RODRIGO MIGUEL RAMOS MOREIRA

Licenciado em Bioquímica

OCCUPATIONAL EXPOSURE TO E-WASTE: HUMAN BIOMONITORING AND BIOMARKERS OF EARLY BIOLOGICAL EFFECTS

MESTRADO EM BIOQUÍMICA

Universidade NOVA de Lisboa

Setembro, 2022

RODRIGO MIGUEL RAMOS MOREIRA

Licenciado em Bioquímica

OCCUPATIONAL EXPOSURE TO E-WASTE: HUMAN BIOMONITORING AND BIOMARKERS OF EARLY BIOLOGICAL EFFECTS

MESTRADO EM BIOQUÍMICA

Universidade NOVA de Lisboa

Setembro, 2022

OCCUPATIONAL EXPOSURE TO E-WASTE: HUMAN BIOMONITORING AND BIOMARKERS OF EARLY BIOLOGICAL EFFECTS

RODRIGO MIGUEL RAMOS MOREIRA

Licenciado em Bioquímica

Orientador:	Doutora Maria João Silva
	Investigadora, Departamento de Genética Humana do Instituto Nacional de Saúde Doutor Ricardo Jorge
Coorientadores:	Doutora Ana Maria Tavares
	Investigadora, Departamento de Genética Humana do Instituto Nacional de Saúde Doutor Ricardo Jorge

Júri:

Presidente:	Professor Doutor Mário Diniz
	Professor, NOVA School of Science and Technology	FCT NOVA
Arguentes:	Doutora Sónia Cristina Namorado
	Investigadora, Departamento de Epidemiologia do Instituto Nacional de Saúde Doutor Ricardo Jorge
Orientador:	Doutora Maria João Silva,
	Investigadora, Departamento de Genética Humana do Instituto Nacional de Saúde Doutor Ricardo Jorge

MESTRADO EM BIOQUÍMICA

Universidade NOVA de Lisboa

Setembro, 2022

Occupational exposure to e-waste: human biomonitoring and biomarkers of early biological effects

Publications

Moreira R, Tavares AM, Louro H, Ladeira C, Viegas S. Exposição a substâncias químicas na indústria da gestão de resíduos elétricos e eletrónicos e seus potenciais efeitos na saúde dos trabalhadores; Boletim Epidemiológico Observações, INSA. 2022; URL: http://repositorio.insa.pt/bitstream/10400.18/8034/1/Boletim_Epidemiologico_Observacoes_N31_2022_artigo3.pdf

A. M. Tavares, I. Alves, H. Louro 1, 2 , C. Ladeira 3 , S. Viegas 4 , S. Loureiro 5 , R. Moreira 1, T. Santonen 6 , T. Göen 7 , A. Kortenkamp 8 , M. Luijten 9 M.J.Silva 1,2 "Occupational exposure to metals and PAHs combining literature-based exposure and in vitro hazard data towards a mixture risk assessement" – EUROTOX 2021 Virtual Congress 26 September – 1 October

Scheepers PTJ, Duca RC, Galea KS, Godderis L, Hardy E, Knudsen LE, Leese E, Louro H, Mahiout S, Ndaw S, Poels K, Porras SP, Silva MJ, Tavares AM, Verdonck J, Viegas S, Santonen T, Hbm Eu E-Waste Study Team*. HBM4EU Occupational Biomonitoring Study on e-Waste-Study Protocol. Int J Environ Res Public Health. 2021 Dec 9;18(24):12987. doi: 10.3390/ijerph182412987. PMID: 34948598; PMCID: PMC8701897.

*Rodrigo Moreira, member of e-waste study team

NOVA
NOVA SCHOOL OF
SCIENCE & TECHNOLOGY

Occupational exposure to e-waste: human biomonitoring and biomarkers of early biological effects

Rodrigo Moreira

2022

AGRADECIMENTOS

Em primeiro lugar, gostaria de devotar um forte agradecimento às instituições Universidade NOVA de Lisboa e ao Instituto Nacional de Saúde Doutor Ricardo Jorge. Obrigado a todos os docentes com que interagi na Faculdade de Ciências e Tecnologias, por todo o trabalho e dedicação aos seus alunos e por me fazerem expandir o meu conhecimento e paixão na área da Bioquímica. Ao diretor do Instituto Nacional de Saúde Doutor Ricardo Jorge e à coordenadora do Departamento de Genética, Doutora Glória Isidro, deixo uma forte gratidão pela oportunidade de poder ter trabalhado neste instituto e por toda a experiência que me foi proporcionada.

Durante o meu percurso académico, tive suporte de várias pessoas, que me disponibilizaram força, incentivo e conhecimento.

Passo a querer agradecer a todo o pessoal do laboratório de Toxicologia Genética. A minha coordenadora Doutora Maria João Silva, pelo excelente acompanhamento e atenciosidade que disponibilizou para me guiar durante todo o projeto, em especial, por me ter facultado a hipótese de adquirir uma bolsa de investigação dentro do projeto HBM4EU. À minha co-coordenadora Doutora Ana Maria Tavares, pela enorme simpatia, sabedoria e trabalho de equipa que tivemos durante o projeto. Às Doutoras Henriqueta Louro e Célia Ventura, por sempre demonstrarem iniciativa na ajuda para o processamento (por natureza, demoroso) de colheitas de linfócitos do sangue periférico, bem como em várias assistências em pesquisas bibliográficas.

Às minhas colegas Dora, Fátima e Nádia, pelo ambiente risonho de trabalho, pelos almoços juntos, pela amizade que formámos, e por todas as ajudas no meu trabalho.

Ao meu amigo e colega João Cadete, por estarmos juntos neste percurso durante o mestrado e pela motivação que ambos partilhámos um com outro para superar os nossos desafios durante a nossa estadia no laboratório. Desejo-te um futuro próspero.

Por fim, mas deveras não menos importante, quero agradecer à minha família pelo extraordinário apoio e força que me deram, e que me continuam a dar. Por me demonstrarem que todos nós, por mais frágeis que sejam certos momentos na nossa vida, encará-los e superá-los torna-se imperativo. Obrigado por serem os melhores exemplos que a vida me poderia dar.

Aos meus amigos, por serem genuínos, por me darem a mão, por me fazerem rir. Obrigado a todos.

"Waste no more time arguing about what a good man should be. Be one."

— Marcus Aurelius, Meditations

RESUMO

Os resíduos de equipamento elétrico e eletrónico (REEE) tornaram-se uma grande preocupação dada sua rápida expansão global, que não mostra indícios de desaceleração. Como a composição dos resíduos eletrónicos é intrinsecamente prejudicial, contendo uma variedade de substâncias perigosas, como metais pesados e retardadores de chama, é certo que representa uma ameaça significativa à saúde humana e ambiental. A exposição ocupacional aos REEE é conhecida por ser uma das formas mais diretas de exposição e, portanto, a biomonitorização eficaz de trabalhadores em atividades de reciclagem de componentes eletrónicos é fundamental.

O ensaio do micronúcleo com bloqueio de citocinese (CBMN) em linfócitos do sangue periférico humano (PBL) é uma ferramenta valiosa para avaliar os efeitos da exposição humana a agentes genotóxicos. Os micronúcleos (MN) demonstraram ser excelentes biomarcadores para medir o nível de dano genético, uma vez que refletem quebra ou perda cromossómica causada pela exposição a agentes genotóxicos, após a divisão das células.

Sob a iniciativa HBM4EU, os objetivos deste projeto foram os seguintes: descrever o estado da arte da exposição ocupacional a REEE e avaliar os efeitos genotóxicos em trabalhadores expostos a esses resíduos, através da análise da frequência de MN em PBL.

A revisão da literatura sublinhou a escassez de estudos de biomonitorização sobre a exposição a REEE que relacionassem a exposição com os possíveis efeitos adversos na saúde, identificando a necessidade de novas abordagens harmonizadas para avaliar os e seus efeitos na saúde dos trabalhadores.

Os resultados do estudo piloto em ambiente ocupacional não mostraram diferenças significativas entre os grupos exposto e controlo na frequência de MN, o que contraria a hipótese de que os trabalhadores envolvidos nesta atividade de gestão de REEE pudessem revelar efeitos genotóxicos em PBL. Foram avançadas várias possibilidades para explicar este resultado, incluindo o pequeno tamanho da amostra, diferenças interindividuais e uso de equipamentos de proteção em algumas das atividades realizadas.

Para concluir, será necessário estender este estudo ocupacional, incluindo um maior número de participantes expostos e outros biomarcadores de efeito, para melhorar a avaliação dos potenciais efeitos biológicos precoces decorrentes da exposição a REEE. Para além disso, será crucial incluir biomarcadores para avaliar a exposição desses trabalhadores a diversos produtos químicos, por forma a, caso haja necessidade, se instituírem medidas mitigadoras para proteger sua saúde.

Palavras-chave: Biomonitorizacão humana; Biomarcadores de efeito; Ensaio dos micronúcleos em células com bloqueio da citocinese; HBM4EU; Resíduos de equipamento elétrico e eletrónico.

ABSTRACT

E-waste has become a major concern given its rapid global expansion, which shows no indications of slowing down. Since the composition of e-waste is intrinsically harmful, containing a variety of hazardous substances such as heavy metals and flame retardants, it is likely that it poses a significant threat to human and environmental health. Occupational exposure to e-waste is known for being one of the most direct forms of exposure. Thus, effective biomonitoring of workers whose jobs include the recycling of electronic components is critical.

The cytokinesis-block micronucleus assay (CBMN) in human peripheral blood lymphocytes (PBL) is a reliable tool to assess the effects of human exposure to genotoxins by measuring the extent of chromosomal damage in cells, both chromosome breakage or chromosome loss in dividing cells.

Under the HBM4EU initiative, the goal of this project was two-fold: to describe the state of the art of occupational exposure to E-Waste and to perform a pilot study to assess the early biological effects from e-waste exposure in workers from E-waste management industries by using the micronucleus assay.

The literature review underlined the scarcity of HBM studies around e-waste that related exposure with the possible adverse health effects and called for new, harmonized approaches to the issue of e-waste and its effects in occupational settings.

The results of the effect biomarkers assessment in exposed workers showed no significant differences between exposed and control groups in the MN frequency, although our assumptions were that e-waste workers would show higher levels of genotoxic damage. Nonetheless, several factors, such as low sample size, interindividual differences and use of protective equipment in e-waste workers might have contributed to these results.

To conclude, there is a need to extend this pilot occupational study by including a higher number of exposed participants and other effect biomarkers, to better assess potential early biological effects from exposure. In addition, from the literature review and considering the design of such human biomonitoring studies, it will be crucial to include biomarkers to assess the exposure of these workers to several chemicals, in order to know whether mitigation measures are needed to protect their health.

Keywords: E-waste; Effect biomarkers; Cytokinesis-block Micronucleus assay; HBM4EU; Human Biomonitoring

TABLE OF CONTENTS

XX

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

Amb3E - Associação Portuguesa de Gestão de Resíduos

As – Arsenic

Ba – Barium

BCEP - Bis(2-chloroethyl) phosphate

BDCIPP - Bis(1,3-dichloro-2-propyl) phosphate

Be - Beryllium

BFRs - Brominated flame retardants

BNC – Binucleated cells

BPhs - Bromophenols

Ca - Calcium

CA- Chromosomal aberrations

CBMN - Cytokinesis-Block Micronucleus Assay

CBPI – Cytokinesis-block Proliferation Index

Cr – Chromium

Co - Cobalt

Cu - Copper

Cyto-B - Cytochalasin-B

DBP - Dibutyl phosphate

DPhP - Diphenyl phosphate

EEE - Electrical and electronic equipment

E-waste - Electronic waste

E2- Estradiol

EU - European Union

ERP - European Recycling Platform

Fe - Iron

FRs - Flame Retardants

FSH - Follicle Stimulating Hormone

GM - Geometric mean

HBM - Human biomonitoring

Hg – Mercury

HQ – Hazard quotient

HUMN - International Collaborative Project on Micronucleus Frequency in Human Populations

In - Indium

INSA - Instituto Nacional de Saúde Doutor Ricardo Jorge

LH - Luteinizing hormone

Li – Lithium

Mg - Magnesium

MDA - Malondialdehyde

MN- Micronucleus

MNBNC - Micronucleated binucleated cells

NBFRs - Novel brominated flame retardants

NBUDs – Nuclear buds

Ni - Nickel

NPBs – Nuclear plasmatic bridges

OH-PCB - Hydroxy-polychlorinated biphenyl

OH-PAH - Hydroxy-polyaromatic hydrocarbons

OPE - Organophosphate ester

PAHs - Polyaromatic hydrocarbons

PHA - Phytohaemagglutinin

Pb - Lead

PBDEs - Polybrominated diphenyl ethers

PBDD/Fs - Polybrominated dibenzo-p-dioxins and dibenzofurans

PBL – Peripheral blood lymphocytes

PCDDs - Polychlorinated dibenzodioxins

PCDDs/Fs - Polychlorinated dibenzo-p-dioxins and dibenzofurans

PCDFs - Polychlorinated dibenzofurans

PCBs - Polychlorinated biphenyls

PFRs - Phosphate flame retardants

PAH – Phytohemagglutinin

POPs - Persistent organic pollutants

REEE - Resíduos elétricos e eletrónicos

ROS – Reactive oxygen species

Se - Selenium

T- Testosterone

tb-DPhP- Tertbutyl diphenyl phosphate

TH - Thyroid hormone

T_4 - Thyroxine

TSH - Thyroid-stimulating hormone

T_3 - Triiodothyronine

WEEE - Waste electrical and electronic equipment

Zn - Zinc

8-OHdG - 8-Hydroxy-2′-deoxyguanosine

1. INTRODUCTION

1.1 E-waste: definition, global overview, and management

Electronic waste (i.e., e-waste) has become a burden and a rising global problem due to not only to its highly generated volumes worldwide, but also to its toxic design, known to cause severe environmental and health implications. In addition, the lack of regulations and modernizations in the e-waste management sector aggravates the problem (Lundgren, 2012).

Currently, there is no standard definition for e-waste, but according to the European Waste Electrical and Electronic Equipment (WEEE) Directive, e-waste can be defined as: "Electrical or electronic equipment which is waste [...] including all components, sub-assemblies, and consumables, which are part of the product at the time of discarding." (Kaya, 2019; THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION, 2012). In other words, electrical and electronic equipment (EEE) of all kinds, and their components that have been thrown out as waste by the owner with no intention of being reused, are collectively referred to as "e-waste." (United Nations University, 2014). Electronic equipment such as refrigerators, personal computers, electronic tools, and medical devices are some examples of equipment that, when discarded, fall into the category of e-waste (Cui & Jørgen Roven, 2011; THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION, 2012).

According to the Global E-waste Monitor of 2020, in 2019 approximately 53.6 million metric tons (Mt) of e-waste were generated and it is estimated that the amount of e-waste generated will exceed 74Mt in 2030 (Forti et al, 2020). E-waste is, therefore, recognized as the fastest-growing category of hazardous waste stream globally (Widmer et al, 2005), in great part due to the relentless manufacturing of new electronic components and the "planned obsolesce", which refers to the ongoing shortening of electrical goods' lifespan as a result of frequent technical advancements. (Bakhiyi et al, 2017).

Around 82.6% of global e-waste flows are not documented (Forti et al., 2020), i.e., the vast majority of e-waste is not formally recycled (Ádám et al., 2021) . This indicates that the majority of e-waste isn't recycled by facilities that process e-waste inside with some level of industrial hygiene, worker protection, and pollution controls, which are ideally licensed and approved operations (Ceballos & Dong, 2016; Forti et al., 2020). Asia produced the highest amount of e-waste in 2019 (24.9Mt), whereas Europe produced the most e-waste per capita (16.2 kg per capita). Additionally, Europe has the highest formal e-waste collection and recycling rate (42.5%) among all continents (Forti et al., 2020). In general, e-waste typically travels from developed to developing nations, with the largest e-waste sites being located in China, Nigeria, Ghana, and India. (Lundgren, 2012).

The development of new strategies for e-waste management has become immensely challenging on a global scale, especially in developing countries (Shittu et al, 2020; Williams, 2016) due to certain key determinants, such as government regulations and financial impacts,

which are shown to influence the e-waste management strategy (Heeks, Subramanian, & Jones, 2015).

In developing nations, the bulk of labor with e-waste is performed in the informal sector, frequently by migrants, children, and other vulnerable populations (ILO - International Labour Organization, 2019). In these countries, crude recycling methods are applied such as open sky incineration, simple smelters, and acid baths to recover precious metals, such as gold (Au) or copper (Cu) from electronics (Osibanjo & Nnorom, 2007). However, disposal of e-waste in landfills remains a common practice in developing countries as opposed to recycling, existing a possibility of leaching of wastes, consequently contaminating the environment and becoming a severe danger to human and animal health (Sivaramanan, 2013). For example, in Nigeria, e-waste recycling and management are minimal, with most of the e-waste ending up in municipal waste dumps where it's incinerated in open air or disposed of in rivers (Andeobu, Wibowo, & Grandhi, 2021). The e-waste flow and main endpoints that can lead up to potential environmental and human health impacts are summarized in figure 1.1.
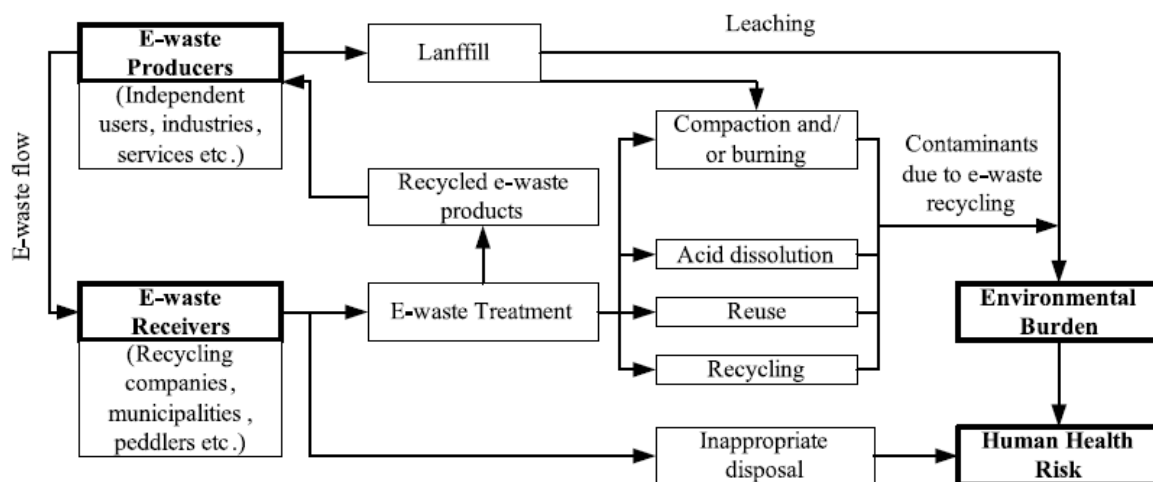
Figure 1.1- E-waste flow and main endpoints (use of image permitted by the author) (Gaidajis, Angelakoglou, & Aktsoglou, 2010)

As of today, only 20% of e-waste generated globally is managed by formal recycling systems (Wagner et al, 2020). The WEEE Directive requires all member states of the European Union (EU) to promote separate collection and resource recovery from e-waste in Europe, where the majority of formal recycling occurs (Parajuly et al, 2020). The EU is at the forefront of hazardous substances policies, which govern the use and recycling of electronic and electrical products (Selin & VanDeveer, 2006). We can set Portugal and Finland as some of the European examples.

Portugal has two entities responsible for the national e-waste management: Amb3E (Associação Portuguesa de Gestão de Resíduos) and the European Recycling Plataform (ERP) of Portugal. Amb3E is a non-profit association that aims at organizing and managing an integrated system to manage electronic waste, and is the founder of the Ponto Eletrão

initiative; ERP Portugal is responsible for new innovative ideas in the waste management sector (European Recyling Plataform, 2020; Marques & Da Silva, 2017). In Finland, the WEEE directive works in harmonization with already pre-existing waste treatment regulations, establishing a symbiotic relationship to facilitate e-waste management (Shittu et al., 2020).

Between 2011 and 2019, the volume of EEE put on the market in the European Union (EU) and the subsequent total of EEE collected, processed, recovered and recycled grew substantially in the designated period (Figure 1.2) (Eurostat, 2021).
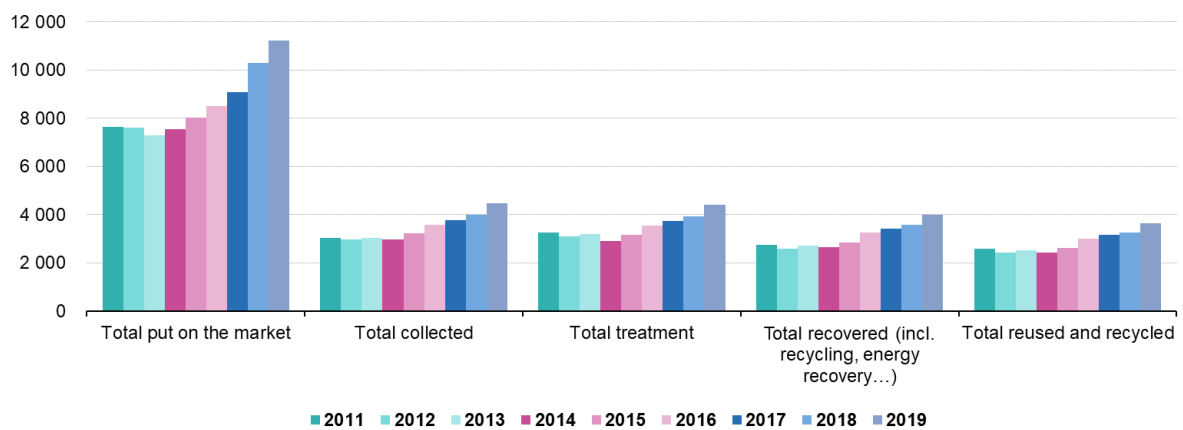
Figure 1.2- Electrical and electronic equipment (EEE) put on the market and waste EEE collected, treated, recovered, recycled, and prepared for reuse in Europe between 2011–2019 (thousand tons) (Eurostat, 2021).

Overall, there are several variables that affect e-waste management. For instance, the effectiveness of e-waste management depends on public knowledge and involvement on the issue (Borthakur & Govind, 2016). Unfortunately, public awareness of the health and environmental threat of e-waste is virtually non-existent in developing countries (Liu et al, 2009), and in developed countries the behaviour regarding proper e-waste recycling and handling, although more adequate, is still far from favourable and remains inconsistent (Bakhiyi et al., 2017). In developing countries, where the e-waste management problem is more severe, the absence of infrastructures destined for e-waste recycling, lack of funds and investments from the governments, and absence of legislation to deal specifically with e-waste aggravates these problems (Time & Sutha, 2020).

Therefore, combating the aforementioned issues remains urgent and a wide range of political, economic, and industrial hygiene measures should be applied. To increase the wellbeing of e-waste recycling workers and the general community, important steps must be taken to improve product recyclability, such as removal of toxicants before recycling, reduce poverty, and manage landfills properly (H. Yang, Ma, Thompson, & Flower, 2017). Furthermore, a collaboration of both informal and formal e-waste management sectors can improve the e-waste collection/recycling rate, ensuring better working conditions for e-waste workers and reducing environmental impacts (Wagner et al., 2020).

1.2 Hazardous components of E-waste

E-waste includes many different components that must be removed and treated separately (Cui & Jørgen Roven, 2011). Key aspects of an electronic equipment, such as the model, manufacturer, and production date all have a significant impact on the impact of e-waste. (Tipre, Khatri, Thacker, & Dave, 2021). E-waste is known to contain more than 1000 different substances, many of which are inherently toxic, such as lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), selenium (Se), hexavalent chromium (Cr (VI)), and flame retardants (FRs) that create dioxin emissions when burned (Widmer et al., 2005).

These hazardous substances have the potential to be released as a mixture of different chemical components. Individuals will then be exposed to a "cocktail" of toxic substances, leading to a variety of adverse health effects that can differ from the effects typically observed for single substances (Pascale, Bares, & Laborde, 2017). Figure 1.3 and Table 1.1 indicate the materials present, in general, in e-waste and the main hazardous substances found in e-waste, respectively.
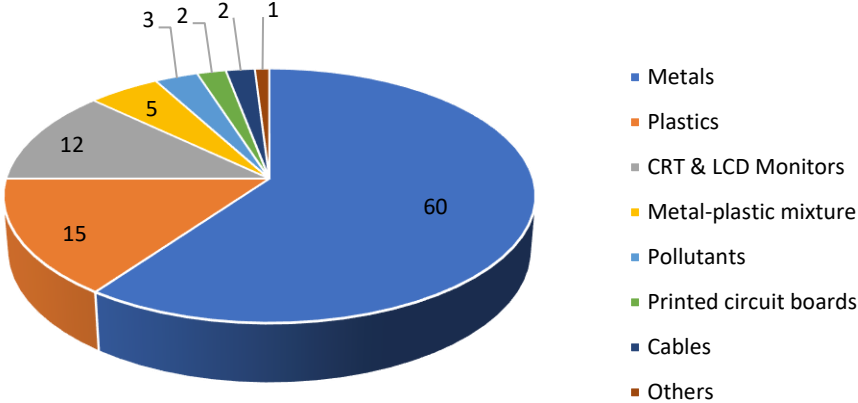
Figure 1.3 - Different contents present in e-waste in percentage (adapted from Andeobu et al., 2021)

Table 1.1- Examples of E-waste hazardous substances (adapted from (Grant et al., 2013; Perkins, Brune Drisse, Nxele, & Sly, 2014))

Persistent organic pollutants	Component of electrical and electronic equipment
Brominated flame retardants (BFRs) Polybrominated diphenyl ethers (PBDEs)	Flame retardants for electronic equipment
Polychlorinated biphenyls (PCBs)	Dielectric fluids, lubricants and coolants in generators, capacitors and transformers, fluorescent lighting, ceiling fans, dishwashers, and electric motors
Combustion by-products	
Polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs)	Released as a combustion by-product
Dioxin-like polychlorinated biphenyls	Released as a combustion by-product but also found in dielectric fluids, lubricants, and coolants in generators, capacitors and transformers, fluorescent lighting, ceiling fans, dishwashers, and electric motors
Polyaromatic hydrocarbons (PAHs)	Released as a combustion by-product
Metals	
Lead (Pb)	Printed circuit boards, cathode ray tubes, light bulbs, televisions (1·5–2·0 kg per monitor), and batteries
Chromium (Cr) or hexavalent chromium (Cr (VI))	Anticorrosion coatings, data tapes, and floppy disks
Cadmium (Cd)	Switches, springs, connectors, printed circuit boards, batteries, infrared detectors, semi-conductor chips, ink or toner photocopying machines, cathode ray tubes, and mobile phones
Mercury (Hg)	Thermostats, sensors, monitors, cells, printed circuit boards, and cold cathode fluorescent lamps (1–2 g per device
Zinc (Zn)	Cathode ray tubes, and metal coatings Batteries
Nickel (Ni)	Batteries
Lithium (Li)	Batteries Cathode
Barium (Ba)	Cathode ray tubes, and fluorescent lamps
Beryllium (Be)	Power supply boxes, computers, x-ray machines, ceramic components of electronics

1.3. Exposure to chemicals in the e-waste management industry and potential health effects on workers

The extremely hazardous and toxic e-waste substances are shown to affect the health not only of the local residents but also of workers at e-waste sites (Yu et al., 2006). Uncontrolled transfer of hazardous components to various ecological compartments during e-waste recycling processes has resulted in substantial environmental contamination, which has an adverse effect on human health as well. (S. Lin et al, 2022).

Multiple biological matrices, such as hair (Huang et al., 2015), nails (Meng et al., 2020), blood (Amankwaa, Adovor Tsikudo, & Bowman, 2017; Z. Li et al., 2021; Q. Wang et al., 2011; Y. Yang, Lu, Li, & Yu, 2013), and urine (Feldt et al., 2013; Julander et al., 2014; Qin et al., 2021; Schecter et al., 2018; Shi et al., 2019; Srigboh et al., 2016; D. Yang et al., 2020) can be used to assess workers' exposure to e-waste chemicals. Occupational studies may include contextual data of individuals, such as social-demographic data (age, gender, education, place of residence, etc.), lifestyle information (alcohol consumption, smoking status, diet, etc.), and details about workers' e-waste recycling activities (dismantling, sorting, or incinerating).

It has been well established that e-waste chemicals released during recycling activities are potential carcinogens by inducing reactive oxygen species (ROS), DNA damage in cells and cytogenetic alterations, including chromosomal aberrations (CA), and increased micronucleus frequency in cells (Issah, Arko-Mensah, Agyekum, Dwomoh, & Fobil, 2021). Liu and colleagues (2009) have shown the obvious detrimental genotoxic effects that e-waste exposure can have in humans. The authors reported a significant increase of MN frequency and CA in lymphocytes of people living near e-waste disposal areas, compared to others living in neighbouring towns without e-waste disposal sites (Liu, Cao, et al., 2009).

Studies have shown that the most susceptible groups to e-waste contaminants are children, women, and workers in archaic recycling sites (Alabi et al. 2021). Compared to more indirect forms of exposure (i.e., environmental exposure), it has been demonstrated that workers in the e-waste industry are more directly exposed to e-waste chemicals, which involves skin contact with hazardous compounds, inhalation of hazardous particles, and ingestion of contaminated dust (Perkins et al., 2014).

Occupational safety and environmental protection are not given priority in the unregulated e-waste recycling industry, which leads to high exposure levels from time spent in landfills or from inadequate ventilation in interior facilities. (Heacock et al., 2016; Kuo et al., 2020). Formal e-waste recycling facilities, however, have modern equipment, designed to safely remove and salvage materials from electronics. Nonetheless, workers from formal or semiformal recycling centres have been shown to still be at risk of occupational exposure (Grant et al., 2013), and comprehensive studies on formal recycling workers exposure are still lacking (Ádám et al., 2021).

Workers may also be exposed to combinations of various substances while recycling e-waste components, since e-waste involves a variety of complex blends of polymers and metals, among other materials, and poor handling may expose workers to extremely dangerous chemical mixtures (Frazzoli et al., 2010). The potential for combination (additivity/potentiation) impacts of e-waste-related substances is also a key consideration. However, in toxicology, elucidating combination effects remains a challenge, as well as a crucial link to "real-life" exposures (Frazzoli et al., 2010).

Additionally, e-waste workers' activity has shown to be impactful in terms of exposure and related health outcomes. In a study by Srigboh and collaborators (2016), workers who incinerated e-waste tended to have the highest biomarker levels for several metals such as Ni, Hg, and cobalt (Co) (Srigboh et al., 2016). Burning e-waste was also correlated with decreased lung function due to the inhalation of particulate matter (PM) in e-waste workers (Srigboh et al., 2016).

Limited knowledge around the risks from exposure among e-waste recycling workers is also prevalent and may exacerbate the problem. A study conducted by Jensen and colleagues (2021) aimed to understand how e-waste recycling workers from a Colombian e-waste site perceived their own risks to mercury exposure, as well from what sources would they acquire information about these risks. The authors found that workers had the tendency to underestimate the risks and frequently gravitated towards informal sources to receive health-related information (Jensen, Combariza Bayona, & Sripada, 2021).

1.3.1. Persistent organic pollutants

Persistent organic pollutants (POPs) can be divided into two main groups: Flame retardants (FRs) and Polychlorinated biphenyls (PCBs).

FRs are known for being applied to materials to slow the spread of fire after it has been ignited, as well as to prevent combustion (van der Veen & de Boer, 2012). In the category of flame retardants, polybrominated biphenyl ethers (PBDEs) are a type of brominated flame retardants (BFRs), which were commonly used in electronics and consist of a family of 209 different congeners (Cai et al., 2020).

BFRs have mostly been banned around the world, being heavily restricted in the EU and voluntarily phased out in the USA, but they can still be found in older electronics (Okeme & Arrandale, 2019; van der Veen & de Boer, 2012). In newer products, novel brominated flame retardants (NBFRs) and organophosphate esters (OPE) are mostly used (Okeme & Arrandale, 2019), but still pose a threat to human health since the evidence shows they might have similar endocrine-disrupting effects compared to PBDEs (Guo et al., 2018).

In general, FRs are known for being endocrine disruptors (Kahn, Philippat, Nakayama, Slama, & Trasande, 2020), affecting ovarian function (X. Wang, Hales, & Robaire, 2021) and the male reproductive system (Hales & Robaire, 2020). Moreover, OPEs were shown to alter DNA repair pathways, including negatively affecting the cell cycle and DNA replication in an

in vivo model (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). PBDEs were also shown to exert genotoxic effects by causing DNA damage in HepG2 cells, as assessed by increased DNA migration and % of the DNA tail in the comet assay (Tchounwou et al., 2012).

During e-waste recycling activities, workers can be exposed to FRs via inhalation and ingestion during the dismantling processes of electronics (Y. Ma, Stubbings, Cline-Cole, & Harrad, 2021). High levels of FRs such as NBFRs, OPEs, and PBDEs can be found in indoor air samples of e-waste recycling facilities, especially PBDE levels, which can be particularly high (Gravel, Lavoué, Bakhiyi, Diamond, et al., 2019). Several studies have reported increased FRs levels in blood and urine of workers compared to other non-exposed groups.

A study conducted by Qu and colleagues (2007) in China evaluated the blood serum levels of 14 PBDE congeners in three distinct groups: e-waste dismantling workers, residents living close to dismantling region, and a control group with no occupational PBDE exposure. Results showed that the levels of all PBDE congeners in e-waste dismantling workers' serum were significantly higher compared to the other groups, especially BDE-209 (median values in ng.g^{-1} of lipid weight (ng g^{-1} l.w): e-waste dismantling group = 83.5 ; residents = 18.5 ; referents = 5.7) (Qu et al., 2007).

In another study, the presence of urinary metabolites of phosphate flame retardants (PFRs) was also evaluated in e-waste workers and children who did not participate in e-waste recycling activities. The levels of di-esters, or the metabolites of phosphate flame retardants (PFRs), were assessed. These included Bis(2-chloroethyl) phosphate (BCEP), dibutyl phosphate (DBP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), and diphenyl phosphate (DPhP). The median concentrations of morning/nightfall urine of BCEP, DBP, BDCIPP, and DPhP in e-waste workers (2.43 - 4.80, 0.09 - 2.65, 0.46 - 0.89 and 0.66 - 1.83 ng/mL, respectively) were higher compared to children's urinary di-ester levels (1.23 - 1.86, 0.08 - 0.12, 0.06 - 0.14, and 0.29 - 0.27 ng/mL, respectively) (Shi et al., 2019).

Other studies not only detected higher levels among workers but also reported several exposed-related endocrine-disrupting effects. Yuan and colleagues (2008) detected higher median levels of PBDEs in e-waste workers' blood (382ng/g lipid weight) compared to the control group (158 ng/g lipid weight). It was found that workers had higher median thyroid-stimulating hormone (TSH) levels, as well as elevated frequencies of micronucleated binucleated cells (MNBNC). The MNBNC frequencies in workers were significantly correlated with previous experience with e-waste, but no other factors were related to thyroid stimulating hormone (TSH) values. However, they concluded that PBDE levels might still affect TSH levels and genotoxic damage among the workers (Yuan et al., 2008).

Graveland and colleagues (2019) studied the e-waste recycling workers' urinary levels of possible endocrine disruptors, which included OPE metabolite diphenyl phosphate (DPhP) and PBDEs. Several hormones were also tested, which included thyroid hormones (TH) (thyroxine [T$_4$], triiodothyronine [T$_3$], thyroid-stimulating hormone [TSH]) as well as sexual hormones (testosterone [T], estradiol [E2], follicle stimulating hormone [FSH], and luteinizing

hormone [LH]). E-waste workers had higher concentrations of BDE209 (a PBDE congener) and DPhP compared to commercial recycling workers (control group). The authors also noticed that a rise in DPhP levels in urine was correlated with lower total T, lower free T, and lower free T/E2 ratio in males. In contrast, an increase in BDE209 was linked to higher levels of total T4 in men. In women, a decrease in free T3 was linked to a 2-fold rise in BDE153. There were no more connections between biomarkers of exposure and biomarkers of effect (Gravel, Lavoué, Bakhiyi, Lavoie, et al., 2019).

Lastly, a class of synthetic organic compounds known as PCBs were often employed as lubricants and coolants in transformers, capacitors, and other electrical equipment (Services, 2000). These were banned in many countries between the 1970s and 1990s, including Sweden, the USA, Norway, Finland and Denmark (Faroon et al., 2003) due to their potential to bioaccumulate in both the body of animals and the environment (Yao et al., 2017). Although they are banned or heavily restricted in many countries around the world, PCBs are still found in old electrical equipment and may be released from e-waste during processing (Okeme & Arrandale, 2019).

PCBs are shown to negatively affect sperm motility and production in men (Jiang et al., 2016), decrease fecundability in women (Han et al., 2016) and induce endocrine-disrupting effects, such as changes in the expression of thyroid hormone-related proteins in children (Guo et al., 2020) and alteration of TH levels in pregnant women (Lignell et al., 2016). Furthermore, PCBs were shown to cause DNA hypomethylation in human peripheral blood monocytes (Tchounwou et al., 2012) and evident oxidative stress in a fish cell line by generating reactive oxygen species (ROS) (Marabini, Calò, & Fucile, 2011).

Studies suggest that exposure to PCBs can occur via inhalation, ingestion, and dermal absorption.

A study by Wang and colleagues (2016) was done to determine the levels of PCBs in the interior and outdoor dust from two informal e-waste recycling facilities, as well as measuring the health risks to the workers. The concentration of PCBs in the gas phase was 85.39 ng/m^3 in the industrial park and the particle phase was 10.31 ng/m^3 in the workshops. Using the hazard quotient, the risk of cancer among e-waste workers was evaluated (HQ), for which the values were considered beyond the acceptable risk levels (Yalin Wang et al., 2016).

Exposure to PCBs can also occur through dermal/hand-to-mouth contact. The finding of PCBs in the hand wipes of e-waste workers suggested that these substances may be present on the skin and that human exposure to PCBs might happen through dermal absorption and hand-to-mouth ingestion (Zhao et al., 2021).

A study conducted by Ma and colleagues (2017) examined the blood of e-waste workers in Taizhou, eastern China, for PCB levels and their hydroxylated metabolites (OH-PCB). Results showed higher levels of PCB and OH-PCB in the workers' blood, with median values of 443.7 and 133.9 ng/g lw, respectively (S. Ma et al., 2017). Furthermore, elevated

concentrations of PCBs have also been found in e-waste workers' hair in another study by Wen and colleagues (2008) (Wen et al., 2008).

Other studies have also detected early health effects caused by workers' exposure to PCBs. A study by Wen and colleagues (2008) reported elevated levels of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) (a biomarker for oxidative damage and oxidative stress), in e-waste recycling workers exposed to several persistent organic pollutants (POPs), including PCBs, were observed. Workers were mostly exposed to the PCB28, 101, 118, 138, 153, 52, and 180 congeners. The levels of urinary 8-OHdG in workers were found to be higher than those in healthy individuals, which suggested that e-waste workers were at higher risk of developing oncological diseases originating from the oxidative stress caused by the exposure to various POPs, including PCBs (Wen et al., 2008).

Furthermore, Eguchi and colleagues (2015) found that in female e-waste workers, blood levels of PCBs and hydroxy-polychlorinated biphenyl (OH-PCB) were positively correlated with total T_3, free T_3, total T_4, and free T_4, but negatively correlated with TSH (Eguchi et al., 2015).

1.3.3 Combustion by-products

Improper disposal and recycling procedures have contributed to the release of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/Fs) and polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) into the environment (Xiao, Hu, Peng, Chen, & Bi, 2016). These pollutants may originate from the conversion of PCBs and PBDEs when incinerated, respectively (Okeme & Arrandale, 2019). Furthermore, polycyclic aromatic hydrocarbons (PAHs) can be found in poisonous gases and ashes that might result from the incineration of computer cases and circuit boards (Xiao et al., 2016). Populations can be exposed to combustion by-products through dietary intake, which is considered to be one of the primary routes of exposure, aside from inhalation of toxic fumes (Fernandes & Falandysz, 2021; Helmfrid et al., 2019).

PCDDs/Fs are known to affect the growth development of children (Z. Wang et al., 2019), decrease lung function, and cause oxidative stress (Zhang et al., 2020). Long-term exposure to PAHs can cause endocrine disruptions and DNA damage, which increases the likelihood of gene mutation in cells, therefore raising the risk of cancer or cardiopulmonary illnesses (Kim, Jahan, Kabir, & Brown, 2013).

Combustion by-products may arise from smelting associated with e-waste recycling and burning or incineration of e-waste (Okeme & Arrandale, 2019), so e-waste workers could be particularly prone to high levels of exposure to this group of chemicals.

Lin and colleagues (2020) evaluated the levels of PAHs and hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) in the hair and urine of e-waste dismantling workers and residents living close to the recycling site. Although the levels of PAHs didn't vary between both groups, the levels of OH-PAHs were 9-37 times higher in e-waste workers' hair than in

that of residents living in surrounding areas. Moreover, e-waste workers were exposed to different congeners of OH-PAHs, more especifically 3-OH-BaP, which is a carcinogenic metabolite that was only detected in e-waste workers' hair, possibly due to endogenous metabolism differences. OH-PAHs levels in urine were similar in both groups (M. Lin et al., 2020).

Elevated concentrations of PCDD/Fs were also found in the hair of workers from a e-waste recycling facility in eastern China. Total PCDD/F concentrations in hair from e-waste workers were approximately 18 times higher than the concentrations measured in hair from the control population (J. Ma et al., 2011).

In a study by Lu and colleagues (2016), PAHs exposure was shown to cause oxidative stress in e-waste recycling workers. Urinary levels of 8-OHdG and malondialdehyde (MDA) were measured to assess the oxidative damage in e-waste workers and bystanders (i.e., people living near e-waste sites). The occupationally exposed e-waste workers not only showed significantly higher urinary OH-PAH concentrations but also showed elevated levels of 8-OHdG and MDA than those found in the other groups (Lu et al., 2016).

1.3.4. Metals

Inhalation, dermal absorption, and ingestion through dietary intake are considered the main routes of the general population exposure to heavy metals (Al osman, Yang, & Massey, 2019). Metals such as Pb, Cu, Cr, Cd, and nickel (Ni) are frequently measured in e-waste sites. Desoldering, shredding, melting, and burning circuit board components can result in heavy metal exposure during the recycling of e-waste. (Okeme & Arrandale, 2019).

Heavy metals exposure is known to cause several health issues, which may include cardiovascular disorders, diabetes, renal injuries and cancer (Rehman et al., 2018). Metals are renowned for their genotoxic properties, since they have the capacity to interact with cell components including DNA and nuclear proteins, promoting DNA damage and structural changes that can result in cell cycle deregulation, carcinogenesis, or cell death (apoptosis). In addition, the generation of ROS and oxidative stress play a major role in the toxicity and carcinogenicity of metals such as As), Cd, Cr, Pb and Hg (Tchounwou et al., 2012).

An occupational study in Sweden measured the levels of 20 potentially toxic metals in personal air samples, paired with whole blood, plasma, and urine samples from workers. The air test revealed that recycling employees who handle e-waste had increased airborne exposure—10 to 30 times higher—to most metals than office workers. The most abundant metal registered in air samples was Fe. Furthermore, the exposure biomarkers showed significantly higher concentrations of Cr, Co, indium (In), Pb, and Hg in the blood, urine, and/or plasma of the recycling workers, compared with in-house office workers (Julander et al., 2014) .

Ha and colleagues (2009) assessed workers' exposure to e-waste by measuring the concentration of several trace elements in workers' hair. Results showed that workers had

higher concentrations of almost all trace elements present in hair samples, particularly Ag (Ha et al., 2009).

Alabi and colleagues (2019) evaluated the DNA damage in exfoliated buccal cells of teenage scavengers at a major e-waste dumpsite through the MN assay in correlation with levels of Pb, Ni, Cd, and Cr. MN frequency in exfoliated buccal cells increased considerably in the exposed group compared to the control group. The blood Pb, Ni, Cd, and Cr levels had a positive link with the generated MN in the buccal exfoliated cells, according to Spearman correlation analysis (Alabi, Adeoluwa, & Bakare, 2019).

Wang and colleagues (2010) also evaluated the levels of heavy metals (Pb, Cu, and Cd) in workers of an e-waste recycling facility and found a positive correlation between MN frequencies and blood lead levels (Q. Wang et al., 2010).

Furthermore, another occupational study on e-waste workers indicated that elevated lead levels in the blood are associated with higher diastolic pressure, which may lead to cardiovascular diseases in the future (Upadhyay, Viramgami, Pagdhune, Balachandar, & Sarkar, 2021).

1.4 Human biomonitoring

Environmental epidemiology studies that analyse potential relations between exposure to chemicals in the environment and changes in health or biological endpoints have found great value in human biomonitoring (HBM) as a technique for exposure assessment (Lum, Chan, & Leung, 2021). HBM can be defined as "the method for assessing human exposure to chemicals or their effects by measuring these chemicals, their metabolites or reaction products in human specimens"(CDC, 2009). HBM studies require certain parameters to be fulfilled, such as: "1. Suitable biological matrices; 2. Suitable parameters that can reflect internal exposure, biochemical or biological effects; 3. Reliable analytical methods and 4. Reference and limit values that enable result interpretation" (Angerer, Aylward, Hays, Heinzow, & Wilhelm, 2011; Bergamaschi, Guseva Canu, Prina-Mello, & Magrini, 2017). Furthermore, HBM provides a comprehensive exposure picture and, more importantly, measures internal exposure levels, when compared to ambient monitoring (e.g., "air monitoring, surface contamination measurement, skin contamination") since it considers aggregated exposures (i.e., "exposure to the same substance from different sources and via different exposure routes")(Viegas et al., 2020).

Another key benefit of HBM data is that it may provide better communication than ambient monitoring data, as seen by the ease with which workers in the e-waste industry sector may get feedback on their exposures (Viegas et al., 2020).

HBM studies generally involve measurements of specific biomarkers in bodily fluids, such as blood, urine, saliva, breast milk, sweat, and other specimens, such as feces, hair, teeth, and nails (CDC, 2009).

A measurement that depicts the interaction between a biological system and a possible hazard—which might be chemical, physical, or biological—is known as a biomarker (World Health Orgnization, 1993). Biomarkers should preferably not be invasive or destructive, and should be relatively easy to measure, and inexpensive (Ladeira & Smajdova, 2017). There are three known classes of biomarkers: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (figure 1.4).

A biomarker of exposure measures an exogenous substance or its metabolite in an organism and it can represent the overall amount absorbed, the amount that reaches a particular tissue or cell, the amount that binds to target molecules, or just the biologically active or effective dose. A biomarker of effect is a measurable modification in an organism's biochemistry, physiology, behaviour, or other aspects that might be connected to a potential health problem or illness. A biomarker of susceptibility evaluates the predicted reaction of the exposed organism to the dosage of the absorbed chemical and elucidates the inter-individual heterogeneity in that response caused, for instance, by different DNA repair genes (Ant et al., 2007; Crawford, Ventii, & Shore, 2014). In the end, when the toxicological relevance of the chemical or its metabolites is well defined, these biomarkers are ultimately useful to understand the toxicokinetic fate of the chemical or its metabolites (exposure biomarkers), the disease/adverse effect mechanism (effect biomarkers), or the intrinsic factors connecting the chemical to the disease/adverse effect (for susceptibility biomarkers) (Manno et al., 2010).
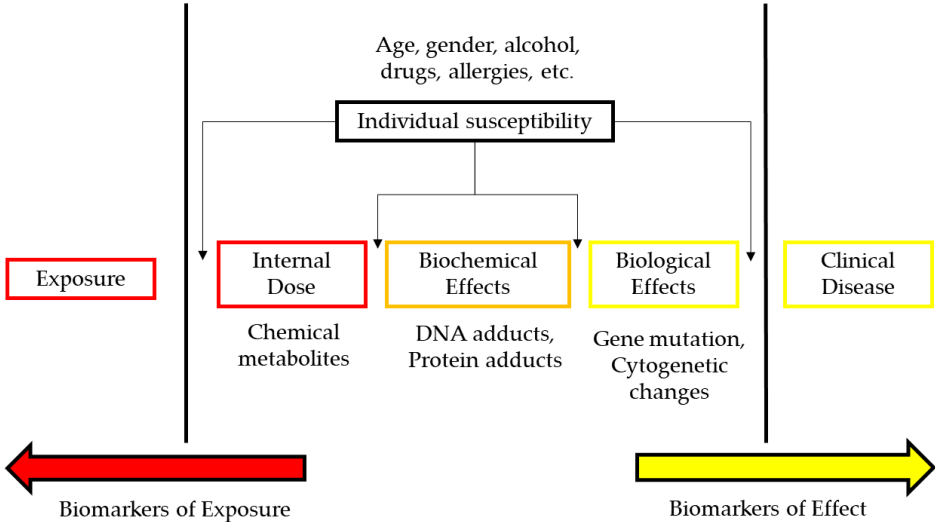

Figure 1.4- Scheme of the different classes of biomarkers (adapted from (Nyberg, Stricklin, & Sellström, 2011))

1.5 Biomarkers of genotoxicity

One of the most prominent groups of effect biomarkers are genotoxicity biomarkers. These biomarkers are often used to assess the genetic impacts of occupational and environmental exposures in cells, in order to evaluate the efficacy of exposure management measures for genotoxic substances, or to identify early disease risk factors (Ladeira & Viegas, 2016). In other words, these biomarkers are of extreme importance in the detection of early biological effects in humans and therefore serve to estimate the effects of the interactions between the environment and the individual (Zare Jeddi et al., 2021).

Several tests, such as CA, MN, and the more modern Comet assay, have been used as genotoxicity biomarkers and are also useful in HBM studies to distinguish non-exposed from exposed populations. These tests are known for being sensitive but not specific, which may result in some difficulty on the interpretation of exposure settings, even if recent approaches, such as the alkaline Comet test, appear to hold promise in discriminating between distinct types of DNA damage (covalent binding vs. oxidative stress) (Manno et al., 2010).

1.5.1 Biomarkers of genotoxicity: Micronuclei

Among the many genotoxicity biomarkers available, MN offers a potential method for evaluating the risks to human health by examining how environmental, occupational, and other variables may contribute to genetic instability. (e.g. dietary habits, age, gender, etc.) (Ladeira & Smajdova, 2017).

MN are amongst the most extensively studied biomarkers of DNA damage and chromosomal instability in humans (Michael Fenech et al., 2020). The MN assay was first developed by Boller and Schmidt in 1970, where they developed a test method to evaluate the frequency of micronucleated erythrocytes among normal erythrocytes, using bone marrow and peripheral blood cells of hamsters treated with a strong alkylating agent (Hayashi, 2016). Since then, due to its ability to accurately evaluate both chromosomal loss and chromosome breakage, MN tests have become one of the most used techniques for evaluating chromosome damage (Michael Fenech, 2008).

MN assays such as the cytokinesis block micronucleus assay (CBMN) have been applied to several human biomonitoring studies, such as in assessing the effects of occupational lead exposure by detecting the extent of genome instability in peripheral blood lymphocytes (PBL) (Kašuba et al., 2020); using exfoliated buccal cells to assess human population exposure to petrochemical industry pollutants (Federico, Vitale, La Porta, & Saccone, 2019), and also in evaluating the effects of chronic exposure to ionizing radiation (Shakeri, Zakeri, Changizi, Rajabpour, & Farshidpour, 2017).

Furthermore, a review by Bonassi and colleagues (2011) found evidence across several human studies that MN frequency in PBL is a valuable tool for prediction of cancer, since MN are related to early events of carcinogenesis (Bonassi, El-Zein, Bolognesi, & Fenech, 2011).

In the specific case of e-waste workers, few studies cover the genotoxic effects potentiated by e-waste handling practices, especially regarding the use of MN as biomarkers. In a systematic review and meta-analysis by Issah and colleagues (2021), nine studies used MN frequency rates as a biomarker of DNA damage associated with e-waste exposure, but only five out of the nine studies targeted occupationally exposed groups (Issah et al., 2021).

Taking this into account, it is plausible to admit that MN may be sensitive to be used in human biomonitoring studies, more specifically in e-waste occupational studies, and be essential to contribute to detect potential alterations that may implicate further modifications of the health status of workers to prevent them from future illness.

MN can therefore be classified as small, intracytoplasmatic corpuscles that are spatially separated from the primary nucleus (Krupina, Goginashvili, & Cleveland, 2021). Despite the fact that they can be of many sizes, they normally range between 1/10th to 1/100th of the size of the original nucleus (Beedanagari, Vulimiri, Bhatia, & Mahadevan, 2014).

Several molecular processes, such as double-stranded DNA breaks, defective DNA replication, impaired DNA repair responses, and crosslinking agents can cause the development of MN (Ye et al., 2019). These phenomena of both structural and numerical chromosome alterations (Russo & Degrassi, 2018) can occur as a result of natural processes, such as metabolism or aging (Sommer, Buraczewska, & Kruszewski, 2020), or can be induced by environmental factors, such as pollutants, radiation, bio-hazard materials, drugs, poisonous chemicals, food/drinking habits, and free-radicals (Samanta & Dey, 2012).

Different genotoxic agents can result in the alteration of DNA structure and affect nuclear integrity by using a variety of different mechanisms, and understanding these mechanisms can be of great significance for the detection of diseases, such as cancer (Luzhna, Kathiria, & Kovalchuk, 2013).

In general, genotoxic agents like aneugens and clastogens are known to produce MN, and an increase in the frequency of MN can reflect exposure to genotoxic agents with clastogenic or aneugenic modes of action (D'Costa, Praveen Kumar, & Shyama, 2019).

Clastogens lead to serious chromosome abnormalities originating from DNA double-strand-breaks, thereby forming acentric fragments. Aneugens can interfere with nuclear division and may disrupt microtubule dynamics (depolymerization/polymerization), or affect mitosis by inhibiting mitotic kinases or motor proteins (Wilde, Queisser, Holz, Raschke, & Sutter, 2019). These effects can result in numerical chromosome changes (aneuploidy), which causes whole chromosomes to lag behind at anaphase (Terradas et al., 2010; Wilde, Queisser, Holz, Raschke, & Sutter, 2019) (Fig 1.5).
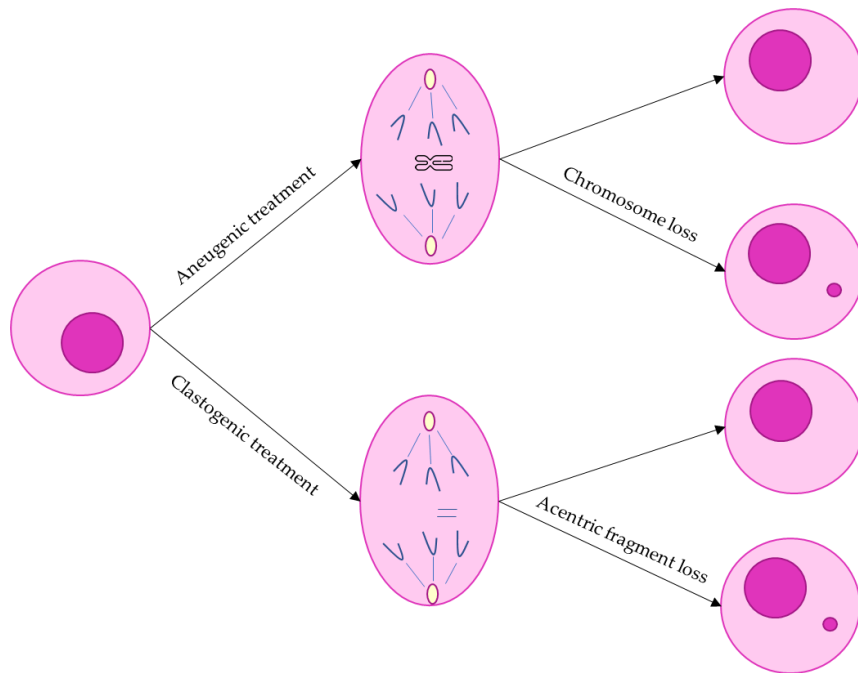
Figure 1.5- Mechanisms of formation of MN: (A) aneugenic agents lead to whole chromosome loss (B) clastogenic agents lead to acentric fragment loss (adapted from (Terradas et al., 2010))

Other nuclear abnormalities seen on the micronucleus assay are nucleoplasmatic bridges (NPBs) and nuclear buds (NBUDs). When the centromeres of dicentric chromosomes are drawn to opposing poles of the cell during mitosis, a process known as anaphase, NPBs are formed. If the anaphase bridge is not broken, the nuclear membrane eventually encircles the daughter nuclei and the anaphase bridge, resulting in the formation of an NPBs. (Fig 1.6). However, NBUDs differ from MN in that they are still attached to the nucleus by a stalk of nucleoplasmic material (M. Fenech et al., 2011).
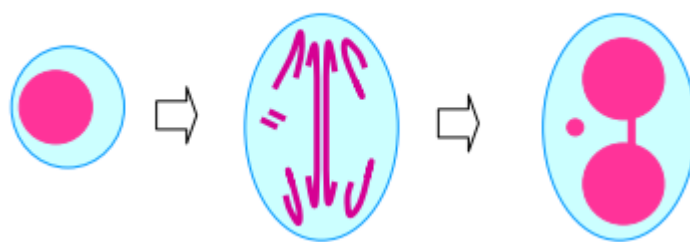
Figure 1.6 - NPB and MN formation (M. Fenech et al., 2011)

1.5.2 The cytokinesis-block micronucleus assay

A comprehensive method for assessing DNA damage, cytostasis, and cytotoxicity in several tissue types, including lymphocytes, is the cytokinesis-block micronucleus (CBMN) assay (Lazarides, 1983).

This assay is used to detect MN in the cytoplasm of interphase cells and is considered to be a robust and effective genotoxicity test compared to other previous MN assays, since it eliminates false negative results produced by the substance being tested inhibiting nuclear division, or because of host factors (e.g., ageing) reducing lymphocyte response to a mitogen. In addition, the CBMN assay provides the proportion of dividing cells to be quantified and becomes possible to score additional nuclear abnormalities (i.e., NPBs and NBUDs) in binucleated cells (BNC) thus making it possible to evaluate genotoxicity in a more thorough manner (Nersesyan et al., 2016).

It is critical to be able to recognize cells that have undergone division at least once during or after treatment, as MN expression is reliant on the cell that is undergoing division. The CBMN test does this by inhibiting cytokinesis without obstructing mitosis using cytochalasin-B (cyto-B), a mycotoxin derived from *Helminthosporium dematioideum* (Fig 1.7 (A and B)). BNC are formed when cells are treated with cyto-B, which makes the identification and scoring of BNC an easier, straightforward process (Doherty, Bryce, & Bemis, 2016).

It is commonly recognised that PBL offer an excellent chance for a unified strategy to research the effects of genotoxicity and cytotoxicity both *in vitro* and *ex vivo*, which is crucial for modelling and predicting effects in humans and other animals (Michael Fenech, 2007). PBL are one of the most utilized biological matrices for evaluation of genotoxic effects in HBM studies, since they are easy to sample, have a relatively long life span, and can be easily damaged by a hazardous substance in any specific target tissue (Ladeira & Smajdova, 2017). The most common technique used in HBM studies to assess exposure to genotoxic chemicals, and assess genetic instability, is the CBMN test in PBL (Bolognesi & Fenech, 2013). The bulk of PBL are long-lived T cells which *in vitro* division will be stimulated by using a mitogen during the CBMN assay (Kirsch-Volders et al., 2014), such as phytohaemagglutinin (PHA), which contains potent cell agglutinating and mitogenic effects, thus promoting cell division (Movafagh, Heydary, Mortazavi-Tabatabaei, & Azargashb, 2011).

However, the CBMN assay is not without its limitations. One serious disadvantage of this assay is the necessity for a cell to undergo mitosis in order for MN to be expressed (Michael Fenech & Morley, 1985). Also, artifactual positive results caused by unfavoured cell culture conditions (e.g. pH level variations in culture medium) can lead to faulty MN scoring in cells, so proper care should be taken to avoid this problem (OECD, 2016). Furthermore, manual scoring of MN can have a lot of drawbacks such as being physically demanding to visually score many samples and the fact of naturally being very time consuming (RAMADHANI & PURNAMI, 2013; Rodrigues, Beaton-Green, Wilkins, & Fenech, 2018).
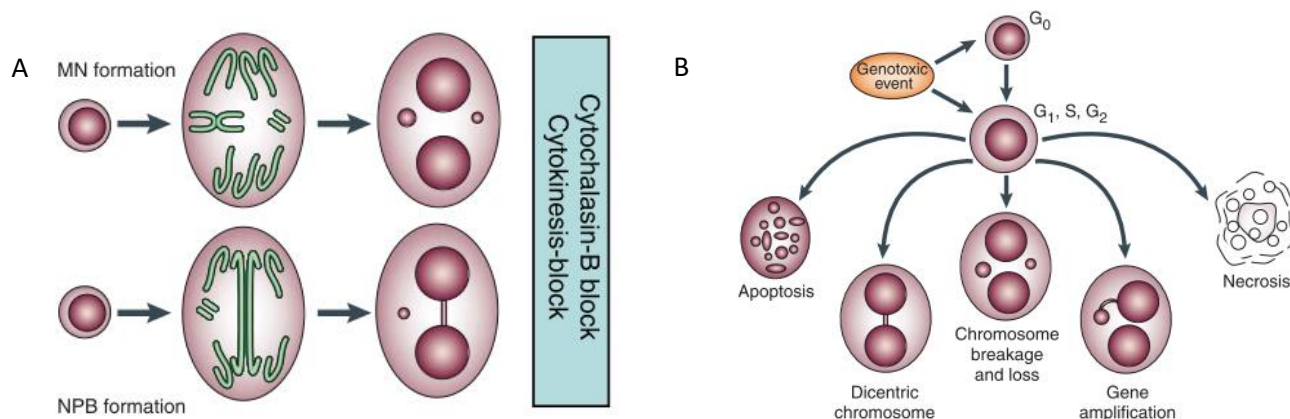
Figure 1.7- (A) Mn and NPB formation in cells undergoing cell division. Cell division is blocked by cyto-B leaving the cell in a binucleated state. (B) The various outcomes of cultured cytokinesis blocked cells (Michael Fenech, 2007).

1.5.3- The HBM4EU initiative

HBM is a cornerstone in current initiatives for evaluating health risk in a more focused manner than environmental monitoring because it looks at the internal dosage and generates an evaluation of the chemical's biologically active body burden (Longo, Forleo, Giampetruzzi, Siciliano, & Capone, 2021). Despite all the benefits of HBM, its importance as an health assessment tool is still overlooked (Viegas et al., 2020).

In 2004, the European Commission acknowledged the importance of human biomonitoring (HBM) and the need for more coordinated methodologies throughout Europe in its Environment and Health Action Plan, allowing for improved comparability of results and more effective use of resources (Gilles et al., 2021).

Therefore, in 2017, the HBM4EU initiative (https://www.hbm4eu.eu) was launched as a joint effort and collaboration between scientists of 30 European countries to provide science-based evidence to further develop new chemical policies and improve chemical management. The primary goal of HBM4EU is to research and investigate current issues in chemical risk assessment and management, with the intention to deliver answers that help policymakers protect human health. This initiative started due to the lack of comparable data at the European level on aggregate exposure to single substances and combinations of chemical substances, all of this concerning citizen's health (Probst-hensch, 2019). More recently an internal subproject of the HBM4EU initiative aimed to investigate, in a harmonized way, the levels of occupational exposure and assess the possible health effects on workers in the WEEE management industry in several European countries, including Portugal (Scheepers et al., 2021). The present study is a pilot study within the referred occupational study that will include a higher number of participants from several European countries and diverse exposure and effect biomarkers.

2. Objectives

The main goal of this work was to contribute to the knowledge on the occupational exposure to e-waste chemicals in European waste management industries and their related early biological effects by following two different approaches:

1. Mini-review on E-waste - Conduct a review to collect information regarding occupational exposure to chemicals in the e-waste management sector and their potential effects on workers' health.

2. Human Biomonitoring study - Assess effect biomarkers, more specifically, genotoxic effects among workers from e-waste recycling facilities and compare with control groups

3. MATERIALS AND METHODS

3.1 Literature Review

3.1.1 Search strategy and selection criteria for the mini review

In order to search for publications regarding occupational exposure to chemicals in the e-waste management sector and their potential effects on workers' health, two databases were consulted: PubMed and Scopus, in July of 2021. The search terms applied to both databases were: ("e-waste" OR "electronic waste" OR "e-waste recycling" OR "waste electrical and electronic equipment" OR "electronic recycling") AND ("occupational" OR "workers" OR "occupational setting" OR "workers exposed" OR "workers exposure" OR "occupational exposure" OR "human health effects " OR "biomarkers" OR "human biomonitoring"). The present research was done during the month of July of 2021.

Articles whose characteristics met the following inclusion criteria were selected (Table 3.1):

Table 3.1- Inclusion and exclusion criteria for the mini-review

Inclusion criteria	Exclusion criteria
Human biomonitoring studies	Not published in English or Portuguese
Studies in an occupational context involving the management of WEEE	Review articles or meta-analysis
Studies including data on biological effects or health effects	Non-occupational studies or studies that did not associate occupational exposure with effects on workers' health.

3.2 HBM study

3.2.1 Study population

Participants in this study received an information leaflet, containing all valuable information and intentions of this study. All individuals who accepted to participate signed an informed consent previously to blood withdrawal. Participants also had to fill out a questionnaire so that contextual data could be collected, such as: sex, age, weight, smoking status, alcohol consumption, etc. (annex 1).

For this study, a total of 171 individuals from Belgium, Finland, Portugal, Netherlands, Luxembourg, and Latvia were recruited. However, only 64 individuals from Portugal and Finland were included in this work. The target population consisted of workers employed by companies involved in the processing of e-waste, including sorting, dismantling, shredding,

and pre-processing of metal and non-metal materials. Both Portuguese and Finnish control groups consisted of individuals that were not involved in e-waste processing (academic tasks) or individuals that were present inside the e-waste recycling facility but weren't directly involved in e-waste processing (office workers).

In addition, participants had to meet certain eligibility requirements, in this case, all individuals who had been exposed to medical analysis (i.e., CT scan or x-ray), or had cancer treatment three months prior to this study, were excluded. After evaluation of the sample total of 64 individuals, 51 individuals fulfilled the inclusion criteria.

The active smokers group also included individuals that smoked regular cigarettes and e-cigarettes. Regular drinkers were considered the ones who would drink daily or weekly; sporadic drinkers incorporated individuals that wouldn't consume alcohol or that would consume it monthly.

3.2.2 Sampling methods

The sample distribution after peripheral blood collection is depicted in Figure 3.1. The blood from each individual was collected by venous puncture and manipulated under sterile conditions. After collection, blood samples were stored in heparin, EDTA, or a mixture of heparin and EDTA tubes. B-01 samples were processed in Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), while the remaining samples were processed by other responsible entities (Scheepers et al., 2021). Finnish and Portuguese B-01 samples were collected at the workers' end-of-shift and placed in heparin tubes to avoid blood coagulation. Finnish samples arrived within 4 days at our laboratory, whereas Portuguese samples arrived on the same day of their collection. All B-01 tubes were stored at 4°C, shielded from light (wrapped in aluminium foil) and immediately shipped to our laboratory.
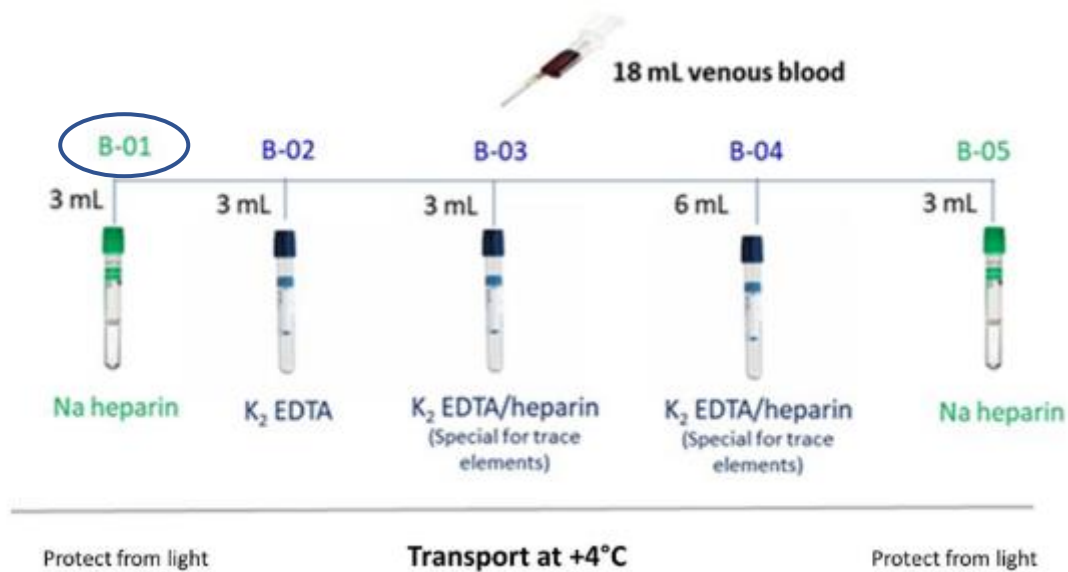
Figure 3.2- Blood sampling method (B-01 samples were processed in INSA and are circled blue)
(Scheepers et al., 2021)

3.2.3 Ethical considerations

The HBM4EU project was conducted under the regulations of the European Commission and the current General Data Protection Regulation (GDPR). Additionally, the project also adheres to the principles of the Declaration of Helsinki and is under national legislation requirements of each participating country, in this case, Finland and Portugal. The study was also submitted and approved by the Ethics Committees for Health of both countries. All the individuals have freely decided to participate in this study and suffered no direct influence in their decision. All personal information was considered confidential.

The study was conducted with respect for all groups in society regardless of race, ethnicity, religion, and culture, and with respect for and awareness of gender or other significant social differences to avoid a situation of unnecessary stigmatization (Scheepers et al., 2021).

3.2.4 Cytokinesis-blocked micronucleus assay in peripheral blood lymphocytes

To analyze DNA damage and chromosomal alterations in PBL of workers from the e-waste industry, the CBMN assay was performed. This assay was conducted as described by Fenech et.al (2007), with some justified alterations to fit our laboratory standards.

T25 flasks were identified with the respective sample code and replicate, including the positive control flasks from two donors. Lymphocyte cultures were carried out by adding 800µl of blood to 5ml of culture medium, containing RPMI-1640 with Glutamax + HEPES (Gibco™, Thermo Fisher Scientific), supplemented with 15% FBS (Gibco™, Thermo Fisher Scientific), 1.5% penicillin-streptomycin (Gibco™, Thermo Fisher Scientific), and 2.5 % phytohaemagglutinin (PHA) (Gibco™, Thermo Fisher Scientific). The flasks were placed at a ~30° angle of inclination in an incubator at 37°C for a 44h incubation.

At 43h, MMC (Sigma-Aldrich), which is an MN-inducing agent (Michael Fenech, 2007), at 0.01µg/µl was applied to the positive internal control cultures. After exposure, the flasks are gently mixed and incubated for 1h.

At 44h, all cultures were treated with cytochalasin B (Sigma-Aldrich) 5µg/ml and subsequently incubated for more 24h.

At 68h, the total volume of each culture flask was transferred to a 15ml centrifuge tube. The tubes were centrifuged at 1200rpm for 5min (centrifuge model: Heraeus Megafuge 1.0). The supernatants were then discarded.

A hypotonic treatment was applied by adding potassium chloride solution at 37ºC(KCl) 0.1M drop-by-drop to every tube while vortexing. The tubes were then centrifuged at 1200rpm for 5min, and the supernatants were discarded.

Cells were then fixed by adding a cold solution containing methanol (MeOH, Merck) and acetic acid (CH$_3$COOH, Merck) (3:1, v/v) drop-by-drop, to every tube while vortexing. The tubes were then centrifuged at 1200rpms for 5min, and the supernatants were discarded. This process is repeated twice.

Second cold fix methanol:acetic acid solution (97:3, v/v) acid was added drop-by-drop, to every tube while vortexing. The tubes were once more centrifuged at 1200rpm for 5min, and supernatants were discarded.

After resuspending the cells, two drops of cell suspension (~30 µL each) were spread onto a microscope slide and air-dried overnight.

After drying, cells were stained with a 4% Giemsa solution (Merck) prepared in Gurr's phosphate buffer (Gibco™). Cells were immersed for 7-8min and washed in the buffer to remove excess staining. Stained microscope slides were air-dried overnight and mounted with Entellan afterward. All slides were coded and scored blindly to avoid bias.

Scoring of lymphocytes was conducted under the microscope at 400x magnification and under the guidelines presented in the HUMN project (Michael Fenech et al., 2002). A total of 2000 binucleated lymphocytes were scored for each individual and obeyed to specific criteria: "1. The cells must be binucleated; 2. The two nuclei in a binucleated cell should have intact nuclear membranes and be within the same cytoplasmic boundary; 3. The two nuclei must be about comparable in size, staining pattern, and intensity; 4. The two main nuclei may touch but ideally should not overlap each other; 5. The cytoplasmic boundary or membrane of a binucleated cell should be intact and discernible from the cytoplasmic boundary of surrounding cells" (Thomas & Fenech, 2011).

Each binucleated cell scored was analysed for the presence of MN, NBUDs, and NPB. For MN scoring, the following criteria were considered: "1. The diameter of a MN should vary between 1/16 and 1/3 of the mean diameter of the main nuclei; 2. MN are round or oval in shape; 3. MN are non-refractile; 4. MN are not linked or connected to the main nuclei; 5. MN may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary; 6. MN staining intensity is similar to the main nuclei" (Michael Fenech et al., 2002).

For NPB scoring, the criteria were: "1. The width of a nucleoplasmic bridge may vary considerably but usually does not exceed one-fourth of the diameter of the nuclei within the cell; 2. NPB should have the same staining characteristics of the main nuclei" (Michael Fenech et al., 2002).
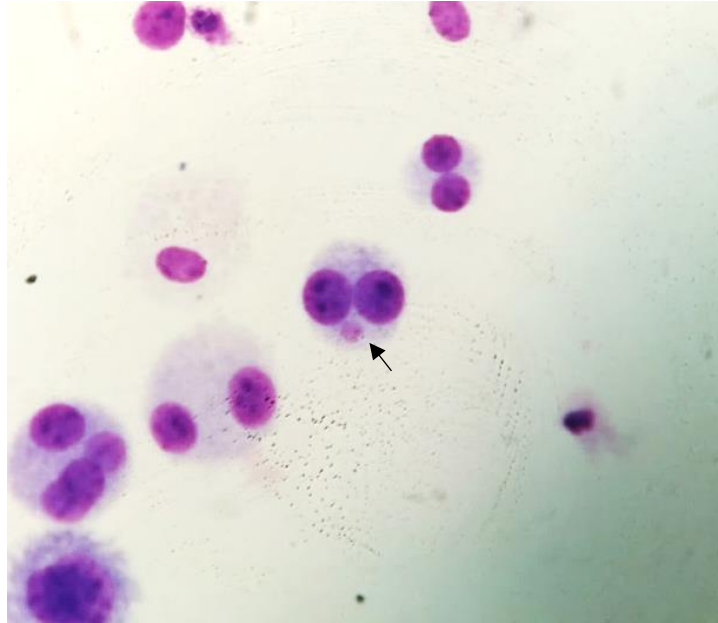
Figure 3.2- Binucleated lymphocyte cell with MN (photographed by the author; ampliation x400)

The cytokinesis-block proliferation index (CBPI) from at least 1000 cells scored per culture was calculated to estimate the cytotoxic and cytostatic effect. The equation for CBPI is indicated below:

$$CBPI = \frac{(Mononucleate\ cells) + (Binucleate\ cells)\ \times 2 + (Multinucleate\ cells) \times 3}{Total\ number\ of\ cells}$$

3.2.5 Statistical analysis

Statistical analysis of results was performed with IBM SPSS Statistics version 27. Shapiro-Wilk test was used to assess the normality of all variables. All esults were considered statistically significant when p-value < 0.05. A Mann-Whitney U test was performed for the CBMN results to compare the frequency of MNBNC/1000, NPBs/1000 BNC, NPBs/1000 BNC and CBPI between groups. The comparison between MNBNC/1000 in MMC treated samples versus samples not treated with MMC was performed by the Wilcoxon test.

4. RESULTS

4.1 Mini review on e-waste

From a total of 572 studies identified on both Pubmed and Scopus, 14 were selected and analysed, after the exclusion of duplicates and application of the inclusion/exclusion criteria (Figure 4.1). The selected studies reported occupational exposure data, obtained through biomarkers in biological samples, and potential health effects, inferred from the analysis of biomarkers of early biological effect.

The studies were carried out in industries responsible for the management of e-waste present in China, Canada, Thailand, and Vietnam. Studies were divided according to their studied biological effects, which included: endocrine disruption, genotoxicity, oxidative stress, and renal and cardiovascular function.
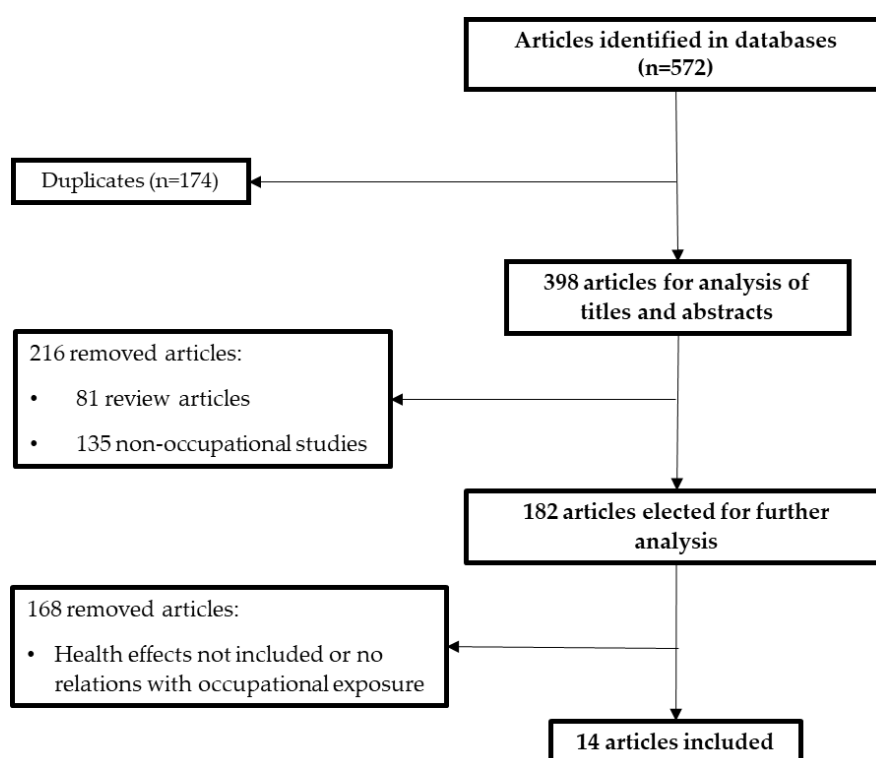
Figure 4.3- Flowchart representative of the selection process of articles obtained through bibliographic research.

Nine of the fourteen studies reported significant correlations between e-waste chemical exposure and health outcomes (Eguchi et al., 2015; Gravel et al., 2019; Lu et al., 2016; Neitzel, Sayler, Arain, & Nambunmee, 2020; Upadhyay et al., 2021; H. Wang, Lv, Li, Liu, & Ke, 2010; Q. Wang et al., 2011; Y. Yang et al., 2014; Zheng et al., 2017), whereas four others found significant correlations between specific confounding variables (namely: industry activity time, working hours, and usage of protective equipment at work) and health outcomes (Neitzel et al., 2020; Yan Wang et al., 2018; Y. Yang et al., 2013; Yuan et al., 2008). The studies differed not only regarding the investigated biological effects, but also in terms of the e-waste chemicals examined, which included PBDEs, PCBs, bromophenols (BPhs), OPEs, PAHs, PCDD/Fs, and several heavy metal elements (Cd, Cu, Pb, Fe, Zn, Ca, Mn, Mg, and Se).

Regarding the endocrine disruption effects, five studies examined the effects of exposure to e-waste on thyroid function (Eguchi et al., 2015; Gravel et al., 2019; Julander et al., 2005; Yuan et al., 2008; Zheng et al., 2017). The findings of the effects on TSH levels were shown to be inconsistent across the five studies, with one study reporting an increased concentration of TSH (Yuan et al., 2008), one reporting a decrease (Eguchi et al., 2015), and three others finding no substantial concentration variations of the hormone (Gravel et al., 2019; Julander et al., 2005; Zheng et al., 2017). Likewise, T_3 and T_4 concentrations can also be conflictual across studies, with some reporting them to be higher (Eguchi et al., 2015; Zheng et al., 2017), lower (Gravel, Lavoué, Bakhiyi, Lavoie, et al., 2019), or no different from the control populations (Julander et al., 2005). PBDEs, PCBs and BPhs were found to have the potential to be directly correlated with changes in TH such as T_4, T_3, and TSH (Eguchi et al., 2015; Gravel, Lavoué, Bakhiyi, Lavoie, et al., 2019; Zheng et al., 2017).

In terms of sex hormones, three studies assessed the levels of T, FSH and LH in exposed groups, all the while comparing the values with control groups (Gravel, Lavoué, Bakhiyi, Lavoie, et al., 2019; Y. Yang et al., 2014, 2013). Gravel and colleagues (2019) found that T levels in men tended to decrease with exposure to e-waste, finding significant correlation between higher levels of the OPE metabolite tertbutyl diphenyl phosphate (tb-DPhP) in urine and 18% lower T values (Gravel et al., 2019), whilst Yan and colleagues (2013) found decreased T levels in men to be correlated with higher Pb concentration levels in blood (Y. Yang et al., 2013) . FSH and LH levels may also be affected by e-waste exposure, with a study finding correlation not so much with exposure itself, but with the use of masks during e-waste recycling activities (Y. Yang et al., 2013). A study found increased FSH levels in men correlated with an increase of Cd, Cu, and Pb levels in blood serum (Y. Yang et al., 2014).

Genotoxic effects, such as increased MN frequency and CA were found across three studies (Q. Wang et al., 2011; Yan Wang et al., 2018; Yuan et al., 2008). Yuan and colleagues (2008) found that higher MN frequency was shown to be correlated with activity time in the e-waste industry, with people who had an history of engaging with e-waste to have significantly higher MN frequencies (Yuan et al., 2008). Wang and colleagues (2018) found that MN and CA frequencies can also be correlated with active duration time at e-waste recycling sites (Yan Wang et al., 2018). Additionally, Wang and colleagues (2011) found that higher MN frequency can also be correlated with increased levels of Pb in blood (Q. Wang et al., 2011).

Potential of e-waste chemicals to cause oxidative stress was also investigated in five studies (Lu et al., 2016; Neitzel et al., 2020; H. Wang et al., 2010; Wen et al., 2008; Yuan et al., 2008). Four studies found changes in urinary 8-OHdG concentration levels, with three of them registering an increase of 8-OHdG levels (Lu et al., 2016; Neitzel et al., 2020; Wen et al., 2008), and one finding a decrease (H. Wang et al., 2010). Increased urinary 8-OHdG levels was found to be positively correlated with the number of working hours (Neitzel et al., 2020), while Lu and colleagues found that urinary 8-OHdG and MDA levels were positively correlated with the levels of PAHs in urine (Lu et al., 2016). Wang and colleagues (2010) found a decrease of

8-OHdG urinary levels in directly exposed groups to be negatively correlated with blood ferrous levels (H. Wang et al., 2010). Yuan and colleagues however have found no variations in neither 8-OHdG or MDA urinary levels (Yuan et al., 2008).

Renal and cardiovascular changes were detected by two studies (Neitzel et al., 2020; Upadhyay et al., 2021). Neitzel and colleagues (2020) found a correlation between higher blood Pb levels and a higher glomerular filtration rate (GFR) in exposed populations (Neitzel et al., 2020). The authors found the latter to differ from the assumption that higher Pb levels would impair renal functions, thus reducing GFR. Upadhyay and colleagues (2020) found higher blood Pb levels to be correlated with higher diastolic pressure, which may lead to cardiovascular diseases such as hypertension (Upadhyay et al., 2020).

Table 4.1- Main exposure and effect biomarkers after occupational exposure to e-waste identified in the literature review (adapted from (Moreira et al, 2022).

Biological effects	Exposure biomarkers	Effect biomarkers	Reference
Endocrine Disruption			
	PBDEs	↑ [TSH] (correlated with active time in the industry)	(Yuan et al., 2008)
	PBDEs*, PCBs	→ [TSH], ↑ [total T$_3$] * ↑ [free T$_3$] ↑[total T$_4$] *	(Zheng et al., 2017)
	PBDEs, PCBs*, BPhs*	↑[total T$_3$]* ↑[free T$_3$]* ↑[total T$_4$]* ↑[free T$_4$]* ↓[TSH]* (in females)	(Eguchi et al., 2015)
	PBDEs*, OPE metabolites*, Hg, Cd, Pb	• ↑[total T$_4$]* ↓[total T]* ↓[free T]* ↓[free T / E2]* (in males) • ↓[free T$_3$]* (in females) • → [TSH]	(Gravel et al., 2019)
	PBDEs	→ [total T$_3$], [free T$_4$] and [TSH]	(Julander et al., 2005)
	Pb*	↑[FSH] ↑[LH] (correlated with the use of masks) ↓[T]*	(Y. Yang et al., 2013)
Genotoxicity	Cd*, Cu*, Pb*, Fe	↑[FSH]* → [LH]	(Y. Yang et al., 2014)
	PBDEs	↑ MNBNC (correlated with active time in the industry)	(Yuan et al., 2008)
	Pb*, Cu, Cd	↑ MNBNC*	(Q. Wang et al., 2011)
	PCBs, Pb, Zn, Ca, Mg, Fe, Se	↑ CA ↑MNBNC (correlated with active time in the industry)	(Yan Wang et al., 2018)
Oxidative stress	PBDEs Cu, Fe*	→ [8-OHdG] → [MDA] ↓ [8-OHdG] *	(Yuan et al., 2008) (H. Wang et al., 2010)
	Pb, Cd, Mn	↑ [8-OHdG] (correlated with the number of working hours)	(Neitzel et al., 2020)
	PAHs*	↑ [8-OHdG] * ↑ [MDA]*	(Lu et al., 2016)
	PCDD/Fs, PBDEs, PCBs	↑ [8-OHdG]	(Wen et al., 2008)
Renal and cardiovascular function	Pb*, Cd, Mn Pb*	↑ glomerular filtration rate * ↑ diastolic blood pressure *	(Neitzel et al., 2020) (Upadhyay et al., 2020)

* - statistically significant correlation between exposure and effect biomarkers
PBDEs - Polybrominated diphenyl ethers ; PCBs - Polychlorinated biphenyls; PAHs - Polycyclic aromatic hydrocarbons; BPhs - bromophenols , OPEs – Organophosphate ; PCDD/Fs – Polychlorinated dibenzo-p-furans; Hg - Mercury; Pb - Lead; Cd - Cadmium; Cu - Copper; Fe - Iron; Mn – Manganese; Zn – Zinc; Ca – Calcium; Mg- Magnesium, Se – Selenium; TSH – Thyroid stimulating hormone; T$_3$ – Triiodothyronine; T$_4$ – Thyroxine; FSH - follicle-stimulating hormone LH - Luteinizing hormone; T - Testosterone; E2 - Estradiol; MNBNC – Micronucleus frequency in binucleated cells; CA - Chromosomal aberrations, 8-OHdG 8-hydroxydeoxyguanosine; MDA = Malondialdehyde.

4.2 HBM study

4.2.1 Population characteristics

During the HBM4EU occupational study, MN analysis in PBL was performed on 30 individuals from Belgium, 23 from Finland, 41 from Portugal, 43 from The Netherlands, 16 from Luxembourg, and 18 from Latvia, making a total of 171 samples. However, in this work only results from Portuguese and Finnish samples are presented because no contextual data was available for samples from the remaining countries. The total number of Portuguese samples (24 exposed and 12 controls) and Finnish samples (12 exposed and 3 controls) was slightly reduced compared with the number of participants recruited since some of the individuals didn't fit the criteria for this study (mentioned in 3.2.1) . Detailed demographic information of the study population is present in Table 4.2.

Table 4.2- Socio-demographic information of the study population

		Exposed	Controls
Population, n (%)		36 (70.6)	15 (29.4)
Gender, n (%)			
	Men	34 (94.4)	10 (66.7)
	Women	2 (5.6)	5 (33.3)
Age (years, mean ± SD)		46.47 ±10.7	44.67±7.49
Age (min - max)		18-63	31-56
Smoking habits, n (%)			
	Current smokers	14 (38.9)	2 (13.3)
	Former and non-smokers	22 (61.1)	13 (86.7)
Alcohol consumption, n (%)			
	Regular drinker	22 (61.1)	7 (46.7)
	Sporadic drinker	14 (38.9)	8 (53.3)
Occupation			
	E-waste recycling	31 (86.1)	(-)
	Maintenance and logistics	5 (16.1)	(-)
	Office work (OC*)		5 (33.3)
	Academic tasks (NOC*)		10 (66.7)
OC - Occupational control; NOC – Non-occupational Control			

The occupationally exposed group (n = 36) consisted of 34 males (94.4%) and 2 females (5.6%), with ages varying between 18-63 years old (mean age, 46.47 ± 10.7). The control group (n=15) consisted of 10 males (66.7%) and 5 females (33.3%), with ages between 31 and 56 years (mean age, 44.67 ± 7.49).

Both the exposed and control groups were divided according to their smoking habits, alcohol consumption and occupation. Of the 36 occupationally exposed individuals, 14 (38.6%) are smokers and 22 (61.1%) are former or non-smokers; 22 (61.1%) are regular drinkers and 14 (38.9%) are sporadic drinkers.

Of the 15 individuals from the control group, 2 (13.3%) are smokers and 13 (86.7%) are former or non-smokers; 7 (46.7%) are regular drinkers and 8 (53.3%) are sporadic drinkers. Furthermore, the groups were divided according to their occupation. In the exposed group, 31 individuals (86.1%) are directly involved with e-waste recycling activities (smelting, dismantling, or sorting) and 5 (13.2%) are from maintenance and logistic jobs; in the control group, 5 individuals (33.3%) are office workers within the e-waste industry, which are considered industrial controls, and 10 (66.7%) have academic tasks (e.g.: professors, IT consultants and researchers) with no relations with the e-waste site.

4.2.2 Cytokinesis-block micronucleus assay

The mean frequency of MNBNC, NBUDs, NPBs per 1000 BNC and CBPI values, as well as the predicator variable impacts for MN frequency in BNC are present in table 4.3 and table 4.4, respectively. For each sample, 2000 BNC were scored and analysed by at least 2 independent readers, giving a total of 102000 BNC scored.

Table 4.3- Frequency of MNBNC, NBUDs, NPBs per 1000 BNC and CBPI values (results in mean ± SD)

	n	MNBNC/1000	NBUDs/1000 BNC	NPBs /1000 BNC	CBPI
Total Exposed	36	9.02±4.41	1.40±1.72*	0.54±0.75*	1.77±0.16*
E-waste recyclers	31	8.40±4.39¥	1.09±4.57¥	0.42±0.69¥	1.78±0.16
Maintenance and logistics (M&L)	5	12.83±1.94	3.33±2.25	1.27±0.72	1.72±0.17
Total Control	15	8.37±4.92	2.20±1.55	0.76±0.51	1.50±0.21
Office work (OC)	5	7.47±4.87	1.47±1.52	0.73±0.56	1.77±0.01
Academic tasks (NOC)	10	8.82±4.88	2.56±1.43	0.78±0.48	1.36±0.09

*Statistically significant *p*-value between total exposed and total control; ¥ Statistically significant *p*-value between e-waste recycling workers and M&L

The total exposed group presented a higher mean frequency of MNBNC compared to the control group (9.02±4.41 vs 8.37±4.92, respectively), but no statistically significant difference was observed. Statistically significant differences were found between total exposed and total control's NBUDs and NPBs frequencies, as well as for CBPI values (for total exposed: NBUDs = 1.40±1.72, NPBs = 0.54±0.75, CBPI = 1.77±0.16 / for total control: NBUDs = 2.20±1.55, NPBs = 0.76±0.51, CBPI= 1.50±0.21).

Relative to the occupational activity, individuals who held maintenance and logistics (M&L) tasks had significantly higher MN frequency compared to e-waste recyclers (12.83±1.94 vs 8.40±4.39, respectively) as well as in other nuclear abnormality frequencies (for M&L: NBUDs = 3.33±2.25, NPBs = 1.27±0.72 / for e-waste recyclers: NBUDs = 1.09±4.57, NPBs = 0.42±0.69). No other statistically significant difference in MN, NBUDs, NPBs frequency or CPBI values was found between office workers (OC) and individuals with academic tasks (NOC) (Table 4.3).

MMC treated samples displayed significant difference in the frequency of MNBNC comparatively to the mean frequency of MNBNC in all samples of individuals without MMC treatment (Figure 4.2).

When considering the individuals categorized by predictor variables that can influence MN formations, such as sex, age, smoking habits, and alcohol consumption, no significant differences in the mean frequency of MNBNC were found (Table 4.4).

Table 4.4- Frequency of MNBNC per 1000 BNC, considering the predictor variables

			MNBNC/1000 (Mean ± SD)			
		n	Exposed	n	Control (OC & NOC)	*p*-value
	Total	36	9.02±4.41	15	8.37±4.92	0.414
Gender						
	Men	34	9.08±4.48	10	7.87±4.17	0.355
	Women	2	8.00±3.00	5	9.37±6.41	1
	p-value		0.863		0.713	
Age						
	≤ 46	16	8.33±4.74	9	9.09±5.35	0.977
	≥ 47	20	9.57±4.44	6	7.29±3.68	0.286
	p-value		0.483		0.555	
Smoking habits						
	Current smokers	14	8.03±5.21	2	6.00±1.5	0.811
	Former and non-smokers	22	9.64±3.68	13	8.73±5.11	0.365
	p-value		0.148		0.798	
Alcohol consumption						
	Regular drinker	22	9.75±4.04	7	8.60±4.69	0.540
	Sporadic drinker	14	7.87±4,72	8	8.17±5.10	0.864
	p-value		0.77		0.908	
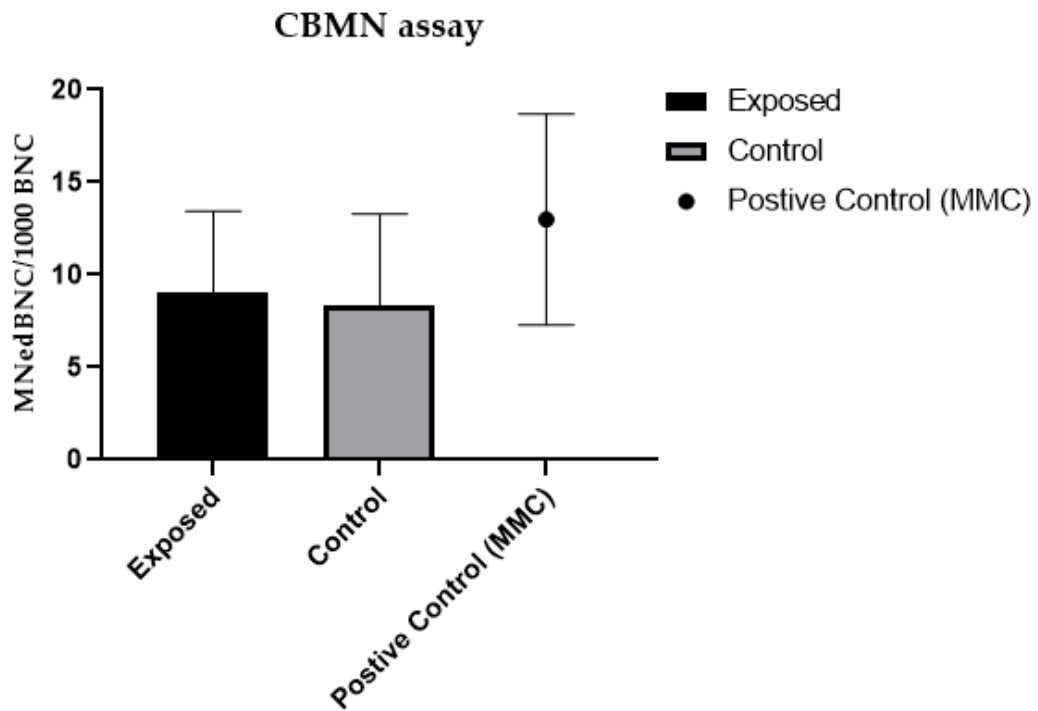
Figure 4.2- MNBNC frequency per 1000 BNC from exposed and non-exposed groups. MMC was used as positive control. Data is shown as mean ± SD. MMC treated samples were shown to have significantly higher frequency of MN when compared to exposed and control groups.

5. DISCUSSION

5.1 Mini review on e-waste

In this mini review, we analysed studies that evidenced how workers in the e-waste recycling sector are exposed to various harmful substances, such as heavy metals and POPs, and how they might correlate with biological effects, which included endocrine disruption, oxidative stress, genotoxic effects, and renal/cardiovascular problems.

The studies mostly showed congruent results between them, with only some degree of heterogeneity on the findings of studies that explored endocrine disrupting effects of e-waste exposure in workers. Not only was it observed that the level of exposure to hazardous e-waste substances can influence a biological effect, but also other variables, such as activity time in the industry or the use protective equipment, might be key aspects to explore in HBM studies as they have shown to also correlate to health effects.

Although there has been little epidemiological research to date on the associations between workplace exposure and illness among e-waste workers, the biological effects present in the review suggest the possibility of development of long-term pathologies, such as metabolic or oncological diseases (Okeme & Arrandale, 2019).

Therefore, the need to create an exposure assessment through reliable exposure biomarkers, quantified by well-controlled analytical methods, together with effect biomarkers that allow the establishment of a relationship between exposure to these substances and the potential development of occupational diseases is fundamental in future HBM studies.

Furthermore, efforts on educating workers, and reducing their occupational exposure via implementing ventilations systems and proper hygiene measures, should become imperative in the near future (Okeme & Arrandale, 2019).

5.2 HBM study

Exposure to waste products from human consumption has historically been recognized to have negative environmental and health repercussions, more specifically the case of e-waste, which contains potentially hazardous substances that may be directly released or generated during the recycling process (Perkins et al., 2014). Workers' who are actively engaged in e-waste recycling are at great risk of exposure, since they are in direct contact with chemicals that can easily be ingested, inhaled, or absorbed through the skin (Okeme & Arrandale, 2019). Occupational exposure levels are also closely related to the e-waste management procedures at work, as crude and rudimentary recycling processes (i.e., informal recycling) have demonstrated to have serious negative consequences to human health, as opposed to a more formal and modernized approach to e-waste recycling (Rautela et al., 2021).

Several HBM studies have been conducted regarding e-waste exposure. Recently, Li and colleagues (2022) assessed the occupational exposure to OPEs in an e-waste recycling plant located in northern China by assessing the levels of OPE metabolites in workers urine samples. The authors found urinary concentrations of OPE metabolites to be significantly higher in

workers (0.910 ng/mL), compared to those who were resident in a nearby town (0.600 ng/mL) (X. Li et al., 2022). Moreover, a systematic review by Cai and colleagues (2020) addressed scientific publications focusing on human exposure to PBDEs, which included occupationally exposed groups. The authors concluded that the groups who worked in the e-waste sector or that lived close to e-waste areas were more exposed to PBDEs compared to other, less vulnerable groups. Furthermore, the authors also observed that the studies that reported PBDE exposure in occupationally exposed groups living in high income countries showed lower PBDE levels in blood (Cai et al., 2020).

Therefore, gathering data related to e-waste chemical exposure, and comprehending their potentially adverse health effects in the human body is paramount, while also considering possible variables that might influence exposure and effects. The primary goal of this work, which takes part in the HBM4EU initiative, is to evaluate the presence of MN in PBL of workers who are actively involved in the recycling of e-waste in Portugal and Finland. To assess the genotoxic effects in workers, the CBMN assay was performed.

Several studies have found that polymorphisms in specific genes with DNA lesion repair functions, or even genes in metabolic enzymes and folate metabolism, to be associated with MN formation (Dhillon et al., 2010). It is crucial to take into account the role of interindividual differences in the susceptibly to adverse health effects caused by chemicals, as polymorphic variants have shown to alter one's body response to xenobiotics (Szyf, 2007), and these variations can be a limiting factor in our acquired results.

Our results showed a slight increase in the average frequency of MNBNC/1000 BNC in the exposed population compared to the control population (9.02±4.41 vs 8.37±4.92), although this difference was not considered significant (Table 4.3). Similar results were also observed in HBM4EU's chromates study, where there was also not found any significant difference between total exposed and total control populations (Tavares et al., 2022). The control population's median MN frequency was 7.0 MNBNC/1000, which agrees with the median MN frequency reported in the International Collaborative Project on Micronucleus Frequency in Human Populations (HUMN) of 6.5 MNBNC/1000 in non-exposed individuals PBL (Kodros et al., 2021).

Most occupational studies find significant differences in MN frequency between exposed and control groups, with exposed groups manifesting a considerable rise in MN frequency. As an example, a study on male e-waste recyclers in China conducted by Wang and colleagues (2011) found a significant increase in the frequency of MN in PBL of those with occupational exposures compared with those with no occupational exposures (26.30 vs. 4.52%, p<0.001) (Q. Wang et al., 2011). Similar results were reported in other studies included in the mini-review mentioned in this work (Moreira et al., 2022).

The use of personal protective equipment (PPE) in the workplace could be a contributor to a lower exposure during e-waste recycling activities, as was shown to be able to protect workers against several threats such as physical, electrical, heat, chemicals, biohazards, and

airborne PM (Juyal, Kaur, & Khatri, 2019). Inhalation is considered a significant route of exposure to toxic chemicals for e-waste workers (Avenbuan et al., 2021), and the use of respiratory protective equipment (RPE) such as face masks and respirators, have shown to be able to filter out PM in the air and other hazardous aerosols, gases, and vapours (Howie, 2005). According to data collected by inquiry, most Portuguese workers were shown to use RPE during activities such as sorting, compacting, burning, and smelting of batteries; most Finnish workers were shown to use RPE during shredding of e-waste.

Although unrelated to MN frequency, a study conducted by Yang and colleagues (2013) found that Pb exposure and it's endocrine disruption effects to be associated with the confounding factor of using masks, with workers who didn't use adequate RPE exhibiting altered levels of FSH and LH in blood (Moreira et al., 2022; Y. Yang et al., 2013). Furthermore, a study conducted by Zeljezic and colleagues (2016) studied the impact of PPE use in pharmaceutical industry workers, who are exposed to a variety of different carcinogenic substances. The authors found that, after 8 months of strict PPE use, MN frequency and comet assay parameters in lymphocytes of pharmaceutical workers significantly decreased compared with prior period of irregular use of PPE (Zeljezic, Mladinic, Kopjar, & Radulovic, 2016).

Another possible reason for this result might have been due to some degree of inaccuracy of the CBMN assay towards reporting increased MN frequency in populations exposed to genotoxic agents. As reported by Speit and colleagues (2012), the authors found that the strong mutagens used in their assay (such as: methyl methanesulfonate, ethyl methanesulfonate and MMC) did not cause increased MN frequencies in human PBL after exposure at the start of the blood cultures, even at high concentrations (except in the case of MMC, which is an extremely efficient inducer of MN). This is due to lymphocytes' ability to efficiently repair most DNA damage brought on by a cell's S-phase. S-phase dependent mutagens, which constitute the majority of mutagens found in the environment or at a particular workplace, are known to be insensitive to MN. The authors went on to explain that it is to be anticipated that the CBMN test utilized in HBM research will be substantially less sensitive in identifying the outcomes of *in vitro* exposure to mutagens and clastogens (Speit, Linsenmeyer, Schütz, & Kuehner, 2012).

Finally, our small sample size was also, and possibly the biggest contributor for non-significant results. A suitable sample size is often necessary for optimal study conditions in order to avoid for a subject's failure to comply in the study, inadequate controls or sample loss as a result of transit or laboratory handling errors (Albertini et al., 2000). In addition, bigger sample sizes are crucial to offer statistical power of a study (Albertini et al., 2000). Therefore, the frequent use of PPE and RPE during e-waste recycling activities, paired with some of the limiting factors present in the CBMN assay and small sample size, might have led to a non-significant difference in MN frequency between exposed and control populations.

Measurements for other nuclear abnormalities of NBUDs and NPBs, as well as the CBPI value for both total exposed and total control populations, was performed (Table 4.3). The total exposed group exhibited significantly lower NBUDs and NPBs frequency and significantly higher mean CBPI value when compared to the total control population. These differences were considered statistically significant and, again, greatly differed from our *a priori* assumptions that the exposed population would suffer more from nuclear abnormalities and cytotoxicity in PBL. It is known that there are still knowledge gaps on the understanding of mechanisms that could lead to NBUD and NPB formations, as the impacts of gender and genetic polymorphisms can greatly affect one's susceptibility to the cell's nuclear malformations. Additionally, the molecular events associated with NBUD and NPB formation are substantially different, which raises the question of which exposure, dietary and lifestyle factors combinations may affect these biomarkers and directly interfere in their molecular mechanisms (M. Fenech et al., 2011). Nonetheless, our small sample size might be a great contributor to these unexpected results, but other variables such as the total control group being naturally more susceptible to DNA damage, or exposed to unknown substances, can still be plausible reasons.

Although its connection to a health consequence is yet unknown, the greater CBPI value for the entire exposed group compared to the total control group may indicate that lymphocytes from the exposed workers have a higher proliferation rate in culture. (Tavares et al., 2022).

Regarding the impacts of the activities in the total exposed and total control groups, workers whose tasks were in maintenance and logistics (M&L), such as technicians and quality control personnel, presented a significantly higher MN, NBUDs and NPBs frequency in PBL compared to e-waste recycling workers (Table 4.3). According to the survey collected from workers regarding their use of PPE and RPE, this group mostly did not wear adequate working clothes or designated respiratory masks. This may explain the considerably higher MN and other nuclear abnormalities frequency in this group, which further proves the importance of worker protection, even if said worker is not directly involved in e-waste recycling. The comparison between both e-waste recycling workers and maintenance and logistics workers can still be unfeasible since both groups differ greatly in terms of number (31 vs 5, receptively). Furthermore, no significant changes in MN and other nuclear abnormality frequencies between office workers at the e-waste site and outside workers (academic tasks) with no relation to the e-waste site.

Certain predictor variables, such as gender, age, smoking habits, and alcohol consumption can be contributors to MN formation (Table 4.4).

Evidence has shown that MN frequency can be sex dependent, with its frequency being higher in females compared to males (Michael Fenech & Bonassi, 2011), with females having 19% higher levels of MN frequency in PBL frequency (Bonassi et al., 2001). This phenomenon has been reported by Tucker and colleagues (1996), that have proven that females have a

higher MN frequency than males due to the X chromosome's increased susceptibility to be lost as an MN compared to other chromosomes, as well as the fact that females have two copies of the X chromosome compared to just one in males (Michael Fenech & Bonassi, 2011; Tucker, Nath, & Hando, 1996). Additionally, most studies reported that MN frequency increases in both males and females as their age progresses. This can be due to a combination of factors, which may include: "1) the cumulative impact of DNA repair-related gene mutations; 2) chromosomal numerical and structural abnormalities brought on by endogenous genotoxins, unhealthy lifestyle choices, or occupational or environmental genotoxins" (Michael Fenech & Bonassi, 2011).

Our results did not show any significant differences of MN frequency in both gender and age variables between exposed and control groups. In terms of gender, comparison between men and women in the exposed groups was shown to be impractical, due to the small sample size of women in the exposed group compared to men (2 vs 34, respectively). Women in the control group have shown exactly 19% higher mean frequency of MN compared to men in the control group (9.37±6.41 vs 7.87±4.17, respectively; 19% difference), which goes according to the value reported in the HUMN international database (Bonassi et al., 2001). As for age, our results didn't show any statistical evidence that age was a precursor for higher MN frequency, which goes in accordance with some results reported in other studies that didn't find any relationship between age and MN frequency (Giorgio et al., 1994; Yan Wang et al., 2018). These results propose that the MN frequency in our studied population was not dependent upon age or gender.

Lifestyle factors are important to consider when it comes to any HBM study, as they have shown to influence MN formation. Such lifestyle factors may include smoking or alcohol consumption.

The effect of tobacco smoking on inducing higher MN frequency in human PBL has been a target of polarizing results amongst studies, as many of them have shown negative or inconclusive results often related to the small sample size (Michael Fenech & Bonassi, 2011). According to the Human MicroNucleus project database on the effect of smoking habit on the frequency of MN in human PBL, the collection of data confirms that smokers generally do not experience any substantial increase in MN frequency, with only heavy smokers (~30 cigarettes per day) showing significant increase in MN frequency (Bonassi et al., 2003; Michael Fenech & Bonassi, 2011). According to our results, there was no statistically significant difference between active smokers and former/non-smokers, which goes in agreement with several studies since most of them failed to observe an increased frequency of MN in PBL in active smokers. The possibility for a body's adaptive response to chronic exposure, as well as the individual susceptibility to the genotoxic effects of tobaccos smoke, should be considered (El-Zein, Etzel, & Munden, 2018; Michael Fenech & Bonassi, 2011; Hiemstra, 2002).

The MN frequency on regular drinkers was higher compared to sporadic drinkers on both exposed and control groups, but this difference was not considered statistically

significant. It has been pointed out by the filled questionnaires that most of the individuals classified as "regular drinkers" would only drink weakly and not daily, with most of them consuming less than 10 drinks per month. This evidence, paired with other interindividual factors, might have contributed to a non-significant difference between regular drinkers and sporadic drinkers. Nevertheless, alcohol consumption is still closely related with higher MN frequency in PBL and is known to contribute to the development of cancer (Michael Fenech & Bonassi, 2011).

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of this pilot study was to assess the effects of e-waste exposure in an occupational setting, using MN as a biomarker for genotoxicity. Thus, the CBMN assay was performed in PBL in a selected group of exposed and non-exposed individuals from Portugal and Finland.

The present results suggested that, overall, there were no significant differences in terms of genotoxicity biomarkers between both exposed and control groups. These results didn't correspond to our inferred assumption that workers who actively participated in e-waste recycling would display higher genotoxic damage and have significantly higher MN, NBUDs and NPBs frequency in lymphocytes. We highlighted some of the factors that might have influenced our results, they will be crucial concerning the future of HBM research. In fact, there is still much to explore in this study as many other countries were not included, which will further develop knowledge around the effects of e-waste in workers of this sector.

In our review article, we emphasized exposure focused HBM studies on occupational activity related to the management of e-waste and its potential effects on workers' health. The analysed studies showed that workers in this sector are exposed to various harmful substances, causing a variety of biological effects, the most consistent being dysregulation of thyroid hormones, oxidative stress, and genotoxic effects. Despite this, we believe there is still a considerable lack of information regarding HBM studies focused on the health effects resulting from occupational activity.

Finally, we emphasize the need to develop further occupational studies, particularly in Europe, including an exposure assessment through reliable exposure biomarkers quantified by well-controlled analytical methods, together with effect biomarkers to relate exposure with the potential development of adverse health effects in populations.

7. BIBLIOGRAPHY

Ádám, B., Göen, T., Scheepers, P. T. J., Adliene, D., Batinic, B., Budnik, L. T., … Au, W. W. (2021). From inequitable to sustainable e-waste processing for reduction of impact on human health and the environment. *Environmental Research*, *194*(November 2020), 110728. https://doi.org/10.1016/j.envres.2021.110728

Al osman, M., Yang, F., & Massey, I. Y. (2019). Exposure routes and health effects of heavy metals on children. *BioMetals*, *32*(4), 563–573. https://doi.org/10.1007/s10534-019-00193-5

Alabi, O. A., Adeoluwa, Y. M., & Bakare, A. A. (2019). Elevated Serum Pb, Ni, Cd, and Cr Levels and DNA Damage in Exfoliated Buccal Cells of Teenage Scavengers at a Major Electronic Waste Dumpsite in Lagos, Nigeria. *Biological Trace Element Research*, *194*(1), 24–33. https://doi.org/10.1007/s12011-019-01745-z

Alabi, O. A., Adeoluwa, Y. M., Huo, X., Xu, X., & Bakare, A. A. (2021). Environmental contamination and public health effects of electronic waste: an overview. *Journal of Environmental Health Science and Engineering*, *19*(1), 1209–1227. https://doi.org/10.1007/s40201-021-00654-5

Albertini, R. J., Anderson, D., Douglas, G. R., Hagmar, L., Hemminki, K., Merlo, F., … Aitio, A. (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutation Research - Reviews in Mutation Research*, *463*(2), 111–172. https://doi.org/10.1016/S1383-5742(00)00049-1

Amankwaa, E. F., Adovor Tsikudo, K. A., & Bowman, J. A. (2017). "Away" is a place: The impact of electronic waste recycling on blood lead levels in Ghana. *The Science of the Total Environment*, *601–602*(December), 1566–1574. https://doi.org/10.1016/j.scitotenv.2017.05.283

Andeobu, L., Wibowo, S., & Grandhi, S. (2021). An assessment of e-waste generation and environmental management of selected countries in Africa, Europe and North America: A systematic review. *The Science of the Total Environment*, *792*, 148078. https://doi.org/10.1016/j.scitotenv.2021.148078

Angerer, J., Aylward, L. L., Hays, S. M., Heinzow, B., & Wilhelm, M. (2011). Human biomonitoring assessment values: approaches and data requirements. *International Journal of Hygiene and Environmental Health*, *214*(5), 348–360. https://doi.org/10.1016/j.ijheh.2011.06.002

Ant, P., Uva, N. I. O. D. E. S., Nacional, E., Nova, U., Uva, S., Nacional, E., & Nova, U. (2007). A utilização de indicadores biológicos em Saúde Ocupacional. *Revista Portuguesa de Saúde Pública*, 45–54.

Avenbuan, O. N., Meltzer, G. Y., Awada, C., Raja, A., Holian, A., & Zelikoff, J. T. (2021). A contemporary review of electronic waste through the lens of inhalation toxicology. *Inhalation Toxicology*, *33*(9–14), 285–294. https://doi.org/10.1080/08958378.2021.1996493

Bakhiyi, B., Gravel, S., Ceballos, D., Flynn, M. A., & Zayed, J. (2017). Has the question of e-waste opened a Pandora's box? An overview of unpredictable issues and challenges. *Environment International*, *110*, 173–192. https://doi.org/10.1016/j.envint.2017.10.021

Beedanagari, S., Vulimiri, S. V., Bhatia, S., & Mahadevan, B. (2014). Genotoxicity biomarkers. In *Biomarkers in Toxicology* (pp. 729–742). https://doi.org/10.1016/B978-0-12-404630-6.00043-9

Bergamaschi, E., Guseva Canu, I., Prina-Mello, A., & Magrini, A. (2017). Adverse Effects of Engineered Nanomaterials (Second Edition). In *Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health: Second Edition* (2nd ed., pp. 125–158). https://doi.org/10.1016/B978-0-12-809199-9.00006-9

Bolognesi, C., & Fenech, M. (2013). Micronucleus Assay in Human Cells: Lymphocytes and

Buccal Cells. In *Methods in Molecular Biology* (Vol. 1044, pp. 191–207). https://doi.org/10.1007/978-1-62703-529-3_10

Bonassi, S., El-Zein, R., Bolognesi, C., & Fenech, M. (2011). Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*, *26*(1), 93–100. https://doi.org/10.1093/mutage/geq075

Bonassi, S., Fenech, M., Lando, C., Lin, Y. P., Ceppi, M., Chang, W. P., … Zijno, A. (2001). HUman MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environmental and Molecular Mutagenesis*, *37*(1), 31–45. https://doi.org/10.1002/1098-2280(2001)37:1<31::AID-EM1004>3.0.CO;2-P

Bonassi, S., Neri, M., Lando, C., Ceppi, M., Lin, Y. P., Chang, W. P., … Zijno, A. (2003). Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the Human MicroNucleus project. *Mutation Research - Reviews in Mutation Research*, *543*(2), 155–166. https://doi.org/10.1016/S1383-5742(03)00013-9

Borthakur, A., & Govind, M. (2016). Emerging trends in consumers' E-waste disposal behaviour and awareness: A worldwide overview with special focus on India. *Resources, Conservation and Recycling*, *117*, 102–113. https://doi.org/10.1016/j.resconrec.2016.11.011

Cai, K., Song, Q., Yuan, W., Ruan, J., Duan, H., Li, Y., & Li, J. (2020). Human exposure to PBDEs in e-waste areas: A review. *Environmental Pollution (Barking, Essex : 1987)*, *267*, 115634. https://doi.org/10.1016/j.envpol.2020.115634

CDC. (2009). Fourth National Report on Human Exposure to Environmental Chemicals. *Fourth National Report on Human Exposure to Environmental Chemicals*, (July), 1–529.

Ceballos, D. M., & Dong, Z. (2016). The formal electronic recycling industry: Challenges and opportunities in occupational and environmental health research. *Environment International*, *95*, 157–166. https://doi.org/10.1016/j.envint.2016.07.010

Crawford, E. D., Ventii, K., & Shore, N. D. (2014). New biomarkers in prostate cancer. *Oncology (Williston Park, N.Y.)*, *28*(2), 135–142. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24701701

Cui, J., & Jørgen Roven, H. (2011). Electronic Waste. In *Waste* (pp. 281–296). https://doi.org/10.1016/B978-0-12-381475-3.10020-8

D'Costa, A., Praveen Kumar, M. K., & Shyama, S. K. (2019). Genotoxicity assays: the micronucleus test and the single-cell gel electrophoresis assay. In *Advances in Biological Science Research* (pp. 291–301). https://doi.org/10.1016/B978-0-12-817497-5.00018-5

Dhillon, V. S., Thomas, P., Iarmarcovai, G., Kirsch-Volders, M., Bonassi, S., & Fenech, M. (2010). Genetic polymorphisms of genes involved in DNA repair and metabolism influence micronucleus frequencies in human peripheral blood lymphocytes. *Mutagenesis*, *26*(1), 33–42. https://doi.org/10.1093/mutage/geq076

Doherty, A., Bryce, S. M., & Bemis, J. C. (2016). The In Vitro Micronucleus Assay. In *Genetic Toxicology Testing* (pp. 161–205). https://doi.org/10.1016/b978-0-12-800764-8.00006-9

Eguchi, A., Nomiyama, K., Minh Tue, N., Trang, P. T. K., Hung Viet, P., Takahashi, S., & Tanabe, S. (2015). Residue profiles of organohalogen compounds in human serum from e-waste recycling sites in North Vietnam: Association with thyroid hormone levels. *Environmental Research*, *137*, 440–449. https://doi.org/10.1016/j.envres.2015.01.007

El-Zein, R. A., Etzel, C. J., & Munden, R. F. (2018). The cytokinesis-blocked micronucleus assay as a novel biomarker for selection of lung cancer screening participants. *Translational Lung Cancer Research*, *7*(3), 336–346. https://doi.org/10.21037/tlcr.2018.05.09

European Recyling Plataform. (2020). *Relatório anual de atividades REEE 2020*.

Eurostat. (2021). Electronic equipment (EEE) put on the market and WEEE processed in the EU. Retrieved January 17, 2022, from https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Electrical_and_electronic_equipment_(EEE)_put_on_the_market_and_waste_EEE_collected,_treated,_recovered,_recycled_and_prepared_for_reuse,_EU,_2011–2018_(thousand_tonnes).png

Faroon, O. M., Keith, L. S., Smith-Simon, C., & De Rosa, C. T. (2003). Concise International Chemical Assessment Document 55: Polychlorinated biphenyls: Human health aspects. *IPCS Concise International Chemical Assessment Documents*, (55).

Federico, C., Vitale, V., La Porta, N., & Saccone, S. (2019). Buccal micronucleus assay in human populations from Sicily (Italy) exposed to petrochemical industry pollutants. *Environmental Science and Pollution Research International*, *26*(7), 7048–7054. https://doi.org/10.1007/s11356-019-04193-3

Feldt, T., Fobil, J. N., Wittsiepe, J., Wilhelm, M., Till, H., Zoufaly, A., … Göen, T. (2013). High levels of PAH-metabolites in urine of e-waste recycling workers from Agbogbloshie, Ghana. *Science of the Total Environment*, *466–467*, 369–376. https://doi.org/10.1016/j.scitotenv.2013.06.097

Fenech, M., Kirsch-Volders, M., Natarajan, A. T., Surralles, J., Crott, J. W., Parry, J., … Thomas, P. (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis*, *26*(1), 125–132. https://doi.org/10.1093/mutage/geq052

Fenech, Michael. (2007). Cytokinesis-block micronucleus cytome assay. *Nature Protocols*, *2*(5), 1084–1104. https://doi.org/10.1038/nprot.2007.77

Fenech, Michael. (2008). The Micronucleus Assay Determination of Chromosomal Level DNA Damage. In *Environmental Genomics* (Vol. 410, pp. 185–216). https://doi.org/10.1007/978-1-59745-548-0_12

Fenech, Michael, & Bonassi, S. (2011). The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. *Mutagenesis*, *26*(1), 43–49. https://doi.org/10.1093/mutage/geq050

Fenech, Michael, Chang, W. P., Kirsch-Volders, M., Holland, N., Bonassi, S., & Zeiger, E. (2002). HUMN project: Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *534*(1–2), 65–75. https://doi.org/10.1016/S1383-5718(02)00249-8

Fenech, Michael, Knasmueller, S., Bolognesi, C., Holland, N., Bonassi, S., & Kirsch-Volders, M. (2020). Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans. *Mutation Research. Reviews in Mutation Research*, *786*, 108342. https://doi.org/10.1016/j.mrrev.2020.108342

Fenech, Michael, & Morley, A. A. (1985). Measurement of micronuclei in lymphocytes. *Mutation Research/Environmental Mutagenesis and Related Subjects*, *147*(1–2), 29–36. https://doi.org/10.1016/0165-1161(85)90015-9

Fernandes, A. R., & Falandysz, J. (2021). Polybrominated dibenzo-p-dioxins and furans (PBDD/Fs): Contamination in food, humans and dietary exposure. *Science of the Total Environment*, *761*(xxxx), 143191. https://doi.org/10.1016/j.scitotenv.2020.143191

Forti, V., Baldé, C. P., Kuehr, R., & Bel, G. (2020). *The Global E-waste Monitor 2020*. Retrieved from http://ewastemonitor.info/

Frazzoli, C., Orisakwe, O. E., Dragone, R., & Mantovani, A. (2010). Diagnostic health risk assessment of electronic waste on the general population in developing countries' scenarios. *Environmental Impact Assessment Review*, *30*(6), 388–399. https://doi.org/10.1016/j.eiar.2009.12.004

Gaidajis, G., Angelakoglou, K., & Aktsoglou, D. (2010). E-waste: Environmental Problems and Current Management Engineering Science and Technology Review. *Journal of Engineering Science and Technology Review*, *3*(1), 193–199. Retrieved from www.jestr.org

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castaño, A., Esteban López, M., … Schoeters, G. (2021). HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity – The HBM4EU survey. *International Journal of Hygiene and Environmental Health*, *237*. https://doi.org/10.1016/j.ijheh.2021.113809

Giorgio, C. Di, De Méo, M. P., Laget, M., Guiraud, H., Botta, A., & Duménil, G. (1994). The micronucleus assay in human lymphocytes: Screening for inter-individual variability and application to biomonitoring. *Carcinogenesis*, *15*(2), 313–317. https://doi.org/10.1093/carcin/15.2.313

Grant, K., Goldizen, F. C., Sly, P. D., Brune, M. N., Neira, M., van den Berg, M., & Norman, R. E. (2013). Health consequences of exposure to e-waste: A systematic review. *The Lancet Global Health*, *1*(6), e350–e361. https://doi.org/10.1016/S2214-109X(13)70101-3

Gravel, S., Lavoué, J., Bakhiyi, B., Diamond, M. L., Jantunen, L. M., Lavoie, J., … Labrèche, F. (2019). Halogenated flame retardants and organophosphate esters in the air of electronic waste recycling facilities: Evidence of high concentrations and multiple exposures. *Environment International*, *128*(May), 244–253. https://doi.org/10.1016/j.envint.2019.04.027

Gravel, S., Lavoué, J., Bakhiyi, B., Lavoie, J., Roberge, B., Patry, L., … Labrèche, F. (2019). Multi-exposures to suspected endocrine disruptors in electronic waste recycling workers: Associations with thyroid and reproductive hormones. *International Journal of Hygiene and Environmental Health*, *225*, 113445. https://doi.org/10.1016/j.ijheh.2019.113445

Guo, L. C., Liu, T., Yang, Y., Yu, S., Gao, Y., Huang, W., … Zhang, Y. (2020). Changes in thyroid hormone related proteins and gene expression induced by polychlorinated biphenyls and halogen flame retardants exposure of children in a Chinese e-waste recycling area. *The Science of the Total Environment*, *742*. https://doi.org/10.1016/J.SCITOTENV.2020.140597

Guo, L. C., Pan, S., Yu, S., Liu, T., Xiao, J., Zhu, B., … Ma, W. (2018). Human Sex Hormone Disrupting Effects of New Flame Retardants and Their Interactions with Polychlorinated Biphenyls, Polybrominated Diphenyl Ethers, a Case Study in South China. *Environmental Science & Technology*, *52*(23), 13935–13941. https://doi.org/10.1021/ACS.EST.8B01540

Ha, N. N., Agusa, T., Ramu, K., Tu, N. P. C., Murata, S., Bulbule, K. A., … Tanabe, S. (2009). Contamination by trace elements at e-waste recycling sites in Bangalore, India. *Chemosphere*, *76*(1), 9–15. https://doi.org/10.1016/j.chemosphere.2009.02.056

Hales, B. F., & Robaire, B. (2020). Effects of brominated and organophosphate ester flame retardants on male reproduction. *Andrology*, *8*(4), 915–923. https://doi.org/10.1111/ANDR.12789

Han, L., Hsu, W. W., Todem, D., Osuch, J., Hungerink, A., & Karmaus, W. (2016). In utero exposure to polychlorinated biphenyls is associated with decreased fecundability in daughters of Michigan female fisheaters: A cohort study. *Environmental Health: A Global Access Science Source*, *15*(1), 1–13. https://doi.org/10.1186/s12940-016-0175-3

Hayashi, M. (2016). The micronucleus test-most widely used in vivo genotoxicity test. *Genes and Environment*, *38*(1), 4–9. https://doi.org/10.1186/s41021-016-0044-x

Heacock, M., Kelly, C. B., Asante, K. A., Birnbaum, L. S., Bergman, Å. L., Bruné, M. N., … Suk,

W. A. (2016). E-waste and harm to vulnerable populations: A growing global problem. *Environmental Health Perspectives*, *124*(5), 550–555. https://doi.org/10.1289/ehp.1509699

Heeks, R., Subramanian, L., & Jones, C. (2015). Understanding e-Waste Management in Developing Countries: Strategies, Determinants, and Policy Implications in the Indian ICT Sector. *Information Technology for Development*, *21*(4), 653–667. https://doi.org/10.1080/02681102.2014.886547

Helmfrid, I., Ljunggren, S., Nosratabadi, R., Augustsson, A., Filipsson, M., Fredrikson, M., … Berglund, M. (2019). Exposure of metals and PAH through local foods and risk of cancer in a historically contaminated glassworks area. *Environment International*, *131*(December 2018), 104985. https://doi.org/10.1016/j.envint.2019.104985

Hiemstra, P. S. (2002). The Adaptive Response of Smokers to Oxidative Stress. *American Journal of Respiratory and Critical Care Medicine*, *166*(5), 635–636. https://doi.org/10.1164/rccm.2205024

Howie, R. M. (2005). Respiratory protective equipment. *Occupational and Environmental Medicine*, *62*(6), 423–428. https://doi.org/10.1136/oem.2002.004424

Huang, Y., Ni, W., Chen, Y., Wang, X., Zhang, J., & Wu, K. (2015). Levels and risk factors of antimony contamination in human hair from an electronic waste recycling area, Guiyu, China. *Environmental Science and Pollution Research*, *22*(9), 7112–7119. https://doi.org/10.1007/s11356-014-3941-1

ILO - International Labour Organization. (2019). Decent work in the management of electrical and electronic waste (e-waste). In *Issues paper for the Global Dialogue Forum on Decent Work in the Management of Electrical and Electronic Waste (E-waste)* (First edit). Retrieved from https://www.ilo.org/wcmsp5/groups/public/---ed_dialogue/---sector/documents/publication/wcms_673662.pdf

Issah, I., Arko-Mensah, J., Agyekum, T. P., Dwomoh, D., & Fobil, J. N. (2021). Electronic waste exposure and dna damage: A systematic review and meta-analysis. *Reviews on Environmental Health*. https://doi.org/10.1515/reveh-2021-0074

Jensen, M., Combariza Bayona, D. A., & Sripada, K. (2021). Mercury exposure among e-waste recycling workers in Colombia: Perceptions of safety, risk, and access to health information. *International Journal of Environmental Research and Public Health*, *18*(17). https://doi.org/10.3390/ijerph18179295

Jiang, L. G., Cheng, L. Y., Kong, S. H., Yang, Y., Shen, Y. J., Chen, C., … Chao, L. (2016). Toxic effects of polychlorinated biphenyls (Aroclor 1254) on human sperm motility. *Asian Journal of Andrology*, *18*(June 2016), 561–566. https://doi.org/10.4103/1008-682X.186876

Julander, A., Karlsson, M., Hagström, K., Ohlson, C. G., Engwall, M., Bryngelsson, I. L., … van Bavel, B. (2005). Polybrominated diphenyl ethers - Plasma levels and thyroid status of workers at an electronic recycling facility. *International Archives of Occupational and Environmental Health*, *78*(7), 584–592. https://doi.org/10.1007/s00420-005-0627-5

Julander, A., Lundgren, L., Skare, L., Grandér, M., Palm, B., Vahter, M., & Lidén, C. (2014). Formal recycling of e-waste leads to increased exposure to toxic metals: AN occupational exposure study from Sweden. *Environment International*, *73*, 243–251. https://doi.org/10.1016/j.envint.2014.07.006

Juyal, A., Kaur, J., & Khatri, A. (2019). Assesment of personal protective equipment usage amongst e-waste handlers. *Journal of Pharmacognosy and Phytochemistry*, 475–477.

Kahn, L. G., Philippat, C., Nakayama, S. F., Slama, R., & Trasande, L. (2020). Endocrine-disrupting chemicals: implications for human health. *The Lancet. Diabetes & Endocrinology*, *8*(8), 703–718. https://doi.org/10.1016/S2213-8587(20)30129-7

Kašuba, V., Milić, M., Zeljezić, D., Mladinić, M., Pizent, A., Kljaković-Gašpić, Z., … Jukić, I. (2020). Biomonitoring findings for occupational lead exposure in battery and ceramic tile workers using biochemical markers, alkaline comet assay, and micronucleus test coupled with fluorescence in situ hybridisation. *Arhiv Za Higijenu Rada i Toksikologiju*, *71*(4), 339–352. https://doi.org/10.2478/aiht-2020-71-3427

Kaya, M. (2019). E-Waste and E-Waste Recycling. In Michael Luby (Ed.), *The Minerals, Metals & Materials Series* (pp. 1–32). https://doi.org/10.1007/978-3-030-26593-9_1

Kim, K. H., Jahan, S. A., Kabir, E., & Brown, R. J. C. (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment International*, *60*, 71–80. https://doi.org/10.1016/j.envint.2013.07.019

Kirsch-Volders, M., Bonassi, S., Knasmueller, S., Holland, N., Bolognesi, C., & Fenech, M. F. (2014). Commentary: Critical questions, misconceptions and a road map for improving the use of the lymphocyte cytokinesis-block micronucleus assay for in vivo biomonitoring of human exposure to genotoxic chemicals-A HUMN project perspective. *Mutation Research - Reviews in Mutation Research*, *759*(1), 49–58. https://doi.org/10.1016/j.mrrev.2013.12.001

Kodros, J. K., O'Dell, K., Samet, J. M., L'Orange, C., Pierce, J. R., & Volckens, J. (2021). Quantifying the Health Benefits of Face Masks and Respirators to Mitigate Exposure to Severe Air Pollution. *GeoHealth*, *5*(9), 1–14. https://doi.org/10.1029/2021GH000482

Krupina, K., Goginashvili, A., & Cleveland, D. W. (2021). Causes and consequences of micronuclei. *Current Opinion in Cell Biology*, *70*, 91–99. https://doi.org/10.1016/j.ceb.2021.01.004

Kuo, L. J., Cade, S. E., Cullinan, V., Schultz, I. R., Arain, A. L., Neitzel, R. L., … Philippe, S. (2020). Novel brominated flame retardants - A review of their occurrence in indoor air, dust, consumer goods and food. *Environment International*, *16*(6), 105559. https://doi.org/10.1016/j.envint.2020.105559

Ladeira, C., & Smajdova, L. (2017). The use of genotoxicity biomarkers in molecular epidemiology: applications in environmental, occupational and dietary studies. *AIMS Genetics*, *04*(03), 166–191. https://doi.org/10.3934/genet.2017.3.166

Ladeira, C., & Viegas, S. (2016). Human Biomonitoring – An overview on biomarkers and their application in Occupational and Environmental Health. *Biomonitoring*, *3*(1), 15–24. https://doi.org/10.1515/bimo-2016-0003

Lazarides, E. (1983). *in Lymphocytes*. *80*(March), 1626–1630. https://doi.org/10.1007/978-1-60327-409-8

Li, X., Wang, L., Wang, Y., Yao, Y., Zhang, P., Zhao, H., & Sun, H. (2022). Occupational exposure to organophosphate esters in e-waste dismantling workers: Risk assessment and influencing factors screening. *Ecotoxicology and Environmental Safety*, *240*(May), 113707. https://doi.org/10.1016/j.ecoenv.2022.113707

Li, Z., Wang, Z., Xue, K., Wang, Z., Guo, C., Qian, Y., … Wei, Y. (2021). High concentration of blood cobalt is associated with the impairment of blood-brain barrier permeability. *Chemosphere*, *273*. https://doi.org/10.1016/J.CHEMOSPHERE.2021.129579

Lignell, S., Aune, M., Darnerud, P. O., Stridsberg, M., Hanberg, A., Larsson, S. C., & Glynn, A. (2016). Maternal body burdens of PCDD/Fs and PBDEs are associated with maternal serum levels of thyroid hormones in early pregnancy: A cross-sectional study. *Environmental Health: A Global Access Science Source*, *15*(1), 1–11. https://doi.org/10.1186/s12940-016-0139-7

Lin, M., Tang, J., Ma, S., Yu, Y., Li, G., Fan, R., … An, T. (2020). Insights into biomonitoring of

human exposure to polycyclic aromatic hydrocarbons with hair analysis: A case study in e-waste recycling area. *Environment International*, *136*(December 2019), 105432. https://doi.org/10.1016/j.envint.2019.105432

Lin, S., Ali, M. U., Zheng, C., Cai, Z., & Wong, M. H. (2022). Toxic chemicals from uncontrolled e-waste recycling: Exposure, body burden, health impact. *Journal of Hazardous Materials*, *426*. https://doi.org/10.1016/J.JHAZMAT.2021.127792

Liu, Q., Cao, J., Li, K. Q., Miao, X. H., Li, G., Fan, F. Y., & Zhao, Y. C. (2009). Chromosomal aberrations and DNA damage in human populations exposed to the processing of electronics waste. *Environmental Science and Pollution Research*, *16*(3), 329–338. https://doi.org/10.1007/s11356-008-0087-z

Liu, Q., Li, K. Q., Zhao, H., Li, G., & Fan, F. Y. (2009). The global challenge of electronic waste management. *Environmental Science and Pollution Research International*, *16*(3), 248–249. https://doi.org/10.1007/s11356-009-0131-7

Longo, V., Forleo, A., Giampetruzzi, L., Siciliano, P., & Capone, S. (2021). Human biomonitoring of environmental and occupational exposures by gc-ms and gas sensor systems: A systematic review. *International Journal of Environmental Research and Public Health*, *18*(19). https://doi.org/10.3390/ijerph181910236

Lu, S. you, Li, Y. xi, Zhang, J. qing, Zhang, T., Liu, G. hua, Huang, M. zhi, … Qiu, R. liang. (2016). Associations between polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress in people living near e-waste recycling facilities in China. *Environment International*, *94*, 161–169. https://doi.org/10.1016/J.ENVINT.2016.05.021

Lum, J. T. S., Chan, Y. N., & Leung, K. S. Y. (2021). Current applications and future perspectives on elemental analysis of non-invasive samples for human biomonitoring. *Talanta*, *234*(June), 122683. https://doi.org/10.1016/j.talanta.2021.122683

Lundgren, K. (2012). The global impact of e-waste: Addressing the challenge. In *International Labour Office*. Geneva.

Luzhna, L., Kathiria, P., & Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: From genetics to epigenetics and beyond. *Frontiers in Genetics*, *4*(JUL), 1–17. https://doi.org/10.3389/fgene.2013.00131

Ma, J., Cheng, J., Wang, W., Kunisue, T., Wu, M., & Kannan, K. (2011). Elevated concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and polybrominated diphenyl ethers in hair from workers at an electronic waste recycling facility in Eastern China. *Journal of Hazardous Materials*, *186*(2–3), 1966–1971. https://doi.org/10.1016/j.jhazmat.2010.12.091

Ma, S., Ren, G., Zeng, X., Yu, Z., Sheng, G., & Fu, J. (2018). Polychlorinated biphenyls and their hydroxylated metabolites in the serum of e-waste dismantling workers from eastern China. *Environmental Geochemistry and Health*, *40*(5), 1931–1940. https://doi.org/10.1007/s10653-017-9958-x

Ma, Y., Stubbings, W. A., Cline-Cole, R., & Harrad, S. (2021). Human exposure to halogenated and organophosphate flame retardants through informal e-waste handling activities - A critical review. *Environmental Pollution*, *268*, 115727. https://doi.org/10.1016/j.envpol.2020.115727

Manno, M., Viau, C., Cocker, J., Colosio, C., Lowry, L., Mutti, A., … Wang, S. (2010). Biomonitoring for occupational health risk assessment (BOHRA). *Toxicology Letters*, *192*(1), 3–16. https://doi.org/10.1016/j.toxlet.2009.05.001

Marabini, L., Calò, R., & Fucile, S. (2011). Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). *Toxicology in Vitro*, *25*(5), 1045–1052.

https://doi.org/10.1016/j.tiv.2011.04.004

Marques, C. G., & Da Silva, V. G. (2017). E-waste in Portugal - A higher education-based study. *Iberian Conference on Information Systems and Technologies, CISTI.* https://doi.org/10.23919/CISTI.2017.7975793

Meng, H. J., Tang, B., Zheng, J., Ma, S. X., Cai, F. S., Zhuang, X., … Yu, Y. J. (2020). Levels and sources of PBDEs and PCBs in human nails from e-waste, urban, and rural areas in South China. *Environmental Science: Processes and Impacts*, 22(8), 1710–1717. https://doi.org/10.1039/d0em00221f

Moreira, R., Tavares, A. M., Louro, H., Ladeira, C., & Viegas, S. (2022). _ *Exposição a substâncias químicas na indústria da gestão de resíduos elétricos _ e eletrónicos e seus potenciais efeitos na saúde dos trabalhadores*.

Movafagh, A., Heydary, H., Mortazavi-Tabatabaei, S. A. R., & Azargashb, E. (2011). The significance application of indigenous Phytohemagglutinin (PHA) mitogen on metaphase and cell culture procedure. *Iranian Journal of Pharmaceutical Research*, 10(4), 895–903. https://doi.org/10.22037/ijpr.2011.1008

Neitzel, R. L., Sayler, S. K., Arain, A. L., & Nambunmee, K. (2020). Metal levels, genetic instability, and renal markers in electronic waste workers in Thailand. *International Journal of Occupational and Environmental Medicine*, 11(2), 72–84. https://doi.org/10.34172/ijoem.2020.1826

Nersesyan, A., Fenech, M., Bolognesi, C., Mišík, M., Setayesh, T., Wultsch, G., … Knasmüller, S. (2016). Use of the lymphocyte cytokinesis-block micronucleus assay in occupational biomonitoring of genome damage caused by in vivo exposure to chemical genotoxins: Past, present and future. *Mutation Research - Reviews in Mutation Research*, 770, 1–11. https://doi.org/10.1016/j.mrrev.2016.05.003

Nyberg, A. G., Stricklin, D., & Sellström, Å. (2011). Mass casualties and health care following the release of toxic chemicals or radioactive material-contribution of modern biotechnology. *International Journal of Environmental Research and Public Health*, 8(12), 4521–4549. https://doi.org/10.3390/ijerph8124521

OECD. (2016). *OECD GUIDELINE FOR THE TESTING OF CHEMICALS, In Vitro Mammalian Cell Micronucleus Test 487*. (July).

Okeme, J. O., & Arrandale, V. H. (2019). Electronic Waste Recycling: Occupational Exposures and Work-Related Health Effects. *Current Environmental Health Reports*, 6(4), 256–268. https://doi.org/10.1007/s40572-019-00255-3

Osibanjo, O., & Nnorom, I. C. (2007). The challenge of electronic waste (e-waste) management in developing countries. *Waste Management & Research : The Journal of the International Solid Wastes and Public Cleansing Association, ISWA*, 25(6), 489–501. https://doi.org/10.1177/0734242X07082028

Parajuly, K., Fitzpatrick, C., Muldoon, O., & Kuehr, R. (2020). Behavioral change for the circular economy: A review with focus on electronic waste management in the EU. *Resources, Conservation & Recycling: X*, 6(February), 100035. https://doi.org/10.1016/j.rcrx.2020.100035

Pascale, A., Bares, C., & Laborde, A. (2017). E-waste: Environmental and health challenges. In *Encyclopedia of the Anthropocene* (Vol. 1–5). https://doi.org/10.1016/B978-0-12-809665-9.10013-8

Perkins, D. N., Brune Drisse, M.-N. N., Nxele, T., & Sly, P. D. (2014). E-waste: A global hazard. *Annals of Global Health*, 80(4), 286–295. https://doi.org/10.1016/j.aogh.2014.10.001

Probst-hensch, N. (2019). *Human Biomonitoring for Europe Program for Switzerland*. 41(0), 1–19.

Qin, R. X., Tang, B., Zhuang, X., Lei, W. X., Wang, M. H., Zhang, L. H., & Hu, K. M. (2021). Organophosphate flame retardants and diesters in the urine of e-waste dismantling workers: Associations with indoor dust and implications for urinary biomonitoring. *Environmental Science: Processes and Impacts*, *23*(2), 357–366. https://doi.org/10.1039/d0em00439a

Qu, W., Bi, X., Sheng, G., Lu, S., Fu, J., Yuan, J., & Li, L. (2007). Exposure to polybrominated diphenyl ethers among workers at an electronic waste dismantling region in Guangdong, China. *Environment International*, *33*(8), 1029–1034. https://doi.org/10.1016/j.envint.2007.05.009

RAMADHANI, D. W. I., & PURNAMI, S. (2013). Automated Detection of Binucleated Cell and Micronuclei using CellProfiler 2.0 Software. *HAYATI Journal of Biosciences*, *20*(4), 151–156. https://doi.org/10.4308/hjb.20.4.151

Rautela, R., Arya, S., Vishwakarma, S., Lee, J., Kim, K. H., & Kumar, S. (2021). E-waste management and its effects on the environment and human health. *Science of the Total Environment*, *773*, 145623. https://doi.org/10.1016/j.scitotenv.2021.145623

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cellular Biochemistry*, *119*(1), 157–184. https://doi.org/10.1002/JCB.26234

Rodrigues, M. A., Beaton-Green, L. A., Wilkins, R. C., & Fenech, M. F. (2018). The potential for complete automated scoring of the cytokinesis block micronucleus cytome assay using imaging flow cytometry. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *836*(March), 53–64. https://doi.org/10.1016/j.mrgentox.2018.05.003

Russo, A., & Degrassi, F. (2018). Molecular cytogenetics of the micronucleus: Still surprising. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *836*, 36–40. https://doi.org/10.1016/j.mrgentox.2018.05.011

Samanta, S., & Dey, P. (2012). Micronucleus and its applications. *Diagnostic Cytopathology*, *40*(1), 84–90. https://doi.org/10.1002/dc.21592

Schecter, A., Kincaid, J., Quynh, H. T., Lanceta, J., Tran, H. T. T., Crandall, R., … Birnbaum, L. S. (2018). Biomonitoring of Metals, Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and Persistent Pesticides in Vietnamese Female Electronic Waste Recyclers. *Journal of Occupational and Environmental Medicine*, *60*(2), 191–197. https://doi.org/10.1097/JOM.0000000000001200

Scheepers, P. T. J., Duca, R. C., Galea, K. S., Godderis, L., Hardy, E., Knudsen, L. E., … Santonen, T. (2021). Hbm4eu occupational biomonitoring study on e-waste—study protocol. *International Journal of Environmental Research and Public Health*, *18*(24). https://doi.org/10.3390/ijerph182412987

Selin, H., & VanDeveer, S. D. (2006). Raising Global Standards: Hazardous Substances and E-Waste Management in the European Union. *Environment: Science and Policy for Sustainable Development*, *48*(10), 6–18. https://doi.org/10.3200/ENVT.48.10.6-18

Services, H. (2000). Toxicological Profile for Polychlorinated Biphenyls (PCBs). In *ATSDR's Toxicological Profiles*. https://doi.org/10.1201/9781420061888_ch129

Shakeri, M., Zakeri, F., Changizi, V., Rajabpour, M. R., & Farshidpour, M. R. (2017). A cytogenetic biomonitoring of industrial radiographers occupationally exposed to low levels of ionizing radiation by using CBMN assay. *Radiation Protection Dosimetry*, *175*(2), 246–251. https://doi.org/10.1093/rpd/ncw292

Shi, Y., Zheng, X., Yan, X., Wang, Y., Xu, R., Wang, M., … Zheng, J. (2019). Short-term variability in levels of urinary phosphate flame retardant metabolites in adults and

children from an e-waste recycling site. *Chemosphere*, *234*, 395–401. https://doi.org/10.1016/j.chemosphere.2019.06.068

Shittu, O. S., Williams, I. D., & Shaw, P. J. (2020). Global E-waste management: Can WEEE make a difference? A review of e-waste trends, legislation, contemporary issues and future challenges. *Waste Management*. https://doi.org/10.1016/j.wasman.2020.10.016

Sivaramanan, S. (2013). E-Waste Management, Disposal and Its Impacts on the Environment. *Universal Journal of Environmental Research and Technology Www.Environmentaljournal.Org*, *3*(5), 531–537. Retrieved from http://www.environmentaljournal.org/3-5/ujert-3-5-1.pdf

Sommer, S., Buraczewska, I., & Kruszewski, M. (2020). Micronucleus assay: The state of art, and future directions. *International Journal of Molecular Sciences*, *21*(4), 7–9. https://doi.org/10.3390/ijms21041534

Speit, G., Linsenmeyer, R., Schütz, P., & Kuehner, S. (2012). Insensitivity of the in vitro cytokinesis-block micronucleus assay with human lymphocytes for the detection of DNA damage present at the start of the cell culture. *Mutagenesis*, *27*(6), 743–747. https://doi.org/10.1093/mutage/ges041

Srigboh, R. K., Basu, N., Stephens, J., Asampong, E., Perkins, M., Neitzel, R. L., & Fobil, J. (2016). Multiple elemental exposures amongst workers at the Agbogbloshie electronic waste (e-waste) site in Ghana. *Chemosphere*, *164*, 68–74. https://doi.org/10.1016/j.chemosphere.2016.08.089

Szyf, M. (2007). The dynamic epigenome and its implications in toxicology. *Toxicological Sciences*, *100*(1), 7–23. https://doi.org/10.1093/toxsci/kfm177

Tavares, A., Aimonen, K., Ndaw, S., Fučić, A., Catalán, J., Duca, R. C., … Silva, M. J. (2022). HBM4EU Chromates Study—Genotoxicity and Oxidative Stress Biomarkers in Workers Exposed to Hexavalent Chromium. *Toxics*, *10*(8), 483. https://doi.org/10.3390/toxics10080483

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Molecular, clinical and environmental toxicicology Volume 3: Environmental Toxicology. *Molecular, Clinical and Environmental Toxicology*, *101*, 133–164. https://doi.org/10.1007/978-3-7643-8340-4

Terradas, M., Martín, M., Tusell, L., & Genescà, A. (2010). Genetic activities in micronuclei: Is the DNA entrapped in micronuclei lost for the cell? *Mutation Research - Reviews in Mutation Research*, *705*(1), 60–67. https://doi.org/10.1016/j.mrrev.2010.03.004

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. (2012). Directive 2011/7/EU of the European Parliament and of the Council. *Official Journal of the European Union*, (July), 38–71. Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:197:0038:0071:en:PDF

Thomas, P., & Fenech, M. (2011). Cytokinesis-block micronucleus cytome assay in lymphocytes. *Methods in Molecular Biology*, *682*, 217–234. https://doi.org/10.1007/978-1-60327-409-8_16/COVER

Time, R. N., & Sutha, A. I. (2020). *European Journal of Molecular & Clinical Medicine The Challenges Of Electronic Waste (E-Waste) Management In India*. *07*(03), 4583–4588.

Tipre, D. R., Khatri, B. R., Thacker, S. C., & Dave, S. R. (2021). The brighter side of e-waste—a rich secondary source of metal. *Environmental Science and Pollution Research*, *28*(9), 10503–10518. https://doi.org/10.1007/s11356-020-12022-1

Tucker, J. D., Nath, J., & Hando, J. C. (1996). Activation status of the X chromosome in human micronucleated lymphocytes. *Human Genetics*, *97*(4), 471–475. https://doi.org/10.1007/BF02267069

United Nations University. (2014). One Global Definition of E-waste. *Step Initiative*, *3576*(June),

1–13. Retrieved from http://www.step-initiative.org/files/step/_documents/StEP_WP_One Global Definition of E-waste_20140603_amended.pdf

Upadhyay, K., Viramgami, A., Pagdhune, A., Balachandar, R., & Sarkar, K. (2021). Hematological and cardiovascular effects of chronic low level lead exposure: A study on e-waste recyclers. *Clinical Epidemiology and Global Health*, *9*(July 2020), 269–274. https://doi.org/10.1016/j.cegh.2020.09.009

van der Veen, I., & de Boer, J. (2012). Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere*, *88*(10), 1119–1153. https://doi.org/10.1016/j.chemosphere.2012.03.067

Viegas, S., Jeddi, M. Z., Hopf, N. B., Bessems, J., Palmen, N., Galea, K. S., … Pasanen-Kase, R. (2020). Biomonitoring as an underused exposure assessment tool in occupational safety and health context—challenges and way forward. *International Journal of Environmental Research and Public Health*, *17*(16), 1–20. https://doi.org/10.3390/ijerph17165884

Wagner, M., Hemkhaus, M., Rabbow, E., Cheng, C., & Ott, D. (2020). *Partnerships between the informal and the formal sector for sustainable e-waste management Authors*. (April).

Wang, H., Lv, S., Li, F., Liu, Q., & Ke, S. (2010). Study on the changes of urinary 8-hydroxydeoxyguanosine levels and burden of heavy metal around e-waste dismantling site. *Science of the Total Environment*, *408*(24), 6092–6099. https://doi.org/10.1016/j.scitotenv.2010.09.019

Wang, Q., He, A. M., Gao, B., Chen, L., Yu, Q. Z., Guo, H., … Yuan, J. (2011). Increased levels of lead in the blood and frequencies of lymphocytic micronucleated binucleated cells among workers from an electronic-waste recycling site. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *46*(6), 669–676. https://doi.org/10.1080/10934529.2011.563176

Wang, X., Hales, B. F., & Robaire, B. (2021). Effects of flame retardants on ovarian function. *Reproductive Toxicology (Elmsford, N.Y.)*, *102*, 10–23. https://doi.org/10.1016/j.reprotox.2021.03.006

Wang, Yalin, Hu, J., Lin, W., Wang, N., Li, C., Luo, P., … Shen, C. (2016). Health risk assessment of migrant workers' exposure to polychlorinated biphenyls in air and dust in an e-waste recycling area in China: Indication for a new wealth gap in environmental rights. *Environment International*, *87*, 33–41. https://doi.org/10.1016/j.envint.2015.11.009

Wang, Yan, Sun, X., Fang, L., Li, K., Yang, P., Du, L., … Hecker, M. (2018). Genomic instability in adult men involved in processing electronic waste in Northern China. *Environment International*, *117*(May), 69–81. https://doi.org/10.1016/j.envint.2018.04.027

Wang, Z., Hang, J. G., Feng, H., Shi, L. L., Dong, J. J., Shen, B., … Sun, X. L. (2019). Effects of perinatal dioxin exposure on development of children: a 3-year follow-up study of China cohort. *Environmental Science and Pollution Research International*, *26*(20), 20780–20786. https://doi.org/10.1007/S11356-019-05362-0

Wen, S., Yang, F. X., Gong, Y., Zhang, X. L., Hui, Y., Li, J. G., … Xu, Y. (2008). Elevated levels of urinary 8-hydroxy-2'-deoxyguanosine in male electrical and electronic equipment dismantling workers exposed to high concentrations of polychlorinated dibenzo-p-dioxins and dibenzofurans, polybrominated diphenyl ethers, and polychlorinat. *Environmental Science and Technology*, *42*(11), 4202–4207. https://doi.org/10.1021/es800044m

Widmer, R., Oswald-Krapf, H., Sinha-Khetriwal, D., Schnellmann, M., & Böni, H. (2005). Global perspectives on e-waste. *Environmental Impact Assessment Review*, *25*(5 SPEC. ISS.),

436–458. https://doi.org/10.1016/j.eiar.2005.04.001

Wilde, S., Queisser, N., Holz, C., Raschke, M., & Sutter, A. (2019). Differentiation of Aneugens and Clastogens in the In Vitro Micronucleus Test by Kinetochore Scoring Using Automated Image Analysis. *Environmental and Molecular Mutagenesis*, *60*(3), 227–242. https://doi.org/10.1002/em.22259

Williams, I. D. (2016). Global Metal Reuse, and Formal and Informal Recycling from Electronic and Other High-Tech Wastes. *Metal Sustainability: Global Challenges, Consequences, and Prospects*, 23–51. https://doi.org/10.1002/9781119009115.ch2

World Health Orgnization. (1993). Biomarkers and risk assessment: concepts and principles. Environmental Health Criteria 155. *Environmental Health Criteria*, (155), 82. Retrieved from http://www.inchem.org/documents/ehc/ehc/ehc155.htm#SectionNumber:1.1

Xiao, X., Hu, J., Peng, P., Chen, D., & Bi, X. (2016). Characterization of polybrominated dibenzo-p-dioxins and dibenzo-furans (PBDDs/Fs) in environmental matrices from an intensive electronic waste recycling site, South China. *Environmental Pollution*, *212*, 464–471. https://doi.org/10.1016/j.envpol.2016.02.029

Yang, D., Liu, Y., Liu, S., Li, C., Zhao, Y., Li, L., & Lu, S. (2020). Exposure to heavy metals and its association with DNA oxidative damage in municipal waste incinerator workers in Shenzhen, China. *Chemosphere*, *250*, 126289. https://doi.org/10.1016/j.chemosphere.2020.126289

Yang, H., Ma, M., Thompson, J. R., & Flower, R. J. (2017). Waste management, informal recycling, environmental pollution and public health. *Journal of Epidemiology and Community Health*, *72*(3), 237–243. https://doi.org/10.1136/jech-2016-208597

Yang, Y., Li, X., Lu, X., Li, M., Yu, Y., & Li, D. (2014). Study on the Correlation Between Heavy Metals and Sex Hormone Levels in Serum of E-Waste Dismantling Area Males. *Asian Journal of Chemistry*, *26*(21), 7301–7306. https://doi.org/10.14233/ajchem.2014.16618

Yang, Y., Lu, X. S., Li, D. L., & Yu, Y. J. (2013). Effects of environmental lead pollution on blood lead and sex hormone levels among occupationally exposed group in An E-waste dismantling area. *Biomedical and Environmental Sciences*, *26*(6), 474–484. https://doi.org/10.3967/0895-3988.2013.06.008

Yao, M., Hu, T., Wang, Y., Du, Y., Hu, C., & Wu, R. (2017). Polychlorinated biphenyls and its potential role in endometriosis. *Environmental Pollution*, *229*, 837–845. https://doi.org/10.1016/j.envpol.2017.06.088

Ye, C. J., Sharpe, Z., Alemara, S., Mackenzie, S., Liu, G., Abdallah, B., … Heng, H. H. (2019). Micronuclei and genome chaos: Changing the system inheritance. *Genes*, *10*(5), 1–21. https://doi.org/10.3390/genes10050366

Yu, X. Z., Gao, Y., Wu, S. C., Zhang, H. B., Cheung, K. C., & Wong, M. H. (2006). Distribution of polycyclic aromatic hydrocarbons in soils at Guiyu area of China, affected by recycling of electronic waste using primitive technologies. *Chemosphere*, *65*(9), 1500–1509. https://doi.org/10.1016/j.chemosphere.2006.04.006

Yuan, J., Chen, L., Chen, D., Guo, H., Bi, X., Ju, Y., … Chen, X. (2008). Elevated serum polybrominated diphenyl ethers and thyroid-stimulating hormone associated with lymphocytic micronuclei in Chinese workers from an e-waste dismantling site. *Environmental Science and Technology*, *42*(6), 2195–2200. https://doi.org/10.1021/es702295f

Zare Jeddi, M., Hopf, N. B., Viegas, S., Price, A. B., Paini, A., van Thriel, C., … Pasanen-Kase, R. (2021). Towards a systematic use of effect biomarkers in population and occupational biomonitoring. *Environment International*, *146*, 106257. https://doi.org/10.1016/j.envint.2020.106257

Zeljezic, D., Mladinic, M., Kopjar, N., & Radulovic, A. H. (2016). Evaluation of genome damage in subjects occupationally exposed to possible carcinogens. *Toxicology and Industrial Health*, *32*(9), 1570–1580. https://doi.org/10.1177/0748233714568478

Zhang, Z., Zhou, M., He, J., Shi, T., Zhang, S., Tang, N., & Chen, W. (2020). Polychlorinated dibenzo-dioxins and polychlorinated dibenzo-furans exposure and altered lung function: The mediating role of oxidative stress. *Environment International*, *137*. https://doi.org/10.1016/J.ENVINT.2020.105521

Zhao, C., Li, J. F. T., Li, X. H., Dong, M. Q., Li, Y. Y., & Qin, Z. F. (2021). Measurement of polychlorinated biphenyls with hand wipes and matched serum collected from Chinese E-waste dismantling workers: Exposure estimates and implications. *Science of the Total Environment*, *799*, 149444. https://doi.org/10.1016/j.scitotenv.2021.149444

Zheng, J., He, C. T., Chen, S. J., Yan, X., Guo, M. N., Wang, M. H., … Mai, B. X. (2017). Disruption of thyroid hormone (TH) levels and TH-regulated gene expression by polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and hydroxylated PCBs in e-waste recycling workers. *Environment International*, *102*, 138–144. https://doi.org/10.1016/j.envint.2017.02.009