

Review Article

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Electronic waste exposure and DNA damage: a systematic review and meta-analysis

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Abstract

Objectives: Inappropriate processing and disposal of electronic waste (e-waste) expose workers and surrounding populations to hazardous chemicals, including clastogens and aneugens. Recently, considerable literature has grown around e-waste recycling, associated chemical exposures and intermediate health outcomes, including DNA damage. Micronuclei (MN) frequency has been widely used as a biomarker to investigate DNA damage in human populations exposed to genotoxic agents. We conducted a systematic review of published studies to assess DNA damage in e-waste-exposed populations and performed a meta-analysis to evaluate the association between e-waste exposure and DNA damage.

Methods: This systematic review with meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement checklist. Articles published in English from January 2000 through December 2020 investigating the associations between e-waste exposure and DNA damage were retrieved from the following three major databases: MEDLINE, ProQuest, and Scopus. Studies that reported the use of MN assay as a biomarker of DNA damage were included for meta-analysis. Studies that also reported other DNA damage biomarkers such as chromosomal aberrations,

comet assay biomarkers, 8-hydroxy-2'-deoxyguanosine (8-OHdG), telomere length, apoptosis rate were reported using narrative synthesis.

Results: A total of 20 publications were included in this review, of which seven studies were within the occupational setting, and the remaining 13 studies were ecological studies. The review found six biomarkers of DNA damage (micronuclei, comets assay parameters (tail length, % tail DNA, tail moment, and olive tail moment), 8-OHdG, telomere length, apoptosis rate and chromosomal aberrations) which were assessed using seven different biological matrices (buccal cells, blood, umbilical cord blood, placenta, urine and semen). Most studies showed elevated levels of DNA damage biomarkers among e-waste exposed populations than in control populations. The most commonly used biomarkers were micronuclei frequency ($n=9$) in peripheral blood lymphocytes or buccal cells and 8-OHdG ($n=7$) in urine. The results of the meta-analysis showed that electronic waste recycling has contributed to an increased risk of DNA damage measured using MN frequency with a pooled estimate of the standardized mean difference (SMD) of 2.30 (95% CI: 1.36, 3.24, $p<0.001$) based on 865 participants.

Conclusions: Taken together, evidence from this systematic review with meta-analysis suggest that occupational and non-occupational exposure to e-waste processing is associated with increased risk of DNA damage measured through MN assay and other types of DNA damage biomarkers. However, more studies from other developing countries in Africa, Latin America, and South Asia are needed to confirm and increase these results' generalizability.

Keywords: biomarker; e-waste; genetic damage; micronucleus.

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Introduction

The techniques used in the informal recycling of e-waste, particularly in lower- and middle-income countries (LMICs), are basic and primitive, with little or no regard for the health and safety of humans and the environment [1].

Recyclers often use basic tools such as a hammer, chisel and occasionally screwdrivers and spanners to dismantle and separate the different components [2] and a long metal rod to rotate/flip burning items such as insulated wires and circuit boards of various sizes [3, 4]. These primitive recycling methods release multiple toxic pollutants into the environment that exposes recyclers and surrounding populations.

Some of the chemicals released into the environment during informal e-waste recycling, such as heavy metals and PAHs, are carcinogens [5]. Recent evidence from developing countries suggests a higher concentration of metals and organic compounds in e-waste recycling sites, among e-waste workers and in people living near e-waste sites than in the general population [6]. In addition, a number of researchers have reported that high concentrations of heavy metals and organic pollutants from e-waste recycling sites are associated with increased cancer risks [1, 7–9].

Occupational and environmental exposure to genotoxic agents mainly through inhalation, ingestion, and dermal contact may damage DNA in a cell, which may be linked to the development of diseases [10–12]. Although studies have reported evidence of an association between crude e-waste disposal and DNA damage [13], to the best of our knowledge, there has not been any systematic synthesis of evidence linking specifically e-waste exposure to DNA damage in human populations as yet.

Recently, considerable literature has grown around e-waste recycling, associated chemical exposures and intermediate health outcomes, including DNA damage and cytogenetic alterations [14–16]. The use of biomarkers such as chromosomal aberrations (CA), micronuclei (MN) frequency, and comet assay parameters (tail length, tail moment, etc.), which indicate biomarkers of early biological effects, may enable researchers to understand the mechanisms or pathways through which e-waste-related chemicals influence their toxicity. In addition, these biomarkers may serve as targets for developing efficient prevention strategies for workers and people living near e-waste sites with uncontrolled exposures and strengthen regulation involving the safe disposal of e-waste in general.

This systematic review was conducted to assess the evidence of genetic alteration associated with e-waste recycling as a penultimate step to analyzing DNA methylation of long interspersed nucleotide element-1 (LINE-1) as a proxy for global DNA methylation among informal e-waste recyclers.

Methods

This systematic review/meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement checklist [17].

Protocol and registration

A review protocol was developed and registered with the International prospective register of systematic reviews (PROSPERO) with registration number CRD42020201149, and it is available from https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=201149.

Eligibility criteria

This review focused on observational studies on human populations exposed to e-waste recycling. We included studies that were original peer-reviewed publications, assessed e-waste exposure and biomarkers of DNA damage, and involved human populations, including women and children. We excluded studies that were not original (e.g., reviews, conference proceedings, letters to the editor, and abstracts) and did not report biomarkers of DNA damage in human populations.

Information sources and search strategy

Articles published in English from January 2000, investigating the associations between e-waste exposure and biomarkers of DNA damage were retrieved through the following three major databases: MEDLINE (Academic Search Complete, CINAHL Complete, Education Research Complete, GreenFILE, Health Source: Nursing/Academic Edition, Library, Information Science & Technology Abstracts), ProQuest, and Scopus. The search terms used included the following keywords: (“electronic waste” OR “e-waste” OR “WEEE”) AND (“DNA damage” OR “chromosomal aberration” OR “DNA strand breaks” OR “micronucl*” OR “Sister chromatid exchanges” OR “oxidative DNA damage” OR “genotox*” OR “oxidative stress”). Reference lists of selected articles were hand-searched for relevant publications that were not captured by the electronic search. The search strategy and results of the various databases are presented in (Supplementary Table 1).

Study selection

The study selection was conducted in 2 phases. In phase 1, two reviewers independently screened titles and abstracts of publications retrieved from the electronic databases and hand searches. Publications that did not meet the inclusion criteria were excluded. After phase 1 screening, 32 publications advanced to phase 2 (see supplementary material), full-text screening. In phase 2, the same reviewers independently examined the full-text publications for inclusion. Any discrepancies between reviewers were resolved by a third reviewer. Studies that were excluded at this stage are presented in Supplementary Table 2 with reasons. Finally, a total of 20 publications met the inclusion criteria.

Diagrammatic representation of study selection

The flowchart representing the process of study selection is presented in Figure 1. In the initial search of three electronic databases, a total of 822 articles were retrieved. Duplicates of 106 were identified and removed, with a total of 717 articles making it to the title and abstract screening

stage. After the title and abstract screening, a total of 685 articles were excluded, allowing 32 articles to advance to the full-text screening stage.

Out of the 32 full-text articles screened, 12 were excluded either because they were not original articles (5), did not meet the inclusion criteria (3), were retracted articles (1) or shared the same population and outcome with another study already included (3) (Supplementary Table 2). A total of 20 publications were included in this review, of which seven studies were within the occupational setting, and the rest were ecological studies. Seven of the 20 studies included in this systematic review were included in the meta-analysis.

Data extraction

The type of data extracted from each of the selected studies was both qualitative and quantitative. The qualitative data include author and year of publication, details of study design (exposure setting and population), the country where the study was conducted, and methods details (samples type, targeted chemicals, outcome measures).

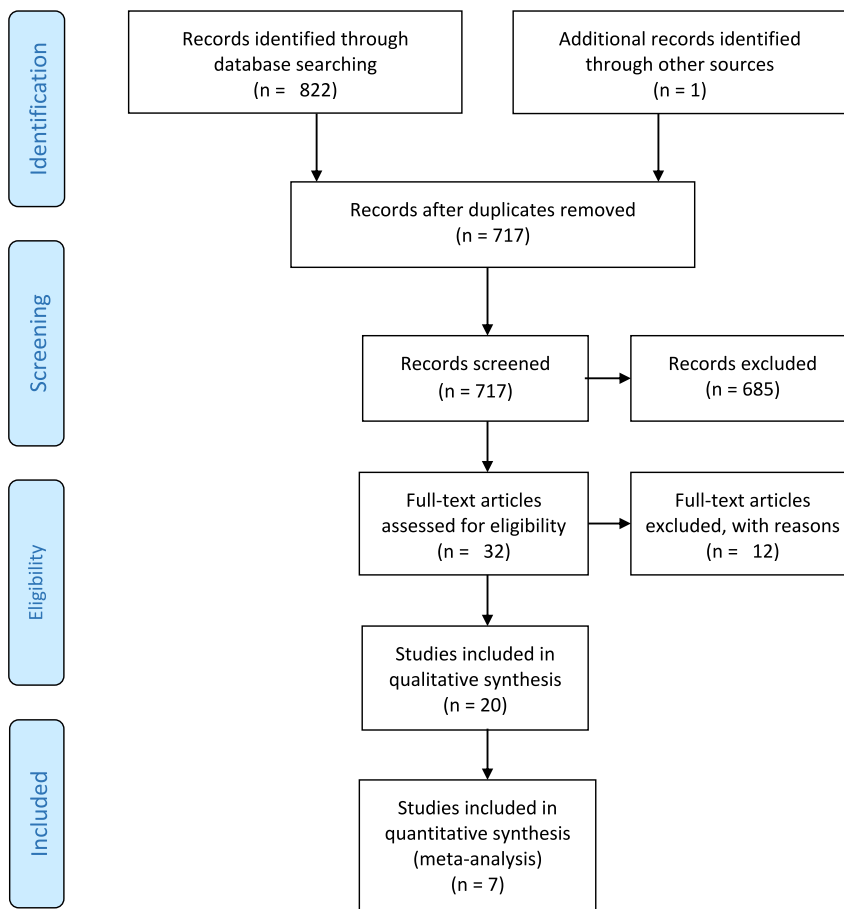


Figure 1: PRISMA flow chart illustrating the process of selecting the studies included in the review.

The quantitative data extracted included the sample size for each study, and the main findings expressed as means and standard deviations if applicable (Table 2). Data extraction was performed by two reviewers. One reviewer extracted the data, and the second reviewer compared the extracted data with the original report.

Risk of bias (quality) assessment

The risk of bias (methodological quality) of each included study was assessed using the modified version of the Newcastle-Ottawa Scale (NOS) for cross-sectional studies developed by Elyasi et al. [18] to ensure that the conclusions and findings of the reviews were based on the best available evidence. The tool was adjusted to include exposure assessment and a comparison group.

The score for each cross-sectional study was calculated based on four categories: group selection (three items), comparability (two items), exposure measurement (one item), and outcome measurement (one item). The items in the first two categories, 'group selection' and 'comparability', were awarded a maximum of two stars and one star for items in the remaining categories. The NOS score ranged from 0 (lowest grade) to 10 (highest grade). Studies that scored above the median were considered high quality [19], that is, >5 in this review. Two reviewers carried out the risk of bias assessment. Any disagreements were addressed by discussion between the two reviewers or by the intervention of a third independent reviewer.

Statistical analysis

The effect size was calculated using standardized mean differences (SMD) since the studies were conducted in different settings (ecological or occupational). Heterogeneity was determined using Cochran's χ^2 test and quantified using the I^2 test. The null hypothesis for heterogeneity was that all studies share a common mean difference for MN frequency. The I^2 describes the percentage of differences across studies attributed to heterogeneity rather than chance [20]. An I^2 value of 25% is considered low heterogeneity, a value between 50 and 75% is moderate, and a value above 75% is considered high heterogeneity [20].

The random effect meta-analysis model with restricted maximum likelihood (REML) method [21] was used to calculate the overall SMD and its 95% confidence interval (CI). The REML method performs well with a small number of studies and produces an unbiased estimate of the

between-study variability owing to differences in study designs and interlaboratory reproducibility. The REML assumes a normal distribution of the random study effect sizes [22]. Forest plots were used to present the results of the meta-analysis.

To further explore heterogeneity between studies, subgroup analyses were performed based on study setting, i.e., whether studies were conducted among e-waste workers vs. non-e-waste workers (occupational) or studies were conducted among residents of an e-waste exposed town/village vs. residents in a neighbouring town/village without e-waste exposure (ecological), and quality, (high quality vs. low quality) as determined by the NOS for cross-sectional studies. All statistical analyses were conducted using Stata version 16.1 (StataCorp LLC, College Station, TX, USA).

Results and discussion

Study characteristics

Design and site

All 20 studies included in this review were cross-sectional studies. The majority of the studies (15 of 20) were conducted in China, and the remaining five studies were conducted in Nigeria [14], Palestine [23], Thailand [16], Vietnam [15], and the Philippines [24].

Populations studied

A total of seven out of the 20 studies included in the review were conducted in occupational settings; the remaining 13 studies targeted people resident in e-waste exposed towns (ecological studies). Only four of the ecological studies targeted children [15, 25] and neonates [26, 27]; the remaining nine studies involved adult populations. A total of 17 of the 20 studies included a comparator group. The comparator groups were mostly residents of non-e-waste recycling towns with no history of e-waste exposure. Two of the occupational-related studies [28, 29] recruited age- and sex-matched farmers as control groups. The sample sizes of the studies included in this review ranged between 48 [30] and 377 [2]. The majority of the studies (16 of 20) had both male and female participants, three studies had all-male participants [28, 31, 32], and one study did not describe gender breakdown [23]. Tables 1 and 2 provide a summary of the study characteristics of the included studies.

Table 1: Summary results of occupational studies including exposure groups, study location, samples used, and key findings.

Author	Exposure setting	Exposed group	Country	Samples type	Exposure	Outcome	Main findings
Alabi et al. [14]	Occupational	Scavengers (95) vs. control group (104)	Nigeria	Blood, buccal cells	Pb, Ni, Cd, and Cr	Micronuclei, binucleated cells, pycnosis, condensed chromatin, karyorrhesis, lobbed nuclei	Micronuclei: mean (168.04 vs. 3.23, $p<0.01$), binucleated cells: (42.20 vs. 0.08, $p<0.01$), pycnosis: (26.02 vs. 0.00, $p<0.01$), condensed chromatin: (13.72 vs. 0.01, $p<0.01$), karyorrhesis: (29.47 vs. 0.00, $p<0.01$), lobbed nuclei: (35.29 vs. 0.00, $p<0.01$). E-waste workers had increased micronuclei compared to the control group.
Berame et al. [24]	Occupational	E-waste recyclers (40) vs. controls (52)	Philippines	Buccal cells	NR	Micronuclei frequency	
Neitzel et al. [16]	Occupational	Informal recycling (120)	Thailand	Urine and blood	Pb, Cd, Mn	8-hydroxy-2'-deoxyguanosine (8-OHdG)	Men who reported working >48 h/week had significantly ($p=0.045$) higher levels of 8-OHdG compared to men working ≤ 48 h/week
Sheng et al. [32]	Occupational	Informal recycling (64)	China	Dust, hair, and urine	PCDD/Fs, PBDEs, and PCBs	8-OHdG	Pre- vs. postworkshift 8-OHdG: mean (range): 6.4 (0.64–95.74) vs. 24.55 (0.37–343.17) $\mu\text{mol/mol creatinine}$, $p<0.05$.
Wang et al. [33]	Occupational	Informal recycling (48) vs. controls (56)	China	TSP, blood and urine	Pb, Cu, and Cd	Micronuclei in binucleated cells	Micronuclei, median (range): median 4.0% (2.0–7.0) vs. 1.0% (0.0–2.0), $p<0.01$. Positive correlation between blood lead and micronuclei in binucleated cells ($r=0.245$, $p<0.01$).
Wang et al. [28]	Occupational	Informal recycling (146) vs. farmers (121)	China	Blood and semen	PCBs, Pb, Cu, Zn, Ca, Mg, Fe and Se	Chromosomal aberration, micronuclei, and DNA damage (DNA TDNA%, T.M. and OTM)	Chromosomal aberration (%): (8.01 vs. 1.80), micronuclei (%): (26.30 vs. 4.52), comet assay (greater DNA damage in exposed than in control group), $p_{\text{all}}<0.001$. duration of exposure is associated with C.A., CBMN, and DNA damage
Yuan et al. [29]	Occupational	Informal recycling (23) vs. farmers (26)	China	Blood and urine	PBDEs	Micronuclei, 8-OHdG	Micronuclei, median (range): 5 (0–96) vs. 0.00 (0–5.00), $p<0.001$, 8-OHdG, mean \pm SD: 69.04 \pm 222.2 vs. 229.97 \pm 210.1 $\mu\text{mol/mol of creatinine}$, $p=0.200$. Working with e-waste is associated with increased micronuclei frequencies OR, 38.85; 95% CI (1–1,358.71), $p=0.044$

Pb, lead; Cd, cadmium; Ni, nickel; Cr, chromium; Mn, manganese; Cu, copper; Zn, zinc; Ca, calcium; Mg, magnesium; Fe, iron; Se, selenium; Hg, mercury; PBDEs, polybrominated diphenyl ethers; PCDD/F, polychlorinated dibenzo-p-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; OH-PAHs, hydroxylated polycyclic aromatic hydrocarbons; TSP, total suspended particles; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; UCB, umbilical cord blood; DNA, deoxyribonucleic acid; TDNA%, % tail DNA; TM, tail moment; OTM, olive tail moment; CBMN, cytokinetic block micronuclei; CA, chromosomal aberration; MNed BNC, micronucleated binucleated cells; NR, not reported; SD, standard deviation.

Table 2: Summary results of ecological studies, including exposure groups, study location, samples used, and key findings.

Author	Exposure setting	Exposed group	Country	Sample type	Exposure	Outcome	Main findings
Chen et al. [34]	Ecological: exposed town vs. control town	Population (n=138) 58 vs. 80	China	Blood	NR	Micronucleated binucleated cells	MNed BNC frequency: (median: 4.0%, IQR: 2.0–7.0%) vs. (median: 1.0%, IQR: 0.0–2.0%), p<0.01
He et al. [30]	Ecological: exposed town vs. control town	Population (n=48) 23 vs. 25	China	Blood	POPs	ROS and micronucleus rate	Micronucleus rate: (16.74 ± 4.17%) vs. (7.8 ± 1.13%), p<0.05
Khlaif et al. [23]	Ecological: exposed town vs. control town	Population (n=61) 45 vs. 16	Palestine	Blood	N.R.	Total chromosome aberrations (CA), tail length relative to tail plus nucleus' length (TL/TL + NL)	Total chromosome aberrations (CA): mean ± SD (4.84 ± 2.9 vs. 0.75 ± 0.931, p<0.001). Comet assay: (TL/TL + NL) mean ± SD (0.7088 ± 0.5595 vs. 0.520 ± 0.0498, p<0.001) Micronucleus rate: (18.27 vs. 7.32%, p<0.05)
Li et al. [35]	Ecological: proximity group vs. remote group	Population (n=58) 30 vs. 28	China	Blood	Ca, Cu, Fe, Pb, Mg, Se, and Zn	Micronucleus rate	
Li et al. [26]	Ecological: exposed town vs. control town	Neonates (n=302) 200 vs. 102	China	UCB	Cr	Comet assay (injury rate (tailing rate) and the lengths of tail)	Cell injury rate (%): 33.20 vs. 10.70, p<0.01, Length of tails (%): 4.49 vs. 2.09, p<0.01
Lin [36]	Ecological: exposed town vs. two control towns (20 and 40 km from the exposed town)	Puerperae (n=320) 227 vs. 93	China	Placenta	Cd, Pb	Placental telomere length	Placental telomere length: negative correlation with placental Cd concentration (r=-0.138, p=0.013).
Liu et al. [37]	Ecological: exposed towns vs. control towns	Population (n=201) 171 vs. 30	China	Blood	NR	Chromosomal aberrations, micronucleus, DNA percentage in the comet tail (TDNA%), tail moment (TM), and olive tail moment (OTM)	CA rates (%): (5.50 vs. 1.70, p<0.001), micronuclear rates (%): (16.92 vs. 3.47, p<0.001), comet assay (mean ± SD): TDNA% (4.27 ± 0.32 vs. 1.18 ± 0.13), TM (0.53 ± 0.09 vs. 0.05 ± 0.01), OTM (0.82 ± 0.09 vs. 0.19 ± 0.02), p _{all} <0.0001.
Lu et al. [38]	Ecological: exposed towns vs. control towns	Population (n=176) 130 vs. 46	China	Urine	OH-PAHs	8-OHdG	8-OHdG, GM order: e-waste area>rural reference>urban reference (16.2>12.3>11.6). Positive association between 8-OHdG and Σ ₁₀ OH-PAHs in e-waste participants (β=0.349; 95% CI: -0.210, 0.488; p<0.001) Comet assay: Tail length (2.07 ± 0.41 vs. 1.78 ± 0.59 μm, p<0.001), olive tail moment (0.16 ± 0.04 vs. 0.14 ± 0.03 μm, p<0.001), Tail DNA (2.67 ± 0.42% vs. 2.22 ± 0.40%, p<0.001). Blood arsenic correlated with Tail length (r=0.244, p<0.05) and Olive Tail Moment (r=0.231, p<0.05)
Ngo et al. [15]	Ecological: exposed town vs. control town	Children (8–14 years) (n=80) 40 vs. 40	Vietnam	Blood	Pb, Cd, Cr, Ni, and As	Comet (tail length (μm), olive tail moment (μm), and %Tail DNA)	UCB plasma 8-OHdG: (median: 179.77 vs. 159.00 ng/mL, p=0.028). 8-OHdG correlated with Cd (r=0.235, p=0.001), Cr (r=0.214, p=0.002), and Ni (r=0.314, p<0.001)
Ni et al. [27]	Ecological: exposed town vs. control town	Neonates (n=201) 126 vs. 75	China	UCB	Pb, Cd, Cr, and Ni	8-OHdG	

Table 2: (continued)

Author	Exposure setting	Exposed group	Country	Sample type	Exposure	Outcome	Main findings
Wang et al. [2]	Ecological: exposed towns vs. control town	Population (n=377) 286 vs. 91	China	Blood and urine	Cu, Fe	8-OHdG	8-OHdG in males (mean \pm SD): $(7.75 \pm 14.39$ vs. 9.73 ± 7.39 , $p < 0.01$) $\mu\text{mol/mol creatinine}$. Blood ferrous associated negatively with 8-OHdG ($\beta = -0.215$, $p = 0.037$)
Xu et al. [25]	Ecological: exposed town vs. control town	Preschool chil-dren (n=118)	China	Blood and urine	Pb, Cd, and Hg	8-OHdG	8-OHdG, median (range): 407.79 (152.05 – 876.26) ng/g creatinine. Higher Pb and Hg exposure are associated with higher 8-OHdG
Yu et al. [31]	Ecological: exposed town vs. control town	Population (n=57) 32 vs. 25	China	House dust, and semen	PBDEs	Tail DNA%, OTM, and apoptosis rate	Comet assay (mean \pm SD): tail DNA% (57.88 ± 6.08 vs. 33.55 ± 6.99 , $p < 0.001$), OTM (12.15 ± 2.52 vs. 5.14 ± 4.86 , $p < 0.001$) TUNEL assay: apoptosis rate (32 ± 19 vs. 20 ± 8 , $p = 0.037$)

Pb, lead; Cd, cadmium; Ni, nickel; Cr, chromium; Mn, manganese; Cu, copper; Zn, zinc; Ca, calcium; Mg, magnesium; Fe, iron; Se, selenium; Hg, mercury; PBDEs, polybrominated diphenyl ethers; PCDD/F, polychlorinated dibenzo-p-dioxins and dibenzofurans; PCBs, polychlorinated biphenyls; OH-PAHs, hydroxylated polycyclic aromatic hydrocarbons; TSP, total suspended particles; 8-OHdG, 8-Hydroxy-2'-deoxyguanosine; UCB, umbilical cord blood; DNA, deoxyribonucleic acid; TDNA%, % tail DNA; TM, tail moment; OTM, olive tail moment; CBMN, cytokinetic block micronuclei; CA, chromosomal aberration; MNed BNC, micronucleated binucleated cells; NR, not reported; SD, standard deviation.

Narrative synthesis of study outcomes

Of the 20 studies included, six (6) biomarkers of DNA damage (micronuclei, comets assay, 8-OHdG, telomere length, apoptosis rate and chromosomal aberrations) were measured in seven (7) different biological matrices (buccal cells, blood, umbilical cord blood (UCB), placenta, urine and semen) (Table 3). Telomere length ($n=1$) and apoptosis rate ($n=1$) were the least biomarkers measured, while the micronuclei frequency rate ($n=9$) was the most commonly measured biomarker. Finally, whereas six studies measured more than one biomarker, the remaining 14 studies measured one biomarker each. Most studies used blood ($n=9$) and urine ($n=7$) as biological matrices in which DNA damage was measured.

Micronuclei frequency

Micronuclei (MN) frequency has been widely used as a biomarker to investigate DNA damage in human populations exposed to genotoxic agents [39]. In this review, nine studies [14, 24, 28–30, 33–35, 37] utilized MN frequency rates as a biomarker of DNA damage associated with e-waste exposure. Five out of the nine studies [14, 24, 28, 29, 33] targeted occupationally exposed groups, and the remaining four were ecological studies. Almost all the studies were conducted in China except for two [14, 24], which were done in Nigeria and the Philippines, respectively.

In the Nigerian study, Alabi et al. [14] evaluated micronuclei frequency among teenage e-waste scavengers in the Alaba International market, a major electronic waste dumpsite in Lagos. The results show that the average MN in exfoliated buccal cells in scavenging e-waste workers ($n=95$) was significantly higher than that in the control group ($n=104$) (168.04 vs. 3.23 , $p < 0.001$). Further analysis of other cytogenetic alterations, such as markers of cell proliferation (cells with condensed chromatin and binucleated chromatin) and parameters of cell death (pycnostotic and karyorrhectic cells), were all significantly higher in the Alaba group than in the control group (all $p < 0.01$). Similarly, Berame et al. [24] measured MN frequency in the buccal epithelium of e-waste recyclers and controls in Payatas, the Philippines. The results showed a significant increase in the number of micronuclei in e-waste workers than controls ($p=0.00$) [24].

All the studies conducted in China reported consistently higher MN in e-waste-exposed populations than in control groups. Wang et al. [33] and Chen et al. [34] reported similar results between e-waste-exposed populations and control

Table 3: Summary of DNA damage biomarkers and samples used. Blue cells indicate biomarkers assessed in the study, and gray cells indicate the type of sample used.

Publications	Sample						DNA damage biomarkers					
	EFBC	Blood	UCB	Placenta	Urine	Semen	MN	CA	Comets*	8-OHdG	TL	A.R.
Alabi et al. [14]	■						■					
Chen et al. [34]		■					■					
He et al. [30]		■					■	■	■			
Khlaif et al. [23]		■					■	■	■			
Li et al. [35]		■					■					
Li et al. [26]			■	■							■	
Lin [36]				■			■	■				
Liu et al. [37]		■					■	■				
Lu et al. [38]					■					■		
Neitzel et al. [16]		■							■	■		
Ngo et al. [15]		■							■	■		
Ni et al. [27]			■							■		
Sheng et al. [32]					■					■		
Wang et al. [2]					■					■		
Wang et al. [33]		■					■					
Wang et al. [28]		■				■	■	■	■			
Xu et al. [25]					■					■		
Yu et al. [31]		■				■			■			■
Yuan et al. [29]		■			■		■			■		
Berame et al. [24]	■						■					
Total	2	9	2	1	6	2	9	3	6	7	1	1

EFBC, exfoliated buccal cells; UCB, umbilical cord blood; MN, micronuclei; CA, chromosomal aberrations; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; TL, telomere length; AR, apoptosis rate; *, comet assay (TDNA%, TM, and OTM).

groups. The median MN frequency was 4% in Guiyu e-waste workers (n=48) compared to 1% in the control group (n=56) ($p < 0.05$) [33]. Similarly, Chen et al. [34] evaluated MN frequency in residents of an e-waste site and reported a higher median MN frequency (4%) in the e-waste residents than in the control group (1%), ($p < 0.01$).

In a study of male e-waste recyclers by Wang et al., a significant increase in the frequency of MN was observed in those with occupational exposures compared with those with no occupational exposures (26.30 vs. 4.52%, $p < 0.001$). In addition, e-waste workers were classified into exposure durations (≤ 3 , 3–6, and > 6 years) to investigate the relationship between e-waste exposure and MN frequency. The results showed a significant positive association between MN and duration working with e-waste [28]. The results from earlier studies [29, 30, 35, 37] all demonstrated a strong and consistent association between e-waste exposure and MN frequency rates. Overall, these studies consistently indicated a link between e-waste exposure and MN frequency in exfoliated buccal cells and peripheral blood lymphocytes (PBLs).

Chromosomal aberrations

Three studies examined the association between e-waste exposure and DNA damage using chromosomal aberration (CA) as a biomarker. All three studies showed significantly higher CA frequency among the e-waste exposed group compared to the control groups. Wang et al. [28] examined DNA damage in peripheral blood lymphocytes among e-waste workers (n=146) and a control group (121) in China. The results of this study showed that the total CA in the e-waste workers was approximately 5-fold higher than that in the control group (8.01 vs. 1.8%, $p < 0.001$). The duration of e-waste exposure was also positively correlated with CA. However, no significant difference in CA was observed between smoking e-waste workers and nonsmoking workers ($p > 0.05$). Similarly, according to Liu et al. [37], individuals (n=171) recruited from three e-waste polluted villages in northern China had significantly increased levels of total CA than those recruited (n=30) from a neighbouring village with no e-waste exposure (5.50 vs. 1.70%, $p < 0.001$). The third study by Khlaif et al. [23] also

found a significant mean difference in total CA among the e-waste-exposed group compared to a control group in Palestine (4.83 vs. 0.75%, $p < 0.001$).

Comet assay parameters

Six studies [15, 23, 26, 28, 31, 37] utilized the comet assay to measure DNA damage associated with e-waste disposal. One study [26] examined the tail injury rates and tail length (TL) of neonates born in Guiyu, an e-waste polluted town, compared to those born in Chaonan, with no history of e-waste exposure. Tail injury rates and TL were observed to be significantly higher in the Guiyu group than in the control group (33.20 vs. 10.7, $p < 0.05$) and (4.49 vs. 2.09, $p < 0.01$), respectively. The measured umbilical cord blood chromium (UCB Cr) level, which was higher in the exposed neonates, correlated positively with DNA damage parameters (tail injury rate and tail length). Similarly, in a recent study in Vietnam, DNA damage in blood cells given as TL, olive tail moment (OTM) and % tail DNA (TDNA%) were measured in children who resided in an e-waste polluted village and children from a control village. The mean \pm standard deviation TL, OTM, and TDNA% of 2.07 ± 0.41 , 0.16 ± 0.04 , and 2.67 ± 0.42 respectively, in the e-waste exposed children were higher than those in children from a control village with 1.78 ± 0.59 , 0.14 ± 0.03 , and 2.22 ± 0.40 respectively ($p_{\text{all}} < 0.001$) [15].

Liu et al. [37] examined DNA damage in residents of e-waste processing sites in China. The results showed that the averages of TDNA% (4.27 vs. 1.18), TM (0.53 vs. 0.05), and OTM (0.82 vs. 0.19) were significantly higher in the e-waste-exposed group than in the control group. Among the exposed group, females had a higher degree of DNA damage than their male counterparts. Another study performed by Khlaif et al. [23] found significantly increased DNA damage parameters (tail and nucleus lengths) in an e-waste-exposed population compared with controls in Palestine.

Yu et al. [31] also examined TL and TDNA% in blood lymphocytes of men recruited from e-waste dismantling areas in south China to assess semen quality associated with e-waste exposure. The results of this study showed that the average TDNA% and OTM of 57.88 vs. 33.55, $p < 0.001$ and 12.3 vs. 5.14, $p < 0.001$ respectively, were significantly higher in the exposed group ($n=32$) than in the control group ($n=25$). The results indicate a higher risk of infertility in the e-waste-exposed group. Similarly, Wang et al. [28] found significantly higher levels of DNA damage (represented by TDNA%, TM and OTM) in spermatozoa and lymphocytes of e-waste-exposed men than in those of the controls. Again, comparing 146 adult men directly and

actively involved in e-waste processing with 121 adult vegetable farmers who resided approximately 50 km away with no history of e-waste exposure, Wang et al. [28] reported the duration of exposure to be significantly positively associated with TDNA% and TM of both spermatozoa and lymphocytes and showed that the e-waste workers were at increased risk of DNA damage compared to the vegetable farmers.

Oxidative DNA damage

Oxidative DNA damage threatens genome stability and has been implicated in the pathogenesis of chronic diseases, including cancers [40, 41]. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the primary product of oxidative DNA damage and is used as a biomarker of genome stability associated with genotoxic exposure [42]. In this review, seven studies [2, 16, 25, 27, 29, 32, 38] examined plasma or urine 8-OHdG as a biomarker of DNA damage associated with e-waste exposure.

The results of these studies were contradictory. Three studies [2, 27, 29] did not find a significant difference between the e-waste-exposed population and the control group. Ni et al. [27] did not find any significant difference in UCB plasma concentration of 8-OHdG in neonates born in Guiyu and those born in a control town (median: 162.9 vs. 153.69 ng/mL, $p=0.117$). However, neonates whose mothers engaged in e-waste recycling activities had higher UCB plasma concentrations of 8-OHdG than neonates to mothers who were non-occupationally exposed (median: 179.77 vs. 159.00 ng/mL, $p=0.028$). In addition, blood Cd, Cr, and Ni were significantly positively associated with UCB plasma 8-OHdG concentration. In contrast, Wang et al. [2] found higher urinary 8-OHdG in the non-occupationally exposed group than in the occupationally exposed group (mean creatinine levels: 3.78 vs. 3.55 nmol/mol, $p < 0.01$). Yuan et al. [29] also reported a statistically insignificant difference in urinary 8-OHdG among 23 e-waste workers and 26 farmers (mean creatinine levels: 69.04 vs. 229.97 $\mu\text{mol/mol}$, $p=0.200$) in China.

Lu et al. [38] examined the urinary concentration of 8-OHdG in people living in and around e-waste dismantling facilities ($n=130$) and in reference populations from rural (24) and urban (22) areas in China. They reported that urinary 8-OHdG concentrations were in the following order: e-waste dismantling area (GM: 16.2 $\mu\text{g/g Cre}$) > rural reference area (GM: 12.3 $\mu\text{g/g Cre}$) > urban reference area (GM: 11.6 $\mu\text{g/g Cre}$). Another study in Thailand found an association between the duration of e-waste exposure and urinary level 8-OHdG. The study found significantly higher urinary 8-OHdG in men who worked ≥ 48 h/week than in

those who worked ≤ 48 h/week [16]. Similarly, Sheng et al. [32] found a sharp increase in the urinary level 8-OHdG from the preworkshift (6.40 ± 1.64 $\mu\text{mol/mol}$) to the post-workshift (24.55 ± 5.96 $\mu\text{mol/mol}$), $p < 0.05$. The rise in postworkshift urinary 8-OHdG levels was attributed to oxidative stress on workers during their work time processing e-waste. Xu et al. [25] also found elevated blood Pb and Hg levels in preschool children living in e-waste-exposed towns to be significantly associated with urinary 8-OHdG concentration.

Telomere length

One study [36] examined 227 placentas of healthy puerperae from Guiyu and 93 placentas from a control group to assess placenta telomere length associated with e-waste exposure. The results showed that placental Cd concentration was negatively correlated with placental telomere length ($r = -0.138$, $p = 0.013$) and was observed to be correlated in a dose-dependent manner.

Apoptosis rate

Regarding the apoptosis rate as a biomarker of DNA damage associated with e-waste exposure, Yu et al. [31] found a significant increase in the apoptosis rate in spermatozoa of residents of e-waste-exposed towns compared to a control group ($32 \pm 19\%$ vs. $20 \pm 8\%$, $p = 0.037$).

Risk of bias (quality) assessment

All studies included in this review were cross-sectional. The risk assessment scores ranged between 3 and 7 (maximum of 10). Of the 20 studies, 12 studies [2, 14, 15, 26–31, 33, 36, 37] scored above the median score of five and were considered high quality. The appraisal details are summarized in Table 4.

Estimate of variance across studies and pooled outcomes

Nine out of the 20 included studies used Micronuclei (MN) frequency assay to measure the risk of DNA damage associated with e-waste exposure. Seven of these studies were included in the meta-analysis. One study [14], which measured MN in exfoliated buccal cells, was excluded from the meta-analysis because it was considered an outlying study that significantly affected the meta-analysis results. The other excluded study was conducted in the Philippines

by Berame et al. [24], which did not report the mean and standard deviation (SD) values. Studies that reported medians and interquartile range (IQR) or range [29, 33, 34] were converted to means and SD using the formulas provided for different sample sizes by Wan et al. [43].

Despite high heterogeneity observed between studies ($I^2 = 95.81\%$), the meta-analysis showed a significantly higher MN frequency among e-waste exposed population than the control population with an overall SMD of 2.30 (95% CI: 1.36, 3.24, p -value < 0.001) (Figure 2a). Potential publication bias was not explored due to the limited number of studies (< 10 studies) included in the meta-analysis.

In a subgroup analysis considering only ecological studies, four studies were included in the analysis. The pooled estimate of the SMD was 1.68 (95% CI: 0.85, 2.51, $p < 0.001$), with high variability between studies ($I^2 = 90.94\%$) (Figure 2b). Considering only studies conducted among e-waste workers (occupational), only three studies were included in the analysis. The combined SMD showed that e-waste recyclers had higher MN than controls (SMD: 3.09, 95% CI: 1.53, 4.66, $p < 0.001$) (Figure 2b). In a further sensitivity analysis stratified by study quality, five studies adjudged “high quality” by the NOS confirmed that MN frequency was higher among the e-waste exposed group compared to the controls (MSD: 2.80, 95% CI: 1.79, 3.81, $p < 0.001$) (Figure 2c). Only two studies were considered “low quality” and did not show a significant difference in MN frequency between the two groups ($p = 0.35$).

Discussion

To the best of our knowledge, this systematic review with meta-analysis is the first to specifically assess the risk of DNA damage associated with e-waste processing/disposal in human populations. We identified and evaluated 20 studies that investigated associations between e-waste exposure and various biomarkers of DNA damage. Nine of these studies measured DNA damage by MN assay of which seven were deemed combinable for meta-analysis. The review provides ample evidence of the deleterious effects of e-waste processing on DNA integrity, as evaluated through well-known DNA damage biomarker assays.

Despite high heterogeneity between studies, the overall SMD estimate showed higher MN frequency among the e-waste exposed group compared with the control group with an effect size (SMD) of 2.30 (95% CI = 1.36, 3.24). Subgroup analysis by study setting revealed that workers who directly recycle e-waste (occupational) had higher MN frequency (SMD: 3.09, 95% CI: 1.53, 4.66, $p < 0.001$)

Table 4: Quality assessment of included studies based on the modified Newcastle–Ottawa Scale for cross-sectional studies.

Publication	Sample selection criteria (four stars)			Comparability (four stars)		Exposure (one star)	Outcome (one star)	Total (10 stars)
	1. Representativeness of sample: a **random; b a *justified and *non-random; c selected groups; d no description 2. Sample size: a *justified and satisfactory; b not justified 3. Non-respondents: a *comparability and response rate satisfactory; b and/or response rate unsatisfactory; c no description 4. Comparison group: a *described by authors as geographically distinct; b *same community; c no comparison group 5. Subjects in outcome study controls for most important confounder; b *study controls for any additional confounder; c study did not control for any confounder. 6. Exposure measurements: a *exposure chemical(s) quantified; b exposure chemicals not reported 7. Outcome measurements: a *validated methods described; b no description of methods.							
Alabi et al. [14]	b*	c	a* b*	a* b*	a*	a*	a*	7/10 (high)
Chen et al. [34]	b*	c	a*	a* b*	b	a*	a*	5/10 (low)
He et al. [30]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Khlaif et al. [23]	b*	c	a*	c	b	a*	a*	3/10 (low)
Li et al. [35]	b*	c	a* b*	c	a*	a*	a*	5/10 (low)
Li et al. [26]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Lin [36]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Liu et al. [37]	a**	c	a*	a* b*	b	a*	a*	6/10 (high)
Lu et al. [38]	b*	c	a*	c	a*	a*	a*	4/10 (low)
Neitzel et al. [16]	b*	c	c	a* b*	a*	a*	a*	5/10 (low)
Ngo et al. [15]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Ni et al. [27]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Sheng et al. [32]	a**	c	c	c	a*	a*	a*	4/10 (low)
Wang et al. [2]	a**	c	a*	a* b*	a*	a*	a*	7/10 (high)
Wang et al. [33]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)
Wang et al. [28]	b*	c	a*	a* b*	a*	a*	a*	6/10 (high)

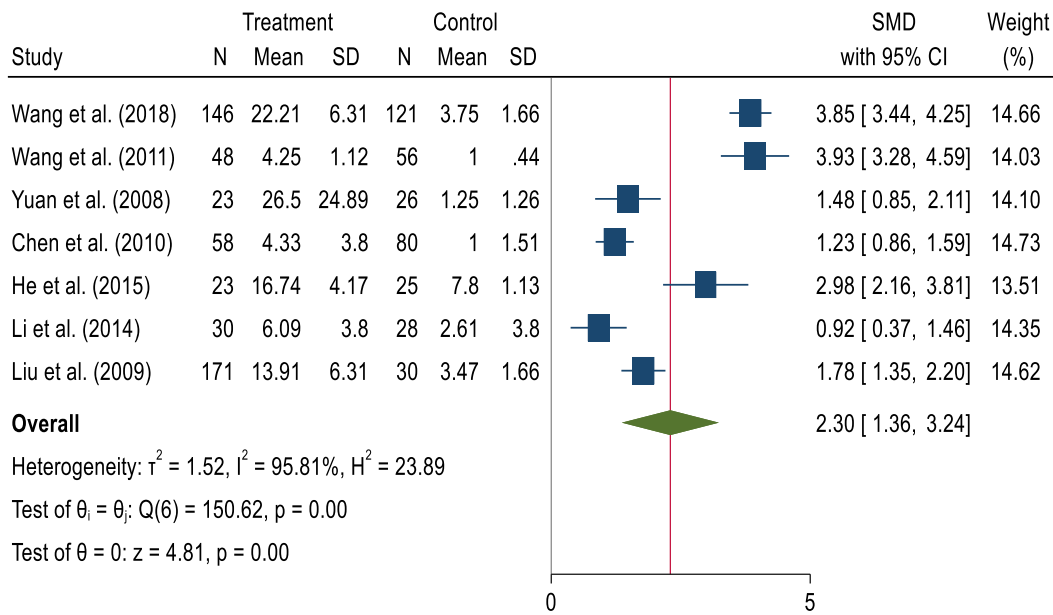
Table 4: (continued)

Publication	Sample selection criteria (four stars)	Comparability (four stars)	Exposure (one star)	Outcome (one star)	Total (10 stars)
	1. Representativeness of sample: a **random; b a *justified and satisfactory; b not justified 2. Sample size: a *justified and satisfactory; b not justified 3. Non-respondents: a *comparability and response rate satisfactory; b comparability and/or response rate unsatisfactory; c no description	4. Comparison group: a *described by authors as geographically distinct; b *same community; c no comparison group 5. Subjects in outcome groups comparable: a *study controls for most important confounder; b *study controls for any additional confounder; c study did not control for any confounder.	6. Exposure measurements: a *exposure chemical(s) quantified; b exposure chemicals not reported	1) Outcome measurements: a *validated methods described; b no description of methods.	
Xu et al [25]	b*	c	a*	a*	5/10 (low)
Yu et al. [31]	b	a*	a*	a*	6/10 (high)
Yuan et al. [29]	b	a*	a*	a*	6/10 (high)
Berame et al. [24]	b	a*	b	a*	5/10 (low)

compared to occupationally unexposed individuals (SMD: 1.68, 95% CI: 0.85, 2.51, $p < 0.001$). A possible explanation for these results might be that e-waste workers in the informal sector are continuously involved in multiple tasks and work in the open using rudimentary tools with little or no use of personal protective equipment [44, 45]. This practice exposes the recyclers to higher levels of toxic chemicals compared to the general population [46]. The frequency of MN is generally used as a biomarker of effect associated with exposure to genotoxic chemicals [47, 48]. Therefore, chronic exposure to these toxic chemicals may result in some degree of DNA damage, as explained by the increased levels of MN in e-waste recyclers compared to the non-occupational exposed group.

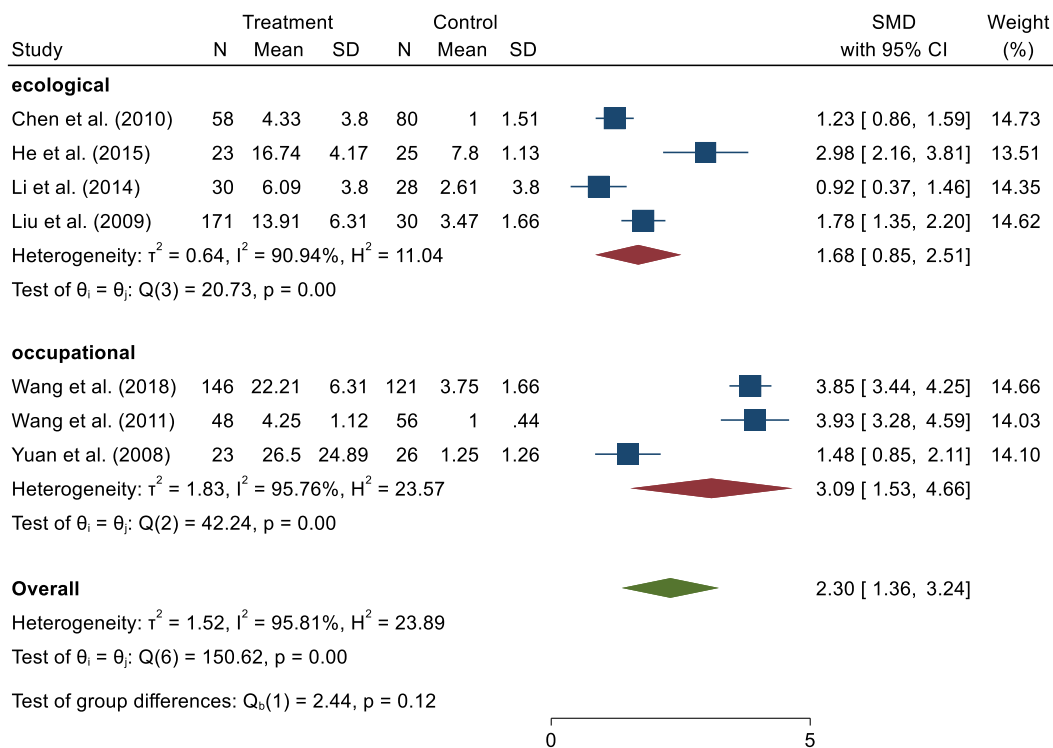
Even though all studies included in the meta-analysis were conducted in China and had similar exposure profiles, substantial heterogeneity still existed. The high variability between studies could be attributed to participant's characteristics such as age, gender, diet, and lifestyle factors (e.g., smoking, alcohol intake, and recreational drugs) which may influence MN frequency in peripheral blood leukocytes (PBL) [49]. Except for the study done by Li et al. [35], which did not control for any confounders, the remaining seven studies controlled for confounding by smoking and other important risk factors through data collection and analysis. However, four studies [28, 29, 33, 37] that controlled for confounding by smoking did not provide quantitative data about smoking, which could result in misleading conclusions when comparing smokers and non-smokers [49]. Given the causal role of MN in cancer development [50] and the evidence of their increased levels shown in this review, MN could be a primary biomarker for assessing DNA damage in e-waste-exposed populations.

The review also found that other DNA damage biomarkers, including chromosomal aberrations, tail length (TL), percent tail DNA (TDNA%), tail moment (TM) and 8-OHdG showed a consistent higher frequency among the e-waste exposed group than the controls. For instance, three studies in this review assessed chromosomal aberrations (CAs) associated with e-waste exposure. Wang et al. [28] demonstrated increased CA levels in the PBL of e-waste workers compared to controls in China. Similarly, Liu et al. [37] showed that individuals living in e-waste polluted villages in northern China had higher CA levels than those living in a neighbouring village with no e-waste exposure. A similar study conducted in Palestine by Khlaif et al. [23] reported increased CA levels in an e-waste-exposed population compared with controls. Induced chromosomal aberrations are useful biomarkers of occupational and environmental exposures to genotoxic agents and are



Random-effects REML model

Figure 2a: Standardized mean difference of micronuclei frequency for e-waste exposed population vs. control.



Random-effects REML model

Figure 2b: Sub-group analysis by study setting.

predictive of future cancer risk [51, 52]. This review’s results are consistent with a recent meta-analysis that concluded that occupational exposure to genotoxic agents such as benzene was associated with CA and MN frequencies [53].

Other studies have found increased frequencies of CA in foundry workers [54] and farmers [55].

Six studies adopted the comet assay (single-cell gel electrophoresis assay) to assess DNA damage associated

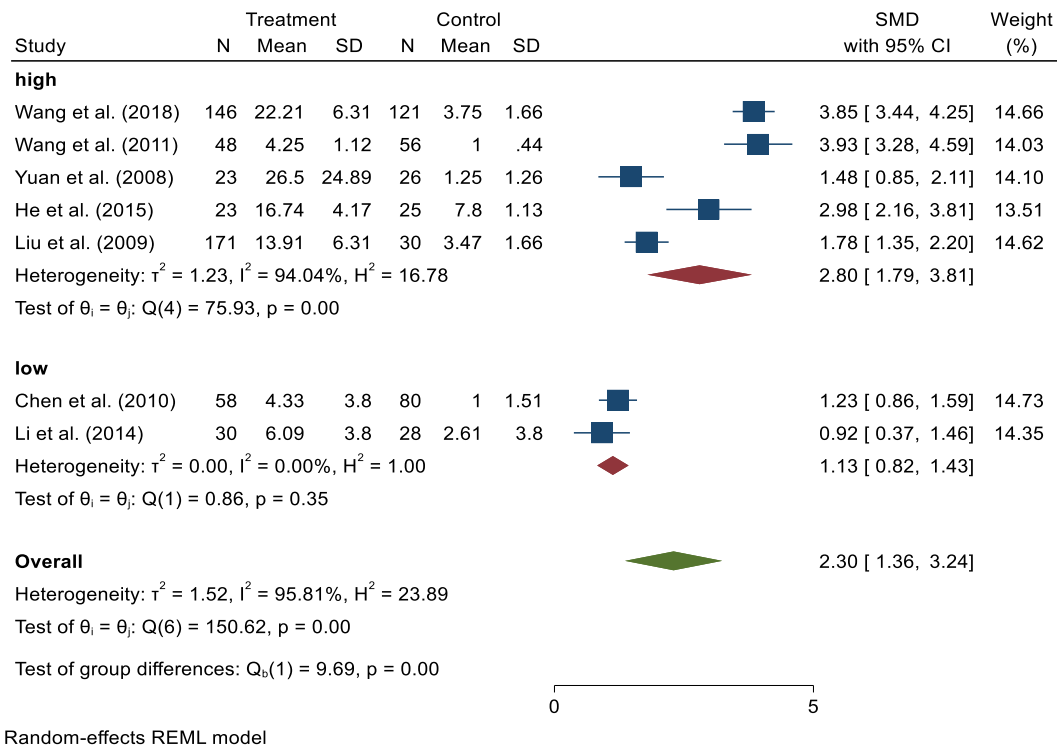


Figure 2c: Sub-group analysis by study quality.

with e-waste disposal. Primary comet assay measurements, including tail length (TL) and a fraction of DNA in the tail (% tail DNA), and derived indices, including tail moment (TM) (tail length \times % tail DNA) and olive tail moment (OTM) (the distance between the centres of gravity of the head and the tail along the x -axis of the comet \times TDNA%) [56], were used to determine DNA damage levels associated with e-waste exposure. The comet assay is a versatile, economical, and fast technique that is widely used in biomonitoring human exposure to mutagenic agents and is considered one of the most reliable biomarkers of early biological effects [57]. However, the available literature does not consider the comet assay to be predictive of cancer risk [58]. Overall, all the studies reviewed demonstrated strong and consistent relationships between e-waste exposure and DNA damage, represented by TL, TDNA%, TM and OTM, as determined by the comet assay. These results may be explained by the fact that the majority of the studies were conducted in and around Guiyu, China. Guiyu is noted for informal e-waste recycling and other industrial activities contributing significantly to environmental pollution [46, 59]. E-waste recyclers and residents may be exposed to high levels of potential clastogens and aneugens, which may damage the DNA, as observed in this systematic review's findings. Genotoxic chemicals released during e-waste recycling

such as metals and other persistent organic pollutants (POPs) are suggested to induce DNA damage through direct interaction with the DNA [60]. The results of this review are similar to those reported by Villarini et al. [61] and Cayir et al. [62], where DNA damage measured by the comet assay was higher among welders exposed to magnetics and farmers exposed to pesticides, respectively, than the general population.

Three of the seven studies [2, 27, 29] that examined DNA damage using 8-OHdG did not find any significant differences between e-waste-exposed populations and the reference populations. Several factors could account for the lack of differences observed in these studies. First, Yuan et al. [29] recruited only 49 (23 exposed and 26 controls) participants in their study. This small sample size may lack the power to detect any differences between the groups. In addition, the biological matrix used to measure 8-OHdG concentration could affect the results of these studies. For instance, urine was widely used to measure 8-OHdG in e-waste workers and controls. Although useful, urinary 8-OHdG is considered a less sensitive and accurate biomarker compared to peripheral blood leukocytes (PBL) 8-OHdG levels [63]. None of the studies in this review measured 8-OHdG in PBL, representing a long-term response to oxidative stress and a more accurate measure of the body burden of DNA damage lesions [63]. However,

direct involvement in e-waste recycling was consistently associated with 8-OHdG levels. For example, neonates of mothers who were directly involved in the processing of e-waste had a significantly higher umbilical cord blood (UCB) plasma 8-OHdG than neonates whose mothers were non-occupationally exposed to e-waste [27]. This could be attributed to the higher concentrations of metals detected in mothers who recycle e-waste, as evidenced by 8-OHdG been positively associated with Cd, Cr, and Ni concentrations ($p_{\text{all}} < 0.05$) [27]. In addition, Neitzel et al. [16] found a significant association between increased work duration and urinary 8-OHdG concentration, while Sheng et al. [32] observed a significant difference between preworkshift and postworkshift urinary levels of 8-OHdG in e-waste workers.

Limitations

The current review is not without limitations. First, because most of the studies included in this review (75%) were conducted in China, results and conclusions may not be transferable to other populations. In addition, the current review cannot demonstrate a causal relationship between e-waste exposure and DNA damage since all the studies included are cross-sectional. Future studies should consider longitudinal studies that will allow researchers to evaluate the causal relationships between e-waste exposure and DNA damage by assessing factors such as temporality and dose-response relationships.

Second, most of the studies included suffered from inadequate sample sizes and non-reporting of response rates and were limited to convenience samples. Only six studies out of the 20 studies had sample sizes > 200 . In addition, only two studies randomly recruited participants, and no study justified the sample sizes used or reported on the response rates. Future studies should consider robust methods that will enable the generalizability of study findings by including sample size calculations and reporting participant's response rates.

Third, to date, far too little attention has been paid to susceptible populations, such as neonates and children, with an increased risk of exposure due to extra exposure routes (breastfeeding and hand-to-mouth behaviour) and lower toxic elimination rates. Only four studies targeted neonates and children in this review. There is, therefore, the need to scale up research involving these groups of people since some of the chemicals released during e-waste recycling are known neurodevelopmental toxicants.

Finally, the use of urinary 8-OHdG concentration to measure DNA damage may not provide an adequate measure of DNA damage. Future studies should consider measuring DNA adducts from the blood, which provides an

integrated measure of exposure to the chemicals of interest, their ability to escape detoxification (metabolic activation) and to be delivered to the target macromolecules in target tissues, and the efficiency of the body's DNA repair pathways [64, 65].

Conclusions

Despite the limitations outlined above, the evidence from this study suggests that occupational and non-occupational exposures to e-waste are associated with an increased risk of DNA damage measured through an MN frequency and other wide range of DNA damage biomarkers. Overall, sensitive, reliable and cost-efficient assays including comet, and micronuclei assays, were used to measure DNA damage. Therefore, the findings of these studies suggest that chronic exposure to e-waste could be predictive of future cancer risk to people who directly process e-waste and residents of e-waste polluted towns. In addition, other DNA modifications, including epigenetic markers such as DNA methylation, post-translational histone modifications and miRNA frequencies, should be considered in future investigations to provide further elucidation on the mechanisms of e-waste induced health effects. We, therefore, propose to conduct a primary study at the Agbogbloshie e-waste recycling site in Accra, Ghana, to analyze DNA methylation of long interspersed nucleotide element-1 (LINE-1) as a proxy for global DNA methylation among informal e-waste recyclers. We also intend to apply robust statistical techniques for estimating the health effects of multi-pollutant mixtures to estimate DNA methylation associated with the mixture of pollutants, which represent the reality of e-waste exposure than estimating the effect of one chemical at a time.

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