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Pesticide Exposure and Oxidative Stress Biomarkers Among West Java Applicators

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Abstract

Pesticide exposure may induce oxidative stress, which subsequently contributes to the onset of chronic diseases. This study aimed to evaluate the association between pesticide exposure intensity and oxidative stress among pesticide applicators using urinary malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) as biomarkers. A total of 74 vegetable farmers who had actively applied pesticides for at least one year were included in this cross-sectional study. The cumulative exposure level (CEL) of pesticides was estimated using a validated quantitative method. Urinary MDA and 8-OHdG were analyzed at an accredited testing laboratory. The Mann-Whitney U test was used to assess differences in MDA and 8-OHdG levels in the study population by individual and occupational characteristics. The mean age was 51.2 years (SD 11.3). Pesticide applicators were exposed to multiple pesticide classes, and the use of complete PPE was low. Higher cumulative pesticide exposure (CEL) was associated with increased oxidative stress, reflected by elevated urinary MDA and 8-OHdG, with different biomarkers capturing distinct exposure dimensions. This finding shows the need for comprehensive preventive strategies that focus on reducing exposure intensity through safer work practices, complemented by the appropriate use of personal protective equipment and ongoing education.

Introduction

Pesticides are widely used worldwide, including in Indonesia, to increase crop productivity. However, agricultural workers' occupational exposure to these chemicals presents a significant public health concern, particularly among smallholders (Casida and Bryant, 2017). Pesticide applicators are frequently exposed through skin contact and inhalation during activities such as mixing, loading, spraying, and cleaning equipment. Such exposures often recur and persist over prolonged periods. Long-term exposure to synthetic pesticides has been linked to increased risk of various adverse health outcomes, including neurotoxicity, cancer, cardiovascular disorders, and endocrine disruption (Liem *et al*., 2024; Ojha *et al*., 2024; Ventura *et al*., 2016; Liem *et al*., 2023b; Ahmed *et al*., 2025). Previous studies have reported that pesticide exposure is associated with multiple adverse health outcomes in agricultural communities. Studies report a high prevalence of occupational skin diseases and peripheral neuropathy (Febriana *et al*., 2023). In addition, a higher risk of hypertension has been observed among exposed populations (Mulyana *et al*., 2020; Liem *et al*., 2023a), highlighting pesticide exposure as a significant public health concern.

A commonly proposed mechanism linking workplace pesticide exposure to health effects is that pesticide exposure increases

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oxidative stress, which subsequently contributes to the onset of chronic diseases. Accumulating evidence indicates that pesticide exposure may induce oxidative stress, a key biological mechanism underlying many pesticide-related health effects (Liu *et al.*, 2024; Janos *et al.*, 2023; Silva *et al.*, 2023; Sule *et al.*, 2022). Oxidative stress arises from an imbalance between oxidants and antioxidants, leading to excessive generation of reactive oxygen species (ROS) or impaired elimination of these radicals. Persistent oxidative stress can lead to lipid peroxidation, oxidative DNA damage, endothelial dysfunction, inflammation, and loss of normal cellular function, contributing to the development and progression of chronic disease. These molecular and cellular alterations are increasingly implicated in the initiation and progression of chronic diseases, including cardiovascular disease, neurodegenerative disorders, and carcinogenesis (Scioli *et al.*, 2020; Ledda *et al.*, 2021).

Oxidative stress is characterized by measurable biochemical changes, making it a useful early indicator of pesticide-induced biological effects. Malondialdehyde (MDA) and 8-hydroxy-2′ -deoxyguanosine (8-OHdG) are widely used biomarkers to assess oxidative stress. MDA reflects lipid peroxidation of cellular membranes, whereas 8-OHdG, which is formed through oxidative modification in DNA bases, indicates oxidative DNA damage. Elevated levels of these biomarkers have been reported in pesticide-exposed populations and are considered indicators of subclinical biological effects (Silva *et al.*, 2023; Liang *et al.*, 2026). Studies in agricultural populations have shown that higher spraying intensity is associated with increased urinary 8-OHdG (Putri *et al.*, 2025), and that pesticide metabolites are positively correlated with oxidative stress biomarkers (Lee *et al.*, 2017). These observations support the biological plausibility of the link between occupational pesticide exposure, oxidative stress, and long-term health risks.

Despite growing evidence linking pesticide exposure to oxidative stress, inconsistencies in the literature remain, partly due to limitations in exposure assessment. Many studies rely on single-exposure indicators that capture only one dimension of exposure. In actual farming environments, however, pesticide exposure is determined by the intensity, frequency, and cumulative duration of pesticide use over the working lifetime. West Java is one of Indonesia's major agricultural regions. Like other farming areas, smallholder farming in West Java is characterized by intensive farming and significant reliance on pesticides. This includes exposure to multiple types of pesticides with varying frequencies and durations, along with inadequate use of personal protective equipment during pesticide-handling activities, as previously reported (Liem *et al.*, 2024; Fizulmi and Wispriyono, 2023).

Evidence on biological effects, particularly oxidative stress biomarkers, remains limited in this population, despite high exposure potential. Therefore, studying pesticide exposure and its biological impacts among applicators in West Java provides important insights into the health risks faced by agricultural communities in Indonesia and similar settings. A cumulative exposure level gives a more comprehensive estimate of lifetime pesticide burden and may better explain oxidative stress–related biological responses. Accordingly, this study aimed to evaluate the association between pesticide exposure intensity and oxidative stress among pesticide applicators using urinary MDA and 8-OHdG as biomarkers. We hope our results will provide valuable information to improve evidence-based preventive strategies that protect agricultural workers' health.

Method

We conducted a cross-sectional study from July to August 2025 in Bandung Barat Regency, a center of vegetable production in West Java, Indonesia. Farming activities are relatively similar throughout the year in this study location; therefore, the timing of recruitment does not affect the assessment of actual exposure. A consecutive sampling method was used, and 74 vegetable farmers who had actively applied pesticides for at least 1 year were included in the study. This number exceeded the minimum sample size requirement of 68, calculated using a two-sample mean-comparison formula with 95% confidence and 80% statistical power. Participants in this study received a small remuneration for their involvement.

Information on sociodemographic characteristics, including age, marital status, educational background, and lifestyle factors such as smoking status, was obtained through interviews. We also collected several occupational characteristics, primarily related to agricultural activities, including the type of pesticide used, pesticide handling, and the use of personal protective equipment (PPE), through a structured interviewer-administered questionnaire. Weight and height were measured and subsequently used to calculate body mass index (BMI) in kg/m2.

The cumulative exposure level (CEL) of pesticides was estimated using a validated quantitative method originally developed by Dosemeci, which is effective in the Agricultural Health Study (Dosemeci *et al*., 2002). In this method, CEL was estimated as a function of pesticide exposure intensity, the lifetime years of pesticide use, and the number of days of spraying per year. Intensity level was measured as a function of several pesticide-handling practices, including application-related activities (e.g., mixing, spraying, washing used equipment), use of personal protective equipment, personal hygiene practices, and spill treatment, as described in the previous study (Dosemeci *et al*., 2002; Liem *et al*., 2021). Participants were categorized into three groups (low, medium, and high exposure) based on tertiles of cumulative exposure. The CEL was calculated according to the following equation:

CEL = IL x Duration x Frequency
IL = Intensity Level of pesticide exposure
Duration = Lifetime years of pesticide use
Frequency = Number of days spraying per year

Urinary malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were analyzed at Prodia Industrial Toxicology Laboratory, an ISO/IEC 17025–accredited testing laboratory in Indonesia. Spot urine samples of study participants were collected. Urine samples were placed in boxes with ice and transported to the laboratory, where they were stored at -20°C before analysis. Urinary malondialdehyde (MDA) was analyzed using Agilent high-performance liquid chromatography with diode-array detection (HPLC–DAD). The analytical method was based on the derivatization of MDA with 2,4-dinitrophenylhydrazine (DNPH) to form a stable chromophore suitable for chromatographic separation and detection. Separation was achieved on a Zorbax Eclipse Plus C18 column under isocratic conditions. The mobile phase consisted of 0.1% acetic acid in Milli-Q water and acetonitrile (1:1), delivered at a flow rate of 1.0 mL/min. Detection was performed at 307 nm with an injection volume of 10 μL. All chemicals were of analytical grade. Reagents used in the analysis included 1,1,3,3-tetraethoxypropane as the MDA precursor standard, acetonitrile, acetic acid, hydrochloric acid, ethyl acetate, methanol, and Milli-Q water.

Calibration standards were prepared from 1,1,3,3-tetraethoxypropane, which was hydrolyzed under acidic conditions to generate MDA. A primary standard solution was prepared and incubated at 60°C to ensure complete hydrolysis. This solution was further diluted to obtain a series of working standards covering a concentration range of 0.125 to 5 ppm. Each standard solution was derivatized with DNPH, incubated at 60°C, extracted with ethyl acetate, and evaporated under nitrogen. The residue was reconstituted in the mobile phase before HPLC analysis. Method validation demonstrated good analytical performance. The calibration curve (Figure 1) was highly linear across the studied concentration range, with a coefficient of determination (R^2) of 0.9982. This level of linearity indicates reliable quantitative performance of the method for determining urinary MDA under the applied analytical conditions. Urine samples were prepared using a procedure similar to that for derivatization and extraction. Briefly, 2.5 mL of the urine sample was mixed with DNPH solution and incubated to allow derivatization. After cooling, the samples were extracted twice with ethyl acetate. The combined organic phase was evaporated under a nitrogen stream, reconstituted with the mobile phase, and transferred into HPLC vials for analysis. Before analysis, the chromatographic column was conditioned with methanol. Quantification of urinary MDA was performed by comparing sample peak areas with those obtained from the calibration curve. Analytical results were expressed as MDA concentrations in urine (mg/L).

Urinary 8-OHdG was quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with an Agilent 1260 Infinity binary liquid chromatography system coupled to an Ultivo triple quadrupole mass spectrometer. Calibration was performed by constructing a calibration curve based on the relationship between instrument response and known concentrations of 8-OHdG. The calibration curve (Figure 2) shows excellent linearity over the concentration range of 1–100 μg/L with a coefficient of determination (R^2) of 0.9987, indicating solid analytical performance and reliable quantification. An analytical standard of 8-OHdG (Sigma-Aldrich) was used to prepare stock and working solutions. A primary stock solution was prepared by dissolving the 8-OHdG standard in Milli-Q water and thoroughly homogenizing the solution. This solution was diluted in series to obtain intermediate and working standards, yielding calibration levels ranging from 1 to 100 ppb. Chromatographic separation was conducted under isocratic conditions using a mobile phase consisting of Milli-Q water and methanol (80:20, v/v) containing 0.1% formic acid. Sample preparation was performed by protein precipitation. Briefly, the urine sample was mixed with acetonitrile, vortex-mixed, and centrifuged. The supernatant was transferred into an LC–MS vial and injected into the LC–MS/MS system for analysis. Before analysis, the analytical column was conditioned with methanol. Quantitative results were expressed as micrograms per liter (μg/L) under optimized LC–MS/MS operating conditions to ensure accurate and reproducible measurements.

Statistical analysis was performed using SPSS 20 for Windows. The characteristics of the study participants were described using

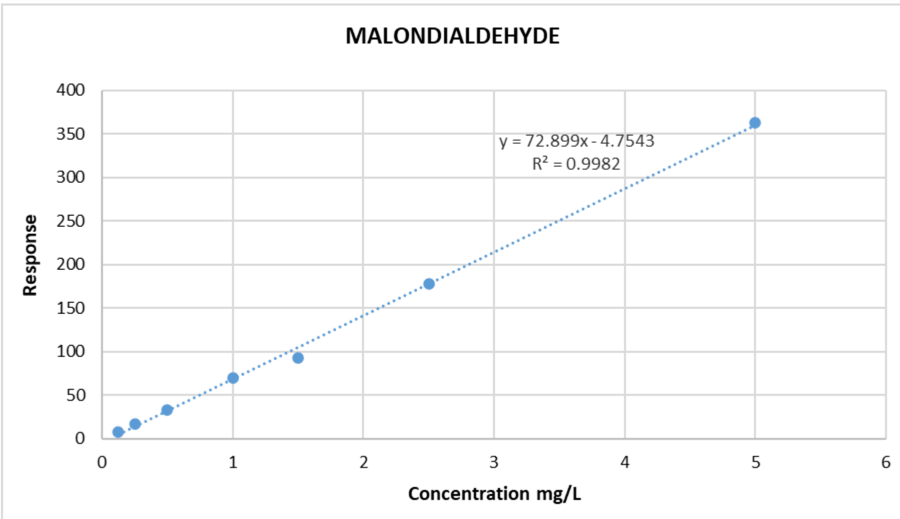

Figure 1. Calibration curve of malondialdehyde (MDA)

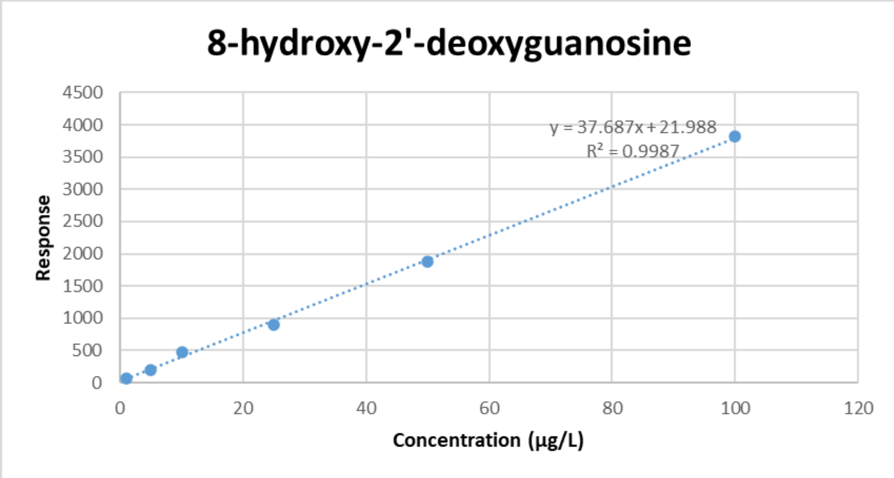

Figure 2. Calibration curve of 8-hydroxy-2′ -deoxyguanosine (8-OHdG)

mean (SD) or median (1st – 3rd quartiles) for continuous variables, and categorical variables were summarized using frequency distributions. The Mann-Whitney U test was used to assess differences in MDA and 8-OHdG levels in the study population by individual and occupational characteristics. All p-values were two-sided, with significance considered at $p < 0.05$. All methods were performed in accordance with the relevant guidelines and regulations. The Medical and Health Research Ethics Committee of the Faculty of Medicine and Health Sciences Universitas Kristen Krida Wacana approved the study protocol on June 11, 2025 (SLKE No: 1960/SLKE/IM/UKKW/FKIK/KEPK/VI/2025). Written informed consent was obtained from all participants.

Results and Discussion

A total of 74 pesticide applicators were included in this study. Most participants were male (95.9%), with only 16.2% having higher education, and 71.6% reporting active smoking. The demographic and occupational characteristics of pesticide applicators were detailed in Table 1.

Table 1. Demographic and Occupational Characteristics of Pesticide Applicators

Characteristics (n =74)	**Description**
Age (years)	51.2 (11.3)
Body mass index (kg/m^2)	23.9 (3.9)
Urinary malondialdehyde (mg/L)	0.15 (0.05 - 0.24)
Urinary 8-hydroxy-2’-deoxyguanosine (µg/L)	4.65 (2.61 - 6.65)
Number of days spraying per year (days)	104 (52 - 104)
Lifetime years of pesticide use (years)	20.0 (9.5 - 30.0)
Weekly duration of pesticide application (hours)	3.0 (2.0 - 4.3)
Number of different pesticides mixed in one application	2 (2 - 3)
Cumulative exposure level (x10^3)	17.52 (7.31 - 33.05)

Values are presented as mean (standard deviation) or median (1st quartile–3rd quartile)

Table 2. Use of Personal Protective Equipment During Pesticide Application

Application equipment	**Rare/never n (%)**	**Frequent/always n (%)**
Hat	5 (6.8)	69 (93.2)
Face mask	55 (74.3)	19 (25.7)
Respirators	74 (100)	0 (0)
Goggles	72 (97.3)	2 (2.7)
Long sleeves	8 (10.8)	66 (89.2)
Long pants	1 (1.4)	73 (98.6)
Chemical gloves	56 (75.7)	18 (24.3)
Boots	2 (2.7)	72 (97.3)

Source: Primary data (2025)

Table 2 shows the proportion of personal protective equipment (PPE) use among pesticide applicators during pesticide application. Overall, the use of complete PPE was low. While some applicators reported frequent use of basic protective items such as hats, boots, or long-sleeved clothing, the consistent use of key protective equipment, including masks or respirators and chemical gloves, was limited. Table 3 presents the types of pesticides used by the study participants. Fungicides were the most frequently used pesticide class, predominantly belonging to the dithiocarbamate and triazole groups, as evidenced by the widespread use of mancozeb and difenoconazole. Among insecticides, compounds from the avermectin, organophosphate, and synthetic pyrethroid classes were most reported. Our results indicate that pesticide applicators were exposed to multiple pesticide classes with distinct modes of action, indicating diverse exposure patterns. As shown in Table 4, participants with a weekly pesticide application duration of ≥4 hours had significantly higher urinary MDA levels. The number of pesticide types used in a single application did not show a statistically significant association with oxidative stress biomarkers, although individuals mixing more than 2 pesticide types tended to have higher 8-OHdG levels. Cumulative exposure level was significantly associated with both oxidative stress biomarkers.

The present study provides evidence of a significant association between pesticide exposure and oxidative stress among pesticide applicators, as demonstrated by higher urinary levels of malondialdehyde (MDA) and 8-hydroxy-2′ -deoxyguanosine (8-OHdG). These two biomarkers represent lipid peroxidation and oxidative DNA damage, respectively, and therefore capture complementary dimensions of oxidative injury. Notably, cumulative exposure level (CEL) showed stronger and more consistent associations with oxidative stress biomarkers than single-exposure indicators, i.e., weekly duration of pesticide application. Distinct patterns were observed for the two biomarkers: MDA was more strongly associated with indicators of recent and short-term exposure intensity, whereas 8-OHdG was more strongly associated with cumulative exposure measures, suggesting differential biological responses to short or recent versus long-term pesticide exposure. These outcomes indicate that different dimensions of pesticide exposure are differentially reflected by oxidative stress biomarkers.

Pesticide exposure has been shown to promote ROS production through multiple mechanisms, resulting in both lipid peroxidation and oxidative DNA damage (Kori *et al.*, 2019). Oxidative stress biomarkers differ in their biological targets and temporal responsiveness to reactive oxygen species (ROS). The finding that weekly pesticide application duration was significantly associated with MDA but not with 8-OHdG suggests that repeated short-term exposure episodes may preferentially induce lipid peroxidation through acute increases in reactive oxygen species. Malondialdehyde is a compound of lipid peroxidation and reflects

Table 3. Types of Pesticides Used by Study Participants

Active ingredient(s)	Utilization	n (%)
Mancozeb	Fungicide	35 (16.6)
Difenoconazole	Fungicide	27 (12.8)
Abamectin	Insecticide	25 (11.8)
Emamectin benzoate	Insecticide	15 (7.1)
Profenofos	Insecticide	15 (7.1)
Broflanilide	Insecticide	13 (6.2)
Propineb	Fungicide	13 (6.2)
Cypermethrin	Insecticide	11 (5.2)
Azoxystrobin	Fungicide	7 (3.3)
Fluxametamide	Insecticide	6 (2.8)
Parakuat diklorida	Herbicide	5 (2.4)
Chlorantraniriprole	Insecticide	4 (1.9)
Chlorotalonil	Fungicide	4 (1.9)
Chlorpyrifos	Insecticide	4 (1.9)
Maneb	Fungicide	4 (1.9)
Chlorfluazuron	Insecticide	3 (1.4)
Hexaconazole	Fungicide	3 (1.4)
Cymoxanil	Fungicide	2 (0.9)
Lambda cyhalothrin	Insecticide	2 (0.9)
Propiconazole	Fungicide	2 (0.9)
Spinosad	Insecticide	2 (0.9)
Thiamethoxam	Insecticide	2 (0.9)
Cartap hydrochloride	Insecticide	1 (0.5)
Chlorfenapyr	Insecticide	1 (0.5)
Dimehypo	Insecticide	1 (0.5)
Fipronil	Insecticide	1 (0.5)
Glyphosate	Herbicide	1 (0.5)
Methomyl	Insecticide	1 (0.5)
Propargite	Insecticide	1 (0.5)

Source: Primary data (2025)

oxidative damage to polyunsaturated fatty acids within cellular membranes (Cordiano *et al*., 2023). Therefore, MDA is widely used as a biomarker of oxidative stress, with levels increasing in response to elevated ROS, as lipid peroxidation can occur rapidly under oxidative conditions, making MDA a sensitive indicator of recent oxidative stress (Ito *et al*., 2019; Silva *et al*., 2023).

In contrast, excess ROS in organisms may also cause oxidative DNA damage. Damage is typically repaired primarily through the base excision repair pathway, and oxidized products are excreted in the urine. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is among the most studied oxidized metabolites and is considered a biomarker of oxidative DNA damage (Martinez-Moral and Kannan, 2019; Wang *et al*., 2018; Graille *et al*., 2020). The accumulation of oxidative DNA damage generally requires persistent ROS generation that exceeds cellular repair capacity over time, and urinary 8-OHdG is therefore commonly interpreted as a marker of longer-term oxidative injury and is more closely linked to sustained or cumulative oxidative stress (Silva *et al.*, 2023; Juan *et al.*, 2021; Sule *et al.*, 2022). Previous studies have similarly reported stronger associations between long-term pesticide exposure and possible chronic effects, including oxidative DNA damage, supporting the importance of cumulative exposure assessment to represent the complexity of the actual condition of occupational pesticide exposure (Ayala *et al.*, 2014; Silva *et al.*, 2023; Putri *et al.*, 2025; Liem *et al.*, 2023b).

Cumulative exposure level (CEL) integrates multiple dimensions of pesticide exposure, including exposure intensity, frequency, and duration of pesticide use over the working lifetime, thereby providing an estimate of total lifetime exposure burden. Higher CEL values in our study may result from greater exposure intensity—such as unsafe handling practices, inadequate use of personal protective equipment, or improper personal hygiene and spill management during application, longer lifetime years of pesticide application, more frequent spraying activities, or a combination of these factors. By capturing accumulated exposure across tasks and time periods, CEL provides a more realistic representation of occupational pesticide exposure than single-exposure indicators that reflect only short-term or isolated events (Liem *et al.*, 2021; Thomas *et al.*, 2010). Similar cumulative exposure modeling approaches have been widely applied in agricultural epidemiology to better approximate real-world pesticide exposure conditions and to improve the assessment of chronic biological effects associated with long-term pesticide exposure (Thomas *et al.*, 2010; Coble *et al.*, 2011).

Table 4. Association between Pesticide Exposure Indicators and Oxidative Stress Biomarkers

Variable(s)	**n**	**MDA***	p^{mw}	**8-OHdG***	p^{mw}
Smoking status					
Smoking	53	0.13 (0.05 - 0.20)	0.257	4.39 (2.51 - 6.58)	0.436
Not smoking	21	0.18 (0.05 - 0.30)		5.02 (2.71 - 6.68)	
Body mass index					
≥ 25 kg/m2	25	0.14 (0.05 - 0.28)	0.818	3.80 (2.22 - 6.05)	0.168
< 25 kg/m2	49	0.15 (0.04 - 0.22)		5.02 (2.66 - 7.21)	
Number of different pesticides mixed in one application					
> 2 types	24	0.17 (0.11 - 0.23)	0.301	5.61 (3.81 - 7.58)	0.064
≤ 2 types	50	0.13 (0.04 - 0.27)		4.23 (2.25 - 6.32)	
Weekly duration of pesticide application					
≥ 4 hours/week	29	0.17 (0.12 - 0.28)	**0.027**	4.39 (3.15 - 6.64)	0.761
< 4 hours/week	45	0.08 (0.04 - 0.22)		5.30 (2.33 - 6.75)	
Cumulative exposure level					
High	24	0.22 (0.10 - 0.45)	**0.010**^{kw,¶}	6.21 (4.44 - 10.00)	**0.004**^{kw,§}
Medium	25	0.13 (0.04 - 0.22)		2.98 (2.10 - 5.51)	
Low	25	0.11 (0.04 - 0.18)		5.30 (2.68 - 6.78)	

* Median (1st quartile - 3rd quartile)

MDA: urinary malondialdehyde (mg/L)

8-OHdG: urinary 8-hydroxy-2'-deoxyguanosine (μg/L)

mw: Mann-Whitney U

The patterns of pesticide use observed among the participants further support the biological plausibility of the associations identified in this study. Fungicides from the dithiocarbamate and triazole classes, along with insecticides from the avermectin and organophosphate groups, were the most commonly used pesticide categories, with additional exposure to pyrethroid compounds also reported. These pesticide classes are widely used in horticultural agriculture and display varying capacities to induce oxidative stress through different toxicological mechanisms, such as inhibition of antioxidant enzymes and activation of inflammatory pathways, thereby generating excessive ROS, which leads to lipid peroxidation, protein oxidation, and oxidative DNA damage (Sule *et al.*, 2022; Gulcin, 2020; Ahamad and Kumar, 2023; Hodosan *et al.*, 2023; Juan *et al.*, 2021). Importantly, the concurrent use of multiple pesticide classes reflects the actual situation in farming operations among smallholders and suggests that pesticide applicators are exposed to complex mixtures rather than to single active ingredients. Although in our study the number of pesticide types mixed in a single application was not significantly associated with oxidative stress biomarkers, a tendency toward increased oxidative DNA damage was observed among workers using multiple pesticide mixtures. Such mixed exposure scenarios may result in additive or synergistic effects on oxidative stress pathways, potentially increasing the overall oxidative burden (Leemans *et al.*, 2019).

Work practices and the use of personal protective equipment (PPE) represent significant modifiers of pesticide exposure in occupational settings and provide important context for the observed oxidative stress findings (Antonangeli *et al.*, 2023; Joko *et al.*, 2020; Putri *et al.*, 2025). From an occupational health perspective, variability in PPE use and work practices can markedly affect exposure intensity, even among workers performing similar tasks, resulting in differences in cumulative exposure. In this study, the generally suboptimal and inconsistent use of PPE suggests that a substantial proportion of pesticide exposure may occur through dermal contact and inhalation during routine activities such as mixing, spraying, and equipment cleaning. Similar patterns of inadequate PPE use and unsafe handling practices have been widely reported among agricultural workers across multiple settings, indicating that this issue is not unique to the study population and represents a broader occupational health challenge associated with agricultural pesticide use (Monger *et al.*, 2023; Tiwari *et al.*, 2022; Joko *et al.*, 2020; Liem *et al.*, 2022). Therefore, inadequate PPE use may not only increase short-term exposure intensity but additionally contribute to higher cumulative exposure over time, possibly exacerbating biological responses such as oxidative stress. Importantly, oxidative stress is recognized as an early biological mechanism that links occupational toxicant exposure to the later development of chronic health conditions. While elevated oxidative stress biomarkers do not necessarily imply that affected individuals will develop specific diseases, they may indicate early biological perturbations that precede clinically apparent outcomes (Sule *et al.*, 2022; Vona *et al.*, 2019; Marrocco *et al.*, 2017).

This study has several strengths. The use of urinary MDA and 8-OHdG, objective biomarkers of oxidative stress, provides biological evidence of early effects associated with occupational pesticide exposure. In addition, pesticide exposure was estimated with a cumulative exposure level (CEL), which integrates exposure intensity, frequency, and lifetime duration, thereby delivering a more comprehensive representation of actual occupational exposure in agricultural settings. Furthermore, the study captures exposure patterns under routine agricultural working conditions, increasing the occupational relevance of the findings. Despite these strengths, interpretation of the results must be balanced with several limitations listed here. First, the cross-sectional design precludes causal inference and limits the ability to assess temporal relationships between pesticide exposure and oxidative stress. Second, biomarker measurements were obtained at a single time point and may not accurately reflect long-term variation in oxidative stress levels. Third, although CEL provides an improved estimate of cumulative exposure, some exposure misclassification is possible due to reliance on self-reported work histories and pesticide use patterns. Fourth, residual confounding arising from factors such as diet, environmental exposures, or lifestyle characteristics cannot be completely excluded despite adjustment for key variables.

Conclusion

In conclusion, this study shows that occupational pesticide exposure is significantly associated with oxidative stress among pesticide applicators, with distinct exposure dimensions differentially reflected in oxidative stress biomarkers. Markers of lipid peroxidation were more closely associated with short-term exposure intensity, whereas oxidative DNA damage was more strongly associated with cumulative exposure. This highlights the cumulative exposure level as an important metric for assessing the early biological effects of pesticide exposure. These findings underscore the need for comprehensive preventive strategies that reduce exposure intensity through safer work practices, complemented by the appropriate use of personal protective equipment and ongoing education. In smallholder agricultural settings, long-term multifactorial exposure requires continuous engagement and capacity-building by relevant stakeholders to ensure healthy, safe, and sustainable agricultural work.

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