Author’s response to reviews

Title: Pesticide exposure assessed through agricultural crop proximity and risk of amyotrophic lateral sclerosis

Authors:

Marco Vinceti (marco.vinceti@unimore.it; mvinceti@bu.edu)
Tommaso Filippini (tommaso.filippini@unimore.it)
Federica Violi (federica.violi@unimore.it)
Kenneth Rothman (kenneth.rothman@gmail.com)
Sofia Costanzini (sofia.costanzini@unimore.it)
Carlotta Malagoli (carlotta.malagoli@unimore.it)
Lauren Wise (lwise@bu.edu)
Anna Odone (anna.odone@mail.harvard.edu)
Carlo Signorelli (carlo.signorelli@unipr.it)
Laura Iacuzio (laurajacq@libero.it)
Elisa Arcolin (elisa.arcolin@virgilio.it)
Jessica Mandrioli (j.mandrioli@ausl.mo.it)
Nicola Fini (n.fini@ausl.mo.it)
Francesco Patti (patti@unict.it)
Salvatore Lo Fermo (totolof@hotmail.com)
Vladimiro Pietrini (vladimiro.pietrini@unipr.it)
Sergio Teggi (sergio.teggi@unimore.it)
Grazia Ghermandi (grazia.ghermandi@unimore.it)
Renato Scillieri (scillieri@tiscali.it)
Caterina Ledda (cledda@unict.it)
Author’s response to reviews:

Revision of manuscript ENHE-D-17-00049 (“Pesticide exposure assessed through agricultural crop proximity and risk of amyotrophic lateral sclerosis”): response to the Editor and the Reviewers

We wish to thank the Editor and the Reviewers of Environmental Health for the thoughtful, comprehensive review of our manuscript. We have done our best to address each concern and we believe that the revisions we have made have strengthened and improved the manuscript. We have also updated the literature through May the 4th and added relevant citations on the basis of the editorial suggestions and the consequent changes to the manuscript. We are grateful to the Environmental Health for the opportunity to submit this revised manuscript.

Reviewer #1:

The question of whether certain environmental exposures are related to the risk of ALS is an important one as so little is presently known about the disease and its triggers. In the present investigation the authors used a matched case control study with four to one matching to consider proximity of historical and present residence to agricultural land and therefore exposures to pesticides routinely used over the years as risk factors for the disease. I believe that there are some questions that should be addressed in order to make this the best possible study for publication.

There were a reasonable number of cases (703) and surveillance and case ascertainment appears to have been exhaustive assuring a representative sample of cases to study. Four primary questions come to mind regarding the methods employed in this study:

1) the first is choice of the control group which on first read sounds reasonable; however, the issue of overmatching always comes to mind, eg. why was the control not chosen at random from the four provinces of study area instead of being matched precisely to the same province? This may have resulted in overmatching on the exposure of interest, proximity to
agricultural exposure to pesticides? I am not sure what you do about this at this point but it should be discussed in the weaknesses and strengths section.

We agree with this concern of the Referee, and we performed new data analyses and generated additional results to clarify this issue. We originally matched on province of residence – in addition to sex and age – since between the Emilia-Romagna region three provinces, and clearly much more between the Emilia-Romagna and the Sicily region provinces – there could be some difference with respect to lifestyle (and possibly genetic factors). This is certainly true for the northern and southern Italy comparison, i.e. between the Emilia-Romagna and Sicily provinces, but it may even be true to some extent for the neighboring provinces of Parma, Reggio and Modena, due to the long-term stability of the populations residing in these provinces and some peculiar food habits of these populations. In addition, we noted that only one of these provinces (Reggio) had ‘historical controls’ available, i.e. controls that could be retrieved in the population directories matched for calendar year to the cases and allowed a sensitivity analysis in this subgroup (now mentioned in the manuscript). Finally, we also point out that all the three Emilia-Romagna provinces have a very similar orography, land use and percentage of residents employed in agriculture and other main activity sectors, as ascertained through the databases of the Emilia-Romagna Region (and now stated in the revised manuscript), thus reducing the risk of overmatching due to subjects’ residence and occupational status when we stratified the study subjects sampling procedure for province. Overall, we took into account the Reviewer’s concerns, which we share, in the revision of the Methods and Discussion section.

With regard to the exposure measure, can you say that you are confident that the residential history was captured XXX years before diagnosis, Perhaps I missed this but I ask this as the authors also indicate that the ALS disease process has an element of various forms of inflammation, oxidative stress, etc so the earlier years of exposure may be particularly important so was an effort made to capture cumulative exposures based on mobility? If not can you comment on how many of the cases and controls stayed within the one residence or is this not possible?

A timeline of exposures by residential history for cases and controls would be important.

The Reviewer makes a valid point, and we recognize not having adequately explained our procedure for selecting the ‘historical’ study subjects, alongside with the related analysis. As reported in the manuscript, we retrospectively identified the oldest residence which was available in the National Revenue Agency (in 1979 for the Emilia-Romagna provinces and in 1989 for the Catania province in Sicily), and we selected the subjects for whom information about residence in that year was available in that database, independently of any subsequent change of residence of these subjects (only requiring two years of stability after that date, as stated in the manuscript). These subjects formed the population that we included in the ‘historical analysis,’ and we ascertained for them the exposure status using the historical land cover. We clarified this issue and the underlying procedure in the Methods.
However, taking into account the Reviewer’s comment and concern, we carried out a new analysis which was restricted to residentially stable subjects, i.e. having the same ‘historical’ residence as their ‘recent’ residence. This analysis clearly helps to clarify whether higher cumulative exposure may favor disease onset even independently on the length of the period of time elapsed from exposure, though in this subgroup we could not be entirely sure that the land use around the residence remained constant over time. We mentioned the results of this analysis in the Results section, and since no substantial deviation of effect estimates emerged, we consider this additional finding supportive of our overall conclusions.

2) Occupational exposure is also an important risk factor, was this asked as part of the study? As it may be that the risk factor, working in the adjacent agricultural location would be important to document. Please comment on availability.

Unfortunately, we did not have detailed information on this important risk factor: we collected some information about occupational activity from 162 study participants with a questionnaire (as we mentioned in Vinceti et al. ‘Pesticides…’, Environ Res 2017;155:261-7), but that study was prone to selection and recall biases and therefore we did not mention here its results. In addition, we could not access data from the National Social Insurance Agency (INPS), as we now stated in the Discussion among the study limitations. However, the population data we could collect through the National Revenue Agency, as mentioned in the manuscript, suggested a higher prevalence of employment in agriculture in residents close to agricultural crops, as largely expected. This suggest that subjects residing near agricultural crops likely experienced a higher occupational exposure to pesticides, in addition to a higher passive exposure, and therefore that the real difference in pesticide exposure compared to subjects not living in the proximity of crops was likely higher than we estimated.

3) Male versus female exposures, as women often have more home related exposures than men, please comment on a stratified analysis to the extent possible sample size wise between cases and controls.

We agree with the Reviewer about the opportunity of such stratified analysis, and therefore we have now implemented it. Its results (not showing substantial sex-related differences in effect estimates) have been now mentioned in the Results.

4) A few descriptive tables would be helpful showing the age, race and gender distribution and some measure of Socioeconomic status (education, income, etc) to show the comparability distribution within cases and controls as well as a map of the four provinces.
We have addressed this comment by inserting a new table (Table 2). However, we were not able to report on the distribution of socioeconomic status (education, income and occupation) because these data were unavailable for the study population. We highlighted this limitation in the Discussion.

Finally, was the rate of ALS similar in these four provinces compared to the rest of the country? In general where does Italy rank regarding the incidence rate of ALS?

With the amount of agriculture in the country, it would be helpful to have some background about the overall rate of disease and how Italy ranks say compared to the US or surrounding countries. I think the null finding is an important contribution to the literature but I believe these questions should be addressed to clarify and strengthen the findings.

We agree with the Reviewer about the utility to put our results in a more general context, taking into account the background ALS rates compared with our Italian regions and the Western countries. We added such information in the Discussion using data from the literature. Overall, the study provinces showed ALS rates comparable to the national average and the US ones as well.

Reviewer #2:

This manuscript reports a case-control study of 703 ALS patients and 2737 age- and sex-matched controls in two regions in Italy. The relationship of ALS risk to residence in proximity to agricultural land was evaluated. Little evidence of an association was observed for either residence at the time of diagnosis or historical residence. The study is large and population-based and the methods for recruiting patients and controls were good.

A major issue is exposure assessment. On the one hand, reasonable evidence indicates that individuals living in proximity to agricultural land are in fact exposed to pesticides. On the other, many features of the exposure measure used may result in substantial misclassification. First, only crops were assessed directly, and pesticide use was inferred. No information on the amounts or types of pesticides used on the land were presented, in contrast to some other studies that used residential proximity as a surrogate for exposure. Second, only data on pesticide classes were presented, but specific pesticides within classes may not have the same relationship to ALS. Third, as the authors point out, individuals living in proximity to agricultural land may be exposed occupationally as well as through proximity, but this is an ecological measure and no data on occupational exposure of individuals were available to control for potential confounding. Finally, no information on the degree of exposure were presented, for example time spent in the residence (as opposed to a workplace) or years lived in the residence; the analysis of historical exposure, although less complete, may provide some information on the latter.
Given the potential for misclassification of exposure, and the relatively small number of exposed cases in some categories, the study may not have the power to support its negative conclusion. This should be acknowledged in the Abstract and final paragraph of the Discussion, and the conclusion should be softened.

The Reviewer makes a valid point, and we clarified and expanded the part related to exposure assessment, its characteristics and its limitations.

We could not systematically overcome the above mentioned limitations in exposure assessment outlined by the Reviewer. i.e. that we had generally available the use of pesticide classes as provided by the Emilia-Romagna and Sicily Region Offices but not of the single chemical compounds included in those classes (apart from the information collected through a survey ad hoc we carried out with the help of the Emilia-Romagna Region Services in a sample of local farms). As far as occupational exposure is concerned, we unfortunately could not retrieve population data (as previously mentioned for Reviewer #1’s comments), as now further highlighted in the Discussion. However, ‘official’ data about agricultural activity obtained from the National Revenue Agency suggest an increased occupational exposure to pesticides in study subjects residing near agricultural crops, suggesting that the null results of our study were not due to confounding from occupational pesticide exposure and further strengthening them. We acknowledge that the amount of time spent in the residence (as opposed to the workplace) was not available, due to the lack of direct contacts with the study subjects according to the study design. However, due to typical life-style habits and socioeconomic characteristics of study population particularly in the 1990s and the 2000s, the amount of time spent at home by females was certainly higher than for males, particularly in the Sicily province, and no excess risk was noted in this sex-specific subgroup. We also mentioned in the revised manuscript the analysis for long-term ‘stable’ residents who remained at the same address, taking into account this note of Reviewer #2 as well as the comments of Reviewer #1. Overall, we softened our conclusions by mentioning the study limitations highlighted by the Reviewer in both the Abstract and the Discussion.

Page 5, line 39: please provide a more explicit reference for the El Escorial criteria

Done.

Page 7, lines 55 & 58: should 'filed' be 'field'?

Corrected.

We hope that our changes fit the indications of the Reviewers’ and the Editor’s expectations, and we wish to thank again for the careful review of our manuscript and for the opportunity to submit our revised version to Environmental Health.