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Epidemiological applications of inflammation and oxidative stress biomarkers to assess the early effects of human exposure to micro and nano-plastics.

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Abstract

Micro- and nano plastics (MNPs) have emerged as one of the most significant environmental challenges of the past decade, raising concerns about their potentially harmful effects on human health. This doctoral thesis aims to investigate the mechanisms of interaction between MNPs and living organisms identifying and quantifying biomarkers suitable for monitoring exposure and early biological effects. This work is divided into two different study lines. In study line I, we critically analysed the available literature performing comprehensive, state-of-art, and systematic reviews on the exposure routes, translocation, fate, and early biological effects of MNPs in vitro, in vivo models, and human studies. In study line II, we performed field epidemiological studies in occupational settings using standardised methodologies enabling the investigation of MNPs effects through human biomonitoring (HBM) and key biomarkers, already analysed in the study line I, reflecting oxidative stress (i.e., Malondialdehyde, 15-f_{2t}-Isoprostane, Total Antioxidant Power), inflammation (Interleukins (IL) IL-1β, IL-6, IL-8, and Tumour Necrosis Factor alpha "TNF-α"), and cito and genotoxicity (Comet assay, Sister Chromatid Exchange, Chromosomal Aberrations). Special attention was given to innovative biological matrices such as exhaled breath condensate (EBC), which can provide precise information on the respiratory tract microenvironment. In the field studies, 80 workers potentially exposed to nanomaterials, were examined assessing their exposure levels through epidemiological questionnaires, environmental measures and HBM. The latter has been implemented on non-invasively collected biological samples (urine, EBC), and by means of advanced analytical methods such as Real-time polymerase chain reaction (PCR), high-sensitivity Enzyme-Linked Immunosorbent Assays (ELISAs), and Nanoparticle Tracking Analysis (NTA). The latter, measures the number of particles in EBC using the NTA instrument, and was implemented and analysed for the first time in a multicentre study to identify an additional internal dose biomarker not yet investigated in the literature. The results revealed significant associations between particle concentration, inflammatory cytokine levels, and oxidative stress in EBC, suggesting that exposure to MNPs can modulate inflammation and oxidative stress levels, with EBC and NTA emerging as promising tools for assessing internal dose and associated risks. Additionally, a second field study was conducted on a cohort of 53 workers potentially exposed to nanomaterials (glass and minerals). Exposure levels were assessed through epidemiological questionnaires, and both urine and EBC samples were collected. Using analytical techniques such as ELISA, qPCR-ELISA, and NTA, inflammatory biomarkers (cytokines) and oxidative stress markers, previously investigated in the prior study, were analysed. This work has not yet been published; the analyses are still ongoing. However, preliminary results have revealed significant associations in the levels of inflammatory cytokines and oxidative stress markers between exposed and non-exposed individuals, respectively in EBC and urine samples. These findings provide a foundation for developing prevention and monitoring strategies for MNPs exposure, contributing to a better understanding of their impact on human health.

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1. Introduction

1.1 Epidemiology and health

Epidemiology is a relatively recent discipline that has evolved alongside societal changes and the emergence of new diseases. This evolution has enabled epidemiology to remain a crucial tool for identifying and understanding diseases and health-related events. Since its inception over a century ago, numerous definitions of this discipline have been proposed (Frerot et al. 2018). The earliest definition dates to 1978, describing epidemiology as "*the study of the prevalence and stages of health within populations*" (Frerot et al. 2018).

The World Health Organization (WHO) later provided a more comprehensive definition, by identifying epidemiology as "*the study of the distribution and determinants of health-related states or events (including diseases) and the application of these studies to control diseases or other health problems*". Epidemiological investigations can utilise various methods, including surveillance or descriptive studies to examine distribution, and analytical studies to explore health determinants (WHO, 2017). According to Frerot et al. (2018), epidemiology is now associated with the study of diseases and, more broadly, with the study of health phenomena. Analysing the definitions of "epidemiology" proposed over the years, key terms such as "population," "control," "study," "disease," and "health" frequently appear, though only a few, like "control" and "health," have remained constant. Similarly, the concept of "health" has undergone significant evolution. Initially equated with the absence of disease, it has expanded to encompass physical, mental, and social well-being. The WHO defines health as "*a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity*" (Terris, 1975). Epidemiology has been instrumental in identifying risk factors related to environmental conditions and lifestyles. Many diseases of varied origin had unknown causes, but through the study of these diseases and outbreaks, numerous previously unidentified etiological factors were uncovered. It became evident that non-communicable diseases, particularly cancers, often result from the combined effects of environmental and genetic factors, rather than genetic factors alone (Marchand, 2005). Consequently, the environment and ecology have become critical concerns for human populations due to their significant impact on public health. The emergence of environmental epidemiology is a clear testament to this. Environmental considerations began appearing in epidemiological definitions in the 2000s, and today, professionals across fields such as health education, environmental health, and occupational health are increasingly required to understand the fundamentals of this discipline (Batty, 1999). In addition to environmental epidemiology, occupational epidemiology has also developed, by applying epidemiological methods to worker populations. Occupational epidemiological studies may focus on workers exposed to chemical, biological, or physical agents (e.g., noise, heat, radiation, etc.) to determine whether these exposures pose health risks. Alternatively, they may examine workers with common adverse health outcomes to identify causal agents (OSHA, 2023). The global demand for goods driven by consumerism has necessitated sustainable production and resource efficiency, giving rise to Industry 4.0 (Stock and Seliger, 2016). The advent of Industry 4.0 has brought new technologies and automation systems that have significantly altered work processes, presenting new challenges for

workers safety (Badri et al., 2018). This trend is expected to lead to different work organisation structures and the production of new materials, which may pose potential long-term risks to human health (Leso et al., 2018). The use of nanotechnology and advanced materials across industries such as agriculture, cosmetics, healthcare, automotive, chemical, and mechanical sectors have brought numerous benefits compared to traditional mass production and heavy machinery. The application of nanomaterials in modern industries is already widespread and will likely become mandatory in all sectors in the future (Waldron et al., 2006). While the advantages of nanotechnology in industry have been widely discussed, its negative or less-explored aspects remain significant. Many risks associated with indiscriminate use are still poorly understood. Because of their small size (ranging from a few nanometers to several micrometers), inhalation or ingestion exposure to airborne particles containing nanomaterial are plausible. Moreover, nanomaterials may agglomerate into bigger particles or longer fiber chains, changing their characteristics and modifying their behavior in the indoor and outdoor settings, as well as their potential exposure and entrance into the human body (Lam et al., 2004; Lee et al., 2007). Further research is crucial to address these concerns within the field of nanotoxicology. Collaboration among researchers, technicians, and industry professionals is recommended to harness nanotechnology effectively. Advances at the nanoscale are essential for a responsible future for this technology. Precautionary measures must address environmental and health concerns, integrating sustainability with nanotechnology to ensure a prosperous future (Schneider, 2007; Malik et al., 2023).

1.2 Micro and nanoparticles

Micro- and nanoparticles represent a novel class of materials, with sizes ranging from 1–1000 µm for the former and 1–100 nm for the latter. Because to their small size, they have a high surface area-to-volume ratio, which greatly increases their chemical and physical reactivity. These particles can be composed of organic materials (polymers, lipids, or biomolecules) or inorganic materials (metals, metal oxides, and carbon-based structures). Their structure, which varies in shape (spherical, cubic, or irregular), surface charge, and functional coatings, making them extremely adaptable for a wide range of uses, including medicine and industry. However, this raises worries about their toxicology (Lopez et al., 2022). Moreover, nanoparticles have the capacity to penetrate biological tissues, overcome barriers such as the blood-brain barrier, and cause inflammatory or oxidative stress, making them potentially harmful to human health and the environment (Suri et al., 2013). The use of new materials and nanoscale processes results in the release of particles that are difficult to detect and exhibit high reactivity due to their small size compared to larger particles (Taran et al., 2021). Within the scale of nanoparticles, defined as materials with at least one dimension between 1–100 nm, fall certain chemicals or materials referred to as nanomaterials (ECHA, 2024). As a result, understanding their physicochemical qualities and biological consequences is critical to ensure their safe and sustainable usage (Wang et al., 2022).

1.2.1 Micro and nano-plastics

According to the revised definition by the European Commission, a nanomaterial is defined as "*a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50% or more of these particles in the number-based size distribution fulfil at least one of the following three conditions: (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm; (b) the particle has an elongated shape, such as a rod, fibre, or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm; (c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm" (The European Commission, 2022/C 229/01). Notably, nano plastics also fall under the category of "nanomaterials*," classified as a specific type of incidental nanomaterial. Nano plastics are now ubiquitous, appearing in numerous processes and finished products, playing an essential role in daily life due to their versatility, durability, and resistance (Dai et al., 2023). However, their excessive use across pharmaceuticals, packaging, agriculture, and various industrial sectors has raised serious environmental safety concerns. Plastics, following their production, use, and exposure to chemical, physical, and biological agents, degrade—albeit slowly—into much smaller fragments, generating micro and nano plastics (MNPs) (Wu et al., 2019). Based on their formation or release processes, these plastics can be categorised as primary or secondary MNPs. Primary MNPs are directly produced and released within peculiar size range of MNPs, while secondary MNPs arise from the fragmentation of larger plastic materials due to degradation processes (Mariano et al., 2021). Additionally, besides their persistence in the environment, nano plastics can act as vectors for organic pollutants, increasing their bioavailability. This raises further concerns for public health and the environment, as significant quantities have been detected in rivers, lakes, seas, and oceans (Covernton et al., 2022; Kumar et al., 2023). Microplastics in the environment can be identified using optical and electron microscopy, and their characterisation can be achieved with techniques such as GC-MS, micro-FTIR, and Raman spectroscopy (Kumar et al., 2023). However, less is known about nano plastics due to the difficulty of their detection, which stems from methodological limitations and analytical constraints (Chen et al., 2020; Ramsperger et al., 2023). At present, knowledge about nano plastics is limited. Thus, current and future scientific investigations should adopt multidisciplinary approaches to develop a broader and more comprehensive understanding of this issue. Advancements in analytical methods for nano plastics identification are particularly critical (Yamamoto et al., 2018; Allan et al. 2021; Zhang et al., 2021).

1.3 Environmental monitoring

Micro- and nanoparticles, are ubiquitous and are continuously released into indoor and outdoor environments through various media, including air, water, and soil, via multiple natural and artificial processes. Natural processes can include physical erosion, such as wave action, which fragments larger particles into smaller ones, generating micro- and nanoparticles as well as MNPs. Artificial processes, on the other hand, are associated with industrial production in sectors such as cosmetics, pharmaceuticals, and automotive manufacturing (Stebounova et al., 2012, El-Kalliny et al., 2023).

Some of these contaminants can persist in the environment for many years. When present in the soil, they may migrate into water resources, posing ecological and potential human health risks (Ho et al., 2005). For this reason, environmental monitoring is essential to protect living organisms and environment from potentially toxic contaminants. It is important to consider that people are mobile and visit different microenvironments daily (home, workplace, school, transportation, etc.), spending most of their time indoors and engaging in various activities that generate particles in their vicinity (Wellenius et al., 2012; Rice et al., 2013). The amount of micro- and nanoparticles produced naturally varies depending on the location where individuals operate or work, making it necessary to characterize exposures (Johannesson et al., 2011). In addition to area-based sampling devices, personal sampling devices can also be used to better characterize individual exposure. These devices were first introduced by Sherwood & Greenhalgh in occupational settings using early instruments equipped with battery-operated pumps, significantly improving the accuracy and precision of actual particle intake by individuals (Sherwood & Greenhalgh, 2016). Over the decades, personal samplers have gained traction in the field of industrial hygiene, providing numerous benefits. With technological advancements, these instruments have become increasingly sensitive (thanks to improved sensors) to detecting micro- and nanoscale particles (inhalable, thoracic, and respirable fractions) (Vincent, 2012). Direct-reading instruments have also been introduced, integrating sensors that provide "real-time" indications of contaminant concentrations, enabling high temporal and spatial resolution measurements of various contaminants when carried by an individual along their daily route. Depending on the type of particles to be investigated, different instruments can be chosen. For example, for fine and ultrafine particulate analysis, instruments like the Lighthouse 3016-IAQ can be used, with a size range of 0.3 µm–10 µm (6 size classes) and a 10-second resolution, measuring particulate concentration in air [n/m^3]. For nanoparticles, devices such as the DiSCmini™ (Testo, Mönchaltorf, Switzerland), with a size range of 10 nm–300 nm and a 1-second resolution, can measure particle concentration in air [n/cm^3].

This thesis focuses primarily on the occupational setting and the integration of environmental monitoring with HBM for workers potentially exposed to micro- and nanoparticles, with particular emphasis on plastics. To accurately assess the risks faced by these workers, it is necessary to combine environmental air monitoring with biological monitoring.

1.4 Human Biomonitoring

Human biomonitoring (HBM) is an extra and supplementary tool for exposure assessment, providing a more focused and multi-level evaluation, in addition to environmental monitoring methods covered in the previous chapter, such as surface sampling and air quality monitoring. HBM supplements exposure assessment by directly measuring a chemical substance, metabolite or biomarker in the biological fluids of individuals or groups exposed to various routes of exposure, including inhalation, ingestion, or dermal absorption (HSE, 1997; Louro et al., 2019; ACGIH, 2020). Biomarkers can be detected and quantified in various biological fluids, such as blood, urine, saliva, and EBC. The analysis primarily focuses on chemical compounds, including elements or their metabolites, or biological biomarkers such as pro- and anti-inflammatory cytokines or other molecules with pro- and antioxidant activity (Strimbu and Tavel, 2010).

HBM plays a crucial role in safeguarding worker health by allowing the assessment of their actual exposure to chemicals used or encountered during industrial processes. Compared to environmental monitoring, HBM has the advantage of measuring the systemic absorption of substances—that is, what has penetrated the body through all possible exposure routes—rather than merely evaluating potential exposure (Needham et al., 2007; Jones, 2020). The optimal preventive strategy in the occupational setting involves an integrated approach that combines exposure assessment with biomonitoring based on biomarkers to identify early alterations in biological systems that may predict adverse health effects (Manno et al., 2010). For this reason, it is advisable to conduct exposure monitoring and biomonitoring campaigns in parallel, where feasible, to correlate the data obtained. Comparing exposure levels with biological endpoints enables estimation of the effective dose of chemicals absorbed by the body, considering all exposure routes, interindividual variability in absorption, metabolism, and excretion processes, and the effectiveness of protective equipment used.

1.4.1 Biological matrices

To conduct HBM appropriately, it is essential to consider various sources, including the stability of the compound of interest as a metabolite, its suitability as a biomarker for assessing exposure to specific compounds, and the selection of the most appropriate biological matrix for its determination. A biological matrix refers to a biological fluid or material used for the quantification of analytes in the context of a biomonitoring investigation (Vorkamp et al., 2021). Biological matrices are classified as invasive or non-invasive depending on the invasiveness of the sampling process. While blood is generally regarded as the reference matrix for analysing the absorption of chemicals, metabolites, and indicators of inflammatory or oxidative processes, its collection involves an invasive procedure. However, advances in new methodologies and analytical techniques have enabled the use of less invasive or non-invasive alternative matrices, such as urine, saliva, sweat, breast milk, faeces, placenta, and EBC. Sampling these matrices is straightforward and rapid, allowing specific analyses to be conducted based on the biomarkers under examination (Esteban and Castano, 2009). The detection of a chemical substance or specific biomarkers in these matrices reflects exposure levels, but it is crucial to establish correlations between the levels found in non-invasive matrices and xenobiotics measured through environmental monitoring. Therefore, the choice of matrix to analyse is of critical importance, and the development of new measurable biomarkers will significantly enhance the quality of the HBM process.

1.4.2 Oxidative stress

Redox processes, such as pH regulation, are fundamental to life and are involved in all essential biological activities, from bioenergetics to metabolism and vital functions (Sies, et al., 2017). In complex systems such as the human body, biochemical processes and reactions continuously lead to the formation of reactive oxygen species (ROS). Naturally, the body has multiple defences, known as antioxidants, to counteract the overproduction of ROS. The balance between pro-oxidants and antioxidants is crucial for understanding oxidative stress for several reasons. First, an imbalance can

result from both an increase in ROS production and a decrease in antioxidant defences. Additionally, ROS are signalling molecules, potentially disrupting normal signal transmission. However, in many cases, excessive perturbation of this balance leads to oxidative stress (Burton and Jauniaux, 2011). This imbalance or shift favouring pro-oxidants over antioxidants, which is defined as oxidative stress, can result in potential damage. Oxidative stress may have a central factor in the pathophysiology of numerous disorders. The severity of the damage caused by such imbalances is often gradual. Mild and transient imbalances may cause minimal harm, whereas more severe and prolonged imbalances can result in significant damage, including cell death. Unfortunately, the distinction between normal physiological changes and pathological conditions is inherently blurred, making it challenging to establish clinical cut-off levels for oxidative stress in a clinical setting (Dröge, 2002). There are many potential sources of ROS, including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and various enzymes whose primary role is to mitigate the toxic effects of these highly reactive molecules and restore physiological balance. Examples of such enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and lactate dehydrogenase (LDH) (Marrocco et al., 2017; Halappanavar and Mallach, 2021). One of the major consequences of oxidative stress is lipid peroxidation, which is the result of oxidative damage to lipid cell membranes. Many indicators of lipid peroxidation have been identified in humans and animals, such as the Isoprostane class, prostaglandin-like compounds derived from the lipid peroxidation of arachidonic acid. Malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) are also widely used as indicators of lipid peroxidation and thus of oxidative stress (Lykkesfeldt, 2007; Spickett et al., 2010).

1.4.3 Inflammation

Inflammation is a complex and essential set of physiological processes activated by the body in response to various harmful stimuli, such as viruses, bacteria, and inorganic particles. This adaptive response involves a series of cellular and molecular events, whose regulation and interactions among the different mediators of inflammation remain areas of active research (Medzhitov, 2008). Depending on the type of stimulus and its duration, inflammation can be classified into two main types: acute and chronic. Acute inflammation is the immediate response to a harmful agent, characterised by a relatively short duration, ranging from a few minutes to several days. This phase is primarily marked by oedema formation and leukocyte migration, predominantly involving neutrophil granulocytes. In contrast, chronic inflammation develops in response to persistent stimuli and has a considerably longer duration. Histologically, it is distinguished by the presence of various types of leukocytes, including lymphocytes and macrophages, as well as by vascular proliferation and the onset of fibrosis or tissue necrosis (King, 2007; Chung et al., 2009).

During an acute inflammatory process, the involved cells migrate to the site of injury/inflammation and are typically sufficient to repair the damage, restore the injured area, or eliminate the responsible pathogen (Germolec et al., 2018). A key role in this process is played by cytokines and chemokines, which are protein-based chemical mediators produced by various cells recruited to the site of inflammation (Vilcek, 2001; Ramesh, et al., 2013). In contrast, chronic inflammation, which persists over time, generates an excessive and continuous stimulus at the injury site, promoting

tissue and fibrotic damage. Chronic inflammation contributes to the onset and progression of various diseases, including asthma, atherosclerosis, arthritis, and many other autoimmune disorders. To date, although in clinical settings the concentrations of inflammatory mediators are measured in blood, it remains challenging to identify or establish cut-off levels for determining an active inflammatory state (Murakami and Hirano, 2012; Antonelli and Kushner, 2017; Germolec et al., 2018; Varela et al., 2018; Roe, 2021).

1.4.4 Biomarkers

A biomarker can be defined as a measurable event, identifiable in the form of a substance, its metabolite, or a process within a biological system, such as the human body. This event can serve as an indicator of normal physiological conditions, pathological states, or responses to therapeutic interventions, allowing the detection of temporary imbalances caused by external stressors, whether organic or inorganic in nature (Atkinson et al., 2001). Biomarkers can be classified into various categories, but they are commonly distinguished as exposure biomarkers, effect biomarkers, and susceptibility biomarkers (Bocca et al., 2024). Additionally, they can be classified as indicators of internal dose, acute inflammation, chronic inflammation, and oxidative stress. Internal dose biomarkers identify exposure to chemicals or external factors, revealing how much of a substance or its metabolites have been detected in the body. Acute inflammation biomarkers, including IL-6 and TNF-α, indicate an early reaction to tissue injury or infections. Conversely, interleukin-1β (IL-1β) indicates chronic inflammation. Finally, biomarkers of oxidative stress include indications of lipid peroxidation such as malondialdehyde and isoprostanes, which represent oxidative damage to cellular membranes (NRC, 2006). They are essential tools for the classification and quantification of environmental exposures and their effects, with numerous applications in toxicological research and epidemiological studies, particularly in occupational settings (Niki, 2014). In clinical settings, biomarkers are used to diagnose diseases, monitor therapeutic interventions, and predict clinical outcomes, often using threshold values (cut-offs). In environmental epidemiology, a biomarker generally represents a reversible change that does not have direct diagnostic purposes but can serve as an indicator of an early modification that could evolve into a disease (i.e., biomarker of effect). One of the goals of environmental epidemiology is to transfer the use of biomarkers from clinical contexts to epidemiological research by identifying, validating, and applying new biomarkers for research and biomonitoring purposes. Despite their potential, the characterisation and validation of many biomarkers still pose a challenge, complicating the monitoring of exposure to environmental chemicals in the workplace, which could be precursors to diseases (Boffetta, 2010). Currently, there is a wide range of clinical biomarkers that can identify or suggest the onset of pathological conditions or confirm an already existing pathological state. In clinical settings, most biomarkers are detected in blood. However, in occupational HBM, the use of this matrix is not always feasible, primarily due to low compliance from workers and companies. Therefore, the use of non-invasive matrices, were feasible, is preferred, as they increase the participants ‘compliance.

1.4.4.1 Internal dose biomarkers

In epidemiological research, biomarkers are frequently used to detect exposure to dangerous compounds that people may be exposed to through their skin, their lungs, or their food. (Martinez-Morata et al., 2023). In these studies, both environmental monitoring, which measures the external dose of exposure, and HBM, which allows the estimation of the internal dose and the exposure-related effects, are performed. This dual approach enhances the accuracy in measuring any risk factor, adding details on internal and external exposure, through HBM and environmental monitoring, respectively. When a substance is detected in tissues or body fluids, under certain specific characteristics it can be considered a biomarker for the internal dose, providing a more accurate estimation of the dose absorbed and metabolised by the body, thus allowing a better understanding of the potential pathophysiological processes associated with exposure. Moreover, the choice of a specific biological matrix for biomarker detection depends on the pharmacokinetic properties of the substance being studied. For example, some chemicals tend to accumulate in adipose tissue, others in blood or urine, while inhaled substances may accumulate in the airways and be detected in EBC (Mayeux, 2004; Yusa et al., 2012).

1.4.4.1.1 Particle Number Concentration (PNC) and Nanoparticles Tracking Analysis (NTA)

Aerosolised particles can access the bloodstream through the alveolar region of the lungs. It is well-established that negative health effects can be associated with the surface chemistry of inhaled particles and their quantity (Kuuluvainen et al., 2016). For this reason, the particle number concentration (PNC) is an important parameter for assessing the effects of airborne particles that may impact public and environmental health (Zhu et al., 2022). Numerous studies have demonstrated that prolonged exposure to elevated concentrations of airborne particles, originating from sources such as combustion and industrial processes (e.g., trimming activities), in both outdoor and indoor environments, is associated with respiratory health issues. These include a reduction in lung function, which can lead to conditions such as asthma and cardiovascular diseases. This association is largely attributed to the inhalation of ultrafine nanoparticles, which can easily penetrate the respiratory system and reach the deepest airways, where they initiate inflammatory responses (Price et al., 2014; Vouitsis et al., 2023). Some devices enabling the analysis of the number of particles aerosolised in the environment include the Partector (Fierz et al., 2014), NanoTracer (Marra, 2011), and DiSCmini™ (Testo, Mönchaltorf, Switzerland). These are direct-reading optical particle counters. Their common uses include assessing individual exposure in susceptible populations (like asthmatics or COPD patients) or in workplaces polluted by particles (such welding fumes and industrial nanoparticles). These instruments have a 1 Hz temporal resolution and monitor the concentration of environmental particles, which is represented as the number of particles per cm^3. The particle size ranges from 10 to 300 nm, and the detection limit ranges from 500 to 1 million particles per cm^3 (Kuuluvainen et al., 2016, Hemmendinger et al., 2023). Moreover, with these devices, it is possible to derive the LDSA (Lung-Deposited Surface Area, expressed as $\mu m^2/cm^3$), which refers to the likelihood of deposition in the pulmonary and alveolar areas of aerosolised

nanoparticles (Oberdörster et al., 2005, Schmid and Stoeger, 2016). In occupational settings where individuals can be exposed to aerosolised particles, HBM is carried out by collecting biological matrices such as urine, blood, and EBC. The latter is essential for conducting localised and specific investigations of the upper airways (Ghelli et al., 2022). Once collected and stored at the correct temperature to limit biochemical processes within the sample, it is possible to analyse it and search for specific biomarkers of oxidative stress, inflammation, and internal dose. Using Nanoparticle Tracking Analysis ZetaView® PMX-120 (Particle Metrix GmbH, Germany) (NTA), it is possible to measure the particle concentration, their size distribution, and their Z-potential in EBC. The Z-potential is an important metric since it represents the surface charge of particles in a liquid media. It is critical for understanding the stability of colloidal suspensions and particle interactions in varied conditions. When paired with NTA, Z-potential measurements offer a complete picture of nanoparticle behaviour. The device features a laser light source with a wavelength of 488 nm and a microscope connected to a camera that records and analyses the Brownian motion of each particle. Furthermore, this instrument allows for the identification of the hydrodynamic diameter of particles based on the Stokes-Einstein relation, derived from the diffusion coefficient obtained, with a range of 30 to 2000 nm. Particle concentration is determined by counting all objects in the field of view and knowing the measured volume. During the method setup for EBC sample analysis, the instrument's background noise was calculated using MilliQ water, considering the type of plastic used to insert and dilute the sample. The limit of detection (LOD) of NTA is 5 x 10^6 nanoparticles/mL (Panizzolo et al., 2024). The combined use of environmental measurement tools with HBM provides an internal dose parameter, although lacking molecular characterisation, and allows for the measurement of inhaled particle concentrations and size distribution in the airways of subjects, thus helping to understand the potential effects of environmental or occupational exposure on them (Shi et al. 1999; Pirjola et al., 2004; Virtanen et al., 2006; Pant and Harrison 2013).

1.4.4.2 Early effect biomarkers

Industrialisation and technological advancements have led to increased pollution levels and occupational exposure to new toxic substances, particularly in urban centres, affecting a significant portion of the population. The literature on the research and validation of new early-effect biomarkers and their use in the prevention of diseases is growing and is crucial for the protection of public health (Gorini et al., 2020). These types of biomarkers assess genomic alterations, such as chromosomal aberrations, sister chromatid exchanges, micronuclei, and potential biochemical changes like oxidative stress caused by excessive ROS production, which damages membrane lipids and generates metabolites. Their use in both environmental epidemiology and clinical settings can improve health risk assessments and contribute to new effective disease prevention policies in environmental and occupational contexts (Bonassi et al., 2001). Therefore, oxidative stress biomarkers are investigated to identify potential imbalances or disruptions in the physiological processes that may lead to disease onset, such as 15-f_{2t}-isoprostane, malondialdehyde through the Thiobarbituric acid reactive substances (TBARS) assay, and total antioxidant power (TAP) (Lykkesfeldt, 2007; Dorjgochoo et al., 2012; Graille et al., 2020). Epidemiological studies also focus

on oxidative damage to lipids, proteins, and DNA. The formation, metabolism, and use of each biomarker are analysed, considering their validity in clinical and animal models, analytical methods, and individual variability. Isoprostanes and 8-oxodG are recommended for monitoring oxidative status over time. Isoprostanes are preferred depending on the individual and the type of matrix being analysed, while urinary levels of 8-oxodG may be influenced by DNA repair capacity (Il'yasova et al., 2012).

1.4.4.2.1 15-f_{2t}-Isoprostane

There are many oxidative stress biomarkers used in the literature to identify alterations in the redox balance. However, many of them have limited use because they are either nonspecific or require invasive methods for matrix collection. Nevertheless, some urinary biomarkers are widely used to study redox balance due to the non-invasive matrix collection, promoting higher compliance in epidemiological/occupational studies, and allowing the identification of oxidative stress markers over a longer period compared to blood or other invasive matrices (Il'yasova et al., 2012). Isoprostanes are prostaglandin-like compounds used to analyse oxidative stress-derived damage, that can be induced by free radicals through arachidonic acid peroxidation. It has been shown that the products of these molecules have significant biological effects, acting as mediators in the pathophysiological processes of many diseases. Therefore, the use of isoprostanes in epidemiology is an excellent tool to analyse in vivo oxidative stress and understand its role in the pathogenesis of human diseases (Montuschi et al., 2004). Specifically, urinary 8-isoprostane is one of the most used F2-isoprostanes in epidemiological studies. Despite its widespread use in the literature, no physiological cut-offs have been identified for using this biomarker in clinical settings (Graille et al., 2020). One commonly used method is a competitive ELISA, which allows the identification of its concentrations within the analysed matrix through colorimetric analysis (Campos et al., 2011). The complete procedure used in this work was described in the Materials and Methods section of 2.2.2 chapter.

1.4.4.2.2 Malondialdehyde

In addition to the class of isoprostanes, there are other metabolites derived from the oxidation of proteins, lipids, and DNA due to elevated levels of oxidative stress. Among them, lipids are the class of biomolecules most involved (Lykkesfeldt et al., 2007). The peroxidation of membrane lipids generates a series of by-products, most of which are excreted through the urinary system. One such by-product is MDA, the primary product of lipid peroxidation (Del Rio et al., 2005). This aldehyde is a highly toxic oxidative stress biomarker, and its interaction with DNA and protein molecules often leads to mutations and consequent cellular damage (Toto et al., 2022). Although no defined levels exist, as with isoprostanes, MDA is used in both in vivo and in vitro studies as an indicator of various

diseases such as diabetes, hypertension, atherosclerosis, and heart failure, and it is considered a valid and reliable indicator (Singh et al., 2015). MDA can be analysed using several biochemical techniques, including high-performance liquid chromatography (HPLC) with spectrophotometric kits, fluorescence detection, and UV photometry (Toto et al., 2022, Tsikas et al., 2023). The complete procedure used in this work was described in the Materials and Methods section of 2.2.2 chapter.

1.4.4.2.3 Total Antioxidant Power

Oxidative stress can lead to various mechanisms that may induce a pathological condition (Siti et al., 2015). The human body has developed several physiological responses (i.e., endogenous antioxidants) to counteract ROS overproduction, able to neutralise these harmful species (Ialongo 2017). Endogenous antioxidants can be soluble in water or lipids and are localised in various cellular compartments (Rivero-Pérez et al., 2007). Currently, there is a growing interest in studying these substances or enzymes and identifying pharmacologically potent compounds with few or no side effects (Ali et al., 2008). The TAP is used as a biomarker of oxidative stress and is quantified in matrices such as plasma, urine, and saliva, as it considers the cumulative action of all antioxidants present, providing an integrated and comprehensive parameter (Ghiselli et al., 2000). TAP is determined in different bodily fluids, offering a convenient means to compare antioxidant defences among patients with acute or chronic inflammatory diseases (Kirschbaum, 2001). It has been shown that the presence of this analyte is influenced by the oral or renal state of the subject, for example following infections (Peluso and Raguzzini, 2016). TAP can be measured using colorimetric assays, allowing for a total measure of the subject's antioxidant power. The Oxford Biomedical Research colorimetric test utilises the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method is an analytical technique used to evaluate the antioxidant capacity of biological, dietary, or chemical materials. It employs the conversion of Cu+2 to Cu+1 due to the reduction potential of the standard or samples, determining a change in the absorption characteristics of the ion. Cu+1 can bind to the chromogenic reagent in a stable 2:1 complex with maximum absorption at 450 nm (Oxford Biomedical Research). The complete procedure used in this work was described in the Materials and Methods section of 2.2.2 chapter.

1.4.4.3 Impaired inflammatory status

Some of the inflammatory cytokines most investigated in the literature (paper II) in the EBC to determine possible pathology are IL-1B, IL-4, IL-6, IL-8, IL-10, TNF-a, C reactive protein (CRP), and Krebs von den Lungen 6 (KL-6) (Panizzolo et al., 2023).

1.4.4.3.1 Interleukin-1β

One of the most well-known members of the interleukin (IL) -1 family, IL-1β exhibits a wide range of biological effects, both positive and negative. It contributes to the start and continuation of inflammation and is produced by phagocytes, especially monocytes. It is a pro-inflammatory cytokine that is a member of a broad protein family and is crucial for controlling activities that are necessary for both the development of illness and good health. One of the functions of this cytokine is the regulation of acute inflammation (Kaneko et al., 2019), acting as a defence mechanism against pathogenic organisms by enhancing the immune response (Baraskar et al., 2021). IL-1β is secreted by macrophages and enters circulation, exerting multiple effects on its targets, such as neurological, metabolic, and haematological changes. Additionally, it contributes to both tissue repair and its destruction, playing a critical role in lymphocyte activation and immune response (Dinarello, 1995).

1.4.4.3.2 Tumour Necrosis Factor - α

Monocytes produce the protein tumour necrosis factor (TNF) -α in reaction to inflammatory cues. When it binds to its receptors, it has a variety of physiological consequences. Furthermore, it modulates apoptosis and cellular survival to control immunological responses (Baraskar et al., 2021). TNF-α plays a significant role in several inflammatory diseases (Bradley, 2008). It is produced by adipose tissue following prolonged inflammation (Alzamil, 2020), and its levels remain elevated in obesity. TNF-α is linked to non-insulin-dependent diabetic mellitus (NIDDM) and insulin resistance in obesity (Tzanavari et al., 2010). Insulin receptor activity is disrupted by TNF-α, which affects the insulin signalling cascade (Hotamisligil and Spiegelman, 1994). According to another research, there is no discernible difference in TNF receptor concentration between normal-weight and obese individuals, while TNF- α concentration is strongly correlated with the degree of obesity (Olszanecka-Glinianowicz et al., 2014). Both TNF-α and its receptor are still increased in the population of obese teenagers and are frequently linked to metabolic disorders (Moon et al., 2004). Also, studies conducted in vitro has shown that prolonged exposure to nanoparticles and microparticles at different doses increases the production of specific inflammatory biomarkers, such as TNF-α. Therefore, elevated levels of this biomarker can be found not only in individuals with obesity or a high BMI but also following exposure to micro and nanoparticles (Park et al., 2008).

1.4.4.3.3 Intereukin-6

An important component of immunological communication, IL-6 is a cytokine that the immune system produces at areas of injury or damage and is linked to several inflammatory illnesses (Tanaka et al., 2014). Both soluble and membrane-bound forms of its receptors can be identified, especially in liver cells and certain white blood cells. Both trans-signaling via soluble receptors and membrane

bound receptors (the classical pathway) are involved in IL-6 signaling. IL-6 exhibits both pro-inflammatory and anti-inflammatory biological activities, sometimes overlapping, and is involved in processes such as regulation of the hepatic acute-phase response, stimulation of B cells (Cronstein, 2007), the balance between regulatory and effector T cells, metabolic regulation, and various neural functions. Blocking IL-6 signalling has shown positive effects in autoimmune diseases, although side effects, including bacterial infections and metabolic alterations, have been reported. However, recent developments in cytokine-blocking therapies aim to reduce these unwanted effects (Naugler and Karin, 2008; Wolf et al., 2014). Effective treatment options are currently limited for an array of lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis, which may be brought on by prolonged exposure to different microorganisms. These diseases require in-depth molecular studies, as cytokines, including IL-6, are often central to the pathological alterations. Indeed, it has been observed that uncontrolled chronic inflammation, with elevated production of cytokines like IL-6, is one of the primary causes of tissue degeneration (Baraskar et al., 2021; Dawson et al., 2021).

1.4.4.3.4 Interleukin-8

Chemokines are a subset of cytokines with chemotactic activity, and interleukin-8 (IL-8), also known as CXCL8, is the prototypical example. IL-8 guides the migration of immune cells to sites of inflammation, facilitating the recruitment of neutrophils. Chemokines are classified into different subcategories based on their protein structure: CXC chemokines have cysteines separated by an amino acid, while CC chemokines have adjacent cysteines. The new nomenclature assigns a number to each chemokine to aid in identification; thus, IL-8 is designated as CXCL8. IL-8 is primarily produced by monocytes and macrophages, and these cells secrete IL-8 in response to stimuli such as lipopolysaccharides, bacteria, and pro-inflammatory cytokines, including TNF-α and IL-1 (Remick, 2005). Experimental studies have shown an association between IL-8 and both acute and chronic inflammatory diseases, with a key role in the pathogenesis of inflammation. Experiments with neutralising antibodies against IL-8 and transgenic or IL-8 receptor knockout animal models demonstrate that this chemokine is crucial in neutrophil recruitment. In cystic fibrosis patients, elevated levels of IL-8 have been detected in epithelial fluids, while in healthy individuals, it was absent. Similarly, in conditions like acute respiratory distress syndrome (ARDS), IL-8 concentrations are elevated, suggesting it significantly contributes to the inflammatory response and the progression of oxidative damage. Interleukin-8 (IL-8) is a key cytokine that plays a fundamental role in both acute and chronic inflammatory responses, due to its ability to recruit neutrophils and prolong its presence at inflammation sites. Unlike other inflammatory cytokines, IL-8 remains active for days or weeks, promoting prolonged inflammation (Matsushima et al., 2022).

1.4.4.3.5 Interleukin-10

IL-10 family consists of 9 members. This family of cytokines is produced by both innate and adaptive immune defences, and their main function is the regulation of the immune system. Their roles are multifaceted, including regulation during inflammation, infections, autoimmune disorders, and cancer (Ouyang and O'Garra, 2019). The human IL-10 gene, located on chromosome 1, consists of five exons and several polymorphisms, particularly single nucleotide polymorphisms (SNPs) in the promoter, which affect its expression. This gene encodes a 178-amino acid protein, with a homodimeric structure of 35 kD, maintained by disulfide bonds that are essential for its stability and function. IL-10 is similar in humans and mice (75% identity) and shares a homologous structure with interferon gamma (IFN-γ) (Sabat et al., 2010). Unlike the cytokines analysed so far, which primarily have pro-inflammatory functions, IL-10 has immunosuppressive effects on monocytes and macrophages, reducing the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and promoting the synthesis of anti-inflammatory molecules (e.g., IL-1 receptor antagonist) (Murray, 2005; Saraiva et al., 2020). Additionally, IL-10 inhibits antigen presentation by reducing the expression of MHC II molecules and other co-stimulatory molecules, thus limiting the adaptive immune response and hindering the differentiation of Th1 and Th17 cells. Paradoxically, IL-10 stimulates phagocytosis by monocytes/macrophages, increasing their ability to eliminate opsonized and non-opsonized pathogens.

Exposure to MNPs has shown, an increase in the production of pro-inflammatory cytokines in lung cells, both in vitro studies (Weber et al., 2022) and in occupational settings (Hemmendinger et al., 2023). In response to this inflammatory effect, a significant increase in IL-10 levels, an anti-inflammatory cytokine, was observed in exposed cells compared to controls, suggesting a negative feedback mechanism aimed at counteracting inflammation (Hemmendinger et al., 2023).

1.4.4.3.6 Krebs von Lungen-6

Type II pneumocytes and bronchial epithelial cells are the main producers and releasers of Krebs von Lungen (KL) -6, a high molecular weight glycoprotein. It has been identified as an effective biomarker indicating both proliferation and damage of the alveolar epithelium. Several investigations have demonstrated elevated KL-6 levels in pathological scenarios, including hypersensitivity pneumonitis, pulmonary sarcoidosis, idiopathic interstitial pneumonia, acute respiratory distress syndrome, and interstitial pneumonia linked to connective tissue disorders. Furthermore, KL-6 is considered an indicator of pulmonary inflammation. Given its ability to reflect lung damage, this protein has gained relevance in the assessment of COVID-19, as it may potentially predict a more severe disease course (Matuszewski et al., 2022). Other studies have also confirmed a correlation between serum levels of KL-6 and severe pneumonia caused by COVID-19. Previous research has already highlighted the potential of this glycoprotein as a key indicator of inflammation and as an early signal of certain diseases, including interstitial pneumonia (Awano et al., 2020).

1.5 State of the art

In light of these considerations, biomonitoring represents an essential starting point for understanding whether exposure to micro- and nanometric airborne materials can have local and systemic effects. These effects can be measured using biomarkers. The approach adopted in this thesis, prior to initiating the biomonitoring phase through field studies, involved an extensive literature analysis, which included the drafting of several in-depth reviews aimed at acquiring knowledge on the state of the art regarding the most frequently studied endpoints in different matrices of organisms exposed to MNPs.

Given the ubiquitous presence of MNPs, numerous studies in the literature have sought to address the potential toxicity of these substances in a wide variety of organisms. Indeed, many investigations have analysed the presence of MNPs in different matrices, such as water, soil, air, and food, through which organisms can come into contact with the particles via inhalation, ingestion, or dermal routes. Other studies have focused on investigating the toxicity arising from acute and chronic exposure to MNPs through in vitro and in vivo approaches, highlighting potential toxic effects, such as an increase in inflammatory biomarkers, oxidative stress, and genotoxicity.

This phase provides a comprehensive understanding of the specific issues and the approaches employed by the scientific community, as well as an overview of the procedures, methodologies, and types of biomarkers analysed in various application contexts. In this regard, this thesis focuses on the exposure to airborne MNPs in different occupational environments. The objective is to identify the biomarkers most predictive of an inflammatory state or oxidative stress following exposure to MNPs, to understand the mechanisms of interaction with organisms, with particular attention to humans, and to determine the most hazardous occupational contexts for human health. Additionally, it aims to explore the methodologies most used for the analysis of these biomarkers.

However, studies conducted in occupational contexts on MNPs are still extremely limited, representing an area of research that requires further exploration. This is especially relevant as technological advancement leads to the increasing use of NMs in industrial settings, with the release of increasingly smaller and harder-to-detect particles (nm), for which no clear regulatory control guidelines currently exist.

The implementation of field studies in occupational settings requires, first and foremost, ethical committee approval of the study, the consent of the companies involved, the selection of easily collectible and non-invasive matrices that enable targeted and specific monitoring based on the analytical goals, and finally, the selection of biomarkers based on the physiological processes under investigation. Based on the literature reviews conducted and the limited studies available in occupational settings, it was possible to select certain inflammatory biomarkers (pro- and anti-inflammatory cytokines) and oxidative stress biomarkers (MDA, TAP, 15-f2t-Isoprostane) that are most reflective of exposure to MNPs. The few studies available in occupational environments have shown that, for the most part, the quantification of biomarkers in exhaled breath condensate (EBC) aims to detect early variations in airway inflammation, which could be associated with an increased risk of developing respiratory diseases. For instance, a study conducted by Sauvain et al. (2014) demonstrated that exposure to micro- and nanometric airborne particles primarily results in local (pulmonary) effects, but when prolonged over time, it may also influence systemic levels of pro-inflammatory cytokines (in the blood) and oxidative stress levels in the urine of exposed individuals. In this thesis, however, the focus was placed on the identification of new biomarkers in non-invasive matrices to be used in occupational settings with the goal of improving workplace safety and monitoring (Carpagnano et al., 2005).

Furthermore, it is important to emphasise that airway inflammation is not solely influenced by exposure to micro- and nanoparticles but can also be altered by other confounding inter-individual factors that may compromise the accuracy of the results obtained (Carpagnano et al., 2003). For example, conditions such as obesity have been observed to affect the inflammatory state: fat accumulation promotes an increase in cytokine release, thereby heightening the risk of respiratory impairments, such as OSAS (obstructive sleep apnoea syndrome), OHS (obesity hypoventilation syndrome), asthma, and COPD (chronic obstructive pulmonary disease).
Moreover, factors such as BMI, habitual tobacco smoking, and alcohol consumption are all elements that significantly increase pro-inflammatory cytokine levels and oxidative stress both locally and systemically (Zammit et al., 2010; Zheng et al., 2018; Habib et al., 2021; Vezir et al., 2021). For these reasons, this thesis focused on the research, identification, and potential validation of biomarkers in non-invasive matrices, such as EBC and urine, primarily in occupational settings where the highest risk conditions and the presence of particles occur, with exposure to micro- and nanoparticles, as well as micro- and nanomaterials such as plastics.

1.6 Thesis objectives

This doctoral thesis aims to provide a comprehensive overview of the biomarkers analysed in non-invasive matrices, such as exhaled breath condensate (EBC) and urine, in relation to exposure to micro- and nanoparticles (MNPs), with particular attention to micro- and nanoplastics. Initially, three literature reviews were conducted. The first, a comprehensive review outlined the state of the art regarding the main sources of MNPs and the mechanisms through which these particles can interact with organisms. The second, defined as a literature review offered an overview of existing studies on MNP exposure, including analyses of in vitro and in vivo models. It also highlighted a lack of human studies and identified commonly used biomarkers, focusing on those most predictive of the early stages of disease. Then the systematic review that through a rigorous approach synthesised numerous studies, providing specific results on the types of biological matrices (e.g., EBC) and biomarkers investigated in healthy non-smoking subjects from various contexts. Additionally, reference values or ranges for the selected biomarkers were established, along with an in-depth analysis of methods for sample collection, storage, and analysis.

Subsequently, two field studies were conducted in occupational settings to investigate the selected biomarkers and identify a new potential biomarker of internal dose, NTA, which has not yet been explored in the literature. This biomarker reflects the number of particles inhaled and reaching the lungs and was measured in EBC using a specifically developed methodology. It is important to note that the validation of non-invasive biomarkers for monitoring MNP exposure in occupational environments poses several challenges. These include difficulties in obtaining company authorisation for worker sampling, issues related to institutional ethics committees, and the need for standardised sampling procedures and tools to ensure the collection of highly reproducible data. Finally, through the collection and analysis of data from various biological matrices, this research aims to develop practical tools for the early diagnosis of inflammatory conditions and to contribute to improving the safety and health of workers at risk of exposure.

2. Materials and methods

2.1 Study line I: Reviews

Three reviews were conducted using three different approaches. Specifically, a comprehensive review, a literature review, and a systematic review were carried out, resulting in three documents whose methodologies are described in detail in the Materials and Methods section below. The first work, the comprehensive review (Paper I), aimed to provide a thorough understanding of the potential sources of contamination and the pathways through which MNPs can access organisms and cells. The second work, the literature review (Paper II), focused on identifying all biomarkers (of oxidative stress, inflammation, and genotoxicity) reported in the literature to assess exposure to MNPs in different models (in vitro, in vivo) and human studies, to define a biomarker panel suitable for field-based epidemiological studies. Finally, the systematic review (Paper III) was conducted to identify the most relevant inflammatory biomarkers analysed in EBC (sample collection, storage and analysis methods).

2.1.1 Comprehensive review (Paper I: Nano- and microplastics: a comprehensive review on their exposure routes, translocation, and fate in humans).

Comprehensive review has become one of the most widely used and increasingly adopted approaches in recent years, following traditional research and it aims to provide extensive coverage of all relevant publications on a given topic. This type of review is not limited to traditional scientific articles but may also include technical reports and grey literature, thereby offering a broad and diversified perspective. The primary objective of a comprehensive review is to integrate existing knowledge, identify research gaps, emerging trends, and potential future directions. However, unlike systematic reviews, it does not necessarily follow strict protocols or specific criteria for the inclusion and exclusion of sources. One of the main advantages of this approach lies in its ability to describe broad contexts or explore emerging fields, where a general overview is essential. Nevertheless, the lack of systematic string construction and search methodology can be a limitation, making it potentially more subjective and less structured compared to more rigorous approaches. Despite this, when the goal is to provide a comprehensive understanding of a field of study about which little is known, it remains an effective choice (Grant et al., 2009; Stratton, 2016).

In Paper I, based on the different chapters analysed, specific search strings, different years, and search terms were chosen. Firstly, a search was conducted to avoid redundancy with other review articles, including only studies from 2015 onward for exposure scenarios. However, no publication year limit was set for describing the potential translocation mechanisms of MNPs from primary exposed organs (lungs, gastrointestinal tract, and skin) to other tissues and secondary organs, as a general understanding of these mechanisms requires foundational literature. Regarding the fate of MNPs in human tissue samples, being a relatively new research field, we included all studies published on this topic. Google Scholar, ISI Web of Knowledge/Web of Science, Scopus, PubMed, and Embase databases were used. The common search terms for all strings were: “microplastic”, “nano plastic”, and “human exposure”. For more specific chapters, we included the following search terms: “drinking water” and “beverages” for “micro- and nano-plastics” in “drinking water”; “meat”, “fish”, “seafood”, “edible tissue”, “vegetables”, “milk”, “egg”, “roots and tubers”, “plants and herbs”, “confectionery”, “honey”, “sugar”, “salt”, “cereal”, “rice”, “maize”, “wheat”, “barley”, “spelt”, “rye”, “oat”, “sorghum”, “millet”, “teabag”, “oil”, “olive oil”, “vegetable oil”, and “palm oil” for the chapter on NMPs in food; “atmosphere”, “atmospheric”, and “air” for the chapter on inhalation of micro- and nano-plastics; and “cosmetics”, “personal care products”, “contraceptives”, “eye”, “contact lenses”, and “ocular surface” for the chapter on personal care products (PCPs). In the chapter on the fate of MNPs, the additional terms used were human tissue and organs. No studies were excluded. My contribution to this study was to search relevant studies relating to the chapter's objectives and write the chapter on MNPs exposure in indoor air and workplaces.

2.1.2 Literature review (Paper II: Biomarkers of oxidative stress, inflammation, and genotoxicity to assess exposure to micro- and nano plastics. A literature review)

To gain a deeper understanding of the risks MNPs substances may pose to humans, the environment, and organisms in general, we conducted a second review, in this case a literature review, albeit with a different focus. A literature review has distinct characteristics compared to a comprehensive review and a systematic review (addressed in the following chapter). The former is particularly useful for research purposes and offers a simpler and less complex methodology compared to the other approaches. This type of review is conducted to understand the state of the art on a specific topic and to expand knowledge regarding analytical and procedural methods applied in certain contexts. As with a comprehensive review, a literature review does not require a bibliographic search following PRISMA methodologies. However, if authors choose to apply them, it adds significant value to the work (Wee and Banister, 2016). An advantage of this type of review is its flexibility and adaptability in introducing complex topics in a concise manner. One of the disadvantages, as also noted in comprehensive reviews, is the lack of methodological rigour, which increases the likelihood of being influenced by author bias (Grant et al., 2009). This second review aims to provide an overview of the current state of research on biomarkers of oxidative stress, inflammation, and genotoxicity explored about exposure to MNPs, using human, cellular, animal, and plant models. Both in vitro and in vivo models suggest that increased oxidative stress and inflammation are the primary mechanisms of action leading to adverse effects such as chronic inflammation, immunotoxicity, and genotoxicity. The identification of these biological endpoints, which represent crucial key initial events (KIEs) for adaptive or adverse outcomes, allows for the definition of a panel of substitute biomarkers to be applied and validated, particularly in occupational settings where exposure levels may be higher.

In Paper II, the literature search was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) methodology across two databases (PubMed and Embase) in figure 14 in results chapter 3.1.2. The search strategy involved filtering publications using a combination of keywords specifying the following MeSH terms and synonyms: "Oxidative Stress," "Inflammation," "Genotoxic," "Biomarkers" (full list of all biomarkers), "Microplastics," "Nanoplastics" (a complete list of MNP is provided in Appendix A). Two reviewers independently assessed the publications, and a third reviewer resolved any disagreements. Following the PRISMA 2020 statement (Page et al., 2021), the articles were initially screened by title and then by abstract. The results were transferred from the databases to a Microsoft Excel spreadsheet, where the inclusion and exclusion criteria were recorded. In both stages, based on the exclusion criteria, studies were excluded if they: I) did not present biomarkers of oxidative stress, inflammation, or genotoxicity, II) focused on micro- and nano-plastic additives, III) were conducted on bacteria, IV) were review articles, V) were full-text articles with unpublished data, VI) were conference abstracts without full texts, VII) were clinical studies (e.g., plastic prosthetic bone integration), and VIII) did not focus on or analyse the potential negative effects of MNP from human (primarily occupational), cellular, animal, and plant models. The included studies are based on methodologies already used to assess the risks associated with nanomaterials and particles of interest. However, so far, only a limited number of studies have directly measured human exposure to MNPs and analysed the relationship between such exposure and its impact on human health.

My contribution to this work as first author was investigation, data curation, formal analysis, visualization and writing the original draft.

2.1.3 Systematic review (Paper III: Inflammatory Biomarkers in Exhaled Breath Condensate: A Systematic Review)

Paper I and Paper II highlighted the need to conduct a targeted study in the occupational field, using biological matrices, after performing a systematic review of inflammatory biomarkers analysed in EBC in healthy individuals.

The systematic review process is structured in several phases, including study selection, critical appraisal, and data extraction, along with the data representation (e.g., by using forest plots able to graphically synthesise data from different studies), all performed in duplicate and independently by multiple reviewers. This approach helps minimise the risk of errors and biases. These rigorous methods are the primary distinguishing feature of systematic reviews compared to traditional literature reviews (such as comprehensive or narrative reviews), which do not require all these mandatory steps. One of the advantages of systematic reviews is the accuracy and meticulousness of the approach, which makes it the greatest standard for evaluating scientific evidence; it is clear and reproducible. On the contrary, it plainly demands a significant amount of time and resources, and it is only appropriate for well-defined concerns rather than general arguments (Munn et al., 2018).

The characteristics of a systematic review are clearly defined and internationally recognised.

The first step is to ensure that the research question has not already been addressed by other authors, which can be done by registering the review protocol, including the title and objectives, in the PROSPERO database. Next, it is necessary to have well-defined objectives and specific research questions, to have established inclusion and exclusion criteria to determine study eligibility, and to create a comprehensive search string able to include all relevant studies, both published and unpublished, which should be represented in a PRISMA flowchart. Furthermore, an evaluation of the quality of the selected studies is required, analysing the validity of the results and documenting exclusions based on quality using standardised scales according to the study design of the individual article. Additionally, a thorough analysis of the data extracted from the included studies is needed, with a clear presentation and summary of the results in a forest plot. Finally, in the final report, the adopted methodology for conducting the review should be transparently indicated in the appropriate section (Aromataris and Pearson, 2014). The protocol of the systematic review (paper III) was registered in the PROSPERO database (Protocol ID = CRD42022316248). Eligible articles were searched and identified in the PubMed, Embase, and Cochrane CENTRAL databases up until February 4, 2022. The search string aimed to find original research articles evaluating the concentration of specific inflammatory biomarkers in EBC, including the following terms: "Cytokines", "Interleukins", "C-reactive protein", "Interleukin-1", "Interleukin-4", "Interleukin-6", "Interleukin-8", "Interleukin-10", "Tumour Necrosis Factor-alpha", "exhaled breath condensate*". The full search strings are provided in Appendix A of the article included in chapter 7. Observational or interventional studies on healthy human subjects (18+ years, non-smokers, without known diseases) measuring the selected biomarkers in EBC were considered potentially eligible. Only

English-language full-text articles were included. Nonquantitative data, reviews, in vitro or animal research, full-text conference abstracts without full text, expert comments, procedures, editorials, and full-text journals containing unpublished data were all excluded. Using the specified inclusion and exclusion criteria, two reviewers separately and blindly went through the article selection process, looking at abstracts and titles. Full-text articles were used for selection where there was insufficient data. A third reviewer discussed and, if required, settled disagreements on the selection of articles. The PRISMA graphic Figure 15 in the findings chapter 3.1.3 provides a summary of the process. Data from the chosen publications was independently retrieved by two researchers and stored in a spreadsheet. Data collected included author name, publication date, title, nation, study design, hiring method, number of participants, subject category, inclusion and exclusion criteria, gender, age, BMI, pre- and post-intervention details, storing methods, α-amylase control, method of analysis, biomarker concentrations, limit of detection (LOD), main results, and any notes. Furthermore, two independent reviewers conducted two steps of quality evaluation of the included papers in this systematic review. The Joanna Briggs Institute (JBI) checklists were used in the first section of the evaluation, which focused on research design, to evaluate the published papers' relevance and dependability. To confirm that study methodologies correspond to the ATS/ERS Task Force guidelines on EBC, the second section focused on the methodological protocol. Based on the examination of several aspects, the quality of the studies was classified as "low", "moderate", or "high". For every study that was included, the final score for every checklist was maintained separately. A third reviewer was consulted if required after any disagreements between the reviewers were discussed. Lastly, the study procedures were especially evaluated to confirm their adherence to the ATS/ERS Task Force guidelines on EBC because of the significant variations in the techniques used in the included studies with respect to sampling, storing, and analysing EBC. The setting up of reference intervals for these biomarkers may make it easier for researchers and clinicians to employ them in clinical and research contexts, not only for monitoring but also, in subsequent longitudinal studies, as indicators of the onset and progression of chronic inflammatory diseases. The Joanna Briggs Institute (JBI) checklists were used in the first section of the evaluation, which focused on research design, to evaluate the published papers' relevance and dependability. To confirm that study methodologies correspond to the ATS/ERS Task Force guidelines on EBC, the second section focused on the methodological protocol. Based on the examination of several aspects, the quality of the studies was classified as "low", "moderate", or "high". For every study that was included, the final score for every checklist was maintained separately. A third reviewer was consulted if required after any disagreements between the reviewers. Lastly, the study procedures were especially evaluated to confirm their adherence to the ATS/ERS Task Force guidelines on EBC because of the significant variations in the techniques used in the included studies with respect to sampling, storing, and analysing EBC. The setting up of reference intervals for these biomarkers may make it easier for researchers and clinicians to employ them in clinical and research contexts, not only for monitoring but also, in subsequent longitudinal studies, as indicators of the onset and progression of chronic inflammatory diseases.

My contribution to this work as co-first author was investigation, data curation, formal analysis, visualization and writing the original draft.

2.2 Study line II: Field studies

This second study line aims to transpose the results obtained from study line I into field studies also addressing eventual gaps identified. In the frame of study line II, we implemented and conducted epidemiological studies trying to overcome the deficiencies highlighted by the literature reviews. The selection of appropriate biological matrices and early biological effect biomarkers to be quantified was based on the literature reviews. To design and conduct field epidemiological studies, we first identified the eligible cohorts of workers to include in the study. Subsequently, a preparatory phase was organised for the collection of the necessary materials for sampling, starting with analyses for the quantification of environmental exposure, the questionnaires to be administered, and the matrices to be collected. The goal of this second line of research was to investigate the association between micro and nanomaterials and innovative biomarkers for the internal dose as well as a panel of early biological effects measured in a cohort of workers potentially exposed to MNPs.

2.2.1 Epidemiolocal study I (Paper IV: Assessing the inhaled dose of nanomaterials by nanoparticle tracking analysis (NTA) of exhaled breath condensate (EBC) and its relationship with lung inflammatory biomarkers)

2.2.1.1 Study design

In the frame of the NanoExplore project supported by the European Commission's LIFE program (Grant LIFE17 ENV/GR/000285) (link: https://www.lifenanoexplore.eu/about/overview). The sample size required to accurately identify differences between subjects was calculated to detect differences in within-subject variations in biomarker concentrations between exposed and non-exposed workers. According to calculations based on studies conducted by Pelclova et al., 2018, a total sample of 120 workers (60 exposed, 60 non-exposed) should enable statistical analyses to detect a significant difference of at least 25% between groups in the within-subject variation of biomarkers of effect. Considering a potential 20% loss to follow-up due to constraints related to biological sampling and staff turnover, a total of 80 exposed workers and 80 non-exposed workers are expected to be recruited. The worker cohort in this project was larger, with 141 subjects enrolled across different campaigns. However, in this study, due to a shortage of EBC samples required to perform complete NTA analyses, only data from 80 subjects were used. The recruited workers are employed in companies located in Italy and Spain that produce paints, adhesives, coatings and construction chemicals.

To characterise the exposure, at each industrial site the nanomaterials present were analysed using transmission electron microscopy (TEM) for shape and size and EDAX spectroscopy for elemental analysis. The companies were anonymised with fictitious names: "A" (paints, adhesives, and coatings), where Carbon, Oxygen, Titanium, Silicon, and Calcium were detected; "B" (chemical building materials), with Aluminium, Silicon, Oxygen, Carbon, Sulphur, Titanium, and Calcium; and "C" (research and development on nanomaterials), where Iron was predominantly detected. Workers from companies' "A" and "B" handle large quantities of materials, while those at "C" manage small amounts for laboratory activities.

2.2.1.2 Environmental exposure assessment

Exposure was monitored using six DISCmini™ particle counters (Testo, DE), which detect airborne particles in the 10–300 nm range with a temporal resolution of 1 second and a detection range of 500–1,000,000 particles/cm^3. These devices are portable and useful for measuring the number of airborne particles in the nanometric size range. The devices were placed near the workers' stations. Finally, based on the results provided by DISCminis, workers were clusterised into high exposed (HE), low exposed (LE) and non-exposed (NE).

2.2.1.3 Biological sampling and quantification of biomarkers of early biological effects

After environmental monitoring, EBC samples were collected using Turbo-DECCS™ condensers (Medivac, Italy) set at -10 °C, following the international guidelines (Horvath et al., 2005). The workers tidally breathed into a disposable condenser circuit until a volume of 90 Liters of condensed air was collected, yielding around 2-3 mL of EBC per subject. Samples, collected at the beginning and end of the working week, were stored at -80 °C until analysis.

I've contributed to the HBM process of sample collection, storage, and analysis. Additionally, as first author, he was responsible for the management, writing, and analysis of Paper IV.

2.2.1.3.1 Biomarkers of internal dose in EBC

The particle concentration and size distribution, as well as the Z potential of particles in the EBC, were determined using ZetaView® PMX-120 (Particle Metrix GmbH, Germany), an NTA equipped with a light source with a wavelength of 488 nm (Figure 1). The NTA uses video to record each particle's Brownian motion. The hydrodynamic diameter of the particles is determined using the Stokes-Einstein equation and the resulting diffusion coefficient (30-2000 nm). Five EBC samples underwent pre-screening to maximize the instrumental parameters and ensure proper sample dilution. The shutter, frame rate, and sensitivity settings were 70%, 100%, and 30%, respectively. For each sample, 3 × 33 one-second videos were recorded by ZetaView V.8.05.14SP7. The EBC samples were diluted 1:5 with double-filtered Milli-Q water. To guarantee precise analysis, a few more concentrated samples were further diluted. The concentration of nanoparticles was also evaluated in the double-filtered Milli-Q water, the plastic materials utilized, and the background noise of the device. The limit of detection (LOD) was 5×10^6 nanoparticles/mL.

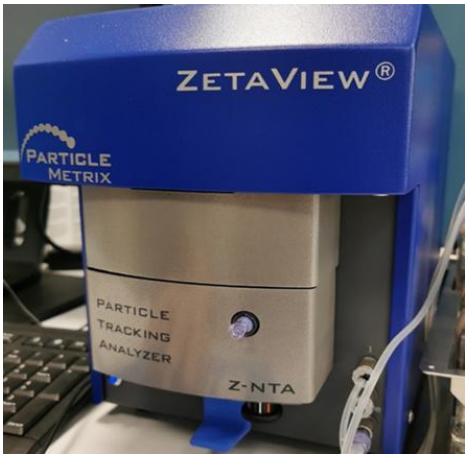

Figure 1. NTA ZetaView® PMX-120 (Particle Metrix GmbH, Germany) device

2.2.1.3.2 Biomarkers of early biological effects in EBC

Inflammatory biomarker analyses (IL-1β, IL-10, TNF-α) were performed using Real-Time PCR combined with ELISA (Invitrogen ProQuantum kits A35574, A35590, and A35601, respectively) from ThermoFisher. These kits are ideal for providing quantitative measurements of multiple human interleukins in small sample volumes with analytes at low concentration. These kits utilise an amplification technology combined with the analytical specificity of the high-affinity antibody-antigen binding, allowing for PCR real-time signal amplification and detection. This results in a simple yet highly effective protein quantification platform. The kits employ two target-specific antibodies that are individually attached to a DNA oligonucleotide. The two DNA oligonucleotides approach one other during the antibody-analyst interaction process, which allows the two strands to attach and create a template strand for amplification. The two conjugated oligonucleotides are in proximity during the first phase of antibody-antigen interaction, which occurs when antibodies attach to two different epitopes on the antigen during an hour-long incubation period. The signal is then amplified using Real-Time PCR in the following phase. During the final stages of kit preparation, a DNA ligase and a third bridging oligonucleotide are added, which allows the two ends of the previously conjugated oligonucleotides to bind, creating a DNA template approximately 100 bases long. The sample underwent 40 cycles of annealing and extension following the ligase's inactivation at 95°C. Fluorescent dyes, which create fluorescence in proportion to the number of PCR product molecules (also known as amplicons) formed, are used to assess the amount of DNA produced following each amplification cycle. A standard curve is produced by plotting the number of cycles needed to attain the fluorescence threshold (also known as the threshold cycle or Ct) versus protein concentration. This method allows for results to be obtained in a few hours with high sensitivity, enabling the detection of low protein levels with higher sensitivity compared to a traditional ELISA. Additionally, due to its wide dynamic range (over 5 logarithmic units), it minimises the need for sample dilutions during analysis. Another advantage of this method is the reduced sample consumption, as only 2 to 5 µL of sample is used per analyte.

Each kit contains a total of 7 components:

- Conjugate A of the analyte in question
- Conjugate B of the analyte in question
- Standard containing lyophilized protein
- Master Mix
- Ligase
- Antibody-conjugate dilution buffer

Then, to correctly perform the kit procedure, two stages are required:

the preparatory phase and the analysis phase. During the preparatory phase, it is necessary to ensure that the cold blocks on which the plates will be placed are at a temperature of 4°C. Next, two separate plates should be prepared. The first will be the working plate, and the second will be the analysis plate, which will be inserted into the PCR. Once the plates are prepared on the appropriate cold blocks, the subsequent steps can proceed.

Preparatory Phase:

The protein standard vial with Assay Dilution Buffer was reconstituted to achieve the optimal concentration for analysis, as specified for the target analyte. The required reconstitution volume for each cytokine kit can be determined from the information provided on the vial label. Then, to ensure complete dissolution, the standard was mixed thoroughly by pipetting. After reconstitution, the standard was incubated at room temperature for 15 minutes.
The antibody conjugate was prepared by combining the components outlined in a sterile 1.5 mL Eppendorf tube. The solution was mixed thoroughly by repeated pipetting.

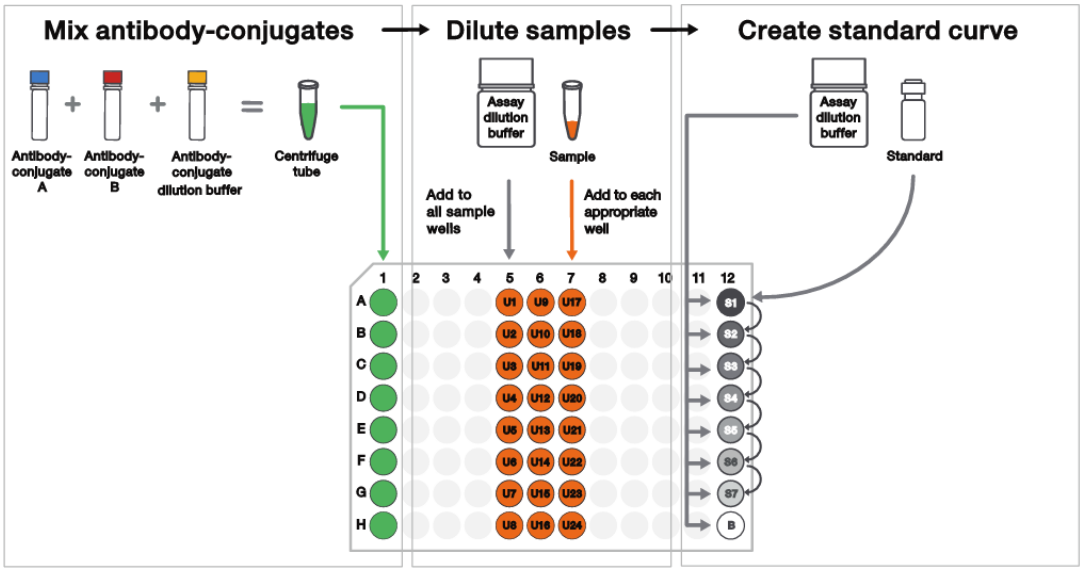

Figure 2. Working plate standards, reagents and samples preparation

An equal or greater amount of 80 µL of the antibody-conjugate was added to each well in one column of the working plate (Figure 2).

The EBC samples were diluted 1:2, combining 5 µL of each sample with 10 µL of Assay Dilution Buffer in the working plate. The mixture was thoroughly homogenised by repeated pipetting.

Following a 15-minute incubation, in the first column of the working plate designated for standards, 80 µL of Assay Dilution Buffer was added to each well from S1 to S7. Next, 20 µL of the Standard protein was transferred into the first well (S1) and mixed by pipetting. Serial dilutions were then performed by transferring 20 µL from S1 to S2, repeating the process down the column through S7. Pipette tips were changed between each transfer to prevent cross-contamination. For well S8, designated as the Blank, only Assay Dilution Buffer was added (Figure 3).

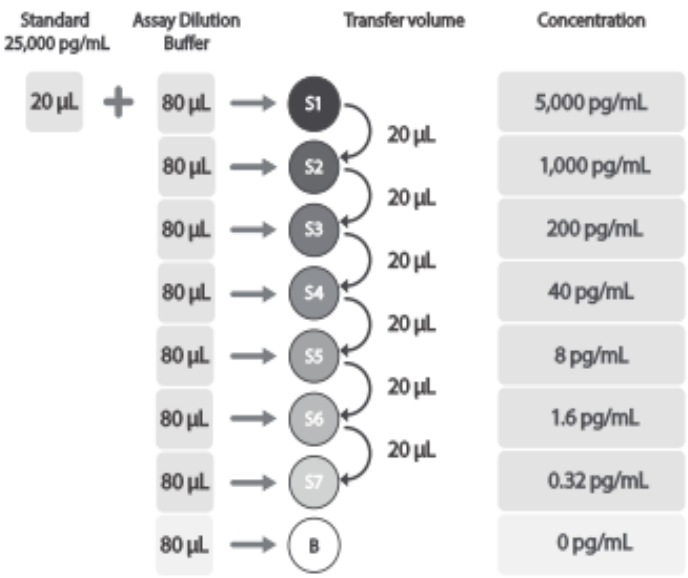

Figure 3. Reconstitution and preparation of the standard curve

After completing the procedures outlined above, the working plate was covered with a protective adhesive film using the plate seal tool. The contents were mixed by tapping the plate laterally three times and then centrifuged at 3000 x g for 1 minute to ensure that all material settled at the bottom of the wells.

Analysis Phase:

During this step, the samples and standards were transferred from the working plate to the analysis plate, where antibody-analyte binding and subsequent signal amplification occur.

Initially, 5 µL of the antibody-conjugate mixture was dispensed into each well of the analysis plate from the working plate using a multichannel pipette. Subsequently, 5 µL of standards and samples were transferred from the working plate to the analysis plate, followed by vigorous mixing through repeated pipetting.

The analysis plate was then sealed with a protective film, gently tapped laterally three times to ensure even distribution, and centrifuged at 3000 × *g* for 1 minute. To facilitate antibody-analyte binding, the plate was incubated at room temperature for 1 hour.

qPCR Analysis:

After the incubation, 40 µL of qPCR mix, including 5 µL of Master Mix and 30 µL of Ligase prepared in a sterile 15 mL Falcon tube, was added to each well using a multichannel pipette.

The plate was sealed with an OPTICAL grade seal, mixed following the same method as in the previous steps, and centrifuged at 3000 x g for 1 minute.

The analysis plate, with wells containing 50 µL of volume, was inserted into the Real-Time PCR machine (CFX96 BioRad, Maestro, BioRad, USA). A new experiment was created according to the methods outlined in Figure 4, and the relevant parameters indicated in Figure 5 were entered.

Parameter	Settings for Applied Biosystems™ instruments
Experiment type	Standard Curve or Quantitation - Standard Curve
Reagents	TaqMan™ reagents
Reporter dye	FAM
Quencher	NFQ-MGB [1]
Passive reference	ROX
Assign wells	Define all wells of the 96-well plate as Unknown
Threshold	0.2
Baseline	3–15

[1] For instruments without this option, enter "None" or "Non-fluorescent".

Figure 4. Settings for Applied Biosystem instruments

Step	Temp (°C)[1]	Time (by block type)		Stage
		Standard	Fast[2]	
Ligation	25	20 min	20 min	Hold
Ligase inactivation	95	2 min	2 min	Hold
Denaturation	95	15 s	1 s	40 cycles
Annealing/extension	60	1 min	20 s	

[1] Set ramp rate to 2°C/sec

[2] Use default values for 7500, 7500 Fast, 7900HT, or non-Applied Biosystems instruments (e.g., 3 s denaturation and 30 s annealing/extension).

Figure 5. Parameters specified for the qPCR

After completing the run, import the results file into the ProQuantum software (cloud version available at apps.thermofisher.com/apps/proquantum). The software allows for the configuration of standard curves, plate layout design, application of a 5-parameter weighted algorithm (5PL), and robust statistical comparisons between groups.

2.2.1.4 Statistical methods

Categorical data are reported as absolute frequencies and relative percentages. Continuous data are expressed as medians with interquartile ranges (IQRs). The data distribution was assessed using the Shapiro-Wilk normality test. Group differences were evaluated based on data distribution and the number of categories: the Mann-Whitney U test was used for comparisons between two groups (NE and Exposed), while the Kruskal-Wallis test was applied for comparisons among three groups (NE, LE, HE). Additionally, correlations between NTA and inflammatory biomarkers were analysed using Spearman's rank correlation test. All statistical analyses were performed using SPSS software, with a significance threshold set at $p < 0.05$.

2.2.2 Epidemiological study II (Paper V, unpublished: Multicentre cohort study on exposure and possible effects that nanomaterials may induce in occupationally exposed workers)

This second epidemiological study was implemented in the frame of the BRIC national project aiming at demonstrating the feasibility of an integrated approach that includes the assessment of exposure to engineered nanomaterials and incidental ultrafine particles through both environmental monitoring and biological monitoring, enabling the evaluation of internal dose and early health effects of exposure to these materials in workers. To carry out this study, the application of a standardised methodology and a harmonised protocol was required, based on case studies defined as occupational scenarios previously performed in the NanoExplore European project. Firstly, as in the previous epidemiological study, it was necessary to identify the different occupational scenarios with presumed or confirmed exposure to nanomaterials and incidental nanoparticles resulting from work processes. In this regard, the power calculation to determine the sample size required for recruitment was performed based on the previous study conducted by Guseva-Canu et al., 2023. Overall, a sample of 120 workers (60 exposed, 60 non-exposed) is expected to allow statistical analysis to detect a significant difference of at least 25% between groups in the variation of biomarkers of effect. Considering a potential 20% loss to follow-up due to constraints related to biological sampling and staff turnover, a total of 80 exposed workers and 80 non-exposed workers are anticipated to be recruited. For the sampling of exposed subjects, a glass manufacturing company from northern Italy was contacted, along with several companies involved in the extraction of minerals and lime from both open-pit and underground quarries. Additionally, a sample of unexposed subjects was enrolled from research unit sectors not handling nanomaterials. All subjects enrolled (n=53) in the study signed informed consent and privacy forms and completed a questionnaire to provide information about their job roles, work shifts, past occupational activities, habits and lifestyle, and health status. Urine and EBC samples were also collected from each worker at the beginning of the working week, then aliquoted into glass/plastic vials and stored at -80°C until analyses. In both matrices, we quantified biomarkers of early biological effects (oxidative stress and inflammation) as described in paragraph 2.2.1.3.2 for EBC), and internal dose biomarkers (NTA, using the methodology described in paragraph 2.2.1.3.1). Additionally, oral mucosal cells were sampled following the methodology described by Ursini et al. (2019) and preserved for subsequent analysis (Ursini et al., 2021) on cyto-genotoxic effects and direct oxidative damage to DNA.

2.2.2.1 Biomarkers of early biological effects in urine: 15-f_{2t}-Isoprostane, Total Antioxidant Power (TAP), and Malondialdehyde (MDA) analysis

Early biological effects in urine were evaluated via the quantification of 15-f_{2t}-Isoprostane, TAP and MDA. 15-f_{2t}-Isoprostane was determined using a competitive ELISA (Oxford Biomedical Research, MI, USA), following the manufacturer's instructions. Briefly, 15-f_{2t}-Isoprostane conjugated to horseradish peroxidase (HRP) and the 15-f_{2t}-Isoprostane found in urine samples or standards competes with the other for binding to a particular polyclonal antibody that has been immobilized

on the plate. When the substrate is added, the HRP enzyme becomes more active, which results in the formation of a colour whose intensity is inversely correlated with the concentration of unconjugated 15-f_{2t}-Isoprostane in the urine samples or standards. The test's LOD is 0.08 ng/mL. After 100 µL of thawed urine samples were placed in Eppendorf tubes, 5 µL of β-glucuronidase was added. After that, the samples were vortexed and incubated at 37 °C for two hours. To minimise interference from nonspecific binding, the samples were diluted 1:4 with the Enhanced Dilution Buffer following incubation. A standard curve with eight points was prepared using a stock solution of 15-f_{2t}-Isoprostane at 1 µg/mL, diluted in EDB to achieve the concentrations according to the scheme showed in the Table 1.

Table 1. 15-f_{2t}-Isoprostane standard curve preparation

Standard	15-Isoprostane F_{2t} Concentration (ng/mL)	Enhanced Dilution Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_7	100	450	50	Standard Stock	300
S_6	50	200	200	S_7	300
S_5	10	400	100	S_6	300
S_4	5	200	200	S_5	300
S_3	1	400	100	S_4	400
S_2	0.1	900	100	S_3	500
S_1	0.05	500	500	S_2	1,000
B_0	0	300	---	---	300

Subsequently, 100 µL of each standard or sample was added to the wells of a 96-well plate, following the scheme in Figure 7.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_7	S_7	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_6	S_6	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_5	S_5	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_4	S_4	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_3	S_3	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_2	S_2	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_1	S_1	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	B_0	B_0	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	RB	RB

Figure 7. 15-f_{2t}-Isoprostane plate layout scheme for standards and samples

Each well, except the Reagent Blank (RB) wells, which were filled with 100 µL of EDB, was then filled with 100 µL of HRP-15-f2t-Isoprostane conjugate, which had been produced and diluted 1:50 with EDB. After that, the plate was left to incubate at ambient temperature for two hours. The plate underwent to three wash cycles following incubation. In each wash, the contents were removed by flipping the plate, using a towel to wipe away any remaining liquid, adding 300 µL of 1x Wash Buffer

(diluted in MilliQ water), and then letting the plate rest for two to three minutes before removing the contents. Following washing, 200 µL of light-sensitive TMB substrate was added to each well. The plate was then incubated for 20–40 minutes, or until the B0 wells started to turn blue. To stop the enzymatic reaction, 50 µL of 3 M sulfuric acid was added to each well, causing a colour change from blue to yellow. The plate was read at 450 nm. By computing the average absorbance values of the RB wells and deducting them from the values of the other wells, the concentration of 15-f_{2t}-Isoprostane in the samples was determined. The %B0 values were calculated by averaging the duplicates of the standards, dividing their values by the mean of the B0 values, and then multiplying the result by 100. A standard curve was then plotted, with %B0 values (linear y-axis) versus standard concentration (logarithmic x-axis), Figure 8. The concentration of 15-f_{2t}-Isoprostane in unknown samples was finally calculated by interpolating the corresponding %B0 value on the standard curve and applying the appropriate dilution factor.

A standard curve was then plotted, with %B0 values (linear y-axis) versus standard concentration (logarithmic x-axis), Figure 8. The concentration of 15-f_{2t}-Isoprostane in unknown samples was finally calculated by interpolating the corresponding %B0 value on the standard curve and applying the appropriate dilution factor.

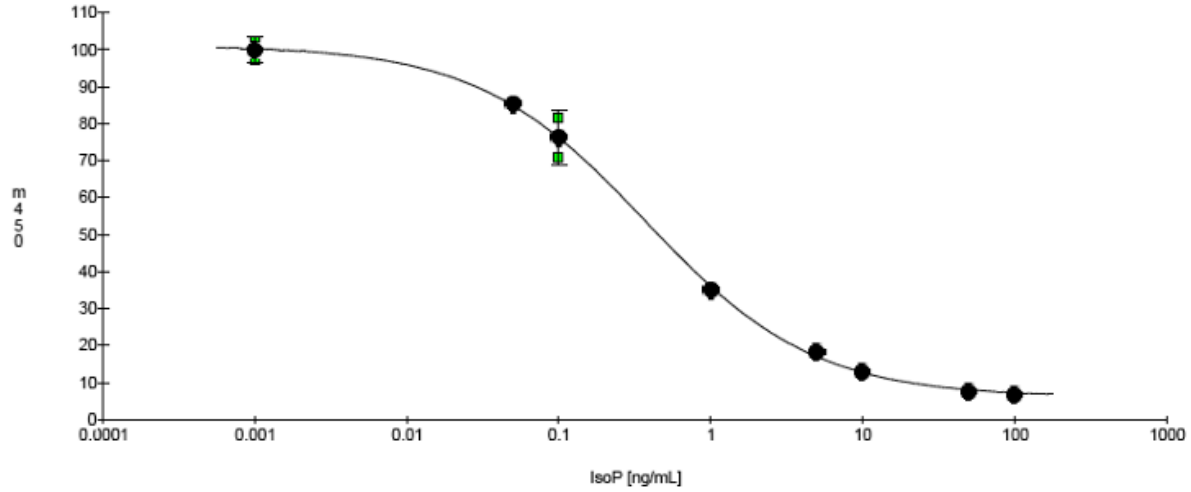

Figure 8. Typical 15-f_{2t}-Isoprostane Standard curve

The test aims to provide a comprehensive assessment of antioxidant capacity, considering a variety of pathways as well as the effects of dietary supplements and lifestyle choices on an individual's antioxidant capacity. The assay's basic idea is the use of the reducing potential of Cu2+ ions to convert them to Cu1+ ions in the presence of samples or standards. The absorption properties of the copper ion are altered because of this action. With a chromogenic reagent, the Cu1+ ion forms a stable 2:1 complex that shows an absorbance peak at 450 nm. The results are displayed in mM Trolox equivalents or µM copper reduction equivalents, and the calibration curve is created using known quantities of Trolox. Specifically, 2 mL of ethanol is added to the Trolox Standard, vortexed

for approximately 1 minute, and is ready for use in creating the 5-point standard curve, as reported in Table 2.

Table 2. TAP standard curve preparation

Standard	Trolox Conc. (mM)	Vol. of Deionized Water (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_5	2.0	-	2000	2 mM Stock	1500
S_4	1.0	500	500	S_5	500
S_3	0.5	500	500	S_4	500
S_2	0.25	500	500	S_3	500
S_1	0.125	500	500	S_2	1000
S_0	0	500	-	-	500

Prior to analysis, thawed urine samples were diluted 1:4 in PBS at pH 7.0. Then, using the supplied Dilution Buffer, both standards and samples were diluted 1:40 (15 µL of sample and 585 µL of Dilution Buffer). A volume of 200 µL of each sample or standard was transferred into the plate, following the scheme shown in Figure 9, while only Dilution Buffer was added to the wells designated as Reagent Blank (BLK).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
B	S_1	S_1	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
C	S_2	S_2	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
D	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
E	S_4	S_4	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
F	S_5	S_5	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}
G	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}	U_{41}	U_{41}
H	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}	BLK	BLK

Figure 9. Plate layout scheme for standard and sample

To provide a baseline measurement, the plate was initially read at 450 nm. Next, 50 µL of copper solution was added to each well, followed by a 3-minute incubation at room temperature, and finally 50 µL of stop solution. The plate was read once more at 540 nm for the second reading. The baseline data was subtracted from the final reading to determine the net absorbance. A typical standard curve was then created by plotting the standards' values (y-axis) against the corresponding Trolox concentrations (x-axis), as seen in Figure 10.

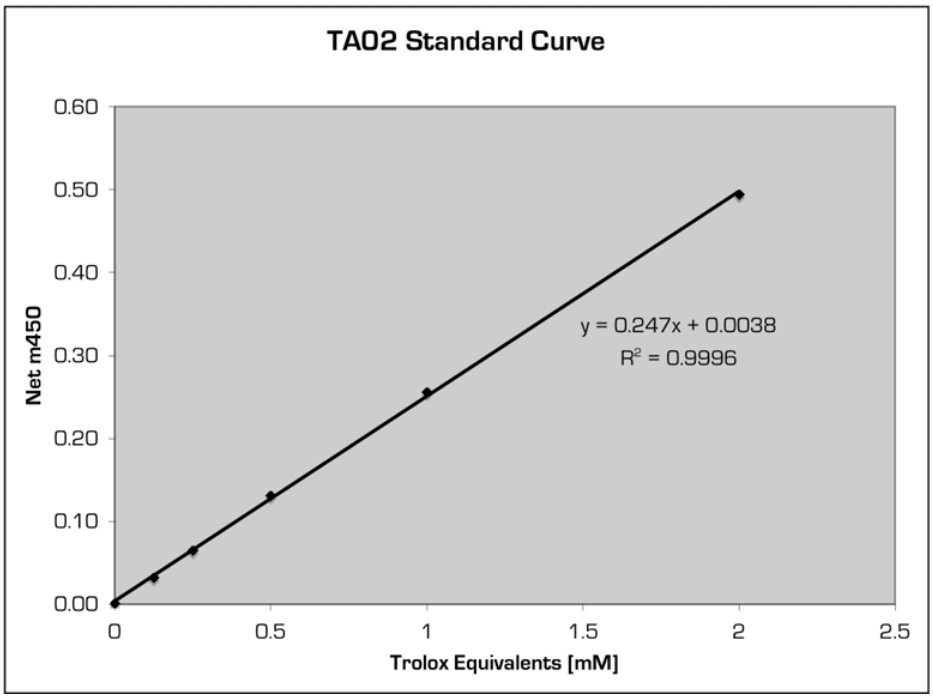

Figure 10. Typical TAP Standard curve graph

Following the manufacturer's instructions, the 2-Thiobarbituric Acid Reactive Substances (TBARS) were quantified using a colorimetric approach (Oxford Biomedical Research, MI, USA) to determine the urine concentration of MDA. Based on the measurement of the MDA-TBA adduct, which is created when MDA and TBA react in an acidic environment at high temperatures, the TBARS test was developed. A chromophore with an absorbance peak between 530 and 540 nm is produced via a Knoevenagel condensation between one MDA molecule and two 2-thiobarbituric acid molecules, as seen in Figure 11.

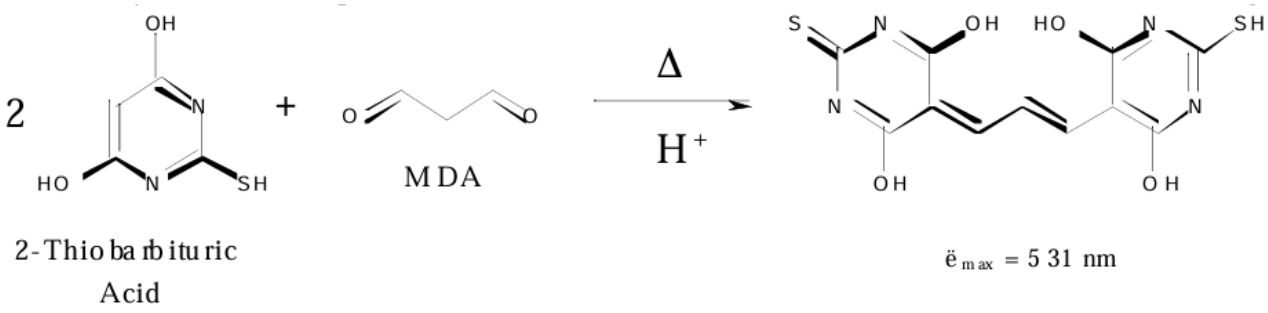

Figure 11. Reaction between the 2-thiobarbituric acid and MDA under acidic conditions

To minimise potential interference from naturally occurring coloured compounds in urine samples, a sample blank was performed for each analysed sample. Starting with a 20 µM stock solution of MDA in the form of MDA tetrabutylammonium salt (MDA-TBA), dissolved in a slightly basic buffer to guarantee MDA stability, an 8-point standard curve was created (Table 3). Acidification produces MDA quantitatively when the stock solution and the acidic indicator solution are combined. Shortly before usage, a 10 mM MDA standard solution was diluted 1:500 in MilliQ water to create the 20 µM stock solution.

Table 3. MDA Standard curve preparation scheme

Standard	MDA Conc. (μM)	Vol. of dH_2O (μL)	Vol. of 20 μM MDA Stock (μL)
S_0	0	400	-
S_1	0.5	390	10
S_2	1.0	380	20
S_3	2.5	350	50
S_4	5.0	300	100
S_5	10.0	200	200
S_6	15.0	100	300
S_7	20.0	-	400

The following chemicals were added to micro-centrifuge tubes and carefully mixed in the appropriate quantities for the standards to quantify the total MDA in both samples and standards: 200 µL each of standard and indicator solution; 200 µL each of sample and indicator solution; 200 µL each of sample and acid reagent for the blank. For forty-five minutes, standards, samples, and blanks were incubated at 65°C. Following the plan shown in Figure 12, 150 µL of each solution was then transferred to the plate and measured at 532 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	SPL_1	SPL_1	SPL_5	SPL_5	SPL_9	SPL_9	SPL_{13}	SPL_{13}	SPL_{17}	SPL_{17}
B	S_1	S_1	SB_1	SB_1	SB_5	SB_5	SB_9	SB_9	SB_{13}	SB_{13}	SB_{17}	SB_{17}
C	S_2	S_2	SPL_2	SPL_2	SPL_6	SPL_6	SPL_{10}	SPL_{10}	SPL_{14}	SPL_{14}	SPL_{18}	SPL_{18}
D	S_3	S_3	SB_2	SB_2	SB_6	SB_6	SB_{10}	SB_{10}	SB_{14}	SB_{14}	SB_{18}	SB_{18}
E	S_4	S_4	SPL_3	SPL_3	SPL_7	SPL_7	SPL_{11}	SPL_{11}	SPL_{15}	SPL_{15}	SPL_{19}	SPL_{19}
F	S_5	S_5	SB_3	SB_3	SB_7	SB_7	SB_{11}	SB_{11}	SB_{15}	SB_{15}	SB_{19}	SB_{19}
G	S_6	S_6	SPL_4	SPL_4	SPL_8	SPL_8	SPL_{12}	SPL_{12}	SPL_{16}	SPL_{16}	SPL_{20}	SPL_{20}
H	S_7	S_7	SB_4	SB_4	SB_8	SB_8	SB_{12}	SB_{12}	SB_{16}	SB_{16}	SB_{20}	SB_{20}

Figure 12. MDA Sample and Standard plate layout

The optical density (OD) readings of duplicate wells were averaged to evaluate MDA in the samples. Using a linear regression technique to generate the equation of the line, the OD values for each standard were plotted in relation to the MDA concentration to create the standard curve. Lastly, the MDA content was determined using the equation from the standard curve (Figure 13) for each sample by subtracting the OD of the blank from the sample OD. 1.0 µM is the LOD.

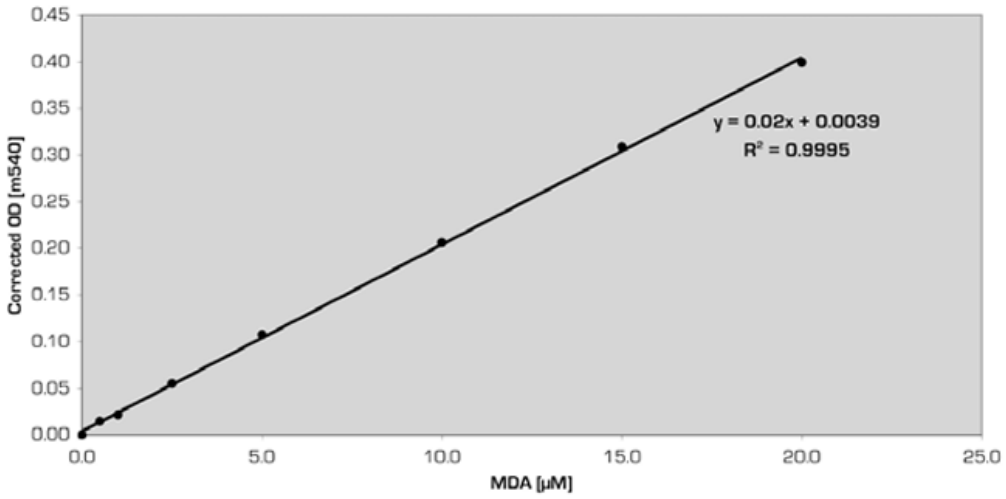

Figure 13. Typical MDA Standard curve

Since all the biomarkers were measured in spot urine, concentration of 15-f_{2t}-Isoprostane, TAP and MDA was normalised to the sample dilution assessed by urine creatinine levels. Creatinine was quantified according to the method of Jaffé et al.,1886 and biomarkers were expressed as ng/mg_{crea} for 15-f_{2t}-Isoprostane, as mmol Trolox equivalents/ $mmol_{crea}$ for TAP and as µmol/$mmol_{crea}$ for MDA.

2.2.2.2 Statistical methods

Categorical data are shown as absolute frequencies and relative percentages. Continuous data are shown as mean and Standard Deviation (SD) or median and IQR. The Shapiro-Wilk normality test was used to assess the data distribution. Then, the Chi-Square test was performed to assess the differences between the percentage among groups. Moreover, based on data distribution, non-parametric Mann-Whitney U-test or t test were used to evaluate median or mean differences among groups. We used SPSS statistical software for all analyses and set statistcal significance at $p < 0.05$.

3. RESULTS

3.1 STUDY LINE I

3.1.1 Paper I

MNPs have been identified in all the sources examined in this review (air, water, personal care products, and food). Numerous studies have investigated the presence of MNPs in the air. In particular, research by Kernchen and colleagues (Kernchen et al., 2021) documented a deposition of 232 tonnes of microplastics in the Weser River area, with concentrations of 500 particles per m^3 even in outdoor environments. However, most studies highlight that indoor air exposure poses a greater risk for MNP inhalation compared to outdoor air (Dris et al., 2017; Liu et al., 2019). Similarly, the Environmental Protection Agency (EPA) reported that indoor chemical concentrations are two to five times higher than outdoor concentrations (Wallace, 1989). It is estimated that daily inhalation of MNPs from indoor air exceeds 48,000 particles (Wieland et al., 2022). In occupational settings, such as during 3D printing processes, exposure to MNPs is an increasing concern due to the release of millions of ultrafine particles, which may pose significant health risks (Stephens et al., 2013; Vance et al., 2017).

The presence of MNPs in drinking water and beverages such as beer and wine suggests that a portion of these particles may originate from packaging contamination (e.g., bottles), which is concerning as it could contaminate food during processing (Zhang et al., 2020). Data have also shown their presence in both processed and unprocessed food, representing a potential source of contamination (EFSA CONTAM Panel, 2016; Food and Authority, 2011).

A significant portion of the global population depends on marine-derived proteins, and numerous studies have identified substantial concentrations of MNPs in seafood, including mussels, crabs, shrimps, prawns, sea urchins, and squid (Akhbarizadeh et al., 2019; Daniel et al., 2020A; Daniel et al., 2020B; Daniel et al., 2021; Feng et al., 2020). However, most MNPs are found in non-edible tissues, such as gastrointestinal tracts, rather than in muscle tissues (Wakkaf et al., 2020; Zhang et al., 2021). For terrestrial protein sources, such as poultry, packaging materials were identified as the primary source of contamination in meat sold for consumption (Huerta et al., 2017).

Regarding other sources, the application of personal care products (PCPs) containing MNPs on the skin may facilitate their penetration into deeper tissues, despite the stratum corneum acting as an effective barrier under healthy conditions (Schneider et al., 2009; Desai et al., 2010). The trans-appendageal pathway, involving hair follicles and glands, represents a potential entry route but accounts for only 0.1–1.3% of the total skin surface (Bos and Meinardi, 2000; Schneider et al., 2009). Particles smaller than 4 nm can penetrate intact skin, whereas those larger than 45 nm cannot (Larese et al., 2015). The size of MNPs in PCPs varies among toothpaste (4–20 µm), shower gels (422 ± 185 µm), and scrubs (~450 µm) (Ustabasi and Baysal, 2019; Lei et al., 2017). Beyond environmental pollution (air and water), MNPs in PCPs applied to mucous membranes can be directly absorbed through the eyes or mouth (Burgener and Bhamla, 2021). Also tampons and other hygiene products release billions of nanoparticles and contribute to environmental dispersion if improperly disposed

of (Munoz et al., 2022). Thus, the ubiquitous presence of MNPs suggests that not only inhalation, especially in specific occupational settings, but also ingestion via food (even though most MNPs in food like poultry or fish are contained in digestive tracts rather than edible tissues) and dermal exposure using PCPs may facilitate the entry of particles smaller than 4 nm into organisms.

3.1.2 Paper II

Once the sources through which organisms and humans might encounter MNPs were identified, it became essential to focus on the matrices and biomarkers most frequently investigated to detect MNP exposure. Additionally, identifying the types of plastics predominantly studied and present in different models was a priority. Following the initial screening for duplicates, the authors double-blinded the titles and abstracts of 5061 publications. A peer assessment determined that 4859 publications were disqualified because they did not contain required information in the research question, such as biomarkers following MNPs exposure. These articles mostly focused on chemically examined components of MNPs, making them unsuitable for our purposes. Alternatively, they presented biomarkers of interest but examined them after being exposed to metal oxides or other compounds that are not classified as MNPs. Finally, this review includes 65 articles, as detailed in the PRISMA plot shown in Figure 14.

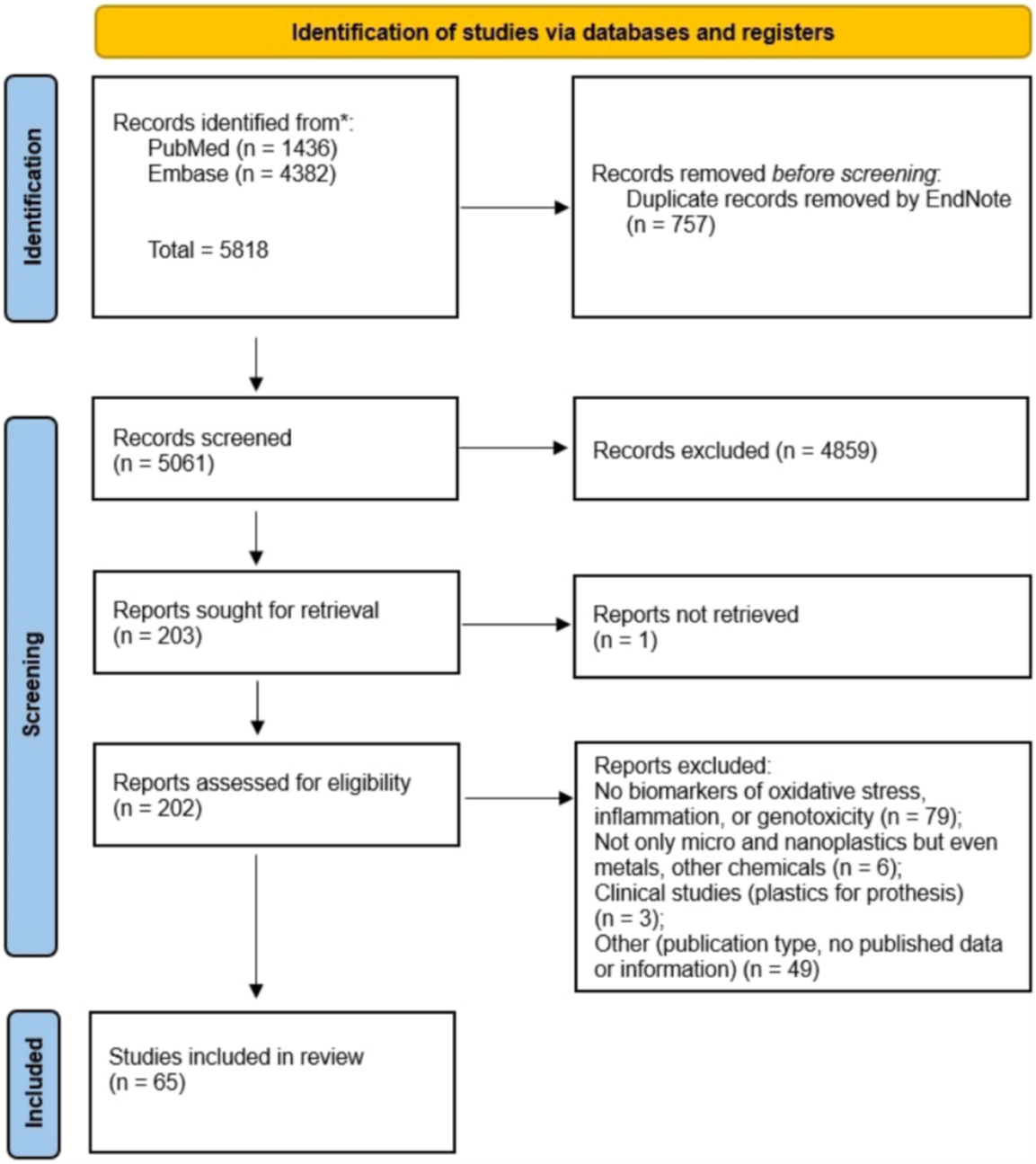

Figure 14. Flowchart of the identification for eligible studies from a search among original articles

Polystyrene (PS) is the most extensively studied MNP in both *in vitro* and *in vivo* analyses. In this review, more than 50% of the studies (43 articles) evaluated the potential adverse effects of PS *in vivo*, with 53.4% focusing on animal models, 9.3% on plant models, and 37.3% on cell cultures. Polyethylene (PE) is the second most investigated polymer, with 17 studies; of these, 70.5% focused on animal models, while 29.5% were *in vitro* studies. It is noteworthy that neither polymer has been studied in humans. Polyvinyl chloride (PVC) was studied in 8 articles, with 62.5% of these investigating cell lines, 25% using animal models, and only 12.5% focusing on occupational exposure in humans. Unspecified polymers (n=5), polypropylene (PP) (n=4), polymethyl methacrylate (PMMA) (n=3), polyethylene terephthalate (PET) (n=2), polyurethane (PUR) (n=1), and poly-lactideco-glycolide (PLGA/PVA) (n=1) were also studied. For more information, see Table 1 in Appendix A of Paper II.

Based on the models used in the included articles, four separate tables reported *in vitro* studies, *in vivo* studies (animal models), *in vivo* studies (plant models), and human studies, respectively. Each table outlines the type of cell, animal, plant, or worker studied, the exposure duration, analytical methods, and investigated biomarkers.

In most *in vitro* studies, lung, liver, and intestinal cells were the primary models. The most frequently investigated biomarkers included MDA, IL-1B, IL-6, IL-8, and TNF-α. Genotoxicity biomarkers assessed included cell viability tests, comet assays, chromatid aberrations (CA), and sister chromatid exchanges (SCE).

In *in vivo* studies involving animals and plants, controlled exposure to MNPs such as PS, PE, and PET ranged from a minimum of 4 hours to several weeks. These studies reported the same biomarkers as *in vitro* studies, with additional markers for oxidative stress, such as TAP, SOD, and CAT. For inflammation, IL-10 was also assessed.

Human studies demonstrated a notable gap, as no epidemiological studies involving workers directly exposed to MNPs were identified. The few studies included focused on workers exposed to PVC, PUR, and acrylonitrile (ACN). Biomarkers primarily investigated in these studies included liver damage markers and SCE in blood-derived cells.

3.1.3 Paper III

This systematic review included 36 research articles. Following the initial screening of 1929 articles, only 303 were deemed eligible for the second selection and re-reading. The main reasons for exclusion in most of the articles were the lack of the biomarkers of interest; furthermore, when these biomarkers were present, their analysis was conducted on matrices different from the one required in the query, EBC. Additionally, the articles deemed eligible after the first screening were read in full, and those that did not meet the characteristics of the epidemiological sample (such as age, health status, or smoking habits), the included biological matrices, and the biomarkers quantified according to the research question were excluded. Moreover, over a hundred articles were excluded for other reasons, including language, the absence of relevant data, or because they could not be classified as full research articles (i.e., conference proceedings). The procedure as well as the resulting number of articles at each stage of screening is summarized in the PRISMA diagram (Figure 15). Subsequently, the included articles were subjected to a quality assessment conducted by two independent reviewers using two different scales. The first assessment was based on the study design, employing the Joanna Briggs Institute (JBI) checklists to evaluate the reliability and relevance of the published articles. The second assessment, developed by the authors and comprising 12 questions, focused on the methodological protocol, particularly assessing the adherence of research protocols to the ATS/ERS Task Force guidelines on EBC. This included evaluating the collection methodology, storage temperature, and analysis procedures.

The full scale can be found in Appendix A of Paper III.

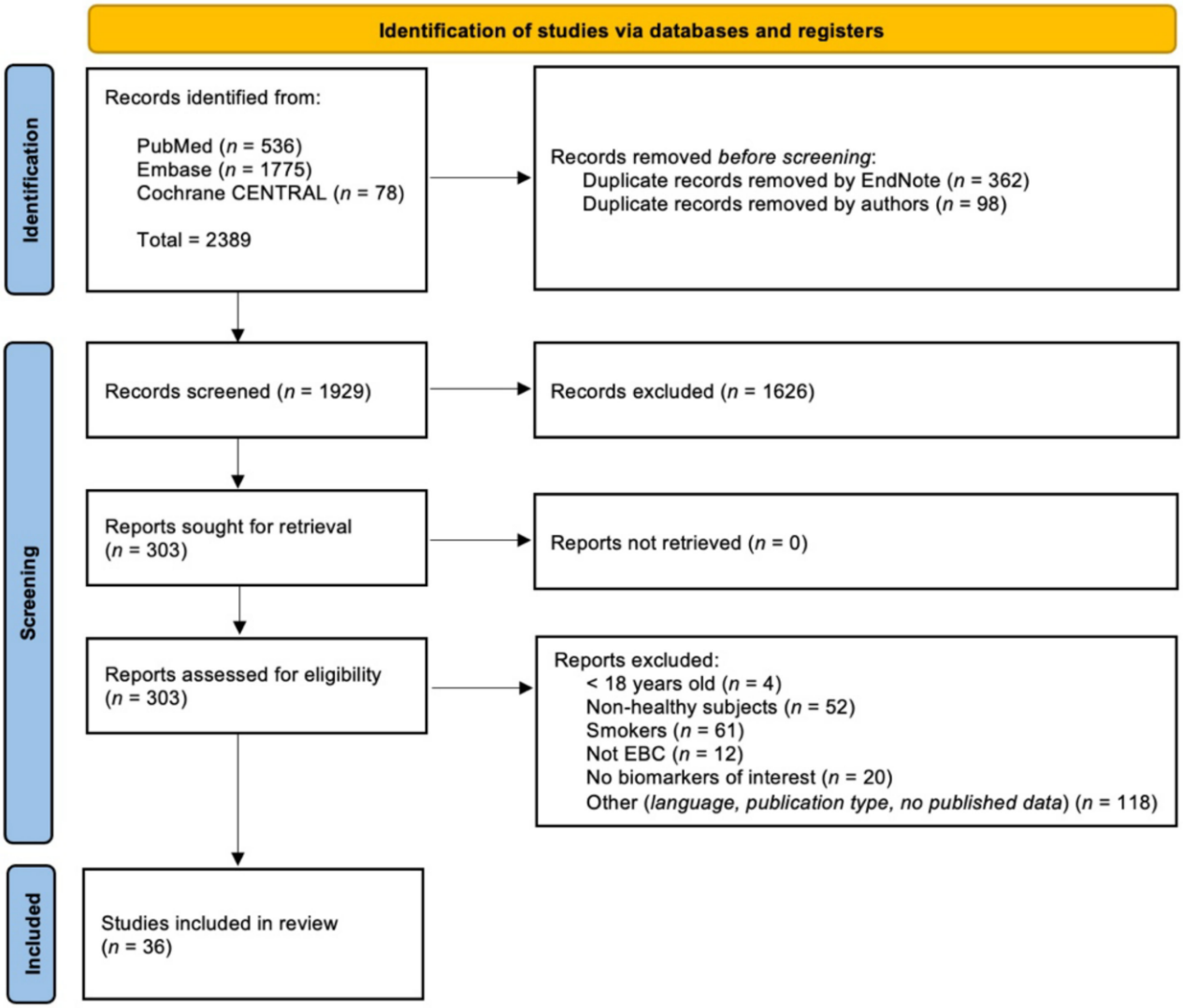

Figure 15. PRISMA flow chart summarising the study selection process and its outputs

Once the quality assessment (risk of bias) was carried out with both scales (JBI and the one of our own design), the most investigated biomarkers were extracted with their respective average levels and dispersion intervals (Table 4).

Table 4. Biomarkers identified in the included articles with the number of studies and percentage of data above and below the LOD

Biomarker	n° of Studies	n° of Studies (%) with Data > LOD	n° of Studies (%) with Data < LOD	n° of Studies (%) without LOD Declared
CRP	3	2 (66.7%)	-	1 (33.3%)
IL-1β	12	2 (16.7%)	5 (41.7%)	5 (41.7%)
IL-4	11	6 (54.5%)	2 (18.2%)	3 (27.3%)
IL-6	19	11 (57.9%)	2 (10.5%)	6 (31.6%)
IL-8	16	5 (31.3%)	4 (25.0%)	7 (43.8%)
IL-10	12	2 (16.7%)	2 (16.7%)	8 (66.7%)
TNF-α	18	6 (33.3%)	3 (16.7%)	9 (50.0%)

Finally, as shown by the forest plot (Figure 16), the levels of the different biomarkers in EBC (mean ±SD) were summarised according to the original study.

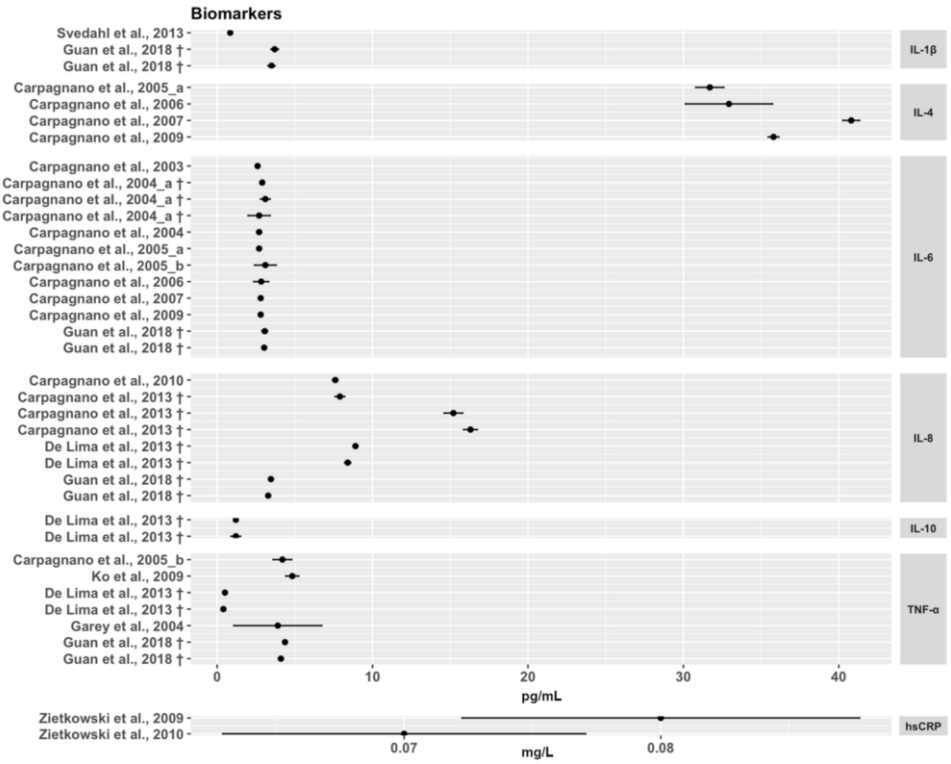

Figure 16. Forest plot of inflammatory biomarkers in EBC according to the original study above the LOD

The main biomarkers of inflammation identified in EBC were IL-1β, IL-4, IL-6, IL-8, IL-10, hs-CRP, and TNF-α. It was highlighted that IL-6 is the most frequently investigated biomarker among the articles included in this study, with as many as 12 out of 36 studies examining this cytokine. Conversely, IL-8, TNF-α, and IL-4 were investigated in 22%, 19%, and 11% of the included articles, respectively. Hs-CRP and IL-1β were assessed in only two studies.

3.2 STUDY LINE II

3.2.1 Paper IV: field study I

In this epidemiological study, implementing the findings from study Line I, 80 subjects were recruited and sampled following the methodologies outlined in the Materials and Methods section of Study Line II.

This study represents the first multicentre investigation to analyse the number of particles in the airways using EBC samples. Based on environmental characterization data obtained using DiSCmini™ (Testo, Mönchaltorf, Switzerland) devices, participants were categorized into three

exposure groups: non-exposed (NE), low exposure (LE), and high exposure (HE), as detailed in Table 5.

Table 5. Characteristic of the subjects according to the exposure ranking

Variables	Non-Exposed	Low-Exposed	High-Exposed
Subjects_n (%)	29 (36)	14 (17)	37 (46)
Age years, min-max (mean± sd)	25-54 (38.6±2.6)	19-60 (36.39±1.9)	22-60 (41.47±1.8)
Male_n (%)	14 (17.50)	14 (17.50)	31 (38.75)
Subjects enrolled in company A n (%)	/	14 (17.50)	3 (3.75)
Subjects enrolled in company B n (%)	/	/	27 (33.75)
Subjects enrolled in company C n (%)	13 (16.25)	/	7 (8.75)
Subjects recruited as non-exposed (universal controls) n (%)	16 (20)	/	/

Subsequently, the number of particles in the airways was analysed in the EBC using the NTA methodology previously described and compared with the environmental data used earlier to categorise the workers. The initial comparison revealed a statistically significant difference, with the number of environmental particles being higher in exposed subjects (LE + HE) compared to non-exposed subjects (NE) (Figure 1, Paper IV attached in full).

A similar result was observed when comparing the number of particles in the EBC of exposed subjects (LE + HE) to non-exposed subjects (NE) ($p < 0.001$) (Figure 1, Paper IV attached in full). Additionally, a correlation was identified between the number of particles in the environment and the number of particles detected in the EBC using NTA. While the correlation showed a weak Rho value, it was statistically significant (Figure 17).

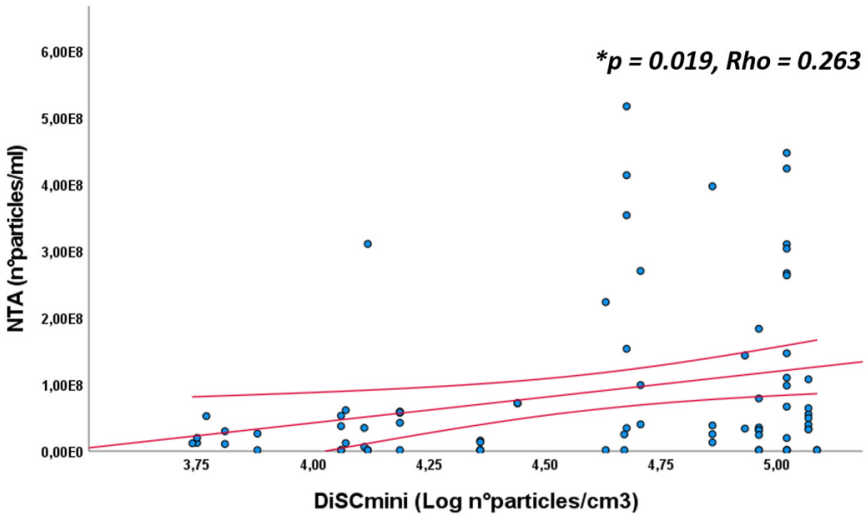

Figure 17. Correlation between the number of particles in the air (DiSCmini™) and the NTA data in EBC

By further categorizing the subjects into HE, LE, and NE groups based on both environmental data from DiSCmini™ (Testo, Mönchaltorf, Switzerland) measurements and NTA data from EBC analysis, significant differences were observed between HE and NE subjects for both measures (environmental and NTA). However, for LE and NE subjects, significant differences were observed only in the environmental data, but not in the NTA data.

Additionally, significant correlations were identified between IL-1β and IL-10 analysed in the EBC, and the particle counts determined using the NTA method (Figure 4, Paper IV attached in full). These findings suggest that elevated levels of airborne particles may impact the airways not only through increased particle deposition but also by amplifying local inflammatory responses, which could represent a potential prodrome of disease.

3.2.2 Work in progress: field study II

The enrolled workers were divided according to their exposure and task into exposed and unexposed. Table 6 describes the demographic characteristics of the epidemiological sample so far recruited whose analyses of exposure and effect biomarkers have been completed.

Table 6. Descriptive analysis of the epidemiological sample

DEMOGRAPHIC CHARACTERISTICS	OVERALL	EXPOSED	UNEXPOSED	P-VALUE[a,b]
N° OF ENROLLED SUBJECTS	53	16	37	–
AGE YEARS MEAN ± S. D. [MIN-MAX]	48.9 ± 10.6 [24-63]	49.7 ± 7.2 [35-63]	48.5 ± 11.9 [24-63]	[a] 0.854
YEARS WORKING MEAN ± S.D. [MIN-MAX]	12.4 ± 11.3 [0-37]	14.5 ± 11.6 [0-37]	11.5 ± 11.6 [0-37]	[a] 0.367

[a]t-student parametric test was performed

NON-SMOKERS N (%)	32 (60.4%)	10 (62.5%)	22 (59.4%)	[b] 0.846
SMOKERS N (%)	21 (39.6%)	6 (37.5%)	15 (40.6%)	[b] 0.582
SUBJECT REPORTING RESPIRATORY SYMPTOMS IN THE LAST 12 MONTHS N (%)	16 (35.2%)	6 (31.3%)	10 (27.1%)	[b] 0.766
SUBJECT REPORTING RESPIRATORY SYMPTOMS IN WINTER N (%)	16 (35.2%)	6 (31.3%)	10 (27.1%)	[b] 0.766
ALLERGIC SYMPTOMS IN THE PAST 3 MONTHS N (%)	YES 17 (32.1%)	YES 4 (25%)	YES 13 (35.1%)	[b] 0.480
INFECTIONS IN THE LAST 3 MONTHS N (%)	**YES 20 (37.7%)**	**YES 4 (25%)**	**YES 16 (43.3%)**	[b] 0.243

[b] chi-square test was performed

Spot urine samples were examined for 15-f_{2t}-Isoprostane, MDA, and TAP, biomarkers of oxidative stress detailed in Table 7. Results from individual determinations, all within the limit of detection (LOD), are presented along with medians and 95% confidence intervals.

Table 7. Oxidative stress levels measured in urine

Biomarker	Whole population	Exposed	Unexposed	P-value[a]
15-f_{2t}-Isoprostane ng/mg_{CREA} median (IQR) [25^{th}-75^{th}]	2.75 (1.07) [2.38-3.45]	3.27 (0.94) [2.552-3.49]	2.66 (1.12) [2.21-3.33]	0.4
MDA µmol Trolox eq/$mmol_{CREA}$ median (IQR) [25^{th}-75^{th}]	0.470 (0.65) [0.36-1.01]	1.13 (0.77) [0.66-1.43]	0.40 (0.26) [0.33-0.59]	**<0.001**
TAP mmol/$mmol_{CREA}$ median (IQR) [25^{th}-75^{th}]	0.39 (0.120) [0.330-0.450]	0.42 (0.132) [0.37-0.50]	0.34 (0.13) [0.29-0.42]	**0.007**

[a]Mann-Whitney non-parametric test was performed

No statistically significant differences were found between exposed individuals and unexposed in the median levels of 8-Isoprostane in urine, while MDA and TAP levels showed statistically significant differences.

The analyses suggested higher levels for two pro-oxidant biomarkers examined.

Regarding inflammatory biomarkers, in the EBC samples from exposed individuals collected at the end of the workweek, a trend towards increased levels of TNF-α was observed, while IL-1β levels were higher in unexposed, both showing highly significant differences.

Detailed information is shown in Table 8.

Table 8. Levels of biomarkers of inflammation measured in the EBC (Real time qPCR)

EBC				
Biomarker	**Whole population**	**Exposed**	**Unexposed**	**P-value[a]**
KL-6 U/mL median (IQR) [25^{th}-75^{th}]	143.38 (32.1) [127.44-159.54]	143.50 (33.34) [127.18-160.52]	143.38 (31.97) [127.57-159.54]	0.8
IL-1β pg/mL median (IQR) [25^{th}-75^{th}]	0.39 (0.34) [0.28-0.620]	0.26 (0.24) [0.15-0.39]	0.44 (0.31) [0.34-0.65]	**<0.001**
IL-10 pg/mL median (IQR) [25^{th}-75^{th}]	3.92 (9.17) [1.73-10.9]	4.46 (5.38) [1.56-6.94]	3.02 (11.65) [1.95-13.6]	**0.04**
IL-17 A pg/mL median (IQR) [25^{th}-75^{th}]	2.76 (1.88) [2.10-3.98]	2.80 (0.93) [2.45-3.39]	2.59 (3.76) [1.98-5.74]	0.7
TNF-α pg/mL median (IQR) [25^{th}-75^{th}]	0.47 (0.32) [0.37-0.69]	0.78 (0.11) [0.71-0.82]	041 (0.13) [0.36-0.49]	**<0.001**

[a]Mann-Whitney non-parametric test was performed

Exposed individuals had significantly higher levels of IL-10, an anti-inflammatory cytokine, than non-exposed individuals.

The mean values of KL-6, although not statistically significant, tend to be higher in the exposed individuals.

In contrast, IL-17 exhibits an unexpected behaviour as a pro-inflammatory cytokine, showing lower levels in exposed individuals compared to controls.

Table 9 reports the medians ± IQR of the average particle size (nm) and the number of particles identified in the samples according to the exposure status of workers.

Table 9. Particle size distribution in EBC assessed by NTA

Particles size nm [min-max]	206 ± 24.9 [137 / 250]		
	Exposed	**Unexposed**	**P-value[a]**
NTA in EBC n° of particles / ml Median (IQR) [25th-75th]	$1.87*10^7$ ($7.34*10^6$) [$1.54*10^7$ – $2.27*10^7$]	$1.93*10^7$($1.1*10^7$) [$1.20*10^7$ -$2.3*10^7$]	0.43

[a]Mann-Whitney non-parametric test was performed

Although there was no statistically significant difference, there were more particles in the exposed group than in the unexposed group.

Preliminary results from this study did not reveal marked effects of the inhalation of micro and nanoparticles or local levels in EBC, but some trends were observed regarding the number of particles detected in the EBC of exposed individuals compared to unexposed subjects. Additionally, levels of IL-10 and KL-6 were generally higher in the exposed compared to the unexposed workers.

4. Discussion of study line I and study line II

MNPs represent a global environmental issue, raising growing concerns for human health due to their ubiquity, small size, and multiple exposure pathways, but because of their unique characteristics such as light weight and strength-to -weight ratio, waterproofness, and thermal and electrical insulation, it is very difficult to date to find a replacement that is up to the mark. (Pilapitiya et al., 2024; Luo et al., 2024). In the study line I "reviews" (paper I and paper II), it emerged that the most studied and widespread plastics in the environment are polystyrene (PS), polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) (Ramsperger et al., 2023; Panizzolo et al., 2023). In paper I, these plastics were found to be the most frequently identified in aquatic organisms, mainly located in the digestive tract rather than in tissues, as well as in personal care products and food, primarily due to packaging. Consequently, it is hypothesised that human interaction occurs predominantly through ingestion and inhalation (Yee et al., 2021), although inhalation exposure is reported to a lesser extent (Lee et al., 2019; Renzi et al., 2019; Tahir et al., 2019; Kim et al., 2018; Inigues et al., 2018; Fisher et al., 2019; Karami et al., 2017; Yang et al., 2015; Hernandez et al., 2019).

Paper II, despite addressing a different research question, confirmed these results by analysing studies conducted in in vitro and in vivo models ,in animals and plants, identifying the same types of MNPs (Maity et al., 2023; Bonanomi et al., 2022; Malinowska et al., 2022; Rouragaard et al., 2022; Palaniappan et al., 2023; Cheng et al., 2022; Weber et al., 2022; Chen et al., 2022; Busch et al., 2021; Vecchiotti et al., 2021; Wang et al., 2021; Cobanoglu et al., 2021; Dong et al., 2020; Rubio et al., 2020; Poma et al., 2019; Espinoza et al., 2018; Schirinzi et al., 2017; Choi et al., 2021; Arikan et al., 2022; Maity et al., 2020; Ni et al., 2023; Liu et al., 2022). Both papers showed that airborne or environmentally dispersed plastics derive from industrial processes such as packaging, moulding, 3D printing, and their resulting waste (Pelegrini et al., 2023).

Literature studies focus on the interaction of MNPs with cells and tissues, highlighting common damage mechanisms such as lipid peroxidation, inflammation, DNA damage, and the production of reactive oxygen species (ROS). In paper II, key biomarkers of oxidative stress and inflammation, such as MDA, TAP, and CAT, were identified in urine, tissues, and cell lysates. Inflammatory markers such as IL-1β, IL-6, IL-10, IL-17, TNF-α, and NF-kB were also identified, whose concentrations significantly increase following exposure to MNPs, as highlighted in the study by Luo et al., 2022. Genotoxicity was studied using tests such as the micronucleus test and the Comet assay, revealing significant DNA damage. However, despite findings from animal and in vitro models suggesting potential human health risks (through the previously mentioned mechanisms), direct correlations in humans remain challenging due to the lack of standardised methods to evaluate exposure, distribution, and toxicological impacts.

To delve deeper into these issues, paper III aimed at identifying sampling methods and analysing local inflammatory biomarkers identifiable in non-invasive matrices such as EBC. Main results (presented in section 3) are referred to specific biomarkers such as IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, and CRP, which seem to be useful for identifying localised inflammatory states in the airways (Siefi et al., 2021; Kim et al., 2023).

Paper IV utilised all the cornerstones provided by the results obtained in papers I, II, and III, adding details on actual NM exposure in a cohort of workers, highlighting significant differences between exposed and unexposed workers in terms of both the number of airborne particles (analysed using DiSCmini™) and the number of particles identified in EBC (NTA). Additionally, significant correlations were identified between the number of airborne particles and the number of particles present in EBC, and between the latter and inflammatory biomarkers (IL-1β and IL-10). The increase in IL-10 may reflect a feedback mechanism to counteract the rise in pro-inflammatory cytokines. Similarly, the study by Sauvain et al., 2017 showed a comparable trend, demonstrating an excellent correspondence between occupational exposure to microparticles (quartz and silica particles) and the number of particles identified in EBC. Additionally, higher levels of TAP in exposed individuals may suggest the activation of the body's feedback mechanisms due to the increased presence of pro-oxidant substances counteracting antioxidant production. Despite the limitations of this study (limited number of participants and matrices investigated, difficulty in recruiting workers, and a restricted number of biomarkers analysed), it represents a significant advancement in understanding the risks associated with MNPs. The review studies conducted using the PRISMA method provide a comprehensive overview of transmission pathways, investigated biological matrices, and the use of NTA as an internal dose biomarker, but at the same time, they highlighted the lack of standardisation in methodologies, both in EBC sampling and analysis. For example, the use of various tools for EBC sampling (i.e., condenser), might have substantially contributed to the observed lack of harmonisation among results from different studies. This, combined with the inconsistent application of methodologies and improper storage at suboptimal temperatures for matrix preservation, represents an additional challenge. Furthermore, the use of different analytical approaches, such as traditional ELISA kits, high-sensitivity ELISA, and Luminex technology, makes results difficult to compare. In fact, discrepancies in many of the data obtained in these review' works are particularly evident for TNF-α and IL-8, due to variations in the analytical methods used. This combined approach, integrating environmental and biological sampling with a common protocol, has improved health risk assessment, particularly in workplaces, underscoring the importance of personal protection to reduce the accumulation of MNPs in tissues. Further studies are essential to standardize sample collection and analysis methods, better understand the mechanisms of translocation and accumulation in human tissues, and foster collaborations between academic and industrial entities to develop effective mitigation and prevention guidelines.

5. Conclusions

Micro- and nanoparticles (MNPs) are ubiquitous and have been identified in nearly all environmental matrices, including water, air, food, and personal care products. Environmental contamination by MNPs is primarily caused by industrial processes, packaging, or atmospheric deposition. This thesis delved into the main pathways through which MNPs can access and interact with biological organisms, spanning in vitro studies on cells, in vivo studies on plants and animals, and studies conducted on humans. Given the scarcity of studies conducted on humans, particularly in occupational settings, which represent a higher risk context for human exposure, it was necessary to draw upon evidence from in vitro and in vivo studies to extrapolate findings to human exposure. This allowed the identification of potential interaction pathways, the most frequently investigated matrices (such as EBC and urine), and a panel of biomarkers of oxidative stress (15-F2t-isoprostane, MDA, TAP) and inflammation (IL-1β, TNF-α, IL-10, IL-17A) that could be best adapted to MNP exposure. Additionally, this thesis identified a novel biomarker of internal dose using NTA, developing a method to estimate the number of particles present in the lungs of exposed workers. Through systematic literature reviews, it was possible to establish that EBC and urine are the most suitable matrices for providing information on the inflammatory status of the airways and lipid peroxidation in individuals under analysis. These biomarkers offer insights into the levels of oxidative stress and inflammation in exposed subjects.

Thus, the multidisciplinary approach implemented in this study, which integrated exposure assessment with the quantification of non-invasive biological biomarkers in specific matrices, confirmed the findings of previous research and represented a significant advancement. This work demonstrated the reliability of particle analysis in EBC, offering a clearer understanding of the actual dose of inhaled particles. These findings were further supported by their association with the inflammatory profile assessed in the same matrix.

In conclusion, the application of NTA for studying the internal dose, combined with the analysis of external exposure and inflammatory profiles, represents a promising approach for investigating the fraction of non-absorbed particles that might contribute to airway inflammation. The integration of exposure assessment with biomonitoring has proven to be the most effective tool for identifying causal relationships and evaluating the potential risks to which workers may be exposed.

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7.1 Scientific contributions at national and international conferences

Panizzolo M., Barbero F., Ghelli F., Squillacioti G., El Sherbiny S., Fenoglio I., Bergamaschi E., Bono R. P373. Nanoparticle Tracking Analyses: A promising tool for estimating the internal dose of nanomaterials of workers occupationally exposed. Poster presentation at the 57th National Congress of the Italian Society of Hygiene (SITI) held from 23-26 October, 2024, in Palermo, Spain.

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Panizzolo M., Gea M., Romano A., Caruso C., Bianchi M., De Castelli L., Carraro E., Bonetta S., Pignata C. Monitoraggio di agenti virali circolanti in reflui civili e zootecnici e non trattati nel territorio piemontese. Poster presentation at the 55th National Congress of the Italian Society of Hygiene (SITI) held from 28 of September to 1 October 2022, in Padova, Italy.

Ghelli F., Bellisario V., Squillacioti G., **Panizzolo M.**, Bono R. Physical activity at work: an agreable way to counteract the oxidative stress burden. Oral presentation at the 24th International Conference on Oxidative Stress Reduction, Redox Homeostasis & Antioxidants held from 22-26 June 2022, in Paris, France.

Squillacioti G., Garzaro G., Bellisario V., **Panizzolo M.**, Antonello G., Bono R., Guseva-Canu I., Bergamaschi E. Early biological effects and respiratory health in workers exposed to nanomaterials. The NanoExplore project. Oral presentation at the 24th International Conference on Oxidative Stress Reduction, Redox Homeostasis & Antioxidants held from 22-26 June 2022, in Paris, France.

Panizzolo M., Ghelli F., Bellisario V., Squillacioti G., Mariella G., Guseva-Canu I., Fito Lopez C., Bono R., Bergamaschi E. Urinary excretion of metals in workers producing technological paints and coatings. Oral presentation at the 19th International Conference on Nanosciences & Nanotechnologies held from 5-8 July, 2022, in Thessaloniki, Greece.

8. Appendix A

Appendix A. Full strings used for item retrieval

PubMed string

#1 "Oxidative Stress"[Mesh] OR oxida*[title] OR antioxida*[title]

#2 "Inflammation"[Mesh] OR inflammat*[title] OR antiinflammat*[title] OR antinflammat*[title]

#3 genotoxic*[title] OR geno-toxic*[title] OR "genetic toxicity"[title] OR toxigeni*[title] OR mutagen*[title]

#4 #1 OR #2 OR #3

#5 "Biomarkers"[Mesh] OR biomarker*[tiab] OR marker*[tiab] OR level*[tiab] OR test[tiab] OR tests[tiab]

#6 "Malondialdehyde"[Mesh] OR malondialdehyde[tiab] OR malonylaldehyde[tiab] OR malonaldehyde[tiab] OR malonyldialdehyde[tiab] OR MDA[tiab]

#7 "8-Hydroxy-2'-Deoxyguanosine"[Mesh] OR "8-hydroxy-2-deoxyguanosine"[tiab] OR "8-hydroxy-deoxyguanosine"[tiab] OR "8-hydroxydeoxyguanosine"[tiab] OR "8-hydroxyguanine"[tiab] OR "8-hydroxy-guanine"[tiab] OR "8-Oxo-2-Deoxyguanosine"[tiab] OR "8-Oxo-Deoxyguanosine"[tiab] OR "8-oxo-dGuo"[tiab] OR 8-Ohdg[tiab] OR 8OHdG[tiab] OR 8-OH-dG[tiab] OR 8-ohg[tiab] OR 8-hydroxy-g[tiab] OR 8-hydroxy-dg[tiab] OR 8-oxodG[tiab] OR 8-oxodGuo[tiab] OR 8-oxo-dG[tiab] OR 8-OH-2dG*[tiab] OR 8-isoprostane*[tiab]

#8 "F2-Isoprostanes"[Mesh] OR IsoP[tiab] OR F2-isoprostane*[tiab]

#9 "Dinoprost"[Mesh] OR dinoprost[tiab] OR 15-f2t-isop[tiab] OR 8-iso-PGF2a[tiab] OR 8-isoprostaglandin-f2[tiab] OR 8-iso-prostaglandin-f2[tiab] OR 8-iso-PGF2a[tiab] OR 8-epi-prostaglandin-F2alpha[tiab] OR 8-epi-prostaglandin-f2alpha[tiab] OR 8-epiprostaglandin-f2alpha[tiab] OR 8-epi-PGF2alpha[tiab]

#10 "Allantoin"[Mesh] OR allantoin*[tiab] OR dioxo-4-imidazolidinyl*[tiab] OR glyoxyldiureide*[tiab] OR 5-ureidohydantoin*[tiab]

#11 "total antioxidant capacity"[tiab] OR "total anti-oxidant capacity"[tiab] OR "total antioxidant power"[tiab] OR TAC[tiab] OR ToAC[tiab] OR "total anti-oxidant power"[tiab] OR TAP[tiab]

#12 "trolox equivalent antioxidant capacity"[tiab] OR "trolox equivalent anti-oxidant capacity"[tiab] OR TEAC[tiab]

#13 "Thiobarbituric Acid Reactive Substances"[Mesh] OR TBARS[tiab] OR "thiobarbituric acid reactive substance*"[tiab]

#14 "Glutathione"[Mesh] OR "Glutathione Peroxidase"[Mesh] OR glutathion*[tiab] OR GSH[tiab] OR GSSH[tiab] OR "GSH/GSSG"[tiab] OR GPX[tiab]

#15 "Uric Acid"[Mesh] OR "uric acid"[tiab] OR UA[tiab]

#16 "Superoxide Dismutase"[Mesh] OR dismutase*[tiab] OR SOD[tiab]

#17 "Lipid Peroxides"[Mesh] OR "lipid peroxid*"[tiab] OR hydroperoxid*[tiab] OR lipoperoxid*[tiab] OR hydro-peroxid*[tiab] OR lipo-peroxid*[tiab]

#18 "ferric reducing"[tiab] OR "ferric ion reducing"[tiab] OR FRAP[tiab]

#19 "oxygen radical absor*"[tiab] OR ORAC[tiab]

#20 "cupric reducing" OR CUPRAC[tiab]

#21 "hydroxyl radical"[tiab] OR HORAC[tiab]

#22 "potassium ferricyanide reducing power"[tiab] OR PFRAP[tiab]

#23 "total peroxyl radical trapping"[tiab] OR "total reactive antioxidant potential"[tiab] OR "total reactive anti-oxidant potential"[tiab] OR TRAP[tiab]

#24 picrylhydrazyl[tiab] OR picryl-hydrazyl[tiab] OR DPPH[tiab]

#25 "total oxyradical scavenging capacity"[tiab] OR TOSC[tiab]

#26 "Fibroblast Growth Factors"[Mesh:NoExp] OR "Fibroblast Growth Factor 2"[Mesh] OR "fibroblast growth factor basic"[tiab] OR "basic fibroblast growth factor"[tiab] OR "fibroblast growth factor-2"[tiab] OR "FGF basic"[tiab] OR bFGF[tiab] OR b-FGF[tiab] OR FGF-b[tiab] OR FGF-2[tiab] OR FGF2[tiab] OR HBGF-2[tiab] OR HBGF2[tiab]

#27 "Chemokine CCL11"[Mesh] OR "Chemokine CCL24"[Mesh] OR "Chemokine CCL26"[Mesh] OR eotaxin*[tiab] OR CCL11[tiab] OR CCL24[tiab] OR CCL26[tiab]

#28 "Granulocyte Colony-Stimulating Factor"[Mesh] OR "granulocyte colony-stimulating factor"[tiab] OR G-CSF[tiab] OR GCSF[tiab] OR "Granulocyte-Macrophage Colony-Stimulating Factor"[Mesh] OR "granulocyte macrophage"[tiab] OR GM-CSF[tiab] OR CSF-2[tiab] OR CSF-GM[tiab]

#29 "Interferon-gamma"[Mesh] OR "interferon-gamma"[tiab] OR "interferon-g"[tiab] OR "gamma-interferon"[tiab] OR "interferon type 2"[tiab] OR "interferon type II"[tiab] OR IFN-gamma[tiab] OR IFN-g[tiab]

#30 "Interleukins"[Mesh] OR "Interleukin 1 Receptor Antagonist Protein"[Mesh] OR interleukin*[tiab] OR IL-1beta[tiab] OR IL-1b[tiab] OR IL1beta[tiab] OR IL1b[tiab] OR IL-1ra[tiab] OR IL1ra[tiab] OR IL-1alpha[tiab] OR IL-1a[tiab] OR IL1alpha[tiab] OR IL1a[tiab] OR IL-2Ralpha[tiab] OR IL-2Ra[tiab] OR IL2Ralpha[tiab] OR IL2Ra[tiab] OR IL-3[tiab] OR IL3[tiab] OR IL-12[tiab] OR IL12[tiab] OR P70[tiab] OR P40[tiab] OR 12p40[tiab] OR IL-16[tiab] OR IL16[tiab] OR IL-2[tiab] OR IL2[tiab] OR IL-4[tiab] OR IL4[tiab] OR IL-5[tiab] OR IL5[tiab] OR IL-6[tiab] OR IL6[tiab] OR IL-7[tiab] OR IL7[tiab] OR IL-8[tiab] OR IL8[tiab] OR IL-9[tiab] OR IL9[tiab] OR IL-10[tiab] OR IL10[tiab] OR IL-13[tiab] OR IL13[tiab] OR IL-15[tiab] OR IL15[tiab] OR IL-17A[tiab] OR IL17A[tiab] OR IL-18[tiab] OR IL18[tiab]

#31 "Chemokine CXCL1"[Mesh] OR "growth regulated oncogene alpha"[tiab] OR "growth regulated oncogene protein alpha"[tiab] OR "growth regulated alpha"[tiab] OR "growth related oncogene alpha"[tiab] OR GRO-alpha[tiab] OR GRO-a[tiab] OR GROalpha[tiab] OR GROa[tiab] OR GRO1[tiab] OR GRO-1[tiab] OR MGSA[tiab] OR FSP[tiab] OR NAP-3[tiab] OR NAP3[tiab] OR "CXC motif chemokine 1"[tiab] OR "C-X-C motif chemokine 1"[tiab] OR "CXC motif chemokine ligand 1"[tiab] OR "C-X-C motif chemokine ligand 1"[tiab] OR CXCL1[tiab] OR SCYB1[tiab]

#32 "Hepatocyte Growth Factor"[Mesh] OR "hepatocyte growth factor"[tiab] OR "scatter factor"[tiab] OR HGF[tiab]

#33 "Interferon alpha-2"[Mesh] OR "interferon alpha-2"[tiab] OR "interferon alpha-ii"[tiab] OR "interferon alpha 2a"[tiab] OR "interferon alpha-2b"[tiab] OR "alpha-2 interferon"[tiab] OR "alpha2 interferon"[tiab] OR "IFN-alpha 2"[tiab] OR "IFNalpha 2"[tiab] OR "IFN-alpha 2a"[tiab] OR "IFNalpha 2a"[tiab] OR "IFN-alpha 2b"[tiab] OR "IFNalpha 2b"[tiab] OR "LeIF A"[tiab] OR "interferon alphaA"[tiab] OR IFN-alphaA[tiab] OR IFNA[tiab] OR IFNA2[tiab] OR IFNA2A[tiab] OR IFNA2B[tiab] OR IFN-A[tiab] OR IFN-A2[tiab] OR IFN-A2A[tiab] OR IFN-A2B[tiab] OR "IFN-A 2A"[tiab] OR "IFN-A 2B"[tiab]

#34 "Leukemia Inhibitory Factor"[Mesh] OR "leukemia inhibitory factor"[tiab] OR "leukemia inhibiting factor"[tiab] OR "differentiation-stimulating factor"[tiab] OR "D factor"[tiab] OR "cholinergic differentiation factor"[tiab] OR LIF[tiab]

#35 "Chemokine CXCL10"[Mesh] OR "inducible protein 10"[tiab] OR "induced protein 10"[tiab] OR "CXC motif chemokine 10"[tiab] OR "C-X-C motif chemokine 10"[tiab] OR "CXC motif chemokine ligand 10"[tiab] OR "C-X-C motif chemokine ligand 10"[tiab] OR CXCL10[tiab] OR C7[tiab] OR IFI10[tiab] OR INP10[tiab] OR IP-10[tiab] OR SCYB10[tiab] OR crg-2[tiab] OR gIP-10[tiab] OR mob-1[tiab]

#36 "Chemokine CCL2"[Mesh] OR CCL2[tiab] OR "monocyte chemoattractant protein-1"[tiab] OR "monocyte chemotactic protein-1"[tiab] OR MCP-1[tiab] OR MCAF[tiab]

#37 "Chemokine CXCL9"[Mesh] OR "CXC motif chemokine 9"[tiab] OR "C-X-C motif chemokine 9"[tiab] OR "CXC motif chemokine ligand 9"[tiab] OR "C-X-C motif chemokine ligand 9"[tiab] OR CXCL9[tiab] OR CMK[tiab] OR HuMIG[tiab] OR MIG[tiab] OR SCYB9[tiab] OR crg-10[tiab]

#38 "Nerve Growth Factor"[Mesh] OR "beta nerve growth factor"[tiab] OR "nerve growth factor beta"[tiab] OR beta-NGF[tiab] OR NGF-beta[tiab] OR NGF-1beta[tiab]

#39 "Chemokine CCL7"[Mesh] OR CCL7[tiab] OR "monocyte chemoattractant protein-3"[tiab] OR "monocyte chemotactic protein-3"[tiab] OR MCP-3[tiab]

#40 "Stem Cell Factor"[Mesh] OR "stem cell factor"[tiab] OR SCF[tiab] OR "KIT ligand"[tiab] OR KITLG[tiab] OR FPH2[tiab] OR FPHH[tiab] OR KL-1[tiab] OR Kitl[tiab] OR MGF[tiab] OR SF[tiab] OR SHEP7[tiab] OR DCUA[tiab] OR DFNA69[tiab] OR SLF[tiab] OR SCGF-beta[tiab] OR "stem cell growth factor-beta"[tiab]

#41 "Chemokine CXCL12"[Mesh] OR "CXC motif chemokine 12"[tiab] OR "C-X-C motif chemokine 12"[tiab] OR "CXC motif chemokine ligand 12"[tiab] OR "C-X-C motif chemokine ligand 12"[tiab] OR CXCL12[tiab] OR "stromal cell-derived factor 1"[tiab] OR "stromal cell-derived factor 1alpha"[tiab] OR SDF-1[tiab] OR SDF-1a[tiab] OR SDF1[tiab] OR IRH[tiab] OR PBSF[tiab] OR SCYB12[tiab] OR TLSF[tiab] OR TPAR1[tiab]

#42 "Chemokine CCL3"[Mesh] OR "macrophage inflammatory protein 1alpha"[tiab] OR "macrophage inflammatory protein 1-alpha"[tiab] OR CCL3[tiab] OR LD78alpha[tiab]

#43 "Chemokine CCL4"[Mesh] OR CCL4[tiab] OR "macrophage inflammatory protein-1 beta"[tiab] OR "macrophage inflammatory protein-1beta"[tiab] OR MIP-1*[tiab]

#44 "Becaplermin"[Mesh] OR becaplermin[tiab] OR "platelet-derived growth factor-BB"[tiab] OR PDGF-BB[tiab]

#45 "Chemokine CCL5"[Mesh] OR CCL5[tiab] OR "normal T cell expressed and secreted"[tiab] OR RANTES[tiab]

#46 "Vascular Endothelial Growth Factors"[Mesh] OR "vascular endothelial growth factor*"[tiab] OR vasculotropin[tiab] OR "vascular permeability factor"[tiab] OR VEGF*[tiab]

#47 "Chemokine CCL27"[Mesh] OR CCL27[tiab] OR "cutaneous T cell-attracting chemokine"[tiab] OR CTACK[tiab]

#48 "Macrophage Migration-Inhibitory Factors"[Mesh] OR "macrophage migration-inhibitory factor*"[tiab] OR "macrophage migration-inhibition factor*"[tiab] OR MIF[tiab]

#49 "Receptors, TNF-Related Apoptosis-Inducing Ligand"[Mesh] OR "TNF-related apoptosis inducing ligand"[tiab] OR (TRAIL[tiab] AND receptor*[tiab]) OR TNFRSF10[tiab] OR TNFRSF-10[tiab]

#50 "Macrophage Colony-Stimulating Factor"[Mesh] OR "colony-stimulating factor"[tiab] OR M-CSF[tiab] OR CSF-1[tiab] OR CSF-M[tiab]

#51 "Lymphotoxin-alpha"[Mesh] OR "tumor necrosis factor beta"[tiab] OR lymphotoxin[tiab] OR TNF-beta[tiab] OR TNF-b[tiab]

#52 "Fatty Acid-Binding Proteins"[Mesh] OR "intestinal fatty acid-binding protein (121-131), human"[Supplementary Concept] OR "intestinal fatty acid-binding protein (1-19), human"[Supplementary Concept] OR "intestinal fatty acid-binding protein (91-107), human"[Supplementary Concept] OR "intestinal fatty acid-binding protein"[tiab] OR "fatty acid binding protein 2" [tiab] OR FABP2[tiab] OR I-FABP[tiab]

#53 "Pancreatitis-Associated Proteins"[Mesh] OR "pancreatitis-associated protein*"[tiab] OR "regenerating islet derived protein"[tiab] OR PAP[tiab] OR REG3[tiab] OR REGIII[tiab]

#54 "Peroxidase"[Mesh] OR myeloperoxidase[tiab] OR MPO[tiab]

#55 "Acute-Phase Proteins"[Mesh] OR "acute-phase protein*"[tiab] OR "acute-phase glycoprotein*"[tiab] OR "acute-phase reactant"[tiab] OR APP[tiab]

#56 "Lithostathine"[Mesh] OR lithostathin*[tiab] OR "pancreatic stone protein"[tiab] OR "pancreatic thread protein"[tiab] OR "regenerating islet-derived 1"[tiab] OR PSP[tiab]

#57 "Mutagenicity Tests"[Mesh] OR "Sister Chromatid Exchange"[Mesh] OR "Lymphocytes"[Mesh] OR lymphocyte*[tiab] OR "white blood cell*"[tiab] OR "exfoliated cell*"[tiab] OR "cytokinesis block*"[tiab] OR "cytokinesis micronucl*"[tiab] OR "cytochalasin-B block*"[tiab] OR CBMN[tiab] OR "nucleoplasmic bridge*"[tiab] OR NPB[tiab] OR "nuclear bud*"[tiab] OR NBUD[tiab] OR "comet assay*"[tiab] OR "single-cell gel electrophoresis"[tiab] OR SCGE[tiab] OR "sister chromatid exchange*"[tiab] OR "micronucleus assay*"[tiab] OR "micronuclei assay*"[tiab] OR "micronucleus test*"[tiab] OR "micronuclei test*"[tiab] OR SCE[tiab] OR SCEs[tiab] OR MN[tiab]

#58 #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52 OR #53 OR #54 OR #55 OR #56 OR #57

#59 "Microplastics"[Mesh] OR microplastic*[title] OR nanoplastic*[title]

#60 micro*[title] OR nano*[title] OR ultrafine[title] OR ultra-fine[title] AND plastic*[title]

#61 "acrylonitrile butadiene styrene"[title]

#62 "poly(lactide)"[Supplementary Concept] OR "polylactic acid"[title] OR polylactide[title] OR polylactate[title] OR "poly(lactide)"[title] OR "poly(lactic acid)"[title] OR "lactic acid polymer"[title]

#63 "Polyethylene Terephthalates"[Mesh] OR "polyethylene terephthalate*"[title] OR "Poly(Ethylene Terephtalate)"[title] OR polyethyleneterephthalate[title] OR "ethylene polyterephthalate*"[title]

#64 "polycarbonate"[Supplementary Concept] OR polycarbon*[title]

#65 "Polystyrenes"[Mesh] OR polystyrene*[title] OR polystyrol*[title]

#66 "Polypropylenes"[Mesh] OR polypropylene*[title] OR "propene polymer*"[title] OR "propylene polymer*"[title] OR prolene[title] OR polypro[title]

#67 "Polyvinyl Chloride"[Mesh] OR polyvinylchloride[title] OR "polyvinyl chloride"[title] OR poly-vinyl-chloride[title] OR polychloroethylene[title] OR "vinyl chloride polymer"[title] OR "chloroethylene homopolymer"[title] OR "chloroethylene polymer"[title] OR polychlorovinyl[title] OR PVC[title]

#68 "Nylons"[Mesh] OR nylon*[title] OR polyamid*[title]

#69 "Polyvinyl Alcohol"[Mesh] OR "polyvinyl alcohol"[title] OR "poly (vinylalcohol)"[title] OR "poly-vinyl-alcohol"[title]

#70 "Polyethylenes"[Mesh] OR polyethylene*[title] OR polythene[title]

#71 "polyether sulfone"[Supplementary Concept] OR "polyether sulfone"[title] OR polyethersulfone[title]

#72 "polyacrylonitrile"[Supplementary Concept] OR polyacrylonitrile[title] OR "poly(acrylonitrile)"[title] OR "polyacrylic acid"[title] OR "poly(acrylic acid)"[title]

#73 "ethylene-vinyl acetate"[title] OR "polyethylene-vinyl acetate"[title] OR "vinyl acetate ethylene"[title]

#74 "Polytetrafluoroethylene"[Mesh] OR polytetrafluoroethylene[title] OR politef[title] OR polytef[title] OR GORE-TEX[title] OR GORETEX[title] OR teflon[title]

#75 polymethylacrylate[title] OR acrylic*[title]

#76 "Polyurethanes"[Mesh] OR polyurethan*[title] OR "urethan polymer"[title] OR "urethane polymer"[title] OR spandex[title] OR elastan*[title] OR lycra[title] OR "thermoplastic elastomer"[title]

#77 "styrene-ethylene-butylene co-polymer"[title] OR "styrene-ethylene-butylene-styrene"[title] OR "poly(styrene-ethylene-butylene-styrene)"[title]

#78 "Polymethyl Methacrylate"[Mesh] OR "polymethyl methacrylate"[title] OR polymethylmetacrylate[title] OR methylpolymetacrylate[title] OR polymethylenemethacrylate[title] OR "polymethylene methacrylate"[title] OR "polymethyl methylacrylate"[title] OR "methyl polymethacrylate"[title] OR plexiglas*[title]

#79 "Polyesters"[Mesh] OR polyester*[title] OR pile[title]

#80 "aromatic polyamide"[title] OR aramid[title] OR polyaramid[title]

#81 kevlar[title] OR viscose[title] OR rayon[title] OR acetate[title] OR lyocell[title] OR modal[title]

#82 #59 OR #60 OR #61 OR #62 OR #63 OR #64 OR #65 OR #66 OR #67 OR #68 OR #69 OR #70 OR #71 OR #72 OR #73 OR #74 OR #75 OR #76 OR #77 OR #78 OR #79 OR #80 OR #81

#83 #4 AND #58 AND #82

#84 "Animals"[Mesh] NOT "Humans"[Mesh]

#85 #83 NOT #84

Embase string

#1 'oxidative stress'/exp OR oxida*:ti OR antioxida*:ti

#2 'inflammation'/exp OR inflammat*:ti OR antiinflammat*:ti OR antinflammat*:ti

#3 'genotoxicity'/exp OR genotoxic*:ti OR geno-toxic*:ti OR 'genetic toxicity':ti OR toxigeni*:ti OR mutagen*:ti

#4 #1 OR #2 OR #3

#5 'biological marker'/exp OR biomarker*:ti,ab,kw OR marker*:ti,ab,kw OR level*:ti,ab,kw OR test:ti,ab,kw OR tests:ti,ab,kw

#6 'malonaldehyde'/exp OR malondialdehyde:ti,ab,kw OR malonylaldehyde:ti,ab,kw OR malonaldehyde:ti,ab,kw OR malonyldialdehyde:ti,ab,kw OR MDA:ti,ab,kw

#7 '8 hydroxydeoxyguanosine'/exp OR '8-hydroxy-2-deoxyguanosine':ti,ab,kw OR '8-hydroxy-deoxyguanosine':ti,ab,kw OR '8-hydroxydeoxyguanosine':ti,ab,kw OR '8-hydroxyguanine':ti,ab,kw OR '8-hydroxy-guanine':ti,ab,kw OR '8-Oxo-2-Deoxyguanosine':ti,ab,kw OR '8-Oxo-Deoxyguanosine':ti,ab,kw OR '8-oxo-dGuo':ti,ab,kw OR 8-Ohdg:ti,ab,kw OR 8OHdG:ti,ab,kw OR 8-OH-dG:ti,ab,kw OR 8-ohg:ti,ab,kw OR 8-hydroxy-g:ti,ab,kw OR 8-hydroxy-dg:ti,ab,kw OR 8-oxodG:ti,ab,kw OR 8-oxodGuo:ti,ab,kw OR 8-oxo-dG:ti,ab,kw OR 8-OH-2dG*:ti,ab,kw OR 8-isoprostane*:ti,ab,kw

#8 'isoprostane derivative'/exp OR IsoP:ti,ab,kw OR F2-isoprostane*:ti,ab,kw

#9 'prostaglandin F2 alpha'/exp OR dinoprost:ti,ab,kw OR 15-f2t-isop:ti,ab,kw OR 8-iso-PGF2a:ti,ab,kw OR 8-isoprostaglandin-f2:ti,ab,kw OR 8-iso-prostaglandin-f2:ti,ab,kw OR 8-iso-PGF2a:ti,ab,kw OR 8-epi-prostaglandin-F2alpha:ti,ab,kw OR 8-epi-prostaglandin-f2alpha:ti,ab,kw OR 8-epiprostaglandin-f2alpha:ti,ab,kw OR 8-epi-PGF2alpha:ti,ab,kw

#10 'allantoin'/exp OR allantoin*:ti,ab,kw OR dioxo-4-imidazolidinyl*:ti,ab,kw OR glyoxyldiureide*:ti,ab,kw OR 5-ureidohydantoin*:ti,ab,kw

#11 'total antioxidant capacity'/exp OR 'total antioxidant capacity':ti,ab,kw OR 'total anti-oxidant capacity':ti,ab,kw OR 'total antioxidant power':ti,ab,kw OR TAC:ti,ab,kw OR ToAC:ti,ab,kw OR 'total anti-oxidant power':ti,ab,kw OR TAP:ti,ab,kw

#12 'trolox equivalent antioxidant capacity'/exp OR 'trolox equivalent antioxidant capacity':ti,ab,kw OR 'trolox equivalent anti-oxidant capacity':ti,ab,kw OR TEAC:ti,ab,kw

#13 'thiobarbituric acid reactive substance'/exp OR TBARS:ti,ab,kw OR 'thiobarbituric acid reactive substance*':ti,ab,kw

#14 'glutathione'/exp OR 'glutathione peroxidase'/exp OR glutathion*:ti,ab,kw OR GSH:ti,ab,kw OR GSSH:ti,ab,kw OR 'GSH/GSSG':ti,ab,kw OR GPX:ti,ab,kw

#15 'uric acid'/exp OR 'uric acid':ti,ab,kw OR UA:ti,ab,kw

#16 'superoxide dismutase'/exp OR dismutase*:ti,ab,kw OR SOD:ti,ab,kw

#17 'antioxidant assay'/exp OR 'lipid peroxide'/exp OR 'lipid peroxid*':ti,ab,kw OR hydroperoxid*:ti,ab,kw OR lipoperoxid*:ti,ab,kw OR hydro-peroxid*:ti,ab,kw OR lipo-peroxid*:ti,ab,kw

#18 'ferric reducing antioxidant power'/exp OR 'ferric reducing':ti,ab,kw OR 'ferric ion reducing':ti,ab,kw OR FRAP:ti,ab,kw

#19 'oxygen radical absorbance capacity'/exp OR 'oxygen radical absor*':ti,ab,kw OR ORAC:ti,ab,kw

#20 'cupric reducing antioxidant capacity'/exp OR 'cupric reducing' OR CUPRAC:ti,ab,kw

#21 "hydroxyl radical":ti,ab,kw OR HORAC:ti,ab,kw

#22 'potassium ferricyanide reducing power':ti,ab,kw OR PFRAP:ti,ab,kw

#23 'total radical-trapping antioxidant parameter'/exp OR 'total peroxyl radical trapping':ti,ab,kw OR 'total reactive antioxidant potential':ti,ab,kw OR 'total reactive anti-oxidant potential':ti,ab,kw OR TRAP:ti,ab,kw

#24 '1,1 diphenyl 2 picrylhydrazyl'/exp OR picrylhydrazyl:ti,ab,kw OR picryl-hydrazyl:ti,ab,kw OR DPPH:ti,ab,kw

#25 'total oxyradical scavenging capacity':ti,ab,kw OR TOSC:ti,ab,kw

#26 'fibroblast growth factor'/de OR 'fibroblast growth factor 2'/exp OR 'fibroblast growth factor basic':ti,ab,kw OR 'basic fibroblast growth factor':ti,ab,kw OR 'fibroblast growth factor-2':ti,ab,kw OR 'FGF basic':ti,ab,kw OR bFGF:ti,ab,kw OR b-FGF:ti,ab,kw OR FGF-b:ti,ab,kw OR FGF-2:ti,ab,kw OR FGF2:ti,ab,kw OR HBGF-2:ti,ab,kw OR HBGF2:ti,ab,kw

#27 'eotaxin'/exp OR 'eotaxin 2'/exp OR 'eotaxin 3'/exp OR eotaxin*:ti,ab,kw OR CCL11:ti,ab,kw OR CCL24:ti,ab,kw OR CCL26:ti,ab,kw

#28 'granulocyte colony stimulating factor'/exp OR 'granulocyte colony-stimulating factor':ti,ab,kw OR G-CSF:ti,ab,kw OR GCSF:ti,ab,kw OR 'granulocyte macrophage colony stimulating factor'/exp OR 'granulocyte macrophage':ti,ab,kw OR GM-CSF:ti,ab,kw OR CSF-2:ti,ab,kw OR CSF-GM:ti,ab,kw

#29 'gamma interferon'/exp OR 'interferon-gamma':ti,ab,kw OR 'interferon-g':ti,ab,kw OR 'gamma-interferon':ti,ab,kw OR 'interferon type 2':ti,ab,kw OR 'interferon type II':ti,ab,kw OR IFN-gamma:ti,ab,kw OR IFN-g:ti,ab,kw

#30 'interleukin derivative'/exp OR 'interleukin 1'/exp OR 'interleukin 1alpha'/exp OR 'interleukin 1beta'/exp OR 'interleukin 1 receptor blocking agent'/exp OR interleukin*:ti,ab,kw OR IL-1beta:ti,ab,kw OR IL-1b:ti,ab,kw OR IL1beta:ti,ab,kw OR IL1b:ti,ab,kw OR IL-1ra:ti,ab,kw OR IL1ra:ti,ab,kw OR IL-1alpha:ti,ab,kw OR IL-1a:ti,ab,kw OR IL1alpha:ti,ab,kw OR IL1a:ti,ab,kw OR IL-2Ralpha:ti,ab,kw OR IL-2Ra:ti,ab,kw OR IL2Ralpha:ti,ab,kw OR IL2Ra:ti,ab,kw OR 'interleukin 3'/exp OR IL-3:ti,ab,kw OR IL3:ti,ab,kw OR 'interleukin 12'/exp OR 'interleukin 12p40'/exp OR 'interleukin 12p70'/exp OR IL-12:ti,ab,kw OR IL12:ti,ab,kw OR P70:ti,ab,kw OR P40:ti,ab,kw OR 12p40:ti,ab,kw OR 'interleukin 16'/exp OR IL-16:ti,ab,kw OR IL16:ti,ab,kw OR 'interleukin 2'/exp OR IL-2:ti,ab,kw OR IL2:ti,ab,kw OR 'interleukin 4'/exp OR IL-4:ti,ab,kw OR IL4:ti,ab,kw OR 'interleukin 5'/exp OR IL-5:ti,ab,kw OR IL5:ti,ab,kw OR 'interleukin 6'/exp OR IL-6:ti,ab,kw OR IL6:ti,ab,kw OR 'interleukin 7'/exp OR IL-7:ti,ab,kw OR IL7:ti,ab,kw OR 'interleukin 8'/exp OR IL-8:ti,ab,kw OR IL8:ti,ab,kw OR 'interleukin 9'/exp OR IL-9:ti,ab,kw OR IL9:ti,ab,kw OR 'interleukin 10'/exp OR IL-10:ti,ab,kw OR IL10:ti,ab,kw OR 'interleukin 13'/exp OR IL-13:ti,ab,kw OR IL13:ti,ab,kw OR 'interleukin 15'/exp OR IL-15:ti,ab,kw OR IL15:ti,ab,kw OR 'interleukin 17'/exp OR IL-17A:ti,ab,kw OR IL17A:ti,ab,kw OR 'interleukin 18'/exp OR IL-18:ti,ab,kw OR IL18:ti,ab,kw

#31 'growth regulated oncogene alpha'/exp OR 'CXCL1 chemokine'/exp OR 'growth regulated oncogene alpha':ti,ab,kw OR 'growth regulated oncogene protein alpha':ti,ab,kw OR 'growth regulated alpha':ti,ab,kw OR 'growth related oncogene alpha':ti,ab,kw OR GRO-alpha:ti,ab,kw OR GRO-a:ti,ab,kw OR GROalpha:ti,ab,kw OR GROa:ti,ab,kw OR GRO1:ti,ab,kw OR GRO-1:ti,ab,kw OR MGSA:ti,ab,kw OR FSP:ti,ab,kw OR NAP-3:ti,ab,kw OR NAP3:ti,ab,kw OR 'CXC motif chemokine 1':ti,ab,kw OR 'C-X-C motif chemokine 1':ti,ab,kw OR 'CXC motif chemokine ligand 1':ti,ab,kw OR 'C-X-C motif chemokine ligand 1':ti,ab,kw OR CXCL1:ti,ab,kw OR SCYB1:ti,ab,kw

#32 'scatter factor'/exp OR 'hepatocyte growth factor':ti,ab,kw OR 'scatter factor':ti,ab,kw OR HGF:ti,ab,kw

#33 'alpha2 interferon'/exp OR 'interferon alpha-2':ti,ab,kw OR 'interferon alpha-ii':ti,ab,kw OR 'interferon alpha 2a':ti,ab,kw OR 'interferon alpha-2b':ti,ab,kw OR 'alpha-2 interferon':ti,ab,kw OR 'alpha2 interferon':ti,ab,kw OR 'IFN-alpha 2':ti,ab,kw OR 'IFNalpha 2':ti,ab,kw OR 'IFN-alpha 2a':ti,ab,kw OR 'IFNalpha 2a':ti,ab,kw OR 'IFN-alpha 2b':ti,ab,kw OR 'IFNalpha 2b':ti,ab,kw OR 'LeIF A':ti,ab,kw OR 'interferon alphaA':ti,ab,kw OR IFN-alphaA:ti,ab,kw OR IFNA:ti,ab,kw OR IFNA2:ti,ab,kw OR IFNA2A:ti,ab,kw OR IFNA2B:ti,ab,kw OR IFN-A:ti,ab,kw OR IFN-A2:ti,ab,kw OR IFN-A2A:ti,ab,kw OR IFN-A2B:ti,ab,kw OR 'IFN-A 2A':ti,ab,kw OR 'IFN-A 2B':ti,ab,kw

#34 'leukemia inhibitory factor'/exp OR 'leukemia inhibitory factor':ti,ab,kw OR 'leukemia inhibiting factor':ti,ab,kw OR 'differentiation-stimulating factor':ti,ab,kw OR 'D factor':ti,ab,kw OR 'cholinergic differentiation factor':ti,ab,kw OR LIF:ti,ab,kw

#35 'gamma interferon inducible protein 10'/exp OR 'inducible protein 10':ti,ab,kw OR 'induced protein 10':ti,ab,kw OR 'CXC motif chemokine 10':ti,ab,kw OR 'C-X-C motif chemokine 10':ti,ab,kw OR 'CXC motif chemokine ligand 10':ti,ab,kw OR 'C-X-C motif chemokine ligand 10':ti,ab,kw OR CXCL10:ti,ab,kw OR C7:ti,ab,kw OR IFI10:ti,ab,kw OR INP10:ti,ab,kw OR IP-10:ti,ab,kw OR SCYB10:ti,ab,kw OR crg-2:ti,ab,kw OR gIP-10:ti,ab,kw OR mob-1:ti,ab,kw

#36 'monocyte chemotactic protein 1'/exp OR CCL2:ti,ab,kw OR 'monocyte chemoattractant protein-1':ti,ab,kw OR 'monocyte chemotactic protein-1':ti,ab,kw OR MCP-1:ti,ab,kw OR MCAF:ti,ab,kw

#37 'CXCL9 chemokine'/exp OR 'CXC motif chemokine 9':ti,ab,kw OR 'C-X-C motif chemokine 9':ti,ab,kw OR 'CXC motif chemokine ligand 9':ti,ab,kw OR 'C-X-C motif chemokine ligand 9':ti,ab,kw OR CXCL9:ti,ab,kw OR CMK:ti,ab,kw OR HuMIG:ti,ab,kw OR MIG:ti,ab,kw OR SCYB9:ti,ab,kw OR crg-10:ti,ab,kw

#38 'nerve growth factor beta subunit'/exp OR 'beta nerve growth factor':ti,ab,kw OR 'nerve growth factor beta':ti,ab,kw OR beta-NGF:ti,ab,kw OR NGF-beta:ti,ab,kw OR NGF-1beta:ti,ab,kw

#39 'monocyte chemotactic protein 3'/exp OR CCL7:ti,ab,kw OR 'monocyte chemoattractant protein-3':ti,ab,kw OR 'monocyte chemotactic protein-3':ti,ab,kw OR MCP-3:ti,ab,kw

#40 'stem cell factor'/exp OR 'stem cell factor':ti,ab,kw OR SCF:ti,ab,kw OR 'KIT ligand':ti,ab,kw OR KITLG:ti,ab,kw OR FPH2:ti,ab,kw OR FPHH:ti,ab,kw OR KL-1:ti,ab,kw OR Kitl:ti,ab,kw OR MGF:ti,ab,kw OR SF:ti,ab,kw OR SHEP7:ti,ab,kw OR DCUA:ti,ab,kw OR DFNA69:ti,ab,kw OR SLF:ti,ab,kw OR SCGF-beta:ti,ab,kw OR 'stem cell growth factor-beta':ti,ab,kw

#41 'stromal cell derived factor 1'/exp OR 'CXC motif chemokine 12':ti,ab,kw OR 'C-X-C motif chemokine 12':ti,ab,kw OR 'CXC motif chemokine ligand 12':ti,ab,kw OR 'C-X-C motif chemokine ligand 12':ti,ab,kw OR CXCL12:ti,ab,kw OR 'stromal cell-derived factor 1':ti,ab,kw OR 'stromal cell-derived factor 1alpha':ti,ab,kw OR SDF-1:ti,ab,kw OR SDF-1a:ti,ab,kw OR SDF1:ti,ab,kw OR IRH:ti,ab,kw OR PBSF:ti,ab,kw OR SCYB12:ti,ab,kw OR TLSF:ti,ab,kw OR TPAR1:ti,ab,kw

#42 'macrophage inflammatory protein 1alpha'/exp OR 'macrophage inflammatory protein 1alpha':ti,ab,kw OR 'macrophage inflammatory protein 1-alpha':ti,ab,kw OR CCL3:ti,ab,kw OR LD78alpha:ti,ab,kw

#43 'macrophage inflammatory protein 1beta'/exp OR CCL4:ti,ab,kw OR 'macrophage inflammatory protein-1 beta':ti,ab,kw OR 'macrophage inflammatory protein-1beta':ti,ab,kw OR MIP-1*:ti,ab,kw

#44 'becaplermin'/exp OR becaplermin:ti,ab,kw OR 'platelet-derived growth factor-BB':ti,ab,kw OR PDGF-BB:ti,ab,kw

#45 'RANTES'/exp OR CCL5:ti,ab,kw OR 'normal T cell expressed and secreted':ti,ab,kw OR RANTES:ti,ab,kw

#46 'vasculotropin'/exp OR 'vascular endothelial growth factor*':ti,ab,kw OR vasculotropin:ti,ab,kw OR 'vascular permeability factor':ti,ab,kw OR VEGF*:ti,ab,kw

#47 'cutaneous T cell attracting chemokine'/exp OR CCL27:ti,ab,kw OR 'cutaneous T cell-attracting chemokine':ti,ab,kw OR CTACK:ti,ab,kw

#48 'macrophage migration inhibition factor'/exp OR 'macrophage migration-inhibitory factor*':ti,ab,kw OR 'macrophage migration-inhibition factor*':ti,ab,kw OR MIF:ti,ab,kw

#49 'tumor necrosis factor related apoptosis inducing ligand receptor'/exp OR 'TNF-related apoptosis inducing ligand':ti,ab,kw OR (TRAIL:ti,ab,kw AND receptor*:ti,ab,kw) OR TNFRSF10:ti,ab,kw OR TNFRSF-10:ti,ab,kw

#50 'colony stimulating factor 1'/exp OR 'colony-stimulating factor':ti,ab,kw OR M-CSF:ti,ab,kw OR CSF-1:ti,ab,kw OR CSF-M:ti,ab,kw

#51 'lymphotoxin'/exp OR 'tumor necrosis factor beta':ti,ab,kw OR lymphotoxin:ti,ab,kw OR TNF-beta:ti,ab,kw OR TNF-b:ti,ab,kw

#52 'fatty acid binding protein 2'/exp OR 'intestinal fatty acid-binding protein':ti,ab,kw OR 'fatty acid binding protein 2':ti,ab,kw OR FABP2:ti,ab,kw OR I-FABP:ti,ab,kw

#53 'pancreatitis associated protein'/exp OR 'pancreatitis-associated protein*':ti,ab,kw OR 'regenerating islet derived protein':ti,ab,kw OR PAP:ti,ab,kw OR REG3:ti,ab,kw OR REGIII:ti,ab,kw

#54 'myeloperoxidase'/exp OR myeloperoxidase:ti,ab,kw OR MPO:ti,ab,kw

#55 'acute phase protein'/exp OR 'acute-phase protein*':ti,ab,kw OR 'acute-phase glycoprotein*':ti,ab,kw OR 'acute-phase reactant':ti,ab,kw OR APP:ti,ab,kw

#56 'lithostathine'/exp OR lithostathin*:ti,ab,kw OR 'pancreatic stone protein':ti,ab,kw OR 'pancreatic thread protein':ti,ab,kw OR 'regenerating islet-derived 1':ti,ab,kw OR PSP:ti,ab,kw

#57 'mutagen testing'/exp OR 'sister chromatid exchange'/exp OR 'cytokinesis blocked micronucleus assay'/exp OR 'lymphocyte'/exp OR lymphocyte*:ti,ab,kw OR 'white blood cell*':ti,ab,kw OR 'exfoliated cell*':ti,ab,kw OR 'cytokinesis block*':ti,ab,kw OR 'cytokinesis micronucl*':ti,ab,kw OR 'cytochalasin-B block*':ti,ab,kw OR CBMN:ti,ab,kw OR 'nucleoplasmic bridge*':ti,ab,kw OR NPB:ti,ab,kw OR 'nuclear bud*':ti,ab,kw OR NBUD:ti,ab,kw OR 'comet assay*':ti,ab,kw OR 'single-cell gel electrophoresis':ti,ab,kw OR SCGE:ti,ab,kw OR 'sister chromatid exchange*':ti,ab,kw OR 'micronucleus assay*':ti,ab,kw OR 'micronuclei assay*':ti,ab,kw OR 'micronucleus test*':ti,ab,kw OR 'micronuclei test*':ti,ab,kw OR SCE:ti,ab,kw OR SCEs:ti,ab,kw OR MN:ti,ab,kw

#58 #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52 OR #53 OR #54 OR #55 OR #56 OR #57

#59 'microplastic'/exp OR 'microplastic pollution'/exp OR microplastic*:ti OR nanoplastic*:ti

#60 micro*:ti OR nano*:ti OR ultrafine:ti OR ultra-fine:ti AND plastic*:ti

#61 'acrylonitrile butadiene styrene'/exp OR 'acrylonitrile butadiene styrene':ti

#62 'polylactic acid'/exp OR 'polylactic acid':ti OR polylactide:ti OR polylactate:ti OR 'poly(lactide)':ti OR 'poly(lactic acid)':ti OR 'lactic acid polymer':ti

#63 'polyethylene terephthalate'/exp OR 'polyethylene terephthalate*':ti OR 'Poly(Ethylene Terephtalate)':ti OR polyethyleneterephthalate:ti OR 'ethylene polyterephthalate*':ti

#64 'polycarbonate'/exp OR polycarbon*:ti

#65 'polystyrene derivative'/exp OR polystyrene*:ti OR polystyrol*:ti

#66 'polypropylene'/exp OR polypropylene*:ti OR 'propene polymer*':ti OR 'propylene polymer*':ti OR prolene:ti OR polypro:ti

#67 'polyvinylchloride'/exp OR polyvinylchloride:ti OR "polyvinyl chloride":ti OR poly-vinyl-chloride:ti OR polychloroethylene:ti OR "vinyl chloride polymer":ti OR "chloroethylene homopolymer":ti OR "chloroethylene polymer":ti OR polychlorovinyl:ti OR PVC:ti

#68 'nylon'/exp OR nylon*:ti OR polyamid*:ti

#69 'polyvinyl alcohol'/exp OR 'polyvinyl alcohol':ti OR 'poly (vinylalcohol)':ti OR 'poly-vinyl-alcohol':ti

#70 'polyethylene derivative'/exp OR polyethylene*:ti OR polythene:ti

#71 'polyethersulfone'/exp OR 'polyether sulfone':ti OR polyethersulfone:ti

#72 'polyacrylonitrile'/exp OR polyacrylonitrile:ti OR 'poly(acrylonitrile)':ti OR 'polyacrylic acid':ti OR 'poly(acrylic acid)':ti

#73 'ethylene vinyl acetate copolymer'/exp OR 'ethylene-vinyl acetate':ti OR 'polyethylene-vinyl acetate':ti OR 'vinyl acetate ethylene':ti

#74 'polytetrafluoroethylene'/exp OR polytetrafluoroethylene:ti OR politef:ti OR polytef:ti OR GORE-TEX:ti OR GORETEX:ti OR teflon:ti

#75 'polymethylacrylate'/exp OR polymethylacrylate:ti OR acrylic*:ti

#76 'polyurethan'/exp OR polyurethan*:ti OR 'urethan polymer':ti OR 'urethane polymer':ti OR spandex:ti OR elastan*:ti OR lycra:ti OR 'thermoplastic elastomer'/exp OR 'thermoplastic elastomer':ti

#77 'styrene-ethylene-butylene co-polymer':ti OR 'styrene-ethylene-butylene-styrene':ti OR 'poly(styrene-ethylene-butylene-styrene)':ti

#78 'poly(methyl methacrylate)'/exp OR 'polymethyl methacrylate':ti OR polymethylmetacrylate:ti OR methylpolymetacrylate:ti OR polymethylenemethacrylate:ti OR 'polymethylene methacrylate':ti OR 'polymethyl methylacrylate':ti OR 'methyl polymethacrylate':ti OR plexiglas*:ti

#79 'polyester'/exp OR polyester*:ti OR pile:ti

#80 'aromatic polyamide'/exp OR 'aromatic polyamide':ti OR aramid:ti OR polyaramid:ti

#81 kevlar:ti OR viscose:ti OR rayon:ti OR acetate:ti OR lyocell:ti OR modal:ti

#82 #59 OR #60 OR #61 OR #62 OR #63 OR #64 OR #65 OR #66 OR #67 OR #68 OR #69 OR #70 OR #71 OR #72 OR #73 OR #74 OR #75 OR #76 OR #77 OR #78 OR #79 OR #80 OR #81

#83 #4 AND #58 AND #82

#84 ('animal'/de OR 'animal experiment'/exp) NOT ('human'/exp OR 'human experiment'/exp)

#85 #83 NOT #84

#86 #85 NOT [conference abstract]/lim

9. Publications in extenso

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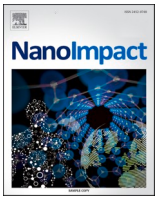

Nano- and microplastics: a comprehensive review on their exposure routes, translocation, and fate in humans

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ABSTRACT

Contamination of the environment with nano-and microplastic particles (NMPs) and its putative adverse effects on organisms, ecosystems, and human health is gaining increasing scientific and public attention. Various studies show that NMPs occur abundantly within the environment, leading to a high likelihood of human exposure to NMPs. Here, different exposure scenarios can occur. The most notable exposure routes of NMPs into the human body are via the airways and gastrointestinal tract (GIT) through inhalation or ingestion, but also via the skin due to the use of personal care products (PCPs) containing NMPs. Once NMPs have entered the human body, it is possible that they are translocated from the exposed organ to other body compartments. In our review article, we combine the current knowledge on the (1) exposure routes of NMPs to humans with the basic understanding of the potential (2) translocation mechanisms into human tissues and, consequently, their (3) fate within the human body. Regarding the (1) exposure routes, we reviewed the current knowledge on the occurrence of NMPs in food, beverages, personal care products and the air (focusing on indoors and workplaces) and found that the studies suggest an abundant presence of MPs within the exposure scenarios. The overall abundance of MPs in exposure matrices relevant to humans highlights the importance of understanding whether NMPs have the potential for tissue translocation. Therefore, we describe the current knowledge on the potential (2) translocation pathways of NMPs from the skin, GIT and respiratory systems to other body compartments. Here, particular attention was paid to how likely NMPs can translocate from the primary exposed organs to secondary organs due to naturally occurring defence mechanisms against tissue translocation. Based on the current understanding, we conclude that a dermal translocation of NMPs is rather unlikely. In contrast, small MPs and NPs can generally translocate from the GIT and respiratory system to other tissues. Thus, we reviewed the existing literature on the (3) fate of

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NMPs within the human body. Based on the current knowledge of the contamination of human exposure routes and the potential translocation mechanisms, we critically discuss the size of the detected particles reported in the fate studies. In some cases, the particles detected in human tissue samples exceed the size of a particle to overcome biological barriers allowing particle translocation into tissues. Therefore, we emphasize the importance of critically reading and discussing the presented results of NMP in human tissue samples.

1. Introduction

The overall increase in single-use throw-away plastic products and packaging has led to a tenfold increase in plastics in municipal solid waste from 1960 until 2005 (Geyer et al., 2017; Jambeck et al., 2015; Lebreton and Andrady, 2019), and has even accelerated during the SARS-CoV-2 pandemic (Klemeš et al., 2020; Vanapalli et al., 2021). This increase in plastic waste is further accompanied by more plastic litter in the environment (GESAMP, 2016; Katare et al., 2022).

Once plastics enter the environment, the properties which make them useful turn into a threat to the environment. For instance, the longevity of plastics leads to plastic accumulation in the environment that is expected to persist for hundreds to thousands of years depending on the plastic type (Barnes et al., 2009). However, due to UV radiation, mechanical and biological degradation, larger plastic items can brittle into ever smaller particles (Barnes et al., 2009). Recently, it has been shown that degradation, for instance of polystyrene (PS), is a two-stage process where photooxidation at the near-surface layer is the first step followed by microcrack formation and particle rupturing, leading to the formation of a multitude of even smaller particles (Meides et al., 2021). Thompson et al., 2004 introduced the term microplastics (MPs), which has later been described as all plastic particles smaller than 5 mm in diameter (Arthur et al., 2009). Although there is no official lower size limit of MPs, 1 μm is widely accepted nowadays, and particles smaller than 1 μm are usually termed nanoplastics (NPs) (Gigault et al., 2018; Hartmann et al., 2019). Although MPs have been detected abundantly in the environment, detection and identification of NPs is still very challenging, mainly due to methodological and analytical limitations for detecting NMPs in environmental samples and biological matrices. This aspect has been comprehensively reviewed elsewhere (e.g., Chen et al., 2020; Möller et al., 2020; O'Connor et al., 2019; Schwaferts et al., 2019).

However, the number of NMPs occurring in nature increases with decreasing particle sizes (Hale et al., 2020). Yet, the overall occurrence of NMPs and their small sizes is a potential health risk for organisms. The risk of accidental ingestion or inhalation is much greater for smaller particles than larger particles. In addition, as particle size decreases, the surface area to mass ratio increases. Consequently, the reactivity and toxicity of particles increases, making subsequent interactions with biological barriers more likely (Buzea et al., 2007). Although NMPs have been present in the environment for several decades (Carpenter and Smith, 1972), they are regarded as a rather newly introduced environmental particulate stressors. Furthermore, as NMPs are a highly diverse group of contaminants with various physicochemical properties, overall conclusions on the potential adverse health effects of NMPs are challenging. However, first attempts to perform a risk assessment of NMPs for humans were conducted, which will be discussed later.

Studies on ingestion and subsequent translocation of NMPs in different organisms in nature (Barboza et al., 2020) and laboratory studies (Galloway et al., 2017; Yong et al., 2020) have raised concern about putative adverse effects of NMPs, even to humans (Prata et al., 2020; Wright and Kelly, 2017). Prata et al. (2020) highlighted that upon exposure and uptake, the potential toxicity of NMPs may result from oxidative stress and inflammation, which consequently could disrupt the immune and nervous system. NMPs from the environment may not solely be coated with an eco-corona which is known for enhancing the cellular uptake (Ramsperger et al., 2020) but also with potentially pathogenic microorganisms (Gkoutselis et al., 2021; Kettner et al., 2019; Kirstein et al., 2016; Weig et al., 2021). The accumulation of pathogens on the surface of NMPs, exceeding the concentration of the surrounding media, may lead to a health threat upon uptake of an increased pathogen load on the particles by organisms.

The number of studies concerning the potential effects of NMPs on an environmental and organismal level steadily increases (Gabriel et al., 2015). In contrast, research on human exposure and toxicity is a relatively new field in NMP research. Nevertheless, there is a growing number of articles addressing the exposure of humans to NMP (Cox et al., 2019; Senathirajah et al., 2021) and their potential health risks (see, e.g. Prata et al., 2020; Rahman et al., 2021; Wright and Kelly, 2017). However, most review articles either focus on a specific exposure route (e.g., Chen et al., 2019; Danopoulos et al., 2020; Mercogliano et al., 2020; Peixoto et al., 2019; Yuan et al., 2022; Zhang et al., 2020) or the potential adverse health effects of NMP to humans upon exposure (Campanale et al., 2020; Danopoulos et al., 2021; Huang et al., 2021; Vethaak and Legler, 2021). In our review article, we combine the current knowledge on the contamination levels of the three major (1) exposure routes of NMPs to humans with the basic understanding of the potential (2) translocation mechanisms into human tissues and, consequently, their (3) fate within the human body. Regarding the (1) exposure scenarios, we reviewed the current knowledge on the occurrence of NMPs in food, beverages, personal care products (PCPs) and the air (focusing on indoors and workplaces). To avoid redundancies to other review articles describing the exposure levels of NMPs to humans, we focused on studies published after 2015. Furthermore, we describe the current knowledge on the potential (2) translocation pathways of NMPs from the primarily exposed organs (skin, gastrointestinal tract (GIT) and lung) into human tissues. Particular attention was paid to the mechanisms that allow particles to translocate into tissues and how likely the translocation from the primary exposed organs to secondary organs is. Based on the presented results of the NMP contamination in the different exposure scenarios and the current understanding of the potential translocation pathways, we critically discuss the significance of the described NMP in the (3) fate studies.

Since there is little to no data on the contamination of the environment and organisms with NPs, we mainly refer to MPs in our review article. We use the abbreviations MPs (5 mm – 1 μm) or NPs (<1 μm) to indicate the size class in the respective sections summarized and discussed. For more general statements, we use the abbreviation NMPs.

2. Methods of literature research

To avoid redundancy to other review articles, we only included studies from 2015 for the (1) exposure scenarios. To describe the potential (2) translocation mechanisms of NMPs from primary exposed organs (lung, GIT and skin) to other tissues and secondary organs, we did not set a threshold for the year of publication since the general understanding of the mechanisms requires fundamental literature. Since the topic of the (3) fate of NMPs in human tissue samples is a relatively new field of research, we included all studies published so far in the sense of NMPs.

We used *Google Scholar, ISI Web of Knowledge/Web of Science, Scopus, PubMed*, and *Embase* as databases. The common search terms for all chapters were: *microplastic*, nanoplastic*,* and *human exposure*. For the more specific chapters, we included the following search terms: *drinking water and beverages* for NMP in drinking water; *meat, fish, seafood, edible tissue, vegetables, milk, egg, roots and tubers, plants and herbs, confectionary, honey, sugar, salt, cereal, rice, maize, wheat, barky, spelt, rye, oat,*

sorghum, millet, teabag, oil, olive oil, vegetable oil, and *palm oil* for the NMP in food chapter; *atmosphere, atmospheric*, and *air* in the NMP inhalation chapter; and *cosmetics, personal care products, contraceptive, eye, contact lenses*, and *ocular surface* for the PCP chapter. We were using the additional search terms *human tissue* and *organs* in the fate chapter. No studies were excluded.

3. Human exposure to NMPs

Since MPs have been detected abundantly in the environment, the exposure of human beings to NMPs is highly likely (Prata et al., 2021). There are numerous routes of exposure through which humans can come into contact with NMPs. Here we summarize the current knowledge on the contamination with NMPs of drinking water and beverages, the most relevant food items, and indoor air. Furthermore, we address polymers intentionally added as ingredient in PCPs designed for direct application on the human body.

3.1. Drinking water and beverages

Water is essential to sustain human life, and we consume water as plain drinking water as well as in other beverages and in food. Although there are guidelines for drinking water quality (WHO, 2017), contamination with NMPs has yet not been implemented. In the report on microplastics in drinking water by the World Health Organization (WHO) (Organization, 2019), it was described that MP should, in principle, be effectively removed since drinking water treatment is designed to remove particulate matter from drinking water sources. However, it is assumed that the contamination of drinking water with MPs could stem from the raw water used for its generation due to inefficient removal of the particles (Pivokonsky et al., 2018). Zhang et al. (2020) described that the efficiency of removing particles >50 μm ranges from 25-–90%, depending on the treatment technologies of the respective drinking water treatment plants. Since many bottled water and other beverages contain filtered municipal tap water, the contamination with particles <50 μm could originate from the drinking water used to produce them. However, Mason et al. (2018) compared bottled water from the same brand available in glass or plastic bottles, and the contribution of the plastic bottle to the NMPs load is larger than that stemming from the water directly. Therefore, another potential source of the NMP contamination of bottled water may derive from the production processes, like packaging (Zhang et al., 2020). Furthermore, one potential reason for the higher contamination of plastic bottled water could be the repeated mechanical stress of opening and closing the bottles, increasing MPs release (Winkler et al., 2019).

Several studies investigated drinking water and beverages contamination with MPs, and other review articles have already summarized the current knowledge of MPs in drinking water (e.g. Danopoulos et al., 2020; Eerkes-Medrano et al., 2018; Koelmans et al., 2019). MPs were detected in drinking water, beverages like beer, refreshments, and wine across the globe (Kankanige and Babel, 2020; Makhdoumi et al., 2021; Mason et al., 2018; Shruti et al., 2021). Schymanski et al. (2018) describe that 80% of the detected particles have a size distribution of 5–20 μm and Oßmann et al. (2018) highlighted that more than 90% of the detected particles in their study were even smaller than 5 μm. Consequently, most MPs in drinking water and beverages are not visible to the naked eye.

However, there is a consensus on the occurrence of MPs in bottled drinking water and beverages produced for human consumption, although the actual amount of NMPs within drinking water is still to be evaluated. Based on 10 publications reviewed, Zhang et al. (2020) calculated a human microplastic intake of up to 4.7×10^3 particles per person per year. Finally, it's worth of note that drinking water is not solely used for direct consumption but also for further food processing. Therefore, it could contribute to the NMP content in processed food items.

3.2. Food

One of the main uptake routes of NMPs by humans is through food. To obtain a comprehensive picture of NMPs contamination in raw and processed food, we used food categories based on a technical report published by the European Food Safety Authority (EFSA) (EFSA CONTAM Panel, 2016; Food and Authority, 2011) and the classification and description system FoodEx2 (revision 2) (European Food Safety Authority, 2015, 2021) (see Table 1).

Amongst the major food commodities for humans are eggs, meat, milk, cereal and roots (FAO, 2013). Approximately 19% of the global population use seafood as their primary source of animal protein, which indicates how heavily reliant humans are on the oceans' life as protein source (Beaumont et al., 2019; Golden et al., 2016). Over the last 70 years, the global fishery capture production increased by a factor of ~5 (1950: 19 million tons living weight; 2019: 94 million tons living weight), whereas the global aquaculture production increased by a factor of ~200 (1950: 6×10^5 tons living weight; 2019: 120 million tons living weight) (FAO, 2020; FishStatJ software v4.02.04, 2022), to meet the increase in protein needs caused by a growing world population. Therefore, we first summarize the current knowledge of NMPs contamination in 'blue meat', a term introduced by Naylor et al. (2021) defining aquatic foods captured from or cultivated in marine and freshwater ecosystems. It must be noted that within this review, we only consider studies focusing on NMPs content in edible parts of the animals, starting with the findings on species consumed as a whole organism.

Mussels are filter feeders and therefore inadvertently ingest NMPs with their food. As a protein source for humans, they thus represent a potential vector of NMPs (Gündoğdu et al., 2020; Nalbone et al., 2021; Ribeiro et al., 2020; Sparks et al., 2021; Kumar et al., 2021; Wakkaf et al., 2020). The contamination of mussels with MPs was mainly stated in MPs per gram of wet weight (MPs/g w.w.) of the mussels and ranged from 0.040 ± 0.003 MPs/g w.w. up to 0.9 ± 0.1 MPs/g w.w. (Gündoğdu et al., 2020; Nalbone et al., 2021; Ribeiro et al., 2020; Sparks et al., 2021; Kumar et al., 2021), whereas one study estimated a higher value of 2.4 MPs/g w.w. (Wakkaf et al., 2020). Different polymer types with different shapes and sizes were detected in mussel tissues (Table 2). Next to mussels, other species consumed in whole may be relevant vectors of NMPs to humans. Ribeiro et al. (2020) analyzed wild and farm seafood (i.e., prawns, squids, sardines) and highlighted a high variability of polymers depending on the studied species. Furthermore, the occurrence of MPs in other commercially relevant marine species was evaluated in edible tissue of crab (Akhbarizadeh et al., 2019; Daniel et al., 2020a; Ribeiro et al., 2020; Zhang et al., 2021), sea urchin (Feng et al., 2020), shrimp (Daniel et al., 2020b, 2021), prawn (Akhbarizadeh et al., 2019; Ribeiro et al., 2020) and squid (Daniel et al., 2021; Ribeiro et al., 2020). Most studies showed that the percentage of MPs in edible tissues is generally lower than in the inedible ones, like the organisms' digestive tract (Daniel et al., 2020a; Wakkaf et al., 2020; Zhang et al., 2021). This implements that animals that are eaten whole, including their digestive

Table 1
Grouping of food categories used in the present chapter.

CATEGORY	subgroup
Cereals	A0EZF, A0EZV
Fruit and Vegetable	A07XJ, A0EZG, A0EZN, A0EZH
Oils	A015E
Roots and Tubers	A00ZS
Other plants and herbs	A010R, A0EZM
Terrestrial Meat	A0EZS, A0EZT
Marine Meat	A0EZR, A0EZQ
Milk	A0BXZ
Eggs	A031E
Confectionery	A04PE
Particular food	A03TD, A03PV, A03RR
Other	A03VA, A042N
isolated purified ingredients	A0BXX

Table 2
Overview of MPs found in selected animal food products. Cellulose Acetate (CA), Cellophane (CE), Ethylene Propylene diene monomer rubber (EPDM), Extruded PS (EPS), Ethylene-vinyl acetate (EVA), Polyamide (PA), Polyacrylamide (PAAm), Polyacrylonitrile (PAN), Polybutylene terephthalate (PBT), Polyethylene (PE), High-density PE (HDPE), Low-density PE (LDPE), Polyethylene terephthalate (PET), Polyethersulfone (PES), Poly(methyl methacrylate) (PMMA), Polypropylene (PP), Polystyrene (PS), Polysulfone (PSU), Polytetrafluoroethylene (PTFE), Polyurethan (PU), Polyvinyl acetate (PVA), Polyvinyl chloride (PVC). ATR-FTIR = Attenuated Total Reflection- Fourier-transform infrared spectroscopy, FPA = Focal Plane Array detector, FE-SEM = Field Emission- Scanning Electron Microscopy, EDX = Energy-dispersive X-ray spectroscopy, Py-GCMS = Pyrolysis–gas chromatography–mass spectrometry. Raw data rounded.

Food matrix	Polymer types found	NMP size	Detected concentrations	Analytical Method	Ref.
Mussel	- PET - Latex - PS-cotton - PVC - CA - EVA - HDPE - Nylon	500 μm – 2000 μm	0.040 ± 0.003 MPs/g wet weight (w.w.) 87% of mussels contained MPs	Stereomicroscope sorting FTIR-ATR	Sparks et al., 2021
Mussel	- PE - PP - PET - PVC	~ 500–1500 μm	Fresh mussels: 0.20 ± 0.24 MPs/g w.w. Processed mussels: 0.9 ± 0.1 MPs/g w.w. 61 % of mussels contained MPs	Stereomicroscope sorting FTIR	Nalbone et al., 2021,
Mussel	- PE - PP - CE	not specified	0.7 ± 0.5 – 3.5 ± 0.3 MPs/g w.w. 97% of mussels contained MPs	Stereomicroscope sorting FTIR	Wakkaf et al., 2020
Mussel	- PE - PP - Nylon - EVA - PET - p-acrylic acid	mean 1.7 ± 0.1 mm	Mean 0.06 MPs/g w.w Range 0.03–0.09 MPs/g w.w. 92% of vendors sold mussels that contained MPs	Stereomicroscope sorting μ-Raman	Gündoğdu et al. (2020)
Mussel	FTIR: - PP - PET - PAN - PE - PA - PU - PS - PBT Raman: - PA - PP - PE - PAN - PU - PET - PS - PMMA	3–60 μm (Raman analysis) Mostly <100 μm (FTIR analysis)	0.63 ± 0.59 MPs/g w.w.	FPA-based μ-FTIR μ-Raman	Kumar et al. (2021)
Mussel	- PVC	not specified	Range 0–24 μg/g	Py-GC/MS	Ribeiro et al. (2020)
Shrimp	Not detected			Stereomicroscope sorting FTIR	Daniel et al. (2021)
Shrimp	- PS - PA - PE - PP	150–1000 μm (72% of total) <500 μm (less than 25%)	0.04 ± 0.07 MPs/g w.w. 31% of the shrimps were contaminated with MPs	Stereomicroscope sorting FTIR	Daniel et al. (2020b)
Prawn	- PVC - PP - PMMA	not specified	PVC: 0–16 μg/g PP: 0-15 μg/g	Py-GC/MS	Ribeiro et al. (2020)
Prawn	not identified	Mainly <50 μm in muscle	0.36 MPs/g w.w. (muscle) 0.77 MPs/g w.w. (gill)	Stereomicroscope sorting FTIR	Akhbarizadeh et al. (2019)
Squid	- PP - PS - PE	~100–400 μm	0.008 ± 0.02 MPs/g w.w.	Stereomicroscope sorting FTIR	Daniel et al. (2021)
Squid	- PVC - PP	not specified	PVC: 0–11 μg/g PP: 0–24 μg/g	Py-GC/MS	Ribeiro et al. (2020)
Crab	- PP - PS - PE	~100–400 μm	0.003 ± 0.01 MPs/g w.w. 13 % of edible tissue contained MPs	Stereomicroscope sorting FTIR	Daniel et al. (2021)
Crab	- CE - PET - PE - PP - PA	20–5000 μm	0.80 ± 1.1 – 23 ± 25 MPs/g w.w. No MPs were found in crab's muscles.	Stereomicroscope sorting μ-FTIR	Zhang et al. (2021)
Crab	- PS - PE	not specified	PS: 0.28–8.1 μg/g PE: 0–40 μg/g	Py-GC/MS	Ribeiro et al. (2020)

(*continued on next page*)

Table 2 (*continued*)

Food matrix	Polymer types found	NMP size	Detected concentrations	Analytical Method	Ref.
	- PVC - PP - PMMA		PVC: 1.2–39 μg/g PP: 2.5–26 μg/g PMMA: 0–4.5 μg/g		
Crab	not identified	Mainly <50 μm in muscle	0.26 MPs/g w.w. (muscle) 0.86 MPs/g w.w. (gill)	Stereomicroscope sorting Hot probe testing SEM-EDX	Akhbarizadeh et al. (2019)
Urchin	- CE - PET:PS - PE - PP - PP:PE - PA - ryon - PAN - PU - PVA:PE	7–1000 μm (60% of total) (range 30–4700 μm)	From 0.16 ± 0.09 MPs/g w.w to 2.3 ± 1.7 MPs/g w.w. ~90% of urchins contained MPs	Stereomicroscope sorting FTIR	Feng et al. (2020)
Fish	-PS -PE -PVC -PP -PMMA	not specified	PS: 0–100 μg/g PE: 0–2400 μg/g PVC: 0–10 μg/g PP: 0–60 μg/g PMMA: 0–30 μg/g	Py-GC/MS	Ribeiro et al. (2020)
Fish	- PE - PP - EPDM - PS	100-200 μm in edible tissue (range 115–210 μm) 200–400 μm in inedible tissue (range 136–4010 μm)	Edible: 0.005 ± 0.02 MPs/g w.w. 7% of fishes had MPs in edible parts. Inedible: 0.05 ± 0.01 MPs/g w.w. 41% of fishes had MPs in inedible parts.	Stereomicroscope sorting FTIR	Daniel et al. (2020a)
Fish	not identified	Mainly < 50 μm in muscle	0.16–0.28 MPs/g w.w. (muscle) 0.25 MPs/g w.w. (gill)	Stereomicroscope sorting Hot probe testing SEM-EDX	Akhbarizadeh et al. (2019)
Fish	- PP - PET - PE - PVC	mean: 1100 ± 940 μm (range 190–3800 μm)	Total 6 MPs found	Stereomicroscope sorting Raman FESEM-EDX	Karami et al. (2018)
Fish	- PP - PE - PS - PET - PA-6	not specified	29 MPs in eviscerated flesh and 7 MPs in organs	Stereomicroscope sorting Raman FESEM-EDX	Karami et al. (2017a)
Chicken	- PE - PS	1–10 mm	Gizzard: mean 46 ± 43 MPs/gizzard Crop: mean 11 ± 15 MPs/crop	Stereomicroscope	Lwanga et al. (2017)
Chicken meat	- EPS - Fibers (not specified)	130–450 μm	4-19 MPs/kg packaged meat	Stereomicroscope sorting ATR-FTIR	Kedzierski et al. (2020)
Milk	- PES - PSU	Fibers and fragments of <500 μm – 5 mm	3–11 MPs/L milk	Stereomicroscope SEM-EDS μ-Raman	Kutralam-Muniasamy et al. (2020)
Milk	- PP - HDPE - LDPE - PAAm	Fibers: 30 – 6740 μm Fragments: 2–180 μm	Fibers: 30–250 MPs/L milk Fragments: 100–280 MPs/L milk	Stereomicroscope sorting FTIR	Diaz-Basantes et al. (2020)
Milk	- PP - PE - PES - PS - PTFE - PU - PSU - PVA	69–99% <50 μm^2	Samples ranged from 800–9700 MPs/L milk	μ-Raman SEM-EDX	Costa Filho et al. (2021)

tract, are a potentially larger vector for NMPs than when only parts of the animals are consumed. For instance, larger fish are usually not eaten whole, but mainly the fillet is consumed by humans. Here, the translocation of MPs from the digestive tract into edible tissues like fish fillet has already been shown in a laboratory study (Zeytin et al., 2020) and also in fish captured in nature for human consumption (Daniel et al., 2020a; Gabriel et al., 2015; Karami et al., 2017a). Therefore, both marine animals eaten as a whole, and saltwater fish fillet consumption can serve as a vector for human consumption of NMPs. However, 12.5% of the total share of captured fish derives from inland freshwater ecosystems (FAO, 2020). Although there are no studies demonstrating NMPs in the fillet of freshwater fish for human consumption, it has been described that freshwater fish also ingest MPs (Galafassi et al., 2021; Parker et al., 2021). Consequently, fillet of freshwater fish might be an additional vector of NMPs to humans.

The total protein requirement of humans is not only met by blue meat but also by a high proportion of meat. Poultry consumption, in particular, has increased over the last 60 years, even overtaking beef consumption (Naylor et al., 2021). However, only little information on MPs levels in meat have been published. First attempts were made to analyze the MPs content in chicken (Huerta Lwanga et al., 2017; Kedzierski et al., 2020). Both studies showed that MPs were attached to chicken tissues. Kedzierski et al. (2020) highlighted that the MPs associated with the washed chicken meat mainly derived from the packaging

itself. Huerta Lwanga et al. (2017) found MPs >1 mm in size in the gizzard of dissected chickens. The authors state that even a thorough washing of the gizzard would not guarantee the complete removal of MPs and calculated possible annual ingestion of 840 MPs per person per year in Mexico. However, to our best knowledge, MPs were not detected within the meat fillet mainly used for human consumption. This lack of knowledge may depend on time- and cost-consuming approaches like enzymatic digestion (Löder et al., 2017) that would be needed prior to analysis of the meat. Recently, Huang et al. (2020) used a non-disruptive method, namely mid-infrared spectroscopy, to detect MPs within chicken meat without destroying the meat matrix. However, the method's sensitivity for detecting MPs is very low (between 1% and 10% (w/w)) and needs to be improved to apply it to real samples.

Another important source of nutrients for humans are milk and dairy products. Milk is not solely used as a raw product but also for many processed food items, like butter, cheese, cream, and ready-made products.

A few studies have already investigated the contamination of MPs in milk (Table 2). For example, Kutralam-Muniasamy et al. (2020) detected MPs in branded milk from Mexico, reporting 3-11 MPs/L, and Diaz-Basantes et al. (2020) reported higher levels of average 40 MPs/L in milk from Ecuador. However, Costa Filho et al. (2021) reported much higher contamination levels, with 88 MPs/L in raw milk and 694 MPs/L in powdered milk. Therefore, although it is premature to conclude on MPs levels in milk, the results of Costa Filho et al. (2021) suggest that MPs' presence increases with milk processing.

In addition, humans consume and also need carbohydrates, with cereals accounting for the largest proportion. The FAO estimates that cereals are mainly produced for direct human consumption (41%) and animal feed (45%), the remaining percentages for industrial applications (brewing, biofuels, etc.). Cereals contribute 55-70% of the total diets of developing countries, with 2/3 represented by corn and wheat. Corn, oats, barley, wheat and sorghum are the main grains used in animal feeding globally (Kleih et al., 2006; World Trade Organization, 2019). Therefore, MP- containing cereals may serve as a direct vector when consumed by humans or indirectly by consuming animal products containing NMPs. There is growing evidence for the contamination of the terrestrial environment, with increasing attention drawn on agricultural soils for food production. However, if this leads to the contamination of cereals is not known to date. Possible transfer of NMPs to cereals may stem from agricultural soils (Harms et al., 2021; Rillig et al., 2017; Steinmetz et al., 2016; Wang et al., 2021), irrigation of cereal crops with contaminated waters (Domenech and Marcos, 2021), and fertilization with sewage sludge and polymer-coated fertilizer (Corradini et al., 2019; Lian et al., 2021; van den Berg et al., 2020; Weithmann et al., 2018). It is not known whether NMPs can enter the crop plant tissue grown on agricultural fields. However, in laboratory studies, it was shown that vascular plants could act as sinks for model NMPs as their surfaces can adsorb them (Taylor et al., 2020) or even be taken up into the plant's tissues (Austen et al., 2022; Bosker et al., 2019; Dong et al., 2021; Li et al., 2021; Lian et al., 2021; Yin et al., 2021; Zhou et al., 2021). Nevertheless, most studies have focused on the potential effects of NMPs on plant physiology (Dong et al., 2020; Pehlivan and Gedik, 2021; Urbina et al., 2020; Wu et al., 2022).

Furthermore, industrial processing and packaging may lead to NMPs contamination of cereals (Dessì et al., 2021). Despite the high proportion of human consumption of cereals, very little data on their contamination by NMPs exists. We observed only one study investigating the MPs contamination of rice produced for human consumption (Table 3). Dessì et al. (2021) investigated the mass concentration of MPs in store-bought rice and found 45-322 μg/g dry weight. The authors found no difference between paper and plastic packaging of the rice. However, washing the rice before further processing reduced the mass of MPs within the samples. Noteworthy, pre-cooked rice contained a fourfold higher concentration of MPs, suggesting that industrial processes may be the primary source of MPs contamination.

Next to cereals, fruits and vegetables contribute to the overall consumption of carbohydrates. There is little information about NMPs' presence in commercial vegetables and fruits produced for human consumption. To our best knowledge, only Oliveri Conti et al. (2020) quantified MPs in several Italian fruits and vegetables produced for human consumption of different contamination levels, with fruit samples being generally more contaminated than vegetables (Table 3). However, the accumulation of NMPs has been described in edible tissues of radish (Tympa et al., 2021) or cucumber (Li et al., 2021) in plants grown under laboratory conditions.

Furthermore, the usual diet of humans also contains processed foods, reported in our used classification system (Table 1) as oil, confectionary, teabags, honey & sugar and salt (Table 4). To date, no studies are available reporting NMPs in confectionary or oil. However, some studies were published investigating NMPs in other processed foods. For instance, Li et al. (2020) detected MPs in packed Nori seaweed, and other edible macroalgae were discussed to be potential vectors for NMPs to humans (Yang et al., 2021). Some studies documented the presence of MPs and other fibers in honey (Diaz-Basantes et al., 2020; Liebezeit and Liebezeit, 2013, 2015; Mühlschlegel et al., 2017) and sugar (Liebezeit and Liebezeit, 2013, 2015) and several studies detected MPs in salt samples (Fadare et al., 2021; Fischer et al., 2019; Gündoğdu, 2018; Iñiguez et al., 2017; Karami et al., 2017b; Kim et al., 2018; Kosuth et al., 2018; Lee et al., 2019; Nithin et al., 2021; Renzi et al., 2019; Renzi and Blašković, 2018; Seth and Shriwastav, 2018; Tahir et al., 2019; Yang et al., 2015) (Table 4). Furthermore, two studies detected the release of MPs from commercial teabags during a typical steeping process (Hernandez et al., 2019; Xu et al., 2021). These results indicate that raw and processed food items may potentially contribute to human exposure to NMPs via ingestion.

Table 3
Overview of MPs found in rice, vegetables and fruits. Cellulose Acetate (CA), Cellophane (CE), Ethylene Propylene diene monomer rubber (EPDM), Extruded PS (EPS), Ethylene-vinyl acetate (EVA), Polyamide (PA), Polyacrylamide (PAAm), Polyacrylonitrile (PAN), Polybutylene terephthalate (PBT), Polyethylene (PE), High-density PE (HDPE), Low-density PE (LDPE), Polyethylene terephthalate (PET), Polyethersulfone (PES), Poly(methyl methacrylate) (PMMA), Polypropylene (PP), Polystyrene (PS), Polysulfone (PSU), Polytetrafluoroethylene (PTFE), Polyurethan (PU), Polyvinyl acetate (PVA), Polyvinyl chloride (PVC). SEM= Scanning Electron Microscopy, EDX= Energy-dispersive X-ray spectroscopy, Py-GCMS= Pyrolysis–gas chromatography–mass spectrometry. Raw data rounded.

Food matrix	Polymer types found	NMP size	Reported concentrations	Analytical methods	Ref.
Rice	- PE - PP - PET	Not determined	Dry rice: 67 ± 26 μg/g dry weight (d.w.) Washed rice: 52 ± 5 μg/g dw Dry instant rice: 280 ± 50 μg/g dw Washed instant rice: 170 ± 41 μg/g dw	Py-GC/MS	Dessì et al. (2021)
Fruit and vegetable	not specified	1.5–2.5 μm	Apples 1.96 × 10^5 ± 1.3 × 10^5 MPs/g Pears 1.90 × 10^5 ± 1.1 × 10^5 MPs/g Broccoli 1.26 x 10^5 ± 8.0 × 10^4 MPs/g Lettuce 5.10 × 110^4 ± 2.5 × 10^4 MPs/g Carrot: 1.02 × 10^5 ± 4.4 × 10^4 MPs/g	SEM-EDX	Oliveri Conti et al. (2020)

Table 4
Overview of MPs found in processed foods. Cellulose Acetate (CA), Cellophane (CE), Ethylene Propylene diene monomer rubber (EPDM), Extruded PS (EPS), Ethylene-vinyl acetate (EVA), Isobutyl Vinyl Ether (IBVE), Polyamide (PA), Polyacrylamide (PAAm), Polyacrylonitrile (PAN), Poly(butyl methacrylate) (PBMA), Polybutylene terephthalate (PBT), Polyethylene (PE), High-density PE (HDPE), Low-density PE (LDPE), Polyetherimide (PEI), Polyethylene terephthalate (PET), Polyethersulfone (PES), Poly(methyl methacrylate) (PMMA), Polyoxymethylene (POM), Polypropylene (PP), Polystyrene (PS), Polysulfone (PSU), Polytetrafluoroethylene (PTFE), Polyurethan (PU), Polyvinyl acetate (PVA), Polyvinyl chloride (PVC). ATR-FTIR = Attenuated Total Reflection- Fourier-transform infrared spectroscopy, FPA= Focal Plane Array detector, FE-SEM = Field Emission- Scanning Electron Microscopy, EDX = Energy-dispersive X-ray spectroscopy, Py-GCMS = Pyrolysis–gas chromatography–mass spectrometry, XPS = X-Ray Photoelectron Spectroscopy, NTA = Nanoparticle Tracking Analysis, NIR = Near-Infrared spectroscopy. Raw data rounded.

Food matrix	Polymer types found	NMP size	Reported concentrations	Analytical method	Ref.
Nori seaweed	- not specified	not specified	0.9–3 MPs/g	Stereomicroscope μ-FTIR	Li et al. (2020)
Honey, Sugar	- not specified	not specified	Honey, fibers 170 ± 150 MPs/kg, fragments 9 ± 9 MPs/kg Sugar, fibers 220 ± 120 MPs/kg, fragments 32 ± 7 MPs/kg Unrefined sugar, fibers 560 MPs/kg, fragments 540 MPs/kg	Stereomicroscope	Liebezeit and Liebezeit (2013)
Honey	- not specified	not specified	Fibers 10–340 MPs/kg, fragments 2–82 MPs/kg.	Stereomicroscope	Liebezeit and Liebezeit (2015)
Honey	- PET	>30 μm	0–8.3 MPs/kg (mean 3.8 MPs/kg)	Raman FTIR-ATR	Mühlschlegel et al. (2017)
Honey	- PP - HDPE/LDPE - PAAm	Fibers 67–2700 μm, fragments 5–230 μm	Fibers 20–180 MPs/L, fragments 190–830 MPs/L.	Stereomicroscope sorting FTIR	Diaz-Basantes et al. (2020)
Salt	- not specified	4–4600 μm	1600–3 × 10^4 MPs/kg	Stereomicroscope sorting μ-FTIR	Renzi and Blašković (2018)
Salt	- not specified	100–5000 μm	47–800 MPs/kg (mean 210 MPs/kg)	Stereomicroscope	Kosuth et al. (2018)
Salt	- PVA - PP - PE	4–4700 μm	0.67 ± 1.2–3.4 ± 4.9 MPs/kg	Stereomicroscope sorting FTIR	Fadare et al. (2021)
Salt	- Nylon - LDPE - PP - PET	not specified	470 ± 120–1600 ± 150 MPs/kg	FTIR	Nithin et al. (2021)
Salt	- PP - PE - PS - PEI - PET - POM	90–1500 μm	9.8 MPs/kg	Stereomicroscope sorting FTIR	Lee et al. (2019)
Salt	- PET - PVC - PA6 - PE - PS - IBVE - PA - PC - PP - PBMA - PU - Viscose	10–150 μm	170–320 MPs/kg (IT); 70–220 MPs/kg (CRO)	FTIR ATR	Renzi et al. (2019)
Salt	- PVA - PE - PS	390–9400 μm	6.7 - 53 MPs/kg	FTIR	Tahir et al. (2019)
Salt	- PES - PS - PA - PE - PET	80% of fragments and fibers were smaller than 500 and 2000 μm resp.	103±39 - 56±49 MPs/kg; 64 μg/kg	Stereomicroscope sorting μ-FTIR	Seth and Shriwastav (2018)
Lake salt, Rock salt, Sea salt	Lake salt: - PP - PE - Teflon - PET Rock salt: - PET - PE - PP Sea salt: - PE - PP - PET	100–5000 μm	Lake salt: 28–460 MPs/kg (mean 250 ± 310 part/kg) Rock salt: 0–150 MPs/kg (mean 38 ± 55 MPs/kg) Sea salt: 0–1700 MPs/kg (mean 680 ± 2600 MPs/kg)	Stereomicroscope sorting FTIR	Kim et al. (2018)
Sea salt, well salt		30–3500 μm	Sea salt: 50–280 MPs/kg Well salt: 120–190 MPs/kg		Iñiguez et al. (2017)

(*continued on next page*)

Table 4 (*continued*)

Food matrix	Polymer types found	NMP size	Reported concentrations	Analytical method	Ref.
	-PET - PP - PE			Stereomicroscope sorting FTIR	
Lake salt, Rock salt, Sea salt	- PET - PES - PE - PB - PP - CE	45–4300 µm	Lake salt: 43–360 MPs/kg Rock salt: 7–200 MPs/kg Sea salt: 550–680 MPs/kg.	Stereomicroscope sorting µ-FTIR	Yang et al. (2015)
Lake salt, Rock salt, Sea salt	Lake salt: - PE - PP - PU - PET - PMMA - PVC - PA-6 Rock salt: - PP Sea salt: - PU - PET - PP - PE - PVC - PA-6	not specified	Lake salt: 8–100 MPs/kg (mean 38 ± 14 MPs/kg) Rock salt: 9–16 MPs/kg (mean 12 ± 1.2 MPs/kg) Sea salt: 16–84 MPs/kg (mean 46 ± 13 MPs/kg).	Stereomicroscope sorting µ-Raman	Gündoğdu (2018)
Salt	- PP - PE - PET - polyisoprene: PS (copolymer) - PAN - PA-6	160–980 µm	10 MPs/kg	Stereomicroscope sorting Raman	Karami et al. (2017b)
Salt	- PP - PET - PE - PS - PVC - PUR - PA - PMMA - PC	-	140-2000 µg/kg	Py-GC/MS	Fischer et al. (2019)
Teabags	- PET - nylon	50−100 µm and 10−400 nm 1−50 µm and 50–600 nm	Estimation of 2.3 million micron-sized and 14.7 billion submicron particles per cup of tea	SEM XPS FTIR NTA	Hernandez et al. (2019)
Teabags	- nylon	500 nm to 100 µm.	Not stated	NIR FTIR	Xu et al. (2021)

3.3. Inhalation

Several comprehensive review articles on the contamination of the atmosphere and breathable ambient air with NMPs already exist (Amato-Lourenço et al., 2020; Bianco and Passananti, 2020; Chen et al., 2019; Wieland et al., 2022; Zhang et al., 2020). A recent study extrapolated wet and dry deposition data to the whole area of the River Weser catchment and reported a total MPs deposition of 232 tons. Furthermore the authors report a MP concentration of 500 MPs per m^3 even in outdoor environments (Kernchen et al., 2021). Although these numbers already seem to be relatively high, most studies indicate that exposure to indoor air seems to comprise a higher likelihood of inhaling NMPs than that of outdoor air (Dris et al., 2017; Liu et al., 2019; Wieland et al., 2022). Interestingly, Liao et al. (2021) reported that the mean values of MPs in indoor air samples were an order of magnitude higher than in outdoor samples. The United States Environmental Protection Agency (EPA) described the concentration of chemicals in indoor environments as 2 to 5 times higher than outdoor concentrations (EPA, 1987). Although the current data suggest that this seems to apply to the concentration of NMP, this needs further investigation. However, since the EPA and the WHO estimate that European citizens usually spend approximately 90 % of their time indoors (Sarigiannis, 2014; US Environmental Protection Agency, 1986), in this review, we focus on the contamination of indoor environments with NMPs.

First attempts to estimate the inhalation of NMPs from indoor air were made using different methods (Table 5). One way to assess the contamination with airborne NMPs is by directly filtering the ambient air (Dris et al., 2017; Liao et al., 2021) or using a breathing mannikin (Vianello et al., 2019). In addition, passive sampling is another approach to assess the contamination with NMPs, for instance, via microparticle sedimentation into openly placed glass wear (Jenner et al., 2021; Soltani et al., 2021) or collecting dust samples (Dris et al., 2017; Zhang et al., 2020). To date, there is no doubt of the presence of NMPs in indoor air, and Wieland et al. (2022) estimated that humans might inhale more than 48,000 MPs per day.

The abundance of NMPs in indoor environments is likely influenced by the use of plastics in diverse human activities. Flooring, synthetic garments, textile and household furniture seem to be the significant determinants for NMPs contamination of the air as reviewed by Facciolà et al. (Facciolà et al., 2021). The highest concentrations of indoor

Table 5
Overview of airborne MPs in indoor environments. Polyamide (PA), Polyacrylonitrile (PAN), Polyethylene (PE), Polyethylene terephthalate (PET), Poly(methyl methacrylate) (PMMA), Polypropylene (PP), Polyvinyl (PV). ATR-FTIR = Attenuated Total Reflection- Fourier-transform infrared spectroscopy, FPA = Focal Plane Array detector, HPLC = High-performance liquid chromatography Raw data rounded.

Indoor sample	Polymer types found	NMP size	Reported concentrations	Analytical method	Ref.
Filtering, passive sampling & dust samples from a vacuum cleaner	- PP - PA-cotton mixture	Dust samples: 4700–4900 μm Indoor air: <3300 μm	Filtering: range 0.4–59 fibers/m^3 with a median value of 5.4 fibers/m^3 Passive sampling: range 2.7 to 20 fibers/day, corresponding to a deposition rate between 1600 and 11,000 fibers/day/m^2 Collected bags of vacuum cleaners: ranged 190 and 670 fibers/mg dust samples.	Stereomicroscope sorting FTIR-ATR	Dris et al. (2017)
Filtering & passive sampling	- PE - PA - PP	Fibers: 60 ± 2.7%: 5–30 μm 29 ± 2.3%: 30–100 μm 11%: >100 μm	Mean concentration: 1600 ± 1200 MPs/m^3	Stereomicroscope sorting μ-FTIR	Liao et al. (2021)
Filtering	- PE - PET - nylon - PP	Fibers: 13% Fragments 87% Size distribution 37-240 μm with a D_{50} of 21-36 μm	Total number of inhaled MPs: 270 MPs The average number of inhaled MPs per unit volume: 9.3 ± 5.8 MP/m^3	FPA-μFTIR-	Vianello et al. (2019)
Passive sampling	- PET - PC	-	PET concentrations in the range of 29–1.1 × 10^5 μg/g dust sample PC concentrations in the range of <0.11–1700 μg/g dust sample	HPLC	Zhang et al. (2020)
Passive sampling	- PET - PA - acrylates - PP - co-polymer blends - PAN - PE - PMMA	Fibers (90%) Fragments (8%) Film (1%) Sphere (1%) Foam (<1%) Size not stated	Mean MPs concentration: 1400 ± 1000 MPs/m^2 per day	μ-FTIR	Jenner et al. (2021)
Passive sampling	- PE - PE:PET - PA - PV	Fibers: - 50–200 μm (5%) - 200–400 μm (19%) - 400–600 μm (17%) Fragments: - 686 μm (average) Films: -100 μm (average)	In total, 7400 fibers, 64 fragments and 18 films were collected. The deposition rate of fibrous MPs ranged from 22 to 6200 fibers/m^2 per day with an average of 3100 fibers/m^2 per day	Stereomicroscope sorting FTIR	Soltani et al. (2021)

airborne MPs (1600 ± 1200 MPs/m^3) were reported by Liao et al. (2021) by active air filtering. They reported that 2/3 of the number of all particles collected were smaller than 30 μm (Liao et al., 2021). Therefore, we can speculate that smaller particles dominate airborne MPs, which is plausible considering that smaller particles remain suspended in the air longer than larger particles. However, to date, there are no data on the occurrence and prevalence of MPs smaller than 5 μm in private indoor environments. Therefore, reliable statements regarding the potential exposure to small MPs or NPs cannot be made.

In some working environments, the potential of being exposed to NMPs generated during mechanical and environmental degradation of plastic goods or by NMPs being added as ingredients to, for example, printer inks, spray paints, injection mouldings, and abrasive may be enhanced (Murashov et al., 2020, https://blogs.cdc.gov/niosh-science-blog/2020/02/19/microplastics/; Bitounis et al., 2022; Getzlaff et al., 2019). However, to date, the occurrence and emission sources of NMPs at workplaces have received little attention. Wieland et al. (2022) compared workplace concentrations of different airborne microparticles and associated occupational diseases. As for many particles and fibers, the physicochemical properties like size, shape, ζ-potential, adsorbed molecules and pathogens, and the MPs' bio-persistence should be regarded as possible drivers of MPs' toxicity (Ramsperger et al., 2020, 2021; Wieland et al., 2022). The US National Institute for Occupational Safety and Health (NIOSH) has defined exposure limits for workers for other airborne particles, such as asbestos or silica dust (Wieland et al., 2022; NIOSH 2020, https://blogs.cdc.gov/niosh-science-blog/2020/02/19/microplastics/). To date, NMPs are considered nuisance dust with a permissible exposure limit (PEL) of 5 mg/m^3 for respirable dust (Bartley and Feldman, 1984, guideline 0600 Issue 3). However, NMP-associated diseases in occupational settings have already been described and summarized (Burkhart et al., 1999; Prata, 2018; Wieland et al., 2022). For instance, the exposure of workers to vinyl chloride monomers used for the production of PVC induce DNA damage in lymphocytes of plastic industry workers (Awara et al., 1998). In addition to the production of the plastic material itself the processing industry may pose a potential hazard to workers. Burkhart et al. (1999) analyzed the workers' particulate exposure during nylon flocking (applying short fibers to adhesive-coated surfaces) and found an average respirable particulate matter of 2.2 mg/m^3. Although this value is below the NIOSH PEL set for nuisance dust, cases of interstitial lung disease were suggested to be linked to the detected respirable particles (Burkhart et al., 1999).

NMPs may be generated via flocking or degradation and from a bottom-up production mechanism during high energy or high heat processes. One example is 3D printing, which is becoming popular in offices and at home, and releases potentially harmful volatile organic compounds and ultrafine particles into the air (Du Preez et al., 2018). Some studies compared the particulate release of 3D printers with PLA

and Acrylonitrile-Butadiene-Styrol-Copolymer (ABS) filaments (Stephens et al., 2013; Vance et al., 2017; Zhang et al., 2019). Zhang et al. (2019) suggested that particles released from PLA filament 3D printers were mainly composed of PLA bulk material, whereas particles from ABS 3D printers differed from the bulk material. In all reported studies investigating the emission of NMPs during 3D printing, several million particles were described to be released. For instance, Stephens et al. (2013) estimated that approximately 2.0×10^{10} and 1.9×10^{11} particles, mainly consisting of particles in the fine to ultrafine range (<0.2–0.1 μm), are released every minute for a 3D printer utilizing a PLA and ABS feedstock, respectively. Although it is currently unclear whether the particles consist purely of the bulk material of the filament, these numbers are alarming, especially given the duration of the printing processes. Next to 3D printers, laser toner printers are known to emit high numbers of nanoparticles, including NP (Bello et al., 2021; Getzlaff et al., 2019). As most of the printing devices are currently sold as standalone devices without any exhaust ventilation or filtering accessories, the results suggest that caution should be taken when operating in inadequately ventilated or unfiltered indoor environments. Especially because the emitted particles are so small that they can deposit in the deep alveolar region of the lungs upon inhalation (Stephens et al., 2013) and were discussed to be a severe health threat (Bello et al., 2021; Bitounis et al., 2022).

3.4. Personal care products (PCPs)

The term PCPs is often used synonymously for cosmetics, although there is a slight but essential difference. The European Commission defined cosmetics as follows: "Any substance or preparation intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours." (European Commission, 2013). However, the term PCPs is not defined by law, but most PCPs are regulated as cosmetics, although some PCPs can be regulated as drugs. For instance, the Food & Drug Administration (FDA) listed PCP drugs as "(…) skin protectants (such as lip balms and diaper ointments), mouthwashes marketed with therapeutic claims, antiperspirants, and treatments for dandruff or acne." (FDA, 2016). Since both PCPs cosmetics and PCPs drugs are intentionally applied onto the human body, we decided to not separate them further concerning NMPs.

The European Commission initiated a restriction procedure on MPs in cosmetics in January 2018. Although an adopted restriction (if agreed by the member states) for the European Union is expected by 2022 (Anagnosti et al., 2021; https://www.europarl.europa.eu/doceo/document/E-9-2021-003388_EN.html), several European countries have already banned the intentional use of MPs in PCPs (Kentin and Kaarto, 2018). However, one of the main difficulties in proposing a general restriction of MPs in PCPs is the lack of a definition of the size range of MPs (Kentin and Kaarto, 2018). In the initiated proposal, the size of MPs was set to be lower than 5 mm in size without a lower threshold (ECHA 2021, https://www.europarl.europa.eu/doceo/document/E-9-2021-003388_EN.html). Although the industry has already responded to the pressure from non-governmental organizations and the concerned public by excluding MPs from several products (Anagnosti et al., 2021), the use of MPs is neither restricted in the European Union nor worldwide. Therefore, PCPs can still contain NMPs.

MPs are intentionally added to PCPs for different functions like viscosity regulators, emulsifiers, glitters, skin conditioning, exfoliants, abrasives, and many more (UNEP, 2015; Yurtsever, 2019). Depending on the desired function of the added MPs to PCPs, different polymer types, shapes, and sizes are used. The most often used polymer type is PE in various shapes and sizes (Gouin and Brunning, 2015; UNEP, 2015). Interestingly, the information on the main size ranges found in the literature is highly heterogeneous and depends on the intended function of the added polymer. For example, Gouin and Brunning (2015) summarized that particles smaller than 60 μm are ineffective as abrasion and exfoliation and the optimum size is around 450 μm. However, Sun et al. (2020) propose that the diameters of MPs added to PCPs range from 24 μm to 2 mm, with more than 95% smaller than 350 μm. The United Nations Environment Programme (UNEP, 2015) highlighted that the primary size of MPs in PCPs lays in between 1 and 50 μm. The size of the added MPs seems to depend on the product type (Sun et al., 2020). For example, in toothpaste, the reported sizes range from 4 - 20 μm (Ustabasi and Baysal, 2019) and 3–145 μm (Praveena et al., 2018). In facial scrubs, sizes were reported between 10–178 μm (Praveena et al., 2018) and 313 ± 130 μm (Lei et al., 2017) and in shower gels of about 422 ± 185 μm (Lei et al., 2017).

Next to the variations in size, MP concentrations are also highly different in PCPs. Variations from less than 1 % (Ustabasi and Baysal, 2019) up to 90 % were reported (UNEP, 2015). Sun et al. (2020) described the concentrations of MPs in PCPs and found the documented concentrations ranging from 2.15 particles per gram up to 3.11×10^6 particles per gram.

Besides the fact that MPs intentionally added to PCPs contribute to overall environmental pollution (Gouin and Brunning, 2015; Praveena et al., 2018), when washed off the body, the direct exposure of humans to the particles is a potential pathway of MPs entering the human body. Especially MPs in toothpaste and other cosmetics applied on mucosa may potentially translocate directly into the human body. For example, swallowing or incomplete rinsing of the mouth after tooth brushing leads to a transfer of MPs into the GIT. Another vulnerable area where PCPs contact the human body is the eye. The skin is relatively thin, and the mucous membrane interacts directly with the environment when the eye is open. Potential contact of the eye's mucous membrane with NMPs can occur through eye shadow and other cosmetic products, contact lenses, and NMPs in the air. As the global PCPs market and the use of contact lenses continue to increase, it is essential to investigate eye and eye care products as a potential gateway for NMPs into our bodies and the environment (https://www.statista.com/statistics/297070/growth-rate-of-the-global-cosmetics-market/; https://www.statista.com/study/48868/contact-lenses-report/). Contact lenses could release NMPs themselves when worn, as they are often made of hydrogel polymers, on the other hand, NMPs from the air could stick to the contact lenses and thus be taken up by ocular surface epithelial cells through prolonged contact time (Burgener and Bhamla, 2021). In addition, glitter, commonly used in eye shadow, can be identified as a primary source of MPs entering the environment and possibly the human body. Glitter, usually in hexagonal form, consists of a core polymer of PET coated with colored aluminum and a transparent polymer, which produces the typical sparkle (Tagg and Ivar do Sul, 2019; Yurtsever, 2019). There are no studies examining the uptake of NMPs by ocular epithelial cells, nor are there any studies showing the presence or accumulation of NMPs in ocular tissues. Hence the relevance of this translocation pathway is unclear.

Other PCPs used by a large part of society are contraceptives and period products. For instance, condoms are a relatively safe, effective, user-controlled contraceptive method that is easy to use and relatively inexpensive. Although the highest share of condom material used on the market are latex, condoms made of polyurethan (PU) or elastomers have already been introduced to the market in the early 1990s (Gallo et al., 2006). Furthermore, Munoz et al. (2022) recently showed that 12 of 24 period products directly in contact with the vaginal wall contained plastic. These products released fibers during *in vitro* tests and fragmented to release up to 17 billion NPs per tampon. A relatively high number of condoms (Lambert et al., 2013) and period products are disposed of down the toilet entering waste water treatment plants or are released to the environments via improper waste disposal, where they may release a substantial number of NMPs. Besides their contribution to environmental pollution with NMPs, it has not been shown whether

condoms made of plastic or plastic containing period products release NMPs during usage and whether potentially released particles may interact with the respective tissues.

4. Translocation of NMPs into human tissues

The translocation of NMPs to our body compartments may occur after applying NMPs-containing PCPs to the skin or after ingestion and inhalation. The potential translocation pathways for the respective primarily exposed organs are described in the following. Since the translocation mechanisms of particulate matter through the human skin is distinct from those within the GUT and lung, we decided to describe the mechanisms separately.

4.1. Human skin

Applying PCPs-containing NMPs onto our skin can directly facilitate the particles translocating from the skin into deeper tissue layers. However, the translocation of particulate matter into the skin is complex (Schneider et al., 2009). The human skin comprises four layers: the stratum corneum, the viable dermis, the dermis and the subcutaneous connective tissue (Desai et al., 2010). The stratum corneum is the outermost layer and provides an effective defensive barrier against particulate matter and pathogens in a healthy status. Schneider et al. (2009) comprehensively reviewed the reported translocation of nanoparticles through the human skin. One potential pathway to how particulate matter could be transported through the skin barrier is via the transappendageal pathway across hair follicles, sebaceous glands, and sweat glands (Desai et al., 2010; Schneider et al., 2009). Vogt et al. (2006) detected a high density of Langerhans cells (dendritic cells) around hair follicles, capable of internalizing nanoparticles of various sizes, whereas the transport across the epidermis was restricted to 40 nm particles in their experimental setup. However, it has to be noted that the transappendageal pathway is restricted to a relatively small area since the total amount of openings amounts between 0.1 and 1.3% of the entire skin (Bos and Meinardi, 2000; Schneider et al., 2009). Nevertheless, keeping in mind the very high concentration of NMPs in some PCPs described above, the translocation of NMPs via the transappendageal pathway might be relevant to consider.

Bos and Meinardi (2000) proposed the 500 Dalton rule by investigating the molecular weight of common contact allergens and topical drugs. They conclude that a molecular weight increasing over 500 Dalton leads to a rapid decline in human skin absorption. Assuming a spherical PS particle with a density of 1.05 g/cm^3, it should not exceed a size of 1.15 nm to be absorbed directly by the skin. However, Schneider et al. (2009) proposed that next to the size, the particles' properties and skin's health status are important factors for translocation. Kohli and Alpar (2004) tested differently charged PS particles of different sizes (50, 100, 200 and 500 nm, positive, negative and neutral charge). They showed that only 50 and 500 nm negatively charged particles penetrated the investigated pigskin. They assume that the density of the negative charges of the 50 and 500 nm particles is higher (50 nm because of the high surface ratio and 500 nm because of a higher number of functional groups) compared to the 100 and 200 nm particles, enabling the interaction and translocation through the skin (Kohli and Alpar, 2004). However, the skin was mechanically stressed, which could impede the barrier function and allow the particles' translocation. Furthermore, the human skin has unique properties, and translocation studies performed in animal models are of limited use for understanding the human skin barrier (Bos and Meinardi, 2000). Larese Filon et al. (2015) comprehensively reviewed the size-dependent translocation of nanoparticles across the human skin. They conclude that nanoparticles can cross the intact skin if their sizes do not exceed 4 nm, nanoparticles between 4–20 nm can potentially cross intact and damaged skin, nanoparticles between 21 and 45 nm can cross only damaged skin, and nanoparticles with sizes >45 nm cannot translocate through the human skin. However, they also highlighted that the material properties (metal or non-metal nanoparticles) are important factors (Larese Filon et al., 2015). No studies are reporting the translocation of NMPs through the human skin to our best knowledge.

4.2. Gastrointestinal tract

NMPs entering the human body via ingestion will encounter different defense mechanisms against tissue translocation. The first line of defense a particle would experience after entering the GIT is the mucus layer produced by the enterocytes in the form of membrane-bound mucins and the goblet cells in the form of secretory mucins. The mucus layer coats the interior surface of the digestive tract and is essential in the maintenance of intestinal homeostasis (Herath et al., 2020). In a healthy GIT, the mucus layer serves as a permeable barrier allowing the absorption of nutrients but limiting the transport of pathogens and microorganisms to the gut epithelial cells (Rackaityte and Lynch, 2020; Vancamelbeke and Vermeire, 2018). However, *in vivo* experiments with mice showed that due to oral exposure to NMPs, the intestinal microbiome's composition can be altered, leading to dysbiosis (Lu et al., 2018). Dysbiosis can change the thickness of the mucus layer and could result in abnormal mucus invasion and epithelial adherence of pathogens (Herath et al., 2020) or may even allow NMPs to interact with the epithelial layer directly. Moreover, the intestinal microbiota is considered a metabolic organ that may contribute to the metabolic health of the human host and, when imbalanced, to the pathogenesis of different disorders. Tamargo et al. (2022) evaluated the effects of the digestion of MPs on the human gut microbiota using feces from healthy donors and the internationally validated Dynamic Gastrointestinal Simulator simgi® model that represents the main functional sections of the digestive tract. The feeding with MPs altered human microbial colonic community composition, promoting the formation of biofilms and MPs biodegradation through digestion by intestinal bacteria (Tamargo et al., 2022).

4.3. Lung

The defense mechanisms associated with the ingestion of NMPs do not seem to depend as closely on particle sizes, as is the case for NMPs inhalation, the first line of defense depends on the particle sizes. The exposure to airborne particles is usually classified by the particles' aerodynamic diameter, with PM_{10} (coarse particles $\leq$ 10 μm), $PM_{2.5}$ (fine particles $\leq$ 2.5 μm) and $PM_{0.1}$ (ultrafine particles $\leq$ 0.1 μm). The occurrence of atmospheric MPs of PM_{10} have already been reported (Kernchen et al., 2021) and the inhalation of NMP is therefore generally possible. PM_{10} are usually trapped in the nasopharyngeal area by hair and mucus, whereas $PM_{2.5}$ can reach the bronchioles and alveoli. $PM_{0.1}$ can directly translocate transcellularly across the alveolar epithelium (Cooper and Loxham, 2019; Schraufnagel, 2020). However, defensive mechanisms against $PM_{2.5\text{-}0.1}$ also occur within the respiratory system. The epithelial layer contains, similar to the GIT, goblet cells contributing to a mucus layer entrapping inhaled particles. By ciliary beating (the so-called mucociliary escalator mechanism), even $PM_{0.1}$ can be transported within the mucus towards the mouth, where the mucus can be expelled or swallowed (Schraufnagel, 2020).

4.4. Transport of NMP across the biological barriers of the GIT and lung

When entrapped within the mucus of the respiratory system or the GIT, a particle can also be transported towards the epithelial layer (Hussain et al., 2001). Here, two potential pathways for the transport from one side of the epithelium to the other can occur. In epithelial cells, small particles (<100 nm) are more easily transported transcellularly through the epithelium by endocytosis than larger particles (in the lower micrometer range), which are transported paracellularly (Boland et al., 1999; Volkheimer, 1975, 1977; Zeytin et al., 2020). The paracellular

transport is mainly regulated through the presence of junctional complexes, like tight junctions, adherence junctions and desmosomes. Tight junctions are the apical-most adhesive complexes sealing the intercellular space (Vancamelbeke and Vermeire, 2018) and make the paracellular transport of particles challenging. However, goblet cells interrupt the network of tight junctions, loosening the tight junctions between epithelial and neighboring goblet cells, consequently allowing the transport of particulate matter in a paracellular manner (Volkheimer, 1977). Within the GIT, the transcellular pathway is also involved in internalizing larger molecules, pathogens and microorganisms (Vancamelbeke and Vermeire, 2018). Once NMPs may have crossed the epithelial layer of the lung, gastrointestinal tract or skin, there is another line of defense. Underneath the dermis of the skin, the interstitium of the lung or the lamina propria in the GIT, i.e. all corresponding tissues directly under the epithelial layer, there are various immune cells such as macrophages, dendritic cells, T and B lymphocytes, eosinophils and mast cells.

The *lamina propria* of the entire GIT is richly populated with diffusely distributed immune cells of different type. Furthermore, it additionally contains situated solitary lymphoid follicles, covered by the so-called follicle-associated epithelium (FAE). Whole aggregates of lymphoid follicles, mainly found in the wall of the ileum and appendix vermiformis, are called aggregated lymph follicles or Peyer's patches. The surface of each follicle is domed by propria tissue and covered with FAE (so-called dome epithelium). Intestinal villi and crypts are missing here, there are no goblet cells, and the mucus is very thin or missing. Instead, M-cells (M = microfold, this cell type is named after its' physiological appearance as the cells have no microvilli but only short microplicae. M-cells can amount 10-15% of the cells in the FAE) are firmly anchored within the epithelium in between enterocytes and can internalize particulate matter, even the size of bacteria (Foged et al., 2005; Hussain et al., 2001; Owen, 1999). M cells transport molecules and particulate matter into pockets, in which migrating lymphocytes, macrophages, and dendritic cells are found (Owen, 1999). With the initiation of an immune response activated B-lymphocytes differentiate into plasma cell precursors on site or in neighboring mesentery lymph nodes where the immune response is further set in motion. The plasma cell precursors differentiate to mature Immunoglobulin A-producing plasma cells that produce an antibody directed against the initial antigen. In addition, dendritic cells push - outside the FAE regions - long projections between the enterocytes into the intestinal lumen to further sense for pathogens or release cytokines (Scott et al., 2005). Furthermore, dendritic cells are in principle capable of internalizing PS particles up to 15 μm in size (Foged et al., 2005).

If, for example, microorganisms or NMP penetrate the mucus and epithelial layer of the GIT, they may be phagocytosed by macrophages in the *lamina propria* (Grainger et al., 2017). These are ideally positioned to ingest and eliminate any bacteria that have passed through (Bain and Schridde, 2018). In principle, macrophages in the lamina propria can trigger the described inflammatory responses, but usually show a silent response to the invader in a healthy organism (Bain and Schridde, 2018; Grainger et al., 2017). However, if specific antigens are perceived or there is increased invasion with pathogens, the immune cells (especially macrophages and dendritic cells) can trigger an inflammatory process by releasing cytokines or migrating into the mesenteric lymph nodes and initiating an immune response. After initiation of the immune response, cells reach the blood circulation via the lymph vessels, lymph nodes and finally the thoracic duct, to be distributed throughout the whole organism (Hampton and Chtanova, 2019; Owen, 1999).

The actual transport of NMPs across biological barriers that may trigger inflammatory responses has not yet been demonstrated. However, *in vitro* experiments showed that macrophages are in principle able to internalize MPs (Ramsperger et al., 2021; Stock et al., 2021), which is even enhanced in the case of environmentally exposed particles coated with an eco-corona (Ramsperger et al., 2020). After particle interaction, NMPs have been shown to trigger inflammatory responses in epithelial cells (Wu et al., 2020) and macrophages (Völkl et al., 2022). The transport of NMPs across more realistic biological barrier models was shown by using single cell culture approaches (Xu et al., 2019) and co-culture of cell lines representing small intestinal barrier models (Stock et al., 2021, DeLoid et al., 2021; Hesler et al., 2019). Furthermore, first attempts were made to estimate the uptake and potential effects of MP on organoid structures of the lung (Song et al., 2022) and intestine (Hou et al., 2022). Here, although MP fibers showed no adverse effects on mature organoids the development of lung organoids was hampered by the presence of MP fibers. The authors state, that the development of lung tissue of young children may be affected by airborne NMP, however, this needs further investigations (Song et al., 2022). The exposure of NP to intestinal organoids resulted in an accumulation of NP mainly in goblet, Paneth and endocrine cells, which consequently induced apoptosis and inflammatory responses (Hou et al., 2022).

Furthermore, *in vivo* studies using mouse model systems revealed the translocation of model nanoparticles from the lungs to the systemic circulation (Campagnolo et al., 2017; Miller et al., 2017; Raftis and Miller, 2019; Stapleton et al., 2012). Miller et al. (2017) and Raftis and Miller (2019) exposed healthy human volunteers to 5 nm gold nanoparticles via inhalation and detected the particles in the blood even three months after exposure. This retention indicates that for small NPs, translocation from the respiratory system in healthy human beings into the blood circulation may be possible. Interestingly, Burkhart et al. (1999) linked the workers' exposure to plastic products with interstitial lung diseases, suggesting that the transport of NMPs and the subsequent inflammatory response are generally possible in human.

To our best knowledge, no empirical *in vivo* studies with volunteer human beings exposed to NMPs either via inhalation, ingestion or dermal exposure were conducted. Therefore, we reviewed the fate of NMPs in different human tissue samples to estimate the amount of NMP present in human tissues and their overall translocation within the human body.

5. The fate of NMPs within the human body

There is a lack of scientific literature documenting the occurrence of NMPs in humans. However, already more than twenty years ago, Pauly et al. (1998) described the presence of fibers in cancerous and non-pathologic human lung tissues. They found fibers in 87% of human lung specimens and discussed that some fibers were made of plastic due to their shape and structure. Since the aim of the study was not to primarily distinguish between natural and plastic fibers, the polymeric composition was not investigated spectroscopically (Pauly et al., 1998). In a more recent study, applying Raman spectroscopy on 20 routine coroner autopsy samples from individuals living in São Paulo, polymeric particles and fibers were detected in 13 samples (Amato-Lourenço et al., 2021). In total, 31 MPs were detected, of which 88% were fragments (mean size: 3.9 ± 0.7 μm) and 13% fibers (mean fiber length: 11 ± 2 μm). Although PM_{10} is usually trapped in the nasopharyngeal region (Cooper and Loxham, 2019; Schraufnagel, 2020), smaller particles may potentially be inhaled, entering deeper lung regions. However, a recent study found MP much larger than PM_{10} in different regions of the human lung (mean particle length: 105.22 ± 92.82 μm, mean particle width: 34.44 ± 22.61 μm) (Jenner et al., 2022). Furthermore, Huang et al. (2022) indirectly measured the contamination of the human lung with NMPs using sputum samples of 22 volunteers. They found different polymer types mainly smaller than 500 μm (median: 75.43 μm). To monitor potential procedural contamination, they conducted one blank sample. Subsequently, the authors corrected the sputum samples with the blank sample value and found a median number of 39.5 MPs/10 mL sputum.

Two pilot studies on the contamination of the human placenta with NMPs were conducted (Braun et al., 2021; Ragusa et al., 2021). Both studies showed the contamination of human placenta samples from vaginal (Ragusa et al., 2021) and cesarean delivery (Braun et al., 2021).

Furthermore, one study investigated MPs in human colon tissue samples (Ibrahim et al., 2021). They found a mean of 28 MPs/g colon sample, with 96% of all MPs being fibers of approximately 1 mm length. Interestingly, the authors found mainly fibers in their samples, whereas in human stool samples, mainly fragment- and film-shaped MPs were detected (Schwabl et al., 2019). A second study confirmed the presence of MPs in human stool samples but unfortunately no information regarding the shape of the MPs were given (N. Zhang et al., 2021). Therefore, we can only speculate that the differences in the observed shapes from colon and stool samples could either derive from differences in the sample collection, procedure, and subsequent measurements or by the fact that fibers are more likely to stick to the colon tissues than fragments and films that are more easily released. However, this is highly speculative and needs further investigation. Just recently, Horvatits et al. (2022) described the presence of MPs in human liver, spleen and kidney samples. Out of 17 tissue samples, the authors found six MPs ranging from 4–30 μm in size. Another study investigated NMPs in human blood samples (Leslie et al., 2022). The authors found a mean NMPs concentration of 1.6 μg/mL of blood by using Py-GCMS. It has to be noted that the particle size distribution is defined by the opening of the venipuncture (0.5 mm, upper limit) and the filter mesh size (700 nm, lower limit). The authors aimed to detect five different polymer types (PET, PE, PS, PMMA and PP). All polymer types were detected except for PP.

At this point, we would like to emphasise that in both the exposure studies and the fate studies different sampling procedures and analytical techniques have been applied while quality assurance and quality control (QA/QC) measures are often lacking. A few studies investigated the quality and reliability of data and whether a proper risk assessment can be performed based on current knowledge. For instance, Koelmans et al. (2019) determined the reliability of studies using nine quality control criteria in a systematic review, including 50 publications on NMPs in freshwater, wastewater and drinking water. They concluded that out of the 50 publications, only 4 scored positive in all criteria and can be considered reliable data. Furthermore, Coffin et al. (2022) aimed to develop and evaluate the feasibility and confidence in deriving a human health-based threshold value for MPs in drinking water. The authors scored the quality of the reviewed publications and concluded that currently, the uncertainties in the data are too high to develop a human health-based threshold for drinking water quality. The conclusion of Coffin et al. (2022) is in great agreement with the WHO report (2022), indicating that "(…) the available data are of only very limited use for assessing the risk of NMP to human health".

Therefore, we would like to highlight that the comparability between studies is challenging and the interpretation of the presented results above should be taken with caution.

6. Reasons why reported studies should be interpreted critically

In our review article, we described the current knowledge of the NMP contamination of the most relevant (1) exposure routes to humans, the potential (2) translocation mechanisms of NMP across biological barriers and summarized the studies of the (3) fate of NMP in human tissues and fluids. Although our review article did not aim to compare contamination levels of NMP in the different studies investigating exposure scenarios and the fate of NMP in human tissues, it is essential to keep several aspects in mind. Other review articles have already addressed the analytical challenges for assessing NMPs in matrices relevant to human exposure and described the crucial steps during sample collection and processing (Alexy et al., 2020; Koelmans et al., 2020; Noventa et al., 2021; Van Raamsdonk et al., 2020; Toussaint et al., 2019; Wright and Kelly, 2017). Especially sufficient QA/QC in NMP analysis are essential. Considering that NMPs are usually found everywhere in the laboratory environment, the possible contamination of a sample (exposure template or human tissues and fluids) should be kept in mind. In brief, using procedural blank samples in every step is critical to monitor potential contamination during sampling and sample processing. Further information on how to sufficiently perform QA/QC in NMP research can be found elsewhere (Brander et al., 2020; Enders et al., 2020; Möller et al., 2020). However, even if QA/QC measures have been addressed, studies must be critically viewed. For instance, in Ragusa et al. (2021), the authors state that they performed procedural blanks and corrected the samples with the blank values; however, the numbers of particles found in the blanks are not stated and therefore, it is hard to interpret the data. Furthermore, they state that they have excluded fibers from their analysis as they could not use laminar airflow cabinets during sample processing. However, NMP fragments also occur in the ambient air and may contribute to the potential airborne contamination of the samples. Another example is the Study of Ibrahim et al. (2021). The authors followed several steps to prevent airborne plastic contamination: E.g. cotton lab wear was worn, liquid reagents were prefiltered before usage (although no mesh sizes were stated), test devices were pre-cleaned, and the use of plastic items for sample processing was kept to a minimum. Here it must be noted that although the authors used blank samples during microscopy, they did not describe the use of blanks during sample collection but have pre-checked the formalin fixative and filters for plastic contamination (Ibrahim et al., 2021).

Given the limitations of state-of-the-art analytical methods, particle numbers and sizes found in exposure matrices and in human tissues and fluids may not reflect accurate numbers. Möller et al. (2020) summarized the advantages and disadvantages of the different techniques used in NMP identification. In brief, visual sorting or hot needle tests are highly error-prone and not recommended. In contrast, vibrational spectroscopy and chromatographic techniques are state-of-the-art and suitable MP identification techniques. Vibrational techniques include Raman or Fourier transform infrared (FTIR) spectroscopy and allow the precise identification of different polymer types. However, it must be noted that a particle's detection limit is at ~1 μm for Raman and ~10 μm for FTIR (depending on the instrument); therefore, smaller MP and NP cannot be detected.

On the other hand, chromatographic techniques such as pyrolysis-gas chromatography-mass spectrometry (py-GCMS) or thermal extraction desorption GCMS (TED-GCMS) can identify MP and even NP within a non-treated sample. However, both methods can only measure relatively small sample sizes and are destructive. Therefore, no information can be given about the number of particles, size and shape (Möller et al., 2020). However, by comparing different particulate contaminants, Wieland et al. (2022) concluded that the size, shape and surface properties play a decisive role in particle toxicity and should be considered. In principle, to determine the size of NMP, the samples could be filtered and therefore grouped in different size classes and subsequently analyzed with py- or TED-GCMS. However, due to the pre-processing of the sample, the decisive advantage that no sample preparation is necessary for chromatographic methods is lost, and the prior processing of the samples create the risk of sample contamination or loss of particles.

Another commonly used method in the presented studies is scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) emission detection. However, an accurate interpretation of the spectra is only possible for flat-polished samples or thin films with irrelevant topography (Girão, 2020). Therefore, due to the different limitations of the various methods as well as the potential contamination of a sample, both the numbers and the polymer types should be critically viewed in the reported studies.

If one considers the translocation mechanisms described earlier in our review article, the size of the particles seems to be one of the driving factors for tissue translocation. For instance, the translocation of particles in healthy human skin is determined by their size, which should not exceed the lower nanometer size range. For the GI and lung, the particles should not exceed sizes of the lower micrometre size range, namely <10 μm or even smaller, with an increasing translocation potential with

decreasing particle sizes. Particulate matter's size-related transport across biological barriers was investigated *in vitro* and *in vivo*. In rodent models, it was shown *in vivo* that radioactive-labelled NPs are more likely to be translocated within the GIT mucosa than MPs. The smaller NPs (50 and 100 nm) showed a higher adsorption rate than 1 μm MP particles (33, 26 and 4.5%, respectively) (Jani et al., 1990). Furthermore, after intratracheal exposure of mice to 20 nm rhodamine-labelled polystyrene NPs the particles could be detected in maternal and fetal tissues (Fournier et al., 2020). However, it has to be noted that it cannot entirely be ruled out that the labelling of the used particles may have leached, and it was not the particles per se being detected. Furthermore, using an *in vitro* model of the small intestinal epithelium, DeLoid et al. (2021) showed significantly higher uptake of small NPs (25 nm carboxylated PS spheres) than larger particles. However, Stock et al. (2019), using a similar epithelial model, demonstrated that the uptake of MP (1, 4 and 10 μm) is generally possible.

Keeping the potential for tissue translocation in mind, most particle sizes detected in the exposure matrices are much larger than the described particle sizes for translocation mechanisms. For instance, the smallest NMP sizes described in the exposure scenario studies presented in this review are in the lower micrometre size range: 1–50 μm (Hernandez et al., 2019), 1.5–2.5 μm (Oliveri Conti et al., 2020), 2–180 μm (Diaz-Basantes et al., 2020), 3–60 μm (Kumar et al., 2021), 3–145 μm (Praveena et al., 2018), 4–20 μm (Ustabasi et al. 2019), <5 μm (Oßmann et al., 2018) and 5–20 μm (Schymanski et al., 2018). However, not all studies present clear evidence that the small fraction of the reported NMP in the exposure matrices are indeed plastic particles. For instance, Praveena et al. (2018) performed FTIR analysis only on the larger fraction of isolated NMPs. Ustabasi and Baysal (2019) did not perform FTIR analysis on single particles but measured a film consisting of particle aggregates. Diaz-Basantes et al. (2020) used FTIR to identify the polymeric composition of 10 particles per sample. The particles must be larger than the instrument's detection limits; therefore, the authors cannot conclude the presence of small NMPs.

In the fate studies, very small MPs (<3 μm) or NPs were also not reported or insufficiently identified. The smallest particles found in human tissues were 2 μm in the lung (Amato-Lourenço et al., 2021), 3.3 μm in liver (Horvatits et al., 2022), and 5–10 μm in human placenta (Ragusa et al., 2021). Horvatits et al. (2022) stained the isolated particulate matter with Nile Red and measured only a few particles with Raman spectroscopy. The authors do not state the size of the identified MP; therefore, no conclusions can be drawn whether all small particles are of polymeric origin.

Next to the size and shape of NMPs, their concentration plays a decisive role. For instance, the concentration of NMP found in blood samples seems to be rather high since concentrations reported in surface waters or bottled waters were by a factor of 22 and 8.300 lower (1.6 μg/mL in blood (Leslie et al., 2022), 0.073 μg/mL in surface waters and 0.000193 μg/mL in bottled drinking water (only PET detected) (Braun et al., 2021). One may assume that the constant exposure of humans to NMP may lead to their accumulation in tissues and blood, even exceeding environmental concentrations. However, whether an accumulation of NMP in human tissues and blood is realistic needs further investigation.

Here would like to emphasise that particle properties other than size or shape are rearly reported in these studies, although different properties can contribute to the particles' potential to cross biological barriers. To date, most studies used model NMP particles, like polystyrene spheres which do not resemble particles present within the exposure matrices. Environmentally relevant NMPs have various sizes and shapes with different surface modifications and are not uniform spherical particles of homogenous sizes. Furthermore, the use of model NMPs in effect studies has been considered insufficient since the choice of the commercial source of the model NMPs can significantly affect the experimental output, and the particles should be characterized in detail (Ramsperger et al., 2021). In contrast, weathered NMPs should be used since it has been shown that an eco-corona (Ramsperger et al., 2020) or the artificial UV-aging of particles (Völkl et al., 2022) alters the surface of the particle leading to differences in the particle-cell interactions and cellular responses. This aspect is also highlighted by the fact that the MP found in human tissue samples is irregular, like fragments or fibres. To date, we have a discrepancy between the studies on the transport of spherical NMP across biological barriers and the properties of the particles described in the fate studies. Therefore, reliable statements of how non-spherical particles can potentially enter the tissues and whether the concentrations found in the tissues are meaningful cannot be made to date.

6.1. Risk assessments of NMP exposure to humans

The presence of NMP may cause oxidative stress and cytotoxicity, either due to the particles' physical or chemical properties or the exposed tissue's response (Prata et al., 2020). Altered metabolism, neurotoxicity, reproductive toxicity, and immune function disruption are also potential health risks (Prata et al., 2020; Rahman et al., 2021). However, these assumptions are predominantly based on observations in animal models or *in vitro* approaches. It remains unclear whether the toxicological effects observed in animal models are transferable to humans (SAPEA, 2019).

In general, it is doubted that without extensive standardization, representative reference materials, and inclusion of physicochemical properties and associated substances, a realistic assessment of human health risks is possible (Brachner et al., 2020; Vethaak and Legler, 2021). Toxic effects may also depend on specific properties such as shape, surface charge or residual monomers of the plastic particles. Kooi and Koelmans, therefore, propose to consider continuous scales for probabilistic risk assessment of microplastics (Kooi and Koelmans, 2019). Ultimately, however, the complex mixtures of different chemicals found in environmental samples of NMPs may present too high a hurdle to separate the different effects of combinations of chemicals and particles (Gouin et al., 2022). Recent studies pointed to the need for adopting tools and models to estimate the exposure and fate of NMPs to perform a risk assessment. For example, modelling human exposure to MP and the associated chemicals needs to consider MPs' characteristics and leaching rates of chemicals in a combined manner for a holistic risk assessment (e.g., Mohamed Nor et al., 2021). Screening and prioritization tools for hazard data are also needed to ensure the use of fit-for-purpose data for risk assessment (Gouin et al., 2022).

Overall, promising steps have been made toward identifying and prioritizing major research needs, limitations in microplastic risk assessment, and the development of the respective tools and models (Gouin et al., 2019; Mehinto et al., 2022). However, a fully operational human health risk assessment is not available to date. Even if only small fractions of NMP can overcome epithelial barriers, the long-term effects of persistent particles and associated chemicals should not be underestimated (Vethaak and Legler, 2021).

7. Conclusion

We describe in this review the various sources and exposure routes of how humans can come into contact with NMPs. We detected three main pathways of how NMPs enter food: First, the contamination of the environment with NMPs determines the contamination of food items (e.g., the contaminated waters determine the contamination of blue meat). Secondly, NMPs can enter food through industrial processing and thirdly, NMPs can enter food through packaging and atmospheric deposition. Concerning the sources, in almost all matrices, NMPs were detected, emphasizing various human exposure sources via drinking water, food, air and PCPs. It is widely accepted that as particle size decreases, interaction with tissue and individual cells increases. From the three exposure routes of NMPs to humans, size-dependent defence mechanisms occur for the skin and inhalation, whereas in principle

NMPs of any size can be ingested. The translocation through the skin is either restricted to particles in the lower nanometer size range or may occur via the transappendageal pathway, restricted to a very small percentage of the skin area (up to 1.3%). As described above, the respiratory system of humans is also equipped with size-dependent defense mechanisms, usually retaining larger NMPs before entering the deeper lung tissue. However, to date, the few studies on the fate of MPs in human tissues, also within the lung, detected particles in a size range of a few micrometers. The fact that it is often not stated in the presented studies which, or if, QA/QC measures were taken, makes it difficult to draw conclusions on the actual exposure level of biologically relevant particle sizes and whether the NMP found in human tissues and fluids are meaningful. Although first studies indicate the presence of small NMP in exposure matrices and human tissues and fluids, we highly recommend, to critically read and interpretate current literature, to not overinterprate the current understanding in NMP research regarding human health. Research into very small MPs and NPs is still in its infancy. Consistently further development of reliable methods for the isolation, purification and analysis of small MPs and NPs is urgently needed to make accurate statements regarding the exposure and fate of NMPs within the human body.

Author statement

All authors contributed to conceptualization, resources and funding acquisition. AFRMR, HK, JB, MGJL, CL, BG, CRL, SP and HPG wrote the introduction. AFRMR, MGJL, CL, HPG and DK wrote the chapter of NMP in drinking water. AFRMR, HK, JB, MGJL, CL, RP, AU, EB, MP, IF, FBa, FBe, MZ, AT and VM wrote the chapter of NMP in food. AFRMR, MGJL, CL, JD and FP wrote the chapter of NMP in PCPs. AFRMR, HK, JB, MGJL, CL, FBe, MZ, MN, HW, AKA, SZN, SEH, TKE, PG, BCB, KCN, EB, MP, IF and FBa wrote the chapter of NMP in indoor air and workplaces. AFRMR, HK, JB, MGJL, CL, FP, HW, AKA, SZN, SEH, TKE, PG, BCB and KCN wrote the chapter of NMP translocation. AFRMR, HK, JB, MGJL, CL wrote the chapter of NMP fate in the human body, abstract and conclusion. AFRM, JB, MGJL, CL, DK, BG, CRL and SP wrote the Reasons why reported studies should be interpreted critically and risk assessment. AFRMR, HK, JB, MGJL, CL wrote the first draft of the manuscript and all authors reviewed and edited the manuscript. JB and AFRMR designed the graphical abstract.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

No data was used for the research described in the article.

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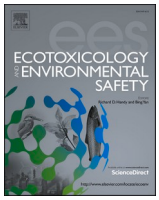

Review

Biomarkers of oxidative stress, inflammation, and genotoxicity to assess exposure to micro- and nanoplastics. A literature review

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ABSTRACT

The increased awareness about possible health effects arising from micro- and nanoplastics (MNPs) pollution is driving a huge amount of studies. Many international efforts are in place to better understand and characterize the hazard of MNPs present in the environment. The literature search was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology in two different databases (PubMed and Embase). The selection of articles was carried out blind, screening titles and abstracts according to inclusion and exclusion criteria. In general, these studies rely on the methodology already in use for assessing hazard from nanomaterials and particles of concern. However, only a limited number of studies have so far directly measured human exposure to MNPs and examined the relationship between such exposure and its impact on human health. This review aims to provide an overview of the current state of research on biomarkers of oxidative stress, inflammation, and genotoxicity that have been explored in relation to MNPs exposure, using

Abbreviations: A-, aged; PS, Polystyrene; AAT, alpha-1 antitrypsin; AChE, acetylcholinesterase; ACN, Acrylonitrile; AHR, aryl hydrocarbon receptor; ALT/AST, alanine aminotransferase, aspartate aminotransferase; CAs, chromosomal aberrations; CAT, catalase; CBA, multiplexing Cytometric Beads Array; CBMN, cytokinesis-block micronucleus; CBPI, cytokinesis-block proliferation index; MCP-1, monocyte chemoattractant protein-1; COL1A1, collagen type I alpha 2 gene; CYP1A, cytochrome P450 Family 1 Subfamily A protein; DCFDA or DCFH-DA assay, 2',7'-dichlorodihydrofluorescein diacetate; DMF, Dimethylformamide; EIA, Enzyme Immuno Assay; ELISA, Enzyme-linked immunosorbent assay; ENAs, extractable nuclear antigens; F, fluorescent-; FP, foam particles; G6DPH, glucose-6-phosphate dehydrogenase; GC, Gas chromatography; GOT/AST, aspartate aminotransferase; GPx, glutathione peroxidase; GR1, gamma response 1 protein; GSH-Px, plasma glutathione peroxidase; GST, glutathione S-transferase; HCA, high content analysis; HDL, high-density lipoprotein; HDPE, high density polyethylene; IL-, Interleukin -; INF-γ, interferon gamma; KIEs, key initiating events; L-, leached; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LDPE, low density polystyrene; LPO, lactoperoxidase; LPS, lipopolysaccharides; MC2R-gene, Melanocortin 2 Receptor; MDA, malondialdehyde; MI, mitotic index; MMP, plasma matrix metalloproteinases; MN, micronuclei; MNPs, micro- and nanoplastics; MOA, mechanism of action; MPO, myeloperoxidase; MPs, generic microplastics polymers; MS, mass spectrometry; MTS, assay (3-(4,5-dimethylthiazol-2-yl)−5-(3-carboxymethoxyphenyl)−2-(4-sulfophenyl)−2H-tetrazolium); MTT, assay (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide); Muc-, muc genes; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; NH2-PS, amino functionalized polystyrene; NLRP3, NOD-like receptor family pyrin domain containing 3; NO, nitric oxide; NPB/NBUD, nucleoplasmic bridge/ nuclear bud; NPs, nanoplastics generic polymers; P-, pristine-; PA, polyamides; PCU, polycarbonate polyurethane; PE, polyethylene; PE-BaP, polyethylene-benzo-a-pyrene; PES, polyester; PET, Polyethylene terephthalate; PI, polyisoprene; PLGA/PVA, polylactide-co-glycolide; PMA, poly methyl acrylate; PMMA, Polymethyl methacrylate; POx, peroxidase; PP, polypropylene; PPAR-α, peroxisome proliferator-activated receptor alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PS-COOH, carboxy functionalized polystyrene; PS-COOH., carboxy functionalized polystyrene.; PS-MP, polystyrene-microplastics; PUR, polyurethane; PVC, polyvinylchloride; qPCR, quantitative Polimerase Chain Reaction; ROS, reactive oxygen species; SCE, sister chromatids exchange; SOD, superoxide dismutase; STAT-3, signal transducer and activator of transcription 3; SULT1A1, sulfotransferase family 1 A member 1 gene; TBARS, Thiobarbituric acid reactive substances; TC, total cholesterol; TEAC, Trolox equivalent antioxidant capacity; TG, triglycerides; TGF-β1, transforming growth factor-beta1; TLR-4, tool-like receptor-4; TNF-α, tumor necrosis factor alfa; TTC assay, triphenyl tetrazolium chloride; U-MDX, metabolites of 4,4'-diphenylmethane di-isocyanate; U-TDX, 2,4- and 2,6-toluene diisocyanate; WST, 1 assay (4-[3-(4-Iodophenyl)−2-(4-nitro-phenyl)−2H-5-tetrazolio]−1,3-benzene sulfonate); ZO-, tight junction protein; α-SMA, alpha-smooth muscle actin.

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human, cellular, animal, and plant models. Both in-vitro and in-vivo models suggest an increased level of oxidative stress and inflammation as the main mechanism of action (MOA) leading to adverse effects such as chronic inflammation, immunotoxicity and genotoxicity. With the identification of such biological endpoints, representing critical key initiating events (KIEs) towards adaptive or adverse outcomes, it is possible to identify a panel of surrogate biomarkers to be applied and validated especially in occupational settings, where higher levels of exposure may occur.

1. Introduction

Synthetic or semi-synthetic materials typically made from polymers derived from petroleum-based are commonly called “plastics”. Despite this oversimplification, plastics are a huge and heterogeneous class of compounds with many industrial and bio-medical applications. There are many types of polymers, but some of the most common types include polyethylene (PE), polystyrene (PS), polypropylene (PP), or polyvinyl chloride (PVC). The formers being the most widely used in the world (Cantor and Watts, 2011). Owing to their properties, these polymers find extensive applications in industrial sectors, such as automotive, in aerospace and electronics. Furthermore, the food industry relies on these polymers for packaging and wrapping purposes (Ncube et al., 2021a; Ncube et al., 2021b).

Plastics can be generated from primary sources including industrial processes, like the production of waterborne paints, medical devices, electronics, coatings, and adhesives. They can also be indirectly produced as secondary materials when larger plastic debris fractures and breakdown through various processes, both natural and non-natural.

Despite the significant increase in plastic production over years, societies have become over-reliant on plastic due to its durability, low cost, and versatility. The consequences of this heightened production include the accumulation of vast amounts of plastic waste that pollutes both terrestrial and aquatic ecosystems. Indeed, as shown in the literature LDPE (low density polystyrene), HDPE (high density polyethylene) and cellulose acetate are the types of plastics most commonly identified in landfills (Afrin et al., 2020). On the other hand, PE, PET (polyethylene terephthalate), PP, PVC, PI (polyisoprene) and PS were identified in sewage, industrial effluents and from the ocean spray (Di Bella et al., 2022; Caracci et al., 2023). The same plastics have also been identified in the atmosphere around urbanised and industrial areas, due to their small size, particles are easily transported by the wind (Pandey et al., 2022). Furthermore, it has been shown in studies by O’Brien and Syversen et al., that the plastics used in the textile and fishing industry are PA (polyamides), PP PE and PES (polyester) (O’Brien et al., 2020; Syversen et al., 2022). Nonetheless, plastic production is expected to still increase in the coming decades (Network; Walker and Fequet, 2023) and it will be a growing need to find alternative eco-friendly materials or solutions to limit their spread in the environment by better educating people (Dube, Grace, 2023). Plastic materials can broadly be classified into five categories based on their sizes which includes; megaplastics (>1 m); macroplastics (<1 m), mescoplastics (<2.5 cm), microplastics (<5 mm); and nanoplastics (<1 μm) (Barnes et al., 2009; Wang et al., 2018a).

Once disposed of, plastic waste is exposed to environmental factors that has the potential to break down into substantial quantities of microplastics (MPs) and nanoplastics (NPs). The breakdown of plastic into smaller particles raises global concerns regarding its possible impacts on the environment and human health (Wagner and Reemtsma, 2019). While MPs have been extensively studied for their environmental impact, our understanding of the quantities, types, and toxicity of NPs and their impacts on human health is limited. It is noteworthy taht a single MP particle can further breakdown into billions of NP particles, indicating the widespread of NPs pollution (Zhang et al., 2023);(Hale et al., 2022). NPs may pose a greater risk than MPs due to their ability to penetrate biological membranes, but whether NPs exposure can affect human health is still debated (Gigault et al., 2016; Hernandez et al., 2017; Ter Halle et al., 2017). The increase in plastic waste represents a health trait to human health as MNPs have been found in many food products, owing to their widespread distribution in aquatic and terrestrial areas (Kolandhasamy et al., 2018; Wagner and Reemtsma, 2019; Wang et al., 2020). MNPs can enter the human body through three primary pathways: inhalation, ingestion, and skin contact (Prata et al., 2020; Rahman et al., 2021). Airborne MPs have been detected in urban dust as a result of synthetic textiles and rubber tire degradation; these particles are typically sub-micronic in size and can be inhaled (Prata, 2018). Ingestion is considered the major route of exposure for the general population, as they are found in the food chain and water sources. Studies have shown that these tiny plastic particles enter the human food chain through various media, including consumption by animals (Santillo et al., 2017), contamination during food production (Karami et al., 2017), and leaching from plastic packaging (Mason et al., 2018). MNPs have been found in a range of food products, including honey, beer, salt, sugar, fish, shrimp, and bivalves, as well as in tap, bottled, and spring water. In fact, a high percentage of tap water sources around the world have been found to contain MPs particles (Kosuth et al., 2018; Mamun et al., 2023).

Although the number of studies about the potential effects of MNPs on living organisms steadily increases (Chang et al., 2020), research on human exposure and toxicity in this context is relatively new. A recent review summarized the current knowledge on the exposure routes of MNPs to humans, and possible pathways for translocation into body compartments (Ramsperger et al., 2023).

Prata et al., 2020 highlighted that following exposure and uptake, the potential toxicity of MNPs may result from oxidative stress and inflammation, which consequently could affect the immune and nervous systems (Prata et al., 2020). Both in-vitro and in-vivo models suggest that increased level of oxidative stress and inflammation are the primarily MOA leading to adverse effects, mainly chronic inflammation, immunotoxicity, and genotoxicity (Poma et al., 2019; Demir, 2021; González-Acedo et al., 2021). While these simplified models are useful for hazard identification, they do not fully reflect the complexity of interactions occurring within human body. However, researchers are still encountering difficulties in assessing the impact of MNPs on human health, owing to the variability of exposure scenarios, the changeable pattern of MNPs along with their constituents and contaminants and the lack of standardized protocols including biomarkers for assessing relevant biological and health endpoints. As a result, until now very few studies have measured human exposure to MNPs and assessed the relationship between MNPs exposure and its effects on human health.

This paper aims to provide a comprehensive review of the current the state of the art of biomarkers investigated following exposure to MNPs in humans, as well as cellular, animal and plant models. Biomarkers are chemicals, metabolites, or products of an interaction between a chemical and some target molecule that is measured in the human body compartments (World Health Organization, 2006). An exposure biomarkers is the concentration of a parent compound or its metabolites in biological matrices (Nieuwenhuijsen et al., 2006), whereas an effect biomarker is a measurable biochemical, physiological, and behavioral effects or other alterations within an organism that, depending on the magnitude, can be associated with an established or possible health impairment or disease (Zare Jeddi et al., 2021). Biomarkers can reveal changes in biological systems resulting from complex pathways of exposure. With the identification of such biological endpoints,

representing the KIEs towards adaptive or adverse outcomes, it should be feasible envisaged a panel of surrogate biomarkers to be applied and validated, especially in occupational settings, where exposure may occur and can be easier characterized.

2. Materials and methods

The search strategy consisted of filtering the publications with a combination of keywords specifying the following mesh terms with synonyms: "Oxidative stress", "Inflammation", "Genotoxic", "Biomarkers" (full list of all biomarkers), "Microplastics", "Nanoplastics" (full list of MNPs). The complete string is provided in the appendix A. We transferred the results from databases to Microsoft Excel spreadsheet where inclusion and exclusion criteria were recorded. Two reviewers evaluated the publications independently and a third reviewer resolved cases of disagreement.

Following PRISMA 2020 Statement (Page et al., 2021), the papers were first screened for title and next for abstract. In both steps, according to the exclusion criteria, we excluded studies (1) without biomarkers of oxidative stress, inflammation, or genotoxicity, (2) investigating micro- nanoplastic's additives, (3) performed on bacteria, (4) all review papers, (5) full texts with unpublished data, (6) correspondences, (7) conferences abstracts without full text and (8) clinical studies (e.g. bone integration of plastic prosthesis).

Studies focused on or analyzing the possible adverse effects of MNPs as result of human mainly occupational, cell, animal, and plant models were considered eligible.

The Fig. 1 summarizes the main steps of the searching strategy.

We reported the following information according to the study types identified: humans, in-vitro and in-vivo: animals and plants. For in-vitro studies, the information reported were the following: author's name, publication time, title, cell type, plastic-type (also size), assessed biomarkers, exposure time, experimental methods, concentration, main results, references, and notes. For in-vivo studies were extracted: author's name, publication time, title, organism type, number of animals or plants, plastic type (also size), matrix (only for animals), assessed biomarkers, exposure time, experimental methods, concentration, main results, references, and notes.

For studies on humans, we reported: author's name, publication time, title, number of subjects, worker's exposure, age, smoking habits, plastic-type (also size), matrix, analytical methods, assessed biomarkers, exposure time, experimental methods, concentration, main results, references, and notes. Data reported by graphs in original studies were extracted by the Web Plot Digitizer software (Rohatgi 2022), version 4.6, Pacifica, California, USA, https://automeris.io/Web PlotDigitizer/ accessed on February 2023).

Among the 5818 studies identified, 757 were duplicates removed by EndNote. The remaining 5061 were screened as title and abstract. Of these, 4849 were excluded and 202 were screened as full text. Finally, 65 articles were included in this state of art review. The exclusion criteria lead to the removal of 137 studies because of the absence of biomarkers of oxidative stress (OS), inflammation, or genotoxicity (n = 79). MNPs

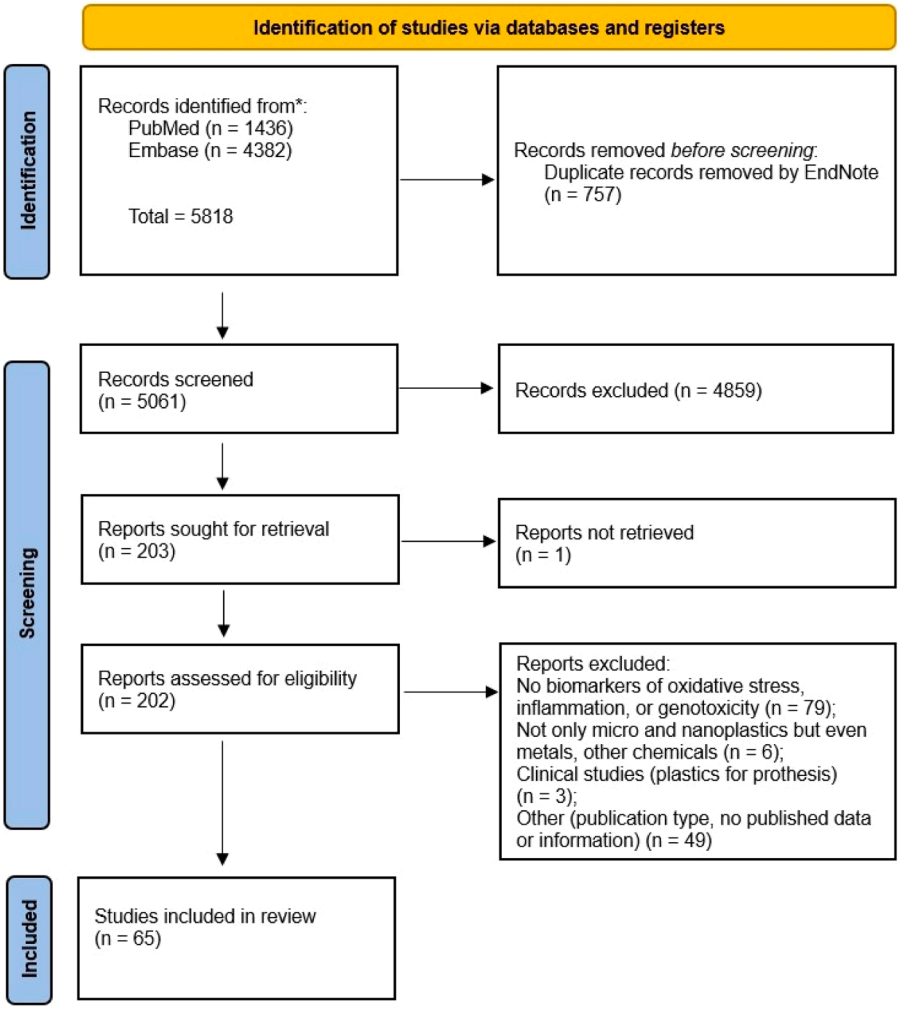

Fig. 1. Flowchart of the identification for eligible studies from a search among original articles.

were not considered as polymers but for their additives or chemicals (n = 6), no data or information published or publication type (n = 49) or were clinical studies (e.g. plastics used for dental or orthopedic prothesis) (n = 3). Data from the 65 included articles were extracted using different templates and organized into spreadsheets according to the type of study. 28 for in-vitro models, 30 for in-vivo studies on animals, 4 for in-vivo studies on plants, and only 3 for studies on humans.

3. Results

Table 1 summarizes the number of eligible articles, according to study type, that investigated the different MNPs. PS is the most widely investigated MNPs in the studies in-vitro and in-vivo. Indeed, among the studies included in this review, 43 articles (>50%), explored the possible adverse effects of PS in-vivo, 53.4% on animals, 9.3% on plant models and 37.3% on cell lines. The second most analyzed polymer is PE being reported in 17 articles. 70.5% investigated the possible effects in animal models, and only 29.5% on in-vitro studies. It is worth mentioning that these two MNPs were not studied in humans. 8 articles explored PVC, 62.5% in cell lines, 25% in animal studies, and only 12.5% in humans, in occupational scenarios. The other MNPs investigated are: not specified polymers (n = 5), PP (n = 4), polymethyl methacrylate (PMMA) (n = 3), polyethylene terephthalate (PET) (n = 2), polyurethane (PUR) (n = 1), and polylactide-co-glycolide (PLGA/PVA) (n = 1).

Table 2 reports the size range of plastics investigated. In in-vitro studies, the plastics size range varies from 0.029 to 150 μm, while in in-vivo the plastics analyzed had a much wider range (from 0.2 μm to 5 mm which mirrors environmental exposure). In occupational studies, since the workers are exposed to mixtures and not to a single particle with defined chemical identity the size range was not provided.

3.1. Biomarkers of oxidative stress, inflammation, and genotoxicity

In the following tables are listed all the biomarkers investigated and the results reported by the included articles.

3.1.1. Oxidative stress

Oxidative stress is a central mechanism of action for both pulmonary and extra-pulmonary health effects of particulate matter (Mills et al., 2009). ROS (reactive oxygen species) are formed as a normal attribute of aerobic life as a by-product of metabolic reactions. Their excessive presence can lead to molecular and tissue damage defined as a result of oxidative stress, i.e. a perturbation of the physiological redox balance that is not balanced by the body's appropriate adaptive responses (Sies, 2015).

Thus, investigating biomarkers of oxidative stress, such as reactive oxygen species (ROS) and their adducts, as well as the enzyme pathways involved in the maintenance of an adequate physiological balance, superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), in biological media, can provide direct evidence of perturbation induced in biological systems (Marrocco et al., 2017; Halappanavar et al., 2021).

Wang et al. studied the adverse effects, following exposure to PS (0.025–0.8 μg/ml) of renal tubule cells by quantifying the release of ROS (Wang et al., 2021). Similarly, Schirinzi et al. who analysed the ROS production following PE (10 ng/ml) and PS (10 μg/ml) exposure using brain and epithelial cell models, found significant increases in ROS levels as compared to untreated controls (Schirinzi et al., 2017).

20 out of 65 studies included in this review reported a possible effect following MNPs exposure. 12 out of 20 showed a statistically significant increase in ROS following MNPs exposure as compared to the untreated control groups, 5 did not show a significant increase, 2 showed no change and only one reported a statistically significant decrease in ROS generation.

Living are endowed with effective defence systems to scavenge and thus counter balance excessive ROS production (Kotha et al., 2022). Enzymes such as SOD and CAT are involved in catalysing the conversion of superoxide anion to oxygen and hydrogen peroxide (Wang et al., 2018b; Sies and Jones, 2020), making the superoxide radical less reactive, by transforming it into molecular oxygen and hydrogen peroxide (H_2O_2). SOD and glutathione peroxidase (GPx) activities are commonly measured as biomarkers of oxidative stress (Lubos et al., 2011). 12 studies included in this review investigated these enzymatic pathways counterbalancing ROS production. Moreover, *lactate dehydrogenase* (LDH) and GPx have been used as biomarkers in in-vitro and in-vivo (animal) studies, whereas H_2O_2 production has only been studied in-vivo (both animal and plant models). Other biomarkers of oxidative stress consistently used in animal models are *glutathione S-transferase* (GST) (n = 10) and *glutathione* (GSH) (n = 11).

Vecchiotti et al. and Chen et al., carried out in-vitro studies where human cell lines were exposed to varying concentrations of PS (from 25 to 1200.0 μg/ml) for 4 h to a maximum of 48 h, showing an early downward trend in SOD enzyme activity, with small increase after 48 h (Vecchiotti et al., 2021; Cheng et al., 2022).

From Table 4 and Table 5, it is argued that similar decreasing trends in SOD enzyme activity are expected in other animal and plant model studies (Xiao et al., 2021; Li et al., 2022; Rodrigues et al., 2022; Ni et al., 2023). Conversely, studies by Cocci et al., found an increasing trend in SOD activity following exposure to PS (Lu et al., 2016; Cocci et al., 2022).

Various articles reported a significant increase in ROS levels by using

Table 1
Number of eligible articles, according to study type, that investigated the different MNPs.

Type of MNPs	Type of study n (%)				
	In-vitro	In-vivo		Occupational	Total
		animals	plants		
PS	16 (37.3)	23 (53.4)	4 (9.3)	/	43
PE	5 (29.5)	12 (70.5)	/	/	17
PVC	5 (62.5)	2 (25.0)	/	1 (12.5)	8
MPs	2 (50.0)	2 (40.0)	/	1 (20.0)	5
PP	2 (50.0)	2 (50.0)	/	/	4
PMMA	3 (100)	/	/	/	3
PET	/	2 (100)	/	/	2
PUR	/	/	/	1 (100)	1
PLGA/PVA	1 (100)	/	/	/	1
Total	34	43	4	3	84

*Some studies investigated more than one plastic type
PS (polystyrene), PE (polyethylene), PVC (polyvinylchloride), MPs (microplastics), PP (polypropylene), PMMA (Polymethyl methacrylate), PET (Polyethylene terephthalate), PUR (polyurethane), PLGA/PVA (polylactide-co-glycolide)

Table 2
Plastics size range analyzed according to the different study types.

Type of MNPs	Plastic size range (μm)		
	In-vitro	In-vivo	
		animals	plants
PS	0.029–2.0	0.2–5000.0	0.1–20.0
PE	0.21–80.0	1.2–5000.0	/
PVC	0.12–150.0	< 0.3	/
MPs	0.1–50.0	38–355.0	/
PP	0.08–0.25	1.2–1000.0	/
PMMA	0.05–10.0	/	/
PET	0.2–0.6	10–250.0	/
PUR	/	/	/
PLGA/PVA	0.2–0.3	/	/

PS (polystyrene), PVC (poly vinil chloride), PE (polyethylene), PP (polypropylene), PET (polyethylene terephthalate), PLGA/PVA (polylactide-co-glycolide), PMMA (polymethyl acrylate), PUR (polyurethane), MPs (generic microplastics polymers).

Table 3
Biomarkers of MNPs exposure analysed in in-vitro studies.

Cell type	Plastic type, size	Concentration	Exposure time	Experimental methods	Biomarkers (Oxidative stress; Inflammation; Genotoxicity and others)	Autors, Year
Onion root cells	PS, 100 nm	25, 50, 100 μg/ml	3 d	TTC and Evans Blue staining; TBARS; qPCR	ROS: ↑dose/dependent; MDA: *↑ vs ctrls; Cell viability: ↑; Comet test: *↑; MI: ↓ vs ctrls	(Maity et al., 2023)
Human intestinal (CCD-18Co) cells	PS, 0.5 and 2 μm	5 or 20 μg/ml	48 h, 28 d and 6 w	DCFDA and flow cytometry	ROS: *↑ vs ctrls; NPs internalization *↑ vs MPs	(Bonanomi et al., 2022)
Human bronchial epithelial cells	PS and NH_2-PS, 100 nm	25, 50, 100, 200, 400 μg/ml	24 h	WST-1 and MTT; DCFH-DA; qPCR	ROS: NH_2-PS *↑ vs PS; IL-1β *↑ expression NH_2-PS vs PS; cytotoxic effects: NH_2-PS *↑ vs PS	(Jeon et al., 2023)
Peripheral blood mononuclear cells	PS, 29, 44 and 72 nm	0.0001–100 μg/ml	24 h	Comet assay; ELISA	8-oxodG: *↑ 0,1 μg /ml- 100 μg /ml vs ctrls; Comet tail: 100 μg /ml: ↑ 23.1%, 29 nm ↑ 13,88%, 44 nm; ↑ 6.9% 72 nm	(Malinowska et al., 2022)
Human lung (A549) cells	wMP, < 50 μm	0.1, 1, 10, 100 μg/ml	24, 48 h	ELISA; DCFDA	ROS: no*↑ vs ctrls; IL-8*↑ vs ctrls; IL-6 ↑ vs ctrls	(Bengalli et al., 2022)
HepG2 cells, Caco-2 cells	PP, 80–250 nm PET, 200–600 nm	PP: 0–175 ng/ml, PET: 0–63 ng/ml and 0.6–7.1 μg/ml	3, 24 h	LDH; WST-1; Comet assay; DCFDA	concentration/dependent; ROS: 3 h no*; DNA damage: ↑; Metabolic activity: no* effects	(Roursgaard et al., 2022)
Human gingival fibroblasts (hGFs)	MP, 100 and 600 nm	Different concentrations	48 h	MTS; qPCR	NFkB *↑ vs ctrls; NLRP3 expression ↓ vs ctrls; Cell viability: ↓ vs ctrls	(Caputi et al., 2022)
Murine fibroblasts and canine kidney epitelial cell lines	PS, 9.5–11.5 μm PE, 1.0–4.0 μm	1, 10, 20 μg/ml	6–24 h	Hemacytometer; MTT; qPCR	SOD: ↓ PS and PE vs ctrls; IL1β, TNF-α: ↑ PS exposure vs ctrls; IL-1β, TNF-α: ↓ PE exposure vs ctrls; Cell viability: ↓ vs ctrls; Metabolic rates: ↑ vs ctrls	(Palaniappan et al., 2022)
Human embryonic stem cell line H1	PS, 1 μm	25 μg/ml	48 h	Commercial kits; P450-Glo assay kit; ELISA	GST activity, GSH, SOD: ↓ vs ctrls; MDA: ↑ vs ctrls; LDH: ↓ vs ctrls; ROS: ↑ vs ctrls; IL-6, COL1A1: ↑regulated dose-dependent; SULT1A1, PPARα, PPARγ: ↓ regulated and ↑ regulated dose-dependent; AST and ALT: *↑ PS-MP exposure; CYP1A: ↓regulated	(Cheng et al., 2022)
Human monocytes and dendritic cells	PS, PMMA, PVC, 50–310 nm	30–300 particles/cell	18, 20 h	ELISA	IL-6, TNF-α and IL-10: ↑ vs ctrls	(Weber et al., 2022)
A549 cells with surface modification	PS, NH_2-PS, PS-COOH, 2 μm and 80 nm	2.5, 5, 10, 25, 50, 100, 200, 400 μg/ml	6, 9, 24 h	MTT; fluorescence microscope; DCFH-DA	ROS: *↑ vs ctrls; MN: ↑, *↑ at ≠ concentrations; Cell viability: ↓ vs ctrls	(Shi et al., 2022)
Human embryonic kidney cells	PS, 3 and 54 μm	3–300 ng/ml	24 h	Phase-contrast microscope; DCFH-DA; Quantibody ® Human Inflammation Array 3 Kit	ROS *↑ vs ctrls; HO-1 expression: no*; NF-κB: No* vs ctrls; NLRP3 expression: *↓ vs ctrls; ZO-2, AAT: ↑ vs ctrls; ↑↓ regulation 33 different cytokines dose-dependent; Cell viability*↓ vs ctrls;	(Chen et al., 2022a)
Caco-2/HT29-MTX-E12/THP-1 cell lines	PS and NH_2-PS, 50 nm; PVC, < 50 μm	1, 5, 10 or 50 μg/cm^2 in 100 μl of medium	24 h	ELISA	IL-1β, IL-6, IL-8 and TNF-α (PS, NH_2-PS): No* vs ctrls; IL-1β (PVC): *↑ vs ctrls; IL-8: ↓; TNF-α and IL-6: No*; Cell viability: *↓ vs ctrls;	(Busch et al., 2021)
Human lung cell lines	PMMA, 120 nm PVC, 140 nm	25, 50, 100, 150, 200 μg/ml	24, 48, 72 h	DCFDA; LDH-Glo cytotoxicity assay	ROS*↑ vs ctrls; LDH*↑ vs ctrls; Cell apoptosis: ↑ vs ctrls	(Mahadevan and Valiyaveettil, 2021)
Colorectal Adenocarcinoma cells (HCT 116)	PS, 100 nm	100, 200, 400, 800, 1200 μg/ml	15, 30, 45 min, 1, 4, 24, 48 h	MTS; Total ROS; Western blot by OECD guidelines	ROS: depending on concentration *↑ vs ctrls; SOD1: ↓, SOD2: ↑, CAT: ↑; GPx1: ↑ depending on concentration vs ctrls; MN: ↑ vs ctrls; Cell viability: ↓ vs ctrls	(Vecchiotti et al., 2021)
Human kidney proximal tubular epithelial cells	PS, 2 μm	0.025, 0.05, 0.1, 0.2, 0.4, 0.8 μg/ml, 0.8 mg/ml	5, 10, 30, 60, 120 min, 3 days	Sulpforhodamine B; MitoSOX Red	ROS: *↑ vs ctrls; Cell viability: *↓ vs ctrls	(Wang et al., 2021)
Human periphral blood lymphocytes	PE, 10–45 μm	25, 50, 100, 250, 500 μg/ml	48 h	CBMN assay with minor modifications	MN: *↑ vs ctrls; NBP and NBUD *↑, CIN: *↑ vs ctrls; CBPI: % index No* vs ctrls	(Çobanoğlu et al., 2021)
Human lung epithelial cells	PS, 1.72 μm	1–1000 μg/cm2	24, 48 h	Trypan blue; DCFH-DA; ELISA	ROS: *↑ vs ctrls; IL-6*↑, IL-8 ↑ vs ctrls; ZO-1, AAT: ↑	(Dong et al., 2020)

(*continued on next page*)

Table 3 (*continued*)

Cell type	Plastic type, size	Concentration	Exposure time	Experimental methods	Biomarkers (Oxidative stress; Inflammation; Genotoxicity and others)	Autors, Year
					expression; Cell viability: *↓ vs ctrls	
Human hematopoietic cells	P-PS, 0.05–0.1 μm F-PS, 0.04–0.09 μm	0–50–100–150–200 μg/ml	24–48 h	Trypan Blue; DCFH-DA; Comet assay	ROS: *↑ vs ctrls in 3 cell lines; Genotoxic damage: *↑ vs ctrls; Cell viability: 3 cell lines ↓ vs ctrls	(Rubio et al., 2020)
Human fibroblast (Hs27) cell line	PS, 100 nm	5, 25, 75 μg/ml	4, 24, 48 h	MTS; Total ROS; CBMN by OECD guidelines	ROS: *↑ vs ctrls; MN: *↑ dose-dependent vs ctrls; CBMN: No* vs ctrls; Cell viability: ↓ vs ctrls	(Poma et al., 2019)
Kidney leucocytes	PVC, 40–150 μm PE, 40–150 μm	1 mg/ml, 10 mg/ml, 100 mg/ml	1 h, 24 h	MTT; flow cytometry; chemiluminescence; colorimetric assay	POx: No* vs ctrls; Cell viability: ↓ vs ctrls; Phagocytic capacity: No* vs ctrls; Burst activity: *↑ vs ctrls;	(Espinosa et al., 2018)
Human cerebral and epithelial cell lines	PE, 3–16 μm PS, 10 μm	10 ng/ml to 10 μg/ml	24, 48 h	HCA	ROS: *↑, ↑ respectively PE, PS vs ctrls; Cell viability: no*↓ vs ctrls	(Schirinzi et al., 2017)
Hamster fibrobast (CHL/IU)	PS, NA	19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 μg/plate	24 h, 48 h	Test di Ames	Test Ames: No* vs ctrls; CA: no* all concentrations vs ctrls; Cell growth: ↑ vs ctrls	(Nakai et al., 2014)
A549 cell line	PLGA/PVA ~ 234 nm, PLGA/CS ~ 233 nm, PLGA/PF68 ~ 229 nm, TiO2 ~ 421 nm, PS ~ 250 nm	0.005–3.5 mg/ml and 0.01–2 mg/ml	48 h	MTT; Non-radioactive Cytotoxicity Assay; multiplexing CBA	LDH: No* effects vs ctrls; IL-6, IL-8 and MCP-1: ↑ vs LPS-treated; IL-1β, TNF-α, IL-10 data were under LOD; Cell viability: ↑ vs ctrls	(Grabowski et al., 2013)
Monocyte cell line TH1 in culture	PE, PE-HM, 2, 3 μm; PCU, 1, 7 μm	Ratio 1:1, 100:1, 500:1 particles/cell	18, 24, 72 h	MTS; TiterZyme EIA assay	IL-1β, TNF-α: ↑ vs ctrls, dose-dependent; Cell viability: No* vs ctrls	(Smith and Hallab, 2010)
Pulmonary cell cultures	PVC, 0.2–2.0 μm, 50 μm	0156 mg/ml	4, 16, 24 and 48 h	ELISA	general cytokines release: ↑; IL-6, and IL-8: *↑	(Xu et al., 2003)
Three human monocytic cell lines (monomac-1, U937 and THP-1)	PE, 0.21, 0.49, 4.3, 7.2, and 88 μm	Cell number ratios: 100:1, 10:1, 1:1 and 0.1:1.	24 h	MTT; ELISA	U937 cells: IL-1β: 0.49 μm *↑ vs 0.21 μm; IL-6: 0.49, 4.3, 7.2 μm* ↑ vs ctrls; TNF-α: (0.21, 0.49, 4.3 μm) *↑ vs ctrl; THP-1 cells: IL-1β: 0.49 μm ↑ vs ctrls, 0.21 and 0.49 μm *↑ vs ctrls; IL-6: *↑ 0.21, 0.49 μm vs ctrls; TNF-α: 0.21, 0.49, 4.3 μm, 0.49 μm *↑ vs ctrls; Cell viability: no* vs ctrls;	(Matthews et al., 2001)
Human monocyte/ macrophages, and fibroblast	PMMA, 1–10 μm	LOW: < 0.05% PMMA, HIGH: > 0.05%	72 h	ELISA	IL6: no co-culture ↑ vs ctrls; IL1β: ↑ co-culture + PMMA vs alone; TNF-α: co-culture + PMMA	(Lind et al., 1998)

PE (polyethylene), PVC (polyvinylchloride), PP (polypropylene), PMMA (Polymethyl methacrylate), PET (Polyethylene terephthalate), PLGA/PVA (polylactide-co-glycolide), P(pristine), F (fluorescent), PS (polystyrene), NH2-PS(amino functionalized polystyrene), PS-COOH (carboxy functionalized polystyrene), PCU (polycarbonate polyurethane), NPs (nanoplastics), MPs (microplastics), MI (mitotic index), MN (micronuclei), ROS (reactive oxygen species), SOD (superoxide dismutase), CAT (catalase), MDA (malondialdehyde), POX (peroxidase), GST (glutathione S-transferase), LDH (lactate dehydrogenase), COL1A1 (collagen type I alpha 2 gene), SULT1A1 (sulfotransferase family 1A member 1 gene), PPAR-α (peroxisome proliferator-activated receptor alpha), PPAR-γ (peroxisome proliferator-activated receptor gamma), ALT/AST (alanine aminotransferase, aspartate aminotransferase), CYP1A (cytochrome P450 Family 1 Subfamily A Member), TNF-α (tumor necrosis factor alfa), IL- (Interleukin -), GPx (glutathione peroxidase), CBMN (cytokinesis-block micronucleus), MCP-1 (monocyte chemoattractant protein-1), LPS (lipopolysaccharides), ZO- (tight junction protein), AAT (alpha-1 antitrypsin), CBPI (cytokinesis-block proliferation index), NPB/NBUD (nucleoplasmic bridge/ nuclear bud), TTC assay (triphenyl tetrazolium chloride), TBARS (Thiobarbituric acid reactive substances), qPCR (quantitative Polimerase Chain Reaction), DCFDA or DCFH-DA assay (2',7'-dichlorodihydrofluorescein diacetate), WST-1 assay (4-[3-(4-Iodophenyl)−2-(4-nitro-phenyl)−2H-5-tetrazolio]−1,3-benzene sulfonate), MTS assay (3-(4,5-dimethylthiazol-2-yl)−5-(3-carboxymethoxyphenyl)−2-(4-sulfophenyl)−2H-tetrazolium), MTT assay (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide), ELISA (Enzyme-linked immunosorbent assay), EIA (Enzyme Immuno Assay), HCA (high content analysis), CBA (multiplexing Cytometric Beads Array), * (significantly), ↓(decreased/inhibited), ↑(increased).

onion root cells (Maity et al., 2023), intestinal (CCD-18Co) cells (Bonanomi et al., 2022), human bronchial epithelial cells (Jeon et al., 2023), and human lung cells (Dong et al., 2020) treated with PS particles (0.5–0.08 μm). *In-vivo* studies in fish (Cocci et al., 2022), broilers (Lu et al., 2023), mice (Wang et al., 2021), and sea worms (Missawi et al., 2020; Lombardo et al., 2022) also showed significant increases in ROS, SOD, and CAT levels compared to controls following exposure to PS and PE (5 mm-1 μm). However, studies in plant organisms (Maity et al., 2020; Liu et al., 2022a; Ni et al., 2023), did show increasing, albeit not significant, trends in CAT compared to controls following treatment with PS (0.01–1 μm).

Among oxidative stress endpoints, MDA has been the most widely investigated (Toto et al., 2022). MDA is a metabolite resulting from the peroxidation of fatty acids. This molecule can interact with nucleic acids and can create DNA adducts generating mutations that might evolve into cancer (Del Rio et al., 2005). Increases in MDA levels were found in 10 out of 14 studies. *In-vitro* studies conducted by Maity et al. and Cheng et al. found higher MDA levels as compared to controls after exposure to 0,01 and 0,1 μm PS, respectively (Cheng et al., 2022; Maity et al., 2023). *In-vivo* animal studies on broilers (Lu et al., 2023) and sea worms (Missawi et al., 2020) showed a significant increase of MDA levels compared to controls after exposure to PS, PE, and PP.

Table 4
Biomarkers of MNPs exposure analysed in animal studies.

Animal model, n°	Plastic type, size	Concentration	Exposure time	Experimental methods	Matrix	Biomarkers (Oxidative stress; Inflammation; Genotoxicity and others)	Autors, Year
Mullus barbatus, Merluccius merluccius, 32	PE, 5–1 mm, 1–05 mm PS, 0.5–0.1 mm	1–20 or 2–15 items/ individual	NA	qPCR	Gut tissues	SOD, CAT expression: ↑ in gut tissue vs ctrls; IL1β, IL-8, and INF-γ expression: ↑ in both species; IL-10: ↑ regulated in gut tissue	(Cocci et al., 2022)
Aeromonas hydrophila, 90	PE, 75–100 μm	0.1, 1, and 10 mg/L	35 days	Commercial kits	Intestinal and muscle tissues	SOD and CAT: * ↓ vs ctrls; GSH, GSH-Px, and GST: initially ↑ trend, then ↓ trend	(Ding et al., 2022)
Charadrius javanicus, 15	PET, 100–250 μm, HDPE, PS, 2 μm, and NH2-PS, 100 nm	(8.1 × 104 fibres/ L), 0.01 mg/L (w/ v), 0.01 mg/L, 0.1 mg/L (w/v), 1 mg/L (w/v)	24 h	Photometric analysis	Tissues	GST: (PET) ↑ vs ctrls; CAT activity: (PET)↑ vs ctrls; GST: *inhibition yellow-HDPE MP; CAT: red-HDPE MP ↓ vs ctrls, blue-HDPE MP, No* , * ↓ vs ctrls	(Esterhuizen et al., 2022)
Gallus gallus domesticus, 120	PS, 5 μm	1 mg/L, 10 mg/L, 100 mg/L	6 weeks	Electron microscopy commercial kits	Lung tissue and serum	CAT, and GSH: ↓ vs ctrls; MDA: * ↑ in all groups; Pathological changes in lung tissue: ↑ damage vs ctrls	(Lu et al., 2023)
Carassius auratus,32	PS, 44 nm	0 −100 μg/L	30 days	Automated laser flow blood cell analyser; optical microscope; EIA	Liver, gut and muscle tissues	ENAs: * ↑ vs ctrls	(Brandts et al., 2022)
Oryzias melastigma, NA	PS, 6.0 μm	1.1 μg/L, 1.1 × 10^3 μg/L, 1.1 × 10^5 μg/ L	14 days	qPCR	Tissues	SOD at T7: * ↑ vs ctrls; CAT, Gpx, AHR and CYP1A1 at any T: No* vs ctrls; CAT, Gpx, AHR and CYP1A1 at any T: = vs ctrls; IL-1β at T3: * ↑ vs ctrls; IL-6, TNF-α, JAK, NF-κB, and STAT-3 at T7: * ↑ vs ctrls; muc7-like at T7: * ↑ vs ctrls; NF-κB at T14: * ↓ vs ctrls; STAT-3 at T7: * ↑ vs ctrls; Il-6, il-1β, NF-κB at T14: * ↓ vs ctrls; IL-8: ↑ vs ctrls; TNF-α: * ↑ vs ctrls; muc13-like at T3: * ↑ vs ctrls; Heg1 and muc5AC-like at T14: * ↓ vs ctrls; muc2 and muc13 at T3: * ↓ vs ctrls	(Chen et al., 2022a)
Cyprinus carpio, 8	PE, NA	1000 ng/L	21 days	Protein determination kit; ELISA	Gill tissues	SOD, AOC, CAT, NO, GSH-Px: * ↓ vs ctrls; MDA: * ↑ vs ctrls; NF-κB/ NLRP3 signal: * ↑; NLRP3, IL-1β: * ↑ vs ctrls; IFN-γ, TNF-α, IL-2 and IL-10: * ↑ vs ctrls; IL-4, IL6 and IL-8: ↑ vs ctrls	(Cao et al., 2023)
Eisenia andrei, 20	PS, < 500 μm Cartyre abrasion, 600 μm	Car tyre,1–1000 mg/kg, PS, 0.1–100 mg/kg	2, 7, 14, 28 days	Fluorescence-based measurements with microplate reader	Tissues	AChE: ↓*Inhibited; ROS, GSH, and GPx: * ↓ vs ctrls; CAT: ↓ vs ctrls	(Lackmann et al., 2022)
Fundulus heteroclitus, Experiment A: 40 Experiment B: 45	Crum rubber, 38–355 μm	Experiment A: 0, 0.059,0.585, 1371, 2.548 g/L Experiment B: 0, 0.01, 0.032, 0.10, and 0.25 g/L	Experiment A: 8/51 exposure days; Experiment B: 9/42 days of 24 h exposure	DNA Damage assay; colorimetric detection kit; Glutathione fluorescent detection Kit	Liver, intestinal tissues, and blood/ plasma	Experiment B: 8-OHdG: ↑ dose-dependent (ρ + 0.27 *); MDA ↓ dose-dependent (ρ − 0.21 *); GSH: ↑ dose-dependent (ρ 0.15 *); Experiment A: CYP1A protein: ↑ vs ctrls	(LaPlaca et al., 2022)
Mus musculus, 44	PS, 5 μm	Intracheal-PS: 1.25 and 6.25 mg/kg, in protective group: 6.25–50 mg/kg	48 h exposure 3x/week for 21 days	Immuno-fluorescence; detection kits; western blot	Lung tissues	SOD: ↓vs ctrls; GSH: ↑ vs ctrls; Pulmonary fibrosis: a-SMA and collagen I * ↑ vs ctrls (dose-dependent)	(Li et al., 2022b)

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Table 4 (*continued*)

Animal model, n°	Plastic type, size	Concentration	Exposure time	Experimental methods	Matrix	Biomarkers (Oxidative stress; Inflammation; Genotoxicity and others)	Autors, Year
Holothuria tubulosa, 30	LDPE 17%, PP 27%, PS 16%, HDPE, PVC 13%, PL 8%, PET 3%, PA 1%	3 different polluted areas	NA	Spectrophotometer; colorimetric assay kit	Gut tissues	CAT, SOD, GST, GSH: * ↑ vs ctrls; AChE, MDA: No* ↑ in all areas vs ctrls	(Lombardo et al., 2022)
Mus musculus, 19	PS and NH2-PS, 100 nm	50 μg/ml x mouse 4 times week	2 weeks	WST-1 and MTT; Duoset ELISA	Serum	IL-1β: ↑ NH2-PS vs PS-MP	(Jeon et al., 2023)
Dicentrarchus labrax, 162	Virgin PVC and incubated PVC, < 0.3 mm	MP environmental concentration 1% w/w	90 days	iQ5 optical System Software v. 2.0	Blood and liver tissue	LPO: both groups 30 days ↓ vs ctrls; 60, 90 days ↑vs ctrls; CAT: 60 days ↑ incubated vs ctrls; 90 days ↓ virgin and Incubated vs ctrls; TNF-α receptor: (30, 60, 90 days) ↓ vs ctrls; PPAR-receptor-α/γ: (30, 60 days) ↑ vs ctrls, (90 days) ↓ vs ctrls	(Pedà et al., 2022)
Scrobicularia plana, 420	LDPE 4–6 μm, 20–25 μm ± Benzo A pyrene (BaP)	1 mg/L	Time 0, 7 days, and 14 days	Colorimetric assay	Gills, and digestive glands	SOD: day 14, all groups ↓ vs ctrls; day 7 PE+BaP * ↑, * ↓ (at ≠concentration and n° exposure days); SOD activity: ↓ digestive glands vs gills; CAT activity: day 7 PE+BaP gills * ↓ vs ctrls, day 14 PE+BaP digestive glands ↓ vs ctrls; GST activity: day 7 PE+BaP, ↑ vs ctrls; AChE: day 14 PE+BaP * ↑ vs ctrls; LPO levels: day 14 PE+BaP ↑ levels vs PE	(Rodrigues et al., 2022)
Coturnix japonica,10	PS, 3293.4 μm	11 MPs particles/ quail/day, 22 MP particles/quail/day, once a day	9 days	ELISA; colorimetric assay	Crop, proventriculus, gizzard, small intestine, muscle (pectoral), brain and liver tissues	H_2O_2: No* vs ctrls; ROS: ↑ vs ctrls; NO: ↓ vs ctrls; MDA: ↑ vs ctrls; SOD activity: ↓ vs ctrls; CAT: No* in ≠ tissues; CAT: ↑ vs ctrls; AChE: No* between groups, trend ↑ in both tissues; Body mass: 9 days (PS-MPs) ↓ vs ctrls	(De Souza et al., 2022)
Rattus norvegicus, 70	PS, < 5 mm	1%, 5% and 10% PS-pellets; 1, 5, 10% FP	90 days	UV/Vis spectrophotometer	Blood (plasma)	TC, TG, HDL: No* vs ctrls; LDL: (1% PS, and 5% PS, and 5%FP) * ↑ vs ctrls; GSH, GPx, GST, SOD, CAT, and MDA: (1% PS, 5%PS, 10% FP) No* vs ctrls	(Nnoruka et al., 2022)
Macrobrachium nipponense, 300	PS-NP, 500 nm	0.04 mg/L, 0.4 mg/ L, 4 mg/L, 40 mg/L	28 days	Commercial kits	Gill, liver, gut, and muscle tissues	H_2O_2: * ↑ vs ctrls; GSH-Px, GSH: * ↑, No* , * ↓ (at ≠ concentrations) vs ctrls; GST: * ↑ vs ctrls; SOD: * ↑, ↓ activity (≠ concentration); CAT ↓, ↑ (≠ concentration)	(Fan et al., 2022)
Sparus aurata, 45	MPs	according to the sea water	120 days	Commercial colorimetric kit	Blood, Plasma, and liver tissues	Liver: SOD, MPO No* vs ctrls; CAT: * ↑ vs ctrls; GPx: * ↑ t60 vs t120; MDA: ↑ vs ctrls; ROS * ↑ vs ctrls; GST: * ↑ vs ctrls Plasma: SOD No* vs ctrls; CAT, MPO: * ↑ vs ctrls; MDA: * ↓ Blood cells: CAT, MPO No* ; SOD: * ↓; MDA, ROS: * ↑ vs ctrls	(Capó et al., 2022)

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Table 4 (*continued*)

Animal model, n°	Plastic type, size	Concentration	Exposure time	Experimental methods	Matrix	Biomarkers (Oxidative stress; Inflammation; Genotoxicity and others)	Autors, Year
Ctenopharyngodon idella, 300	PS, 32–40 μm	100 μg/L, 1000 μg/L	21 days	ELISA	Liver tissues	SOD: * ↓ vs ctrls; CAT: PS-1000 μg/L * ↓ vs PS-100 μg/L; CYP1A: ↑ (liver) dose-dependent	(Chen et al., 2022b)
Goniopora columna, 198	PE-MP, 40–48 μm	5, 10, 50, 100 and 300 mg/L	7 days	Commercial kits	Tissues	MDA, GST, CAT, GSH, SOD: * ↑ vs ctrls; GPx: * ↑, * ↓ (≠ exposure time and ≠concentration); GSH and GST: No* vs groups	(Liu et al., 2022b)
Caenorhabditis elegans, NA	PS, 1 μm	0.1, 1.0, 10, and 100 mg/L	48 h	Fuorescence microscope; qPCR	Tissues	ROS: * ↑ vs ctrls; Clk-1, ctl-1, SOD-3, SOD-4, and SOD-5 in F0: * ↑ vs ctrls; SOD-3: * ↑ vs ctrls in the F3 and F4 generations; Metabolic activity: * ↓ vs ctrls;	(Chen et al., 2021)
Caenorhabditis elegans, 400	PS, 20–100 nm	0,1–100 μg/L	6,5 days	DCFDA	Tissues	ROS: ↑ vs ctrls; Locomotion behaviour, brood size: No* changes vs ctrls;	(Liu et al., 2021)
Dicentrarchus labrax, NA	PSNP + HA (humic acid), 30–70 nm	0.02 mg/L and 20 mg/L PSNPs ± 1 mg/L of HA	96 h	qPCR; commercial kits; spectrophotometric method; TEAC	Skin mucus, Blood, and Head kidney tissues	TNF-α, IL-10: * ↑ vs ctrls; IL-1β, IL-6, IL-8 (TNF-α): No* vs ctrls (≠concentration); TGFb: all exposure conditions * ↑ vs ctrls; TG, TC, TAC: No* vs ctrls; MC2R gene: * ↑ vs ctrls; GR1: * ↑ vs ctrls;	(Brandts et al., 2021)
Mus musculus, 24	PS, 5 μm	0.1 mg/day	90 days	Optical microscope; qPCR	Liver tissues	ROS: ↓ vs ctrls; MMP: ↓ vs ctrls; Liver lesions: hepatic tissue rupture vs ctrls	(Pan et al., 2021)
Mus musculus, NA	PS, 2 μm	0.2 and 0.4 g/day twice a week	4–8 weeks	Shandon HistoCentre 3; western blot	Kidney tissues	ROS: ↑; kidneys lesions: ↑	(Wang et al., 2021)
Acropora sp., NA	PET, PE, 10–40 μm	250 mg/100 ml	24 h, 96 h	Commercial kits	Tissues	LDH: 24 h ↓, 96 h values * ↓ vs ctrls; TAC: 24 h * ↑, 48 h, 72 h ↓ vs ctrls; T-SOD: 24 h ↑, 96 h ↓ vs ctrls; GSH: 24 h ↑ vs ctrls, 96 h↓ vs ctrls; NO: 96 h * ↑ vs ctrls; G6DPH: 24 h * ↓vs ctrls	(Xiao et al., 2021)
Mus musculus, 24	PS-MPs 1, 4, 10 μm	10, 50 and 100 μg/ml/day	14 days	Protein assay kit; western blot	Mid colon tissues	NLRP3, NF-κB, TNF-α, IL-6, IL-1 β, IL-10, and TGF-β1: * ↑ vs ctrls	(Choi et al., 2021)
mytillus spp., Exposure 1: 8 Exposure 2: 8	PS, 20 μm and 50 nm, PMA, 10 × 30 μm	PS, 500 ng/L PMA, 500 ng/L,	24 h, 7 days	Commercial kits; comet assay	Digestive glands and gills tissues	SOD: * ↑ vs ctrls; TBARS: * ↓ vs ctrls; MN: No* vs ctrls; Comet assay: No* vs ctrls	(Cole et al., 2020)
Poecilia reticulata, 60	PS, 32–40 μm	100 μg/L, 1000 μg/L	28 days	Different methods according to different studies	Gut tissues	TNF-α, IFN-γ, TLR4, and IL-6: * ↑ vs ctrls; TNF-α: no* between two MP-treated groups; TLR4: * ↑ vs ctrls (higher conc. Vs lower); Histopathological changes: in gut MPs exposed changed vs ctrls,	(Huang et al., 2020)
Hediste diversicolor, NA	PE, PP, HDPE, LDPE, PAPEVA, 1 mm to 1.2 μm	Areas with different plastic pollution	NA	Different methods according to different studies	Tissues	CAT, GST, AChE, MDA: ↑ vs ctrls	(Missawi et al., 2020)
Corbicula fluminea, NA	PS, 80 nm	0.1, 1 and 5 mg/L	96 h	ELISA	Visceral mass, gills, and mantles tissues	MDA, SOD, CAT, GSH-Px, GST, GSH: * ↑ vs ctrls; AchE and GPT: * ↓ vs ctrls; GOT: No* vs ctrls;	(Li et al., 2020)
Danio rerio, 180	PS, 5, 20 μm and 70 nm	20 mg/L	4 h, 12 h, 1, 2, 7 days (every 48 h new PS solution)	Commercial kits	Liver tissues	SOD and CAT: * ↑ dose-dependent	(Lu et al., 2016)

PE (polyethylene), HDPE (high density polyethylene), LDPE (low density polystyrene), PVC (polyvinylchloride), PP (polypropylene), PET (Polyethylene terephthalate), PMA (polymethyl acrylate), PS (polystyrene), NH2-PS(amino functionalized polystyrene), PS-COOH (carboxy functionalized polystyrene), PS-MP (polystyrene-microplastics), NPs (nanoplastics), MPs (microplastics), ROS (reactive oxygen species), SOD (superoxide dismutase), CAT (catalase), TBARS (Thiobarbituric acid reactive substances), POX (peroxidase), GST (glutathione S-transferase), GSH (glutathione), GSH-Px (plasma glutathione peroxidase), PPAR-α (peroxisome proliferator-activated receptor alpha), PPAR-γ (peroxisome proliferator-activated receptor gamma), CYP1A (cytochrome P450 Family 1 Subfamily A protein), TNF-α (tumor necrosis factor alfa), IL- (Interleukin -), MDA (malondialdehyde), ENAs (extractable nuclear antigens), GPx (glutathione peroxidase), MCP-1 (monocyte chemoattractant protein-1), LPS (lipopolysaccharides), INF-γ (interferon gamma), AHR (aryl hydrocarbon receptor), muc- (muc genes), NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT-3 (signal transducer and activator of transcription 3), TAP/TAC (total antioxidant capacity), NO (nitric oxide), NLRP3 (NOD-like receptor family pyrin domain containing 3), AChE (acetylcholinesterase), α-SMA (alpha-smooth muscle actin), PE-BaP (polyethylene-benzo-a-pyrene), LPO (lactoperoxidase), H2O2 (hydrogen peroxide), TC (total cholesterol), TG (triglycerides), HDL (high-density lipoprotein), LDL (low-density lipoprotein), MC2R-gene (Melanocortin 2 Receptor), GR1 (gamma response 1 protein), G6DPH (glucose-6-phosphate dehydrogenase), TGF-β1 (transforming growth factor-beta1), TLR-4 (tool-like receptor-4), GOT/AST (aspartate aminotransferase), MPO (myeloperoxidase), FP (foam particles), MMP (plasma matrix metalloproteinases), qPCR (quantitative Polimerase Chain Reaction), DCFDA or DCFH-DA assay (2',7'-dichlorodihydrofluorescein diacetate), WST-1 assay (4-[3-(4-Iodophenyl)−2-(4-nitrophenyl)−2H-5-tetrazolio]−1,3-benzene sulfonate), MTT assay (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide), ELISA (Enzyme-linked immunosorbent assay), EIA (Enzyme Immuno Assay), TEAC (Trolox equivalent antioxidant capacity) *(significantly), ↓(decreased/inhibited), ↑(increased).

Table 5
Biomarkers of MNPs exposure analysed in plants studies.

Plant model, n°	Plastic type, size	Concentration	Exposure time	Experimental methods	Biomarkers (Oxidative stress and others)	Autors, Year
Skeletonema costatum, NA	P-PS, A-PS, L-PS, 0.1–1 μm	0, 5, 10 and 50 mg/L	0, 24, 48, 72, 96 h	Commercial kits	SOD: (P-PS) ↓ dose-dependent; CAT: (1 μm) ↑ vs (0.1 μm P-PS); MDA: ↑dose-dependent; Growth: all three groups *↓ vs ctrls;	(Ni et al., 2023)
Apostichopus japonicus, 360	PS, 20 μm-100 nm	100 mg/kg	60 days	Different methods according to different studies	ROS, MDA: (PS 20 μm) *↑ vs (PS 100 nm) and vs ctrls; SOD, CAT: 10 days (PS 20 μm) ↑ vs ctrls; Growth rate: < (PS, 100 nm) vs > (PS 20 μm)	(Liu et al., 2022a)
Lemna minor L., NA	PS, 230 and 260 nm	100 and 200 mg/L	NA	Different methods according to different studies	SOD, CAT, and POX activity: ↑ vs ctrls; low PS, H_2O_2 scavenging by regulating the redox state and enzyme/non-enzyme; Growth: 100 mg/L No* toxicity effects on growth	(Arikan et al., 2022)
Allium cepa, NA	PS, 100 nm	25, 50, 100, 200 and 400 mg/L	24, 48, 72 h	Different methods according to different studies	SOD: 72 h ↑ dose-dependent vs ctrls; MDA: 72 h No* vs ctrls; Lipidic peroxidation: 72 h*↓ vs ctrls; CAs index: 72 h*↑ vs ctrls; Root growth: ↓ vs ctrls	(Maity et al., 2020)

P-(pristine), A-(aged), L-(leached), PS (Polystyrene), SOD (superoxide dismutase), CAT (catalase), MDA (malondialdehyde), POX (peroxidase), ROS (reactive oxygen species), H2O2 (hydrogen peroxide), CAs (chromosomal aberrations), * (significantly), ↓(decreased/inhibited), ↑(increased).

Table 5 summarizes the biomarkers of effect of MNPs reported in plant studies analysed. Studies in *A. japonicus* (Liu et al., 2022a) and *S. Costatum* (Ni et al., 2023) following PS exposure point to a similar trend of MDA levels as in-vivo animal studies.

Similarly to MDA, total antioxidant capacity (TAC) or total antioxidant power (TAP) has been used to assess the cumulative effects of the antioxidants (Suresh et al., 2009). In animal models, Xiao et al. observed a notable rise in TAC levels within 24 h of exposure to PET and PE (Xiao et al., 2021). Conversely, a separate study conducted by Brandts et al. on fish exhibited no alteration in micronuclei (MN) of liver and muscle tissues after PS (0,04 μm) exposure (Brandts et al., 2021).

3.1.2. Inflammation

Inflammation is a physiological condition carried out by living organisms in response to external stimuli, such as pathogens, inorganic or organic particles, such as plastic (Pahwa et al., 2023). Based on the time course of the inflammatory response, we can distinguish acute and chronic inflammation. The mediators used in both types of responses are cytokines that play a pleiotropic function in mediating and regulating the immune response: on one side, they stimulate the cytokine production and thus increase inflammation levels; on the other side they reduce the production in order to limit the inflammatory response (Ghelli et al., 2022).

Table 3 and Table 4 summarize the studies that have investigated cytokines, such as IL (interleukin)−1β, IL-6, IL-8, and IL-10 as biomarkers of inflammation in cell lines and animal studies.

The modulation of immune response has been investigated by the evaluation of transcription factors, e.g. the nuclear transcription factor NF-kb, endowed with a central role in the inflammatory response, and NLRP3, one of the proteins involved in inflammation, which is expressed on the membrane of macrophages to initiate the inflammatory response.

Moreover, 7 articles analyzed the NLRP3 multi-protein complex and the MY88D protein responsible for the activation of the innate immune response (Table 3).

NFKb was investigated in 4 studies. For instance, Caputi et al. showed a significant increase in Nf-kb levels and an increase in NLPR3 protein expression in cell cultures of human gingival fibroblasts exposed to MP (0.1–0.6 μm) (Caputi et al., 2022). Similarly, Chen et al., showed an increase in Nf-kb levels in human embryonic kidney cells exposed to PS (3.54 μm) but at the same time, a significant decrease in NLPR3 protein expression following a 24-hour exposure (Chen et al., 2022a).

In-vivo studies in fishes exposed to PE for 21 days (Cao et al., 2023) and in mice following 14 days of PS exposure (Choi et al., 2021), showed inflammasome activation with significant increases in NfKb and NLPR3 levels compared to untreated controls.

The papers included in this review have analysed different cytokines, like the pro-inflammatory IL-1β (n = 12), lL-6 (n = 14), TNF-α (tumor necrosis factor alfa) (n = 14) and INF -γ ((interferon gamma) (n = 3) (Zhang and An, 2007). Of 10 articles dealing with IL-1β levels, five of them showed significant increases in IL-1β after exposure to PE or PS in in-vitro models.

Exposure to MNPs consisting of PS, PMMA, and PVC (ranging from 50 to 310 nm) led to elevated levels of IL-6, as indicated by 11 studies, which demonstrated slight changes compared to untreated controls. Additionally, 9 studies reported increased levels of TNF-α, while 3 studies showed elevated INF-γ levels.

IL-8 and IL-10 have a dual function, in stimulating the production of other cytokines and limiting their production. Increased levels have been shown in all investigated papers. This suggests that MPs can affect the regulation of pro-inflammatory cytokine production through negative feedback (Zhang and An, 2007).

In-vitro studies report the inflammatory biomarkers as the main indicators of the perturbation occurring in biological systems and cell cultures challenged with different types of MNPs. Weber et al., analysed the exposure of human monocytic dendritic cells to PMMA and PVC (0,05–0310 μm), highlighting an increasing trend of IL-10 and decreasing trend for IL-6 and TNF-α compared to controls (Weber et al., 2022). Conversely, Bengalli et al. showed a statistically significant increasing trend of IL-6 and IL-8 in human lung cells exposed to MP (<50 μm) compared to controls (Bengalli et al., 2022). Cheng et al., showed a dose-dependent increase of IL-6 in cell medium of human embryonic cell lines after exposure to PS (1 μm) (Cheng et al., 2022). Palaniappan et al. tested L929 cells after exposure to PE (1–4 μm), PS (9, 5–11,5 μm) showing dose-dependent trend of IL-1β and TNF-α (Palaniappan et al., 2022). On the contrary, Busch et al., didn't show any changes in levels of both pro-inflammatory cytokines in Caco2 cells exposed to PS micro particles, though IL-1β levels were significantly higher after exposure to PVC (50 nm) as compared to controls (Table 3) (Busch et al., 2021).

In animal models, exposure to particulate matter (PM) has been shown to induce increased levels of IL-1β, IL-8, IL-10 in various tissues, such as gut, mucous membranes, blood and kidney cells, compared to controls. Cocci et al., reported progressively increasing levels of IL-1β, IL-8, IL-10, TNF- α, and INF-γ following exposure to PE and PS with respective sizes of 5–1 mm, 1–0.5 mm, and 0.5–0.1 mm (Table 4) (Cocci et al., 2022). In the gills of carp exposed to PE for 21 days, the levels of IL-2, IL-10, INF-γ, and TNF-α were significantly higher than in controls, while IL-4, IL-6, and IL-8 showed a non-significant increase compared to non-exposed individuals (Cao et al., 2023). In the renal tissues of sea bass following exposure to PS (30–70 nm), no statistical differences were observed in the levels of IL-1β, IL-6, and IL-8 compared to controls. Conversely, significantly higher levels of TNF-α were detected in exposed fish compared to non-exposed individuals (Brandts et al., 2021). In mice exposed to PS (1, 4, 10 μm) at a concentration of 50–100 mg/cm^2, the levels of IL-1 β, IL-6, IL-10, TNF-α, and Nf-kB significantly increased compared to controls. Similarly, Huang et al. showed in intestinal tissues of fish exposed to PS (32–40 μm) at 100–1000 mg/ml an increasing trend of TNF-α, IL-6 and INF-γ compared to controls (Huang et al., 2020). Regarding the studies included in this work, carried out in humans and plants, no cytokines were analysed.

3.1.3. Genotoxicity

The DNA damaging potential of MNPs is known or suspected, and has been investigated, both in-vitro and in-vivo. Oxidative stress and inflammation can lead to oxidative damage to nucleic acids.

In the present paper we found evidence of genotoxicity from MNPs following exposure to known polymers. DNA strand breaks and MN were the main biomarkers used to assess this endpoint. MNs and chromosomal aberrations (CA) were investigated both in-vitro and in-vivo (animal models) as well as in few human studies (blood nucleated cells). MNs derive from whole chromosomes or acentric fragments that do not migrate to the poles during anaphase and are not incorporated into the main nucleus, giving rise to smaller accessory nuclei (Heddle et al., 1991). In this review, 5 articles (4 on cell cultures and 1 on experimental animal models) investigated the presence of MN reporting an increase compared to controls following prolonged exposure to MNPs. In addition, CA (n = 3) and sister chromatid exchanges (SCE) (n = 1) were investigated.

Maity et al., and Malinowska et al., tested the genotoxicity of 0.1 μm, 0.029 μm, 0.044 μm, 0.072 μm PS particles in exposed cells by Comet test showing an increasing dose-dependent trend in DNA damage as compared to non-exposed (Maity et al., 2020; Malinowska et al., 2022). MNs were investigated in lung carcinoma epithelial cells by Shi et al., highlighting an increasing trend of MN formation after exposure to 0.08–2.0 μm PS compared to controls (Shi et al., 2022). Conversely, Cole et al. who conducted a study on mussels exposed to PS (0.05–20.0 μm), and PMA (10.0 ×30.0 μm) did not show statistical differences for MN formation between exposed and not exposed mussels (Cole et al., 2020).

Roursgaard et al., analysed PP (0,08–0,25 μm) and PET (0,2–0,6 μm) exposure toxicity, on hepatocellular carcinoma and colorectal adenocarcinoma epithelial cell lines, assessing an increasing DNA damages in a dose-dependent manner after exposure to PET compared to controls (Table 3) (Roursgaard et al., 2022). In a study carried out on fish by Laplaca et al., DNA damages (8-oxo-dG) increased in a dose-dependent manner after exposure to crumb-rubber (38–355.0 μm) compared to controls underlining a positive correlation (Rho=0,27 *) (Table 4) (LaPlaca et al., 2022).

One study carried out in workers exposed to MP analysed CA and SCE on blood cells and it was demonstrated trend towards an increase of CA in those exposed to Acrylonitrile (ACN), and a similar number of SCE compared to controls (Major et al., 1998) (Table 6). Maity et al., found significant increases of CA in plants after exposure to PS (0,1 μm) at different concentrations (Table 5) (Maity et al., 2020).

Whereas mechanistic studies in plant species seem irrelevant for human exposure, many in-vivo studies in rodents suggest at least three endpoints relevant for human beings, although the dose levels are, in many cases, far behind the likelihood of exposure for humans. Inflammation in gut tissues, gill, mid colon, liver, and muscle tissues may lead to alterations of lipid metabolism, and reduction of antioxidant defence system that can be defined as either oxidative stress, inflammation or general toxicity biomarkers summarized in Tables 3–6.

4. Discussion

MP are ubiquitous in the environment and have been detected in different environmental media, raising concerns about human exposure through different pathways. While there is limited evidence suggesting MPs, excluding their chemical constituents or contaminants, migh have adverse effects on human health, there is a growing consensus among stakeholders and heightened public awareness to reduce exposure to MNPs.

Numerous in-vivo and in-vitro studies indicate that exposure to MNPs can lead to inflammation, ROS production, genomic instability and immune system dysfunction. These findings are consistent across living species, suggesting common pathways of disease and MOA shared with other foreign particulates, resulting in biochemical changes and subtle dysfunctions. Key biomarkers assessed in these studies often reflect imbalances in antioxidant defence system, including markers like lipid peroxidation, membrane damage, ROS, SOD, CAT, MDA, GST, GSH, GPx, and TAP.

Inflammation is one of the probable outcomes investigated following MNPs exposure; in particular, IL-1β, IL-6, IL-8, IL-10, TNF-α, NF-kB were the cytokines most frequently investigated as an index of an inflammatory condition in clinical, environmental, and occupational studies are the same as those investigated in the papers included in this review. For genotoxicity, biomarkers such as MNs, cytokinesis-block proliferation index, Comet test index, CA, and SCE have been frequently studied as indicators of DNA damage, which is crucial for human health (Çobanoğlu et al., 2021). Recent critical reviews have provided insight into the possible mechanisms that can lead to initiation and progression of cancer pathogenesis in the body (Alimba et al., 2021; Domenech et al., 2023). The potential mechanisms underlying the development of cancer caused by MNPs revolve around the individual and/or interactive effects of ROS, the induction of oxidative stress, genome instability, and chronic inflammation. However, it is yet to be explored whether these mechanisms hold relevance for human health through dedicated studies on human subjects.

There are concerns about the potential of MNPs impact the entire

Table 6
Biomarkers of MNPs exposure analysed in human studies.

Subjects, n°	Plastic type	Concentration	Exposure time	Experimental methods	Matrix	Biomarkers (Inflammation, genotoxicity, and others)	Autors, Year
exposed workers, 889	PVC	1000 ppm x year	1 year	Sonography and enzymatic assays	Blood test, liver imaging	liver lesions 39,5% (BMI<27)	(Mastrangelo et al., 2004)
14 exp symptomatic, 15 exp asymptomatic, 9 non-exposed	PUR	NA	24 h	GC-MS	Urine, plasma, nasal lavage fluid	Ctrl: U-MDX [0,28[nq-2,3]], U-2,4-TDX [0,32[nq-0,6]], U-2,6-TDX [0,27[nq-0,6]]; exposed: U-MDX[0,35 [nq-0,6]],U-2,4-TDX [nq [nq-1.0]],U-2,6-TDX [0,27 [0,35 [nq-0,7]]]	(Littorin et al., 2002)
26 exposed, 26 non-exposed	MPs, ACN, DMF	NA	20 months	GC	Urine and Blood	ACN* ↑ vs ctrls; CA ↑ vs ctrls; SCE= (No*) vs ctrls	(Major et al., 1998)

ACN: Acrylonitrile, DMF (Dimethylformamide), PVC (polyvinil chloride), PUR (polyurethane), MPs (generic microplastics polymers), SCE (sister chromatids exchange), U-MDX (metabolites of 4,4'-diphenylmethane di-isocyanate), U-TDX (2,4- and 2,6-toluene diisocyanate), GC (Gas chromatography), MS (mass spectrometry), *(significantly), ↓(decreased/inhibited), ↑(increased).

ecosystem (Mateos-Cárdenas et al., 2019),as they are found in both indoor and outdoor environments, spread by atmospheric events like rain and wind, and even transferred between marine species in aquatic ecosystems. (Zhang et al., 2020). For this reason, numerous studies have focused on aquatic ecosystems by investigating their presence and possible trophic transfer from aquatic plants to animal organisms. (Welden et al., 2018) and (Nelms et al., 2018) in their work noted a transfer of MNPs between prey-predator marine species (Welden et al., 2018);Nelms et al., 2018). While transfer to humans via plants has been suggested (Schwabl et al., 2019), it remains poorly understood, and the route of intake, whether through the food chain or other exposures including occupational, is unclear due to the lack of standardized methods and procedures for identifying and interpreting results. (Toussaint et al., 2019). Research on the effects of MNPs has primarily been conducted in controlled settings, indicating growth reductions at the cellular and apical level, lower biomass yields, and increased levels of OS and inflammation in exposed animals (Pan et al., 2021; Cocci et al., 2022), but the extent of trophic transfer has mostly been studied in laboratory models (He et al., 2021). Limited studies have explored the impact of MNPs exposure in occupational settings, potentially leading to increased intake and effects primarily observed in in-vitro or in-vivo models with animals or plants.

Inflammatory biomarkers play a crucial role in biomonitoring the effects of MNPs exposure.p This review summarizes the types of MNPs studied, their sizes and the biomarkers used in in-vitro, in-vivo, and occupational studies. To assess the risks to human health, more studies considering various exposure scenarios and the size distribution of airborne plastic particles, including those reaching the alveolar region of the lungs, are necessary. Workplace studies can offer insights into dose-response relationships and overcome the limitations of in-vitro tests. The identification of reliable biomarkers should support field studies and epidemiological investigations, aiding in understanding the potential risks of MNPs and the development of mitigation strategies (Mastrangelo et al., 2004).

Therefore, the biomarkers summarised in this review may be a good starting point for investigating effects in the occupational setting to provide a complete scenario and the necessary knowledge on the adverse effects that MNPs may have on humans. Therefore, the biomarkers summarised in this review may be a good starting point for investigating effects in the occupational setting to provide a complete scenario and the necessary knowledge on the adverse effects that MNPs may have on humans. These findings suggest a minimum set of biomarkers to be assessed in biological matrices of volunteers and workers with potential exposure to MNPs should help clarifying its relationship with health outcomes. This can be further complemented with recently validated biomarkers reflecting long-term endpoints, such as chronic inflammation and fibrosis, as well as cardiovascular endpoints, considering possible interference in lipid metabolism. This consideration is based on what we know from field investigations of nanomaterials, in which the successful implementation of a harmonized protocol allowed to demonstrate the feasibility of similar research projects in the future, facilitating further studies in target populations, and inform stakeholders of regulatory aspects targeting occupational exposure to MNPs (Bergamaschi et al., 2015; Guseva Canu et al., 2023).

Human studies on MNPs exposure remain limited. Although some recent investigations have found MNPs in stool (Schwabl et al., 2019) and in induced sputum samples (Huang et al., 2022) further research is needed to clarify the implications of MNPs presence in human biological samples. Challenges include aggregating data from various studies using different analytical methods and considering factors like plastic shape, which can significantly influence the harm caused by MNPs. As pointed out in the work of Suman et al., smaller plastics in the μm range are more bioavailable in both in-vitro and in-vivo models by increasing levels of OS, inflammation, and possible genotoxicity (Suman et al., 2021). Moreover, different studies showed that plastics with an irregular shape were the ones most ingested by organisms; this combined with the small particle size makes them more harmful (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017; Schwabl et al., 2019). Considering these aspects, it seems reasonable to take advantage of what we already know about particles toxicology, working under the assumption that different nanoparticles may lead to the same pathway for disease, or share common mechanisms (e.g. inflammation).

5. Conlusions

Data on biomarkers of effect after inhalation or dietary exposure for characterizing the hazard of MNPs remain relatively scarce, primarily restricted to studies with model particles, such as polystyrene beads. These model particles typically fall within the regulatory size range (e.g., < 10 μm) as defined by the World Health Organization (WHO). These investigations underscore the need of more comprehensive data on the impacts of MNPs, considering factors beyond mere size, including aspects like shape, polymer composition and other attributes representative of environmentally relevant MNPs.

Despite the limited characterization of MNPs' hazards, especially concerning human health, existing literature findings suggest that MNPs may yield adverse effects akin to those observed with other extensively studied solid and insoluble particles, presumably through comparable modes of action. Nevertheless, the available data fall short of providing a definitive link between MNP exposure and specific illnesses, both directly and indirectly. Quality control concerns in published studies, as highlighted by the WHO in 2006, have not been adequately addressed. Biomarkers of effect are valuable tools in the early detection of subclinical changes before the onset of disease, aiding in the anticipation of potential adverse effects associated with engineered nanomaterials and the elucidating of dose–effect relationships. However, their practical

utility in environmental and occupational exposure monitoring and health surveillance remains limited.

Author contributions statement

Marco Panizzolo: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Vitor Hugo Martins**: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Federica Ghelli**: Visualization, Methodology, Data curation, Writing – review & editing. **Giulia Squillacioti**: Visualization, Methodology, Writing – review & editing. **Valeria Bellisario**: Visualization, Validation, Writing – review & editing. **Giacomo Garzaro**: Visualization, Writing – review & editing. **Davide Bosio**: Visualization, Writing – review & editing. **Nicoletta Colombi**: Conceptualization, Investigation, Validation, Methodology. **Roberto Bono**: Supervision, Conceptualization, Resources, Writing – review & editing. **Enrico Bergamaschi**: Funding acquisition, Conceptualization, Project administration, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115645.

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Review

Inflammatory Biomarkers in Exhaled Breath Condensate: A Systematic Review

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Abstract: Inflammation is a comprehensive set of physiological processes that an organism undertakes in response to a wide variety of foreign stimuli, such as viruses, bacteria, and inorganic particles. A key role is played by cytokines, protein-based chemical mediators produced by a broad range of cells, including the immune cells recruited in the inflammation site. The aim of this systematic review is to compare baseline values of pro/anti-inflammatory biomarkers measured in Exhaled Breath Condensate (EBC) in healthy, non-smoking adults to provide a summary of the concentrations reported in the literature. We focused on: interleukin (IL)-1β, IL-4, IL-6, IL-8, IL-10, tumour necrosis factor-alpha (TNF-α), and C reactive protein (CRP). Eligible articles were identified in PubMed, Embase, and Cochrane CENTRAL. Due to the wide differences in methodologies employed in the included articles concerning EBC sampling, storage, and analyses, research protocols were assessed specifically to test their adherence to the ATS/ERS Task Force guidelines on EBC. The development of reference intervals for these biomarkers can result in their introduction and use in both research and clinical settings, not only for monitoring purposes but also, in the perspective of future longitudinal studies, as predictive parameters for the onset and development of chronic diseases with inflammatory aetiology.

Keywords: inflammation; cytokines; exhaled breath condensate; non-invasive; reference values; non-smoking healthy adults

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1. Introduction

Inflammation is a comprehensive set of physiological processes that an organism undertakes in response to a foreign stimulus, including human pathogens, such as viruses and bacteria, and inorganic particles [1]. Depending on the duration of these processes, it is possible to distinguish between two inflammatory response types: acute and chronic [2]. In both cases, a key role is played by cytokines, protein-based chemical mediators produced by a broad range of cells, including the immune cells recruited in the inflammation site. These polypeptides are pleiotropic molecules that elicit their effects in an autocrine or paracrine manner, binding to specific receptors on cell walls and regulating their activation [3]. Cytokines can be classified according to their role as pro-inflammatory, anti-inflammatory, or chemotactic. The pro-inflammatory cytokines owe their name to their role in orchestrating the early immune response to infection/injury by recruiting immune cells to the infection site and activating them [4]. They are often released in a cascade, and the lack of control over their release/activity can lead to damage to host tissues as well as pathogens [4]. The main cytokines with a pro-inflammatory role are interleukin (IL)-1β, IL-6, and tumour necrosis factor α (TNF-α). Anti-inflammatory cytokines, instead, such as IL-4 and IL-10, play a crucial role in controlling the regulation of pro-inflammatory cytokines. Finally,

chemokines are a cytokine subgroup whose main role is the activation and recruitment of leukocytes, as, for instance, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1a, MIP-1b and IL-8 [5]. Another non-cytokine polypeptide, named C-reactive protein (CRP), is an acute inflammatory protein that increases its concentration at sites of inflammation or infection [6]. It may be considered a useful diagnostic tool in the assessment of early inflammation, such as in acute-phase diseases [7]. Most biomarkers of inflammation and oxidative stress (OS) are often investigated in clinical settings using invasive biological matrices, such as blood and broncho-alveolar lavage (BAL).

Molecular epidemiology studies, especially when involving children and the elderly, can reliably rely on biological matrices collected by non-invasive methods such as Exhaled Breath Condensate (EBC) and urine [8,9]. Cytokine profiling analyses play a crucial role in the early detection and follow-up of inflammatory processes. Among non-invasive matrices, EBC is a validated method for assessing volatile markers and inflammatory mediators. This methodology allows collecting droplets from airway lining fluid by the condensation of warm, humid breath onto a cold surface in a condensing device [10]. To date, a variety of both commercial and homemade devices for the collection of EBC are available. The most widely used commercial devices are EcoScreen™, RTube™, and TurboDECCS™ [8]. The samplers differ in the cooling system type (pre-cooled sleeve or electric cooling system), providing temperatures ranging from 0 °C to −20 °C in the tube covering materials and in the electrical power [11]. In non-clinical studies, there is a greater effort to provide standardisation of non-invasive sampling methods and to provide reference values of OS and inflammation biomarkers in the general population, with the purpose of identifying a range that can highlight a possible onset of disease [12]. Therefore, the aim of this systematic review is to compare baseline values of pro/anti-inflammatory biomarkers measured in EBC in healthy, non-smoking adults to provide a summary of the concentrations reported in the literature. A further goal is to highlight possible methodological issues preventing the definition of reference intervals, to employ them not only in clinical scenarios but even in environmental and occupational settings. We focused on the most searched biomarkers quantified in EBC: interleukin 1β (IL-1β), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin IL 10 (IL-10), tumor necrosis factor-alpha (TNF-α) and C reactive protein (CRP).

2. Materials and Methods

The present systematic review protocol is registered on PROSPERO database (Protocol ID = CRD42022316248). The registration underwent only the basic automated checks for eligibility to enable the PROSPERO team to focus on COVID-19 submissions. The study is reported in accordance with the PRISMA 2020 Statement [13].

2.1. Study Selection

Eligible articles were searched and identified in PubMed, Embase, and Cochrane CENTRAL up to 4 February 2022.

The search string aimed to find original research articles evaluating the concentration of some inflammatory biomarkers in EBC, including the following terms: “Cytokines”, “Interleukins”, “C-Reactive Protein”, “Interleukin-1”, “Interleukin-4”, “Interleukin-6”, “Interleukin-8”, “Interleukin-10”, “Tumor Necrosis Factor-alpha”, “exhaled breath condensate*”. Full strings are reported in Appendix A (Table A1). Table 1 summarises the pathophysiological role of these biomarkers.

Table 1. Most searched biomarkers in EBC.

Biomarkers	Role	Description
CRP	Pro-inflammatory	Detection of bacteria and damaged human cells and complement activation. Circulating concentration rises in response to infection and is associated with risk of coronary heart disease [6].
IL-1β	Pro-inflammatory	Response to exogenous and endogenous noxious stimuli and induction of IL-6 and IL-8 secretion by bronchial epithelial cells [14,15].
IL-4	Anti-inflammatory	Response to allergic airway inflammation [16].
IL-6	Pro-inflammatory	Response to several stimuli, including exercise, allergens, and respiratory viruses [5].
IL-8	Pro-inflammatory	Neutrophil recruitment with an important role in pathological and physiological conditions [15,17].
IL-10	Anti-inflammatory	Immune-suppressive cytokine, which reduces the recruitment of effector T cells and counteracts the effects of TNF-α and IL-1β Response to allergic challenge [18].
TNF-α	Pro-inflammatory	Pleiotropic immune activator, involved in many airway disorders [19].

2.2. Inclusion and Exclusion Criteria

Observational or interventional original research studies on healthy humans (18+ years, non-smoking, no known disease) measuring the selected biomarkers in EBC were considered potentially eligible. Only full texts written in English were considered suitable for inclusion.

Non-quantitative data, full texts with unpublished data, reviews, non-human and in vitro studies, correspondence, conferences, abstracts without full text, expert opinions, protocols, and editorials were excluded.

Two reviewers completed the article selection in blind process, screening titles and abstracts according to the inclusion and exclusion criteria declared. In case of insufficient data, the selection was based on the full texts. Disagreements on article selection were discussed and eventually submitted to a third reviewer. The procedure is summarised in the PRISMA diagram [13] reported in Figure 1.

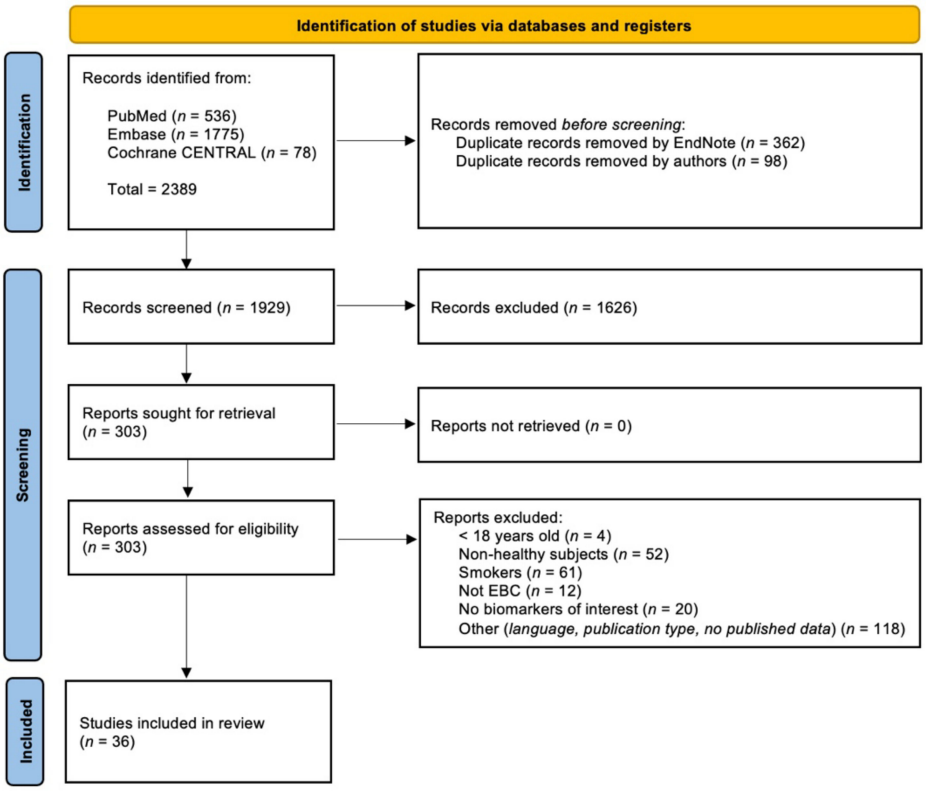

Figure 1. PRISMA flow chart summarising the study selection process.

2.3. Data Extraction

Two researchers independently extracted the data from the selected articles by filling in a spreadsheet. We reported the following information: author's name, publication time, title, country, study design, recruitment method, number of subjects, subject category, inclusion and exclusion criteria, male (n°), female (n°), age, BMI, timing (pre- and post-intervention), collection details (device, temperature, and time), storage temperature, α-amylase control, analytical methods, biomarker concentrations, Limit of Detection (LOD), main results and notes. Data reported by graphs in original studies were extracted by the WebPlotDigitizer software (Rohatgi (2021), version 4.5, Pacifica, California, USA, https://automeris.io/WebPlotDigitizer/ accessed on 25 July 2022).

2.4. Quality Assessment

The quality assessment of the included articles was performed by two independent reviewers in a two-step process. The first part of the assessment was carried out according to the study design by the proper Joanna Briggs Institute (JBI) checklists to assess the reliability and relevance of the published articles. The second part was focused on the methodological protocol, specifically to test the adherence of the research protocols to the ATS/ERS Task Force guidelines on Exhaled Breath Condensate [20,21]. The checklist is reported in Appendix B (Table A2). Each study was awarded a Completeness of Reporting (COR) score according to the number of items met in each of the two checklists employed. The score was calculated as COR (%) = ("satisfied"/("satisfied" + "not satisfied/unclear")) × 100). Quality was then defined as "poor" (COR < 50%), "moderate" (COR = 50–74%) or "high" (COR ≥ 75%) [22]. The final ranking due to each checklist has been kept separate for each of the included studies. Any discrepancy between reviewers was discussed, and if required, a third reviewer was consulted.

2.5. Statistical Analysis

Categorical variables have been reported as frequency (*n*), while continuous variables were reported as Mean ± Standard Deviation (SD) or Mean ± Standard Error of the Mean (SEM) or Median and Interquartile Range (IQR), as reported in the original research articles. For studies declaring the analytical LOD, arithmetic mean and SE of data above this parameter were approximated in order to obtain a graphical representation [23]. The forest plot was created by R Studio (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA, USA).

3. Results

3.1. Qualitative Synthesis

Among the 2389 items initially identified, 460 duplicates were removed before screening by EndNote and manually. The remaining 1929 were screened, and 36 research articles were included in the systematic review [7,24–58]. The exclusion criteria lead to the removal of 267 articles. Among these, 117 papers were excluded because of the epidemiological sample characteristics (juveniles subjects (*n* = 4), non-healthy subjects (*n* = 52), smoking subjects (*n* = 61)), 12 because they did not include the EBC matrix, 20 for not assessing the biomarkers included in the string, and 118 were excluded because they were not in English, they were not research articles, or they had a lack of data. The procedure is summarised in the PRISMA diagram reported in Figure 1.

3.2. Study and Participant Characteristics

Appendix C reports the quality assessment scores (Figures A1–A3). All the included studies were assessed by adopting the proper JBI checklists according to the study design (cross-sectional studies (28), quasi-experimental studies (7), and randomised controlled trials (1)). A total of 50% of the studies were awarded a "High" quality score, while 50% with a "Moderate" quality score. Furthermore, due to the lack of questions assessing the methodological approach in those tools, we created an additional checklist for the objective assessment of

the analytical methods applied in the included studies. According to this second evaluation, 10 of the studies were awarded a "High" quality score, 16 with a "Moderate" quality score, and 10 with a "Low" quality score.

3.3. Inflammation Biomarkers in EBC

Table 2 reports the characteristics of the studies specifying the absence or presence of LOD and, in this case, the percentage of determinations above the assay sensitivity.

Table 2. Frequency of studies reporting or not reporting value above the LOD. Some studies analysed more than one biomarker.

Biomarker	n° of Studies	n° of Studies (%) with Data > LOD	n° of Studies (%) with Data < LOD	n° of Studies (%) without LOD Declared
CRP	3	2 (66.7%)	-	1 (33.3%)
IL-1β	12	2 (16.7%)	5 (41.7%)	5 (41.7%)
IL-4	11	6 (54.5%)	2 (18.2%)	3 (27.3%)
IL-6	19	11 (57.9%)	2 (10.5%)	6 (31.6%)
IL-8	16	5 (31.3%)	4 (25.0%)	7 (43.8%)
IL-10	12	2 (16.7%)	2 (16.7%)	8 (66.7%)
TNF-α	18	6 (33.3%)	3 (16.7%)	9 (50.0%)

The forest plot (Figure 2) summarises the biomarker concentrations reported in papers declaring the assay LOD and the measurements above it. The values measured in Edmè et al., 2008 have not been included because the concentration declared was not divided by the concentration factor. As well, we did not include the quantification assessed by Matsunaga et al., 2006 because the authors reported only the relative intensity concentrations expressed as percentages. The concentrations extracted are reported in Table 3, while the details of data reported in those articles not declaring the assay LOD or reporting measurements lower than this parameter are reported in Supplementary Materials (Table S1).

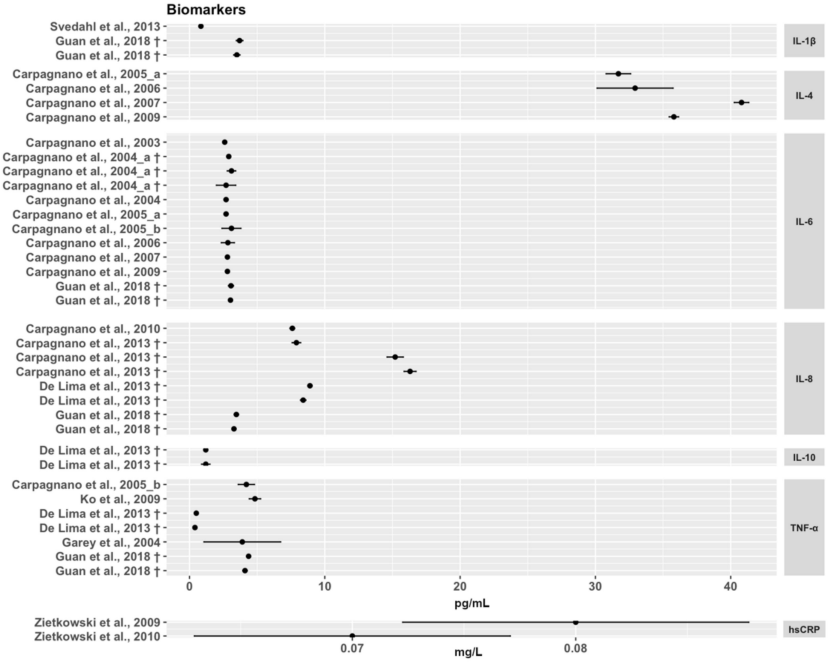

Figure 2. Forest plot summarising the concentration of the selected biomarkers in the articles where the sensitivity of the employed assays, and the measurements above the LOD were reported. † More subjects groups were analysed in the same article. The "a" and "b" following the indication of articles with the same first author and year are referred to the order of the articles in the bibliography paragraph [7,27–37,41,44,45,54,58].

Table 3. Data extracted from articles reporting data above the declared assay LOD. Data are expressed as: Geometric mean = †; Mean ± SD; Median (IQR); Median ("25°th–75°th"); Median [min–max].

Authors, Year	Country	n° Subjects (M;F)	Age	Collection Device	Collection Temperature	Storage Temperature	Analytical Method	Data	LOD	SCORE Quality Assessment JBI	SCORE Authors' Quality Assessment
CRP											
Zietkowski et al., 2009 [7]	Poland	15 (6;9)	33.13 (6.71) †	EcoScreen; Eric Jaeger GmbH, Hoechberg, Germany	0 °C	−80 °C	highly sensitive CRP assay (Konelab, Waltham, MA, USA)	0.08 ± 0.03 mg/L	0.05 mg/L	77.78 High	45.45 Low
Zietkowski et al., 2010 [58]	Poland	8 (4;4)	29.9 (7.1) †	EcoScreen; Eric Jaeger GmbH, Hoechberg, Germany	0 °C	−80 °C	highly sensitive CRP assay (Konelab, Waltham, MA, USA)	0.07 ± 0.03 mg/L	0.02 mg/L	88.89 High	72.73 High
IL-1β											
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.71 (2.31) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.34 (2.26) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Svedahl et al., 2013 [54]	Norway	24 (14;10)	23.8 ± 2.5	ECoScreen; Jager, Wurzburg, Germany	NA	−70 °C	Quantikine HS from R&D Systems (Minneapolis, MN, USA)	0.84; CI= 0.64–1.10 pg/mL	0.05 pg/mL	77.78 High	63.64 Medium
IL-4											
Carpagnano et al., 2005_a [30]	Italy	15 (5;10)	35 ± 6	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	31.7 ± 3.5 pg/mL	20 pg/mL	77.78 High	90.91 High
Carpagnano et al., 2006 [32]	Italy	17 (8;9)	37 ± 9	EcoScreen (Jaeger, Wurzburg, Germany)	On ice	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	31.6 (27.5–39.7)pg/mL	20 pg/mL	50.00 Medium	63.64 Medium
Carpagnano et al., 2007 [33]	Italy	10 (5;5)	44 ± 8	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	40.8 ± 1.7 pg/mL	15 pg/mL	75.00 High	54.55 Medium
Carpagnano et al., 2009 [34]	Italy	10 (-;-)	43 ± 9	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−80 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	35.8 ± 1.1 pg/mL	20 pg/mL	85.71 High	63.64 Medium
Edmè et al., 2008 * [39]	France	19 (-;-)	38.3 ± 13.6	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	Cytometric Bead Arrays (CBA) Becton Dickinson, San Jose, CA	32.1 (23 76) † pg/mL	5 pg/mL	66.67 Medium	66.67 Medium
Matsunaga et al., 2006 [47]	Japan	10 (3;7)	34.4 ± 6.6	EcoScreen, (Jaeger, Germany)	−20 °C	−70 °C	Human Inflammation Antibody III (ray Biontec Inc, Norcross, GA, USA)	5.2 ± 1.7 pg/mL	1pg/mL	57.14 Medium	72.73 Medium
IL-6											
Carpagnano et al., 2003 [27]	Italy	14 (8;6)	45 ± 6	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.6 ± 0.2 pg/mL	1.5 pg/mL	87.50 High	81.82 High
Carpagnano et al., 2004_a [28]	Italy	18(5;13)	46 ± 6	EcoScreen (Jaeger, Wurzburg, Germany)	On ice	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.9 ± 0.6 pg/mL	1.5 pg/mL	77.78 High	81.82 High
Carpagnano et al., 2004_a [28]	Italy	5 (2;3)	47 ± 3	EcoScreen (Jaeger, Wurzburg, Germany)	On ice	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	3.1 ± 0.6 pg/mL	1.5 pg/mL	77.78 High	81.82 High
Carpagnano et al., 2004_b [29]	Italy	15 (8;7)	48 ± 7	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.7 ± 0.6 pg/mL	1.5 pg/mL	62.50 Medium	54.55 Medium

Table 3. *Cont.*

Authors, Year	Country	n° Subjects (M;F)	Age	Collection Device	Collection Temperature	Storage Temperature	Analytical Method	Data	LOD	SCORE Quality Assessment JBI	SCORE Authors' Quality Assessment
Carpagnano et al., 2005_a [30]	Italy	15 (5;10)	35 ± 6	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.7 ± 0.6 pg/mL	1.5 pg/mL	77.78 High	90.91 High
Carpagnano et al., 2005_b [31]	Italy	7 (5;2)	42 ± 5	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	3.1 ± 0.7 pg/mL	1.5 pg/mL	77.78 High	90.91 High
Carpagnano et al., 2006 [32]	Italy	17 (8;9)	37 ± 9	EcoScreen (Jaeger, Wurzburg, Germany)	On ice	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.6 (1.9-4.0) pg/mL	1.5 pg/mL	50.00 Medium	63.64 Medium
Carpagnano et al., 2007 [33]	Italy	10 (5;5)	44 ± 8	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.8 ± 0.1 pg/mL	1.5 pg/mL	75.00 High	54.55 Medium
Carpagnano et al., 2009 [34]	Italy	10 (-;-)	43 ± 9	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−80 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	2.8 ± 0.1 pg/mL	1.5 pg/mL	85.71 High	63.64 Medium
Edmè et al., 2008 * [39]	France	19 (-;-)	38.3 ± 13.6	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	Cytometric Bead Arrays (CBA) Becton pg/mL Dickinson, San Jose, CA, USA	111.7 (70-362) † pg/mL	5 pg/mL	66.67 Medium	66.67 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.09 (3.08) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.08 (2.03) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Matsunaga et al., 2006 [47]	Japan	10 (3;7)	34.4 ± 6.6	EcoScreen, (Jaeger, Germany)	−20 °C	−70 °C	Human Inflammation Antibody III (ray Biontec Inc, Norcross, GA, USA)	5.2 ± 1.2 pg/mL	1 pg/mL	57.14 Medium	72.73 Medium
IL-8											
Carpagnano et al., 2010 [35]	Italy	8 (5;3)	42 ± 4	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA kit (Human Interleukin-8, Bender med-Systems, Vienna, Austria)	7.6 ± 0.5 pg/mL	1.3 pg/mL	85.71 High	90.91 High
Carpagnano et al., 2013 [36]	Italy	10 (5;5)	26 ± 4.9	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	7.9 ± 1.0 pg/mL	1.5 pg/mL	71.43 Medium	90.91 High
Carpagnano et al., 2013 [36]	Italy	10 (4;6)	52 ± 5.9	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	15.2 ± 1.9 pg/mL	1.5 pg/mL	71.43 Medium	90.91 High
Carpagnano et al., 2013 [36]	Italy	10 (5;5)	67 ± 4.6	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	16.3 ± 1.4 pg/mL	1.5 pg/mL	71.43 Medium	90.91 High
De lima et al., 2013 [37]	Brazil	73 (73;0)	42 ± 7	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	8.9 ± 1.8 pg/mL	3.50 pg/mL	85.71 High	81.82 High
De lima et al., 2013 [37]	Brazil	14 (14;0)	30 ± 5	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	8.4 ± 0.9 pg/mL	3.50 pg/mL	85.71 High	81.82 High

Table 3. *Cont.*

Authors, Year	Country	n° Subjects (M;F)	Age	Collection Device	Collection Temperature	Storage Temperature	Analytical Method	Data	LOD	SCORE Quality Assessment JBI	SCORE Authors' Quality Assessment
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.58 (1.95) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	3.15 (1.95) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Matsunaga et al., 2006 [47]	Japan	10 (3;7)	34.4 ± 6.6	EcoScreen, (Jaeger, Germany)	−20 °C	−70 °C	Human Inflammation Antibody III (ray Biontec Inc, Norcross, GA, USA)	5.4 ± 1.8 pg/mL	1 pg/mL	57.14 Medium	72.73 Medium
IL-10											
De lima et al., 2013 [37]	Brazil	14 (14;0)	30 ± 5	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	1.0 (1.4) pg/mL	0.50 pg/mL	85.71 High	81.82 High
De lima et al., 2013 [37]	Brazil	73 (73;0)	42 ± 7	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	1.2 (1.6) pg/mL	0.5 pg/mL	85.71 High	81.82 High
Edmè et al., 2008 * [39]	France	19 (-;-)	38.3 ± 13.6	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	Cytometric Bead Arrays (CBA) Becton Dickinson, San Jose, CA, USA	24.3 (13-492) † pg/mL	5 pg/mL	66.67 Medium	66.67 Medium
TNF-α											
Carpagnano et al., 2005_b [31]	Italy	7 (5;2)	42 ± 5	EcoScreen (Jaeger, Wurzburg, Germany)	−20 °C	−70 °C	EIA (Cayman Chemical, Ann Arbor, MI, USA)	4.2 ± 0.6 pg/mL	1.5 pg/mL	77.78 High	90.91 High
De lima et al., 2013 [37]	Brazil	14 (14;0)	30 ± 5	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	0.4 (0.2) pg/mL	0.20 pg/mL	85.71 High	81.82 High
De lima et al., 2013 [37]	Brazil	73 (73;0)	42 ± 7	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	High sensitivity enzyme-immunoassays (Quantikine HS, R&D Systems Inc. Minneapolis, MN, USA)	0.5 (0.4) pg/mL	0.106 pg/mL	85.71 High	81.82 High
Edmè et al., 2008 * [39]	France	19 (-;-)	38.3 ± 13.6	EcoScreen (Jaeger, Wurzburg, Germany)	NA	−80 °C	Cytometric Bead Arrays (CBA) Becton Dickinson, San Jose, CA, USA	44.6 (32-91) † pg/mL	5 pg/mL	66.67 Medium	66.67 Medium

Table 3. *Cont.*

Authors, Year	Country	n° Subjects (M;F)	Age	Collection Device	Collection Temperature	Storage Temperature	Analytical Method	Data	LOD	SCORE Quality Assessment JBI	SCORE Authors' Quality Assessment
Garey et al., 2004 [41]	USA	9 (5;4)	22.0 ± 1.9	Breath condensate was collected using a novel method where the subject inspires repeatedly to TLC and exhales into 1.5 m Teflon perfluoroalkoxy (PFA) tubing with 0.5 cm internal diameter	Immersed in ice	−70 °C	ELISA (R&D System Minneapolis, MN)	3.9 ± 8.5 pg/mL	2 pg/mL	71.43 Medium	54.55 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	4.36 (1.79) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Guan et al., 2018 [44]	China	15 (7;8)	20 ± 1	ECOScreen (Jager, Germany)	NA	−80 °C	BD Cytometric Bead Array, BD-Biosciences, San Jose, CA, USA	4.14 (2.56) pg/mL	2.4 pg/mL	84.62 High	54.55 Medium
Ko et al., 2009 [45]	China	14 (9;5)	75.2 ± 4.1	EcoScreen (VIASYS Healthcare, Conshohochen, PA, USA)	NA	−70 °C	BioSource International, Camarillo, CA, USA	4.84 (3.86-5.81) pg/mL	0.09 pg/mL	71.43 Medium	81.82 High

The various biomarkers analysed are highlighted in bold. (*) In the study of Edmé et al., the concentrations declared were not divided by the concentration factor.

4. Discussion

The analysis of inflammatory biomarkers in EBC in both occupational and environmental studies is increasingly topical. The primary aim of the selected papers was to detect early changes in airway inflammatory status that could be related to a higher risk of developing pulmonary disorders [30]. The lack of established reference values in the general healthy non-smoking population, however, makes such achievement difficult.

Despite the easiness and non-invasiveness of sampling, our review highlights the lack of a standardised analytical protocol among researchers, making any inter-studies comparison challenging. These issues mainly concern the criteria used when selecting groups in epidemiological studies, sampling and storage protocols, as well as the comparability of analytical methods and eventual pre-treatment procedures.

Therefore, we established to carry on the quality assessment not only on the basis of the study design but also on a detailed evaluation of their methodological quality. The most common critical issue highlighted by the JBI checklists concerns the lack of a detailed description of subjects enrolled, with the subsequent poor characterisation of eventual confounding factors able to influence their inflammatory status. Obesity, for example, is associated with both systemic and airway inflammation [27]. Even though the underlying mechanisms have not been clearly elucidated and contrasting results have been reported, some authors suggest that the release of cytokines by the adipose tissue may be related to respiratory disorders such as obstructive sleep apnea syndrome (OSAS), obesity hypoventilation syndrome (OHS), asthma or chronic obstructive pulmonary disease (COPD) [59–62]. Only 16 of the included studies reported the BMI of the subject enrolled. Indeed, most of the studies included in the present review consist of small age-matched control groups from clinical studies, who are described only as healthy and non-smokers. Airways or systemic inflammation can increase with ageing [63]; thus, a detailed characterisation of this status should be performed in subgroups of the population using EBC, which allows repeated measurements over time [28].

The methodological assessment was based on compliance with the guidelines issued by the American Thoracic Society/European Respiratory Society Task Force in 2005 and 2015 [20,21]. To date, some of the critical issues highlighted are still unsolved. Concerning the EBC collection, the characteristics of the collection device may influence the biomarker concentration in the final sample [64]. In our systematic review, most of the articles included using Ecoscreen™ sampling devices. In many studies, the ventilation pattern sustained by subjects during the sampling is not declared, despite the importance of sampling during tidal breathing to avoid an alteration in the biomarker composition, especially for those biomarkers that may be sensitive to the respiratory pattern [65]. Inflammatory markers are produced in both the airway and the alveolar compartments, defining, at least partially, a possible flow-rate dependence of their concentration in EBC [66].

Wearing a nose clip was often not reported or not in use (56%). Albeit slightly uncomfortable, it is recommended to minimise the contamination with the nasal airway lining fluid and make subjects exhale strictly through the mouth [20]. The salivary contamination, which could determine a contribution to the inflammatory biomarker levels in EBC, was generally prevented by saliva-trap on sampling devices or by mouth rinses before the sampling. Some researchers also quantified the amylase levels, even though this method can be affected by some false positives [20]. Concerning the EBC storage, on the contrary, the vast majority of the included studies did not report the duration of the sample storage, assuming the concentration of cytokines remained stable over time. In frozen plasma samples, most cytokines are stable for up to two years, with the exception of IL-1β, IL-6, and IL-10, which undergo a degradation process up to 50% within 2–3 years of storage [67]. Further studies aiming to assess the cytokine stability in EBC would thus be recommended.

The main critical issue in the quantification of inflammatory biomarkers levels, however, concerns the analytical methods. Cytokines in EBC are often quantified by ELISA or Cytometric Bead Array (CBA) assays, according to the manufacturer's guidelines. However, as previously pointed out by Horvath et al., EBC is a diluted matrix and the cytokine

concentration is generally around the assay LOD, where assay variability is higher. Information about the assay validation for this matrix or any reason justifying the assay choice was generally not provided. Moreover, 33% of the articles did not report the assay LOD declared by the manufacturers, whereas in some cases, the quantification declared was lower than the assay LOD. The lack of this information significantly affects the reliability of these measurements, preventing the possibility of comparing data with those obtained from other studies. In both cases, we considered those data as potentially biased, and thus we excluded them from the summarising forest plot. The assays, indeed, appear to be more sensitive in discriminating large differences in cytokine levels due to acute vs chronic inflammatory states, while in healthy conditions, smaller magnitudes of cytokine levels were observed [34]. In some studies, EBC was concentrated lyophilising samples to improve the assay performance, despite this being a complex and expensive method [68]. This methodology could be a source of bias when comparing data from different studies.

Another current critical issue is the normalisation of biomarker levels in EBC to take into account the inter-individual variability in droplet formation, resulting in samples being variously diluted. To overcome this problem, in some studies, data were reported both raw and normalised for the total protein concentration in EBC, even if this is not a widely accepted method [39,42,43]. Moreover, EBC collection involves a large variability in the volume exhaled for each breath over time. Thus, the American Thoracic Society (ATS) has suggested standardising the concentrations of biomarkers in EBC by registering the total volume of exhaled air and stopping the exhalation collection when the set volume has been accomplished. Thus, EBC collection will consider the volume of exhaled breath, the volume of condensation collected from the exhaled volume, and the collection time must be correlated in order to evaluate the effectiveness of the collection of EBC. To achieve this goal, a volume-meter can be enclosed in line with the DECCS circuit, thus allowing measuring the total volume of air exhaled (e.g., 90 L) during an EBC collection session.

To provide a complete description of the more studied inflammatory mediators measured in EBC, we focus on IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, and CRP (as determined by the high sensitive assay).

The data retrieved in this review present some limits, actually preventing the possibility of considering them as truly reference values. First of all, the vast majority of the selected studies describe small epidemiological samples representing the control group in clinical studies, an aim that does not match the purpose of our review. The frequent absence of a detailed description of those subjects in terms of demographic and health-related data hampers the analysis of sources of variability in biomarker concentration, which would inform the need for partitioning when summarising the reference values and the reference interval. Secondly, methodological discrepancies and the lack of standardisation in sampling and analysis protocols make it difficult to compare data obtained in different settings.

The strength of our systematic review can thus be identified in the research string that results are very sensitive, even though non-specific, allowing us to obtain a comprehensive set of articles to screen and to highlight the main criticisms still affecting the evaluation of the inflammatory profile in EBC.

5. Conclusions

In conclusion, EBC is a useful tool to characterise the airway inflammatory state due to the easiness and non-invasiveness of sampling. However, to obtain consistent reference values, more efforts are needed. Firstly, the creation of datasets with measurements obtained from vast epidemiological samples suitably selected according to health criteria and with repeated measurements would be strongly recommended. Secondly, qualitative criteria requested from the study design must be integrated with the criteria proposed by the ATS/ERS Task Force guidelines on Exhaled Breath Condensate in 2005 and 2015 [20,21,68].

The development of reference intervals for these biomarkers can result in their introduction and use in both research and clinical settings, not only for monitoring purposes

but also, in the perspective of future longitudinal studies, as a predictive parameter for the onset and development of chronic diseases with inflammatory aetiology.

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Appendix A

Table A1. Search strings.

PubMed	
1	"Tumor Necrosis Factor-alpha" [Mesh]
2	"tumor necrosis factor-alpha" [tiab] OR "tumor necrosis factor-a" [tiab] OR "TNF-alpha" [tiab] OR TNFalpha [tiab] OR TNF-a [tiab] OR TNFa [tiab] OR "tumor necrosis factor (TNF)-alpha" [tiab]
3	"C-Reactive Protein" [Mesh]
4	"C-Reactive Protein" [tiab] OR CRP [tiab]
5	"Cytokines" [MESH:noexp]
6	"Interleukins" [MESH:noexp]
7	cytokines [tiab] OR interleukins [tiab]
8	"Interleukin-1" [Mesh]
9	"interleukin-1beta" [tiab] OR "interleukin-1 beta" [tiab] OR "interleukin-1 b" [tiab] OR "interleukin-1b" [tiab] OR "IL-1beta" [tiab] OR "IL-1 beta" [tiab] OR "IL1beta" [tiab] OR "IL1 beta" [tiab] OR "IL-1b" [tiab] OR "IL-1 b" [tiab] OR "IL1b" [tiab] OR "IL1 b" [tiab] OR "interleukin (IL)-1beta" [tiab] OR "interleukin (IL)-1 beta" [tiab]
10	"Interleukin-4" [Mesh]
11	"interleukin-4" [tiab] OR "IL-4" [tiab] OR IL4 [tiab] OR "interleukin (IL)-4" [tiab]
12	"Interleukin-6" [Mesh]
13	"interleukin-6" [tiab] OR "IL-6" [tiab] OR IL6 [tiab] OR "interleukin (IL)-6" [tiab]
14	"Interleukin-8" [Mesh]
15	"interleukin-8" [tiab] OR "IL-8" [tiab] OR IL8 [tiab] OR "interleukin (IL)-8" [tiab]
16	"Interleukin-10" [Mesh]
17	"interleukin-10" [tiab] OR "IL-10" [tiab] OR IL10 [tiab] OR "interleukin (IL)-10" [tiab]
18	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
19	"exhaled breath condensate *" [tiab] OR EBC [tiab] OR EBCs [tiab] OR "exhaled breath" [tiab] OR "breath condensate *" [tiab]
20	"Breath Tests" [Mesh]
21	"Exhalation" [Mesh]
22	#19 OR #20 OR #21
23	#18 AND #22

Table A1. *Cont.*

24	"Animals" [Mesh]
25	"Humans" [Mesh]
26	#24 NOT #25
27	#23 NOT #26
28	"Adolescent" [Mesh]
29	"Child" [Mesh]
30	"Infant" [Mesh]
31	#28 OR #29 OR #30
32	"Adult" [Mesh]
33	#31 NOT #32
34	#27 NOT #33
Embase	
1	'tumor necrosis factor'/exp
2	'tumor necrosis factor-alpha':ti,ab,kw OR 'tumor necrosis factor-a':ti,ab,kw OR 'TNF-alpha':ti,ab,kw OR TNFalpha:ti,ab,kw OR TNF-a:ti,ab,kw OR TNFa:ti,ab,kw OR 'tumor necrosis factor (TNF)-alpha':ti,ab,kw
3	'C reactive protein'/exp
4	'C-Reactive Protein':ti,ab,kw OR CRP:ti,ab,kw
5	'cytokine'/de
6	'interleukin derivative'/de
7	cytokines:ti,ab,kw OR interleukins:ti,ab,kw
8	'interleukin 1'/exp
9	'interleukin-1beta':ti,ab,kw OR 'interleukin-1 beta':ti,ab,kw OR 'interleukin-1 b':ti,ab,kw OR 'interleukin-1b':ti,ab,kw OR 'IL-1beta':ti,ab,kw OR 'IL-1 beta':ti,ab,kw OR 'IL1beta':ti,ab,kw OR 'IL1 beta':ti,ab,kw OR 'IL-1b':ti,ab,kw OR 'IL-1 b':ti,ab,kw OR 'IL1b':ti,ab,kw OR 'IL1 b':ti,ab,kw OR 'interleukin (IL)-1beta':ti,ab,kw OR 'interleukin (IL)-1 beta':ti,ab,kw
10	'interleukin 4'/exp
11	'interleukin-4':ti,ab,kw OR 'IL-4':ti,ab,kw OR 'IL4':ti,ab,kw OR 'interleukin (IL)-4':ti,ab,kw
12	'interleukin 6'/exp
13	'interleukin-6':ti,ab,kw OR 'IL-6':ti,ab,kw OR 'IL6':ti,ab,kw OR 'interleukin (IL)-6':ti,ab,kw
14	'interleukin 8'/exp
15	'interleukin-8':ti,ab,kw OR 'IL-8':ti,ab,kw OR 'IL8':ti,ab,kw OR 'interleukin (IL)-8':ti,ab,kw
16	'interleukin 10'/exp
17	'interleukin-10':ti,ab,kw OR 'IL-10':ti,ab,kw OR 'IL10':ti,ab,kw OR 'interleukin (IL)-10':ti,ab,kw
18	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
19	'exhaled breath condensate'/exp
20	'exhaled breath condensate *':ti,ab,kw OR EBC:ti,ab,kw OR EBCs:ti,ab,kw OR 'exhaled breath':ti,ab,kw OR 'breath condensate *':ti,ab,kw
21	'breath analysis'/exp
22	'exhalation'/exp
23	#19 OR #20 OR #21 OR #22
24	#18 AND #23
25	'animal'/de
26	'animal experiment'/exp
27	'nonhuman'/de
28	#25 OR #26 OR #27
29	'human'/de
30	#28 NOT #29
31	#23 NOT #30
32	'adolescent'/exp
33	'child'/exp
34	#32 OR #33

Table A1. *Cont.*

35	'adult'/exp
36	#34 NOT #35
37	#31 NOT #36
Cochrane CENTRAL	
#1	MeSH descriptor: [Tumor Necrosis Factor-alpha] explode all trees
#2	("tumor necrosis factor-alpha" OR "tumor necrosis factor-a" OR "TNF-alpha" OR TNFalpha OR TNF-a OR TNFa):ti,ab,kw
#3	MeSH descriptor: [C-Reactive Protein] explode all trees
#4	("C-Reactive Protein" OR CRP):ti,ab,kw
#5	MeSH descriptor: [Cytokines] this term only
#6	MeSH descriptor: [Interleukins] this term only
#7	(cytokines OR interleukins):ti,ab,kw
#8	MeSH descriptor: [Interleukin-1] explode all trees
#9	("interleukin-1beta" OR "interleukin-1 beta" OR "interleukin-1 b" OR "interleukin-1b" OR "IL-1beta" OR "IL-1 beta" OR "IL1beta" OR "IL1 beta" OR "IL-1b" OR "IL-1 b" OR "IL1b" OR "IL1 b"):ti,ab,kw
#10	MeSH descriptor: [Interleukin-4] explode all trees
#11	("interleukin-4" OR "IL-4" OR IL4):ti,ab,kw
#12	MeSH descriptor: [Interleukin-6] explode all trees
#13	("interleukin-6" OR "IL-6" OR IL6):ti,ab,kw
#14	MeSH descriptor: [Interleukin-8] explode all trees
#15	("interleukin-8" OR "IL-8" OR IL8):ti,ab,kw
#16	MeSH descriptor: [Interleukin-10] explode all trees
#17	("interleukin-10" OR "IL-10" OR IL10):ti,ab,kw
#18	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
#19	("exhaled breath condensate *" OR EBC OR EBCs OR "exhaled breath" OR "breath condensate *"):ti,ab,kw
#20	MeSH descriptor: [Breath Tests] explode all trees
#21	MeSH descriptor: [Exhalation] explode all trees
#22	#19 OR #20 OR #21
#23	#18 AND #22.

(*) The asterisk was employed to retrieve any variations of the indicated terms.

Appendix B

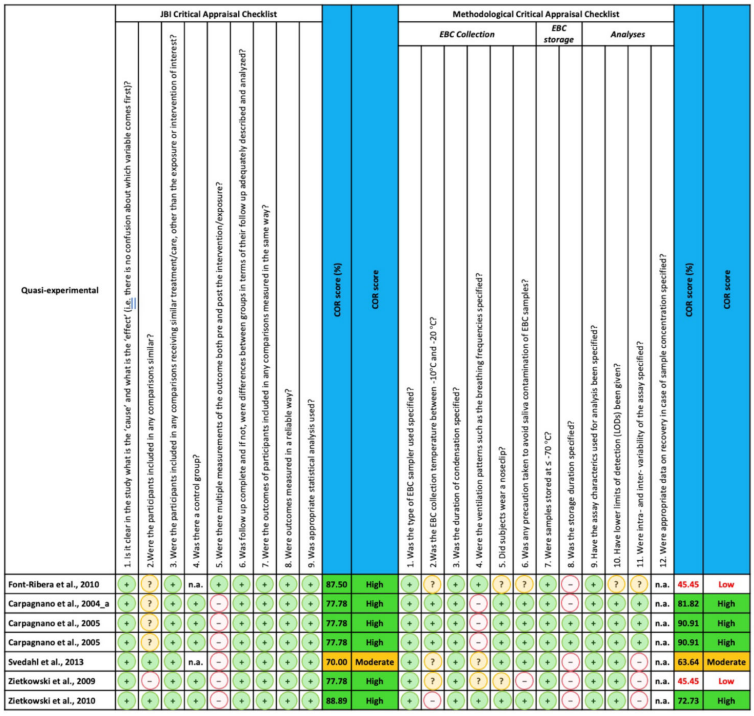
Table A2. Authors quality assessment additional questions.

1	Was the type of EBC sampler used specified?
2	Was the EBC collection temperature between -10 °C and -20 °C?
3	Was the duration of condensation specified?
4	Were the ventilation patterns such as the breathing frequencies specified?
5	Did subjects wear a noseclip?
6	Was any precaution taken to avoid saliva contamination of EBC samples?
7	Were samples stored at ≤ -70 °C?
8	Was the storage duration specified?
9	Have the assay characteristics used for analysis been specified?
10	Have lower limits of detection (LODs) been given?
11	Were intra- and inter-variability of the assay specified?
12	Were appropriate data on recovery in case of sample concentration specified?

Appendix C

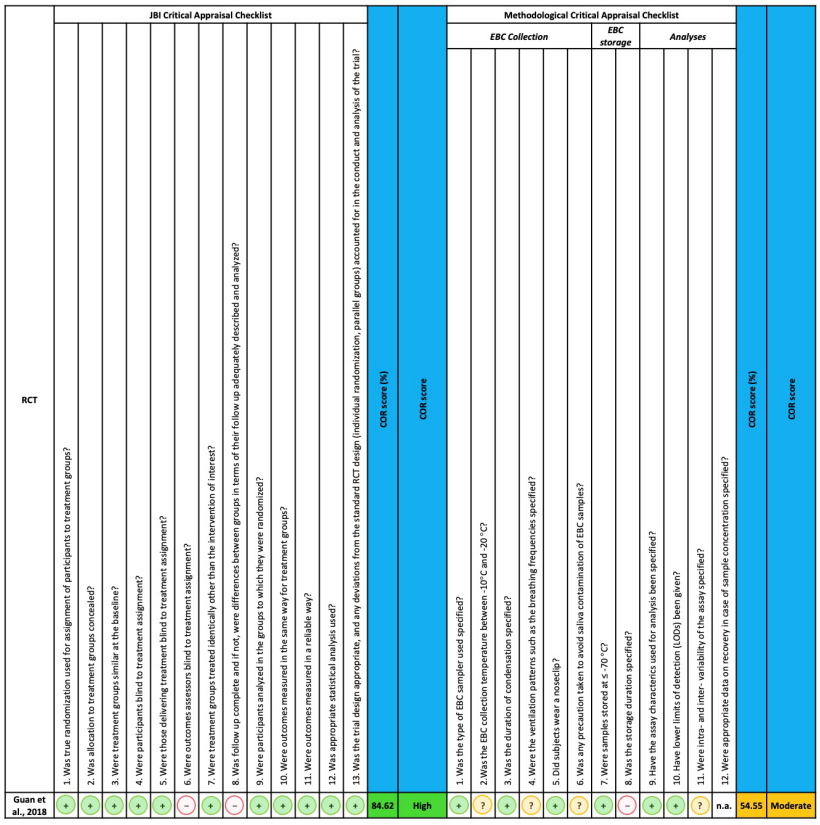
Cross-sectional	JBI Critical Appraisal Checklist										Methodological Critical Appraisal Checklist													
											EBC Collection						*EBC storage*		*Analyses*					
	1. Were the criteria for inclusion in the sample clearly defined?	2. Were the study subjects and the setting described in detail?	3. Was the exposure measured in a valid and reliable way?	4. Were objective, standard criteria used for measurement of the condition?	5. Were confounding factors identified?	6. Were strategies to deal with confounding factors stated?	7. Were the outcomes measured in a valid and reliable way?	8. Was appropriate statistical analysis used?	COR score (%)	COR score	1. Was the type of EBC sampler used specified?	2.Was the EBC collection temperature between -10 °C and -20 °C?	3. Was the duration of condensation specified?	4. Were the ventilation patterns such as the breathing frequencies specified?	5. Did subjects wear a noseclip?	6. Was any precaution taken to avoid saliva contamination of EBC samples?	7. Were samples stored at ≤ -70 °C?	8. Was the storage duration specified?	9. Have the assay characterics used for analysis been specified?	10. Have lower limits of detection (LODs) been given?	11. Were intra- and inter- variability of the assay specified?	12. Were appropriate data on recovery in case of sample concentration specified?	COR score (%)	COR score
Aquino-Santos et al., 2020	+	+	?	+	+	?	+	+	75.00	High	+	?	+	–	?	–	+	–	+	?	–	n.a.	33.33	Low
Ausin et al., 2017	+	+	n.a.	+	+	?	+	+	85.71	High	+	?	+	?	?	?	?	–	+	?	?	n.a.	27.27	Low
Brandao-Ragel et al., 2021	+	?	n.a.	+	+	–	+	+	71.43	Moderate	+	+	+	?	?	+	+	–	+	?	?	n.a.	54.55	Moderate
Carpagnano et al., 2003	+	?	+	+	+	+	+	+	87.50	High	+	+	+	–	+	+	+	–	+	+	+	n.a.	81.82	High
Carpagnano et al., 2004_b	+	?	?	+	+	?	+	+	62.50	Moderate	+	?	+	–	?	–	+	–	+	+	+	n.a.	54.55	Moderate
Carpagnano et al., 2006	+	?	?	+	–	–	+	+	50.00	Moderate	+	?	+	–	?	+	+	–	+	+	+	n.a.	63.64	Moderate
Carpagnano et al., 2007	+	+	+	+	–	–	+	+	75.00	High	+	?	+	–	+	?	?	–	+	+	+	n.a.	54.55	Moderate
Carpagnano et al., 2009	+	+	n.a.	+	+	?	+	+	85.71	High	+	+	+	–	–	?	+	–	+	+	+	n.a.	63.64	Moderate
Carpagnano et al., 2010	+	?	n.a.	+	+	+	+	+	85.71	High	+	+	+	+	+	+	+	–	+	+	+	n.a.	90.91	High
Carpagnano et al., 2013	+	?	n.a.	+	+	–	+	+	71.43	Moderate	+	+	+	+	+	+	+	–	+	+	+	n.a.	90.91	High
De Lima et al., 2013	+	+	+	n.a.	+	?	+	+	85.71	High	?	+	+	+	+	+	+	+	+	+	–	n.a.	81.82	High
Diez-pina et al., 2009	+	?	–	+	+	+	+	+	75.00	High	+	?	+	+	–	?	+	–	+	?	–	n.a.	45.45	Low
Edmè et al., 2008	+	?	n.a.	+	?	–	+	+	66.67	Moderate	+	?	+	–	?	+	+	–	+	+	+	+	66.67	Moderate
Garey et al., 2004	+	+	+	n.a.	?	?	+	+	71.43	Moderate	?	?	+	+	–	?	+	–	+	+	+	n.a.	54.55	Moderate
Gessner et al., 2005	+	?	n.a.	+	?	–	+	+	57.14	Moderate	+	+	+	+	?	+	?	–	+	?	+	+	66.67	Moderate
Gessner et al., 2007	+	?	n.a.	+	?	–	+	+	57.14	Moderate	+	+	+	+	?	+	?	–	+	?	+	+	66.67	Moderate
Ko et al., 2009	+	?	n.a.	+	+	?	+	+	71.43	Moderate	+	?	+	+	+	+	+	–	+	+	+	n.a.	81.82	High
Liu et al., 2014	+	?	n.a.	+	+	–	+	+	71.43	Moderate	?	–	+	+	–	+	+	–	?	–	?	n.a.	36.36	Low
Matsunaga et al., 2006	+	–	n.a.	+	–	–	+	+	57.14	Moderate	+	+	+	–	+	–	+	+	+	+	?	n.a.	72.73	Moderate
Mazur et al., 2009	+	+	+	+	+	+	+	+	100.00	High	+	+	+	–	?	?	+	–	+	?	?	n.a.	45.45	Low
Nielepkowicz-Goździńska et al., 2013	+	?	n.a.	+	?	?	+	+	57.14	Moderate	+	?	+	+	?	+	+	–	+	+	?	n.a.	63.64	Moderate
Nielepkowicz-Goździńska et al., 2014	+	+	n.a.	+	?	?	+	+	71.43	Moderate	+	?	+	+	?	+	+	–	+	+	?	n.a.	63.64	Moderate
Radulovich et al., 2015	+	–	–	+	+	+	+	+	75.00	High	+	+	+	?	?	?	+	–	+	?	–	n.a.	45.45	Low
Rolla et al., 2016	+	?	n.a.	+	?	?	+	+	57.14	Moderate	+	+	+	+	+	+	+	–	+	?	?	n.a.	72.73	Moderate
Sack et al., 2006	+	–	n.a.	+	–	–	+	+	57.14	Moderate	+	?	+	?	?	?	?	–	+	+	+	n.a.	45.45	Low
Tufvesson et al., 2006	+	?	n.a.	+	?	?	+	+	66.67	Moderate	+	?	+	+	+	+	+	–	+	+	–	–	66.67	Moderate
Vergara et al., 2015	+	?	+	n.a.	+	?	+	+	83.33	High	+	+	+	+	+	+	+	–	+	+	+	n.a.	90.91	High
Yan et al., 2019	+	?	n.a.	+	+	–	?	+	66.67	Moderate	–	?	–	–	?	?	?	–	?	–	–	n.a.	0.00	Low

Figure A1. Quality Assessment for cross-sectional studies, according to JBI critical appraisal tool and to the ATS/ERS Task Force guidelines on EBC [20,21]. (+) means "yes"; (-) means "no"; (?) means "unclear"; (n.a.) means "not applicable".



Quasi-experimental	JBI Critical Appraisal Checklist													COR score (%)	COR score	Methodological Critical Appraisal Checklist												COR score (%)	COR score
																EBC Collection						*EBC storage*		*Analyses*					
	1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	2. Were the participants included in any comparisons similar?	3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	4. Was there a control group?	5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?	6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	7. Were the outcomes of participants included in any comparisons measured in the same way?	8. Were outcomes measured in a reliable way?	9. Was appropriate statistical analysis used?							1. Was the type of EBC sampler used specified?	2. Was the EBC collection temperature between -10°C and -20 °C?	3. Was the duration of condensation specified?	4. Were the ventilation patterns such as the breathing frequencies specified?	5. Did subjects wear a noseclip?	6. Was any precaution taken to avoid saliva contamination of EBC samples?	7. Were samples stored at ≤ -70 °C?	8. Was the storage duration specified?	9. Have the assay characterics used for analysis been specified?	10. Have lower limits of detection (LODs) been given?	11. Were intra- and inter- variability of the assay specified?	12. Were appropriate data on recovery in case of sample concentration specified?		
Font-Ribera et al., 2010	+	?	+	n.a.	+	+	+	+	+	87.50	High	+	?	+	+	?	?	+	-	+	?	?	n.a.	45.45	Low
Carpagnano et al., 2004_a	+	?	+	+	-	+	+	+	+	77.78	High	+	+	+	-	+	+	+	+	+	+	+	n.a.	81.82	High
Carpagnano et al., 2005	+	?	+	+	-	+	+	+	+	77.78	High	+	+	+	-	+	+	+	+	+	+	+	n.a.	90.91	High
Carpagnano et al., 2005	+	?	+	+	-	+	+	+	+	77.78	High	+	+	+	-	+	+	+	+	+	+	+	n.a.	90.91	High
Svedahl et al., 2013	+	+	+	n.a.	-	+	+	+	+	70.00	Moderate	+	?	+	?	+	+	+	-	+	+	-	n.a.	63.64	Moderate
Zietkowski et al., 2009	+	-	+	+	-	+	+	+	+	77.78	High	+	?	+	?	?	-	+	-	+	+	-	n.a.	45.45	Low
Zietkowski et al., 2010	+	+	+	+	-	+	+	+	+	88.89	High	+	-	+	+	+	+	+	-	+	+	-	n.a.	72.73	High

Figure A2. Quality Assessment for quasi-experimental studies, according to JBI critical appraisal tool and to the ATS/ERS Task Force guidelines on EBC [20,21]. (+) means "yes"; (-) means "no"; (?) means "unclear"; (n.a.) means "not applicable".



RCT	JBI Critical Appraisal Checklist													COR score (%)	COR score	Methodological Critical Appraisal Checklist												COR score (%)	COR score
																EBC Collection						*EBC storage*		*Analyses*					
	1. Was true randomization used for assignment of participants to treatment groups?	2. Was allocation to treatment groups concealed?	3. Were treatment groups similar at the baseline?	4. Were participants blind to treatment assignment?	5. Were those delivering treatment blind to treatment assignment?	6. Were outcomes assessors blind to treatment assignment?	7. Were treatment groups treated identically other than the intervention of interest?	8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	9. Were participants analyzed in the groups to which they were randomized?	10. Were outcomes measured in the same way for treatment groups?	11. Were outcomes measured in a reliable way?	12. Was appropriate statistical analysis used?	13. Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?			1. Was the type of EBC sampler used specified?	2. Was the EBC collection temperature between -10°C and -20 °C?	3. Was the duration of condensation specified?	4. Were the ventilation patterns such as the breathing frequencies specified?	5. Did subjects wear a noseclip?	6. Was any precaution taken to avoid saliva contamination of EBC samples?	7. Were samples stored at ≤ -70 °C?	8. Was the storage duration specified?	9. Have the assay characterics used for analysis been specified?	10. Have lower limits of detection (LODs) been given?	11. Were intra- and inter- variability of the assay specified?	12. Were appropriate data on recovery in case of sample concentration specified?		
Guan et al., 2018	+	+	+	+	+	-	+	-	+	+	+	+	+	84.62	High	+	?	+	?	+	?	+	-	+	+	?	n.a.	54.55	Moderate

Figure A3. Quality Assessment for randomised-control trial studies, according to JBI critical appraisal tool and to the ATS/ERS Task Force guidelines on EBC [20,21]. (+) means "yes"; (-) means "no"; (?) means "unclear"; (n.a.) means "not applicable".

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Assessing the inhaled dose of nanomaterials by nanoparticle tracking analysis (NTA) of exhaled breath condensate (EBC) and its relationship with lung inflammatory biomarkers

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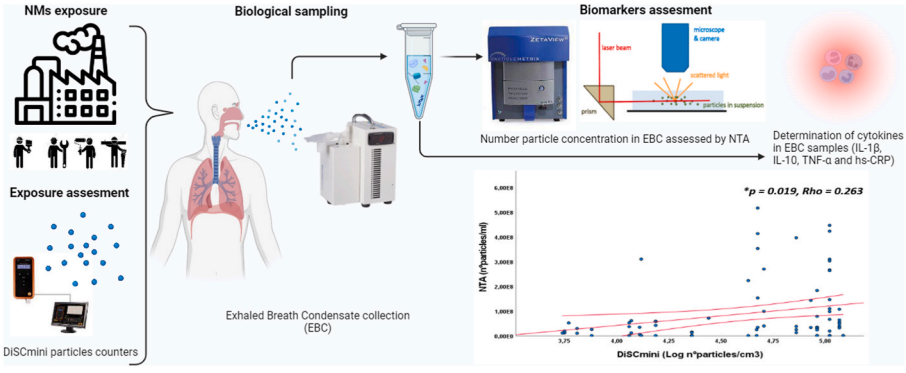
HIGHLIGHTS (5 BULLET POINTS)

- The first multi-center occupational study on a cohort of 80 workers exposed to NMs.
- Increasing exposure to NMs revealed increasing particles number in EBC assessed by NTA.
- Nanoparticle Tracking Analysis (NTA) is a possible biomarker of internal dose.
- An increased number of particles in EBC correlates significantly with IL-1β and IL-10.
- This study underlines a lack of occupational studies on non-invasive matrices.

GRAPHICAL ABSTRACT



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ABSTRACT

The widespread and increasing use of nanomaterials has resulted in a higher likelihood of exposure by inhalation for nanotechnology workers. However, tracking the internal dose of nanoparticles deposited at the airways level, is still challenging.

To assess the suitability of particle number concentration determination as biomarker of internal dose, we carried out a cross sectional investigation involving 80 workers handling nanomaterials. External exposure was characterized by portable counters of particles DISCminiTM (Testo, DE), allowing to categorize 51 workers as exposed and 29 as non-exposed (NE) to nanoparticles. Each subject filled in a questionnaire reporting working

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practices and health status. Exhaled breath condensate was collected and analysed for the number of particles/ml as well as for inflammatory biomarkers.

A clear-cut relationship between the number of airborne particles in the nano-size range determined by the particle counters and the particle concentration in exhaled breath condensate (EBC) was apparent. Moreover, inflammatory cytokines (IL-1β, IL-10, and TNF-α) measured in EBC, were significantly higher in the exposed subjects as compared to not exposed. Finally, significant correlations were found between external exposure, the number concentration of particles measured by the nanoparticle tracking analysis (NTA) and inflammatory cytokines. As a whole, the present study, suggests that NTA can be regarded as a reliable tool to assess the inhaled dose of particles and that this dose can effectively elicit inflammatory effects.

Acronyms/abbreviations

EBC	(Exhaled Breath Condensate)
FFP2	(filtering face piece type 2)
HE	(High-exposed)
CRP	(C-reactive Protein)
IL-	(Interleukins)
LDSA	(lung deposited surface area)
LE	(Low-exposed)
NE	(Non-exposed)
NMs	(Nanomaterials)
NTA	(Nanoparticle Tracking Analysis)
PM	(airborne particulate)
PPE	(personal protective equipment)
TNF	(Tumour Necrosis Factor)

1. Introduction

In the last decades, the use of nanotechnologies has experienced significant growth in many industrial sectors, leading to increased nanomaterials (NMs) production and handling with the subsequent likelihood of occupational exposure in Companies and Laboratories (Ghafari et al., 2020; Luo et al., 2022). However, many dry powders used for industrial products (e.g. in paints) can fall under the EU NM definition given by the European Commission (European Commission, 2022; Bergamaschi et al., 2022).

Owing to the low number of human studies, much of our knowledge regarding the potential toxicity of NMs has been evaluated through in vivo and in vitro studies (Gonzalez and Kirsch-Volders, 2016). It has been demonstrated that particles, fine and ultrafine in size, present a large specific surface area, a low coordination of atoms at the surface with other atoms, a high curvature radius, and can have a colloidal nature, all these properties make NMs very reactive (Barbero et al., 2021). Moreover, NMs present ability to penetrate inside the organism and, in his way, interact with biomolecules, potentially inducing local inflammation (Barbero et al., 2017). The main way of penetration of particles into the organism is breathing (Borm et al., 2006; Yah et al., 2012; O'Shaughnessy, 2013) and the quantification of their intake needs to be explored more in depth (Wittmaack, 2007; Ferdous and Nemmar, 2020). Although exposure assessment for nanomaterials has dramatically improved over the last years, relying on innovative approaches, as well as on devices allowing the sampling in the breathing zone of workers and personal monitors translating the aerosol characteristics in relevant metrics, such as the lung deposited surface area (LDSA) (Iavicoli et al., 2018), there is the need to assess both the internal dose and possible effects at the target organ (Bergamaschi et al., 2015). Exposure assessment combined with biomonitoring seems the most useful tool for identifying the causal relationships and the potential risks that workers can be exposed (Bergamaschi et al., 2015). Providing objective demonstration of the absorption of chemicals in the body, exposure biomarkers can be useful in occupational toxicology for a more accurate risk assessment, reducing misclassification in health studies (Mutti, 2001). Thus, a particle exposure assessment based on the dose deposited in the lungs would be the gold standard for the evaluation of any resulting health effects. Measuring particles in exhaled breath could help to evaluate particle retention in the lungs. By cooling a subject's exhaled breath in a non-invasive way, it is possible to collect a liquid composed mainly of water and a very small amount of airway lining fluids. Exhaled breath condensate (EBC), a non-invasive matrix predominantly composed of water vapour and small droplets from various regions of the respiratory tract, including the bronchial and alveoli regions, is considered a valuable biological to monitoring matrix when traditional matrices, like blood or induced sputum are not feasible (Goldoni et al., 2004, 2006; Hunt, 2007). It is thought that EBC might be a useful biological monitoring matrix where either biological monitoring is currently not possible using traditional biological matrices such as urine or blood (e.g., for dusts or respirable crystalline silica) or where the interpretation of elemental species is difficult in a biological sample (e.g., for hexavalent and trivalent chromium) (Forest et al., 2021; Marie-Desvergne et al., 2022; Forest and Pourchez, 2023).

Innovative techniques and tools, such as nanoparticle tracking analysis (NTA) have been used for the quantification of particles in biological matrices, such as EBC, and can thus support the assessment of internal dose of particles as an exposure biomarker (Guseva Canu et al., 2021).

The aims of the present study are: i) to quantify the number of particles in EBC of workers occupationally exposed to nanomaterials by NTA and ii) to assess the relationship between the number of particles in EBC and the number particles concentration quantified by real time monitoring devices and iii) to explore the relationships between different particle metrics and the pro- and anti-inflammatory biomarkers, which are commonly analysed for characterizing the severity of respiratory diseases (Montuschi, 2007) as well as effect biomarkers for nanomaterial exposure (Ghelli et al., 2022).

2. Material and methods

2.1. Study protocol

The study protocol was approved by the NanoExplore Consortium and the EU monitor in charge of the NanoExplore project. Moreover, approvals have been obtained from the local ethics regulation organs: the Swiss Ethics in Switzerland (approval 2020–01098); the Bio-ethical Committee of the University of Torino in Italy (approval 336577 August 8, 2020); and the Health and Safety Board of the Catalan Institute of Nanoscience and Nanotechnology in Spain (approval ICN2-22-03-2022). This work was supported by the European Commission LIFE program (Grant LIFE17 ENV/GR/000285).

2.2. Study participants & companies involved

A subgroup of workers potentially exposed to nanomaterials belonging to a larger group of subjects recruited for the LIFE NanoExplore project (Grant LIFE17 ENV/GR/000285) was recruited. These workers belong to different companies where paints, adhesives, coatings, construction chemicals are handled and produced. Each company

was identified by a fictitious name based on the type of materials used in their work. Moreover, the presence of the nanomaterials at each company site was investigated by analyzing the filters held in the particle samplers by transmission electron microscopy (TEM) for size and shape and subsequently by energy dispersive X-ray (EDAX) for elemental analysis, as reported more specifically in the study by Hemmendinger et al. (2023); Guseva Canu et al., 2023. In "Company A," paints, adhesives, and coating materials are produced, and filter analysis identified the following elements Carbon, Oxygen, Titanium, Silicon, and Calcium. These elements also correspond to the main nanomaterials used in manufacturing products such as paints and varnishes. "Company B″ produces construction materials of chemical origin. The elements detected at this company site as the main components of the nanoparticles produced in the plant were Aluminium, Silicon, Oxygen, Carbon, Sulfur, Titanium and Calcium. Elements very similar to those found in Company A. Finally, "Company C″ is involved in NM research and development, and the material most commonly found in filters was Iron. Whereas people working in companies A and B handled large quantities of materials, the subjects recruited in Company C were mainly involved in research and development, handling small quantities of materials needed for experiments in the research labs.

2.3. Exposure monitoring

Exposure monitoring was performed using six particle-size concentration counters DISCmini™(Testo, DE), placed near different types of workstations (near field measurements). These devices measure the number of airborne particles in the nanometric size range from 10 to 300 nm and the resulting data are expressed as number of particles/cm^3. Particle size is expressed in nanometers with a time resolution of 1 s. The detection range of the DISCmini™(Testo, DE)is around 500–1,000,000 particles/cm^3. Based on the results obtained from the DISCmini™ (Testo, DE)sampling, the LDSA was determined, a metric based on the size-dependent deposition of particles within the lung (Schmid and Stoeger, 2016).

2.4. EBC sampling collection

EBC samples were collected using a Turbo-DECCS™ condenser (Medivac, Parma, Italy) set at −10 °C equipped by a flow meter (VOLTMET 20 Medivac, Parma, Italy), to comply with the American Thoracic Society and the European Respiratory Society Task Force guidelines and normalize the volume of exhaled air collected from different subjects. Workers were required to breathe into the condenser circuit at tidal volume while wearing a nose clip until the air volume of 90 L. This allowed to collect 2–3 ml of EBC. EBC was divided into aliquots of 300 μL and stored at −80 °C until analysis.

The biological sample collection, handling and storage were operated by a dedicated operator in a closed clean room, in different buildings. In this study, EBC sampling was done both at the beginning and at the end of the working week (time A and B), with the aim of identifying possible washout during the weekend or an accumulation over the working week.

2.5. NTA-fine tuning methodology

Particle concentration, size distribution and Z potential analysis were performed by the ZetaView® PMX-120 (Particle Metrix GmbH, Germany) nanoparticle tracking analyser, equipped with a light source set to a wavelength of 488 nm. NTA captures the Brownian motion of each particle in a video. The hydrodynamic diameter of the particles is determined based on the Stokes-Einstein relation starting from the obtained diffusion coefficient (size range 30–2000 nm). The particle concentration is determined by counting all objects in the field of view and knowing the measured volume. To optimize the instrumental parameters and the correct sample dilution, a pre-screening on 5 EBC samples was necessary. The sensitivity, the shutter and the frame rate were finally set at 70, 100 and 30, respectively; 3 × 33 videos of 1 s for each sample were recorded. The dilution of the EBC samples in double-filtered Milli-Q water was set at 1:5, optimal for almost all the analysed samples. Few samples - the most concentrated - were further diluted to carry out a correct analysis. The background noise of the instrument, of the double-filtered Milli-Q water and of the used plastic ware was determined too. A LOD of 5 × 10^6 NPs/mL was calculated.

2.6. Inflammation analyses

Cytokines concentrations in EBC, namely IL-1β, IL-10, and TNF-α were determined by Real-Time PCR – linked ELISA (Invitrogen), whereas the C-reactive protein (CRP) was investigated with high-sentivity ELISA kit (MyBioSource). Real-Time PCR linked ELISA was chosen because cytokine levels in EBC are often highly diluted, resulting in typical concentrations at the pg/mL and a highly sensitive test for their quantification is needed.

2.7. Statistical analyses

All environmental and biological data were uploaded and integrated into a database to perform the statistical analysis using SPSS software. Subjects were classified into different groups based on environmental data obtained from DiSCmini™ (Testo, DE)), and their profiles were juxtaposed the number of particles (ZetaView® PMX-120 (Particle Metrix GmbH, Germany) nanoparticle tracking analyser) and inflammatory biomarkers in EBC analysing the variance using the non-parametric Kruskal-Wallis's test.

Additionally, bivariate correlations were established, using "Spearman's" coefficient depending on the parametric nature of the data, to explore the relationships between DiSCmini™ (Testo, DE) measurements, NTA, and inflammatory markers."

3. Results

According to the results of DiSCmini™ (Testo, DE) devices, were identified three subgroups of workers. Subjects exposed to a number concentration (log 10) ranged 3,30–3.88 were classified as non-exposed (NE), workers exposed to NMs ranging between 4.12 and 4.71 were classified as low-exposed (LE), and workers exposed to NMs ranging from 4.92 to 5.74 were classified as high-exposed (HE) group (Hemmendinger et al., 2023). Thus, from the whole epidemiological sample, were identified 51 NM-exposed workers, of whom 37 were categorized as HE subgroup, 14 LE subgroup and 29 workers with no apparent occupational exposure to NMs were included as controls (NE). Table 1 summarizes the characteristic of the subgroups according to the exposure ranking.

Particle number concentrations recorded by DISCmmini were significantly higher in exposed workers as compared to NE ($p < 0.001$)

Table 1
Characteristic of the subgroups according to the exposure ranking.

Variables	NE	LE	HE
Subjects n° (%)	**29 (36.25%)**	**14 (17.50%)**	**37 (46.25%)**
Age min-max (mean ± sd)	**25–54 (38,6 ± 2,6)**	**19–60 (36,4 ± 2,0)**	**22–60 (41,5 ± 1,8)**
Male n° (%)	**7 (8,75%)**	**22 (27,50%)**	**30 (37.50%)**
Female n° (%)	**9 (11.25%)**	**6 (7.50%)**	**6 (7.50%)**
Subjects from company A n° (%)	/	**14(17.50%)**	**3 (3.75%)**
Subjects from company B n° (%)	/	/	**27(33.75%)**
Subjects from company C n° (%)	**13 (16.25%)**		**7 (8.75%)**
Subjects recruited as controls (NE) n° (%)	**16 (20%)**	/	/

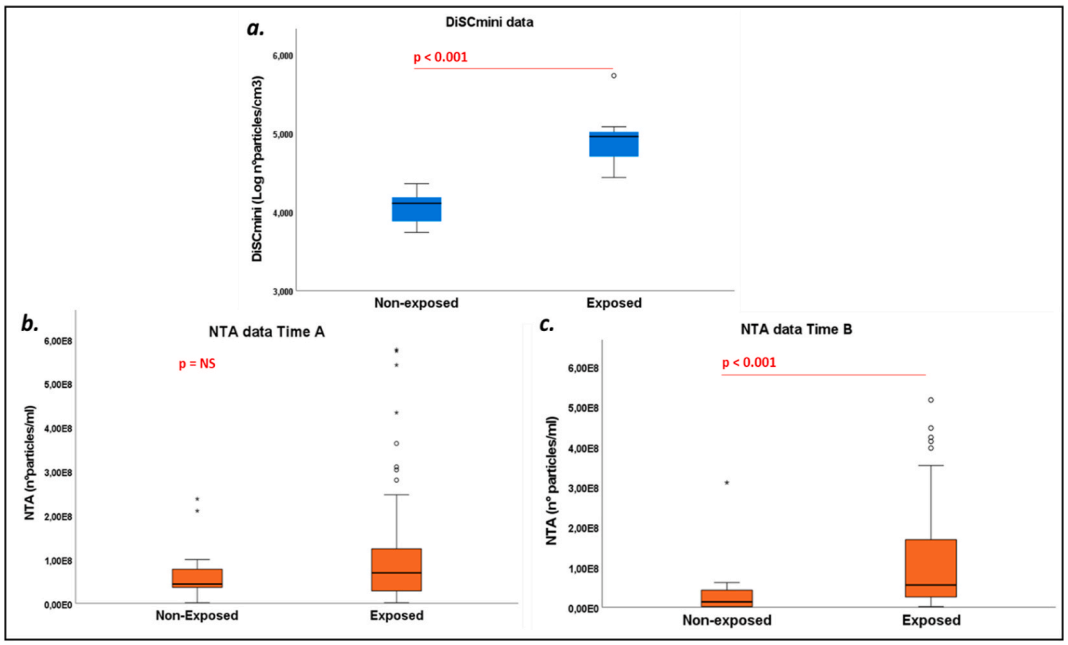

Fig. 1. DiSCmini™ (Testo, DE) data expressed as logarithmic mean of exposed and non-exposed subjects (part a.); Part b illustrates the NTA data expressed as the average particle number concentration of exposed and non-exposed subjects at the beginning of the week (Time A) and at the end (Time B). Blue = DISCmini data; Orange = NTA data). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 1, a).

The NTA data were consistent with the external exposure data. Particle number quantified by NTA revealed a tendency towards higher values, though not statistically significant, in the exposed subgroup at the beginning of the working week (Fig. 1, b) and a statistically significant difference between NE and the whole group of exposed at the end of the working week (Fig. 1, c).

Both HE and LE subgroups showed significantly higher external exposure values as compared to the NE ($p < 0.001$; $p = 0.004$, respectively). Moreover, HE demonstrated a significantly higher concentration than LE ($p < 0.001$). (Fig. 2, a).

This categorization applied to the NTA data in EBC revealed that subjects belonging to the HE and LE had a significantly higher number of particles than NE ($p = 0.007$ and $p < 0.001$, respectively). However, and unexpectedly, the median value was higher in LE subjects than in HE subjects.

As a whole, a higher concentration of airborne particles at workplace is consistently associated with a greater number of particles in the EBC of exposed subjects as compared to NE subjects.

Considering the epidemiological sample, a positive and significant correlation was apparent between airborne particle number and number of particles measured by NTA in EBC (Rho = 0.263; $p = 0.019$), showed a relatively low Rho correlation coefficient, though significant (Fig. 3). The number of particles measured by NTA in EBC was also consistently associated with LDSA (Rho = 0.288, $p = 0.009$). The association between the number of particles to which workers are exposed and their presence in exhaled breath appeared weak but statistically significant.

As revealed by the part a. of Fig. 4, the correlation between IL-1β and NTA data, was statistically significant (Rho = 0.283; $p = 0.012$). The part b. of Fig. 4 shows the correlation between NTA data and IL-10 levels, indicating a weakly positive relationship (Rho = 0.239; $p = 0.035$). Lastly, the part c. of Fig. 4 suggests a positive trend between particle count and TNF-α data but without reaching a significant level.

To further explore any relationships between DiSCmini™ (Testo, DE) and NTA data, it was chosen to aggregate the data according to the different materials produced i.e. aggregating by companies recruited. Statistically significant differences in particle concentration between workers exposed to different occupational settings (A, B, and C) and

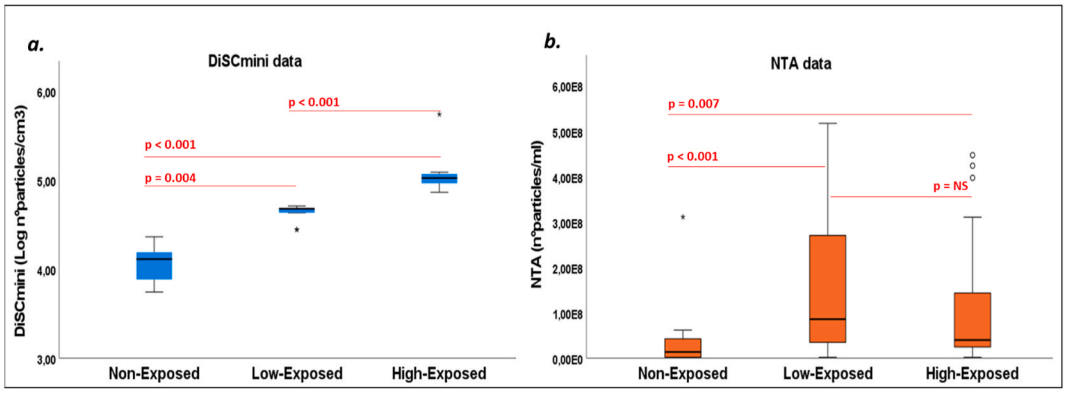

Fig. 2. DiSCmini™ (Testo, DE) data expressed as Log mean divided into NE, LE, and HE (*a.*); NTA data expressed as mean particle number concentration of NE, LE, and HE subjects (*b.*); Blue = DISCmini data; Orange = NTA data). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

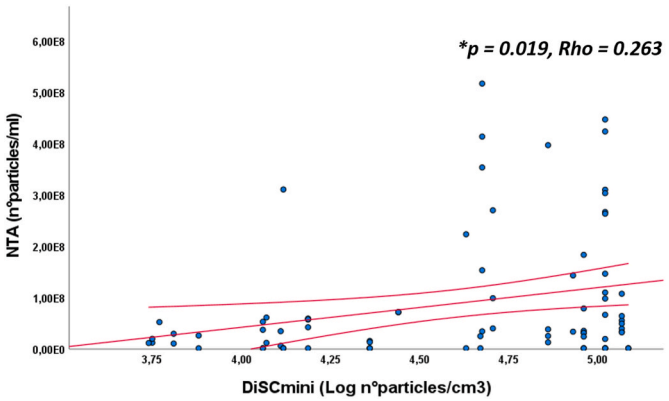

Fig. 3. Correlation between DiSCmini™ (Testo, DE) data and NTA data.

subjects recruited as controls (NE) were observed (Fig. 5, a; $p < 0.001$). When examining the panel b. of Fig. 5, the distributions of NTA across different locations were positively and significantly different in NE subgroup as compared to the exposed workers from company A ($p < 0.009$) and company B ($p < 0.036$) but not significantly different with company A. It is worth mentioning that, whereas the companies are located in different geographical areas, they produced different materials, different amount of dusty materials as well as they undertook different types of processes, workers belonging to the companies A and B resulting more exposed to particulate matter as compared to those working in company C. These results demonstrate that an increased presence of airborne particles can also be found at the airway level (NTA), even if this finding is not observed in the workers of company C.

4. Discussion

Exposure assessment for nanomaterials has dramatically improved over the last years, now relying on innovative industrial hygiene approaches, new devices for sampling in the breathing zone of workers and new personal monitors translating the aerosol characteristics in relevant metrics. Devices such as DiSCmini™ (Testo, DE), in addition to providing the PNC (particle number concentration) of an aerosol, also provide the LDSA, which is the surface area of particles deposited in the lungs. The latter has been recognized as a more accurate metric for understanding the toxicity of nanoparticles compared to the more commonly used particle mass concentration. LDSA concentrations can be obtained through direct measurements or calculations based on empirical lung deposition models and measurements of the particle size distribution, with the unit of measurement being $\mu m^2/cm^3$. However, it is important to note that neither LDSA measurements nor size distribution measurements are mandatory or regulated by governmental authorities (Fung et al., 2022). Nevertheless, LDSA has been suggested as a crucial predictor for health outcomes resulting from aerosol exposure, particularly for low- and poorly soluble spherical NPs, as it stands

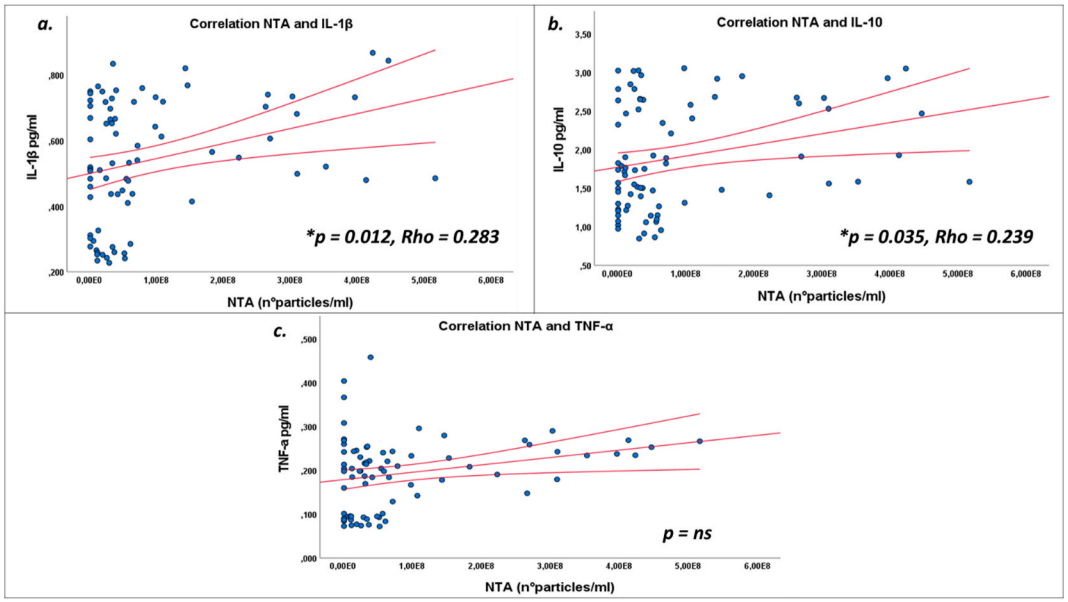

Fig. 4. Correlation graphs between IL-1β (*a.*), IL-10 (*b.*), TNF-α (*c.*) and NTA.

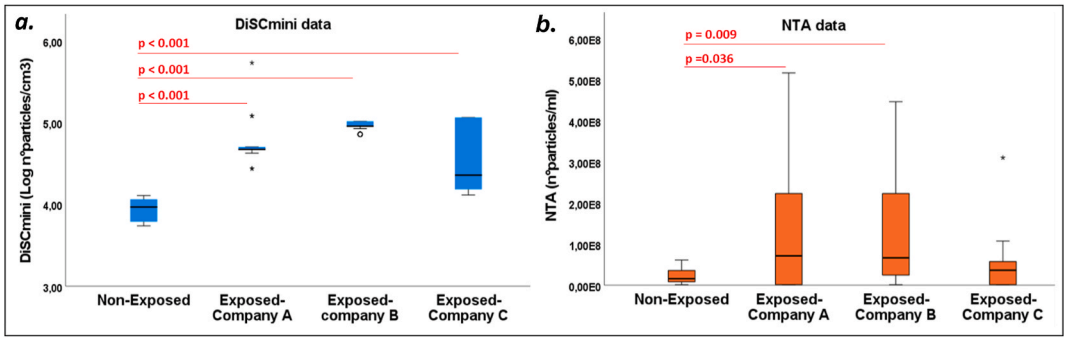

Fig. 5. DiSCmini™ (Testo, DE) data (*a.*) and NTA data (*b.*) analysed *per* company.

out as one of the most effective dose metric for acute pulmonary inflammation (Schmid and Stoeger, 2016).

However, whereas the characterization of external exposure has been improved, there is the need to assess both the internal dose and possible effects at the target organ. Exposure, i.e. the contact between a foreign substance and the body surface to a given chemical, usually results in uptake and leads to an internal dose. For traditional chemicals, the internal dose is usually assessed by both the amount of the substance and/or its metabolites or as a product of interaction with biomolecules in biological fluids. This definition of exposure biomarker cannot simply apply to nanomaterials (Bergamaschi et al., 2017). Available biokinetic data suggest that translocation rates of nanoparticles from the portal-of-entry - the respiratory tract - to secondary organs, is size- and charge-dependent (Choi et al., 2010), but the amount of particles reaching the systemic circulation is actually very low (Kreyling et al., 2002, 2009). As a result, the quantification of particles in biological matrices from lung airways can be regarded as a complementary approach to the definition of exposure and related local effects (Bergamaschi et al., 2017; Marie-Desvergne et al., 2022; Forest et al., 2021; Forest and Pourchez, 2023).

Using light scattering, the NTA technique can detect particles in liquid matrices providing their number-based concentration (Filipe et al., 2010). Therefore, the quantification of breathed particles based can allow an esteem of the dose deposited in the lungs (Sauvain et al., 2017; McCormick et al., 2021). Several studies are strongly focused on the diagnosis of lung disease (asthma, silicosis, asbestosis, etc.) carried out observing the relationship between exposure to particulate through the respiratory route and increased levels of inflammatory cytokine (Greenberg et al., 2007; Leung et al., 2013; Bhattacharjee et al., 2016).

Various investigations have established a correlation between the number of particles in the air and various health-related indicators, such as particle number concentration and certain biomarkers. Consequently, this metric proves promising for assessing potential health risks associated with particle exposure in both environmental and occupational settings (Chang et al., 2022; Lepistö et al., 2022).

Cytokines play a primary role in the inflammatory process, and their analysis in non-invasive matrices such as EBC is optimal for occupational sampling (Ghelli et al., 2022). Therefore, combining the measurement of particle number in the EBC using NTA, to an analysis of the inflammatory spectrum in the same matrix, could aid in assessing particle retention in the lungs, bridging the gap from exposure to inflammation, and playing a crucial role in primary prevention in occupational settings (Sauvain et al., 2014; Gubala et al., 2018).

In our study, involving a relevant number of workers from three different exposure scenarios, the NTA data were consistent with the external exposure data. Particle number quantified by NTA revealed a tendency towards higher values, though not statistically significant, in the exposed subgroup at the beginning of the working week and a statistically significant difference between NE and the whole group of exposed at the end of the working week. This probably occurred because at the beginning of the week the accumulation process has just begun while, at the end of the working week, this process has progressively occurred, allowing evidence of accumulation during the working week among the workers exposed to particle.

The categorization applied to the NTA data in EBC revealed that subjects belonging to the HE and LE had a significantly higher number of particles than NE. However, and unexpectedly, the median value was higher in LE subjects than in HE subjects. The LE workers are white collars or technical employees working in the same companies. This suggests that workers directly involved in operations with dusty materials, but wearing personal protective equipment (FFP2) are more protected than workers less or not directly involved (LE) who are not used to wear personal protective equipment (PPE), with the likelihood to result more exposed by inhalation.

This study shows that a higher concentration of airborne particles at workplace is consistently associated with a greater number of particles in the EBC of exposed subjects as compared to NE subjects. In the study by Hemmendinger et al. there were demonstrated strong positive correlations between the airborne particle count, defined by LDSA, and inflammatory cytokines in EBC (Hemmendinger et al., 2023).

As revealed by Fig. 4, the correlation between IL-1β and NTA data was statistically significant, whereas the correlation between NTA data and IL-10 levels was weak (Rho = 0.239; p = 0.035). Finally, a positive trend between particle count and TNF-α data was observed, though not statistically significant.

Both IL-1β and IL-10 are cytokines, with the former possessing pro-inflammatory properties and the latter acting as an anti-inflammatory agent. Through a negative feedback mechanism, IL-10 helps regulate the synthesis of cytokines, achieving a balance. Additionally, C-reactive protein (CRP), produced by the liver, is employed to identify systemic inflammatory states (Sproston and Ashworth, 2018). Even so, our results reinforce the hypothesis that the number of particles in the EBC is representative of environmental exposure and is associated with and increased level of inflammatory mediators.

It is worth mentioning that, whereas the companies are located in different geographical areas, they produced different materials, different amounts of dusty materials as well as they undertook different types of processes, workers belonging to the companies A and B resulting more exposed to particulate matter as compared to those working in company C. Company C is mainly involved in the research and development of NMs, while the other facilities are involved in the production of paints, adhesives, coating materials, and construction materials. Workers belonging to the companies A and B result more exposed to particulate matter as compared to those working in company C. Company C presents just 7 subjects on 20 categorized as exposed, while the other company workers were all part of the exposed category (Table 1). This discrepancy is undoubtedly influenced by the specific processing activities conducted in these companies and the nature and quantity of materials handled. Although DiSCmini™ (Testo, DE) data of company C result significantly higher than the NE subjects (Fig. 5, a), the distribution of NTA in company C subjects exhibits comparable levels to those of NE subjects (Fig. 5, b). The partial discrepancy between DiSCmini™ (Testo, DE) data and NTA in company C can be explained by the overall less exposure, the different materials handled, different working procedures and/or more careful use of PPE. This result further highlights the importance of the determination of internal dose biomarker.

As recently observed by Luo and co-workers (Luo et al., 2022), there are still some shortcomings about the use of EBC as suitable matrix for biomonitoring purposes. However, our study shows a significant increase in the number of airborne-derived particles in EBC of the exposed subjects, which is suggestive of a higher deposition of a portion of these particles in their airways. The demonstrated concordance between the environmental and the biological measures, is consistent with other studies which reported an increased number of particles in workers exposed to silica when compared to controls (Sauvain et al., 2017; Hemmendinger et al., 2023).

Furthermore, the burden of deposited particles in the airways is associated with an increased cytokine inflammation.

Therefore, in order to better understand the actual exposure of workers, non-invasive methodologies can be used to improve workers' compliance with biological sampling.

Only a few studies have used EBC to investigate the inflammatory profile in workers. Workers exposed to nanocomposites demonstrated through their EBC samples, an increase of concentrations in biomarkers associated with oxidative stress and inflammation. Several of these biomarkers showed significant changes, although the analysis did not include the quantification of the number of particles present in the samples. Another study revealed an increase in leukotrienes, both at the beginning and end of the work shift, while a follow-up study conducted two years later detected a significant increase in certain cytokines such as IL-4, IL-10, IL-13, and TNF-α after exposure to nanomaterials (Pelclova et al., 2017, 2020). In the EBC samples, in addition to the NTA

analysis, was also measured the inflammatory profile, in particular interleukins 1β, IL-10, and TNF-α.

The exposed subjects exhibited significantly higher levels of all three cytokines when compared to the NE group. Similar trends were also highlighted in the serum in a study conducted by Ursini, which reported an increase in IL-6, IL-8, and TNF-α levels in workers exposed to nanomaterials such as graphene (Hunt, 2002; Ursini et al., 2021). Furthermore, positive correlations were observed between NTA measured in EBC and two of the three inflammatory cytokines: IL-1β, IL-10. Instead, no correlation was found comparing TNF-α and particles measured in EBC with NTA, but only an increasing trend with increasing exposure resulted. A similar answer of TNF-α was found in pathological subjects, in controls after exposure to airborne particulate (PM) (Ghozikali et al., 2022) and in subjects exposed to titanium dioxide. An increase inflammatory levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α in plasma was observed as a result of professional exposure (Zhao et al., 2018). To the best of our knowledge, our study represents the initial attempt to quantify the particles in the air analysed using DiSCmini™ (Testo, DE) and compare them with those found in the exhaled breath of a multicenter cohort of workers exposed to nanomaterials, incorporating inflammatory biomarkers. Our study aims to provide a starting point for the identification of an internal dose marker that can reflect the actual uptake of particles present in the workplace environment.

However, this study presents some limitations, such as the small size of the epidemiological sample which would be useful to expand to acquire greater statistical power and, at the same time, to challenge the above approach against different occupational. Furthermore, an important future perspective will be to discriminate between inorganic and organic particles, to provide an even more accurate measure of particle uptake by the subjects.

5. Conclusions

In recent years, the advancement of technology has result in an increasing use of NMs across various industrial and technological sectors. This trend has raised concerns in the scientific community about the toxicological properties of these substances and possible short- and long-term health effects. Consequently, there is a pressing need for a multidisciplinary approach that integrates exposure assessment and quantification of non-invasive biological markers in specific matrices (Schulte et al., 2018; Bergamaschi et al., 2017). Identifying suitable biomarkers reflecting actual exposure to these substances is crucial. This study confirms previous studies and represents a further step in demonstrating the reliability of the analysis of particles in EBC to quantify the number of particles present (using NTA) in subjects recruited from an international multicentre study. The health significance of such internal dose is reinforced by the association with an inflammatory profile analysed in the same matrix. In conclusion, the use of NTA as a tool to investigate the internal dose, integrating the assessment of external exposure, with the inflammatory profile, represents a valid starting point for assessing the fraction of unabsorbed particles that could increase the levels of airways inflammation. Exposure assessment combined with biomonitoring seems the most useful tool for identifying the causal relationships and the potential risks that workers can be exposed (Bergamaschi et al., 2015; Hemmendinger et al., 2023). Further investigations will be needed on a larger sample of workers in diverse company settings.

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CRediT authorship contribution statement

Marco Panizzolo: Writing – original draft, Investigation, Formal analysis, Data curation. **Francesco Barbero:** Writing – original draft, Investigation, Formal analysis, Data curation. **Federica Ghelli:** Writing – review & editing, Visualization, Methodology, Data curation. **Giacomo Garzaro:** Validation, Visualization, Writing – review & editing. **Valeria Bellisario:** Validation, Visualization, Writing – review & editing. **Irina Guseva Canu:** Writing – review & editing, Visualization. **Ivana Fenoglio:** Writing – review & editing, Visualization, Conceptualization. **Enrico Bergamaschi:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Roberto Bono:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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