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Short Communication

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Occupational exposure to pesticides and neurobehavioral outcomes. Impact of different original and recalled exposure measures on the associations

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Abstract

Background Several measures of occupational exposure to pesticides have been used to study associations between exposure to pesticides and neurobehavioral outcomes. This study assessed the impact of different exposure measures for glyphosate and mancozeb on the association with neurobehavioral outcomes based on original and recalled self-reported data with 246 smallholder farmers in Uganda.

Methods The association between the 6 exposure measures and 6 selected neurobehavioral test scores was investigated using linear multivariable regression models. Exposure measures included original exposure measures for the previous year in 2017: (i) application status (yes/no), (ii) number of application days, (iii) average exposure-intensity scores (EIS) of an application and (iv) number of EIS-weighted application days. Two additional measures were collected in 2019: (v) recalled application status and (vi) recalled EIS for the respective periods in 2017.

Results Recalled applicator status and EIS were between 1.2 and 1.4 times more frequent and higher for both pesticides than the original application status and EIS. Adverse associations between the different original measures of exposure to glyphosate and 4 neurobehavioral tests were observed. Glyphosate exposure based on recalled information and all mancozeb exposure measures were not associated with the neurobehavioral outcomes.

Conclusions The relation between the different original self-reported glyphosate exposure measures and neurobehavioral test scores appeared to be robust. When based on recalled exposure measures, associations observed with the original exposure measures were no longer present. Therefore, future epidemiological studies on self-reported exposure should critically evaluate the potential bias towards the null in observed exposure–response associations.

Key words: exposure assessment; exposure misclassification; farmers; glyphosate; mancozeb; neurobehavioral outcomes; pesticides; recall; Uganda.

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What’s Important About This Paper?

The determination of exposure-response relationships can be influenced by bias and metrics of exposure. This study found that crude exposure measures (e.g. yes/no applicator) yield similar associations as the more complex exposure measures such as exposure days or exposure-intensity scores in the context of pesticide exposure and neurobehavioral outcomes. The study also shows that recalled exposure (in this case 2 years) might lead to a potential bias towards the null (no effect), and hence, should be critically evaluated in epidemiological studies.

Introduction

There are several different occupational exposure measures available to assess the associations of pesticide exposure with different health effects (Ohlander et al. 2020). These range from crude (e.g. job title or binary application status) to complex (e.g. exposure-intensity scores [EIS] based on algorithms that reflect different exposure pathways) and from indirect (e.g. questionnaire-based) to direct measurements of external exposure (e.g. biomarkers of exposure) (Freeman 2020). Further, the quality of the exposure measurements can change when data is recalled (Bell et al. 2019; Mueller et al. 2022). To inform future epidemiological studies about which exposure measures to apply in the analysis with health data, comparative assessments are needed to understand their implication on interpreting the measured associations (e.g. exposure to magnetic fields (Kromhout et al. 1997; Loomis et al. 1998) or air pollutants(Szpiro et al. 2011a, 2011b; Szpiro and Paciorek 2013) but are rarely done for the exposure–response analyses of pesticides and health outcomes (Fuhrimann et al. 2023).

This short communication aims to understand better how well-existing pesticide exposure measures perform when applied within epidemiological studies focusing on neurobehavioral health outcomes. For this comparative endeavor, previously published data from Ugandan smallholder farmers focusing on the association between glyphosate and mancozeb exposure and neurobehavioral test scores were used (Fuhrimann et al. 2020).

Methods

Data availability and study population

Previously published data were used from 246 Ugandan smallholder farmers who were enrolled in the “PESticide use in TROPical settings” (PESTROP)-Uganda study between September and November 2017 (Fuhrimann et al. 2019; Staudacher et al. 2020). In addition, recalled data for the same exposures and study population collected 2 years later, between September and November 2019, were used (Mueller et al. 2022). The described comparative assessment of the impact of alternative exposure assessment methods is part of the IMPRESS study (Jones et al. 2020). The 42 participants who were lost to follow up in the 2019 survey had similar pesticide use characteristics but had 14% more women included (Supplementary Table S1).

Selection of studied pesticides

The assessment of the associations between glyphosate and mancozeb with neurobehavioral outcomes was chosen because both pesticides were applied by more than half of our study population in product formulations that are usually applied independently from other pesticides. In addition, in our previous publication (Fuhrimann et al. 2021), an adverse association between yearly glyphosate application days weighted by EIS and impaired visual memory was observed. However, the same analysis showed no association between mancozeb exposure and neurobehavioral outcomes.

Exposure assessment methods and measures

Six different exposure measures, which represent glyphosate and mancozeb exposure in the year before the original survey, were assessed (Fig. 1a) (Fuhrimann et al. 2021). Four original exposure measures based on information collected in 2017 indicated exposure for the previous year: (i) application status (yes/no), (ii) number of application days, (iii) average EIS of an application derived from a semi-quantitative exposure algorithm and (iv) yearly EIS (i.e. number of EIS-weighted application days per year). In addition, recalled information collected in 2019 resulted in two additional measures: (v) re-called application status and (vi) recalled EIS for the respective periods in 2017 (i.e. recalculated for exposure measures 1 and 3).

A detailed description of the algorithm to calculate the EIS was previously published (Fuhrimann et al. 2020, 2021). The EIS predicts the intensity of an average application over the past year with a range from 0 (no exposure) to 13 (highest exposure score), estimated using 5 exposure-modifying factors (mixing, applying, personal protective equipment [PPE] use, time interval between pesticide application and change of clothes, and time interval between application and shower).

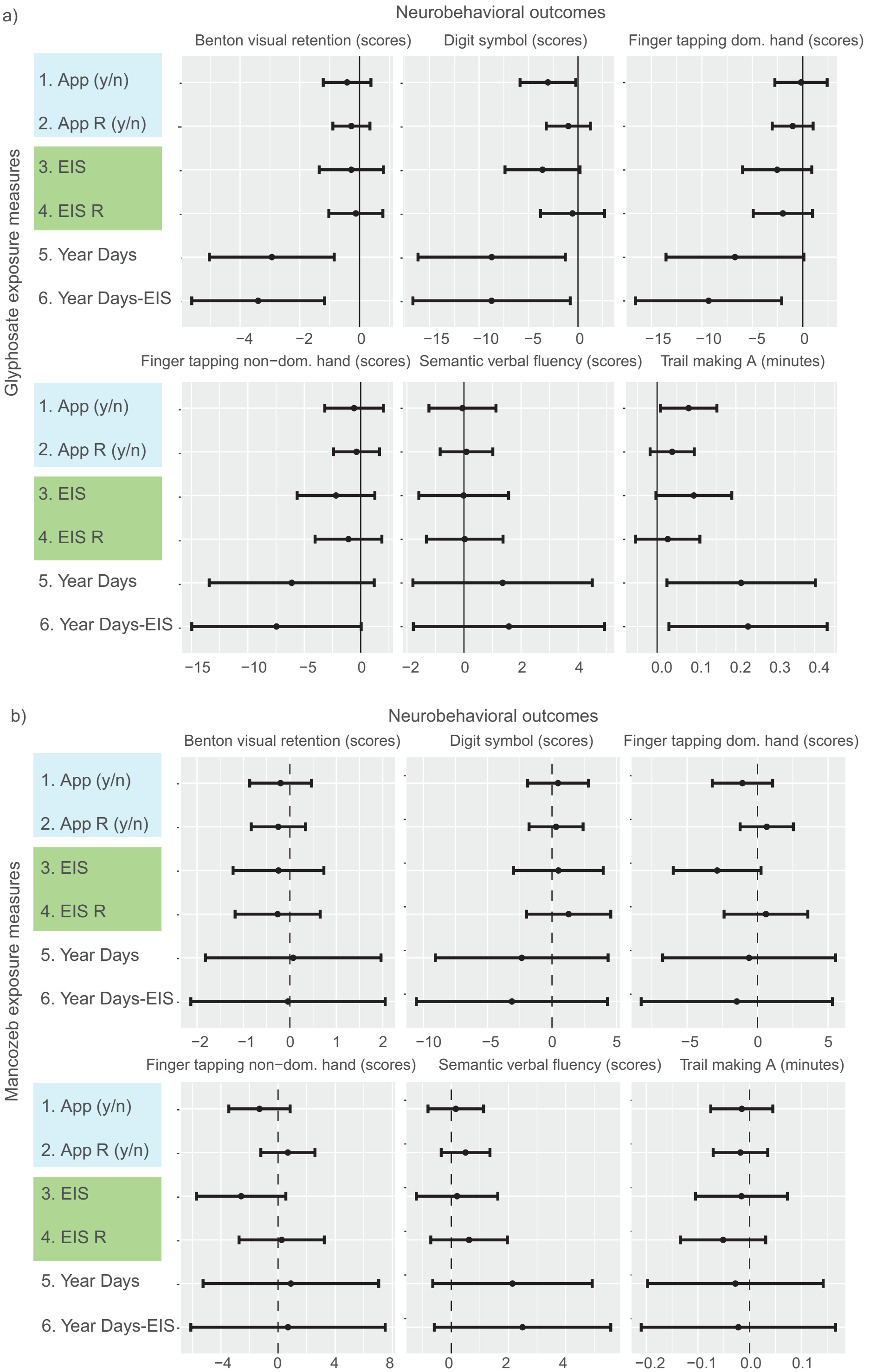

Fig. 1. (a) Summary table of the exposure assessment measures among the 246 smallholder farmers in Uganda. (b) and (c) Forest plots showing beta coefficients and 95% confidence intervals (95%CI) estimated for the 6 glyphosate (b) and mancozeb (c) exposure measures and 6 neurobehavioral outcome scores. To conduct the regression analysis, continuous exposure assessment measures (#3-6) were normalized on a scale between 0 and 1 (x—min(x))/ (max(x) - min(x)) before the analysis. App = application (yes); R = Recall; EIS = exposure-intensity scores. Outcomes: lower socres indicate a worse direction; outcome Trail Making A longer test performance (minutes) indicates a worse performance.

Health outcomes

Six neurobehavioral outcome variables as assessed by 2 trained psychometricians in 2017 were used, these being: Benton Visual Retention Test, Finger tapping dominant hand (scores) and non-dominant hand (scores), Trail making A log10 (minutes), Digit symbol (scores) and Semantic verbal fluency (scores) (Fuhrimann et al. 2021). Originally, associations with 14 outcome variables using a Bayesian model-averaging approach were studied (Fuhrimann et al. 2021). For this paper, a sub-set of 6 outcome variables was chosen for which an adverse association with exposure to specific active ingredients (i.e. glyphosate) was shown.

Statistical analyses

Associations were compared of the 6 glyphosate and mancozeb exposure measures with the 6 neurobehavioral outcome variables using linear multivariable regression models adjusted for the following confounders: age group (19–42, 43–55, and >55 years), education (<7th year versus >=7th year), the language of assessment (Luganda versus English), sex (male versus female), literacy (yes versus no), alcohol use (never versus ever), co-exposures (i.e. application of any pesticides in the past year; yes versus no) and the precision variables psychometrician (A versus B), history of head injury (never versus ever), and HIV status (positive versus negative).

To compare associations, all continuous exposure measures were standardized (x—min(x))/ (max(x) - min(x)) before the regression analysis. Comparisons of the impact of the different exposure measures were based on forest plots that allowed a visual comparison of the associations (i.e. beta coefficients, standard errors, 95% confidence intervals [95% CI, *P*-values and FDR-corrected *P*-values]). Statistical analyses were done in R (Foundation for Statistical Computing, version 3.6.3, RStudio version 1.2).

Ethical clearance

All participants signed informed consent. The studies were approved by the Higher Degrees, Research and Ethics Committee of Makerere University School of Public Health (2017 study reference no. 522; 2019 study reference no. 719).

Results

Exposure assessment measures

Figure 1a features a summary table of the 6 exposure measures. Out of 288 participating smallholder farmers in 2017 (Fuhrimann et al. 2021), 246 farmers were enrolled in our follow-up survey in 2019. In 2017, there were more glyphosate applicators than mancozeb applicators (58% versus 39%; Supplementary Table S2). On average (median), the applicators reported 2 glyphosate application days (interquartile range [IQR] 2) and 8 application days of mancozeb (IQR 14) in the year before the study. Recalled exposure showed a fair to moderate agreement and was generally more frequent for application status (proportional increase of 1.25 and 1.37 for glyphosate and mancozeb, respectively) and higher for recalled EIS (1.24 and 1.18 for glyphosate and mancozeb, respectively).

Exposure–response relationships

Figure 1b and c visualizes the associations between the 6 exposure measures and the 6 neurobehavioral outcomes. Tables 1 and 2 show the details of the obtained associations. Associations for 3 out of 6 different glyphosate exposure measures with 4 neurobehavioural test scores were observed (Benton visual retention, digital symbol, finger tapping dominant hand, and trail making A) with betas pointing in a direction indicating a worse performance in the respective neurobehavioural test. EIS-weighted application days were also associated with the above-mentioned 4 tests and showed the strongest relationships with the neurobehavioral tests, followed by a number of application days (associated with 3 tests), application (yes/no; associated with two tests), and EIS (associated with one test). The recalled application status and recalled-EIS for glyphosate showed estimates closer to the null, but overall with slightly lower standard errors. Adverse associations were no longer present. The finger tapping non-dominant hand and semantic verbal fluency tests showed no association with any measure of glyphosate exposure. Also, all 6 measures of exposure to mancozeb were not related to any of the 6 neurobehavioral outcomes and hence were not further interpreted within that paper.

Discussion

This paper shows what the effect of recalled information has on the pesticide exposure–response association in a cross-sectional study. As an example, the impact of 6 different measures of glyphosate and mancozeb exposure on the associations with neurobehavioral outcomes in a cohort of smallholder farmers in Uganda was studied. EIS-weighted application days resulted in the strongest associations with several neurobehavioural outcomes, indicating that this exposure measure may perform better compared to the other tested exposure measures. There were relationships between the crude number of application days with several neurobehavioural outcomes, while the exposure information on application status and EIS alone yielded fewer associations. These findings

Table 1. Detailed results of the different regression models for the 6 glyphosate-specific exposure measures and 6 neurobehavioral outcome scores (beta coefficients, standard errors, 95% confidence intervals [95% CI], and *P*-values).

Neurobehavioral outcome	Exposure assessment	Beta coef.	Std. Error	95% CI		*P*-value	*P*-value FDR
Benton visual retention (scores)	1. App. 17	−0.42	0.41	−1.22	0.38	0.306	0.517
	2. App. Recall	−0.27	0.32	−0.89	0.35	0.388	0.599
	3. EIS	−0.28	0.55	−1.35	0.80	0.614	0.806
	4. EIS Recall	−0.12	0.46	−1.03	0.78	0.787	0.908
	5. Year Days	−2.94	1.07	−5.03	−0.85	0.006	0.022
	6. Year EIS	−3.39	1.13	−5.62	−1.17	0.003	0.011
Digit symbol (scores)	1. App. 17	−3.04	1.44	−5.85	−0.22	0.036	0.098
	2. App. Recall	−0.97	1.14	−3.21	1.26	0.394	0.606
	3. EIS	−3.59	1.94	−7.38	0.21	0.065	0.160
	4. EIS Recall	−0.55	1.65	−3.79	2.69	0.738	0.887
	5. Year Days	−8.72	3.80	−16.17	−1.27	0.023	0.066
	6. Year EIS	−8.73	4.06	−16.69	−0.78	0.032	0.091
Finger tapping dom. hand (scores)	1. App. 17	−0.18	1.32	−2.77	2.42	0.894	0.944
	2. App. Recall	−1.00	1.03	−3.03	1.02	0.334	0.545
	3. EIS	−2.54	1.75	−5.97	0.89	0.148	0.304
	4. EIS Recall	−1.97	1.50	−4.91	0.97	0.190	0.377
	5. Year Days	−6.72	3.49	−13.56	0.11	0.055	0.141
	6. Year EIS	−9.33	3.69	−16.57	−2.09	0.012	0.038
Finger tapping non-dom. hand (scores)	1. App. 17	−0.59	1.33	−3.19	2.01	0.658	0.832
	2. App. Recall	−0.37	1.04	−2.41	1.67	0.722	0.877
	3. EIS	−2.19	1.76	−5.63	1.25	0.214	0.415
	4. EIS Recall	−1.08	1.51	−4.04	1.87	0.473	0.681
	5. Year Days	−6.11	3.73	−13.42	1.20	0.103	0.225
	6. Year EIS	−7.46	3.83	−14.97	0.04	0.053	0.135
Semantic verbal fluency (scores)	1. App. 17	−0.05	0.60	−1.23	1.12	0.930	0.961
	2. App. Recall	0.09	0.47	−0.83	1.01	0.852	0.930
	3. EIS	−0.01	0.80	−1.58	1.56	0.991	0.993
	4. EIS Recall	0.03	0.68	−1.31	1.37	0.969	0.982
	5. Year Days	1.35	1.60	−1.79	4.49	0.399	0.610
	6. Year EIS	1.57	1.71	−1.77	4.92	0.358	0.574
Trail making A (minutes)	1. App. 17	0.08	0.04	0.01	0.15	0.030	0.084
	2. App. Recall	0.04	0.03	−0.02	0.09	0.181	0.361
	3. EIS	0.09	0.05	0.00	0.19	0.059	0.148
	4. EIS Recall	0.03	0.04	−0.05	0.11	0.519	0.724
	5. Year Days	0.21	0.10	0.02	0.40	0.027	0.078
	6. Year EIS	0.23	0.10	0.03	0.43	0.025	0.073

App = applicator (yes); EIS = exposure-intensity scores. Continuous exposure assessment measures (#3-6) were normalized on a scale between 0 and 1 (x—min(x))/ (max(x) - min(x)) before the analysis. FDR = *P*-value corrected for false discovery rate; Green = *P*-value > 0.05; yellow = *P*-value > 0.1.

suggest that the frequency of application over 1 year (i.e. number of application days) is a stronger predictor for adverse neurobehavioral outcomes than the average intensity of an application (i.e. EIS) in a smallholder context in Uganda. Indeed, exposure intensity is high in most farmers as they lack appropriate PPE and hygienic practices (Staudacher et al. 2020). These are key exposure variables in the applied exposure algorithm, and hence, the resulting exposure contrast of the EIS is minimal (Fuhrimann et al. 2020; Mueller et al. 2024).

Table 2. Detailed results of the different regression models for the 6 mancozeb-specific exposure measures and 6 neurobehavioral outcome scores.

Neurobehavioral outcome	Exposure assessment	Beta coef.	Std. Error	95% CI		*P*-value	*P*-value FDR
Benton visual retention (scores)	1. App. 17	−0.20	0.34	−0.87	0.46	0.553	0.756
	2. App. Recall	−0.25	0.30	−0.83	0.33	0.404	0.614
	3. EIS	−0.25	0.50	−1.22	0.73	0.623	0.812
	4. EIS Recall	−0.26	0.47	−1.18	0.65	0.574	0.776
	5. Year Days	0.07	0.97	−1.82	1.96	0.941	0.965
	6. Year EIS	−0.04	1.07	−2.13	2.05	0.970	0.982
Digit symbol (scores)	1. App. 17	0.47	1.21	−1.90	2.83	0.699	0.861
	2. App. Recall	0.32	1.07	−1.78	2.41	0.768	0.904
	3. EIS	0.50	1.78	−2.99	3.98	0.780	0.907
	4. EIS Recall	1.29	1.67	−1.98	4.56	0.439	0.654
	5. Year Days	−2.35	3.42	−9.06	4.36	0.493	0.700
	6. Year EIS	−3.11	3.79	−10.54	4.31	0.412	0.622
Finger tapping dom. hand (scores)	1. App. 17	−1.07	1.09	−3.21	1.07	0.326	0.536
	2. App. Recall	0.65	0.96	−1.24	2.54	0.500	0.706
	3. EIS	−2.87	1.59	−5.98	0.25	0.072	0.173
	4. EIS Recall	0.59	1.52	−2.38	3.56	0.696	0.858
	5. Year Days	−0.60	3.13	−6.74	5.53	0.848	0.930
	6. Year EIS	−1.47	3.46	−8.26	5.31	0.671	0.839
Finger tapping non-dom. hand (scores)	1. App. 17	−1.30	1.10	−3.45	0.84	0.235	0.448
	2. App. Recall	0.69	0.97	−1.21	2.58	0.479	0.685
	3. EIS	−2.58	1.60	−5.71	0.54	0.107	0.233
	4. EIS Recall	0.25	1.52	−2.73	3.24	0.869	0.937
	5. Year Days	0.90	3.14	−5.25	7.05	0.775	0.907
	6. Year EIS	0.69	3.47	−6.11	7.50	0.842	0.926
Semantic verbal fluency (scores)	1. App. 17	0.16	0.50	−0.81	1.13	0.751	0.893
	2. App. Recall	0.50	0.43	−0.35	1.35	0.251	0.469
	3. EIS	0.20	0.73	−1.22	1.63	0.781	0.907
	4. EIS Recall	0.62	0.68	−0.72	1.96	0.364	0.580
	5. Year Days	2.14	1.42	−0.65	4.93	0.134	0.278
	6. Year EIS	2.49	1.57	−0.59	5.58	0.115	0.247
Trail making A (minutes)	1. App. 17	−0.02	0.03	−0.08	0.04	0.618	0.809
	2. App. Recall	−0.02	0.03	−0.07	0.03	0.509	0.714
	3. EIS	−0.02	0.05	−0.10	0.07	0.727	0.880
	4. EIS Recall	−0.05	0.04	−0.13	0.03	0.223	0.431
	5. Year Days	−0.03	0.09	−0.20	0.14	0.750	0.893
	6. Year EIS	−0.02	0.10	−0.21	0.17	0.820	0.918

App = applicator (yes); EIS = exposure-intensity scores (beta coefficients, standard error, 95% confidence intervals (95% CI) and *P*-values). Continuous exposure assessment measures (#3-6) were normalized on a scale between 0 and 1 (x—min(x))/ (max(x) - min(x)) before the analysis. FDR = *P*-value corrected for false discovery rate; Green = *P*-value > 0.05; yellow = *P*-value > 0.1.

We no longer observed associations when the application status and information to derive EIS was recalled 2 years later. This is rather surprising as a good recall of glyphosate and mancozeb application status and the most commonly used PPE items in the recalled data was found (e.g. overall agreement >70% and area under the curve values > 0.7) (Mueller et al. 2022). However, over-reporting in the recalled data of glyphosate and mancozeb was found (prevalence ratio 1.28 and 1.33, respectively), which might have attenuated the associations.

To decrease such exposure bias, smallholder farmers should be encouraged to keep spray records, enabling them to assess historical pesticide use. At the same time, this should go hand in hand with supporting professionalism within these smallholder farming systems while increasing education and literacy levels.

Strengths and limitations

This paper presents a comparison of different exposure assessment methods based on self-reported information on the association of neurobehavioral outcomes in smallholder farmers in a low-income context. Also, the effect of recalled exposure estimates 2 years after the initial survey with a response of 84% of the initial study population is featured.

There are 2 noteworthy limitations: (i) only the effect of single pesticide exposure (glyphosate and mancozeb in separate models) was assessed and only adjusted for co-exposure of any pesticide application in the past year while not specifically addressing the confounding by other specific pesticide active ingredients. Indeed, there might be mixture effects, which were addressed in our earlier publication using Bayesian Model Averaging models (Fuhrimann et al. 2021), but these did not facilitate an easy comparison between different exposure measures. Of note, the prediction direction did not change between the single active ingredients models featured here and what was previously published; and (ii) only the effect of recalled application status (yes/no) and recalled EIS was assessed because use frequency could not be added to the questionnaire for time reasons in 2019; (iii) original estimates for mancozeb exposures are not showing any evidence of an effect; hence, the measurement error impact is much more challenging to discern and was therefore not further discussed.

Conclusion

The exposure–response associations between self-reported glyphosate exposure and neurobehavioral test scores pointed in the same direction across the different exposure measures (e.g. binary). However, exposure estimates based on recalled information resulted in associations no longer different from the null despite a recall period of only 2 years. Future epidemiological studies on the topic may consider multiple exposure assessments of shorter recall periods (e.g. 1 year or the past growing season) and critically evaluate the potential impacts of exposure bias in the observed exposure–response associations when exposure is self-reported.

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Conflict of interest

No conflicts of interest are declared. The contents, including any opinions and/or conclusions expressed in this manuscript, are those of the authors alone and do not necessarily reflect the opinions or policies of the organizations to which they are employed.

Data availability

The full data underlying this article cannot be shared publicly for ethical reasonas to protect the privacy of individuals that participated in the study. *Summary data or selected anonymized data can be shared on reasonable request to the corresponding author.*

Supplementary material

Supplementary material is available at *Annals of Work Exposures and Health* online.

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