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Association between global DNA methylation (LINE-1) and occupational particulate matter exposure among informal electronic-waste recyclers in Ghana

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ABSTRACT

This study examined the associations between PM (2.5 and 10) and global DNA methylation among 100 e-waste workers and 51 non-e-waste workers serving as controls. Long interspersed nucleotide repetitive elements-1 (LINE-1) was measured by pyrosequencing. Personal $PM_{2.5}$ and PM_{10} were measured over a 4-hour work-shift using real-time particulate monitors incorporated into a backpack . Linear regression models were used to assess the association between PM and LINE-1 DNA methylation. The concentrations of $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers than the controls (77.32 vs 34.88, $p < 0.001$ and 210.21 vs 121.92, $p < 0.001$, respectively). $PM_{2.5}$ exposure was associated with increased LINE-1 CpG2 DNA methylation (β = 0.003; 95% CI; 0.001, 0.006; p = 0.022) but not with the average of all 4 CpG sites of LINE-1. In summary, high levels of $PM_{2.5}$ exposure was associated with increased levels of global DNA methylation in a site-specific manner.

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DNA methylation; LINE-1; $PM_{2.5}$; PM_{10}; e-waste; e-waste workers

Introduction

Particulate matter (PM) is a heterogeneous mixture of chemicals and fine/ultrafine particles suspended in the air that originate from multiple sources, such as combustion products from biomass burning for household use, vehicular emissions, and soil particles from road dust (Pipal et al. 2011). In addition, occupational activities such as agriculture, construction, and mining present another essential source of PM exposure (Garcia et al. 2013; Kim et al. 2015; Gautam et al. 2016). Furthermore, the informal electronic waste (e-waste) recycling industry; where proper regulation and controls are lax or absent and worker protection is often inadequate, has emerged as a significant source of occupational particulate matter (PM) air pollution, especially in developing countries (Bi et al. 2010).

Particulate matter varies significantly in composition and particle size. The major components of PM include nitrates; sulfates; elemental and organic carbon; organic compounds (e.g. PAHs); biological compounds (e.g. endotoxin, cell fragments); and metals (e.g. iron, copper, nickel, zinc, and vanadium) (Kim et al. 2015; Gao et al. 2018). Based on particle size, PM is categorized into three major fractions: (i) coarse PM with aerodynamic diameter ≤ 10 μm (PM_{10}), defined as inhalable particles that can penetrate the lungs, (ii) fine PM with aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$),

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defined as respirable and can enter the alveoli, and (iii) ultrafine PM with aerodynamic diameter ≤ 0.1 μm ($PM_{0.1}$) (Pipal et al. 2011; Sun et al. 2018). The toxicity of PM is highly correlated with particle size, where smaller particles are associated with increased toxicity (Stanek et al. 2011).

Combustion products from e-waste burning generate fine PM, which is linked to pulmonary and cardiovascular diseases (Franchini and Mannucci 2012; Jin et al. 2015; Kuang et al. 2019). A recent study revealed elevated ambient PM levels at the Agbogbloshie e-waste site over background levels (Kwarteng et al. 2020). Another study by Amoabeng Nti et al. (2020) showed an association between PM and adverse respiratory effects in e-waste workers compared to controls. However, the biological Pathway for PM-related morbidities is not fully understood, and epigenetic alterations such as DNA methylation may partly mediate the adverse effects of PM on health (Wang et al. 2012; Li et al. 2017).

DNA methylation, the covalent addition of a methyl group to the 5′ carbon of cytosine in a CpG dinucleotide, is the most stable and best-studied epigenetic mark that often responds to environmental stimuli and is critical in regulating gene expression and maintaining chromosomal integrity (Jamebozorgi et al. 2018; Lei et al. 2018). Most studies have focused on the influence of PM on either global methylation or gene-specific methylation (Baccarelli et al. 2009; Madrigano et al. 2011; Kile et al. 2013). The Long interspersed nucleotide elements-1 (LINE-1) are often used as surrogates for global DNA methylation measurement, given their relatively uniform spread across the genome (Yang et al. 2004). They are repetitive elements or transposons, constitute approximately 18% of the human genome, and are usually heavily methylated to suppress retrotransposition (Perera et al. 2020). Because DNA methylation marks are labile and respond to environmental stimuli, LINE-1 methylation is often used as an epigenetic biomarker of effect where low methylation levels correlate with genome instability (Rozek et al. 2014).

Available evidence suggests that alterations in DNA methylation pathways such as inhibition, DNMTs activity, or promotion of ten-eleven translocation (TET) enzyme activity may contribute to DNA methylation changes induced by PM. For example, $PM_{2.5}$ decreased LINE-1 DNA methylation, decreased DNMT1 and increased DNMT3B (Ruiz-Hernandez et al. 2015). Additionally, a decrement in global DNA methylation (%5mC) and DNMT3B was associated with elevated PM levels in coke oven workers (Wang et al. 2020). Furthermore, elderly men from the Normative Aging Study (n = 549), who were deficient in methyl-nutrients, were more susceptible to PM-induced global DNA hypomethylation and subsequent adverse health outcomes (Baccarelli et al. 2008). Demographic and lifestyle factors such as age, cigarette smoking, and alcohol consumption may also influence DNA methylation efficiency (Rakyan et al. 2010; Teschendorff et al. 2010). Available data suggest that LINE-1 hypomethylation is associated with various pathological conditions, including cardiovascular diseases and cancer (Kemp and Longworth 2015; Hossain et al. 2017). However, recent evidence also suggests that global DNA hypermethylation is associated with myopia (Hsi et al. 2019) and Head and Neck Squamous Carcinoma (Akinmoladun et al. 2020).

The Agbogbloshie e-waste recycling site in Ghana has been cited as one of the largest and most studied e-waste sites in Africa (Pascale et al. 2018). Open burning of e-waste at low temperatures to recover valuable materials is a major recycling activity at the Agbogbloshie site, which usually produces PAHs via incomplete combustion (Kwarteng et al. 2020). The composition of PM is complex, as adsorbed compounds such as PAHs can influence DNA methylation (Alvarado-Cruz et al. 2017). For example, among male non-smoking coke-oven workers in Poland, PAH exposure and (BaP) DNA adducts were positively associated with LINE-1 methylation (Pavanello et al. 2009). E-waste workers and individuals who live near e-waste recycling sites are directly exposed to these air pollutants mainly through inhalation (Leung 2019).

Although considerable research has been done on the role of PM on global DNA methylation, data regarding associations of occupational PM exposure and PM-related chemicals such as PAH and DNA methylation are limited (Sun et al. 2018). Furthermore, it is still not known whether occupational PM causes hyper- or hypomethylation globally (Tarantini et al. 2009; Fan et al. 2014). For example, Tarantini et al. (2009) found an inverse association between PM_{10} and both LINE-1

Figure 1. Burning activity at Agbogbloshie. (a) worker was wearing a backpack for monitoring his breathing zone PM pollution.

and Alu methylation among steelworkers in Italy. However, Fan et al. (2014) reported a positive association between $PM_{2.5}$ and LINE-1 methylation among occupational welders in the U.S. Additionally, less attention has been paid to the informal e-waste recycling industry where there are no proper pollution control measures. The primary exposure among e-waste recyclers results from burning e-waste in open air to recover metals and other valuable materials (Figure 1). In these informal settings, exposures are likely to be two to three times higher than in more formal settings, where modern industrial processes are used. Furthermore, PM's composition generated due to e-waste recycling may be different from PM from other sources.

This study has two primary objectives: 1. to measure and compare the concentrations of personal $PM_{2.5}$ and PM_{10} between e-waste workers and controls, 2. to determine whether $PM_{2.5}$ and PM_{10} are associated with global (LINE-1) DNA methylation. Therefore, in the present study, we hypothesized that $PM_{2.5}$ and PM_{10} concentrations were significantly higher among e-waste workers than among controls and were associated with the alteration of global DNA methylation measured through the LINE-1 gene.

Materials and methods

Study area

The study was conducted in two locations: the e-waste site in Agbogbloshie and in Madina Zongo, a control site. These study sites were previously described (Laskaris et al. 2019; Amoabeng Nti et al. 2020). Briefly, the Agbogbloshie e-waste recycling site is one of the busiest sites of its kind in the world and has become a hub of the informal e-waste sector in Ghana and many other formal and informal businesses (Srigboh et al. 2016; Simon 2018). It is located in central Accra and rated as the most contaminated site on earth (Amoyaw-Osei et al. 2011; Blacksmith Institute 2013). The e-waste workers are mostly young men who migrated from the northern part of Ghana in search of employment opportunities. These workers' main job types include collecting, dismantling, and open burning of the e-waste (Acquah et al. 2019). These activities are carried out with little or no worker protection, exposing workers to multiple pollutants, including PM of varying sizes.

Madina Zongo is an area of greater Accra, more than 10 km North-East of the Agbogbloshie e-waste recycling site. Previous studies have successfully recruited control participants from Kwabenya North, a suburb of Accra. Kwabenya residents are similar to the e-waste workers and residents of Madina Zongo with respect to age, length of time residing in the greater Accra area (and

region of the country from where they moved), socioeconomic position, religion and culture (Wittsiepe et al. 2017). There are no e-waste recycling activities in the area, and the individuals recruited were not involved in any e-waste work.

Study population

The study population originated from the GeoHealth-II study, a longitudinal cohort study, with the broad aims of increasing multidisciplinary understanding of the health risks at the Agbogbloshie e-waste site in central Accra, Ghana, and to use study findings to inform evidence-based policy development and implementation at the national, regional, and international level. The study population has been described previously (Takyi et al. 2020). Briefly, in March 2017, we organized a community assembly to engage and inform leaders of the study sites and potential study participants about the study. Leaders and potential participants were given a detailed explanation of the study, its objectives and procedures, and the benefits and possible risks of participating in the study. If willing to be enrolled, participants were asked to provide written consent. The inclusion criteria were adult males aged 18 years and above who have worked at the e-waste site for at least six months. Similarly, participants from the control site (Madina Zongo) must have lived in the area for at least six months. The formula for calculating sample size for the analysis of longitudinal data by Diggle et al. (2002) was used to calculate the sample size required to achieve the desired power of 80% and α – level of 0.05 for the broader GEOHealth II study. A total of 151 participants were enrolled for the study at baseline, of which 100 were e-waste workers, and the remaining 51 served as the control group. For this current study, we restricted our analyses to the baseline sample. The Ethical and Protocol Review Committee at the College of Health Sciences, University of Ghana (protocol identification number CHS-ET/M.4-P 3.9/2015-2016) approved the GeoHealth-II study protocol.

Questionnaire survey

All participants answered a questionnaire that was administered by trained staff. Interviews were conducted in English and other local languages (Dagbani, Twi and Hausa). The interview included demographics (age, gender, religion/ethnicity, education, measures of socioeconomic position, location of birth and childhood, and location of all residences), information to assess past and current potential exposures to air pollutants (use of tobacco, exposure to indoor cooking using biomass fuels, type of housing, detailed job history), personal and family medical history (diagnosed illnesses, reported symptoms), and other related anthropometric measurements such as weight, height, and blood pressure.

Exposure assessment

A detailed description of personal PM measurements was previously described by Laskaris et al. (2019). In summary, personal PM of varying sizes (1, 2.5, 4, and 10) was collected simultaneously every minute from all participants using a battery-operated 5-channel optical particle counter (OPC; Aerocet 831, Met One Instruments, Inc, Oregon, USA) at a flow rate of 2.8 L/min. In addition, $PM_{2.5}$ was collected on a 47 mm Teflon filters with a high flow (SKC, 10 L/min) impact sampler. The sampling pumps were carried in a backpack and worn by participants during the work shift. The inlet of the sampling pump was placed on a shoulder strap of the backpack to collect PM from each participant's breathing zone. Five backpacks were used, allowing only five participants to be studied daily. The sampling time was set to 4 hours during peak working hours (8:00 am – 12:00 noon) to ensure uninterrupted exposure assessment.

PM measurement for the controls was conducted in the same manner as with the e-waste workers. They wore the backpacks for 4 hours, usually between 8 am to 12 noon, while going about their routine activities. The overall sampling period lasted for six weeks (March 2017 to May 2017).

Before deployment, OPC batteries (including an auxiliary battery) were charged, flow rates were set and confirmed using a flowmeter (VFB-67, Dwyer Instrument Inc, IN, USA) connected to a HEPA capsule filter (Pall Gelman Science, Ann Arbor, Mi, USA), which also confirmed the 'zero' test. After sampling, flow rates were rechecked, and OPC data were downloaded to a laptop.

Blood sample collection

Each participant provided 10 ml of whole blood for DNA extraction and methylation analysis. Blood was collected by an experienced phlebotomist following sterile procedures via venepuncture of the antecubital fossa into EDTA tubes. The blood samples were aliquoted into 2.5 ml cryotubes and placed in a cooler with ice blocks and later transported to the University of Ghana for storage at −80°C until later transported on dry ice to the University of Michigan, USA for DNA extraction and methylation analysis.

Extraction of DNA from whole blood for LINE-1 methylation

DNA was extracted in the laboratory at the University of Michigan School of Public Health using the Qiagen DNA Blood Mini Kit (Qiagen, Valencia, C.A), following the manufacturer's instructions. The purity and quantity of DNA samples were assessed with the Qubit Broad Range Double-stranded DNA assay and Nanodrop Spectrophotometer through the University of Michigan DNA Sequencing Core. The extracted DNA was then stored at −20°C until LINE-1 methylation analysis was conducted.

LINE-1 methylation analysis

Sodium bisulfite conversion was performed on 300 ng of extracted genomic DNA using the Qiagen EpiTect Bisulfite Kit per the manufacturer's protocols. PCR amplification was performed for the promoter region of LINE-1 using a previously published assay (Yang et al. 2004). In summary, 15 µL of HotStarTaq Master Mix (Qiagen, Valencia, CA), water, and 15 µL desalted forward and reverse primers were combined to create a PCR master mix. Finally, 3 µL of bisulfite-converted DNA was added to each well to bring the final primer concentration to 0.2 mM and the total reaction volume to 30 µL. PCR cycling conditions were 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s for 35 cycles. PCR product quality was confirmed using 2% agarose gels and gel red staining. Following amplification, 12 µL of PCR product was combined with each sequencing primer and analyzed for CpG-specific methylation using the PyroMark MD System (Qiagen, Valencia, CA). Four bisulfite conversion controls (EpigenDX) and four pyrosequencing controls (Qiagen) were prepared at methylation levels of 0%, 30%, 60% and 100%. CpG site-specific methylation percentages (0–100%) were generated for each of the four CpG sites included in the assay. All samples on a plate were rerun if any of the controls failed, and samples were measured in duplicate.

Statistical analysis

The Shapiro-Wilk test was used to test the normality of PM2.5, PM10, and LINE-1 DNA methylation. Demographic and other characteristic differences between e-waste workers and controls were presented as mean ± SD for continuous variables that exhibited normal distribution (age, BMI, hours worked/day, days worked/week) and compared by student t-test. All categorical variables were presented as numbers (percentages) and compared using the chi-squared test.

$PM_{2.5}$ and PM_{10} were not normally distributed and were therefore presented as median (IQR) and compared between e-waste workers and controls by the non-parametric Mann-Whitney U test. To determine whether the specific job-tasks performed by e-waste workers were associated with PM exposure levels, the e-waste workers were categorized into three main groups based on their

primary job tasks as burners, dismantlers, and collectors to identify high-risk worker groups. We relied on self-reported primary job tasks since there were no documented job titles or task protocols at the informal e-waste site. They were categorized into the different job categories if they reported having been performing a specific task for approximately 70% of their time for the past month. Kruskal-Wallis test was used to compare the concentrations of PM across the primary job-tasks. The concentrations of $PM_{2.5}$ and PM_{10} were compared to air quality guidelines reference values set by the World Health Organization (WHO) and the Ghanaian reference values set by the Ghana Standards Authority (GSA) (GSA, 2019). Differences between LINE-1 DNA methylation and specific CpG sites methylation of the LINE-1 gene were compared between the e-waste workers and controls using student t-test. Pearson correlation analysis was used to assess specific CpG sites' correlations within the same repetitive element (LINE-1). We further performed bivariate analysis to evaluate the relationships between anthropometric and lifestyle factors, and LINE-1 methylation levels.

We used ordinary least square (OLS) regression models with robust standard errors to assess the association between the average breathing zone $PM_{2.5}$ and PM_{10}, and LINE-1 DNA methylation. Both mean percent methylation of four CpG sites of LINE-1, and methylation of specific CpG sites were modelled. Sensitivity analyses were performed using different variants of the outcome model (robust, and least absolute shrinkage and selection operator (LASSO) regressions) to compare with the results of OLS. Covariates included in the multivariable regression models (indoor use of biomass fuel for cooking, alcohol consumption, age, smoking status, and BMI) were based on evidence of their association with DNA methylation from previous studies (Alegría-Torres et al. 2011). All statistical analyses were performed using Stata v15.1 (STATA Corp LLC. Texas, USA), and GraphPad Prism v8.3.1 was used to generate graphs. The results were considered statistically significant if p-values were ≤ 0.05.

Results

Characteristics of e-waste workers and controls

The demographic characteristics of the study participants have been described previously (Laskaris et al. 2019). Briefly, the e-waste workers were significantly younger (mean age = 25.4 years) compared to the controls (mean = 32.5 years). Even though the BMI of both the e-waste and the controls were within normal weight according to the World Health Organization (WHO) parameters, the BMI of the controls (mean = 23.8) was significantly higher than that of the e-waste workers (mean = 21.6, Table 1). The e-waste workers worked for an average of 9 hours per day, 6 days per week, with 59.8% of them living and working on the e-waste site while the rest lived off-site, but within 1 km of Agbogbloshie (40.2%). Significant differences were observed in educational level between the e-waste workers and the controls: 25.0% of the e-waste workers had no formal education at all (vs 13.7% of the controls), 33.0% had up to middle/junior high school (vs 25.5% of the controls), and only 16.0% had secondary school education or higher (vs 51.0% of the controls). The majority of the participants were Muslims, and over 80% earned 20–80 Ghanaian Cedi (GHS); the equivalence of 5–15 USD per day. The prevalence of smoking was not statistically different between the e-waste workers (28.0%) and non-e-waste workers (15.7%, Table 1). The indoor use of biomass for fuel was significantly higher in the controls (31.4%) than the e-waste workers (14.0%).

Particulate matter (PM) exposure of e-waste workers and controls

The median concentrations of $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers than the controls ($PM_{2.5}$: median (interquartile range) 77.32(34.08) $\mu g/m^3$ vs 34.88 (16.55) $\mu g/m^3$, $p < 0.001$ and PM_{10}: median (interquartile range) 210.21 (93.32) $\mu g/m^3$ vs 121.92 (82.93) $\mu g/m^3$, $p < 0.001$, respectively) (Figure 2(a,b)). The median concentrations of

Table 1. Characteristics of e-waste workers (n = 100) and controls (n = 51) enrolled for the study, March 2017-May 2017 at Agbogbloshie and Madina Zongo, Accra, Ghana.

	Total	E-waste workers	Controls	p-value
Characteristics	n = 151	n = 100 [c]	n = 51 [c]	
BMI (kg/m^2), mean(±SD)	22.4(3.2)	21.6(2.7)	23.8(3.5)	**<0.001**[a]
Age (years), mean(±SD)	27.8(8.6)	25.4(6.3)	32.5(10.4)	**<0.001**[a]
Workdays/week, mean(±SD)	NA	6.0(1.0)	NA	
Hours work/day, mean(±SD)	NA	9.3(2.5)	NA	
Sleep location, n(%)	NA	58(59.8)	NA	
On the site	NA	39(40.2)	NA	
≤1 km off-site				
Education, n(%)	32(21.2)	25(25.0)	7(13.7)	**<0.001**[b]
No formal education	31(20.5)	26(26.0)	5(9.8)	
Primary	46(30.6)	33(33.0)	13(25.5)	
Middle/JHS	42(27.8)	16(16.0)	26(51.0)	
Secondary/SHS+				
Marital status, n(%)	73(48.7)	43(43.4)	30(58.8)	0.074[b]
Single	77(51.3)	56(56.6)	21(41.2)	
Married				
Income, n(%)	120(80.5)	81(81.8)	n = 50	0.059[b]
GHC 20–80	10(6.7)	9(9.1)	39(78.0)	
GHC 81–140	19(12.8)	9(9.1)	1(2.0)	
> GHC 140			10(20.0)	
Indoor use of biomass	30(19.9)	14(14.0)	16(31.4)	**0.011**[b]
Yes	121(80.1)	86(86.0)	35(68.6)	
No				
Alcohol use, n(%)	18(1.9)	15(15.0)	3(87.7)	0.183[b]
Regular	12(8.0)	9(9.0)	3(8.2)	
Former	121(80.1)	76(76.0)	45(6.1)	
Never				
Smoking, n(%)	36(23.8)	28(28.0)	8(15.7)	0.093[b]
Yes	115(76.2)	72(72.0)	43(84.3)	
No				
E-waste job category, n(%)	NA	32(32.0)	NA	
burners	NA	49(49.0)	NA	
dismantlers	NA	19(19.0)	NA	
collectors/sorters				

Abbreviations: SD = standard deviation, N = Total number of participants, n(%) = frequency(percent frequency), JHS = junior high school, SHS = senior high school. [a] p-values obtained by t-test, [b] p-values obtained by chi-square test, [c]some figures may not add up to the total numbers because of missing values, **bold** p-values are statistically significant

PMs obtained for both the e-waste and the controls exceeded the World Health Organization (WHO) Air Quality Guideline (AQG) for short-term exposure of 25 μg/m^3 and 50 μg/m^3 for $PM_{2.5}$ and PM_{10} respectively (Table S1). Further comparison with the Ghanaian Ambient Air Quality Standards for short-term exposure, the median concentration of $PM_{2.5}$ and PM_{10} among the e-waste workers exceeded the reference values of 35 μg/m^3 and 70 μg/m^{3,} respectively. However, the concentrations of $PM_{2.5}$ (34.88 μg/m^3) in the controls was lower than the Ghanaian standards 35 μg/m^3, whereas PM_{10} (121.92 μg/m^3) concentration was higher than the Ghanaian standards of 70 μg/m^3 (Table S1).

Particulate matter exposure of e-waste workers by primary job-tasks performed

Comparing the e-waste workers based on their primary job-tasks, participants were categorized into three main groups: burners, dismantlers, and collectors. There was a statistically significant difference in the median $PM_{2.5}$ and PM_{10} exposure between the groups as determined by the Kruskal-Wallis test (p = 0.013 and p = 0.027, respectively). Dunn's post hoc test revealed that the

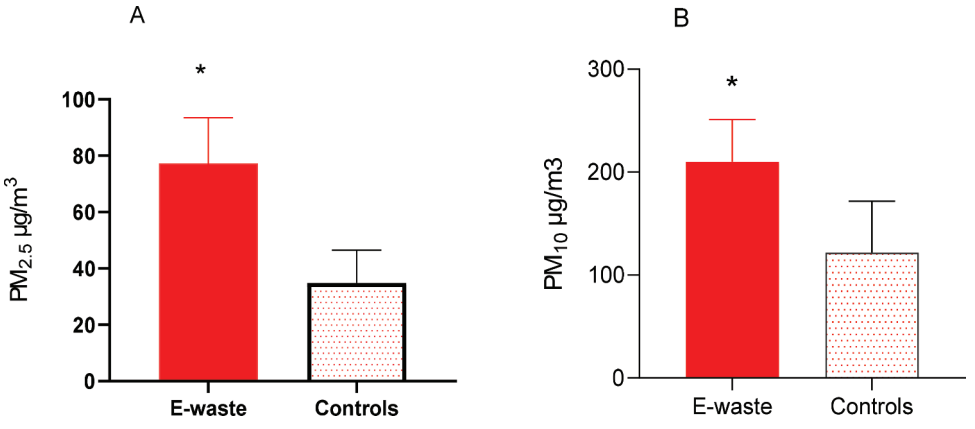

Figure 2. Particulate matter exposure between e-waste and controls. Data are presented as the median (interquartile range). (a) $PM_{2.5,}$ and (b) $PM_{10,}$ * = p ≤ 0.05, p-values are obtained by Mann-Whitney-U test.

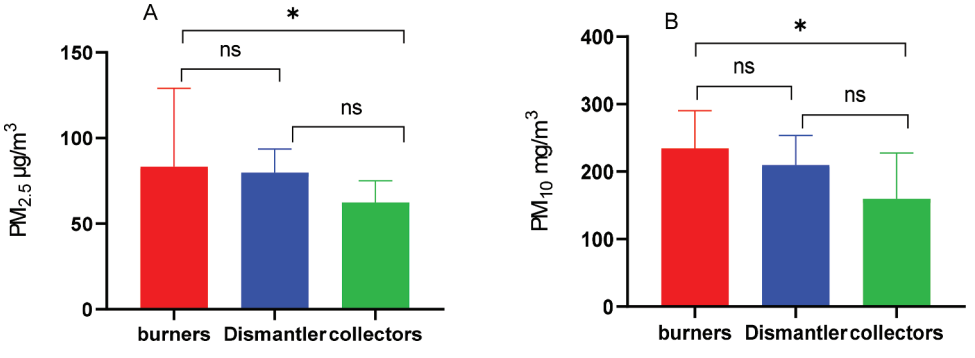

Figure 3. Particulate matter concentration across primary job tasks performed by e-waste workers showing high median concentration in burners, (a) $PM_{2.5,}$ (b) PM_{10}. Data are presented as the median (interquartile range), * = p ≤ 0.05 and ns = non-significant. P-values obtained by the Kruskal-Wallis and Dunn's post-hoc tests.

medians of both $PM_{2.5}$ and PM_{10} were significantly higher among burners than among collectors (p = 0.009 and p = 0.029, respectively). No significant differences were observed when burners were compared to dismantlers for $PM_{2.5}$ and PM_{10} exposure (Figure 3(a,b)).

LINE-1 DNA methylation in e-waste workers and controls

Overall, there were no significant differences (p = 0.950) between LINE-1 methylation (%) in the e-waste workers and controls (Figure 4(a)). An average of 85.16 ± 1.32% was obtained for the e-waste workers compared to 85.17 ± 1.11% for the controls. However, non-e-waste workers had significant hypomethylation compared to e-waste workers at the CpG1 site (p = 0.034), but not at other CpG sites in LINE-1 (Figure 4(b)).

The correlation of methylation among CpG sites for LINE-1 was explored (Table S2). The results showed a trend in positive significant correlation between consecutive CpG sites. A positive correlation was observed between CpG1 and CpG2 (r = 0.43), CpG2 and CpG3 (r = 0.39), and then CpG3 and CpG4 (r = 0.30), all P ≤ 0.05.

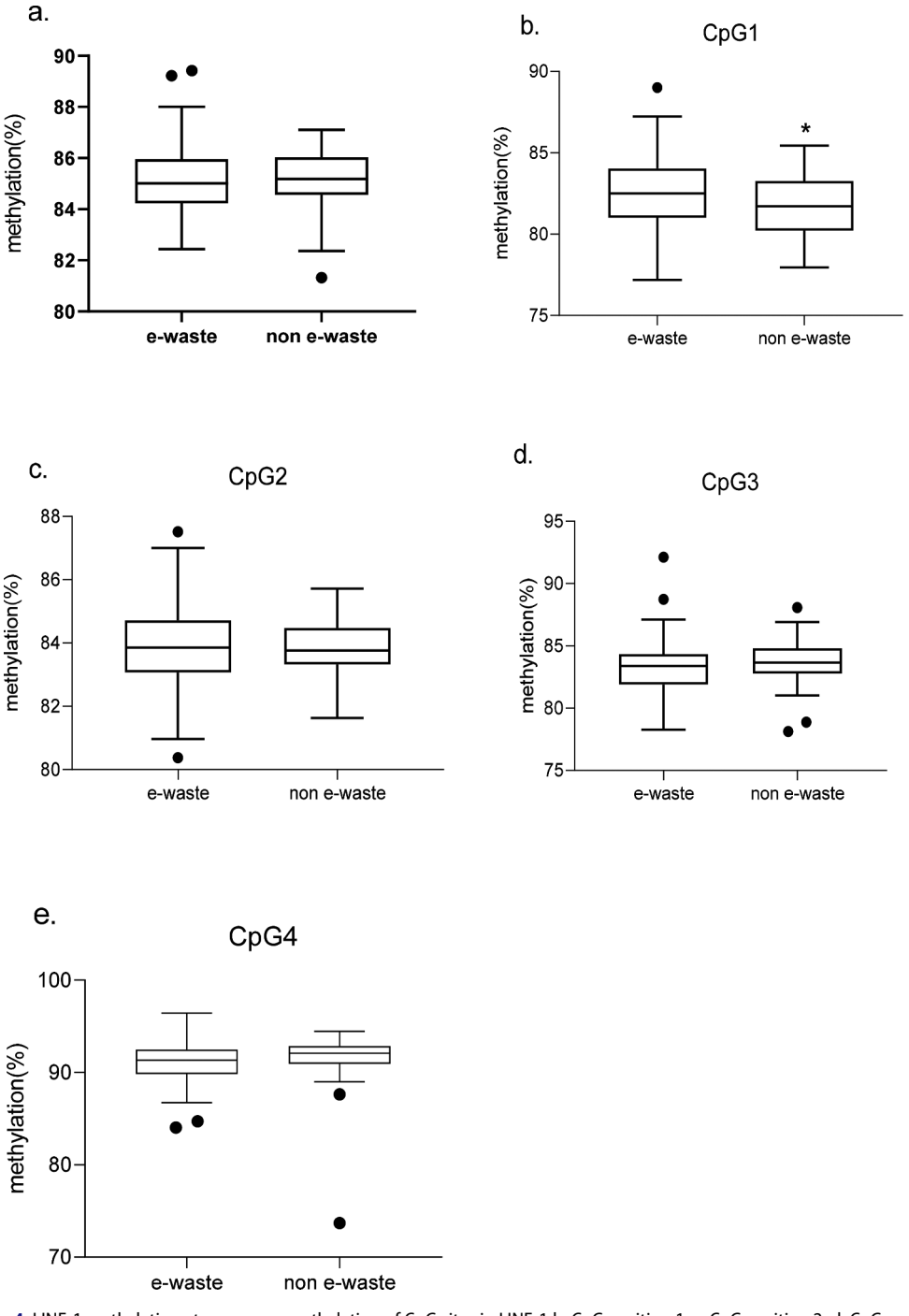

Figure 4. LINE-1 methylation at a: average methylation of CpG sites in LINE-1 b: CpG position 1, c: CpG position 2, d: CpG position 3, and e: CpG position 4; among e-waste workers at Agbogbloshie and controls at Madina Zongo. * = $p \leq 0.05$ between groups.

Table 2. Relationship between LINE-1 methylation and anthropometric and lifestyle factors between e-waste workers at Agbogbloshie (n = 100) and controls at Madina Zongo (n = 51).

	LINE-1 methylation		
Variable	E-waste workers (n = 100)	Controls (n = 51)	p-value
Age (years)	85.2(1.2)	85.4(0.6)	0.817
≤20	85.1(1.4)	85.3(1.3)	0.520
21–30	85.3(1.3)	84.8(1.2)	0.399
31-40	85.73(2.0)	85.2(1.0)	0.541
>40			
Smoking	85.2(1.3)	85.1(0.7)	0.805
Yes	85.2(1.3)	85.2(1.2)	0.892
No			
Alcohol intake	85.4(1.5)	85.6(1.3)	0.797
occasional/regular	84.2(2.5)	85.7(1.2)	0.339
former	85.2(1.3)	85.1(1.1)	0.744
never			
BMI (kg/m^2)	84.6(1.2)	85.2(0.5)	0.492
Low weight	85.2(1.3)	85.2(1.1)	0.796
Normal weight	84.9(1.3)	85.5(1.1)	0.307
Overweight	85.7(0.0)	84.2(1.3)	0.364
Obesity			
Indoor use of biomass	85.3(1.5)	84.9(1.1)	0.370
Yes	85.2(1.3)	85.3(1.1)	0.574
No			
Job category	85.3(1.3)	NA	
Burners	85.2(1.4)	NA	
Dismantlers	84.6(1.0)	NA	
Collectors/sorters			

Body Mass Index (BMI) according to World Health Organization (WHO) parameters: low weight (≤18.5 kg/m^2); normal weight (> 18.5 kg/m^2 and ≤24.9 kg/m^2); overweight (> 24.9 kg/m^2, and ≤29.9 kg/m^2), and obesity (≥30 kg/m^2), P-values were obtained by the ANOVA test, NA: Not applicable. LINE-1 methylation presented as mean(±SD)

Relationship between LINE-1 methylation and anthropometric and lifestyle factors

The mean methylation of LINE-1 between e-waste workers and controls was assessed based on anthropometric and lifestyle factors such as age, BMI, smoking, alcohol consumption, and indoor use of biomass fuel for cooking via one-way analysis of variance (ANOVA) (Table 2). LINE-1 methylation was not related to age, BMI, smoking, alcohol consumption, or indoor use of biomass fuel for cooking amongst either group ($p_{all} > 0.05$).

Associations between global DNA methylation and PM exposure

In linear regression models controlling for confounders (indoor use of biomass fuel for cooking, age, cigarette smoking, alcohol consumption, smoking status, location of study (Agbogloshie or Madina Zongo), and BMI), the DNA methylation levels were not significantly different between the e-waste workers and controls when the total sample was analyzed. The associations between both $PM_{2.5}$ and $PM_{10,}$ and LINE-1 DNA methylation were positive but not statistically significant ($\beta_{PM2.5}$ = 0.003; 95% CI; −0.001, 0.009, p = 0.159), and (β_{PM10} = 0.002; 95% CI; −0.001, 0.004, p = 0.121), respectively (Table 3). However, a significant positive association was observed between $PM_{2.5}$ and LINE-1 CpG2 when the specific CpG sites were evaluated (β = 0.003; 95% CI; 0.001, 0.006; p = 0.022). This estimate suggests that a 1 μg/m^3 increase in $PM_{2.5}$ resulted in a an increase of 0.003 in percent LINE-1 CpG2 DNA methylation (Table 3). Sensitivity analyses using different variants of the outcome model (robust and cross-fit partialling-lasso regressions) all showed similar trends in the associations between DNA methylation and PM exposure (Table 3). In addition, linear

Table 3. Associations between total and specific CpG sites DNA methylation of LINE-1 and levels of PM exposure in total sample (n = 151).

			Sensitivity analysis	
		Linear regression with robust standard errors from OLS	Robust regression	Cross-fit partialling-out LASSO regression
PM exposure	Outcomes (LINE-1) DNA methylation	β(95% CI)	β(95% CI)	β(95% CI)
$PM_{2.5}$				
	Average of 4 CpGs	0.003(−0.001, 0.009)	0.003(−0.001, 0.006)	0.004(−0.001, 0.009)
	CpG1	0.001(−0.008, 0.009)	−0.001(−0.007, 0.005)	0.002(−0.007, 0.010)
	CpG2	0.003(0.001, 0.006)*	0.004(0.001, 0.007)*	0.003(0.001, 0.006)*
	CpG3	0.009(−0.001, 0.018)	0.004(−0.002, 0.010)	0.008(−0.001, 0.017)
	CpG4	0.002(−0.002, 0.007)	0.001(−0.004, 0.007)	0.001(−0.002, 0.005)
PM_{10}				
	Average of 4 CpGs	0.002(−0.001, 0.004)	0.002(−0.000, 0.003)	0.002(−0.001, 0.004)
	CpG1	0.002(−0.002, 0.005)	0.001(−0.002, 0.005)	0.001(−0.003, 0.005)
	CpG2	0.002(−0.000, 0.003)	0.002(0.000, 0.004)*	0.001(−0.000, 0.003)
	CpG3	0.003(−0.002, 0.008)	0.000(−0.002, 0.003)	0.004(−0.001, 0.008)
	CpG4	0.001(−0.001, 0.004)	0.001(−0.002, 0.004)	0.001(−0.001, 0.003)

P-value notation: *p < 0.05, β: average DNA methylation change, CI: Confidence Interval, **$PM_{2.5}$** = particulate matter ≤ 2.5 μm in aerodynamic diameter, **PM_{10}** = particulate matter ≤ 10 μm in aerodynamic diameter; LINE-1: long interspersed nucleotide element; CpG: cytosine guanine dinucleotide. All models adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking, study location and BMI.

Table 4. Relationship between e-waste recycling activities and LINE-1 DNA methylation non-e-waste workers as reference.

Covariates	Linear regression with robust standard errors from OLS		Cross-fit partialling-out Lasso regression	
	β(95% CI)	P value	β(95% CI)	P value
Job category				
Ref = control group				
Burners	−0.165(−0.865, 0.534)	0.641	0.114(−0.492, 0.721)	0.712
Dismantlers	−0.153(−0.740, 0.433)	0.606	0.026(−0.498, 0.550)	0.923
Collectors	−0.668(−1.266, 0.069)	0.029	−0.651(−1.200, −0.102)	0.020

*p < 0.05, β: average DNA methylation change, CI: Confidence Interval. Note: models adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking, $PM_{2.5}$, PM_{10} and BMI

regression models stratified by e-waste exposure status (e-waste workers/controls) showed similar estimates of the associations between $PM_{2.5}$ and PM_{10} exposure and LINE-1 DNA methylation among only the e-waste workers (Table S3).

Associations between global DNA methylation and specific job tasks performed by e-waste recycler

We further evaluated the relationship between specific e-waste recycling tasks (burners, dismantlers, and collectors) and LINE-1 methylation using OLS with robust SE and lasso regression models. We used the control group as a reference, while we controlling for age, bmi, smoking, alcohol use, indoor biomass fuel use, and PM exposure (Table 4). Results from the analyses showed that, compared to the controls, e-waste collectors had significantlty decreased LINE-1 methylation (β = −0.667; 95% CI; −1.266, −0.069; p = 0.029). This estimate suggest that, working as an e-waste collector results in 0.67 decrease in percent LINE-1 methylation compared to the non-e-waste workers.

Discussion

The current study examined the association between PM (2.5 and 10) exposure and global (LINE-1) DNA methylation among e-waste workers at Agbogbloshie and a control group at Madina Zongo in Accra, Ghana. We found significantly higher concentrations of breathing zone $PM_{2.5}$ and PM_{10} in e-waste workers than the control group. Overall, LINE-1 methylation did not differ between the e-waste workers and controls. There was no significant association between LINE-1 DNA methylation PM (2.5 and 10) exposure. However, $PM_{2.5}$ showed a significant positive relationship with LINE-1 CpG2.

As expected, based on previous studies by Zhang (2017) and Zheng et al. (2015), $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers compared to the controls. We found substantially higher PM (2.5 and 10) exposure in workers who primarily burn e-waste compared to those engaged in other recycling activities such as collection. This finding is consistent with the finding of Bungadaeng et al. (2019), where open burning of e-waste in the Buriram Province of Thailand resulted in elevated levels of the average concentrations of $PM_{2.5}$ and PM_{10} of 2774 μg/m^3 and 3215 μg/m^3, respectively, in e-waste workers (N = 33). These high levels of PM among e-waste workers, particularly e-waste burners, might be attributed to the release of fumes and smoke from e-waste combustion as well as other mechanical processes (dismantling, sorting, shredding and transportation), which also releases dust into the ambient air (Kwarteng et al. 2020). The biomass burning of a nearby refuse pile, burning wood for commercial cooking near the e-waste site, as well as vehicle exhaust emission contribute to the higher levels of PM at the Agbogbloshie e-waste recycling site (Kwarteng et al. 2020). However, the fractions contributed by each activity at the site were not evaluated. Notably, almost all the PM measured in the controls exceeded the WHO

Ambient Air Quality Guidelines (WHO 2006). The high concentration of PM measured in the control group suggests a high background concentration of PM in urban Ghana. This can be attributed to the over-reliance on solid fuels for cooking, biomass burning, vehicular emissions, and road dust (Ofosu et al. 2012; Health Effects Institute 2019).

Global DNA methylation can provide insight into epigenetic regulation and further improve the mechanistic understanding between environmental or occupational PM exposure and disease, especially respiratory health effects development (Suhaimi et al. 2021) . In this study, we used LINE-1 methylation status as a proxy for global methylation. LINE-1 methylation has been linked to human diseases, especially cancers and other cardiopulmonary diseases, by decreasing genome stability with decreasing methylation levels. However, previous studies have linked both hypermethylation and hypomethylation of LINE-1 to disease (Bollati et al. 2011; Liao et al. 2011; Kitkumthorn et al. 2012; Kerachian and Kerachian 2019).

Overall, LINE-1 methylation did not differ significantly between the e-waste and controls. Our data show that LINE-1 was heavily methylated in whole blood of e-waste workers (mean ±SD: 85.16 ± 1.32%) and in controls (mean ± SD: 85.17 ± 1.11%) as compared to previous occupational exposure studies such as dental professionals (mean ± SD: 68.4 ± 3.7%) (Goodrich et al. 2013), coke oven workers (median: 59.0%) (Duan et al. 2013), and workers exposed to multi-wall carbon nanotubes (mean ±SD: 74.2 ± 1.4%) (Ghosh et al. 2017). The possible explanation for the lack of difference in LINE-1 methylation between the e-waste workers and controls might be the choice of our comparator group since the categorization of hyper- or hypomethylation is dependent on the methylation levels of the comparator group (Phetliap et al. 2018). For example, the differences in age between the e-waste workers and controls could affect our comparison of their methylation levels. However, the average age of the e-waste workers (25.4 ± 6.3 years) and controls (32.5 ± 10.4 years) are not in the category of vulnerable window including the elderly (Bollati et al. 2009), and children and adolescents (Burris et al. 2011), where environmental exposures greatly affect the epigenome.

In the present study, the association of PM(2.5 and 10) and LINE-1 methylation was not statistically significant, even though positive trends were observed. Our findings are consistent with a previous study where the investigators examined a cohort of 38 male welders in the USA, and found that occupational exposure to $PM_{2.5}$ was not significantly associated with LINE-1 and Alu methylations (Kile et al. 2013.). Our inability to detect significant associations may be due to low statistical power to detect any differences. In addition, our analysis was based on DNA methylation derived from whole blood which may not be the most appropriate target tissue for assessing the effect of PM exposure compared to DNA derived from other tissues such as the lung. However, the collection of such specific organ tissues are invasive (McCullough et al. 2017); therefore, DNA methylation derived from blood is a widely used surrogate tissue (Gondalia et al. 2019).

A similar study performed by Fan et al. (2014) among 66 welders found a significant positive association between $PM_{2.5}$ and LINE-1 methylation. In addition, Mishra et al. (2021) in their pilot study found an increase in LINE-1 DNA methylation among individuals exposed to high levels of air pollution. However, several other studies have reported inverse relationships between PM and DNA methylation in both occupational and environmental settings. For instance, Tarantini et al. (2009) found a decrease in LINE-1 methylation with PM_{10} exposure in peripheral blood samples of steel production plant workers in Italy. A similar association has been reported in Thailand, where decreased LINE-1 methylation was associated with working in an industrial estate with elevated air pollution levels (Peluso et al. 2012). Additionally, a decrement in LINE-1 methylation was reported in a cohort of 718 elderly participants exposed to $PM_{2.5}$ in the Boston area Normative Aging Study (Baccarelli et al. 2009). A recent systematic review and meta-analysis by Wu et al. (2021) found an inverse association between $PM_{2.5}$ and global DNA methylation in adults. The variability of results seen in studies where PM alter DNA methylation could be attributed to PM's source and composition. For example, Tarantini and co-workers measured PM in steelworkers (Tarantini et al. 2009), Fan et al. (2014) measured PM concentrations in welders, while this present study measured PM

from e-waste recyclers. Different sources of may result in different complex mixtures of toxic organic chemicals which in turn might have different biological effects (Peluso et al, 2012). For example, welders may be exposed to high levels of metals (Ding et al. 2016), while PM generated through e-waste recycling comprises a relatively lower concentration of metals (Bi et al. 2010). The discrepancies in findings may also reflect the variability of epigenetic responses to environmental exposure since factors such as ethnicity, occupation, or tissue type may influence the effect of the same pollutant on the epigenome (Jamebozorgi et al. 2018). Also worthy of note is that DNA methylation may be dependent on othe factors such as timing and length of exposure, routes of exposure, and host genetics (Ji and Hershey 2012), and thefore could contribute to the variability observed from different studies. The mechanism of these contributory factors therefore require further studies and clarification.

Previous studies have shown that LINE-1 specific CpG sites by default differ in methylation levels (Sharma et al. 2019) and respond differentially to environmental factors (Goodrich et al. 2015). This suggests that a site-specific approach to assessing environmental pollutants and epigenetic modifications is critical in understanding the biological mechanism of exposure-outcome relationships (Hanna et al. 2012; Goodrich et al. 2015). This present study found a significant positive association between $PM_{2.5}$ and LINE-1 CpG2 site. The results suggest that a 1 $\mu g/m^3$ resulted in a 0.003 increase in LINE-1 CpG2 DNA methylation percent. This finding should be taken with caution because, even though specific CpG sites methylation levels provide a limited assessment of the region of interest (Hanna et al. 2012), the evidence available suggests that alteration of one of the four CpG sites may alter the overall LINE-1 DNA methylation (Hata and Sakaki 1997).

We also evaluated the relationship between specific e-waste job tasks performed by e-waste workers and LINE-1 methylation. We found a significant decline in LINE-1 methylation among e-waste collectors compared to those in the control group. This finding should be taken with caution since the difference could be derived from the relative small sample size in each job category, which may increase the likelihood of chance finding due to the effect of multiple comparison analysis.

The mechanism linking PM and DNA methylation is not fully understood. Exposure to high PM concentrations increases reactive oxygen species (ROS) generation (Gurgueira et al. 2002), and subsequent DNA damage, such as strand brakes (James et al. 2003). DNA damage increases DNA methyltransferase (DNMT) activities with high affinity to DNA repair sites that methylate adjacent CpG nucleotides (James et al. 2003; Cuozzo et al. 2007). The increased DNMT activity secondary to DNA damage may increase LINE-1 CpG methylation levels (Bollati et al. 2011), as observed in the increased level of LINE-1 CpG2 methylation in this study. Recent reports showed that cells exposed to PM resulted in the upregulation of all three DNA methyltransferases (DNMT1, 3a 3b), positively corresponding with a considerable increase in global DNA methylation (Sunil et al. 2017; Bhargava et al. 2019; Mishra et al. 2021).

This study has some limitations. First, dietary intake data on folic acid, choline, vitamin B12, and betaine, which could affect DNA methylation (Terry et al. 2011), was not measured in this study. In addition, we measured PM for 4 hours of the worker's work-shift, which may not be representative of the overall 8-hour occupational exposure. However, since there were no regulations and documented work schedules in place, the 4-hours monitoring in the informal e-waste site was sufficient to monitor participants during their active work hours. Furthermore, workers involved in e-waste recycling are faced with a myriad of exposures, including PAHs (Feldt et al. 2014), metals (Wittsiepe et al. 2017), and other persistent organic pollutants (POPs) (Wittsiepe et al. 2015). We are, therefore, not able to rule out the effects of other exposures other than PM on LINE-1 in our population.

Our study's significant strength is that it is one of the first to examine the association between PM and repetitive elements DNA methylation in electronic waste recyclers in Ghana. Furthermore, the Agbogbloshie e-waste recycling site is arguably one of the most contaminated, best researched, and most easily accessible site worldwide and therefore represents a good place to investigate pollution exposure to workers and associated adverse health outcomes.

Conclusions

In conclusion, we did not observe a significant difference in the methylation of the repetitive element (LINE-1) between the e-waste and controls. Our results showed that PM is was not significantly associated with LINE-1 methylation. However, $PM_{2.5}$ exposure was positively associated with LINE-1 CpG2 methylation. These findings suggest that alteration in DNA methylation of transposable elements such as LINE-1 with environmental exposures is multidirectional and may vary by specific sites. Future studies should examine other epigenetic modifications such as histone modifications or gene-specific methylations and further assess whether these associations mediate PM's effects on adverse health outcomes.

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Disclosure Statement

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