

Answers to reviewers' comments

Dear Recommender,

We warmly thank the editorial board of PCI Ecotox Env Chem for providing us the opportunity to revise and re-submit our manuscript.

We also thank the reviewers for their fruitful remarks and advices.

We have reworked the manuscript according to the comments of the reviewers.

Please find hereafter our answers to reviewer's comments. In the revised version of the manuscript, changes are highlighted using the "track changes" option. Please note that the numbers of lines cited hereafter refer to the version of the manuscript with track changes.

The final "clean" one is provided on HAL.

We thank you for considering our new version.

Best,

Clémentine Fritsch

Revision round #2 - Reviewer's comments and responses

[Review by anonymous reviewer 3, 28 Oct 2024 01:13](#)

I appreciate the author's diligence in thoroughly revising the manuscript to address all reviewers comments.

The comment about feedback on the dose reconstruction was directed at the Editor to notify them this reviewer did not have the expertise to review that section of the manuscript. This was not directed towards the authors.

I have no additional comments as the feedback was addressed and the manuscript revised appropriately or the authors provided excellent rational as to why they opted to not change the sections.

[Review by Sabrina Tartu, 06 Sep 2024 14:32](#)

The authors have taken into account all my comments and most comments of the two other reviewers. The manuscript has greatly improved, and I am completely satisfied with this revised version. I therefore recommend this manuscript for the PCI Ecotoxicology and Environmental safety.

- We thank the two reviewers for their help in improving our manuscript and for the recognition of the efforts made to revise the manuscript.

Review by anonymous reviewer 2, 03 Nov 2024 20:58

This is a nice, and a very interesting work, however despite the work carried out after the previous revision, it is still too long, and hard to read, and therefore to follow. I agree with other reviewers that it could give rise to two papers. Anyway, if authors prefer to keep it as a single manuscript, I strongly urge to summarise it as much as possible. I have described some crucial aspects that, in my opinion, should be clarified to improve the manuscript before publication.

- We acknowledge that the manuscript is quite long and we have justified the reasons why we prefer to publish altogether both the results about exposure and about the related risk in responses to the first review round. We have already addressed the comments and recommendations of the 3 reviewers during the previous round, with the aim of shortening and changing the structure of the article in order to facilitate the reading and improve fluency. The two other reviewers acknowledged that the manuscript has been improved, that comments have been addressed overall, and that the justification about having one instead of two manuscripts is convincing. They finally conclude that they are satisfied by the revisions and have no further recommendation. In this context, making further changes in the structure and writing of the manuscript would not meet the comments of the two other referees and our aim towards the presentation of our research work.
- We tried again to simplify the text as far as possible, and consider the issue of clarity and need to summarize when addressing the comments of the reviewer. The document is composed now of 69 pages while the previous version was composed of 72 pages.

Sampling

- A) I think the authors have made an important effort to capture the micromammals, however, the sampling, and sampling effort is very confused. As I can understand, they used 1 trapline (composed by 25 traps) for each of the 78 plots related to 40 sampling squares of 1km² (around two plots per square). In this sense traps deployment were: 78 sampling plots x 1 trapline/sampling plot x 25 traps/trapline = 1950 traps deployed, it is correct?. Then in results authors stated that animals were captured in 29 of the 78 traplines deployed, which authors specify that correspond to 25 of the 40 sampling squares selected for this study, this mean that animals were only collected in 25 of the 40 squares?, these 25 squares (Fig B) appear to be in adjacent areas, in relation to Fig. A, then nothing else was captured in the rest of the study area?, because it was tried, right?. Regarding these numbers, in Fig. 1, there are 44 sampling squares, the number of sampling plots is difficult to count, but the number of traplines in Fig 1B are 27 and not 29. I think that an additional effort must be done to clearly describe the sampling carried out to avoid confusion.

- As requested we have simplified as much as possible the description of the sampling design and the sampling process (Lines 154-167 and 177-1940). We have deleted detailed information about the trapping effort in order to focus on the animals from which hair samples that are considered in this study have been collected.

B) In addition, regarding sampling, the authors indicate that some animals died during sampling, and that hair was collected from these animals after thawing, but nothing more is indicated afterwards. This issue also raises doubts about how many animals were found dead in the traps?

- We have added the information Line 193 (The survival rate to capture and handling was 91.6%.)

, if was checked the effect of freezing in the residues analysed?

- This issue has not been checked since all hair samples have been stored frozen as stated line 190.

Furthermore, reading that, one might think that the authors would take advantage of it to analyse internal tissues, but was not the case at least in this manuscript.

- Thank you for this interesting comment but this goes beyond the scope of the present article.

In this context, I think that authors could choose to provide more information on this, or to avoid providing such incomplete information.

- We have trimmed as requested to focus on essential information.

C) Figure 1: Despite that this figure can be very useful to know the study area, it should have a better resolution as it is difficult to read. Also, taking advantage of the figure, and being a composite figure, I think it could be called more often in the text.

- We have modified Figure 1 in order to focus on essential information and to improve the resolution. (See page 19). The figure is now made of only panel, and is cited in M&M.

Chemical analysis and concentration of residues

I am aware of the effort made by the authors to try to follow the recommendations given in the previous review regarding limits of detection/quantification, however, due to the LOQ is defined as the lowest possible concentration of the analyte that can be quantified by the method in a reliable way, it is still strange to see in Table 1 that median concentrations are below to the LOQ for GLY and AMPA, but not for GLUF.

- Values below the LOQ are values for which variability and accuracy are higher than 25%, which is the criteria to establish the LOQ. Thus, compounds are detected, and quantified, although with a lower accuracy than if above the LOQ. As explained lines 279 – 280 on the basis of the reference Keizer et al. 2015, "*The use of concentrations below the LOQ has been shown to be a suitable method that may be preferred to the absence of data or other established approaches such as imputation-based methods*". Median concentrations were not below the LOQ for GLUF because the concentrations measured in our samples were all, including the minimum detected value, above the LOQ value. This was not the case for the two other compounds for which the concentrations reached often levels lower than the LOQ but above the LOD.

- In order to make sure that readers will be aware of how the LOQ was defined when reading Table 1, we have added this information lines 272-273.

Statistical analysis and Results

Due to the length of this manuscript, I think it would be very useful if both the description of the statistical analyses and the results followed the same order and even the same sections.

- It is the case overall: the description of the statistical analyses broadly follows the order of the results with (1) description of tests about the range of values and correlations between compounds, (2) then about detection frequency and (3) finally about concentrations. The first section of 2.6 describes the general approach that apply to all the tests. Lines 350-353

correspond to 3.2 (there are no statistics associated to 3.1). Lines 363-372 describe the preliminary analyses that shape the final chosen models for the analyses of the rest of the data. Lines 373-378 correspond to the tests on detection frequency, and Lines 379-384 and Lines 385-392 correspond to the tests on concentrations. Results about detection frequency and concentrations are similarly described separately and sequentially in the section “Results”.

- Within the section 2.6, the order of subsections is defined according firstly to the structure of the Results, and then according to the statistical methods that are used. These methods vary according to the type of response variable (i.e. detection frequency, concentrations when detected, and concentrations including non-detects), not according to the explanatory factors tested.

- The section “Results and Discussion” have been organized to show and discuss both detection frequency and then concentrations, as it is organized in *Data analysis and Statistics*. Then, subsections have been added on the basis of results about differences between species, habitats or PPP treatments. This was chosen (1) to align with available literature that is used to discuss and interpret the results, and (2) to fulfil the recommendations dealing with clarity and constancy in review round 1, which ended up in more paragraphs.

- Following tightly the same order in the description of the statistical analyses would lead to several redundancies and duplications. Changing again the subsections in Results to strictly align with Statistics would adversely impact the fluency and comprehensibility of the discussion.

- Please note that this section has already been reworked according to review round 1 and following the recommendations of the reviewers.

Results

A) Overall, results must be shortened, for example those described in 3.4.

- Please note that in this manuscript the structure gathers results and discussion together (we have 4 main sections: 1. Introduction, 2. Materials and Methods, 3. Results & Discussion, 4. Conclusion).

- About 3.4 (now 3.3), the length of the part about the results on differences between species in both detection frequency and concentration of the compounds is around half a page (lines 562-576). There is no redundancy with other sections or other materials (Tables, Figures). No values or detailed statistical outputs are presented, the text systematically refers to Appendix, Figures and Tables. The figures extend on 1 page; they are quite large since we have followed the recommendation of the previous review round asking for better resolution and greater size of the figures.

- Considering the changes made to simplify M&M (see above) and this remark, we have described the sample size in M&M (Lines 210-221), thus allowing to trim the Results. We do not see how to further revise in order to meet the reviewer’s recommendation without losing information.

B) The analysis of hair or other keratinised samples to determine exposure to metals is well established, however the usefulness or information provided by hair analysis is not clear for these compounds. On the one hand authors stated that “measurements in hair represent a temporally integrative exposure assessment during the time of hair growth” (lines 922-923) but on the other hand that “the highest concentrations found here may correspond to animals exposed to GLY soon after its application” (lines

1680-1681), all this when the range of concentrations detected is in the order of pg/mg. I think that this issue must be coherent and consistent throughout the manuscript.

- It is indeed stated that *“measurements in hair represent a temporally integrative exposure assessment during the time of hair growth”* lines 454-456, and that *“the highest concentrations found here may correspond to animals exposed to GLY soon after its application”* lines 519-521. Residues in hair are accumulated during the growth of hair. In the case the hair grew soon after a GLY application, when the environmental concentrations are the highest and therefore exposure level can be the highest, this would explain high concentrations in samples. Contrarily, hair grown when animals are exposed to lower environmental levels, longer after GLY application, might show lower concentrations. Because of the relatively low environmental persistence of GLY and the rapid decrease in tissues and viscera shown in the ref cited line 454 (Newton et al. 1984), levels of exposure should indeed be lower as should be concentrations accumulated in hair.

- To consider for potential misinterpretation and revise according to this comment, we have detailed the explanation lines 507-515.

Other comments

Line 43: include the units, please.

- We have added “pg/mg” four more times.

Lines 204: why it is negligible?

- We have already addressed this remark during the previous round of review, the sentence has been rewritten. AMPA and GLUF are not likely to be sprayed: AMPA is a transformation product and GLUF is no longer authorized as a PPP in France. Therefore, the exposure through this pathway should not be an issue.

- To make sure that the statement is understandable, we have reworded (Line 128-136).

Lines 407-409: include the units for quantities sold, please.

- After checking in the different versions, final or with track changes, we did not find lines numbered around 407-409 corresponding to a statement dealing with quantities sold. We thought that this comment echoes the same comment on the previous version *“Lines 331-332, quantities, was this grams, kgs, etc?”* We have already addressed this comment: the values provided are ratio, therefore they are unitless. The units are provided in M&M (Line 245) and Table 1.

- Nevertheless, we have modified line 431 by adding “(in kg)” in the sentence.

Line 490: For clarity, please change a.s. by active substance in “expressed as daily doses in mg a.s per kg body weight”.

- Done. Line 287.

Table 1: Please, check the numbers for not treated or treated animals, NT=41?, and T=20 it is not the contrary??

- Potentially treated plots are conventional cropped fields and fields in transition from conventional to organic farming = 20 plots where hair have been collected. Not-targeted areas are organic farming cropped fields and hedgerows or woodlots. Therefore, the values provided are correct.
- We have changed the title of the line in Table 1 to clarify, and detailed when describing the sample size in M&M.

Table 1: Sales are given in kg? this are sale for township? How many townships?

- We have added the unit in Table 1. Details about townships are already provided in M&M and can be seen in the new Figure 1. As the length of the manuscript and the lack of simplicity are considered concerns in this work, we did not add further complexity in Table 1 by adding an information already mentioned elsewhere.

Lines 878-883: you have tested the correlation between sales of Gly and Gluf and % of animals with residues?

- Yes indeed, it has been done, please see lines 661-669, Table A2 and Fig. A4.

Line 884: indicate what is PING, please.

- "PING" is the acronym of the research project for which this study was conducted.
- We have detailed Line 157.
- We have added "(see M&M)" line 438 when the project is mentioned in the discussion.

Lines 917-919: Maybe that can be also due to season effect, due to hunted animals are usually collected from October to January, while here sampling was during spring, may and June, in addition can be interesting include the concentrations detected.

- After checking in the different versions, final or with track changes, we did not find lines numbered around 917-919 corresponding to hunted animals. We supposed that this comment concerns the study on Iberian hares conducted by Martinez-Haro et al. (2022). The issue related to seasonal effects on levels of exposure was already addressed in the manuscript in another section, we have moved and reworded these parts:
 - lines 448-462 we have detailed the issue of seasonality in the section "*Detection frequency of GLY, AMPA and GLUF in the hair of small mammals*".
 - Lines 683-688 we have focused on differences between treated/non-treated areas in the section "*Influence of habitat, farming practices, and proxies of treatment intensity on the detection frequency of the compounds*".
 - The concentrations found in gastric contents are reported and discussed lines 501-503 in the section "*Concentrations of GLY, AMPA and GLUF in the hair of small mammals*" together with concentrations found in viscera in Newton et al (1984).
 - We have added lines 828-834 the daily dose estimated from these concentrations.

Line 1788. What do authors mean with reference? I suppose that it is referred to the model, but in order to avoid confusion, reference could be replaced with another term.

- Not sure about the line numbering, but it should be in the results about odds ratios. We have simplified the sentence to remove the use of "reference" (Lines 564).

The reference Pelosi et al. (2022- Agric Ecosyst Environ 305:107167), was eliminated, and I think that due to that was carried out in the same study area of the present study, it represents an interesting work that is worth commenting/discussing because small mammals can be also potentially exposed to many other active substances not included in this manuscript but also with ecotoxicological implications.

- This sentence focused only on neonicotinoids that are not the focus of this paper, we have deleted indeed during the 1st review round to shorten and simplify the manuscript as requested by the reviewers. We therefore removed the reference Pelosi et al 2021 in Agric Ecosyst Environ (305:107167).
- Please note that the reference Fritsch et al (2022), which reports concentrations of about 140 compounds screened in hair of small mammals captured over the same area, have been kept and is used to discuss the results (lines 725, 754, 798, 893, 971).

Overall, there are many very long sentences, made up of several lines of text, often difficult to follow (87-95,....). In this sense, I think that more effort must be done to make the text fluid and easy to understand.

- Troubles with line numbering to identify the sentence of concern lines 87-95. These lines do not correspond to only one long sentence, neither in the version with track changes nor in the re-submitted "clean" version.
- We have carefully reviewed the text and shorten or split the sentences as far as possible (ex: lines 71-79, line 148, line 315, line 344, line 453, line 474, line 500, line 625, line 671, line 688, line 724).