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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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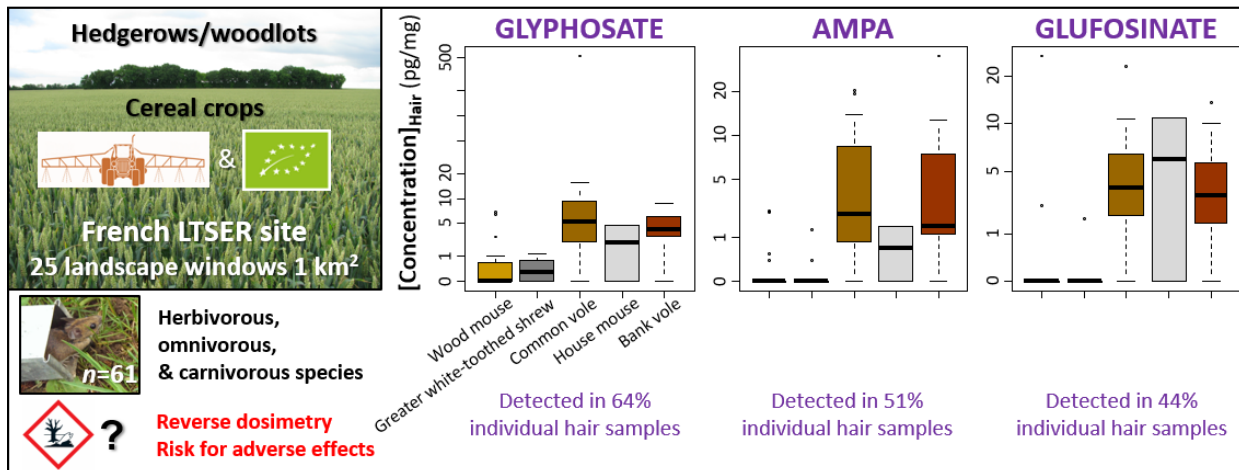
30 **Abstract**

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

54

55

56 Graphical abstract



57

58

59 **Keywords:** agroecosystem; bioaccumulation; currently used pesticides; landscape ecotoxicology;

60 wildlife toxicology

61

62

63 **1. Introduction**

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

72 Initially, GLY was marketed as an “environmentally friendly” herbicide owing to its low expected
73 environmental persistence (easily biodegraded), low predicted toxicity to wildlife and humans
74 (absence of the shikimate pathway in animals, which is found only in plants and microorganisms and
75 is the target pathway of GLY in plants), and lack of potential for bioaccumulation (hydrophilic, low log
76 octanol–water partition coefficient $K_{ow} = -3.12$), poorly metabolized and rapidly eliminated in
77 mammals) (EFSA 2015a; Gandhi et al. 2021; Kissane and Shephard 2017; Richmond 2018). The log K_{ow}
78 is a measure to assess whether a substance is more or less hydrophobic (and/or lipophilic, fat solubility)
79 or hydrophilic (and/or lipophobic, water solubility). A high bioaccumulation potential may be expected
80 for log $K_{ow} > 3$, and K_{ow} is used as one of the regulatory criteria to classify a compound as potentially
81 bioaccumulative if log $K_{ow} > 4.5$. However, concerns about the environmental safety and health risks
82 associated with GLY have increased within recent decades, especially in the context of its continuously
83 increasing use (Gandhi et al. 2021; Székács and Darvas 2018; Van Bruggen et al. 2018; Vandenberg et
84 al. 2017). GLY and AMPA are more persistent in the environment than initially assessed, as shown by
85 the ubiquitous occurrence of residues in soil, water, and air (Bai and Ogbourne 2016; Gandhi et al.
86 2021; Richmond 2018; Van Bruggen et al. 2018; Xu et al. 2019). They can accumulate in environmental
87 compartments, crops, and the human food chain (Bai and Ogbourne 2016; Richmond 2018). They are
88 associated with chronic low-dose exposure risks in animals and humans, and can be more harmful to

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

96 In the USA and EU, registration reviews concerning GLY use as a plant protection product (PPP)
97 identified potential risks to terrestrial and aquatic plants and nontarget terrestrial arthropods, and
98 risks to mammals and birds (EFSA 2015b, 2015c, 2015a; US EPA 2020). In the EU assessment for
99 vertebrates, the taxa identified as the most at risk were mammals, particularly herbivorous mammals
100 (EFSA 2015b, 2015a). Glufosinate (GLUF), like GLY, is an organophosphorus compound used as a broad-
101 spectrum nonselective contact herbicide with a systemic action and was considered “*one of the very*
102 *few alternatives to glyphosate*” (European Commission 2017). However, risks for herbivorous and
103 insectivorous mammals associated with the herbicidal use of GLUF have been reported in
104 environmental risk assessment (ERA) processes (EFSA 2005, 2012). GLUF was classified as a candidate
105 for substitution in the EU according to Commission Regulation EC No 1107/2009 (PPP Regulation) in
106 2015, and withdrawal was scheduled at its end of approval in the EU in 2018 (Robin and Marchand,
107 2023). However, GLUF is still used in many countries worldwide.

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

115 regulatory decisions with respect to the objectives of reducing the risks and impacts of pesticide use
116 on human health and the environment (e.g. Directive 2009/128/EC). Assessing and characterizing the
117 exposure of wildlife to GLY, AMPA and GLUF is therefore a critical issue for environmental protection
118 and biodiversity conservation.

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

141 differences between small mammal species (rodents and shrews) with various ecological traits were
142 investigated. Herbivorous voles are expected to exhibit greater exposure than are granivorous,
143 omnivorous or insectivorous species according to the higher daily dietary doses estimated during ERA
144 processes (EFSA 2005, 2012; European Commission 2015). Moreover, herbivorous voles are less
145 mobile than omnivorous mice and voles or shrews are (van den Brink et al. 2011; Wijnhoven et al.
146 2005) and may not avoid treated areas. We expect species that feed on animal matter (i.e. omnivores,
147 carnivores, insectivores or vermivores) to also be exposed. Indeed, GLY residues have been found in
148 small carnivorous and omnivorous mammals in a forest ecosystem (Newton et al. 1984), and residues
149 of GLY, AMPA and GLUF have been found in earthworms collected from the study area, a taxa that can
150 be prey of rodents and shrews (Pelosi et al. 2022).

151 **2. Materials and methods**

152 *2.1. Study site and design*

153 The study site was the Long-Term Socio-Ecological Research (LTSER) “Zone Atelier Plaine & Val de
154 Sèvre” (ZAPVS, <https://za-plaineetvaldesevre.com/>) in mid-western France (see Bretagnolle et al.
155 (2018) for a general site description). The study was conducted within the framework of a larger project
156 which acronym was “PING”, for which the sampling design was based on about 40 sampling squares
157 of 1 km², selected on the basis of landscape features (gradient of seminatural habitats such as
158 hedgerows and a gradient of organic farming surfaces), in which sampling was conducted in cropped
159 and semi-natural habitats (cf. Pelosi et al. 2022). The trapping of small mammals was conducted in the
160 1 km² sampling squares in both an arable cereal field (winter cereals) and a woody patch (hedgerow
161 or woodlot edge) as close as possible to the sampled arable field (Fig. 1). Cropped cereals and
162 hedgerows are referred to as “habitats” hereafter. Cropped fields were cultivated either under
163 conventional farming (CF) or organic farming (OF). The farming practices in the organic fields followed
164 the rules of the “AB (Agriculture Biologique) France label” and were under OF for at least three years
165 at the time of sampling. A few fields were in transition from CF to OF and were referred to as “OF/CF”.

166 2.2. *Sampling process and sample size*

167 Sampling was conducted in spring 2018 (May-June). Small mammals were captured using traplines of
168 nonlethal traps (25 traps, 2 m apart) with dormitory (Le Quilliec and Croci 2006) filled with hay and
169 food (i.e. pieces of carrots and apples, sunflower seeds, cat food and peanut butter). The hay,
170 vegetables, fruits and seeds originated from organic agriculture. The traps were checked every day,
171 rebaited and refilled or replaced as necessary. The traplines were set during one to four nights
172 depending on logistics and capture success (i.e. longer duration, if possible, in case of an absence of
173 capture during the first night(s)). Species were determined during the handling of individuals on the
174 basis of morphometrics and confirmed by molecular analyses in case of doubt. *Microtus arvalis* and
175 *Microtus agrestis* being morphologically similar species, the identification of *Microtus* voles was
176 confirmed by molecular analyses (Sup Info Annex 1. Text A1). The suspected *Mus* spp individuals were
177 also confirmed genetically. For that, a tiny piece of ear was sampled (using medical clean and
178 disinfected scissors, followed by disinfection of the ear) and stored by freezing (-20 °C) until DNA
179 extraction. A sample of 50-100 mg of hair was taken by shaving, wrapped in aluminium foil and stored
180 in plastic zip-lock bags at -20 °C until analysis. Individuals were released after handling. The survival
181 rate to capture and handling was 91.6%. In case of recapture, the individuals were immediately
182 released without any measurement or sampling (recapture rate around 3%).

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

190 equipped with a mobile anaesthesia unit and all of the materials and equipment required for animal
191 welfare).

192 The sample size resulted in hair samples obtained from 61 individuals captured in 29 traplines
193 distributed over 25 squares of 1 km² (Fig. 1, Table 1; further details about the sample sizes are provided
194 in Sup Info Annex 1. Table A1). Individual samples of 18 wood mice (*Apodemus sylvaticus*), 14 greater
195 white-toothed shrews (*Crocidura russula*), 16 common voles (*Microtus arvalis*), 11 bank voles (*Myodes*
196 *glareolus*), and two house mice (*Mus musculus*) were analysed. A total of 41 samples were collected
197 from individuals captured in plots from CF farming category, 10 from OF/CF category, and 10 from the
198 OF category. A total of 39 samples were collected from hedgerows or woodlots, and 22 were collected
199 from cereal fields. Forty-one samples came from habitats classified as non-targeted by PPP treatments
200 and 20 from plots classified as targeted by PPP treatments.

201 *2.3. Proxies related to the intensity of treatments at various spatial scales*

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

208 For the proxies of treatment intensity, at the plot scale (arable field or woody patches where trapping
209 was realized), a general proxy of potential for pesticide treatment was computed. The fields cultivated
210 under OF and hedgerows or woodlots were considered not intentionally targeted by treatments (NT),
211 whereas the arable fields cultivated under CF and transitional fields cultivated from CF to OF were
212 considered possibly targeted (T). A second proxy to characterize the intensity of potential treatments
213 was computed at the landscape scale. The locations and boundaries of cultivated plots in 2018 were
214 obtained from the official French “Registre Parcellaire Graphique”

215 (<https://www.data.gouv.fr/fr/datasets/parcelles-en-agriculture-biologique-ab-declarees-a-la-pac>).

216 The software Quantum GIS (QGIS 3.16) was used to calculate the proportion of OF plots in the

217 landscape, which was computed in each sampling square of 1 km² as the proportion of the total surface

218 occupied by OF arable fields. The final proxy resulted in a two-level factor, with a category “OF

219 dominant” with proportions varying from 50 to 100% ([49-79], 6 sampling squares), in which treatment

220 intensity at the landscape scale was expected to be lower since arable fields not targeted by pesticide

221 treatments prevailed within the landscape mosaic. The second category, “CF dominant”, gathered

222 squares with landscape OF proportions ranging from 0 to 50% ([0-49], 19 sampling squares). Finally, to

223 consider the intensity of potential treatments over the range of several farmlands, a proxy at the

224 township scale was computed. The sales of active substance (a.s., data provided in kg) were obtained

225 from the “Banque Nationale des ventes de produits phytopharmaceutiques par les Distributeurs

226 agréés (BNV-D Traçabilité)” as released to the public by official agencies at the township level (i.e.

227 according to postal code, <https://www.data.gouv.fr/fr>). These data were extracted for 2017 and 2018

228 (the year before and the year when sampling was conducted). The sales of GLY and GLUF were

229 associated with each trapping location on the basis of township positioning. The sales of GLY were

230 highly correlated between years at the township level ($R^2=0.950$), rendering it impossible to study the

231 influence of potential use intensity separately in the year before or in the year of sampling. The

232 marketing authorisation of pesticides containing GLUF was withdrawn in France by October 2017. The

233 quantities sold in 2017 were very low (i.e. 0.75 kg) and sporadic (i.e. only one quantity reported as sold

234 in the townships studied) whereas in 2018, no sales of GLUF were reported in the townships of the

235 study area of concern. The quantities sold in 2017 and 2018 were thus summed for each a.s. and

236 classified into two categories. For GLUF, the categories were “0” and “0.75”. For GLY, the median of

237 the summed quantities in the dataset was calculated at 7300, and the categories were set at “lower

238 than 7000” ([4138-7000[, 2 townships) and “higher than 7000” ([7000-9366[, 3 townships).

239 *2.4. Chemical analyses of glyphosate, AMPA and glufosinate*

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

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

251 The concentrations are expressed as pg/mg hair. The limits of quantification (LOQ, established as the
252 lowest concentration level presenting variability and accuracy < 25%.) and lowest detected values
253 (Minimum) are presented in Table 1. We did not perform specific experiments and calculations to
254 compute limits of detection (see Sup Info Annex 1. Text A2). Importantly, only one concentration value
255 was reported below the lowest concentration in quality controls (the minimum GLY concentration, see
256 Table 1). All values above the lowest analytical detected value were included as “detects” contrary to
257 “nondetects”. All reported concentration values, including values between the LOQ and the lowest
258 detected value, were included as continuous data. The use of concentrations below the LOQ has been
259 shown to be a suitable method that may be preferred to the absence of data or other established
260 approaches such as imputation-based methods (i.e. efficiency in terms of bias and precision)(Keizer et
261 al. 2015).

262 *2.5. Dose reconstruction to assess the risk of deleterious effects in small mammals*

263 Given the concentrations of GLY, AMPA and GLUF in the keratinized tissues of small mammals, our
264 study highlights the exposure of these animals. However, the toxicological meaning of such hair
265 concentrations is hard to interpret with respect to toxicity benchmarks (which are expressed as daily
266 doses in mg active substance per kg body weight) because relationships between residues in hair and
267 exposure doses or between residues in hair and in those other organs are not known. To overcome
268 this gap and assess whether exposure levels could reach toxic levels, approaches of reverse dosimetry
269 have been applied to estimate levels of exposure in terms of daily dose intake, to obtain data
270 comparable to toxicological thresholds. Different types of data (i.e. concentrations in mammal body
271 fluids and hair, residues in viscera and body burden in small mammals, toxicokinetics in small mammal
272 blood, pharmacokinetics of fast-elimination chemicals) that may be used for such purposes have been
273 collected from the literature (Sup Info Annex 1. Text A3 for full description), leading to different
274 approaches of back-calculations computed under several assumptions when there was a lack of
275 available data. Five different approaches could be computed for GLY and one for GLUF (Tables 2 and
276 3).

277 The objective of our risk assessment was to determine the overall orders of magnitude of daily dose
278 intake relative to the concentrations measured in hair to evaluate whether wild small mammals may
279 experience deleterious toxicological effects. The lack of data and assumptions that had to be applied
280 hamper the accuracy of the calculated dose values. The interpretation of the findings was thus
281 developed to consider ranges and global patterns rather than refined (but biased) toxicological
282 assessments. The doses calculated were compared with toxicity data using the usual and regulatory
283 procedure of toxicity-exposure ratio (TER) calculation, with associated triggers of 5 for chronic toxicity
284 data and of 10 for acute toxicity data. The doses were reconstructed for each species, using four levels
285 of residue concentrations measured in hair: minimum, median, 3rd quartile and maximum values (only
286 one value available in house mice for GLY and in house mice and shrews for GLUF). The dose of GLY
287 was compared to five toxicological thresholds: the short-term dietary no observed effect level (short-

288 term dietary NOEL), the chronic 21-day no observed adverse effect level (chronic NOAEL, which is the
289 same value as the reproductive NOAEL), the long-term NOAEL, the no mortality dose to the Oregon
290 vole, and a “chronic low dose” limit. Details about the values of the thresholds are provided in the Sup
291 Info Annex 1. Text A3. The dose of GLUF was compared to four available toxicological thresholds: the
292 acute oral median lethal dose, the short-term dietary NOEL, the chronic 21-day NOAEL, and the long-
293 term NOAEL.

294 The process ended with the TER values computed for each approach, under different scenarios of
295 assumption, for the different levels of residue concentrations measured in hair, for each species, for
296 the different toxicological thresholds (see Tables 2 and 3 and details in Sup Info Annex 2). Finally, the
297 number of cases where TER exceeded the triggers, which highlights the risk for toxic effects in small
298 mammals, and the number of individuals involved were summarized.

299 *2.6. Data analysis and statistics*

300 Analyses were performed using R (3.3.1), with the additional packages “AICcmodavg”, “effects”,
301 “EnvStats”, “forestmodel”, “ggeffects”, “ggplot2”, “gtsummary”, “multcomp”, “pgirmess”, and
302 “questionr”. Since more than 2 individuals were obtained from the same trapline in only 8 out of the
303 29 where the animals were captured, pseudoreplication was not considered an issue. Moreover, the
304 traplines extended over 50 metres, which should limit the influence of spatial autocorrelation between
305 individuals. The statistical approaches were designed to handle left-censored data within a situation
306 where the smallest observations of data are nondetects, with high proportions of nondetects, skewed
307 distributions, and small sample sizes, which hampered the use of methods considered “gold standards”
308 to manage left-censored data (ITRC 2013; Shoari and Dubé 2018). Following recommendations to
309 analyse datasets including nondetected and left-censored observations, we (1) used tests dedicated
310 to censored data when possible, (2) computed descriptive statistics separately on detection
311 frequencies and on detected concentrations (for which median, interquartile range, and outer
312 percentiles were reported), (3) performed group comparisons separately on detection frequencies and

313 on detected concentrations, and (4) preferred nonparametric tests to analyse the combined dataset,
314 including uncensored data and nondetects with the latter being substituted by 0 (ITRC 2013; Shoari
315 and Dubé 2018). Compared with other methods (i.e. imputation- and nonimputation-based
316 approaches), the traditional substitution approach has been shown to be appropriate for handling
317 nondetects in a study that investigated a dataset of pesticide concentrations in pollen and nectar to
318 assess the exposure of bumble bees (Adjei and Stevens 2022). Substitution with a half limit of detection
319 outperformed the other approaches in the study of Adjei and Stevens (2022), which used parametric
320 tests (nested ANOVA). However, we preferred to use 0 because (1) we used all reported values,
321 including those between the LOQ and lowest detected values, instead of data left-censored by the limit
322 of detection (Keizer et al. 2015), (2) the lowest detected values reached low values that would have
323 brought the value of half of the LOD close to 0, (3) this approach allows easy identifying nondetects in
324 the databases and graphics, and (4) nonparametric tests were used to compute statistics on both
325 nondetects and quantified values.

326 Comparisons between the distributions of the compounds were performed using Tarone-Ware two-
327 sample test for censored data (i.e. GLY vs. AMPA, GLY vs. GLUF, and AMPA vs. GLUF), with nondetects
328 set to the corresponding lowest detected value of the corresponding compound as needed in such
329 tests, for all species together (the sample sizes were too small to run the tests by species). Correlations
330 between raw values with nondetects set to 0 of the concentrations of the three compounds were
331 investigated using Spearman's rank correlation tests. The relationships between the concentrations of
332 each compound were further studied according to species via generalized linear models (Gaussian
333 family) on log-transformed data ($\log(x+1)$) to meet the assumptions of normality and variance
334 homogeneity. The models were checked for homogeneity of variance, normality of error and
335 linearity/additivity and leverage was checked (i.e. standardized residuals vs. leverage). An extreme
336 outlier of GLY concentration was identified (one *Microtus* vole) as having overinfluence on the
337 estimated parameters (i.e. Cook distance greater than 1). This outlier was removed from the dataset
338 to compute model outputs and graphics in this part of the analyses.

339 Then, statistical analyses were performed on (1) GLY, AMPA or GLUF frequencies of detection and (2)
340 GLY, AMPA or GLUF concentrations quantified above the lowest detected value (i.e. in samples where
341 the compound was detected, without nondetects). According to the preliminary analyses showing
342 differences between species, further analyses were conducted taking species into account in statistics
343 using the factor “species” first in the models with the other factors. Full models including all
344 explanatory factors and first-order interactions could not be used due the large number of NAs.
345 Preliminary tests performed on combinations of factors, including interactions, revealed that none of
346 the interactions were statistically significant and that models without interactions better fit the data
347 (according to corrected Akaike information criterion (AICc) comparisons). The final models used to
348 compute the statistics were therefore run without interactions.

349 We tested whether the detection of GLY, AMPA or GLUF in hair of small mammals was dependent on
350 species, habitat, farming practices and proxies for treatment intensity using binomial GLMs (link =
351 logit). The detection was coded as “0” for nondetected and “1” when detected. The models were built
352 using species alone and species with each of the other variables as explanatory factors. The significance
353 of the factors was checked by the *chi-squared* test. Odds ratios with confidence intervals were
354 computed to characterize differences between levels.

355 We tested whether the concentrations of GLY, AMPA or GLUF in the hair of small mammals were
356 dependent on species, habitat, farming practices and proxies for treatment intensity using GLMs on
357 log-transformed values above the lowest detected value (i.e. without nondetects) to meet the
358 assumptions of normality and variance homogeneity. The models were built using species alone and
359 species with each of the other variables as explanatory factors. The significance of the explanatory
360 factors was checked via an *F* test, and statistical differences between levels were via a *t* test.

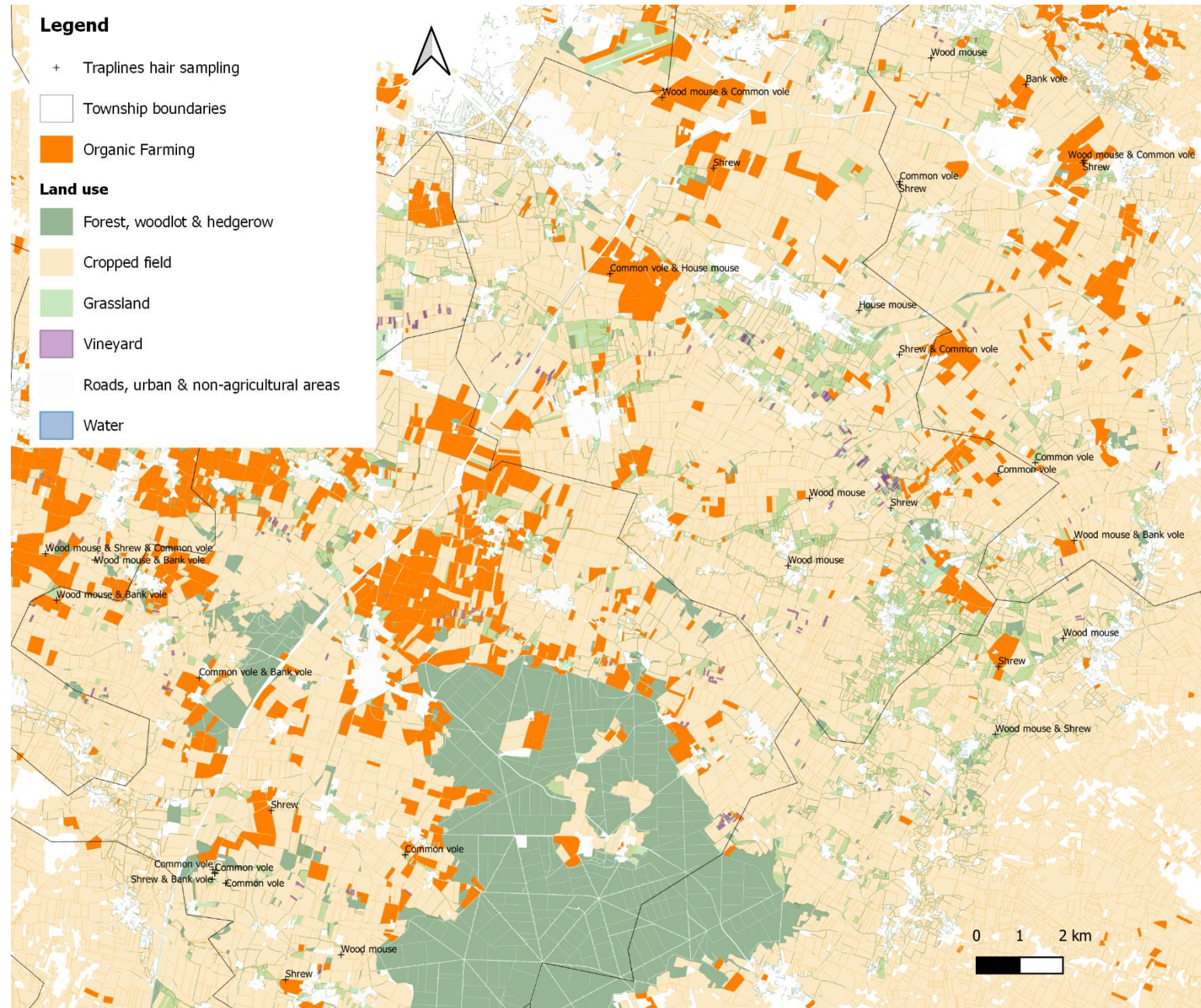
361 To support the results and interpretation, both the detection and concentration data were analyzed
362 via nonparametric tests on all of the data, with nondetects included and set to 0. The differences
363 between species, habitats, farming practices, and the proxies of treatment intensity were tested

364 adequately via the Wilcoxon-Mann-Whitney test or the Kruskal-Wallis test adequately depending on
365 the number of levels of the factors (i.e. two or more). Post hoc multiple comparison tests after Kruskal-
366 Wallis tests were used to investigate pairwise comparisons when relevant. Correlations between
367 concentrations and landscape OF proportions or sales of a.s. used as raw continuous data were tested
368 via Spearman's rank correlation test.

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370 **Figure 1. Map of the traplines where hair samples were collected across the study area.**

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378 **3. Results & Discussion**

379 *3.1. Detection frequency of GLY, AMPA and GLUF in the hair of small mammals*

380 Considering all of the species together, GLY, AMPA and GLUF were detected in 64%, 51%, and 44% of
381 the individuals, respectively (Table 1). Compared with the quantities sold (in kg) in the townships
382 studied, the quantities of GLUF were 2484 to 4844 times lower than those of GLY in 2018 and 1653 to
383 4548 times lower in 2017. Indeed, after its ban in 2017, GLUF could only have been used until stock
384 depletion or usage derogation. Focusing on the part of the region where the study was carried out (i.e.
385 “Département des Deux-Sèvres”), the purchases of GLUF rapidly decreased after 2015 and reached 0
386 in 2019 (BNV-D”). Despite its low intensity of application compared with that of GLY, GLUF was
387 detected in approximately half of the small mammal individuals. Within the “PING” research
388 programme (see M&M), the part of the study conducted over the same year over the same sampling
389 sites as the present one detected GLY in 88% of the soil samples and 74% of the earthworm samples,
390 AMPA in 58% of the soil samples and 38% of the earthworms, and GLUF in 35% of the soil samples and
391 12% of the earthworms (Pelosi et al. 2022). These detections in soil, earthworms and small mammals
392 collected from arable fields and hedgerows, both in conventional and organic farming fields, revealed
393 a generalized occurrence of GLY, AMPA and GLUF in the agricultural environment and fauna across this
394 typical French agricultural area. In comparison, in three county-level hair pools of carcasses of big
395 brown bats (*Eptesicus fuscus*; an insectivorous species weighing 15 to 26 g) in Missouri (USA), GLY was
396 detected in all three analysed sample pools (100% detection frequency) (Hooper et al. 2022). The
397 frequency of exposure to GLY in a farmland herbivorous mammal, the Iberian hare (*Lepus granatensis*)
398 via analyses of residues in samples of gastric content, reached 45% of individuals found dead in March-
399 April due to roadkill or unknown causes (Martinez-Haro et al. 2022), which is comparable to our results.
400 In hunted hares the frequency of detection in gastric content ranged between 9% and 22% in animals
401 from pesticide-treated areas. These later values in hunted hares look lower than the frequencies found
402 in rodents in our study. This, possibly because residues in gastric content represent a snapshot of
403 recent exposure whereas measurements in hair represent a temporally integrative exposure

404 assessment during the time of hair growth, and because of the seasonal difference. Indeed, the hunted
405 Iberian hares were collected from October to January, while our sampling session was conducted
406 during springtime. The authors indicated that the frequency of detection in hares progressively
407 increased over the hunting season, being 0% and 11% in October and November and 50% in December
408 and January (Martinez-Haro et al. 2022). Issues related to local contamination may further explain the
409 lower detection frequency in hunted hares.

410 *3.2. Concentrations of GLY, AMPA and GLUF in the hair of small mammals*

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

418 In county-level hair pools from big brown bats in Missouri, Hooper et al. (2022) reported
419 concentrations of GLY varying from 5.7 to 4505.2 pg/mg. These maximum concentrations are much
420 higher than those measured here in shrews (median = 0.8 pg/mg) and almost ten times higher than
421 the maximum concentration measured in our study in a vole (i.e. 522 pg/mg). Several nonexclusive
422 hypotheses may be proposed to explain such high levels found in bats: analytical issues, physiological
423 and ecological traits of the species enhancing exposure and/or bioaccumulation (Fritsch et al. 2024),
424 and issues related to the amount of GLY used and the timing of exposure of the animals after GLY
425 application.

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

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Descriptive statistics on concentrations were computed when the given compound was detected, i.e. nondetects (samples whose concentrations were below the lowest detected value (Minimum)) were excluded. Home range size were obtained from Chambers et al. (2000) and Wijnhoven et al. (2005). The bank vole is often considered a granivorous species but recent studies have shown omnivory. Abbreviations: OF=Organic farming, CF=Conventional farming, PPP=plant protection product, NT=Not targeted by treatments, T=Targeted by treatments, LOQ= Limit of 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	
Targeted or not by PPP treatments						
« 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 (kg)						
< 7000	25	6	4	11	2	2
> 7000	36	12	10	5		9
Sales GLUF (kg)						
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	3.79	3.65
Q3	5.77	4.83	0.950	8.13		5.63
Maximum	522	5.92	1.22	522		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	1.37	1.61
Q3	6.45	2.03	1.05	7.65		6.51
Maximum	33.6	2.07	1.27	19.4		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	1.48	4.05	9.75	3.01
Q3	7.25	19.7		5.80		4.87
Maximum	25.5	25.5		21.8		12.6

433

434 Newton et al. (1984) studied the fate and behaviour of GLY and AMPA in forest brush field ecosystems
435 in the Oregon Coast Range (USA) following aerial application of GLY at doses comparable to the
436 recommended GLY application rate in the EU (i.e. 3.3 kg a.s./ha there, maximum field application of
437 4.32 kg a.s./ha in the EU (European Commission 2015)). They measured the residues of GLY and AMPA
438 in the viscera and body of free-ranging small mammals captured at the sites before treatment and over
439 the 55 days posttreatment. As in the present work, GLY was detected in all of the trophic groups (LOD
440 of 0.10 mg/kg = 100 pg/mg). However, AMPA was detected in two voles only at 130 and <160 pg/mg
441 (LOD of 100 pg/mg) (Newton et al. 1984), which is far lower than in our study. This may be because
442 the lowest detected value in our work was much lower (i.e. 0.240 pg/mg) and because different tissues
443 were used to measure residues.

444 Interestingly, considering these previous findings of Newton et al. (1984) in forest small mammal
445 tissues, the concentrations varied over the same order of magnitude as the maximum concentrations
446 measured in our hair samples from agricultural landscapes. They reported concentrations in viscera
447 ranging from < 100 pg/mg (LOD) to 1690 pg/mg in shrews, 5080 pg/mg in deermice and 1700 pg/mg
448 in voles. They reported concentrations in body tissues ranging from < 100 to 410 pg/mg in shrews, 400
449 pg/mg in deermice and 250 pg/mg in voles. As a comparison, Martinez-Haro et al. (2022) reported GLY
450 concentrations ranging from 0.11 to 16 µg/g (110 to 16000 pg/mg) in the gastric content of Iberian
451 hares found dead or hunted in Spain. Newton et al. (1984) showed that the concentrations were the
452 highest during the first days following herbicide treatment both in the viscera and body tissues. Body
453 concentrations decreased under the LOD by 2 weeks after treatment while they were still detectable
454 in the viscera, and concentrations in the viscera decreased to nondetectable concentrations or near
455 the LOD by day 55 after treatment (Newton et al. 1984). The environmental persistence of GLY is
456 estimated to vary around 43 days (reported values ranged from 1 to 180 days, up to 197 days) (Gandhi
457 et al. 2021; Kissane and Shephard 2017). Although residues may be detected several months after
458 application following spraying and rainfall, the binding to soil and biodegradation of glyphosate is
459 estimated between 4 to 11 days post-treatment (Kissane and Shephard 2017). In their forest study,

460 Newton et al. (1984) reported GLY half-lives ranging from 10 to 27 days in foliage and litter, half-life of
461 40 days for exposed soil, and half-life of 29 days for litter-covered soil. The GLY residues overall
462 dropped under 20-30% of the initial deposit within 7-14 days (Newton et al. 1984)). This pattern of GLY
463 concentration decrease over time in the environment and in small mammal body and viscera following
464 a single GLY application, together with differences in the matrices analysed, may explain the overall
465 lower GLY levels in our study in comparison to the one of Newton et al. (1984). This also suggests that
466 the highest concentrations found here may correspond to animals highly exposed to GLY soon after its
467 application when environmental concentrations were the highest, and/or repeatedly exposed during
468 the growth of hair. Our samples were collected during springtime, from May-June, whereas glyphosate
469 is usually applied to winter cereals during late summer or fall (often in October). The seasonal moult
470 of small mammals is expected to be completed mostly by May-June, when they were captured, and
471 the sampled fur might have partly or even totally grown within the period of February-May (Abad
472 1991; López-Fuster et al. 1986; Sealander 1972). However, the postwinter moult may be incomplete,
473 depending on the age of individuals and the species of concern, allowing the possibility for hair grown
474 during autumn to be included in the analysed fur sample (Beltran et al. 2018).

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

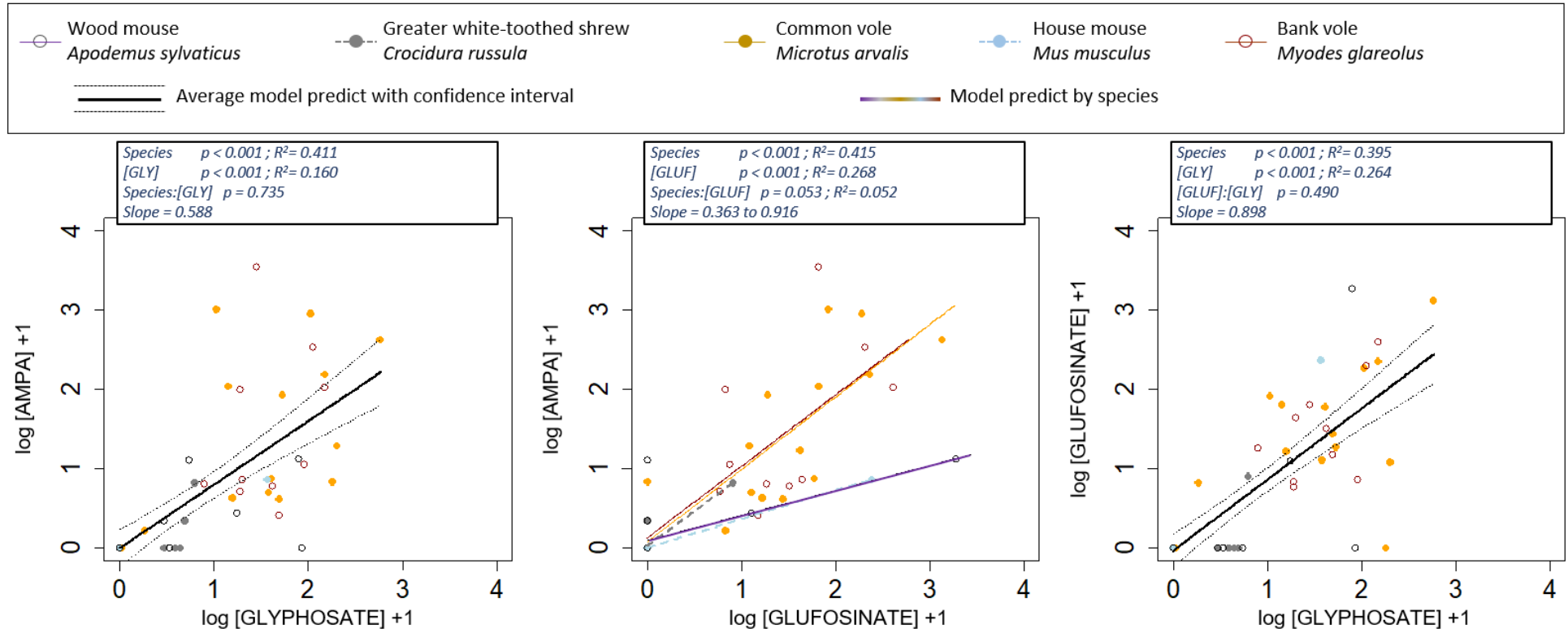
476 *The concentrations are expressed in pg/mg. The statistical outputs of general linear models are provided on graphs. Predictions are plotted according to species when the interaction*
 477 *between the factors is significant, and average predictions are provided when the interaction is not significant.*

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

507 *3.3. Differences between species in detection frequency and concentration of the compounds*

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

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

523 In their forest study where residues of GLY and AMPA were measured in several free-ranging
524 mammalian herbivores, carnivores, and omnivores, Newton et al. (1984) reported that GLY was
525 present in all trophic groups, whereas AMPA was present in only a few herbivorous voles. Our results
526 are in line with these findings, with confirmed exposure to GLY (and AMPA) in both herbivorous and
527 granivorous or omnivorous rodents and insectivorous shrews and greater levels of AMPA in
528 herbivorous mammals (note that the bank vole was often considered a granivore, but recent literature
529 has shown omnivory (Balčiauskas et al., 2022)).

530 **Figure 3. Probabilities of the detection of glyphosate, AMPA and glufosinate in the hair of small mammals according to species.**
 531

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

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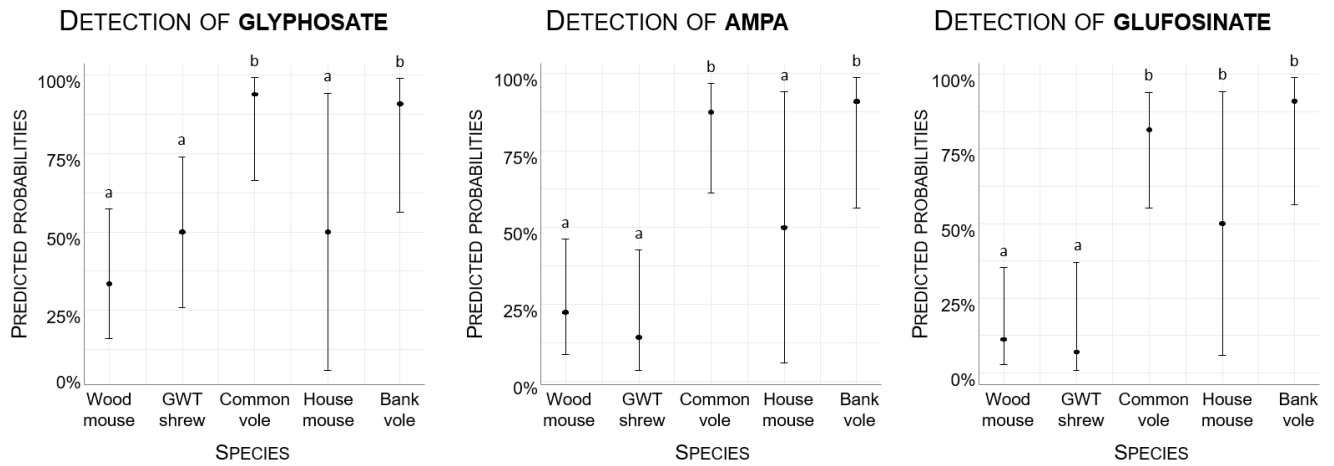
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545 **Figure 4. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals according to species.**

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

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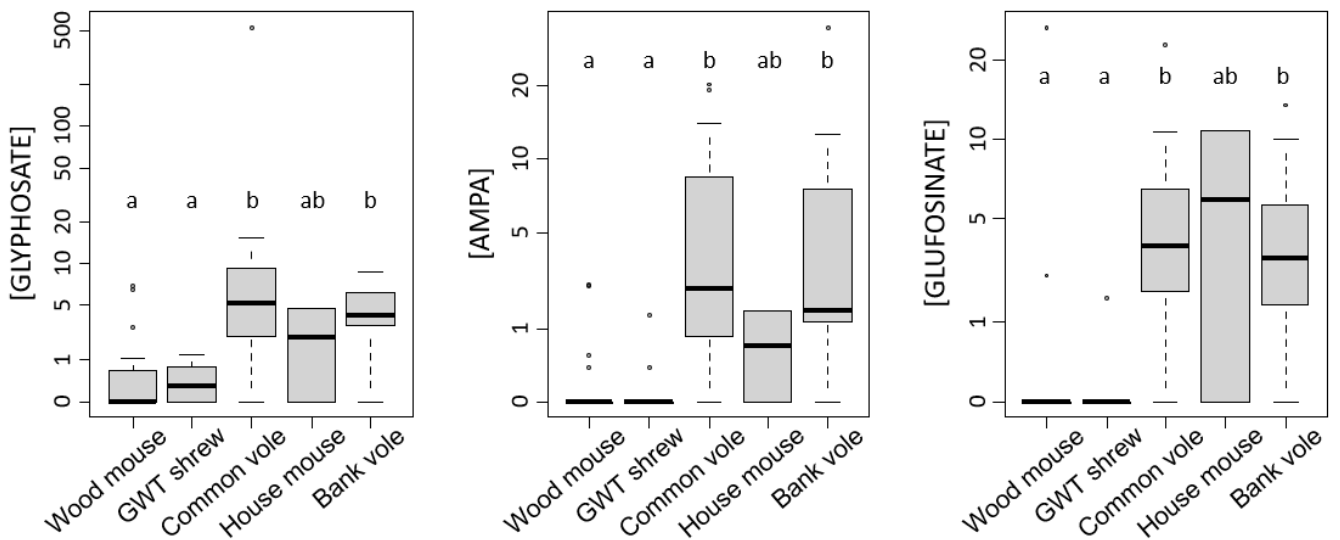
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558 Newton et al. (1984) emphasized that exposure to and accumulation of GLY in mammals varied
559 according to diet preference, since omnivores (deermice) had the highest concentrations of GLY in the
560 viscera during the 2 weeks posttreatment, suggesting a higher initial intake of GLY in omnivores, but
561 such visceral levels decreased the fastest in comparison to carnivores (shrews and weasel) and
562 herbivores (squirrel, vole, chipmunk). The detailed data revealed greater body concentrations in
563 shrews and deermice (0.35 to 0.41 mg/kg) than in squirrels and voles (0.13 to 0.25 mg/kg) within the
564 first days posttreatment. Our results similarly revealed differences in the accumulation or uptake of
565 GLY and AMPA between trophic groups or species, but various trends were identified according to
566 trophic preferences. Overall, we found lower detection frequencies and concentrations in insectivores,
567 whereas the highest detection frequencies and concentrations were observed in herbivorous and
568 omnivorous voles. Both the detection probability and the concentrations were greater for the two
569 species of voles than for the omnivorous wood mice and house mice in our study. The differences in
570 the matrices analysed and in the timing of exposure after application may explain the discrepancies
571 between the findings of Newton et al. (1984) and ours regarding the influence of diet on accumulation.
572 Moreover, differences in the identities of the species (i.e. European vs. American species) and
573 ecosystems (i.e. agrosystem vs. forest) may explain the discrepancies. The physiological and metabolic
574 traits affecting uptake and accumulation in body tissues/fluids and the ecological traits affecting
575 exposure may shape the differences found here between species or trophic groups, but their role is
576 difficult to disentangle. The species exhibiting the lowest detection frequency differ in terms of
577 phylogeny, since both *Apodemus* and *Mus* mice of the subfamily Murinae as well as Crocidurinae
578 *Crocidura* shrews were represented (Sup Info Annex 1. Fig. A4). They also differ in their ecological traits
579 since they are omnivorous and insectivorous. The most herbivorous and, to a lesser extent, the least
580 mobile species, which are the two species of voles studied here, presented the highest detection
581 frequencies and greatest concentrations. *Microtus* and *Myodes* voles are classified into two different
582 genera, but both belong to the same subfamily, Arvicolinae (Sup Info Annex 1. Fig. A2). The correlation
583 between the levels of GLUF and AMPA varied under species-specific patterns, as detailed above. The

584 wood mouse and the house mouse, which are both omnivorous Murinae species, presented a slope
585 different from that of the herbivorous common vole, the omnivorous bank vole and the insectivorous
586 greater white-toothed shrew which presented a steeper slope. The bank vole is usually classified as
587 showing intermediate features of traits between the wood mouse and the common vole in terms of
588 mobility and homerange size, habitat selectivity, and diet breadth (van den Brink et al. 2011).
589 According to ecotoxicological studies where the dietary route is the predominant exposure pathway,
590 its responses are expected to be intermediate or closer to the trends exhibited by the wood mouse
591 than by the common vole (Baudrot et al. 2018; van den Brink et al. 2011). Although the importance of
592 toxicokinetics on accumulation in hair cannot be ignored (Faÿs et al. 2023), our results may again
593 suggest the involvement of different origins of the compounds and of a different contribution of the
594 various exposure routes in shaping interspecies accumulation dissimilarities. The differences found
595 here between species and between animals captured in different habitats within the landscape
596 highlights the need to include both ecological and physiological features in the wildlife risk assessment
597 process and postregistration surveys of the unintentional effects of PPP (Morrissey et al. 2023).
598 Importantly, these results highlight the need for additional research to gain knowledge about the traits
599 that shape the exposure and uptake of PPP in wildlife and about all the interacting mechanisms
600 involved.

601 *3.4. Influence of habitat, farming practices, and proxies of treatment intensity on the* 602 *detection frequency of the compounds*

603 The frequency of detection did not differ according to habitat or farming practices (Sup Info Annex 1.
604 Table A2). Accordingly, the study of Pelosi et al. (2022), which was done within the same research
605 project as the present study (same location of sampled plots and sampling in 2018) did not show
606 differences in GLY detection frequency in soils sampled from cereal fields or hedgerows. However, in
607 earthworms, GLY and AMPA were detected more frequently in cereal fields than in hedgerows.

608 The frequency of detection of GLY, AMPA and GLUF in small mammals was not significantly dependent
609 on the proxies of treatment intensity at the plot, landscape and township scales, except in the case of

610 GLUF sales (Sup Info Annex 1. Table A2, Fig. A3, Fig. A4). However, such an effect of a.s. sales on GLUF
611 detection probabilities was negative, i.e. the detection probabilities were lower in townships where
612 GLUF sales were the highest, and the differences between categories were not significant (Sup Info
613 Annex 1. Table A2, Fig. A4). To investigate this paradoxical result further and avoid any interpretation
614 bias, a model with GLUF sales as the only explanatory factor (i.e. without the factor “species” first in
615 the model formula) was run, revealing an absence of significant differences (Sup Info Annex 1. Table
616 A2, Fig. A4). Hedgerows, woodlots and fields cultivated under OF are not supposed to be targeted by
617 pesticide treatments, but they may be unintentionally contaminated due to drift or run-off (Gandhi et
618 al. 2021). Furthermore, the home range of small mammals can overlap wooded patches, nontarget
619 crops and surrounding cropped habitats. In agricultural topsoil samples from conventional and organic
620 farms across Europe screened for chemicals, GLY and AMPA were among the compounds with the
621 highest frequency of detection and the greatest concentrations (Geissen et al. 2021). Moreover, AMPA
622 was the residue most often detected in both conventional and organic fields, with 96% occurrence in
623 conventional systems and 83% in organic systems (Geissen et al. 2021). AMPA and GLY were also the
624 most commonly detected pesticides in soils under various land uses sampled over mainland France, in
625 which 111 pesticide residues were screened (Froger et al. 2023). The detection frequency was 83% for
626 AMPA and 70% for GLY, and the two compounds were found both in treated cultivated fields and
627 nontargeted habitats, as they occurred both in conventional and organic farming plots, and in different
628 types of land uses: arable lands, orchards, forests, grasslands, and brownfields (Froger et al. 2023).
629 Such a global occurrence of these two compounds, including in organic fields and grasslands or forests,
630 echoes the pervasive exposure found here in small mammals. Martinez-Haro et al. (2022) reported
631 results contrasted with the present findings about the influence of farming practices on exposure to
632 GLY: the prevalence was 9–22% in the gastric content of hunted Iberian hares and 45% in hares found
633 dead in pesticide-treated areas, whereas no residues were detected in hares from organic crops
634 (pesticide-free areas). As stated above, this pattern may be due to the differences in the matrices
635 analysed which reflect different temporal integration of exposure.

636 Finally, the intensity and extent of the GLY treatments may have been greater in our study region, and
637 the OF and CF plots may have been geographically closer in our case. In addition to the study of GLY
638 levels in environmental matrices such as soil or biota, more research is needed to characterize the
639 patterns of exposure of wildlife to GLY, AMPA and GLUF and quantify the underlying processes and
640 spatiotemporal dynamics.

641 *3.5. Influence of habitat, farming practices, and proxies of treatment intensity on the* 642 *concentrations of the compounds*

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

649 This may be a confirmation of the different contributions of exposure pathways for GLY in comparison
650 to AMPA and GLUF. This pattern may indeed be related to a contribution of overspray in addition to
651 dietary exposure because of direct spraying and/or elevated GLY deposits on hedgerow vegetation (1)
652 during spraying due to drift and/or (2) due to (wet) deposition (Gandhi et al. 2021). GLY is known to
653 have a low volatility but is found in air and rainfall in intensively treated areas, at relatively high
654 frequencies and concentrations, and is subjected to wet deposition (Chang et al. 2011). Compared with
655 those in open habitats, contaminant deposits, including PPP, on vegetation in hedgerows are known
656 to be enhanced by larger surfaces of interception and by “edge effect” (Davis et al. 1994; Fowler et al.
657 2004; Lazzaro et al. 2008; Ould-Dada et al. 2002). Mayer et al. (2020) reported for European hares
658 (*Lepus europaeus*) that pesticide uptake by foraging could be 7-fold lower than uptake via
659 overspray/oral grooming. Moreover, GLY has been found to persist in vegetation for up to one year
660 following treatments in perennial forested areas, representing a potent dietary source of exposure for
661 wildlife (Edge et al. 2021). In a previous study addressing the exposure of small mammals to banned

662 and currently used PPP by residue screening in hair, which was based on animals sampled from the
663 same study area and another region in France, we reported greater contamination (in terms of the
664 number of compounds and concentrations) in individuals captured in hedgerows than in those
665 captured in grasslands. However, the contamination did not differ between animals captured in
666 hedgerows and those sampled from cereal crops (Fritsch et al. 2022). In Pelosi et al. (2022), GLY and
667 AMPA concentrations in both soils and earthworms were, however, higher in samples from cereal
668 fields than in those from grasslands and hedgerows. This may be an argument in favour of the
669 contribution of processes related to overspraying in determining small mammal exposure to GLY. The
670 role of external exposure to herbicides in small mammals and the importance of oral exposure by
671 grooming remain to be investigated. The contribution of nondietary routes to wildlife exposure is still
672 an overlooked field of research that needs to be addressed.

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

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682 **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**
683 **account statistically in modelling.**

684 *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*
685 *GWT shrew stands for the greater white-toothed shrew. The sample sizes are provided in Table 1 and Table A1.*

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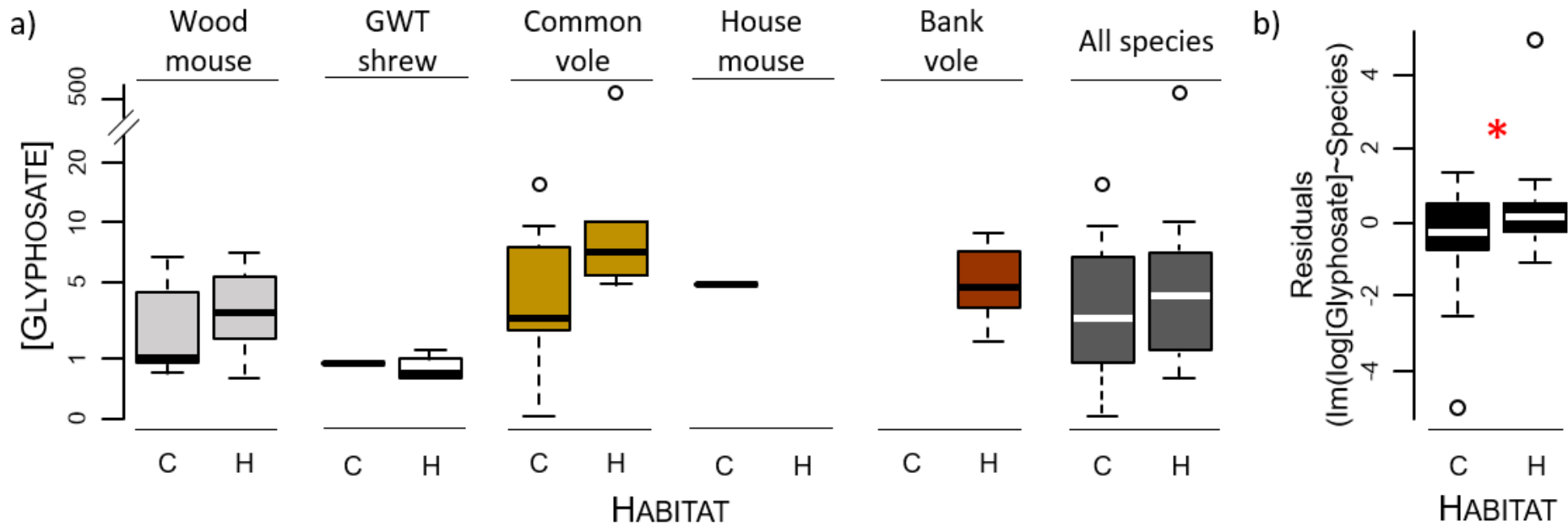
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697 **Figure 6. Concentrations of glyphosate, AMPA, and glufosinate in hair of small mammals with nondetects set to 0,**
 698 **according to habitat where captured (a), farming practices where captured (b), and proxies of treatment intensity at**
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

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

703

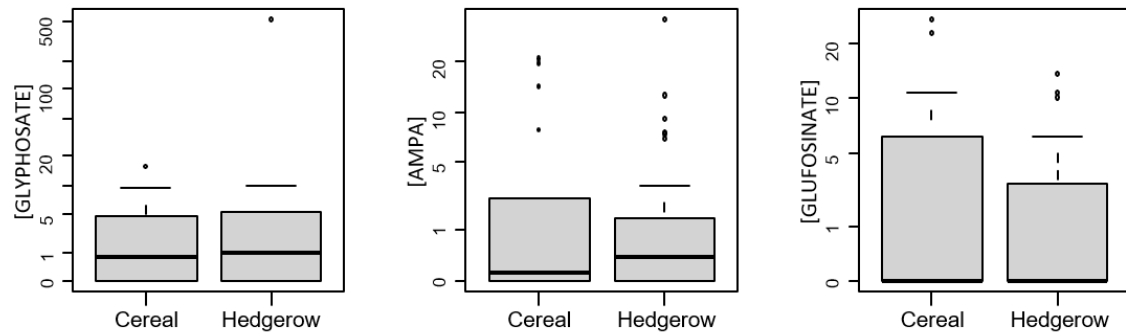
a) HABITAT

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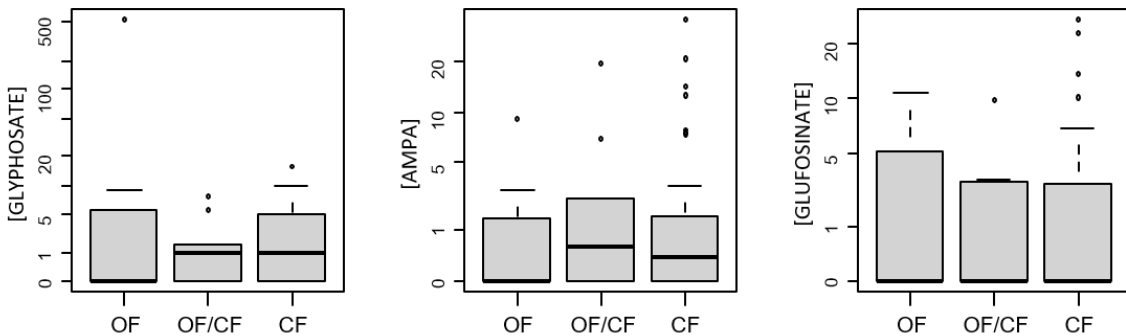
b) FARMING PRACTICES (ORGANIC FARMING 'OF', CONVENTIONAL FARMING 'CF')

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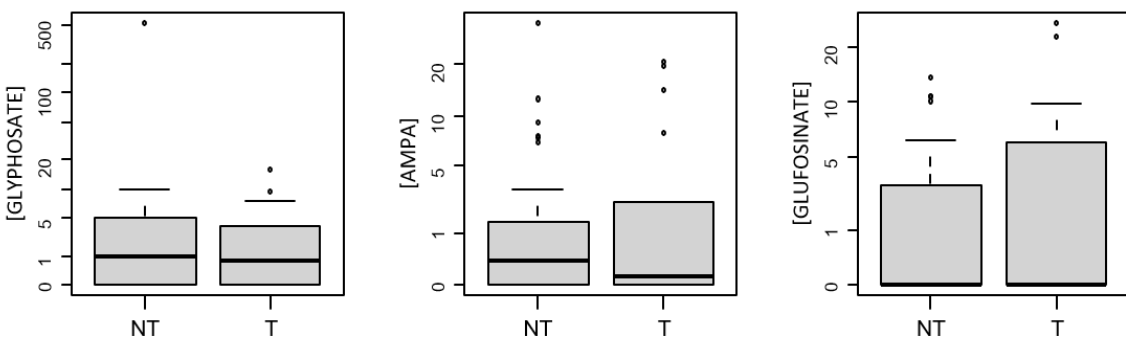
c) TREATMENT BY PESTICIDES (NOT TARGETED 'NT', TARGET 'T')

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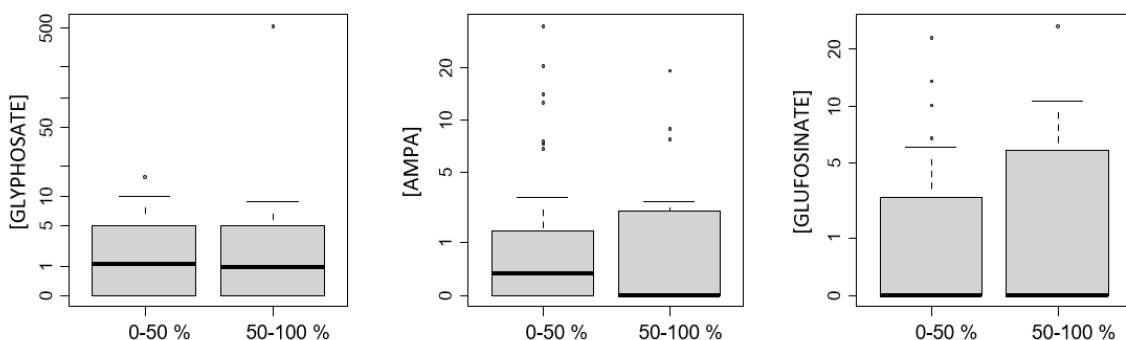
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d) PROPORTION OF ORGANIC FARMING IN LANDSCAPE

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

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

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

751 *3.6. Dose reconstruction for risk assessment*

752 To evaluate the risk of unintentional impacts in small mammals by comparing exposure dose values
753 estimated from back-calculation using the concentrations measured in hair to toxicological data, we
754 found that toxic thresholds could be exceeded for both GLY and GLUF. In the case of GLY, at least one
755 of the five considered toxicological thresholds was exceeded according to the trigger of concern for at
756 least one species in all of the approaches (Table 2). The potential for toxicological impairment was thus
757 observed in all of the approaches developed. This finding suggests a risk for small mammals, especially
758 rodents, and particularly for herbivorous voles (Table 2). Often, only the maximum value measured in
759 *Microtus* voles reached toxic levels (10 times out of 16 scenarios). In five of the 16 scenarios computed
760 via the different approaches, all of the species were found to be at risk, with maximum but also median
761 and minimum concentrations measured being involved. At the population level, in the “best-case”
762 scenario, toxicological effects related to GLY exposure impaired at least 6% of individuals in the
763 common voles studied here, which represents a risk of effects in 2.1% of the rodents and in 1.6% of
764 the small mammal community studied, including insectivorous species (Table 2). In the worst-case
765 scenario, toxicological impairment occurred in 87.5% of the common voles, 88.9% of the voles (bank
766 voles and common voles), 66% of all of the rodent species, 50% of the shrews and 62.3% of the
767 individuals within the small mammal community (Table 2). Up to 93.8% of the common voles and
768 92.6% of the individuals of all vole species may be impacted if individuals close to the trigger value of
769 5 are included in the calculation (Table 2).

770 From GLY concentrations in gastric content, Martinez-Haro et al. (2022) calculated a daily dose for
771 Iberian hares of 0.2-32.8 mg of GLY/kg body weight/day, with a median estimated at 0.36 mg GLY/kg
772 body weight/day. We found here estimated daily doses varying from 0.00002 to 1231 mg GLY/kg body

773 weight/day (Table 2). The median for all approaches was 0.373 mg GLY/kg body weight/day (1st quartile
774 = 0.037 mg GLY/kg body weight/day, 3rd quartile = 1.84 mg GLY/kg body weight/day). One may notice
775 the congruence between these estimated median values of exposure dose in Iberian hares and in small
776 mammals here.

777 For GLUF, estimated daily doses varied from 0.080 to 6.38 mg GLUF/kg body weight/day (Table 3). The
778 median for all approaches was 0.811 mg GLUF/kg body weight/day (1st quartile = 0.314 mg GLUF/kg
779 body weight/day, 3rd quartile = 1.57 mg GLUF/kg body weight/day). The four toxicological thresholds
780 considered were exceeded in the two scenarios for all rodent species, including the maximum
781 concentrations measured in our samples but also the third quartile, median and even minimum values
782 (Table 3). This would translate, on the basis of our captured individuals, into a possible impairment
783 related to GLUF exposure in 31.3 to 75% of the common voles, 25.9 to 70.4% of the vole individuals,
784 19.1 to 46.8% of individuals within the rodent population and 14.8 to 36.1% of individuals within the
785 small mammal community (Table 3). This result suggests that many of the free-ranging nontargeted
786 small mammals could be at risk for toxicological effects related to exposure to GLUF, especially
787 herbivorous and granivorous or omnivorous species, at the time of sampling (i.e. 2018), although the
788 use of this compound in France has largely decreased since 2015.

789 Thus, our findings are consistent with the first conclusions of the risk assessments performed during
790 the registration processes, which identified potential threats for both GLY and GLUF to herbivorous,
791 granivorous and insectivorous terrestrial vertebrates, especially herbivorous voles (EFSA 2005, 2012,
792 2015a). Note that the regulatory assessments were based on dietary exposure, but wildlife may be
793 exposed to PPP - and this is especially the case for GLY when used through spraying - via additional
794 routes such as oral uptake by grooming, inhalation, dermal contact, and/or ocular contact, as
795 mentioned for human populations (Gandhi et al. 2021; Mayer et al. 2020; Mineau 2011).

796 **Table 2. Summary of risk assessment for glyphosate based on reverse dosimetry approaches.**

797 *Details are provided in Sup Info Annex 1 and Annex 2. Abbreviations: a.s. active substance; Min minimum; Max maximum; Med median; Nb number; Ind individuals; L-T NOAEL*
 798 *long-term no observed adverse effect level; S-T NOEL short-term dietary no observed effect level; Ac NOAEL acute NOAEL; Ch NOAEL chronic NOAEL; CLD chronic low dose threshold;*
 799 *Apsy Apodemus sylvaticus (wood mouse); Crru Crocidura russula (greater white-toothed shrew); Miar Microtus arvalis (common vole); Mumu Mus musculus (house mouse); Mygl*
 800 *Myodes glareolus (bank vole); Max maximum; Q3 3rd quartile; Med median; Min minimum; PK pharmacokinetics.*

Approach	Estimated dose (mg a.s./kg/day) Min-max [med]	Cases when toxicity/exposure ratio exceed trigger value (of 10 for acute toxicity data or of 5 for chronic 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
(including individuals close to the trigger*)													
Approach 1													
[GLY] in human urine and hair													
Scenario 1.1													
Head hair													
Scenario 1.1.a	0.0008 - 23.9 [0.186]	3	L-T NOAEL, S-T NOEL, CLD	Miar	Max	1	1		2	2	4	6	
Urinary excretion 10%													
Scenario 1.1.b	0.0004 - 11.9 [0.093]	2	L-T NOAEL, CLD	Miar	Max	1	1		2	2	4	6	
Urinary excretion 20%													
Scenario 1.1.c	0.0082 - 239 [1.86]	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)
Urinary excretion 1%													
Scenario 1.2													
Pubic hair													
Scenario 1.2.a	0.0042 - 123 [0.956]	12	L-T NOAEL, S-T NOEL, Ac NOAEL, Ch NOAEL, CLD	Miar, Apsy, Mygl	Max, Q3, Med	16 (18)	16 (17)	(1)	26 (30)	34 (36)	48 (48)	50	(7)
Urinary excretion 10%													
Scenario 1.2.b	0.0021 - 62 [0.478]	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Urinary excretion 20%													
Scenario 1.2.c	0.0424 - 1230.84 [9.56]	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
Urinary excretion 1%													
Approach 2													
Body burden in small mammals 7 days after dose													
Scenario 2.1	0.0018 - 52.4 [0.366]	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Proportions hair/body burden													
Scenario 2.2	0.0018 - 52.2 [0.365]	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
[C] _{whole body} = [C] _{hair}													

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

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804 **Table 2. continued**

Approach Scenarios Type of data / Specific data / Assumptions	Estimated dose (mg a.s./kg/day) Min-max [med]	Cases when toxicity/exposure ratio exceed trigger value (of 10 for acute toxicity data or of 5 for chronic 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
(including individuals close to the trigger*)													
Approach 3													
Toxicokinetics in small mammal blood													
Scenario 3.1 Anadon et al. (2009)													
Scenario 3.1.a GLY residues	0.0016 - 45.2 [0.316]	4	L-T NOAEL, S-T NOEL, Ac NOAEL, CLD	Miar	Max	1	1		2	2		6	
Scenario 3.1.b AMPA residues	0.2307 - 32.3 [1.55]	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	<0.0001 - 8.54 [0.013]	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	0.0001 - 3.82 [0.0031]	1	CLD	Miar	Max	1	1		2	2		6	
Scenario 4.2 Average ratio viscera/body residues	<0.0001 - 8.54 [0.0069]	1	CLD	Miar	Max	1 (2)	1 (2)		2 (3)	2 (4)	(7)	6 (13)	
Scenario 4.3 Max ratio viscera/body residues	0.0004 - 11.4 [0.019]	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	0.921 - 523 [4.55]	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	62 (64)	66 (68)	89 (93)	88 (94)	50
Scenario 5.2 AMPA residues	0.824 - 34.2 [1.95]	20	L-T NOAEL, S-T NOEL, CLD	Miar, Apsy, Mumu, Mygl, Crru	Max, Q3, Med, Min	30 (31)	28 (29)	2	49 (51)	60 (62)	85 (89)	81 (88)	14

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

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808 **Table 3. Summary of risk assessment for glufosinate based on reverse dosimetry approaches.**

809 *Details are provided in Sup Info Annex 1 and Annex 2. Abbreviations: a.s. active substance; Min minimum; Max maximum; Med median; Nb number; Ind individuals; L-T NOAEL*
 810 *long-term no observed adverse effect level; S-T NOEL short-term dietary no observed effect level; Ac NOAEL acute NOAEL; Ch NOAEL chronic NOAEL; CLD chronic low dose threshold;*
 811 *Apsy Apodemus sylvaticus (wood mouse); Crru Crocidura russula (greater white-toothed shrew); Miar Microtus arvalis (common vole); Mumu Mus musculus (house mouse); Mygl*
 812 *Myodes glareolus (bank vole); Max maximum; Q3 3rd quartile; Med median; Min minimum; PK pharmacokinetics.*

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Approach Scenarios Type of data / Specific data /Assumptions	Estimated dose (mg a.s./kg/day) Min-max [med]	Cases when toxicity/exposure ratio exceed trigger value (of 10 for acute toxicity data or of 5 for chronic 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
(including individuals close to the trigger*)													
Approach 1													
Toxicokinetics in small mammal blood													
Scenario 1.1 Values in males	0.290 - 6.38 [1.33]	18	L-T NOAEL, S-T NOEL, Acute LD50, Chronic NOAEL	Miar, Apsy, Mumu, Mygl	Max, Q3, Med, Min	22 (23)	22	(1)	36 (38)	47	70	75	(7)
Scenario 1.2 Values in females	0.080 - 1.76 [0.368]	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	

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

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820 Importantly, in the study on small mammals carried out in the same area and mentioned above (Fritsch
821 et al. 2022), 112 different residues of banned and currently used PPP (parent chemicals and
822 metabolites) were detected in hair samples from wood mice and greater white-toothed shrews, with
823 32 to 65 residues detected per individual. This means that “cocktail effects” are likely to occur, which
824 can ultimately increase the risk of GLY- or GLUF-induced toxicity assessed here owing to mechanisms
825 of additivity and synergy (Martin et al. 2021). In their review, Martin et al. (2021) reported that
826 additivity was the interaction that most often occurred in chemical mixtures, followed by synergistic
827 interactions. For example, Brodeur et al. (2014) reported synergic toxic impacts in the common South
828 American toad (*Rhinella arenarum*) of binary mixtures of GLY-and cypermethrin-based commercial PPP
829 products; two compounds that have been detected in more than half of the small mammal individuals
830 in our studies. They reported that the degree of synergy ranged from 2 to 9 times depending on the
831 mixture tested (Brodeur et al. 2014).

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

846 wild taxa may be highly sensitive to GLY, AMPA or GLUF, more than expected from laboratory studies
847 on models such as rats and mice, and commercial formulations can be more toxic than a.s. alone
848 (Fritsch et al., 2024). For example, the mortality rate of postmetamorphosis juveniles of the species
849 *Rana sylvatica*, *Bufo woodhousii fowleri*, and *Hyla versicolor* reached 68-86% when they were exposed
850 via spraying to a GLY-based commercial herbicide at the recommended dose (Relyea 2005). The
851 exposure of spined toads (*Bufo spinosus*) to AMPA at environmental concentrations affects hatchling
852 and tadpole morphology and decreases embryonic and tadpole survival (Cheron and Brischoux, 2020,
853 2023).

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

867 Our findings warrant further research to confirm their interpretation and gather knowledge about the
868 ecological impacts of the use of GLY and GLUF as PPP. There is a need to characterize the toxicokinetics
869 and toxicodynamics of these compounds in wild species better and to provide data to relate
870 concentrations in hair with exposure dose, accumulation in other tissues, and toxicological responses.
871 Furthermore, more research is needed to assess whether the potential risks computed here actually

872 translate into deleterious effects in free-ranging populations, which would require field surveys and
873 monitoring of wildlife exposure and health. The dynamics of GLY, AMPA and GLUF transfer and their
874 impacts on food webs crucially need to be investigated. Here, we provide lines of evidence to support
875 the identification of the organophosphate chemicals GLY and GLUF as ‘emerging organic
876 contaminants’, as emphasized by Kissane and Shephard (2017). Given the pervasive exposure of
877 wildlife to GLY, AMPA and GLUF and suggesting chronic exposure at sublethal levels on the basis of
878 nondestructive sampling in biosentinel species, our study highlights the need for research to fill gaps
879 in knowledge about early warning signals of impacts as highlighted by Kissane and Shephard (2017).

880 **4. Conclusion**

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

891 Our study relied on measurements of residues in hair samples, a method that is developing in human
892 epidemiology, and looks a promising nondestructive approach to study the field ecotoxicology of PPP
893 and monitor wildlife exposure or detect early signals in wild species. Although this approach should be
894 strengthened by further assessment of exposure doses and measurements of effects in animals, such
895 surveys may represent valuable opportunities for the assessment of regulatory measures and
896 postregistration monitoring and provide support for phytopharmacovigilance and toxicovigilance

897 schemes (Vijver et al., 2017). For example, the presence of pesticides in the hair of small mammals
898 reported in Fritsch et al. (2022) was reported as an exposure warning for nontarget species by the
899 French department of the agency ANSES in charge of phytopharmacovigilance.

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

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910 preparing or contributing to the field and laboratory work. We especially acknowledge the help of
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918 **Author’s contribution**

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

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

923 Bertrand Colette: Conceptualization, Project administration, Investigation, Data curation, Writing -
924 review & editing

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934 review & editing

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937 **Data accessibility**

938 The raw dataset can be accessed at DOI: [10.25666/DATAUBFC-2024-08-29](https://doi.org/10.25666/DATAUBFC-2024-08-29)

939 **Supplementary information**

940 List of information in the appendix document:

941 *Annex 1.*

942 Text A1: Molecular identification of small mammal species

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944 glufosinate

945 Text A3: Approaches of reverse dosimetry to assess the risk for deleterious effects in small
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963

964 *Annex 2.*

965 Computation of reverse dosimetry approaches (calculation spreadsheets).

966

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1158 **Appendix – Annex 1**

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1180 the concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals. 68

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1183 *Text A1: Molecular identification of small mammal species*

1184 Total genomic DNA was extracted from 10-25 mg of ear tissue using the DNeasy Blood and
1185 Tissue kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations (pp 28-30,
1186 version 07/2006). Each sample was processed independently in an automated manner using the
1187 QIAcube robot (Qiagen). The DNA concentration was then measured using a NanoVue Plus
1188 spectrophotometer (Biochrom). The DNA extracts were stored at -20 °C until DNA amplification.

1189 All of the DNA extracts were then amplified into an approximately 900 bp-long fragment
1190 (excluding primers) of the *cytochrome b* gene. We used the primers CytB Uni fw 5' –
1191 TCATCMTGATGAAAYTTYGG – 3' and CytB Uni rev 5' – ACTGGYTGDCCBCCRATTCA – 3' published in
1192 Schlegel et al. (2011). Amplifications were performed in 50 µL reactions containing 1× HotStart Taq
1193 Master Mix (Qiagen), 0.32 µM of each primer, 0.5 ng/µL of bovine serum albumin, 0.75 mM of MgCl₂,
1194 and 5 µL of extracted DNA (10-70 ng/µL). The PCR program consisted of an activation step of 15 mn at
1195 95 °C, followed by 40 cycles of denaturation at 94 °C during 30 s, annealing at 53 °C during 45 s, and
1196 extension at 72 °C during 1 min. A final extension was performed at 72 °C for 10 min. Amplification
1197 reactions were performed using an Eppendorf Mastercycler DNA Engine.

1198 The PCR products were separated and visualized using the QIAxcel device and a QIAxcel DNA
1199 Screening kit (Qiagen). The amplified products were purified using the Illustra GFX PCR DNA and Gel
1200 Band Purification Kit (GE Healthcare) according to the manufacturer's instructions. Direct sequencing
1201 of the PCR products was performed with an automated sequencer (Applied Biosystems Seqstudio
1202 Genetic Analyzer). All of the samples were sequenced with the primers employed for the PCR
1203 reactions. The obtained DNA sequences were submitted to GenBank via the BLAST algorithm
1204 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A query sequence was then assigned to a given species when
1205 identity and coverage matched the database sequence with a threshold of at least 99% for only one
1206 species.

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1208 **References**

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1212

1213 *Text A2: Reagents and procedures used for the chemical analyses of glyphosate, AMPA and*
1214 *glufosinate*

1215 **Chemicals and Reagents**

1216 Methanol Pesti-S grade, acetonitrile, ammonium acetate and water ULC-MS grade were purchased
1217 from Biosolve (Dieuze, France). Sodium dodecyl sulfate, Fmoc chloride, sodium tetraborate
1218 decahydrate and glyphosate, glufosinate ammonium, aminomethylphosphonic acid and glyphosate-
1219 1,2-¹³C₂,¹⁵N standards were purchased from Sigma Aldrich (Diegem, Belgium).

1220 **Sample preparation**

1221 To differentiate compounds biologically incorporated into hair from chemicals externally deposited on
1222 the hair surface due to contaminated air or dust, the samples were decontaminated with sodium
1223 dodecyl sulfate (SDS) solution and methanol (Duca et al. 2014). This procedure was developed to be
1224 as “universal” as possible, and was tested on a large panel of chemicals with different physicochemical
1225 properties including rather hydrophilic chemicals. The two steps (SDS and methanol) were found to be
1226 the best compromise to remove both lipophilic and hydrophilic chemicals from hair surface.
1227 Importantly, SDS solution being an aqueous solution (5% SDS / 95% water), it is therefore well adapted
1228 to remove hydrophilic compounds such as GLU, GLY and AMPA.

1229 Note that in other studies where hair samples were washed prior to analyses of GLY, Hooper et al.
1230 (2022) decontaminated hair of bats using ultrapure (MilliQ) water and subsequently isopropanol, and
1231 Alvarez et al. (2022) used dichloromethane twice to wash human hair samples.

1232 **Analysis of glyphosate, glufosinate and AMPA in hair**

1233 After decontamination, the samples (50 mg) were spiked with 5 µL of internal standard solution at 1
1234 mg/L, and 1 mL of water was added before overnight extraction at 40 °C with 350 rpm agitation. The
1235 supernatants were evaporated to dryness at 50 °C under N₂ flow, and 100 µL of borate buffer and
1236 Fmoc-Cl were added before derivatization and injection into the LC-MS/MS Waters system Acquity
1237 UPLC H-class system equipped with a Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 µm particle

1238 size) and with a binary mobile phase consisting of ammonium acetate 10mM in water (A) and
1239 acetonitrile (B). The flow rate was initially set at 0.4mL/min with 98% A and 2% B and reached 2% A
1240 and 98 B after 6 min. From 6.0 to 9min the column was re-equilibrated to 98% A and 2% B. The UPLC
1241 system was coupled to Waters Xevo TQ-S triple quadrupole mass spectrometer. With each analytical
1242 run, quality controls consisting of hair samples supplemented at several concentrations (0.1, 0.2, 0.5,
1243 1, 2, 5, 10, 20, 50, and 100 pg/mg) were used to control for possible sensitivity drift, and a blank sample
1244 (sample without the target chemicals) was used to verify possible cross-contamination. Since field
1245 samples were not available in sufficient amount to conduct method validation, it has to be conducted
1246 on blank samples from laboratory animals supplemented with standards. The slopes of the calibration
1247 curves were 13.56, 6.31 and 10.68 for GLU, GLY and AMPA respectively. The corresponding R² values
1248 were all > 0.998.

1249 The concentrations are expressed as pg/mg hair. The limits of quantification (LOQ) and lowest detected
1250 values (Minimum) are presented in Table 1. The LOQ was determined as the lowest concentration level
1251 presenting variability and accuracy < 25%. The estimation of the limit of detection (LOD, the lowest
1252 concentration of a substance that can be reliably detected by the analytical method) can be done
1253 through various experimental and computational approaches, and choices must be made according to
1254 the type and the objective of the analytical method (Evard et al. 2016b, 2016a). We did not apply
1255 specific experiments and calculations to compute the LOD in this study owing to several reasons: (1)
1256 the purpose of the study was not focused on an analytical method development and validation; (2)
1257 high performance and resolution of current instrumentation and modes hamper the use of methods
1258 based on signal-to-noise to estimate the LOD; (3) the analyses were not run within a framework
1259 requiring a specific guideline to be followed; and (4) no critical decision has to be based on the LOD.
1260 As described above, the analytics were based on up-to-date protocols and materials and followed the
1261 general principles of metrology (such as inclusion of blank samples and over 5 calibration or quality
1262 control levels, among others). The accuracy and sensitivity of the method were not fully computed but
1263 the inclusion of blanks and pretty low concentrations in standards (i.e. 0.1 and 0.2 pg/mg) limits the

1264 potential for bias towards false positive. All values above the lowest analytical detected value were
1265 included as “detects” contrary to “nondetects”. Importantly, only one concentration was reported
1266 below the lowest concentration in quality controls (the minimum GLY concentration, see Table 1). All
1267 reported concentration values, involving values between the LOQ and the lowest detected value, were
1268 included as continuous data. The use of concentrations below the LOQ has been shown to be a suitable
1269 method preferable to the absence of data, which shows better efficiency in terms of bias and precision
1270 than several other established methods (Keizer et al. 2015).

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1287

1288 *Text A3: Approaches of reverse dosimetry to assess the risk for deleterious effects in small*
1289 *mammals*

1290 In this part of the work, reverse dosimetry approaches have been applied to estimate the daily dose
1291 intake of small mammals from the concentrations of glyphosate (GLY), AMPA or glufosinate (GLUF)
1292 measured in hair, and several types of data that may be useful for applying dose reconstruction
1293 processes have been used. Concentrations in mammal body fluids and hair, residues in viscera and
1294 body burden in small mammals, and toxicokinetics in small mammal blood have been collected from
1295 the literature. Several approaches of back-calculations were thus applied depending on the type of
1296 input data and the different approaches were computed under several assumptions in cases with a
1297 lack of available data.

1298 For GLY, four different approaches have been applied on the basis of data about (1) concentrations in
1299 human urine and hair (Alvarez et al. 2022), (2) body burden in rats 7 days after dose administration
1300 (Brewster, 1991), (3) toxicokinetics in rat blood (Anadón et al. 2009; Kim et al. 2023), (4) residues of
1301 GLY in the viscera and body of wild small mammals in a field study (Newton et al. 1984), and (5)
1302 equations for fast-elimination chemicals that relate concentrations in hair in rats and the level of
1303 exposure, taking pharmacokinetics into account (Faÿs et al. 2023). For GLUF, one approach could be
1304 proposed on the basis of toxicokinetics in rat blood (Joint FAO/WHO Meeting on Pesticide Residues
1305 (JMPR) et al. 2012). The body weights of small mammals were obtained from actual morphometrics
1306 measured in our experiment by weighing individuals in which residues of GLY, AMPA and GLUF were
1307 measured (Sup Info Annex 2). Data concerning urinary excretion percentage of GLY were obtained in
1308 documents released by the EFSA and recent reviews (Connolly et al. 2020; EFSA 2015a; Peillex and
1309 Pelletier 2020). The concentrations of GLY in the viscera and body reported in Newton et al. (1984) for
1310 shrews (carnivores), deermice (omnivores) and voles (herbivores) were used separately according to
1311 diet preference to compute reverse dosimetry in shrews (carnivores), wood mice, house mice and bank
1312 voles (omnivores), and common voles (herbivores), respectively. The food intake of the different

1313 species was computed using the mean food intake of the different items provided by Crocker et al.
1314 (2002). The mean food intake of arthropods and cereal seeds provided for the wood mouse has been
1315 used for the wood mouse and the house mouse; the mean food intake of arthropods and earthworms
1316 provided for the common shrew has been used for the greater white-toothed shrew; and the mean
1317 food intake of grasses and forbs provided for the field vole has been used for the common vole (Crocker
1318 et al. 2002). The assumptions that had to be made to allow the calculations were that the distribution
1319 was equivalent between body parts and hair, that the concentration in hair was equivalent to the
1320 concentration in plasma/blood and proportional to the administered dose, and that the concentration
1321 in viscera reflected the concentration in the diet. These assumptions, if not verified, might, however,
1322 be considered acceptable according to current knowledge about GLY pharmacokinetics. It is known
1323 that *“absorbed GLY is poorly metabolised, widely distributed in the body, does not undergo*
1324 *enterohepatic circulation and is rapidly eliminated”* (EFSA 2015a). Like GLY, GLUF is distributed
1325 ubiquitously in the body rapidly after administration and is mostly excreted in faeces and urine (Joint
1326 FAO/WHO Meeting on Pesticide Residues (JMPPR) et al. 2012). Chemicals are mostly incorporated into
1327 living hair bulb cells from the bloodstream, and residue concentrations in hair are representative of
1328 the internal dose during the time of hair sample growth (Appenzeller et al. 2017; Faÿs et al. 2023). In
1329 a field study where GLY was aerially applied at 3.3 kg a.s./ha in a forest, the concentrations measured
1330 in the remainder of small mammal bodies (without viscera) were of the same order of magnitude as
1331 the maximum concentrations measured here in hair; owing that in the EU the maximum field
1332 application of GLY in cropped fields is comparable (4.32 g a.s./ha) (Newton et al. 1984). Newton et al.
1333 (1984) considered concentrations in viscera as reflecting intake and reported that *“GLY in the viscera*
1334 *of herbivores was roughly equal to or somewhat below concentrations found in litter and ground*
1335 *cover”*.

1336 The dose of GLY was compared with five toxicological thresholds. Three of these thresholds were
1337 regulatory thresholds: the short-term dietary NOEL (no observed effect level, 150 mg active
1338 substance/kg body weight), the chronic 21 day NOAEL, which has the same value as the reproductive

1339 NOAEL (no observed adverse effect level, 351 mg a.s./kg bw/d), and the long-term NOAEL (maternal
1340 and developmental NOAEL, which is the same than long-term NOAEL for terrestrial vertebrates, 50 mg
1341 a.s./kg bw/d) (EFSA, 2015a; PPDB, <http://sitem.herts.ac.uk/>). The No Mortality Dose to the Oregon
1342 vole (NOAEL Acute toxicity (Intraperitoneal), 450 mg a.s./kg bw) was also used to consider a wild
1343 species (McComb et al. 2008). Some more recent publications highlighted that the harmful effects of
1344 GLY were detected below regulatory limits (e.g. within the spectrum of acceptable daily intake) (Cai et
1345 al. 2017; Clair et al. 2012; Mesnage et al. 2015). We therefore added a “chronic low-dose” limit, with
1346 a value of 5 mg a.s./kg bw/d obtained from studies showing effects on reproductive development,
1347 endocrine disruption, maternal behaviour, neuroplasticity and the gut microbiota in rats (Dechartres
1348 et al. 2019; Romano et al. 2010).

1349 The dose of GLUF was compared to four available toxicological thresholds: the acute oral LD50 (median
1350 lethal dose; 416 mg/kg bw), the short-term dietary NOEL (64 mg a.s./kg bw), the chronic 21 days NOAEL
1351 (6.3 mg a.s./kg bw/d) and the long-term NOAEL (2 mg a.s./kg bw/d) (Joint FAO/WHO Meeting on
1352 Pesticide Residues (JMPR) et al. 2012; PPDB, <http://sitem.herts.ac.uk/>).

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- 1402
- 1403

1404 *Text A4: Statistical outputs of the Tarone-Ware tests*

```

1405 > twoSampleLinearRankTestCensored(x = TAB$GLY, x.censored = TAB$GLY.censored,
1406 +                               y = TAB$AMPA, y.censored = TAB$AMPA.censored,
1407 +                               test = "tarone-ware")
1408 Results of Hypothesis Test
1409 Based on Censored Data
1410 -----
1411 Null Hypothesis:                Fy(t) = Fx(t)
1412 Alternative Hypothesis:        Fy(t) != Fx(t) for at least one t
1413 Test Name:                     Two-Sample Linear Rank Test:
1414                               Tarone-Ware Test
1415                               with Hypergeometric Variance
1416 Censoring Side:                left
1417 Censoring Level(s):           x = 0.018
1418                               y = 0.24
1419 Data:                          x = TAB$GLY
1420                               y = TAB$AMPA
1421 Censoring Variable:           x = TAB$GLY.censored
1422                               y = TAB$AMPA.censored
1423 Sample Sizes:                 nx = 61
1424                               ny = 61
1425 Percent Censored:             x = 36.1%
1426                               y = 49.2%
1427 Test Statistics:              nu      = 58.055344
1428                               var.nu = 1504.925791
1429                               z        = 1.496527
1430 P-value:                      0.1345163
1431
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1434 > twoSampleLinearRankTestCensored(x = TAB$GLY, x.censored = TAB$GLY.censored,
1435 +                               y = TAB$GLUF, y.censored = TAB$GLUF.censored,
1436 +                               test = "tarone-ware")
1437 Results of Hypothesis Test
1438 Based on Censored Data
1439 -----
1440 Null Hypothesis:                Fy(t) = Fx(t)
1441 Alternative Hypothesis:        Fy(t) != Fx(t) for at least one t
1442 Test Name:                     Two-Sample Linear Rank Test:
1443                               Tarone-Ware Test
1444                               with Hypergeometric Variance
1445 Censoring Side:                left
1446 Censoring Level(s):           x = 0.018
1447                               y = 1.161
1448 Data:                          x = TAB$GLY
1449                               y = TAB$GLUF
1450 Censoring Variable:           x = TAB$GLY.censored
1451                               y = TAB$GLUF.censored
1452 Sample Sizes:                 nx = 61
1453                               ny = 61
1454 Percent Censored:             x = 36.1%
1455                               y = 55.7%
1456 Test Statistics:              nu      = 13.9088437
1457                               var.nu = 1304.2735588
1458                               z        = 0.3851294
1459 P-value:                      0.7001415
1460
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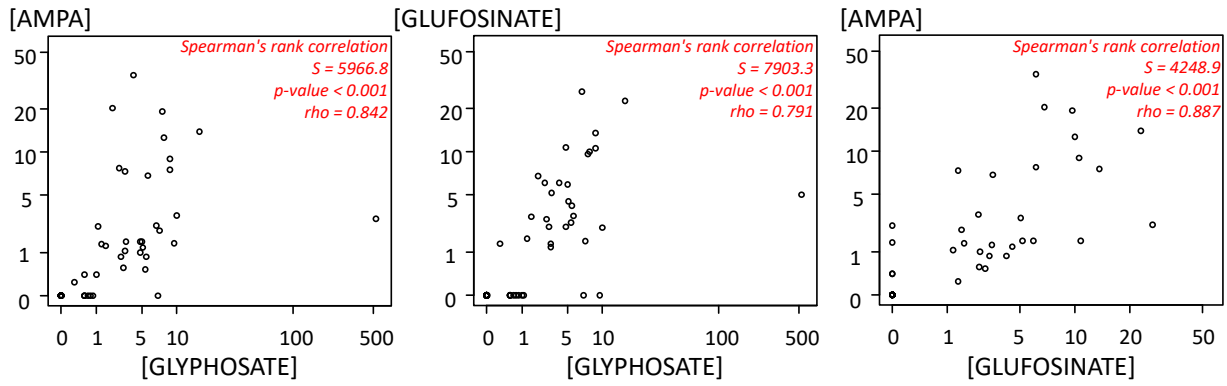
1462 > twoSampleLinearRankTestCensored(x = TAB$AMPA, x.censored =
1463 TAB$AMPA.censored,
1464 + y = TAB$GLUF, y.censored = TAB$GLUF.censored,
1465 + test = "tarone-ware")
1466
1467 Results of Hypothesis Test
1468 Based on Censored Data
1469 -----
1470 Null Hypothesis: Fy(t) = Fx(t)
1471 Alternative Hypothesis: Fy(t) != Fx(t) for at least one t
1472 Test Name: Two-Sample Linear Rank Test:
1473 Tarone-Ware Test
1474 with Hypergeometric Variance
1475 Censoring Side: left
1476 Censoring Level(s): x = 0.24
1477 y = 1.161
1478 Data: x = TAB$AMPA
1479 y = TAB$GLUF
1480 Censoring Variable: x = TAB$AMPA.censored
1481 y = TAB$GLUF.censored
1482 Sample Sizes: nx = 61
1483 ny = 61
1484 Percent Censored: x = 49.2%
1485 y = 55.7%
1486 Test Statistics: nu = -33.2689131
1487 var.nu = 1197.6199535
1488 z = -0.9613446
1489 P-value: 0.3363789
1490
1491

```

1492 **Figure A1. Correlations between the concentrations of glyphosate, AMPA, and glufosinate in small mammal**
1493 **hair.**

1494 *The concentrations are expressed in pg/mg. Nondetects are set to 0.*

1495

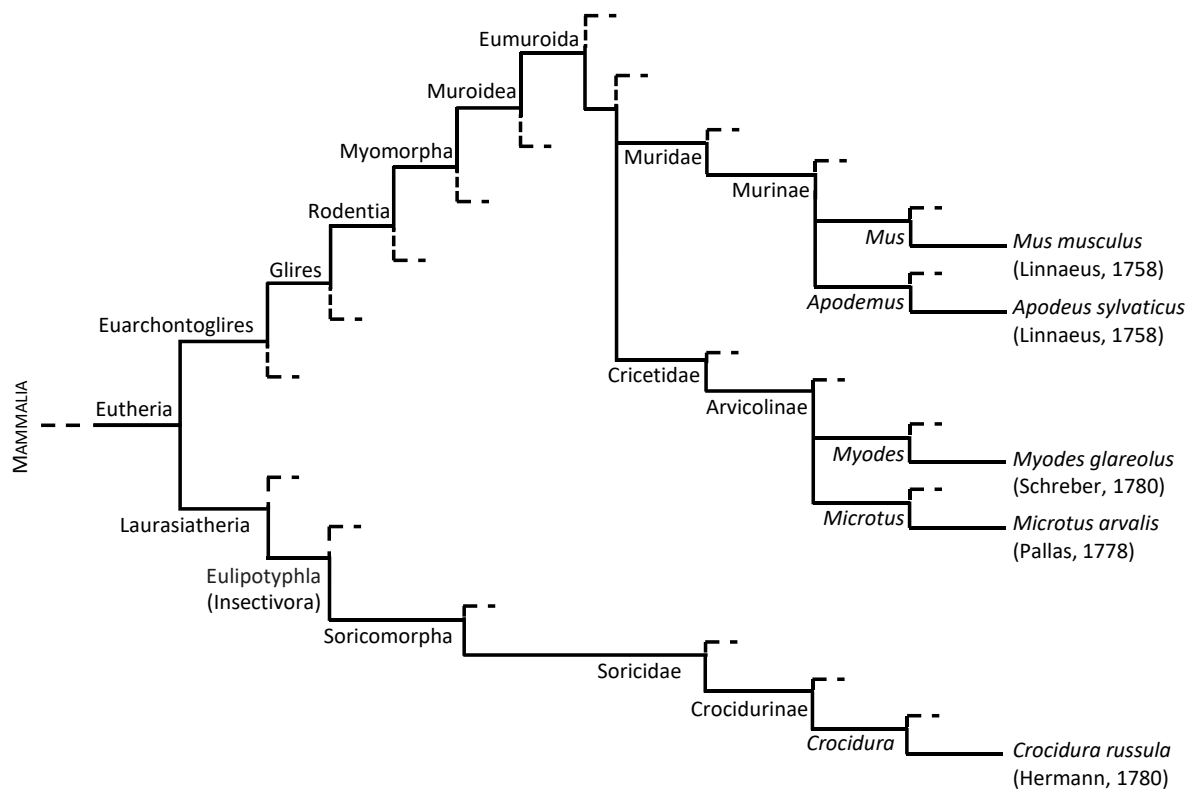


1496

1497 **Figure A2. Simplified phylogenetic tree of the rodents and shrews included in the study.**

1498 *Adapted from (Brace et al. 2016; Springer 2004; Swanson et al. 2019).*

1499



1500 **References**

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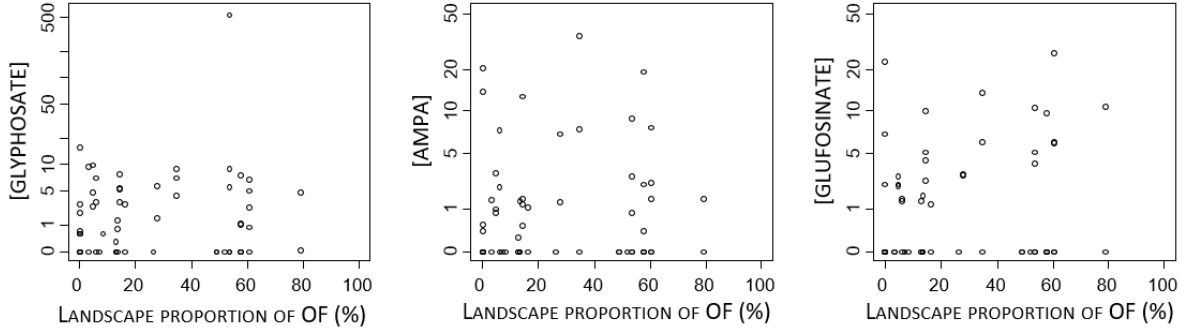
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1508

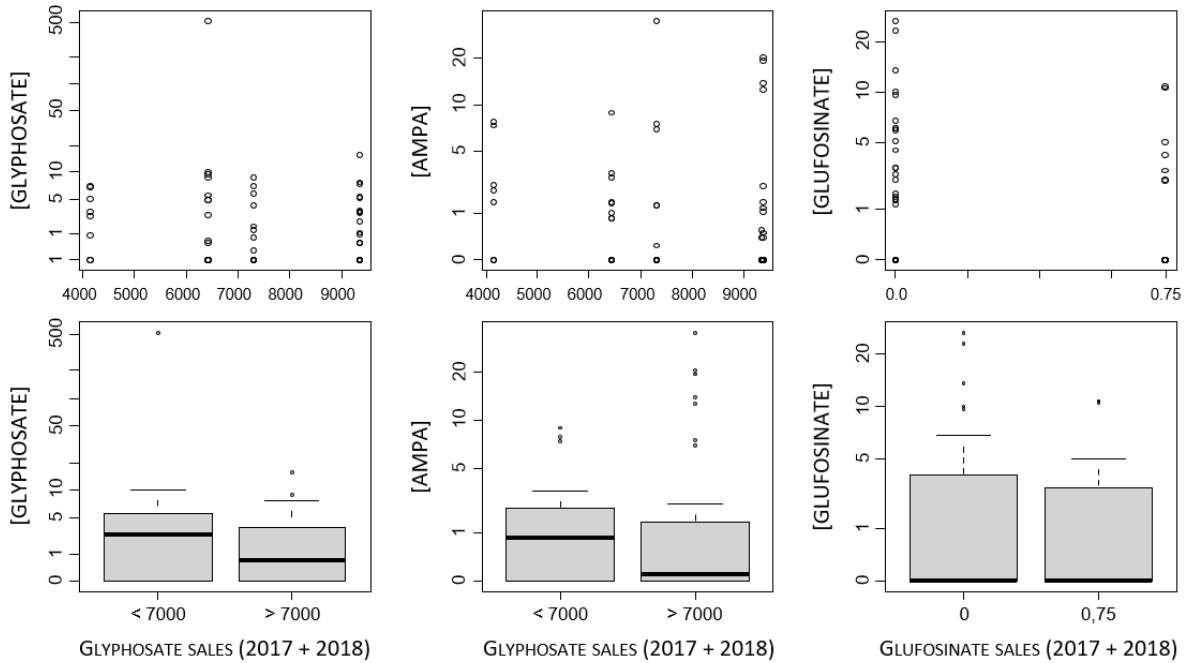
1509 **Figure A3. Concentrations of glyphosate, AMPA, and glufosinate in the hair of small mammals with nondetects**
 1510 **set to 0 according to the proportion of organic farming in the landscape as a continuous factor (a) and to the**
 1511 **proxy of treatment intensity at township scale as a continuous factor and as a categorical factor (b).**

1512 *The concentrations are expressed in pg/mg. Nondetects are set to 0. Statistics were performed via the Wilcoxon-*
 1513 *Mann-Whitney test or the Kruskal-Wallis test depending on the number of levels of the factor of concern and*
 1514 *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



1515

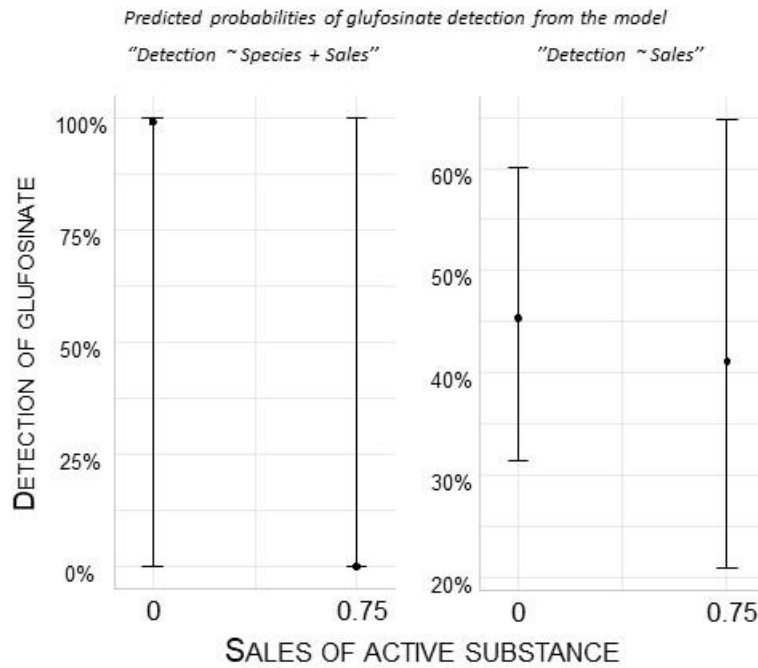
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1518 **Figure A4. Probabilities of detection of glufosinate in the hair of small mammals according to the sales of the**
1519 **active substance at the township scale.**

1520 *The predicted probabilities of detection were obtained from the statistical binomial models "Detection ~ species +*
1521 *sales"(left panel) and "Detection ~ sales" (right panel).*

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1546 **Table A1. Overview of sample size.**

1547 *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

1548

1549 **Table A2. Statistical outputs of binomial GLMs used to investigate the influence of explanatory factors on the detection frequencies of glyphosate, AMPA, and glufosinate**
 1550 **in the hair of small mammals.**

1551 *The binomial models were computed as “Detection ~ Species” and “Detection ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AS sales”. **
 1552 *alternative model with the factor “glufosinate sales” alone without the factor “species”. Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus*
 1553 *(Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew), Miar=Microtus arvalis (Common vole), Mumu=Mus musculus (House mouse), Mygl=Myodes glareolus (Bank*
 1554 *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			0.662	Reference	---				Reference	---					Reference	---		
Hedgerows		39				0.71	(0.14, 3.31)	0.663			1.12	(0.22, 6.59)	0.893				1.50	(0.24, 12.3)	0.673	
Farming			3.273	54	0.195				3.364	54	0.186			1.496	54	0.473				
OF		10				Reference	---				Reference	---					Reference	---		
OF/CF		10				6.98	(0.57, 217)	0.169			12.7	(0.79, 4745)	0.102				5.83	(0.35, 133)	0.200	
CF		41				6.96	(0.84, 174)	0.119			6.35	(0.65, 172)	0.161				2.49	(0.30, 27.1)	0.400	
Treatment			0.000	55	0.999				0.030	55	0.863			0.016	55	0.900				
NT		41				Reference	---				Reference	---					Reference	---		
T		20				1.00	(0.22, 4.47)	0.999			1.15	(0.23, 5.63)	0.863				0.90	(0.14, 5.12)	0.900	
Landscape OF			0.331	55	0.565				0.031	55	0.860			0.013	55	0.908				
0-50%		40				Reference	---				Reference	---					Reference	---		
50-100%		21				0.67	(0.16, 2.60)	0.568			1.14	(0.24, 5.18)	0.860				0.91	(0.15, 4.88)	0.908	
AS sales			0.009	55	0.925				1.257	55	0.262			5.266	55	0.022				
Low category		25				Reference	---				Reference	---					Reference	---		
High category		36				1.07	(0.26, 4.58)	0.925			2.66	(0.51, 21.9)	0.289				0.00	NA	> 0.900	
																	(0.84)*	(0.26, 2.59)*	(0.800)*	

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1557 **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**
 1558 **of small mammals.**

1559 *The models were computed as “log(concentration) ~ Species” and “log(concentration) ~ Species + Habitat or Farming or Target treatment or Landscape OF proportion or AI sales”.*
 1560 *Abbreviations: OF=Organic Farming, CF=Conventional farming, Apsy=Apodemus sylvaticus (Wood mouse), Crru=Crocidura russula (Greater white-toothed shrew), Miar=Microtus*

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

Factor	GLYPHOSATE										AMPA						GLUFOSINATE										
	Factor		Mode			Levels					Factor		Model		Levels		Factor		Model		Levels						
	Levels	n	F	Df	p	R ²	R ² R ² Adj	Estimate	SE	p	F	Df	p	R ²	R ² R ² Adj	Estimate	SE	p	F	Df	p	R ²	R ² R ² Adj	Estimate	SE	p	
Species		1.643	4	0.186	0.162	0.162					1.110	4	0.373	0.146	0.146				1.008	4	0.425	0.155	0.155				
	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					0.750	1	0.395	0.025	0.171				2.759	1	0.112	0.098	0.253				
						0.148									0.005							0.075					
	Cereal	14					Reference	---	---						Reference	---	---					Reference	---	---			
	Hedger	25					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					0.725	2	0.495	0.049	0.195				0.087	2	0.917	0.007	0.162				
						0.006									0.000							0.000					
	OF	5					Reference	---	---						Reference	---	---					Reference	---	---			
	OF/CF	6					-0.184	1.05	0.861						0.800	0.988	0.426					-0.194	0.750	0.799			
	CF	28					-0.146	0.890	0.871						0.022	0.838	0.979					-0.252	0.606	0.682			
Treatment		0.232	1	0.633	0.006	0.168					0.750	1	0.395	0.025	0.171				2.759	1	0.112	0.098	0.253				
						0.042									0.005							0.075					
	NT	27					Reference	---	---						Reference	---	---					Reference	---	---			
	T	12					-0.289	0.600	0.633						0.521	0.602	0.395					0.716	0.431	0.112			
Landscape OF		0.151	1	0.700	0.004	0.166					0.754	1	0.393	0.025	0.171				3.454	1	0.077	0.119	0.274				
						0.039									0.005							0.101					
	0-50%	27					Reference	---	---						Reference	---	---					Reference	---	---			
	50-100%	12					0.227	0.582	0.700						0.496	0.570	0.393					0.790	0.425	0.077			
AS sales		0.104	1	0.749	0.003	0.165					0.595	1	0.448	0.020	0.166				0.884	1	0.358	0.034	0.189				
						0.038									0.000							0.000					
	Low category	17					Reference	---	---						Reference	---	---					Reference	---	---			
	High category	22					-0.172	0.535	0.749						0.416	0.539	0.448					-0.454	0.483	0.358			

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