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Self-reported and urinary biomarker-based measures of exposure to glyphosate and mancozeb and sleep problems among smallholder farmers in Uganda

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ABSTRACT

Objective: We aim to showcase the impact of applying eight different self-reported and urinary biomarker-based exposure measures for glyphosate and mancozeb on the association with sleep problems in a study among 253 smallholder farmers in Uganda.

Methods: The questionnaire-based exposure measures included: (1) the number of application days of any pesticide in the last 7 days (never, 1–2; >2 days) and six glyphosate and mancozeb-specific measures: (2) application status over the last 12 months (yes/no), (3) recent application status (never, last 7 days and last 12 months), (4) the number of application days last 12 months, (5) average exposure-intensity scores (EIS) and (6) EIS-weighted number of application days in last 12 months. Based on 384 repeated urinary biomarker concentrations of ethylene thiourea (ETU) and glyphosate from 84 farmers, we also estimated (7) average biomarker concentrations for all 253 farmers. Also in the 84 farmers the measured pre-work and post-work biomarker concentrations were used (8). Multivariable logistic regression models were used to assess the association between the exposure measures and selected Medical Outcomes Study Sleep Scale (MOS-SS) indices (6-item, sleep inadequacy and snoring).

Results: We observed positive associations between (1) any pesticide application in the last 7 days with all three MOS-SS indices. Glyphosate application in the last 7 days (3) and mancozeb application in the last 12 months (3) were associated with the 6-item sleep problem index. The estimated average urinary glyphosate concentrations showed an exposure–response association with the 6-item sleep problem index and sleep inadequacy in the same direction as based on self-reported glyphosate application in the last 7 days. In the analysis with the subset of 84 farmers, both measured and modelled post-work urinary glyphosate concentration showed an association with snoring.

Conclusions: Self-reported, estimated average biomarker concentrations and measured urinary biomarker exposure measures of glyphosate and mancozeb showed similar exposure–response associations with sleep outcomes.

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1. Introduction

Occupational exposure to pesticides can be assessed via different methods (Ohlander et al. 2020), which can result in an array of possible exposure measures that can be used in epidemiological analysis studying associations with detrimental health effects. These measures can include indirect questionnaire-based exposure measures which range from crude (e.g., job title or binary application status (yes/no)) to complex (e.g., exposure-intensity scores (EIS) based on algorithms that reflect different exposure pathways) as well as direct measurement of human external and internal exposure concentrations.

To inform future epidemiological studies about which exposure measures to apply in the design and analysis of health data, comparative assessments are needed to understand their implication on interpreting the measured associations. Such sensitivity analyses have been successfully performed for other occupational exposures (Kromhout et al. 1997, 1999; Loomis et al. 1998) but are absent for occupational exposure to pesticides and health outcomes.

This study aims to compare the performance of a variety of pesticide exposure assessment methods and resulting exposure measures when applied in an epidemiological study focusing on acute sleep perturbations. We used published data from Ugandan smallholder farmers focusing on the association between a single self-reported exposure measure for glyphosate and mancozeb exposure and Medical Outcomes Study Sleep Scale (MOS-SS) sleep perturbations (Fuhrimann et al. 2022).

2. Methods

2.1. Study design

We used previously published data from 253 Ugandan smallholder farmers who were enrolled in a follow-up survey of the "PESticide use in TROPical settings" (PESTROP)-Uganda cohort between October and December 2019 (Fuhrimann et al. 2022). PESTROP-Uganda cohort was initiated with 305 smallholder farmers in 2017. The aim of PESTROP is to assess the possible gaps between pesticide use-related environmental exposure, human health effects, and institutional determinants in two tropical agricultural settings in Uganda and Costa Rica (Winkler et al., 2019). Compared to the previous study, we compare the performance of several exposure measures based either on self-reported information or estimated average urinary biomarker concentrations of glyphosate and ethylene thiourea (ETU; a biomarker of mancozeb), which were not available at the time of the initial publication. This assessment of the performance of alternative exposure measures from different assessment methods is part of the IMPRESS study (Ohlander et al. 2020, Jones et al. 2020; Mueller et al. 2022a; Mueller et al., 2022b). A sister manuscript focuses on several pesticide exposure measures based on self-reports and the effect of recalled information on chronic neurobehavioral outcomes in the same population in Uganda (Fuhrimann et al. under review).

We chose to assess glyphosate and mancozeb associations with sleep problems for the following reasons: (1) in our previous publication (Fuhrimann et al. 2022) we observed increased ORs for the association between the sleep problem index 6-item and mancozeb exposure during the past year 2.28 [1.12–4.71] and past week 2.51 [0.86–7.55] and glyphosate exposure during the past week 3.75 [1.24–11.8] compared to non-applicators; (2) both pesticides were applied by more than half of our study population (Fuhrimann et al. 2022); and (3) Mancozeb's metabolite ETU was associated with a disruption of thyroid functioning which could result in a disrupted circadian rhythm (Costa et al. 2008; Leemans et al. 2019; Richardson et al. 2019). Glyphosate exposure was suggested to cause neurotoxic effects, including depressive behavior (Ait Bali et al. 2017; Cattani et al. 2017). Important to note is also that we assess two active ingredients which are fundamentally different in terms of their application and physical–chemical properties (e.g., half-life in soil) or metabolism in the human bodies (excretion rates and biomarkers). These differences are inherited when assessing pesticide exposure and health outcomes, and hence, mancozeb and glyphosate are representative of this (PPDB 2023).

2.2. Questionnaire interview

All farmers responded to a face-to-face interview-administered questionnaire conducted at their farm, by a trained research assistant, in the farmer's preferred language (English or Luganda). Information was obtained on socio-demographic variables (e.g., sex, age, education), their pesticide use frequency and different exposure modifying factors (e.g., use of personal protective equipment (PPE) and hygienic practices) in the last 7 days and 12 months before the interview, relevant to construct a pesticide exposure-intensity score (Fuhrimann et al. 2020, 2021). The questionnaire was previously developed, adapted, and used in different contexts in low-to-middle income countries (LMICs), including a survey with the same study population in 2017 (Fuhrimann et al., 2021, Hansen et al., 2019, 2021, 2020; Staudacher et al., 2020).

2.3. Urine collection and analysis

384 urine samples were collected from a sub-sample of 84 farmers in the morning (pre) and the evening (post) of a working day with and without specific pesticide application. The 84 farmers were selected based on their intention to spray the pesticides glyphosate, mancozeb, chlorpyrifos or any pyrethroids during the study period (Jones et al. 2020). We used common urine sample collection and analytical methods, which were described in the IMPRESS study protocol (Jones et al., 2020). Urine samples were immediately stored in the participant's refrigerator before being collected by researchers in the field and then frozen by the local research teams prior to courier shipment to the UK for analysis. On receipt, samples were stored frozen (<-15 °C) until analysis. Samples were shipped to the UK with temperature loggers and all samples remained frozen during transit.

Samples were analysed at the Health and Safety Executive (HSE), in the UK for the biomarkers glyphosate and ethylenethiourea (ETU, a marker for mancozeb and other ethylene dithiocarbamates as well as ethylenethiourea itself). Biomarker levels were adjusted for creatinine concentrations. The methods of biomarker analysis were established previously: ETU adapted from Jones et al. (2010) and glyphosate was as described in Connolly et al. (2018). Urinary excretion half-lives have been reported to range from 20 (Lindh et al. 2008) to 100 h (Kurttio et al. 1990) and 5.5 (Connolly et al. 2019) to 9.0 h (Zoller et al. 2020) hours for ETU and glyphosate, respectively. The limit of quantification (LoQ), the minimum concentration at which the compound can be measured reliably, was set at 0.5 μg/L for both compounds. The HSE laboratory has established internal quality control systems for all the proposed methods and, in addition, has successfully participated in external quality assurance at environmental levels (https://www.g-equas.de) for glyphosate and other pesticides. There is currently no ETU biomarker quality assurance scheme. Published data, quality control or sample stability data show that all analytes are stable at < -15 °C for more than two years.

The analytical method for ETU is presented in supplementary material (S1), along with sample stability data. Glyphosate was analysed according to the method of Connolly et al. (2018) and sample stability data has been reported in Mueller et al (under review).

2.4. Exposure assessment methods and measures

We assessed eight different exposure measures based on either self-reported information or urinary biomarker measurements. The questionnaire-based exposure measures included: (1) number of application days of any pesticide in the last 7 days (never, 1–2; >2 days) and six glyphosate- and mancozeb-specific measures: (2) application status over the last 12 months (yes/no), (3) recent application status (never, last 7 days and last 12 months), (4) number of application days last 12

months, (5) average exposure-intensity scores (EIS) derived from a semi-quantitative exposure algorithm and (6) EIS-weighted number of application days in last 12 months. Based on modelled 384 repeated urinary biomarker concentrations of ethylene thiourea (ETU) and glyphosate from 84 farmers we also estimated (7) average biomarker concentrations for all 253 farmers. Finally, (8) in a sensitivity analysis with the 84 farmers who provided urine, the measured pre-work and post-work biomarker concentrations were used from the study visit which was 7 days before the sleep problem outcome assessment.

2.4.1. Measured and estimated exposure based on urinary biomarker levels

Descriptive analyses (boxplots) were performed by exposure groups and potential exposure affecting factors like "application timing" (over last 12 months, over last 7 days, on the day of sample collection, none), "personal protective equipment (PPE)", "age", "sex" and "literacy". Consequently, linear mixed models were developed with log-transformed creatinine-adjusted ETU and glyphosate concentrations in urine as dependent variables based on the 384 biomonitoring measurements from 84 farmers. For these models, biomonitoring measurements below LoQ were imputed. Imputation was done using maximum-likelihood based on log10 transformed pesticide values applying the R package "survival" (Lubin et al. 2004). Only statistical significant ($p < 0.05$) exposure affecting factors were kept in the multivariable linear mixed models. Based on the linear mixed models pre-work ETU levels and post-work glyphosate levels were estimated for each of the 253 study participants based on their personal characteristics. For the subgroup of 84 farmers we additionally used their actually measured biomarker values.

2.4.2. Exposure algorithm to estimate exposure-intensity scores

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 last 12 months with a range from 0 (no exposure) to 13 (highest exposure score), estimated using five exposure-modifying factors: (i) mixing of pesticide (MIX; score 5); (ii) applying pesticides outdoors using manual handheld knapsack sprayers, which is the case for all self-reported pesticide applications in our study (APPLICATION; score 8); (iii) overall average protection achieved by PPE use, covering different body areas and accounting for differences in application frequency (PPE, score 0.14 to 1); (iv) time interval between pesticide application and change of clothes (CHANGE; score 0.7 to 1); and (v) time interval between application and shower (SHOWER; score 0.7 to 1).

2.5. Sleep problem outcomes

Three MOS-SS sleep problem indices (Hays and Stewart 1992) were used with a 1-week recall period. The MOS-SS is a 12-items questionnaire to assess key constructs of sleep. For this paper, we selected 6-item sleep problem index, as well as the sleep dimensions, sleep inadequacy (2-items) and snoring (1-item), which were associations with pesticide exposure observed in our previous paper, see for a detailed description Fuhrimann et al. (2022). The usual 1-month measurement period of the MOS-SS, was adapted to 1-week to investigate the potential acute effects of pesticide exposure. The 1-week recall period was validated and showed to be reliable in other studies (Sadosky et al. 2009). For example, as follows: "How often during the past four weeks did you …" was changed to "How many days in the last week did you …". The original 6-point Likert scale from "not at all" to "all the time" was changed to an 8-point Likert scale from 0 to 7 days. To calculate the indices and dimensions, the number of days reported for each of the questions (0 to 7 days) was proportionally transformed to a scale from 0 to 100, with a high score indicating sleep problems. Scores were then dichotomized for statistical analysis, and a cut-off value of 30 was used based on the distribution of our data (considered symptomatic).

2.6. Statistical analyses

We described exposure measures using descriptive statistics and compared continuous exposure measures between each other using spearman correlation coefficients (Table S2 and Table S3). We compared the associations of the pesticide exposure days during the last 7 days and the six glyphosate and mancozeb exposure measures with the three sleep problem indices using multivariable logistic regression models. Glyphosate and mancozeb measures were always added in the same model and hence mutually adjusted. All models were adjusted for the following confounders: age (split in tertiles: $\leq$44, 45–57, and $\geq$ 58 years), sex, alcohol consumption (non-current drinker and current drinker), body mass index (BMI; $<$ 18.5, 18.5 – 24.9 and $\geq$ 25.0), and sleep disruption during the past week (yes/no).

As a sensitivity analysis, we applied the same models also to the subpopulation of 84 farmers who provided a urine sample. For this sub-set, the association with the actual measured pre- and post-work urine levels collected 7-days before the health assessment were also used as measures of exposure.

To compare associations, continuous measures were standardized ((x – quantile(Q)2)/(Q3-Q1)) before the 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., odds ratio (OR), standard errors, 95 % confidence intervals (95 % CI, *p*-values). Across all analyses, *p*-values below 0.05 were considered statistically significant, and *p*-values below 0.1 were considered noteworthy). Statistical analyses were done in R (Foundation for Statistical Computing, version 3.6.3, RStudio version 1.2).

2.7. Ethical clearance

The study was approved by the Higher Degrees, Research and Ethics Committee of Makerere University School of Public Health, Uganda (reference number 719). Each participant signed a written informed consent form.

3. Results

3.1. Sociodemographic characteristics of the study population

A total of 253 farmers were enrolled in the study in 2019 (Table 1). The study population consisted of 41 % females, 70 % of the whole study population did not consume alcohol and 66 % had a normal BMI (18.5–24.9). The median (interquartile range (IQR)) age was 52 (19)

Table 1
Socio-demographic characteristics of participating the 253 farmers and the subset of 84 farmers who provided urine samples in Wakiso, Uganda, 2019 [n (%)].

Characteristics		All 253 farmers	Sub-set 84 farmers
Number of farmers		253 (100)	84 (100)
Sex	Male	149 (58.9)	66 (78.6)
	Female	104 (41.1)	18 (21.4)
Age (years)	$\leq$ 39	85 (33.6)	36 (42.9)
	40–49	86 (34)	33 (39.3)
	$\geq$ 50	82 (32.4)	15 (17.9)
Currently consuming alcohol	No	178 (70.4)	55 (65.5)
	Yes	75 (29.6)	29 (34.5)
Body Mass Index (BMI)	Normal (18.5 – 24.9)	167 (66)	61 (72.6)
	Low ($<$18.5)	14 (5.5)	5 (6.0)
	High ($\geq$25.0)	72 (28.5)	18 (21.4)
Sleep disruption during assessment week*	No	64 (25.3)	20 (23.8)
	Yes	189 (74.7)	64 (76.2)

*Sleep disruption variable consists of six reasons for sleep disruption (yes/no): mosquitos, bedbugs, noise, infectious disease, wearing activity meter and other reasons.

years. The subset of 84 farmers who provided urine samples had a similar distribution for most characteristics. Of note, there were only about half as many female participants (21.4 %) in the sub-set than in the complete data set (41.1 %).

3.2. Exposure assessment measures

3.2.1. Self-reported pesticide exposure measures

There were more glyphosate applicators than mancozeb applicators among those smallholder farmers (56 % versus 38 %) in the last 12 months before the study (Table 2). Of these applicators, 31 (12.3 %) and 32 (12.6 %) applied the active ingredients also in the week before the visits, respectively. On average (median), the applicators reported one glyphosate application day (IQR 1) and eight application days of mancozeb (IQR 22) in the year before the study. In the subpopulation, there were considerably more glyphosate (85 %) and mancozeb (71.2 %) applicators in last 12 month before the study as well as in the week before the study, 49.4 % and 34.1 %, respectively than in the total population. Average EIS did not differ in glyphosate and mancozeb applicators, both had a median score of 0.45 (IQR 0.22).

3.2.2. Estimated average biomarker concentrations

Within the 380 urine samples, 83 % ETU concentrations were above the LoQ of 0.5 μg/l. ETU pre-work levels were 37 % higher than post-work levels (Table 3a). Frequent users of mancozeb (>12 days per year) had three times higher levels of ETU than non-users, and less frequent users (1–12 days per year) had 50 % higher levels than non-users (Table 4). Farmers older than 50 years had 35 % lower levels of ETU, while males had 21 % lower levels than females. Literate farmers had 51 % lower levels of ETU in their urine than illiterate farmers. No effect was seen for the use of PPE per body part (i.e. protection of hands, upper-body, legs, feet's, moth; yes/no) or calculated as PPE intensity scores as calculated for the EIS, acreage of agricultural land, number of years working as an applicator and type of application equipment (all knapsacks).

Of the glyphosate samples, 41.3% were above the LoQ of 0.5 μg/l (Table 3a). For glyphosate, post-work levels were 35 % higher than pre-work levels. Frequent users of glyphosate (>1 days per year) resulted in six times higher levels of glyphosate than non-users, and less frequent users (1 day per year) had four times higher levels than non-users (Table 5). Males had 21 % lower levels compared to females and literate farmers 68 % lower levels than illiterate farmers. As for ETU no effect was seen for PPE use, acreage, number of years working as an applicator and type of application equipment.

From Table 3b it can be observed that average post-work glyphosate concentrations varied up to a factor of 80 while for ETU concentrations it was somewhat less (47-fold). However the within-day variability was by far the largest component followed by between visits variability.

3.3. Associations between pesticide exposure measures and sleep problem indices

The forest plots in Fig. 1 visualize the associations between the different exposure measures and the three sleep problem indices. Table S4 shows the details effect numbers of the obtained associations. We observed positive relationships between the application of any pesticide use in the last 7 days and all three sleep problem indices. For active ingredient specific exposure measures the results were somewhat less clear-cut. For glyphosate, a positive association was observed for exposure in the preceding 7 days (OR 4.0; CI 95 % 1.6 and 10.1), while for mancozeb, a positive association with the 6-item sleep problem index was observed for exposure over the last 12 months (OR 2.4; CI 95 % 1.2 and 4.7) as well as for the last 7 days (OR 2.5; CI 95 % 1.0 and 5.9). Other, noteworthy associations with the 6-item sleep problem index were seen for the modelled post-shift urinary glyphosate concentrations (OR 1.6; CI 95 % 1.0 and 1.3) as well as for self-reported mancozeb EIS

Table 2
Self-reported and biomarker-based exposure measures for 253 smallholder farmers and a sub-population of 84 farmers who provided urine samples in Wakiso, Uganda.

Exposure 1	Abbreviation	Unit	253 Farmers			Sub-sample 84 farmers		
			Any pesticides	Glyphosate	Mancozeb	Any pesticides	Glyphosate	Mancozeb
			N (%)	N (%) or median (IQR)	N (%) or median (IQR)		N (%) or median (IQR)	N (%) or median (IQR)
1) Any pesticide spraying days over the last 7 days		0 days	135 (53.3)			0 (0)		
		>2 days	32 (12.6)			54 (64.3)		
		1 or 2 days	86 (33.9)			30 (35.7)		
Active ingredient-specific exposure measures								
2) Application	Last 12 months	Yes		142 (56.1)	97 (38.3)		72 (85.0)	61 (71.2)
3) Application	Last 7 days	Yes		31 (12.3)	32 (12.6)		42 (49.4)	29 (34.1)
	Last 12 months but not last 7 days	Yes		111 (43.9)	65 (25.7)		30 (35.3)	32 (37.6)
4) Yearly application days	Year Days	Days		1 (1)	8 (22)		2 (2)	9 (21)
5) Exposure intensity scores (EIS)	EIS	Scores 0–13		0.45 (0.22)	0.44 (0.22)		0.45 (0.19)	0.44 (0.19)
6) Yearly exposure-intensity scores	Year Days EIS	EIS weighted days		7.2 (7.1)	35.7 (115.1)		8.3 (0.4)	51.7 (135.6)
7) Estimated average biomarker concentrations (based on 380 repeated samples from the 84 farmers)*		Levels (μg/g creatinine)		0.1 (0.07)[A]	4.4 (4.1)[B]		0.1 (0.3)[A]	6.6 (6.9)[B]
8) Measured biomarker concentration (84 farmers urine samples taken one week before sleep assessment)*								
Pre-work (only visit 1, N = 84)							0.2 (0.5)	7.3 (11.3)
Post-work (only visit 1, N = 84)							0.3 (2.8)	4.3 (11.4)

*Detail summary statistics of all collected 380 urine samples which were used for estimating average biomarker concentrations, can be found in Table 3.
[A] post-work, [B] pre-work.

Table 3
(a) summary of measured urinary biomarker concentrations of the three separate visits resulting in a total of 380 samples (i.e., 190 days with pre- and post-work urine samples) of the 84 smallholder farmers in Wakiso, Uganda. **(b)** shows **the nested random components (farmer, visit within farmer and pre-post work (within-day))**.

(a) Biomarker	Visit	Work shift	No. samples	% above LOD	Mean	SD	Median	IQR	Max
Mancozeb (ETU)	Total samples		380	83.4	12.81	18.19	6.54	13.24	143.08
	1	POST-WORK	84	15.5	11.57	20.52	4.25	11.48	143.08
		PRE-WORK	84	14.3	12.70	17.59	7.29	11.25	98.28
	2	POST-WORK	80	17.5	12.19	16.93	5.75	12.22	69.36
		PRE-WORK	80	18.8	13.44	18.75	6.32	13.64	82.59
	3	POST-WORK	26	19.2	11.53	12.38	7.91	13.48	51.40
		PRE-WORK	26	15.4	18.37	19.59	13.04	19.94	86.03
Glyphosate	Total samples		390	41.3	11.50	171.39	0.20	0.66	3361.34
	1	POST-WORK	84	38.1	8.30	24.20	0.29	2.79	156.30
		PRE-WORK	84	66.7	1.24	3.62	0.15	0.53	24.23
	2	POST-WORK	80	57.5	1.86	7.40	0.24	0.93	62.65
		PRE-WORK	80	68.8	41.79	371.12	0.19	0.38	3361.34
	3	POST-WORK	26	53.8	1.72	4.31	0.20	0.49	20.06
		PRE-WORK	26	76.9	0.50	1.23	0.10	0.34	6.07
(b)		K = 84; N = 380	**Glyphosate**			**Mancozeb (ETU)**			
			between farmer	1.25	21 %	between farmer	0.96	34 %	
			between visit (nested)	1.69	29 %	between visit (nested)	1.17	41 %	
			within-day	2.96	50 %	within-day	0.72	25 %	
			total	5.90		total	2.86		
			GSD_{total}	11.34		GSD_{total}	5.42		
			$GSD_{betwfarm}$	3.06		$GSD_{betwfarm}$	2.67		
			$_{bf}R_{95}$	80		$_{bf}R_{95}$	47		
			ICC	0.2		ICC	0.3		

GSD = geometric standard deviation; $_{bf}R_{95}$ = between farmer fold range in average biomarker concentration;ICC = Intraclass Correlation Coefficient.
For the 253 study participants, pre-work ETU levels and post-work glyphosate levels were estimated based on the linear mixed models described above, taking into account frequency of use and literacy for both ETU and glyphosate and sex and age for ETU only. It resulted in distinct differences in assigned exposures with larger contrasts in biomarker levels for glyphosate than ETU (Tables 4 and 5).
The correlation between modelled average post-work levels and individual post work levels of glyphosate was low ($r_s = 0.09$) (see S2). Also for pre-work ETU levels the correlation between modelled and individual pre-work levels was low ($r_s = 0.18$) (See S2).

Table 4
Estimated pre-work ETU levels (μg/g creatinine) by application frequency, sex, age group and literacy status.

			ETU μg/g creatinine	
Spray frequency	Gender	Age group	Illiterate	Literate
Not sprayed	female	<50 years	8.7	4.4
Last 12 months		≥50 years	5.5	2.8
	male	<50 years	6.4	3.2
		≥50 years	4.1	2.8
Low (1–12 days per year)	female	<50 years	18.5	9.3
		≥50 years	11.7	5.9
	male	<50 years	13.7	6.9
		≥50 years	8.7	4.4
High (>12 days per year)	female	<50 years	27.9	14
		≥50 years	17.7	8.9
	male	<50 years	20.6	10.4
		≥50 years	13.1	6.6

Table 5
Estimate post-work urinary glyphosate levels (μg/g creatinine) by application frequency and literacy status.

	Glyphosate μg/g creatinine	
Spray frequency	Illiterate	literate
Not sprayed Last 12 months	0.13	0.06
Low (1 day per year)	0.24	0.1
High (>1 days per year)	0.9	0.4

(OR 1.3; CI 95 % 1.0 and 2.5). Modelled pre-work urinary ETU concentrations were positively associated with the 6-item sleep problem index but did not reach statistical significance (OR 1.4; CI 95 % 1.0 and 2.0). Furthermore, self-reported glyphosate use during the preceding 7 days indicated a positive association with snoring that did not reach statistical significance (OR 3.52; CI 95 % 1.0 and 12.6). Modelled pre-work urinary ETU concentrations appeared to be associated with sleep inadequacy (OR 1.5; CI 95 % 1.0 and 2.2).

In the sensitivity analysis with the subset of 84 farmers (Fig. 2 and Table S5), both actual measured and modelled post-work urinary glyphosate concentration showed an association with snoring (OR 4.4 (CI 95 % 1.2 and 19.4) and OR 1.1 (CI 95 % 1.1 and 1.3) respectively).

4. Discussion

We compared the impact of different self-reported and biomarker-based exposure measures of glyphosate and mancozeb, on the associations with sleep problems among smallholder farmers in Uganda.

We observed pesticide-specific associations for different exposure time windows. For example, for glyphosate, the association with the 6-item sleep problem index was stronger for recent exposure (application over the last 7 days), while for mancozeb the association was stronger with exposure over a longer period (exposure over the last 12 months but not in the preceding seven days). The more detailed exposure measures (number of application days, algorithm-based exposure intensity scores) did not result in statistically significant associations. The modelled urinary glyphosate and ETU concentrations both showed positive associations with the 6-item sleep problem index but these associations did not reach statistical significance. In addition, in the sensitivity analysis, only higher pre-work urinary ETU concentrations were associated with higher odds for sleep inadequacy.

These findings can be partly explained by the frequency of use and the application purpose of the two pesticides. Mancozeb is used frequently (median eight applications per year) and is also used for post-harvest applications (e.g., on tomatoes (Atuhaire et al. 2017)). Exposure of smallholder farmers is assumed to be high via direct (application), and via indirect routes of exposure (re-entry work, food consumption) and will be relatively constant over time, which will make disentangling of recent exposure from longer-term exposure difficult. For the herbicide glyphosate the picture is quite different. It is less frequently used (median of one application per year, mainly at the beginning of the season to

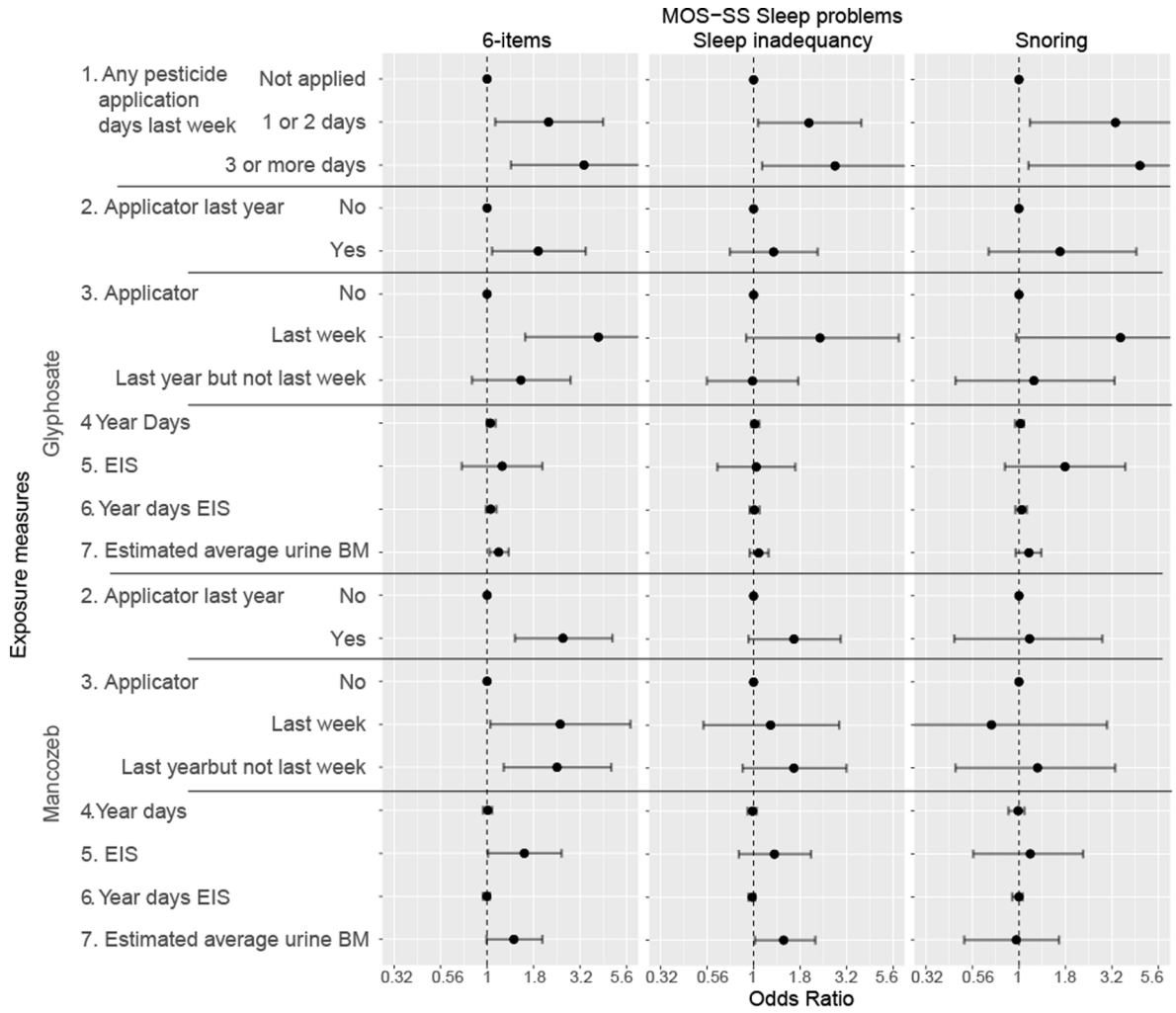

Fig. 1. Forest plot showing odds ratios and 95 % confidence intervals (on a log10 transformed scale) estimated for different multivariable models for all 253 farmers, including the number of any pesticide spray days in the last 7 days and six other glyphosate and mancozeb-specific self-reported and predicted exposure measures and their associations with three MOS-SS sleep problems indices. EIS = exposure-intensity scores; BM = biomarker. Continuous exposure measures (#4 to 7) were standardized ((x – quantile(Q)2)/(Q3-Q1)) before the analysis.

clear the field from weeds (Staudacher et al. 2020)) and therefore, indirect exposure via re-entry work is less likely.

Including longer-term cumulative exposure measures (such as number of application days or EIS) in the statistical analysis did not result in stronger associations between exposure and outcomes. This might be partly explained by the more acute nature of sleep problems. This is different for more chronic health effects like neurobehavioral outcomes, which we assessed in a parallel publication and for which we observed the strongest association for the more detailed longer-term exposure measures (yearly pesticide use days and EIS-adjusted yearly pesticide use days; Fuhrimann et al. under review).

The modelled urinary biomarker concentrations confirmed the associations with the 6-item sleep problem index and sleep inadequacy. Sensitivity analyses with the actual pre- and post-workday biomarker concentrations revealed trends pointing in the same direction as based on self-reported glyphosate application in the last 7 days. The predicted and measured urinary glyphosate biomarkers performed in general better than the ETU biomarkers most likely to larger between farmer differences in glyphosate exposure than in mancozeb exposure in this population.

4.1. Strengths and limitations

We compared the performance of a comprehensive set of different exposure measures based on self-reported exposure methods and biomonitoring in the analysis of the association of exposure to glyphosate and mancozeb and sleep problems. The comparison clearly showed how different exposure measures can result in different interpretations of the observed epidemiological effects. Overall, results for the glyphosate and mancozeb exposure measures showed similar associations with sleep outcomes as observed with self-reported and urinary biomarker-based exposure measures. Particularly using estimated average biomarker concentrations based on modelled urinary pesticide biomarkers from a sub-population and self-reported information on observed determinants of urinary biomarkers enabled estimation of biomarker-based estimates of exposure for the entire population. This group-based approach is known to result in Berkson-type error, resulting in unbiased risk estimates but with less precision (Armstrong, 1998; Tielemans et al. 1998). Our sensitivity analysis, in which we compared modelled with measured urine biomarker exposure measures, clearly showed this effect (Fig. 2). These estimated long-term average biomarker concentrations (i.e., as a proxy for cumulative long-term exposure) based on modelled urinary biomarker concentrations could be employed in more future epidemiological studies of occupational exposure to pesticides. It will overcome the predominance of temporal variability and minimize non-differential exposure misclassification (Preller et al. 1995). In most occupational pesticide exposure scenarios, the temporal component of exposure variability will outweigh the between study subject variability, as shown

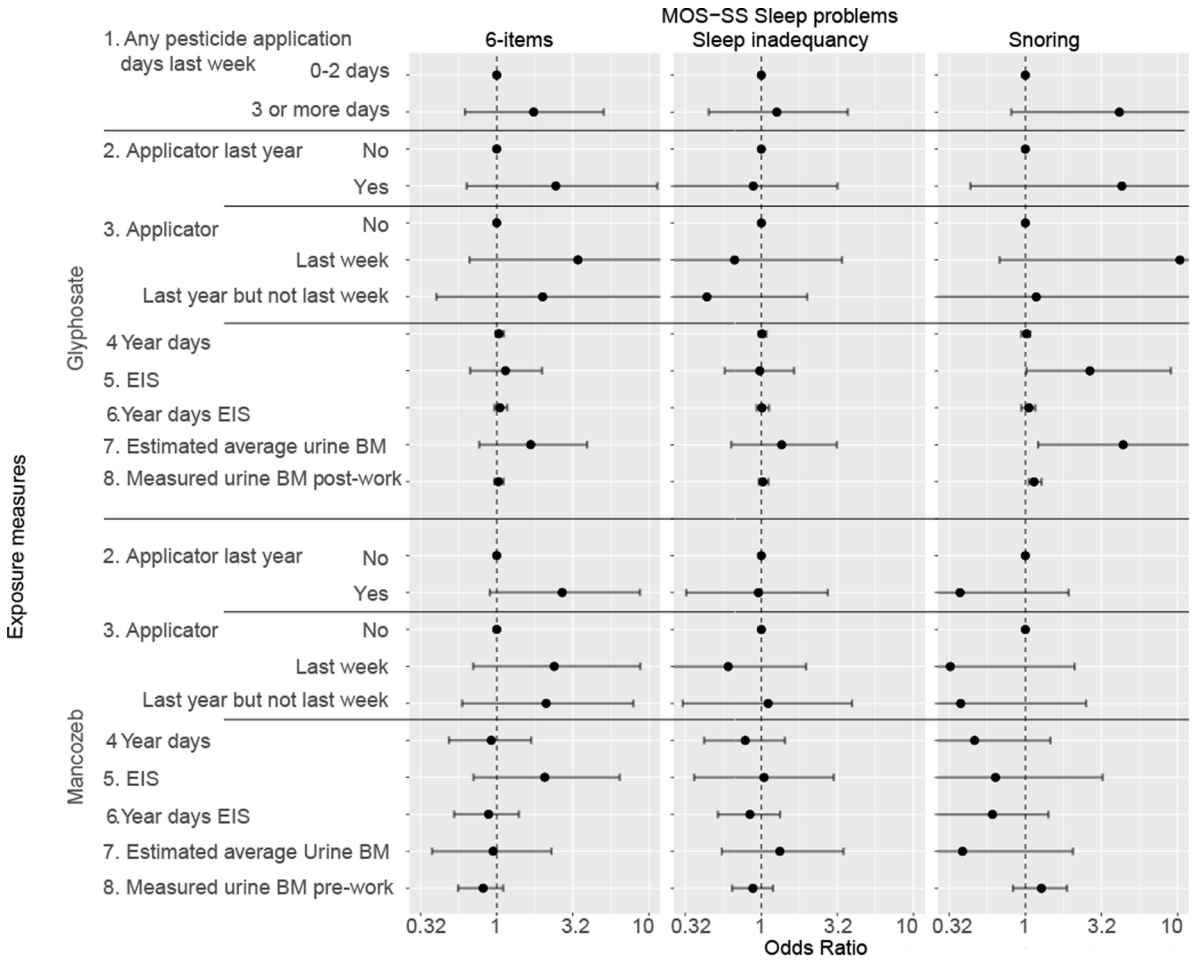

Fig. 2. shows odds ratio and 95 % confidence intervals (on a log10 transformed scale) estimated for different multivariable models based on the sub-set of 84 farmers who provided urine samples. The plot includes the number of pesticide spray days in the last 7 days and eight different glyphosate and mancozeb-specific self-reported exposure of measures, predicted, pre- and post-shift measured biomarker levels and their association with three MOS-SS sleep problems indices. EIS = exposure-intensity scores; BM = biomarker. Continuous exposure measures (#4 to 8) were standardized ((x – quantile(Q)2)/(Q3-Q1)) before the analysis.

here for glyphosate and mancozeb. A model approach with group-based exposure assignment will be necessary when enrolling a study population.

A limitation of our study is that we only assessed the associations of specific exposure to glyphosate and mancozeb but not for other pesticides used in that week or year (e.g., organophosphates or pyrethroids) (Fuhrimann et al. 2022).

5. Conclusion

Using more detailed exposure measures based on self-reported exposure methods or estimated urinary biomarker concentrations did not unravel other associations with sleep problems than those solely based on reported frequency of any pesticide or specific pesticides over the last seven days.

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CRediT authorship contribution statement

Samuel Fuhrimann: Conceptualization, Visualization, Data curation, Formal analysis, Writing - original draft. **William Mueller:** Conceptualization. **Aggrey Atuhaire:** Conceptualization, Data curation, Writing - review & editing. **Johan Ohlander:** Conceptualization. **Aggrey Atuhaire:** Conceptualization, Data curation, Writing - review & editing. **Ruth Mubeezi:** Conceptualization. **Andrew Povey:** Conceptualization. **Ioannis Basinas:** Conceptualization, Funding acquisition, Writing - review & editing. **Martie van Tongeren:** Conceptualization, Funding acquisition, Writing - review & editing. **Kate Jones:** Conceptualization, Data curation, Funding acquisition, Writing - review & editing. **Craig Sams:** Data curation, Writing - review & editing. **Karen S. Galea:** Conceptualization, Funding acquisition, Writing - review & editing. **Hans Kromhout:** Conceptualization, Visualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no conflicting interests. The authors report funding from CropLife Europe, however, CropLife Europe did not influence the presentation of the findings or review the manuscript .

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108277.

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