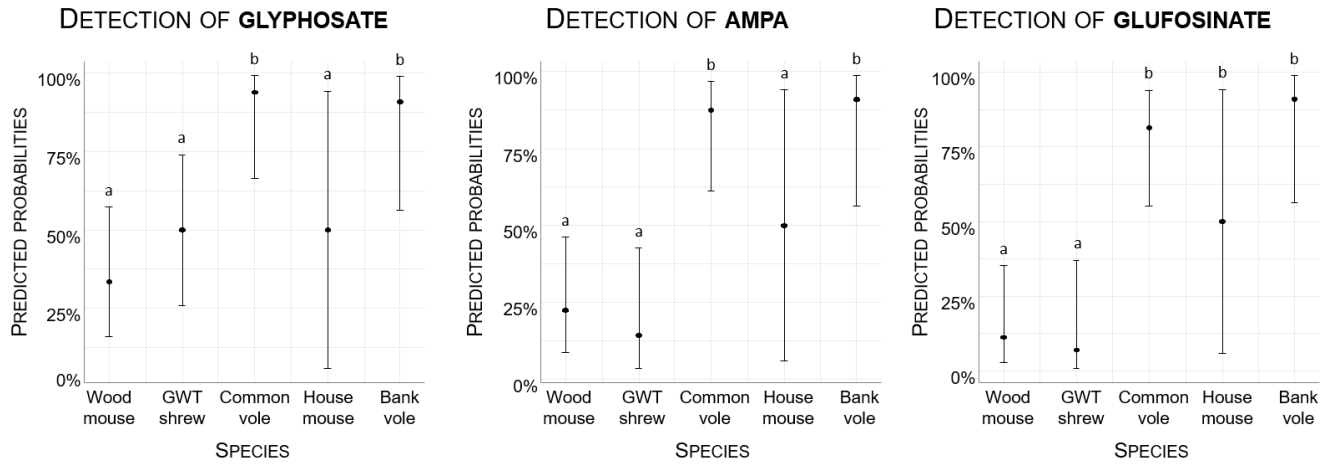
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531 **Figure 4. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals according to species.**

532 *The concentrations are expressed in pg/mg. Nondetects are set to 0. Statistical significance is indicated with letters, and different letters between*
 533 *levels indicate significant differences. Statistics were performed via the Kruskal-Wallis test followed by posthoc multiple comparison tests to*
 534 *investigate pairwise differences. Abbreviations: GWT shrew stands for greater white-toothed shrew. The sample sizes are provided in Table 1.*

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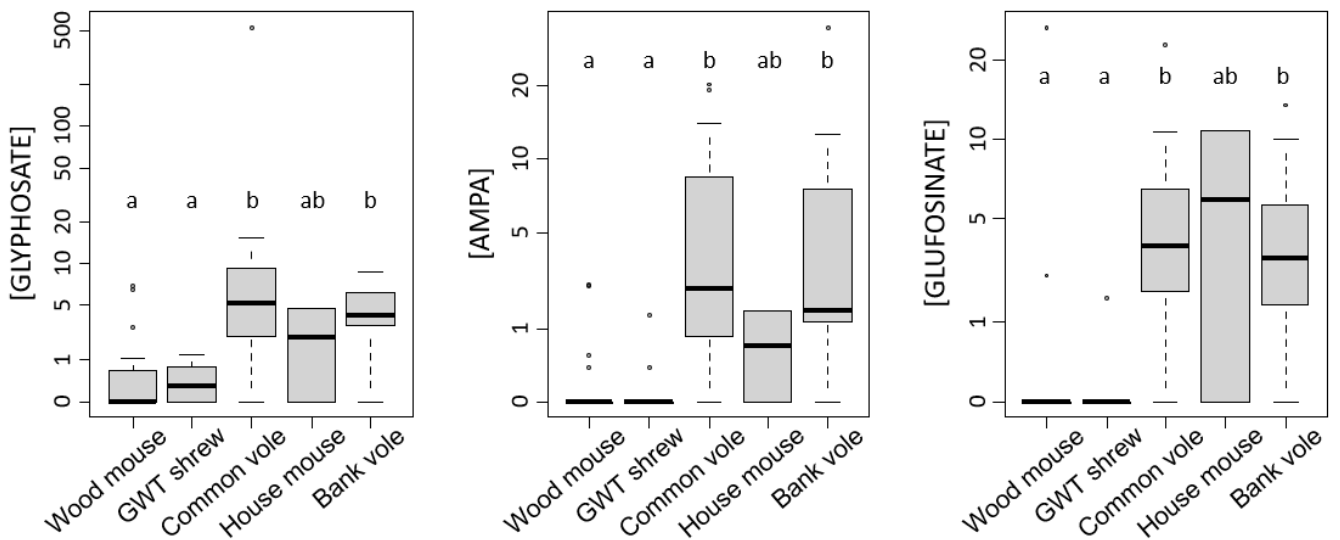
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544 In their forest study where residues of GLY and AMPA were measured in several free-ranging
545 mammalian herbivores, carnivores, and omnivores, Newton et al. (1984) reported that GLY was
546 present in all trophic groups, whereas AMPA was present in only a few herbivorous voles. Our results
547 are in line with these findings, with confirmed exposure to GLY (and AMPA) in both herbivorous and
548 granivorous or omnivorous rodents and insectivorous shrews and greater levels of AMPA in
549 herbivorous mammals (note that the bank vole was often considered a granivore, but recent
550 literature has shown omnivory (Balčiauskas et al., 2022)). Newton et al. (1984) emphasized that
551 exposure to and accumulation of GLY in mammals varied according to diet preference, since
552 omnivores (deermice) had the highest concentrations of GLY in the viscera during the 2 weeks
553 posttreatment, suggesting a higher initial intake of GLY in omnivores, but such visceral levels
554 decreased the fastest in comparison to carnivores (shrews and weasel) and herbivores (squirrel, vole,
555 chipmunk). The detailed data revealed greater body concentrations in shrews and deermice (0.35 to
556 0.41 mg/kg) than in squirrels and voles (0.13 to 0.25 mg/kg) within the first days posttreatment. Our
557 results similarly revealed differences in the accumulation or uptake of GLY and AMPA between
558 trophic groups or species, but various trends were identified according to trophic preferences.
559 Overall, we found lower detection frequencies and concentrations in insectivores, whereas the
560 highest detection frequencies and concentrations were observed in herbivorous and omnivorous
561 voles. Both the detection probability and the concentrations were greater for the two species of
562 voles than for the omnivorous wood mice and house mice in our study. The differences in the
563 matrices analysed may explain the discrepancies between the findings of Newton et al. (1984) and
564 our findings regarding the influence of diet on accumulation, in addition to differences in the
565 identities of the species (i.e. European vs. American species) and ecosystems (i.e. agrosystem vs.
566 forest). The physiological and metabolic traits affecting uptake and accumulation in body
567 tissues/fluids and the ecological traits affecting exposure may shape the differences found here
568 between species or trophic groups, but their role is difficult to disentangle. The species exhibiting the
569 lowest detection frequency differ in terms of phylogeny, since both *Apodemus* and *Mus* mice of the

570 subfamily Murinae as well as Crocidurinae *Crocidura* shrews were represented (Sup Info Annex 1. Fig.
571 A4). They also differ in their ecological traits since they are omnivorous and insectivorous. The most
572 herbivorous and, to a lesser extent, the least mobile species, which are the two species of voles
573 studied here, presented the highest detection frequencies and greatest concentrations. *Microtus* and
574 *Myodes* voles are classified into two different genera, but both belong to the same subfamily,
575 Arvicolinae (Sup Info Annex 1. Fig. A2). The correlation between the levels of GLUF and AMPA varied
576 under species-specific patterns, as detailed above. The wood mouse and the house mouse, which are
577 both omnivorous Murinae species, presented a slope different from that of the herbivorous common
578 vole, the omnivorous bank vole and the insectivorous greater white-toothed shrew which presented
579 a steeper slope. The bank vole is usually classified as showing intermediate features of traits between
580 the wood mouse and the common vole in terms of mobility and homerange size, habitat selectivity,
581 and diet breadth (van den Brink et al. 2011). According to ecotoxicological studies where the dietary
582 route is the predominant exposure pathway, its responses are expected to be intermediate or closer
583 to the trends exhibited by the wood mouse than by the common vole (Baudrot et al. 2018; van den
584 Brink et al. 2011). Although the importance of toxicokinetics on accumulation in hair cannot be
585 ignored (Faÿs et al. 2023), our results may again suggest the involvement of different origins of the
586 compounds and of a different contribution of the various exposure routes in shaping interspecies
587 accumulation dissimilarities. The differences found here between species and between animals
588 captured in different habitats within the landscape highlights the need to include both ecological and
589 physiological features in the wildlife risk assessment process and postregistration surveys of the
590 unintentional effects of PPP (Morrissey et al. 2023). Importantly, these results highlight the need for
591 additional research to gain knowledge about the traits that shape the exposure and uptake of PPP in
592 wildlife and about all the interacting mechanisms involved.

593 *3.5. Influence of habitat, farming practices, and proxies of treatment intensity on the*
594 *detection frequency of the compounds*

595 The frequency of detection did not differ according to habitat or farming practices (Sup Info Annex 1.
596 Table A2). Accordingly, the study of Pelosi et al. (2022), which was done within the same research
597 project as the present study (same location of sampled plots and sampling in 2018) did not show
598 differences in GLY detection frequency in soils sampled from cereal fields or hedgerows. However, in
599 earthworms, GLY and AMPA were detected more frequently in cereal fields than in hedgerows. The
600 frequency of detection of GLY, AMPA and GLUF was not significantly dependent on the proxies of
601 treatment intensity at the plot, landscape and township scales, except in the case of GLUF sales (Sup
602 Info Annex 1. Table A2, Fig. A3, Fig. A4). However, such an effect of a.s. sales on GLUF detection
603 probabilities was negative, i.e. the detection probabilities were lower in townships where GLUF sales
604 were the highest, and the differences between categories were not significant (Sup Info Annex 1.
605 Table A2, Fig. A4). To investigate this paradoxical result further and avoid any interpretation bias, a
606 model with GLUF sales as the only explanatory factor (i.e. without the factor “species” first in the
607 model formula) was run, revealing an absence of significant differences (Sup Info Annex 1. Table A2,
608 Fig. A4). Hedgerows, woodlots and fields cultivated under OF are not supposed to be targeted by
609 pesticide treatments, but they may be unintentionally contaminated due to drift or run-off (Gandhi
610 et al. 2021), and the home range of small mammals can overlap wooded patches, nontarget crops
611 and surrounding cropped habitats. In agricultural topsoil samples from conventional and organic
612 farms across Europe screened for chemicals, GLY and AMPA were among the compounds with the
613 highest frequency of detection and the greatest concentrations. (Geissen et al. 2021). Moreover,
614 AMPA was the residue most often detected in both conventional and organic fields, with 96%
615 occurrence in conventional systems and 83% in organic systems (Geissen et al. 2021). AMPA and GLY
616 were also the most commonly detected pesticides in soils under various land uses sampled over
617 mainland France, in which 111 pesticide residues were screened (Froger et al. 2023). The detection
618 frequency was 83% for AMPA and 70% for GLY, and the two compounds were found both in treated
619 cultivated fields and nontargeted habitats, as they occurred both in conventional and organic farming

620 plots, and in different types of land uses: arable lands, orchards, forests, grasslands, and brownfields
621 (Froger et al. 2023). Such a global occurrence of these two compounds, including in organic fields and
622 grasslands or forests, echoes the pervasive exposure found here in small mammals. Martinez-Haro et
623 al. (2022) reported results contrasted with the present findings about the influence of farming
624 practices on exposure to GLY: the prevalence was 9–22% in the gastric content of hunted Iberian
625 hares and 45% in hares found dead in pesticide-treated areas, whereas no residues were detected in
626 hares from organic crops. This pattern may be due to the differences in the matrices analysed, with
627 an assessment of recent short-term exposure in the case of gastric content versus past integrated
628 measures of exposure in the case of hair. Furthermore, the Iberian hares from conventional and
629 organic crops were collected by hunting from October to January, while our sampling session was
630 conducted during springtime. The authors indicated that the frequency of detection in hares
631 progressively increased over the hunting season, being 0% and 11% in October and November and
632 50% in December and January (Martinez-Haro et al. 2022). The hares found dead were collected in
633 March-April (45% positive for GLY). Finally, the intensity and extent of the GLY treatments may have
634 been greater in our study region, and the OF and CF plots may have been geographically closer in our
635 case. In addition to the study of GLY levels in environmental matrices such as soil or biota, more
636 research is needed to characterize the patterns of exposure of wildlife to GLY, AMPA and GLUF and
637 quantify the underlying processes and spatiotemporal dynamics.

638 *3.6. Influence of habitat, farming practices, and proxies of treatment intensity on the* 639 *concentrations of the compounds*

640 In terms of the concentrations of the compounds when quantified, the GLY concentrations differed
641 among the small mammals captured in the different habitats: they were greater in individuals from
642 hedgerows than in those from cereal fields (Fig. 5, Sup Info Annex 1. Table A3, Fig. A3). Considering
643 both nondetects set to 0 and quantified concentrations, the trends were similar but not significant
644 (Fig. 6). Such a pattern was not observed in the cases of AMPA and GLUF (Fig. 6, Sup Info Annex 1.
645 Table A3).

646 This may be a confirmation of the different contributions of exposure pathways for GLY in
647 comparison to AMPA and GLUF. This pattern may indeed be related to a contribution of overspray in
648 addition to dietary exposure because of direct spraying and/or elevated GLY deposits on hedgerow
649 vegetation (1) during spraying due to drift and/or (2) due to (wet) deposition (Gandhi et al. 2021).
650 GLY is known to have a low volatility but is found in air and rainfall in intensively treated areas, at
651 relatively high frequencies and concentrations, and is subjected to wet deposition (Chang et al.
652 2011). Compared with those in open habitats, contaminant deposits, including PPP, on vegetation in
653 hedgerows are known to be enhanced by larger surfaces of interception and by “edge effect” (Davis
654 et al. 1994; Fowler et al. 2004; Lazzaro et al. 2008; Ould-Dada et al. 2002). Mayer et al. (2020)
655 reported for European hares (*Lepus europaeus*) that pesticide uptake by foraging could be 7-fold
656 lower than uptake via overspray/oral grooming. Moreover, GLY has been found to persist in
657 vegetation for up to one year following treatments in perennial forested areas, representing a potent
658 dietary source of exposure for wildlife (Edge et al. 2021). In a previous study addressing the exposure
659 of small mammals to banned and currently used PPP by residue screening in hair, which was based
660 on animals sampled from the same study area and another region in France, we reported greater
661 contamination (in terms of the number of compounds and concentrations) in individuals captured in
662 hedgerows than in those captured in grasslands; however, the contamination did not differ between
663 animals captured in hedgerows and those sampled from cereal crops (Fritsch et al. 2022). In Pelosi et
664 al. (2022), GLY and AMPA concentrations in both soils and earthworms were, however, higher in
665 samples from cereal fields than in those from grasslands and hedgerows. This may be an argument in
666 favour of the contribution of processes related to overspraying in determining small mammal
667 exposure to GLY. The role of external exposure to herbicides in small mammals and the importance
668 of oral exposure by grooming remain to be investigated. The contribution of nondietary routes to
669 wildlife exposure is still an overlooked field of research that needs to be addressed.

670

671 **Figure 5. Concentrations of glyphosate in the hair of small mammals without nondetects (a) according to species and habitat and (b) according to habitat taking species into**
672 **account statistically in modelling.**

673 *The concentrations are expressed in pg/mg in hair. The statistical significance is depicted by a red asterisk. The habitats are represented as "C" for cereals and "H" for hedgerows. The*
674 *GWT shrew stands for the greater white-toothed shrew. The sample sizes are provided in Table 1 and Table A1.*

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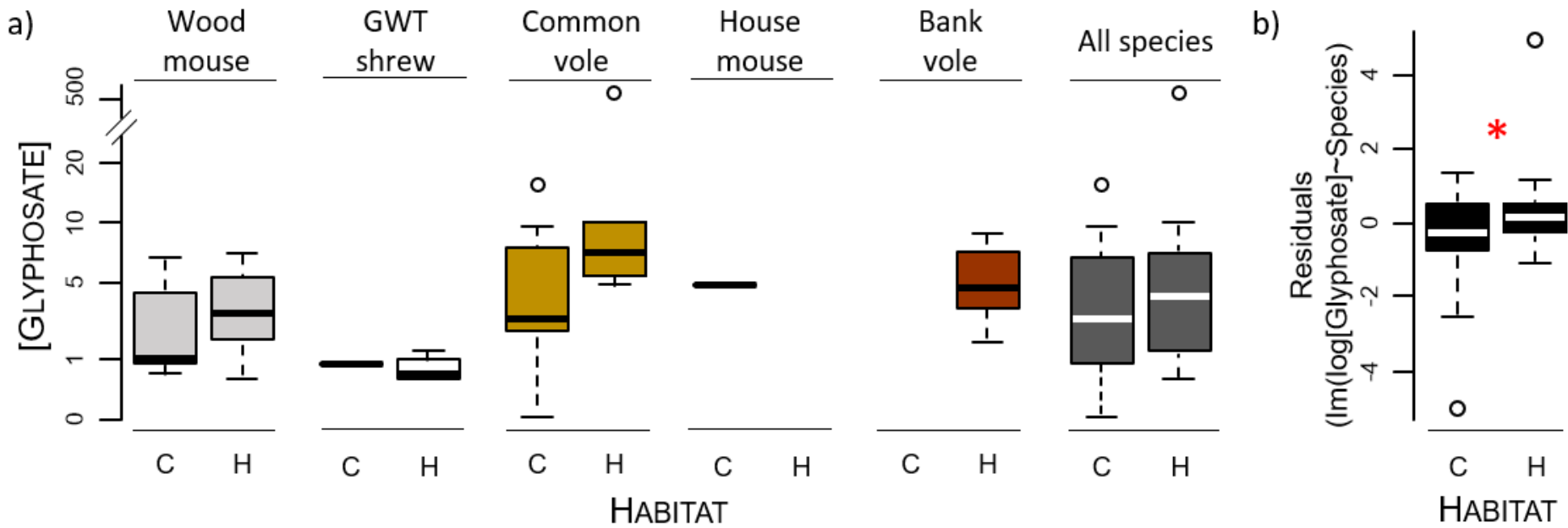
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686 No differences in the quantified concentrations of GLY, AMPA or GLUF in the hair of small mammals
687 were detected according to farming practices or proxies of pesticide treatment intensity at the
688 different scales (Sup Info Annex 1. Table A3). Running the analyses on the basis of concentrations and
689 including nondetects as 0 provided similar results (Fig. 6, Sup Info Annex 1. Fig. A3). Previously, we
690 also highlighted the absence of differences in contamination by currently used pesticides other than
691 GLY between small mammals captured in conventional or organic farming plots (neither the number
692 of compounds nor their concentrations in individuals) (Fritsch et al. 2022).

693 **Figure 6. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals with nondetects set to 0,**
 694 **according to habitat where captured (a), farming practices where captured (b), and proxies of treatment intensity at**
 695 **the plot scale (c) and at the landscape scale (d).**

696 *The concentrations are expressed in pg/mg. Statistics were performed via the Wilcoxon-Mann-Whitney test or the*
 697 *Kruskal-Wallis test depending on the number of levels of the factor of concern. Posthoc multiple comparison tests after*
 698 *Kruskal-Wallis tests were used to investigate pairwise comparisons when relevant. No significant differences were found.*

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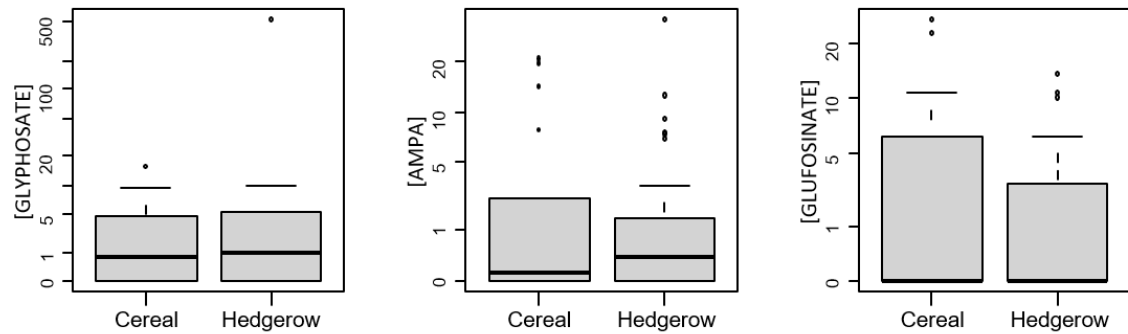
a) HABITAT

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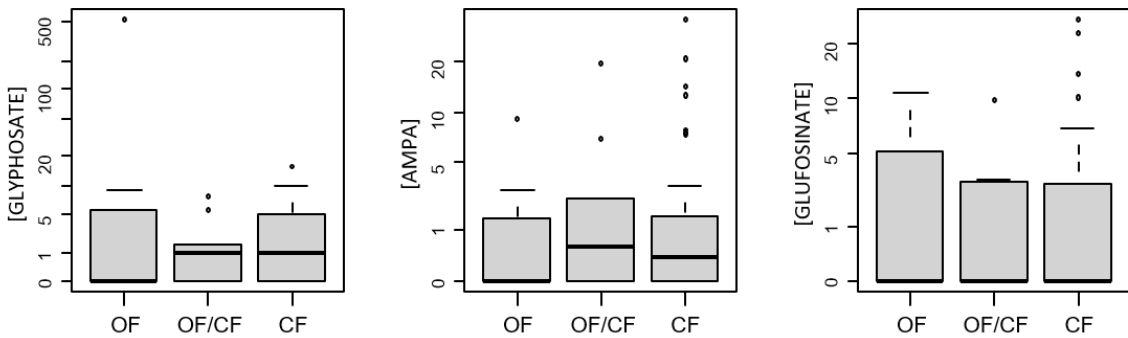
b) FARMING PRACTICES (ORGANIC FARMING 'OF', CONVENTIONAL FARMING 'CF')

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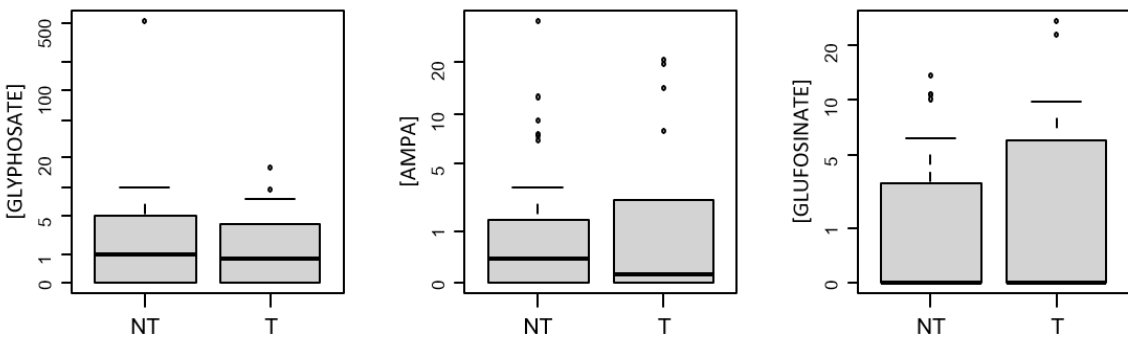
c) TREATMENT BY PESTICIDES (NOT TARGETED 'NT', TARGET 'T')

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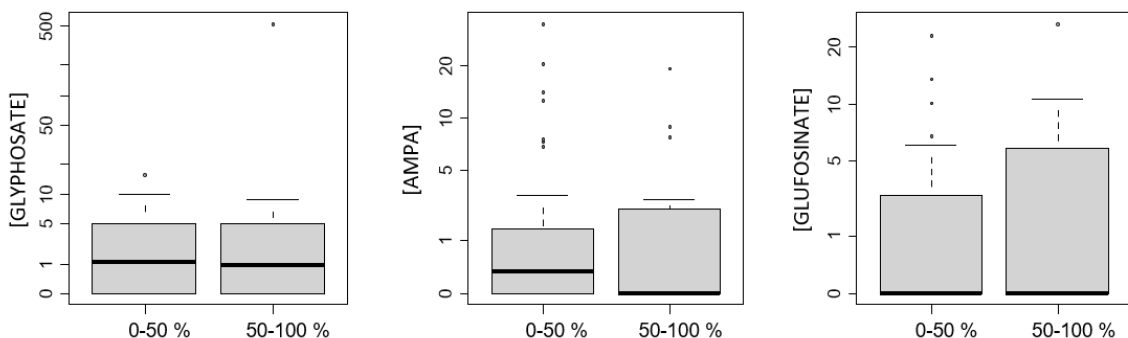
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d) PROPORTION OF ORGANIC FARMING IN LANDSCAPE

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718 In soils and earthworms sampled within the ZAPVS, Pelosi et al. (2022) also reported no significant
719 differences in GLY, AMPA or GLUF levels according to pesticide use (i.e. comparing treated
720 conventional fields to nontreated habitats such as grasslands, hedgerows and organic fields) or
721 according to farming systems in cropped fields (i.e. fields cropped under conventional or organic
722 farming). The ubiquity of exposure to GLY, AMPA and GLUF in arable landscapes, both in crops and
723 agroecological infrastructure, was therefore shown in recent studies for soils and for both terrestrial
724 invertebrates and vertebrates belonging to various taxa and of different trophic levels. The transfer
725 of GLY, AMPA and GLUF in food webs is thus questioned and requires further attention. This
726 widespread occurrence of GLY, AMPA and GLUF in the environment and biota, regardless of the
727 farming practices and intensity of use at the landscape scale, indicates pervasive contamination that
728 may reflect the magnitude of repeated GLY-based (and formerly GLUF-based) herbicidal use over
729 large surfaces in French agrosystems.

730 On the basis of our results and recent literature, the beneficial role of agroecological infrastructures
731 (e.g. hedgerows) and nontreated crops as refuges or recovery areas towards pesticide treatments in
732 agroecosystems under the current agricultural model is questioned. Our findings highlight that the
733 current surfaces of nontreated habitats within the agricultural landscapes studied are insufficient to
734 mitigate the exposure of wildlife to GLY, AMPA and GLUF. We reached similar conclusions when we
735 studied the exposure of small mammals to 73 residues of fungicides, herbicides (excluding GLY,
736 AMPA and GLUF), insecticides, and some of their metabolites in Fritsch et al. (2022). With respect to
737 the mitigation of unintentional effects of PPP on wildlife, these results call for a reassessment of the
738 relevance and efficiency of the current procedures and a strengthening of national and regional
739 initiatives for agri-environmental schemes. Further investigations into the pathways of wildlife
740 exposure to PPP and responses at the individual and population levels are needed to understand and
741 predict the role of agroecological infrastructures in shaping the direct and indirect effects of PPP. The
742 beneficial role of hedgerows in limiting inputs of PPP on non-target plots around treated fields can be
743 leveraged in agri-environmental schemes, but this desirable use of agroecological infrastructures to

744 protect the environment and biodiversity may turn seminatural habitats into “ecotoxicological
745 traps”. These issues deserve attention and should be addressed in both future research studies and
746 monitoring schemes.

747 *3.7. Dose reconstruction for risk assessment*

748 To evaluate the risk of unintentional impacts in small mammals by comparing exposure dose values
749 estimated from back-calculation using the concentrations measured in hair to toxicological data, we
750 found that toxic thresholds could be exceeded for both GLY and GLUF. In the case of GLY, at least one
751 of the five considered toxicological thresholds was exceeded according to the trigger of concern for
752 at least one species in all of the approaches (Table 2). The potential for toxicological impairment was
753 thus observed in all of the approaches developed. This finding suggests a risk for small mammals,
754 especially rodents, and particularly for herbivorous voles (Table 2). Often, only the maximum value
755 measured in *Microtus* voles reached toxic levels (10 times out of 16 scenarios). In five of the 16
756 scenarios computed via the different approaches, all of the species were found to be at risk, with
757 maximum but also median and minimum concentrations measured being involved. At the population
758 level, in the “best-case” scenario, toxicological effects related to GLY exposure impaired at least 6%
759 of individuals in the common voles studied here, which represents a risk of effects in 2.1% of the
760 rodents and in 1.6% of the small mammal community studied, including insectivorous species (Table
761 2). In the worst-case scenario, toxicological impairment occurred in 87.5% of the common voles,
762 88.9% of the voles (bank voles and common voles), 66% of all of the rodent species, 50% of the
763 shrews and 62.3% of the individuals within the small mammal community (Table 2). Up to 93.8% of
764 the common voles and 92.6% of the individuals of all vole species may be impacted if individuals
765 close to the trigger value of 5 are included in the calculation (Table 2).

766 For GLUF, the four toxicological thresholds considered were exceeded in the two scenarios for all
767 rodent species, including the maximum concentrations measured in our samples but also the third
768 quartile, median and even minimum values (Table 2). This would translate, on the basis of our

769 captured individuals, into a possible impairment related to GLUF exposure in 31.3 to 75% of the
770 common voles, 25.9 to 70.4% of the vole individuals, 19.1 to 46.8% of individuals within the rodent
771 population and 14.8 to 36.1% of individuals within the small mammal community (Table 2). This
772 result suggests that many of the free-ranging nontargeted small mammals could be at risk for
773 toxicological effects related to exposure to GLUF, especially herbivorous and granivorous or
774 omnivorous species, at the time of sampling (i.e. 2018), although the use of this compound in France
775 has largely decreased since 2015.

776 Thus, our findings are consistent with the first conclusions of the risk assessments performed during
777 the registration processes, which identified potential threats for both GLY and GLUF to herbivorous,
778 granivorous and insectivorous terrestrial vertebrates, especially herbivorous voles (EFSA 2005, 2012,
779 2015a). Note that the regulatory assessments were based on dietary exposure, but wildlife may be
780 exposed to PPP - and this is especially the case for GLY when used through spraying - via additional
781 routes such as oral uptake by grooming, inhalation, dermal contact, and/or ocular contact, as
782 mentioned for human populations (Gandhi et al. 2021; Mayer et al. 2020; Mineau 2011).

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

784 *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*
 785 *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*
 786 *russula (greater white-toothed shrew); Miar Microtus arvalis (common vole); Mumu Mus musculus (house mouse); Mygl Myodes glareolus (bank vole); Max maximum; Q3 3rd*
 787 *quartile; Med median; Min minimum; PK pharmacokinetics.*

Compounds Approach Scenarios	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] _{hair} of concern	Nb ind	Nb rodents	Nb shrews	% All species	% Rodents	% Voles	% Microtus	% Shrews
GLYPHOSATE													
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 (1)	26 (30)	34 (36)	48 (48)	50 (50)	7 (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] _{whole body} = [C] _{hair}	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1	2	2		6		

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794 **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] _{hair} of concern	Nb ind	Nb rodents	Nb shrews	% All species	% Rodents	% Voles	% Microtus	% Shrews
GLYPHOSATE													
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	1		2	2		6	
						(2)	(2)		(3)	(4)	(7)	(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)
GLUFOSINATE													
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

797 Importantly, in the study on small mammals carried out in the same area and mentioned above
798 (Fritsch et al. 2022), 112 different residues of banned and currently used PPP (parent chemicals and
799 metabolites) were detected in hair samples from wood mice and greater white-toothed shrews, with
800 32 to 65 residues detected per individual. This means that “cocktail effects” are likely to occur, which
801 can ultimately increase the risk of GLY- or GLUF-induced toxicity assessed here owing to mechanisms
802 of additivity and synergy (Martin et al. 2021). In their review, Martin et al. (2021) reported that
803 additivity was the interaction that most often occurred in chemical mixtures, followed by synergistic
804 interactions. For example, Brodeur et al. (2014) reported synergic toxic impacts in the common South
805 American toad (*Rhinella arenarum*) of binary mixtures of GLY-and cypermethrin-based commercial
806 PPP products; two compounds that have been detected in more than half of the small mammal
807 individuals in our studies. They reported that the degree of synergy ranged from 2 to 9 times
808 depending on the mixture tested (Brodeur et al. 2014).

809 From terrestrial wildlife and biodiversity conservation perspectives, our findings may be worrisome.
810 As mentioned earlier, GLY concentrations were found at higher levels in bats than in this study
811 (Hooper et al. 2022). The authors emphasized that GLY reached the highest concentrations measured
812 in their study, where 8 compounds, including neonicotinoids and several systemic or selective
813 herbicides, were analysed. These authors suggested that extremely high environmental inputs of GLY
814 may counteract the role of its short half-life in mitigating risks to wildlife (Hooper et al. 2022). A
815 growing body of evidence raises concerns about the lethal and sublethal toxic effects of GLY in biota
816 and highlights endocrine disruption and reproductive impairment in vertebrates chronically exposed
817 to environmental concentrations (Fritsch et al., 2024). Recent studies have shown the toxic effects of
818 GLUF at environmentally relevant concentrations and suggest that it has deleterious consequences
819 on biota health under chronic exposure scenarios (Santovito et al., 2024). For example, genomic
820 instability and cellular damage *in vitro* in cultured human lymphocytes and *in vivo* in *Lymnaea*
821 *stagnalis* haemocytes following low-dose exposure to glufosinate-ammonium have been reported, as
822 have negative impacts on reproductive health in *L. stagnalis* (Santovito et al., 2024). Furthermore,

823 some wild taxa may be highly sensitive to GLY, AMPA or GLUF, more than expected from laboratory
824 studies on models such as rats and mice , and commercial formulations can be more toxic than a.s.
825 alone (Fritsch et al., 2024). For example, the mortality rate of postmetamorphosis juveniles of the
826 species *Rana sylvatica*, *Bufo woodhousii fowleri*, and *Hyla versicolor* reached 68-86% when they were
827 exposed via spraying to a GLY-based commercial herbicide at the recommended dose (Relyea 2005).
828 The exposure of spined toads (*Bufo spinosus*) to AMPA at environmental concentrations affects
829 hatchling and tadpole morphology and decreases embryonic and tadpole survival (Cheron and
830 Brischoux, 2020, 2023).

831 Risks of indirect effects of GLY on wildlife via disturbances in natural habitats and dietary resources
832 due to effects on nontarget plants and arthropods [from offsite spray drift] have been noted in risk
833 assessments (EFSA 2015c; US EPA 2020). Following GLY treatments, altered habitat and/or food
834 resources have been associated with reduced (breeding) populations and negative effects on
835 reproduction in birds (Lazaran et al. 2013; Ojelade et al. 2022), a reduction in the frequentation of elk
836 populations (Milner et al. 2013), and a reduced abundance of small mammals (especially insectivores
837 *Soricidae* and herbivores *Microtinae*) (Santillo et al. 1989). Our results show a pervasive exposure, and
838 they question the magnitude of positive compensatory effects (i.e. refuges and connectivity
839 recolonization) expected from untreated areas within the agricultural landscape mosaic (see, for
840 instance, Dalkvist et al. (2013) about the role of landscape features in the impacts of pesticides on
841 vole population dynamics). Moreover, recurring treatments over seasons and years and use over
842 large surfaces may lead to pseudopersistence within the environment (Hvězdová et al. 2018) and
843 thus likely limit the possibility for recovery. Indirect effects may thus occur in addition to direct toxic
844 effects.

845 Our findings warrant further research to confirm their interpretation and gather knowledge about
846 the ecological impacts of the use of GLY and GLUF as PPP. There is a need to characterize the
847 toxicokinetics and toxicodynamics of these compounds in wild species better and to provide data to
848 relate concentrations in hair with exposure dose, accumulation in other tissues, and toxicological

849 responses. Furthermore, more research is needed to assess whether the potential risks computed
850 here actually translate into deleterious effects in free-ranging populations, which would require field
851 surveys and monitoring of wildlife exposure and health. The dynamics of GLY, AMPA and GLUF
852 transfer and their impacts on food webs crucially need to be investigated. Here, we provide here
853 lines of evidence to support the identification of the organophosphate chemicals GLY and GLUF as
854 ‘emerging organic contaminants’, as emphasized by Kissane and Shephard (2017). Given the
855 pervasive exposure of wildlife to GLY, AMPA and GLUF and suggesting chronic exposure at sublethal
856 levels on the basis of nondestructive sampling in biosentinel species, our study highlights the need
857 for research to fill gaps in knowledge about early warning signals of impacts as emphasized by
858 Kissane and Shephard (2017).

859 *5. Conclusion*

860 Our results provide new insights into the ecological risks of GLY- and GLUF-based herbicides, showing
861 that in addition to the evidence related to environmental and human health issues, these PPP are of
862 concern for wildlife exposure and may be of concern for impacts and further trophic cascading
863 effects. The exposure of wild small mammals to GLY, AMPA and GLUF was shown to be widespread
864 in all trophic groups or taxa and in treated and nontreated habitats within the studied agricultural
865 area and was unrelated to the intensity of the treatments at the plot or landscape scale. These
866 results support the most recent findings concerning pervasive GLY environmental contamination,
867 occurrence in food webs, and associated ecological risks. Our results warrant further research on the
868 ecological safety of the use of GLY and GLUF as PPP and provide support for assessing their potential
869 as ‘emerging organic contaminants’.

870 Our study relied on measurements of residues in hair samples, a method that is developing in human
871 epidemiology, and looks a promising nondestructive approach to study the field ecotoxicology of PPP
872 and monitor wildlife exposure or detect early signals in wild species. Although this approach should
873 be strengthened by further assessment of exposure doses and measurements of effects in animals,

874 such surveys may represent valuable opportunities for the assessment of regulatory measures and
875 postregistration monitoring and provide support for phytopharmacovigilance and toxicovigilance
876 schemes (Vijver et al., 2017). For example, the presence of pesticides in the hair of small mammals
877 reported in Fritsch et al. (2022) was reported as an exposure warning for nontarget species by the
878 French department of the agency ANSES in charge of phytopharmacovigilance.

879 This study highlights the need for research on the pathways of wildlife exposure to PPP and the
880 toxicokinetics and toxicodynamics of GLY, AMPA and GLUF and, more broadly, of PPP initially
881 assessed as not persistent and not bioaccumulative on the basis of criteria established on legacy
882 compounds. Our findings strongly suggest that current agri-environmental schemes do achieve the
883 objectives of ecological risk mitigation. They call for additional research and monitoring of the role of
884 agroecological infrastructure in shaping PPP impacts on biodiversity and provide support for an
885 overall reflection, if not an overhaul, of agroenvironmental policies.

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889 preparing or contributing to the field and laboratory work. We especially acknowledge the help of
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892 Chrono-Environnement Department, which manages the equipment used for the experiments and
893 for molecular analyses.

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897 **Author's contribution**

- 898 Clémentine Fritsch: Conceptualization, Funding acquisition, Project administration, Supervision,
899 Investigation, Data curation, Formal analysis, Validation, Visualization, Writing - original draft
- 900 Brice Appenzeller: Project administration, Investigation, Methodology; Supervision, Validation,
901 Writing - review & editing
- 902 Bertrand Colette: Conceptualization, Project administration, Investigation, Data curation, Writing -
903 review & editing
- 904 Coeurdassier Michael: Conceptualization, Investigation, Writing - review & editing
- 905 Driget Vincent: Investigation, Methodology, Resources
- 906 Hardy Emilie: Methodology, Investigation
- 907 Palazzi Paul: Methodology, Investigation
- 908 Schaeffer Charline: Methodology, Investigation
- 909 Goydadin Anne-Claude: Methodology, Investigation
- 910 Sabrina Gaba: Conceptualization, Project administration, Supervision, Writing - review & editing
- 911 Vincent Bretagnolle: Conceptualization, Project administration, Supervision, Writing - review &
912 editing
- 913 Céline Pelosi: Conceptualization, Funding acquisition, Project administration, Supervision, Writing -
914 review & editing

915 **Additional Information**

- 916 Competing financial interests: The authors declare that they have no competing financial interests.

917 **Data accessibility**

- 918 The raw dataset can be accessed at <https://search-data.ubfc.fr/>. DOI : XXXXXX

919 **Supplementary information**

- 920 List of information in the appendix document:

921 *Annex 1.*

922 Text A1: Molecular identification of small mammal species

923 Text A2: Reagents and procedures used for the chemical analyses of glyphosate, AMPA and
924 glufosinate

925 Text A3: Approaches of reverse dosimetry to assess the risk for deleterious effects in small
926 mammals

927 Text A4: Statistical outputs of the Tarone-Ware tests

928 Figure A1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in
929 small mammal hair.

930 Figure A2. Simplified phylogenetic tree of the rodents and shrews included in the study.

931 Figure A3. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals
932 with nondetects set to 0 according to the proportion of organic farming in the landscape as a
933 continuous factor (a) and to the proxy of treatment intensity at township scale as a continuous factor
934 (b) and as a categorical factor (c).

935 Figure A4. Probabilities of detection of glufosinate in hair of small mammals according to the
936 sales of the active substance at the township scale.

937 Table A.1. Overview of sample size.

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

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

943

944 *Annex 2.*

945 Computation of reverse dosimetry approaches (calculation spreadsheets).

946

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1141 **Appendix – Annex 1**

1142 Text A1: Molecular identification of small mammal species 53

1143 Text A2: Reagents and procedures used for the chemical analyses of glyphosate, AMPA and
1144 glufosinate 55

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1149 Figure A1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in small
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1151 Figure A2. Simplified phylogenetic tree of the rodents and shrews included in the study. 65

1152 Figure A3. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals with
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1155 a categorical factor (c)..... 66

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1157 sales of the active substance at the township scale. 69

1158

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1160 Table A2. Statistical outputs of binomial GLMs used to investigate the influence of explanatory
1161 factors on the detection frequencies of glyphosate, AMPA, and glufosinate in the hair of small
1162 mammals..... 71

1163 Table A3. Statistical outputs of the GLMs used to investigate the influence of explanatory factors on
1164 the concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals..... 71

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1167 *Text A1: Molecular identification of small mammal species*

1168 Total genomic DNA was extracted from 10-25 mg of ear tissue using the DNeasy Blood and
1169 Tissue kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations (pp 28-
1170 30, version 07/2006). Each sample was processed independently in an automated manner using the
1171 QIAcube robot (Qiagen). The DNA concentration was then measured using a NanoVue Plus
1172 spectrophotometer (Biochrom). The DNA extracts were stored at -20 °C until DNA amplification.

1173 All of the DNA extracts were then amplified into an approximately 900 bp-long fragment
1174 (excluding primers) of the *cytochrome b* gene. We used the primers CytB Uni fw 5' –
1175 TCATCMTGATGAAAYTTYGG – 3' and CytB Uni rev 5' – ACTGGYTGDCCBCCRATTCA – 3' published in
1176 Schlegel et al. (2011). Amplifications were performed in 50 µL reactions containing 1× HotStart Taq
1177 Master Mix (Qiagen), 0.32 µM of each primer, 0.5 ng/µL of bovine serum albumin, 0.75 mM of MgCl₂,
1178 and 5 µL of extracted DNA (10-70 ng/µL). The PCR program consisted of an activation step of 15 mn
1179 at 95 °C, followed by 40 cycles of denaturation at 94 °C during 30 s, annealing at 53 °C during 45 s,
1180 and extension at 72 °C during 1 min. A final extension was performed at 72 °C for 10 min.
1181 Amplification reactions were performed using an Eppendorf Mastercycler DNA Engine.

1182 The PCR products were separated and visualized using the QIAxcel device and a QIAxcel DNA
1183 Screening kit (Qiagen). The amplified products were purified using the Illustra GFX PCR DNA and Gel
1184 Band Purification Kit (GE Healthcare) according to the manufacturer's instructions. Direct sequencing
1185 of the PCR products was performed with an automated sequencer (Applied Biosystems Seqstudio
1186 Genetic Analyzer). All of the samples were sequenced with the primers employed for the PCR
1187 reactions. The obtained DNA sequences were submitted to GenBank via the BLAST algorithm
1188 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A query sequence was then assigned to a given species
1189 when identity and coverage matched the database sequence with a threshold of at least 99% for only
1190 one species.

1191

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1197 *Text A2: Reagents and procedures used for the chemical analyses of glyphosate, AMPA and*
1198 *glufosinate*

1199 **Chemicals and Reagents**

1200 Methanol Pesti-S grade, acetonitrile, ammonium acetate and water ULC-MS grade were purchased
1201 from Biosolve (Dieuze, France). Sodium dodecyl sulfate, Fmoc chloride, sodium tetraborate
1202 decahydrate and glyphosate, glufosinate ammonium, aminomethylphosphonic acid and glyphosate-
1203 1,2-¹³C₂,¹⁵N standards were purchased from Sigma Aldrich (Diegem, Belgium).

1204 **Sample preparation**

1205 To differentiate compounds biologically incorporated into hair from chemicals externally deposited
1206 on the hair surface due to contaminated air or dust, the samples were decontaminated with sodium
1207 dodecyl sulfate (SDS) solution and methanol (Duca et al. 2014). This procedure was developed to be
1208 as “universal” as possible, and was tested on a large panel of chemicals with different
1209 physicochemical properties including rather hydrophilic chemicals. The two steps (SDS and methanol)
1210 were found to be the best compromise to remove both lipophilic and hydrophilic chemicals from hair
1211 surface. Importantly, SDS solution being an aqueous solution (5% SDS / 95% water), it is therefore
1212 well adapted to remove hydrophilic compounds such as GLU, GLY and AMPA.

1213 Note that in other studies where hair samples were washed prior to analyses of GLY, Hooper et al.
1214 (2022) decontaminated hair of bats using ultrapure (MilliQ) water and subsequently isopropanol, and
1215 Alvarez et al. (2022) used dichloromethane twice to wash human hair samples.

1216 **Analysis of glyphosate, glufosinate and AMPA in hair**

1217 After decontamination, the samples (50 mg) were spiked with 5 µL of internal standard solution at 1
1218 mg/L, and 1 mL of water was added before overnight extraction at 40 °C with 350 rpm agitation. The
1219 supernatants were evaporated to dryness at 50 °C under N₂ flow, and 100 µL of borate buffer and
1220 Fmoc-Cl were added before derivatization and injection into the LC-MS/MS Waters system Acquity
1221 UPLC H-class system equipped with a Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm particle

1222 size) and with a binary mobile phase consisting of ammonium acetate 10mM in water (A) and
1223 acetonitrile (B). The flow rate was initially set at 0.4mL/min with 98% A and 2% B and reached 2% A
1224 and 98 B after 6 min. From 6.0 to 9min the column was re-equilibrated to 98% A and 2% B. The UPLC
1225 system was coupled to Waters Xevo TQ-S triple quadrupole mass spectrometer. With each analytical
1226 run, quality controls consisting of hair samples supplemented at several concentrations (0.1, 0.2, 0.5,
1227 1, 2, 5, 10, 20, 50, and 100 pg/mg) were used to control for possible sensitivity drift, and a blank
1228 sample (sample without the target chemicals) was used to verify possible cross-contamination. Since
1229 field samples were not available in sufficient amount to conduct method validation, it has to be
1230 conducted on blank samples from laboratory animals supplemented with standards. The slopes of
1231 the calibration curves were 13.56, 6.31 and 10.68 for GLU, GLY and AMPA respectively. The
1232 corresponding R^2 values were all > 0.998.

1233 The concentrations are expressed as pg/mg hair. The limits of quantification (LOQ) and lowest
1234 detected values (Minimum) are presented in Table 1. The LOQ was determined as the lowest
1235 concentration level presenting variability and accuracy < 25%. The estimation of the limit of
1236 detection (LOD, the lowest concentration of a substance that can be reliably detected by the
1237 analytical method) can be done through various experimental and computational approaches, and
1238 choices must be made according to the type and the objective of the analytical method (Evard et al.
1239 2016b, 2016a). We did not apply specific experiments and calculations to compute the LOD in this
1240 study owing to several reasons: (1) the purpose of the study was not focused on an analytical
1241 method development and validation; (2) high performance and resolution of current instrumentation
1242 and modes hamper the use of methods based on signal-to-noise to estimate the LOD; (3) the
1243 analyses were not run within a framework requiring a specific guideline to be followed; and (4) no
1244 critical decision has to be based on the LOD. As described above, the analytics were based on up-to-
1245 date protocols and materials and followed the general principles of metrology (such as inclusion of
1246 blank samples and over 5 calibration or quality control levels, among others). The accuracy and
1247 sensitivity of the method were not fully computed but the inclusion of blanks and pretty low

1248 concentrations in standards (i.e. 0.1 and 0.2 pg/mg) limits the potential for bias towards false
1249 positive. All values above the lowest analytical detected value were included as “detects” contrary to
1250 “nondetects”. Importantly, only one concentration was reported below the lowest concentration in
1251 quality controls (the minimum GLY concentration, see Table 1). All reported concentration values,
1252 involving values between the LOQ and the lowest detected value, were included as continuous data.
1253 The use of concentrations below the LOQ has been shown to be a suitable method preferable to the
1254 absence of data, which shows better efficiency in terms of bias and precision than several other
1255 established methods (Keizer et al. 2015).

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1274 *Text A3: Approaches of reverse dosimetry to assess the risk for deleterious effects in small*
1275 *mammals*

1276 In this part of the work, reverse dosimetry approaches have been applied to estimate the daily dose
1277 intake of small mammals from the concentrations of glyphosate (GLY), AMPA or glufosinate (GLUF)
1278 measured in hair, and several types of data that may be useful for applying dose reconstruction
1279 processes have been used. Concentrations in mammal body fluids and hair, residues in viscera and
1280 body burden in small mammals, and toxicokinetics in small mammal blood have been collected from
1281 the literature. Several approaches of back-calculations were thus applied depending on the type of
1282 input data and the different approaches were computed under several assumptions in cases with a
1283 lack of available data.

1284 For GLY, four different approaches have been applied on the basis of data about (1) concentrations in
1285 human urine and hair (Alvarez et al. 2022), (2) body burden in rats 7 days after dose administration
1286 (Brewster, 1991), (3) toxicokinetics in rat blood (Anadón et al. 2009; Kim et al. 2023), (4) residues of
1287 GLY in the viscera and body of wild small mammals in a field study (Newton et al. 1984), and (5)
1288 equations for fast-elimination chemicals that relate concentrations in hair in rats and the level of
1289 exposure, taking pharmacokinetics into account (Faÿs et al. 2023). For GLUF, one approach could be
1290 proposed on the basis of toxicokinetics in rat blood (Joint FAO/WHO Meeting on Pesticide Residues
1291 (JMPR) et al. 2012). The body weights of small mammals were obtained from actual morphometrics
1292 measured in our experiment by weighing individuals in which residues of GLY, AMPA and GLUF were
1293 measured (Sup Info Annex 2). Data concerning urinary excretion percentage of GLY were obtained in
1294 documents released by the EFSA and recent reviews (Connolly et al. 2020; EFSA 2015a; Peillex and
1295 Pelletier 2020). The concentrations of GLY in the viscera and body reported in Newton et al. (1984)
1296 for shrews (carnivores), deermice (omnivores) and voles (herbivores) were used separately according
1297 to diet preference to compute reverse dosimetry in shrews (carnivores), wood mice, house mice and

1298 bank voles (omnivores), and common voles (herbivores), respectively. The food intake of the
1299 different species was computed using the mean food intake of the different items provided by
1300 Crocker et al. (2002). The mean food intake of arthropods and cereal seeds provided for the wood
1301 mouse has been used for the wood mouse and the house mouse; the mean food intake of
1302 arthropods and earthworms provided for the common shrew has been used for the greater white-
1303 toothed shrew; and the mean food intake of grasses and forbs provided for the field vole has been
1304 used for the common vole (Crocker et al. 2002). The assumptions that had to be made to allow the
1305 calculations were that the distribution was equivalent between body parts and hair, that the
1306 concentration in hair was equivalent to the concentration in plasma/blood and proportional to the
1307 administered dose, and that the concentration in viscera reflected the concentration in the diet.
1308 These assumptions, if not verified, might, however, be considered acceptable according to current
1309 knowledge about GLY pharmacokinetics. It is known that *“absorbed GLY is poorly metabolised, widely
1310 distributed in the body, does not undergo enterohepatic circulation and is rapidly eliminated”* (EFSA
1311 2015a). Like GLY, GLUF is distributed ubiquitously in the body rapidly after administration and is
1312 mostly excreted in faeces and urine (Joint FAO/WHO Meeting on Pesticide Residues (JMPR) et al.
1313 2012). Chemicals are mostly incorporated into living hair bulb cells from the bloodstream, and
1314 residue concentrations in hair are representative of the internal dose during the time of hair sample
1315 growth (Appenzeller et al. 2017; Faÿs et al. 2023). In a field study where GLY was aerially applied at
1316 3.3 kg a.s./ha in a forest, the concentrations measured in the remainder of small mammal bodies
1317 (without viscera) were of the same order of magnitude as the maximum concentrations measured
1318 here in hair; owing that in the EU the maximum field application of GLY in cropped fields is
1319 comparable (4.32 g a.s./ha) (Newton et al. 1984). Newton et al. (1984) considered concentrations in
1320 viscera as reflecting intake and reported that *“GLY in the viscera of herbivores was roughly equal to
1321 or somewhat below concentrations found in litter and ground cover”*.

1322 The dose of GLY was compared with five toxicological thresholds. Three of these thresholds were
1323 regulatory thresholds: the short-term dietary NOEL (no observed effect level, 150 mg active

1324 substance/kg body weight), the chronic 21 day NOAEL, which has the same value as the reproductive
1325 NOAEL (no observed adverse effect level, 351 mg a.s./kg bw/d), and the long-term NOAEL (maternal
1326 and developmental NOAEL, which is the same than long-term NOAEL for terrestrial vertebrates, 50
1327 mg a.s./kg bw/d) (EFSA, 2015a; PPDB, <http://sitem.herts.ac.uk/>). The No Mortality Dose to the
1328 Oregon vole (NOAEL Acute toxicity (Intraperitoneal), 450 mg a.s./kg bw) was also used to consider a
1329 wild species (McComb et al. 2008). Some more recent publications highlighted that the harmful
1330 effects of GLY were detected below regulatory limits (e.g. within the spectrum of acceptable daily
1331 intake) (Cai et al. 2017; Clair et al. 2012; Mesnage et al. 2015). We therefore added a “chronic low-
1332 dose” limit, with a value of 5 mg a.s./kg bw/d obtained from studies showing effects on reproductive
1333 development, endocrine disruption, maternal behaviour, neuroplasticity and the gut microbiota in
1334 rats (Dechartres et al. 2019; Romano et al. 2010).

1335 The dose of GLUF was compared to four available toxicological thresholds: the acute oral LD50
1336 (median lethal dose; 416 mg/kg bw), the short-term dietary NOEL (64 mg a.s./kg bw), the chronic 21
1337 days NOAEL (6.3 mg a.s./kg bw/d) and the long-term NOAEL (2 mg a.s./kg bw/d) (Joint FAO/WHO
1338 Meeting on Pesticide Residues (JMPR) et al. 2012; PPDB, <http://sitem.herts.ac.uk/>).

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1390 *Text A4: Statistical outputs of the Tarone-Ware tests*

```

1391 > twoSampleLinearRankTestCensored(x = TAB$GLY, x.censored =
1392 TAB$GLY.censored,
1393 + y = TAB$AMPA, y.censored =
1394 TAB$AMPA.censored,
1395 + test = "tarone-ware")
1396 Results of Hypothesis Test
1397 Based on Censored Data
1398 -----
1399 Null Hypothesis: Fy(t) = Fx(t)
1400 Alternative Hypothesis: Fy(t) != Fx(t) for at least one t
1401 Test Name: Two-Sample Linear Rank Test:
1402 Tarone-Ware Test
1403 with Hypergeometric Variance
1404 Censoring Side: left
1405 Censoring Level(s): x = 0.018
1406 y = 0.24
1407 Data: x = TAB$GLY
1408 y = TAB$AMPA
1409 Censoring Variable: x = TAB$GLY.censored
1410 y = TAB$AMPA.censored
1411 Sample Sizes: nx = 61
1412 ny = 61
1413 Percent Censored: x = 36.1%
1414 y = 49.2%
1415 Test Statistics: nu = 58.055344
1416 var.nu = 1504.925791
1417 z = 1.496527
1418 P-value: 0.1345163
1419
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1421
1422 > twoSampleLinearRankTestCensored(x = TAB$GLY, x.censored =
1423 TAB$GLY.censored,
1424 + y = TAB$GLUF, y.censored =
1425 TAB$GLUF.censored,
1426 + test = "tarone-ware")
1427 Results of Hypothesis Test
1428 Based on Censored Data
1429 -----
1430 Null Hypothesis: Fy(t) = Fx(t)
1431 Alternative Hypothesis: Fy(t) != Fx(t) for at least one t
1432 Test Name: Two-Sample Linear Rank Test:
1433 Tarone-Ware Test
1434 with Hypergeometric Variance
1435 Censoring Side: left
1436 Censoring Level(s): x = 0.018
1437 y = 1.161
1438 Data: x = TAB$GLY
1439 y = TAB$GLUF
1440 Censoring Variable: x = TAB$GLY.censored
1441 y = TAB$GLUF.censored
1442 Sample Sizes: nx = 61
1443 ny = 61
1444 Percent Censored: x = 36.1%
1445 y = 55.7%
1446 Test Statistics: nu = 13.9088437
1447 var.nu = 1304.2735588

```

```

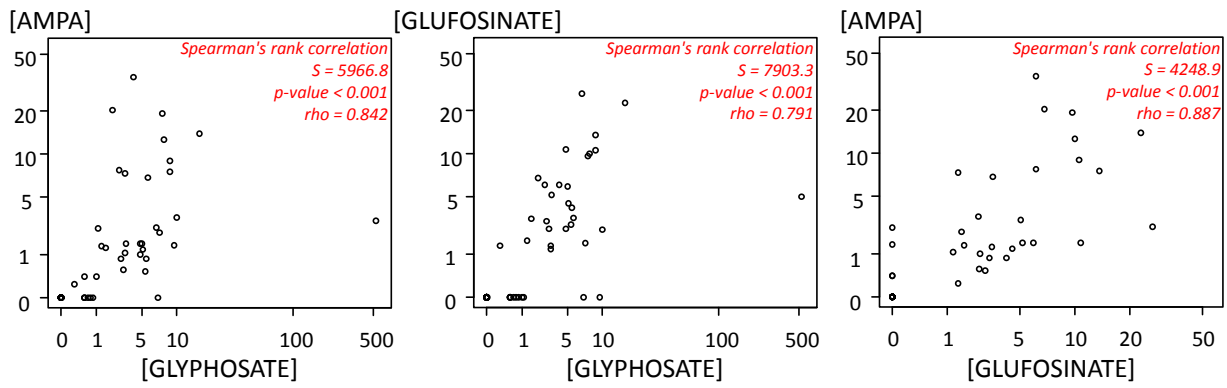
1448          z          =      0.3851294
1449 P-value:          0.7001415
1450
1451
1452 > twoSampleLinearRankTestCensored(x      =      TAB$AMPA,      x.censored      =
1453 TAB$AMPA.censored,
1454 +                                y      =      TAB$GLUF, y.censored      =
1455 TAB$GLUF.censored,
1456 +                                test = "tarone-ware")
1457
1458 Results of Hypothesis Test
1459 Based on Censored Data
1460 -----
1461 Null Hypothesis:          Fy(t) = Fx(t)
1462 Alternative Hypothesis:  Fy(t) != Fx(t) for at least one t
1463 Test Name:              Two-Sample Linear Rank Test:
1464                        Tarone-Ware Test
1465                        with Hypergeometric Variance
1466 Censoring Side:         left
1467 Censoring Level(s):    x = 0.24
1468                        y = 1.161
1469 Data:                  x = TAB$AMPA
1470                        y = TAB$GLUF
1471 Censoring Variable:    x = TAB$AMPA.censored
1472                        y = TAB$GLUF.censored
1473 Sample Sizes:         nx = 61
1474                        ny = 61
1475 Percent Censored:     x = 49.2%
1476                        y = 55.7%
1477 Test Statistics:      nu      = -33.2689131
1478                        var.nu = 1197.6199535
1479                        z      = -0.9613446
1480 P-value:              0.3363789
1481
1482

```


1483 **Figure A1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in small mammal**
1484 **hair.**

1485 *The concentrations are expressed in pg/mg. Nondetects are set to 0.*

1486

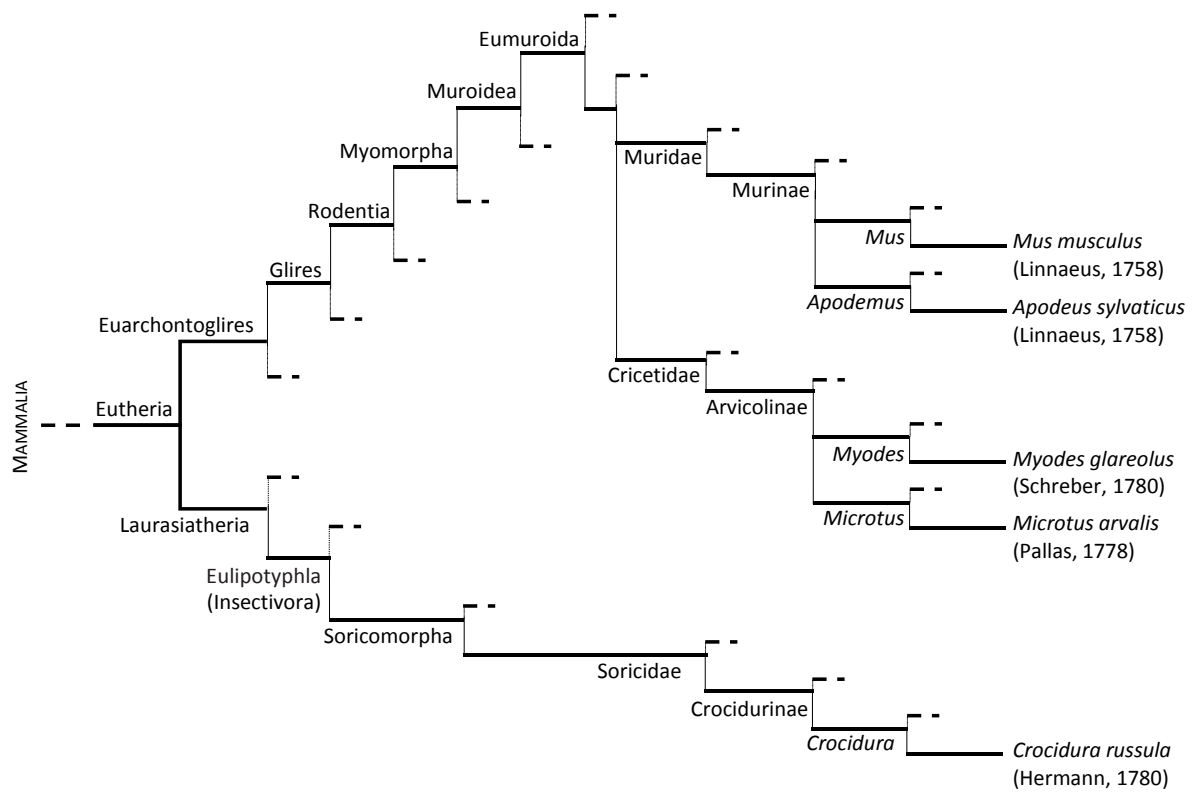


1487

1488 **Figure A2. Simplified phylogenetic tree of the rodents and shrews included in the study.**

1489 *Adapted from (Brace et al. 2016; Springer 2004; Swanson et al. 2019).*

1490



1491 **References**

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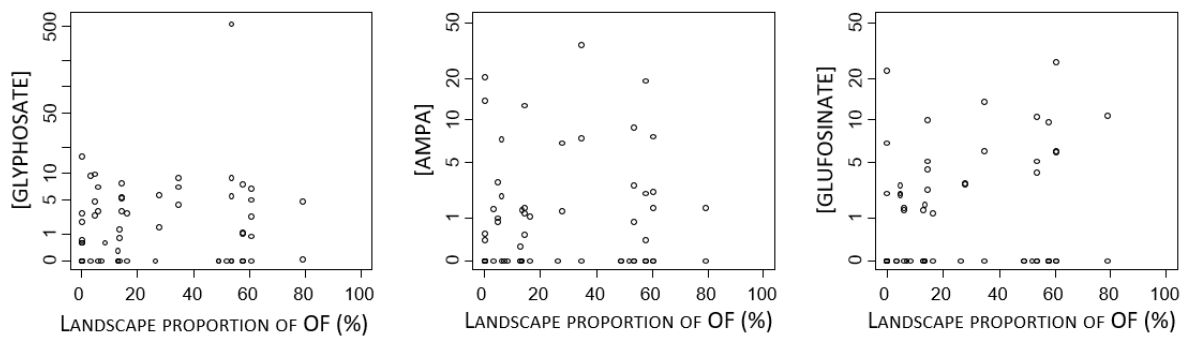
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1499

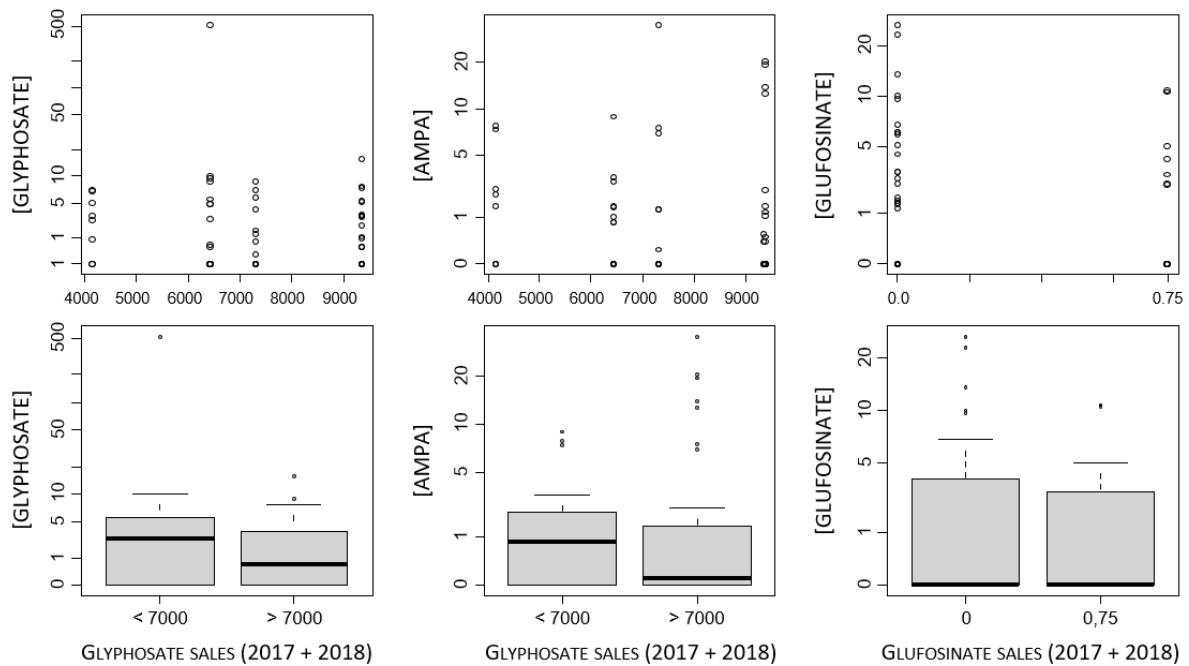
1500 **Figure A3. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals with**
 1501 **nondetects set to 0 according to the proportion of organic farming in the landscape as a continuous factor (a)**
 1502 **and to the proxy of treatment intensity at township scale as a continuous factor and as a categorical factor**
 1503 **(b).**

1504 *The concentrations are expressed in pg/mg. Nondetects are set to 0. Statistics were performed via the Wilcoxon-*
 1505 *Mann-Whitney test or the Kruskal-Wallis test depending on the number of levels of the factor of concern and*
 1506 *Spearman's rank correlation test for continuous factors. No significant differences were found.*

a) PROPORTION OF ORGANIC FARMING (OF) IN LANDSCAPE as a continuous factor



b) SALES OF ACTIVE SUBSTANCE IN THE TOWNSHIP as a continuous factor and by category



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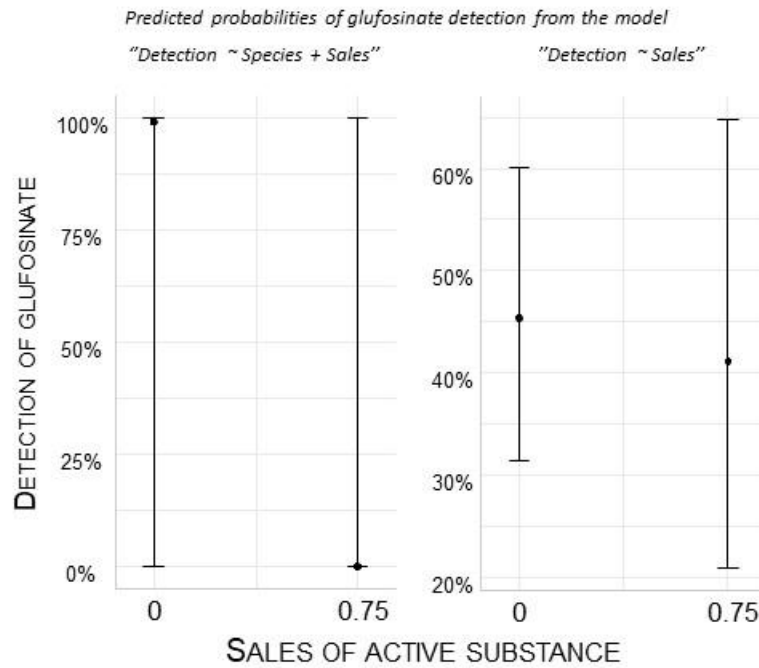
1511

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1514 **Figure A4. Probabilities of detection of glufosinate in the hair of small mammals according to the sales of the**
1515 **active substance at the township scale.**

1516 *The predicted probabilities of detection were obtained from the statistical binomial models "Detection ~ species*
1517 *+ sales"(left panel) and "Detection ~ sales" (right panel).*

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1542 **Table A1. Overview of sample size.**

1543 *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
Farming							
Cereal							
	CF	5	2	7		1	15
	OF/CF	4		1			5
	OF			1		1	2
	Total	9	2	9		2	22
Hedgerow or woodlot							
	CF	7	7	2	10		26
	OF/CF		3	1	1		5
	OF	2	2	4			8
	Total	9	12	7	11		39
	Total CF	12	9	9	10	1	41
	Total OF/CF	4	3	2	1		10
	Total OF	2	2	5		1	10
	Total « NT »	9	12	8	11	1	41
	Total « T »	9	2	8		1	20
Landscape OF proportion							
	0-50%	11	9	8	11	1	40
	50-100%	7	5	8		1	21
AS sales GLY							
	< 7000	6	4	11	2	2	25
	> 7000	12	10	5	9		36
AS sales GLUF							
	0	15	11	7	11		44
	0.75	3	3	9		2	17
	Total	18	14	16	11	2	61

1544

1545 **Table A2. Statistical outputs of binomial GLMs used to investigate the influence of explanatory factors on the detection frequencies of glyphosate, AMPA, and**
 1546 **glufosinate in the hair of small mammals.**

1547 *The binomial models were computed as “Detection ~ Species” and “Detection ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AS sales”. **
 1548 *alternative model with the factor “glufosinate sales” alone without the factor “species”. Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus*
 1549 *sylvaticus (Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew), Miar=Microtus arvalis (Common vole), Mumu=Mus musculus (House mouse), Mygl=Myodes*
 1550 *glareolus (Bank vole), NT=Not targeted, T=Targeted, AS=Active substance, p=p-value. Significant p-values are indicated in bold.*

Factor	Levels	n	GLYPHOSATE				AMPA				GLUFOSINATE									
			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	<0.001				32.464	56	<0.001				39.079	56	<0.001			
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)	0.003				24.5	(4.56, 208)	<0.001				34.7	(6.05, 321)	<0.001
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)	0.010				35.0	(4.73, 753)	0.003				80.0	(9.16, 1999)	<0.001
Habitat			0.191	55					0.018	55	0.892				0.182	55	0.669			
Cereal	22					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)*	0.022 (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)*

1551

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1553 **Table A3. Statistical outputs of the GLMs used to investigate the influence of explanatory factors on the concentrations of glyphosate, AMPA, and glufosinate in the hair**
 1554 **of small mammals.**

1555 *The models were computed as “log(concentration) ~ Species” and “log(concentration) ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AI*
 1556 *sales”. Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus (Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew),*

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1558

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

Factor	Levels	GLYPHOSATE								AMPA						GLUFOSINATE											
		Factor			Model		Levels			Factor			Model		Levels			Factor			Model		Levels				
		n	F	Df	p	R ²	R ² _{Adj}	Estimate	SE	p	F	Df	p	R ²	R ² _{Adj}	Estimate	SE	p	F	Df	p	R ²	R ² _{Adj}	Estimate	SE	p	
Species		1.643	4	0.186	0.162	0.162	0.063			1.110	4	0.373	0.146	0.146	0.014				1.008	4	0.425	0.155	0.155	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	0.044	0.098	0.260	0.148			0.750	1	0.395	0.025	0.171	0.005				2.759	1	0.112	0.098	0.253	0.075			
Cereal	14					Reference	---	---							Reference	---	---						Reference	---	---		
Hedgerows	25					1.20	0.572	0.044							-0.521	0.602	0.395						-0.716	0.431	0.112		
Farming		0.017	2	0.983	0.001	0.162	0.006			0.725	2	0.495	0.049	0.195	0.000				0.087	2	0.917	0.007	0.162	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	0.042			0.750	1	0.395	0.025	0.171	0.005				2.759	1	0.112	0.098	0.253	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	0.039			0.754	1	0.393	0.025	0.171	0.005				3.454	1	0.077	0.119	0.274	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	0.038			0.595	1	0.448	0.020	0.166	0.000				0.884	1	0.358	0.034	0.189	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		

