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Coordinatrice Prof.ssa Triassi

"OCCUPATIONAL EXPOSURE TO ULTRAFINE PARTICLES AND ENGINEERED NANOPARTICLES"

Relatore

Chiar.mo Prof. **Ivo Iavicoli**

Candidato

Dott. **Mauro Fedele**

Introduction

Nanomaterials

Nanomaterials are chemicals or materials composed of particles with at least one size between 1 and 100 nanometers. The European Commission in 2011 proposed the following definition of nanomaterials as a "natural material, derived or manufactured containing particles in the free state, aggregated or agglomerated, and in which, for at least 50% of the particles in the numerical dimensional distribution, one or more external dimensions are between 1 nm and 100 nm. In specific cases, and where concerns for the environment, health, safety and competitiveness justify it, the threshold of 50% of the numerical size distribution can be replaced by a threshold between 1% and 50%" (CE: 2011/696 / EU).

Between 2013 and 2021, the Commission carried out a review of the above mentioned Recommendation, addressing the objective, scope, clarity and use of its definition of nanomaterial. The review in particular focused on whether the particle number-based size distribution threshold of 50% should be increased or decreased and whether to include materials with internal structure or surface structure in the nanoscale such as complex nanocomponent materials including nanoporous and nanocomposite materials that may be used in specific sectors. Indeed, in 2022, the European Commission adopted the following recommendation (2022/C 229/01): "'Nanomaterial' means 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 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."

Nanomaterials occur in nature, may be an incidental product of human activity, e.g. welding fumes, (incidental nanoparticles), or deliberately manufactured and engineered to exhibit novel characteristics such as increased strength, chemical reactivity or conductivity compared to the same material without nanoscale features (engineered nanoparticles).

Incidental nanomaterials

Incidental nanomaterials or “ultrafine particles” are unintentionally produced through direct or indirect human influences or anthropogenic (e.g., mechanical or industrial) processes, such as vehicle exhaust gases, welding gases, solid fuel heating (home heathers), and combustion during cooking (Barhoum et al. 2022).

Incidental nanoparticle exposure in welding activities

Welding activities may characterize a possible source of incidental nanoparticle occupational exposure. Welding is used to join metal pieces by intense heat where consumable electrodes are frequently applied to improve the assembly of the larger parts. Welding fume is a complex mixture of metals, gases, and other compounds. In addition, it comprises very small particles, including ultrafine matter (Berlinger et al., 2011). Fumes, in fact, consist of predominantly fine solid particles with an aerodynamic diameter of less than 1 μm and are a complex mixture of particles from the wire or electrode, base metal, or any coatings on the base metal. They consist mainly of metal oxides, silicates, and fluorides (IARC Monograph 118, 2018). Particularly, a great proportion (up to 63%) of the total particles emitted during welding operations has been demonstrated to be in the nano size range (Iavicoli et al. 2013). From a qualitative perspective, these are generally characterized by metal complexes, with Fe, Cr and Ni being the most common metals present (Antonini et al., 2013; Newton et al. 2021). Nanoparticles type and concentrations are strongly influenced by the welding materials, operative techniques and measures adopted to control the emission source. Concerning possible health effects induced by welding particle exposure, inflammation, lung defence suppression, oxidative stress, DNA damage, and genotoxic effects were observed after exposure to both mild and stainless-steel welding fumes in cellular and animal models (Riccelli et al., 2020; Présumé et al. 2016). The international Agency for Research on Cancer (IARC) classified welding fumes as carcinogenic to humans (Group 1). As for all particles, the toxicological profile of welding fumes is not only dependent on the material and concentration, but also on the particle size distribution and surface characteristics (IARC Monograph 118, 2018).

Area samplings carried out during welding activities performed at 33 German companies in the period between May 2007 and October 2009 determined a median concentration of particles with a diameter between 14 and 673 nm of 120,000

particles/cm^3. The Tungsten Inert Gas welding technique was found to be responsible for the emission of particles with a smaller aerodynamic diameter, <100 nm, while other types of welding, including Gas Metal Arc welding induced the production of agglomerates with a diameter greater than 100 nm (Lehnert et a coll. 2012). A survey conducted by Iavicoli et al. (2013) in an engineering industry, demonstrated that ultrafine particles characterized more than half of the total particles dispersed during welding activities with a numerical concentration of particles, with a diameter between 7 and 94 nm, greater than 60,000 particles/cm^3. This occurred on days when large metal pieces were welded, thus impossible to work on the counter and welded in the center of the room by several operators simultaneously, with a mobile dust extraction system that did not guarantee adequate containment of particulate dispersion. In relation to the role of that the welding technique may have on the emission of ultrafine particulate matter, Brand et al. (2013) have shown how the metal inert gas (MIG) and the metal active gas (MAG) techniques produce particles that tend to agglomerate and reach dimensions greater than 100 nm with a percentage of 10-15% of particles with a < 50 nm in diameter. A more recent study (Adamec and coll. 2020), evaluated the number concentration of fine and ultrafine particles produced during various work phases performed with and without ventilation, as well as during the break from work. The concentration of the ultrafine particles was lower before starting the welding activity (about 7000 particles/cm^3) with a significant increase at the beginning of the welding process. The concentration of ultrafine particles during welding with the ventilation systems in operation was around 13500 particles/cm^3. When these systems were switched off the concentration of the particles showed a constant increase, up to ten times the concentration determined during the work with active exhaust ventilation systems on. Even during the break, when ventilation was not functioning, the concentration of particles was particularly high. In relation to the aerodynamic diameter of the particles, only a small proportion had a diameter greater than 800 nm, while most of the particles had an aerodynamic diameter <300 nm. Qualitative scanning electron microscope analysis highlighted the presence of iron, silica and manganese particles in line with the technique used and the composition of the processed pieces.

Engineered nanomaterials

Hundreds of products containing nanomaterials are already in use. Examples are novel sensors, medical devices, batteries, coatings and anti-bacterial clothing. Around 1.5% of all new cosmetic products notified in Europe contain nanomaterials. Analysts expect markets to grow considerably in the near future. Nano- innovation is seen in many sectors including public health, employment and occupational safety and health, information society, industry, innovation, environment, energy, transport, security and space. Due to a larger specific surface area per volume, nanomaterials can have different characteristics than the same material without the nanoscale characteristics. Consequently, the physicochemical properties of nanomaterials may differ from those of bulk substances or larger particles (ECHA, 2020):

- Chemical properties: the high surface/volume ratio of nanomaterials makes them highly reactive, increasing their effectiveness as catalysts in certain chemical reactions;
- Electrical properties: increased electrical conductivity in ceramics and magnetic compounds; increased electrical resistance in metals;
- Mechanical properties: greater hardness and resistance of metals and alloys, ductility and plasticity of ceramics;
- Optical Properties: Increased conversion efficiency of light energy into electrical in photoelectric devices;
- Biological Properties: Increased permeability through biological barriers (membranes, blood brain barrier, placenta).

Physico-chemical properties

Nanomaterials can differ in a number of aspects, including the dimensional range, the chemical composition, the shape, the crystalline structure, the surface reactivity, the state of agglomeration. Based on the chemical composition, they are classified into carbonaceous and non-carbonaceous. Carbonaceous include fullerenes, carbon nanotubes, and carbon black. Nanotubes are tubular structures having a tube diameter ranging from a few nanometers to a few tens of nanometers. Carbon nanotubes consist solely of carbon atoms arranged on a single graphite sheet wrapped around a cylindrical axis. Carbon black, produced by the incomplete combustion of petroleum products, is widely used in the automotive industry. Among non-carbonaceous nanomaterials, metal-based or metal-oxide-based nanoparticles are included. Their use is very wide, among the most used are: aluminum and zinc oxides, silicon and titanium dioxide, silver

and iron nanoparticles. Nanodots are crystalline structures of metal nanoparticles; when these crystalline structures come into contact with water or other dispersions, they can change their organization (INAIL, 2011).

The nanoparticle characteristics that can influence their toxicological profile also include the crystalline structure. In particular, as regards titanium dioxide, evidences show that only in the mineral form of anatase, the nanoparticles exhibit a marked secondary toxic activity due to the high catalytic activity of this form (Sayes et al. 2006). It is important, however, to underline that the crystalline structure is susceptible to variation in relation to the interaction with biogical fluids and tissues (Zhang et al. 2003). The shape of nanomaterials is onother parameter that can influence their toxicological and toxicodynamic behavior. In particular, with regard to titanium dioxide nanoparticles,"belts" or nanofibers (Allegri et al. 2016), rather than the particle form, are associated with greater cytotoxic and pro-inflammatory effects (Hamilton et al. 2009; Xia et al. . 2013).

The development of weak bonds such as Van der Waals forces and electrostatic attractions allow the arrangement of particles in *agglomerates*, i.e. nuclei of particles that retain their spatial individuality, while being affected by the attraction exerted by forces from neighboring nuclei. Very stable forces are instead at the basis of the spatial arrangement of the particles in *aggregates*, a single heterogeneous nucleus within which we find different nanoparticles strongly bound or fused. The state of agglomeration/aggregation is fundamental in the toxicokinetic evaluation of nanoparticles, influencing their deposition capacity as a function of changes in the aero-dynamic diameter and surface area. This aspect is fundamental as it influences the biological reactivity of the nanomaterials themselves (Nel et al. 2006; Yang et al. 2008). The bio-molecular characteristics of the medium in which the nanoparticles are found or with which they interact, including the pH and the various biological components dissolved in the medium, can influence the surface coating of the nanoparticles, resulting in the acquisition of different physicochemical properties compared to those of the particle in its primary form (Christian et al. 2008). In fact, once adsorbed nanomaterials interact with the molecular components of the biological fluids, in particular with proteins, which therefore can completely coat the substance creating a "protein crown", thus changing thei r biological reactivity (Monopoli et al. 2012). It is possible that the effects of different types of nanoparticles are partly mediated by the type of coating they acquire in the organism. The relationship between the physic-

chemical characteristics of a substance and its toxicokinetic properties is therefore at the basis of the type of interaction with biological systems (Aillon et al. 2009; Bernstein et al. 2005; Utembe et al. 2015).

Uses and commercial sectors

The sectors in which the nanotechnological approach has already been launched for some production processes are: health, information technology, materials science, manufacturing industry, energy, safety, aerospace science, optics, acoustics, chemistry, food and the environment. Based on the products already developed and the potential that can be glimpsed, it is realistic to say that nanotechnologies will significantly improve the quality of life, the competitiveness of the manufacturing industry and sustainable development.

Among the sectors in which nanomaterials have found their greatest use is certainly the electronics sector. The excellent thermoelectric properties of carbon nanotubes have allowed them to be used in the construction of flat screen monitors and capacitors and batteries (McCarthy et al. 2011). Furthermore, these nanomaterials, together with TiO_2 and ZnO nanoparticles, are used in photovoltaic plants (Saito et al. 2008; Chaar et al. 2011; Bakhshayesh et al. 2013). For the production of solar panels, nanomaterials based on silicon, metal oxides and more recently graphene have been used (Casaluci et al. 2016; Ding et al. 2015).

The uses in the textile industry of nanoparticles, nanocomposites, nanocapsules, nanospheres and nanostructures make it possible to give traditional textiles special features such as antibacterial properties, water repellency, dirt resistance, antistaticity, non-flammability, anti-IR characteristics, dyeability, mechanical resistance. Examples of applications of nanomaterials and their properties in the textile sector include (Dubas et al. 2006; Zhang et al. 2009; Sawhney et al. 2008; Bae et al. 2009):

- Nano-silver: antimicrobial property;
- Titanium dioxide: resistance to UV rays;
- Amorphous silica: anti-stain action;
- Aluminum oxide: abrasion resistance;
- Carbon nanotubes: abrasion and tear resistance and flexibility.

One sector in which nanomaterials are widely used is that of cosmetics. Currently, the nanomaterials actually used in cosmetics in Europe are represented by some mineral oxides used as sunscreens or pigments and nanoparticles (for example silicon) used in

some toothpastes. Titanium dioxide and zinc oxide nanoparticles, thanks to their photo-protective properties, are widely used in anti-UV skin protection creams (Popov et al. 2005; Dréno et al. 2019). The advantages provided by these nanomaterials are low density compared to traditional creams and the absence of sensitization or irritation phenomena (Antoniou et al. 2008). Furthermore, they do not penetrate the skin barrier. Hydroxyapatite, on the other hand, is used in modern toothpastes for its remodeling and restorative effect on tooth enamel, as well as inhibiting the process of caries development (Najibfard et al. 2011). Regulation (EC) no. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products was the first regulatory act to provide specific provisions for nanomaterials, ensuring a high level of human health protection for each cosmetic product containing nanomaterials.

The application of nanomaterials in the field of medicine and related sciences deserves a separate discussion. The most evident advantages of using substances in nanoparticle form are represented by the more precise and safe administration of therapies, the use of new diagnostic techniques, the application in the field of prosthetic processes, the microbicidal activity of some substances on a scale nanometer. There are essentially two lines of research and applications from which the greatest advantages of nanomedicine are expected: nanodiagnostics and nanotherapy (intelligent drug delivery).

In this sector, nanoparticles can act as carriers are: liposomes, polymer carriers, dendrimers. Liposomes allow substances to reach the target site quickly and in an elective manner; the direct consequence of this selective action is the reduction of the drug concentration to obtain the same effect. A major benefit is therefore the reduction of side effects (Kraft et al. 2014; Mody et al. 2014). In the diagnostic field, nanomaterials can be used as a contrast medium together with gadolinium to improve the accuracy of MRI, or in ultrasound with contrast medium (CEUS) forming nanobubbles for the identification of tumor pathologies. Carbon nanotubes can be used as biomarkers for the detection of cancer cells. The use of gold nanoparticles, during brachyro-radiotherapy cycles, has so far given excellent results as it has been shown that a more homogeneous and precise distribution of the radioactive dose is obtained within the tumor mass (Laprise-Pelletier et al. 2018). Among the properties of silver in nanometric dimensions, the antibacterial activity stands out (Kim et al. 2007). Silver also exhibits antibacterial activity in association with antibiotics. The antimicrobial function of silver has also been demonstrated on some viruses, including respiratory

syncytial virus, Herpes Virus, monkeypox virus, HIV type 1 and hepatitis B virus (Taylor et al. 2005; Lu et al. 2008; Rogers et al. 2008; Baram-Pinto et al. 2009). This action would be related both to the release of ionic species and to the specific inhibition of some viral enzymes (Taylor et al. 2005; Lara et al. 2010).

Nanomaterials find another sector of use in the production, conservation and packaging of food (Morones et al. 2005). Titanium dioxide and graphene oxide, assembled in structures of the polymeric structure, are used to avoid the deterioration of foods (Xu et al. 2016). They are therefore used in the composition of the surface of the films that are used for food preservation; meat, fruit and vegetables. Silver can also be stored in polymeric structures dedicated to conservation (Addo Ntim et al. 2015; Metak et al. 2015). It would seem that some nanomaterials can increase the bioavailability of some nutrients present in foods such as vitamins, coenzymes, carotenoids, fatty acids, calcium (Joye et al. 2014; Oehlke et al. 2014; Katouzian et al. 2016; Salvia-Trujillo et al. 2016). The use of nanomaterials in the production of food containers also brings the advantage of providing greater mechanical, thermal and UV exposure resistance.

As far as the construction sector is concerned, reasons for the diffusion of nanomaterials concerned the benefits brought to the more rational use of resources, to the reduction of costs in the life cycle of products, to the production of new materials with high performance levels, to the improvement of efficiency. and the durability of the products. In particular, nanomaterials are used in paints and coatings, for example, to improve durability and impart new functionality. Among these, the ease of cleaning thanks to the stain and water repellent properties, the resistance to microbes or scratches. Currently the most popular nanomaterials in paints and coatings are titanium dioxide and silicon dioxide nanoparticles. The former are used in coatings for their photocatalytic activity which makes surfaces self-cleaning. The addition of amorphous synthetic silica can improve the solidity and resistance of the paint to abrasion, scratching and atmospheric agents. In hospital construction, the application of silver nanoparticles has been widely considered for its inhibiting action on bacterial growth (Gunell et al. 2017).

The use of nanomaterials in the building sector has led to a number of improvements in terms of thermal insulation. Nanomaterials are used above all with the function of insulating structures from the thermal point of view and fortifying the materials used for construction. Silicon dioxide and titanium dioxide are also used in the building sector: the first guarantees greater mechanical resistance; the second is used to increase the

self-cleaning and purification properties of the air with which the cement comes into contact (Folli et al. 2012; Bossa et al. 2016). Calcium carbonate and nano-clays are also used to increase resistance (Kawashima et al. 2013), while carbon nanotubes include electrical resistance and resistance to compressive and bending stimuli among their main characteristics (Mendes et al. 2015). The woodworking sector has benefited enormously from the introduction of nanoparticles in the various processing and preservation processes, in particular in the use of amorphous silica, titanium dioxide, zirconium and silicon, given their ability to give wood fireproof and hydrophobic properties, (Chang et al. 2015; Bueno et al. 2014)

In the chemical field, nano-sized metal nanoparticles are excellent catalysts as they can be reused in multiple reactive cycles, shorten reaction times, and are also less expensive than non-nano-sized particles. To guarantee the excellent catalytic properties of the nanoparticles is the presence of free atomic species on the surface with respect to the total number of atoms, a characteristic that increases the reactivity of the compound (Navalòn et al. 2016). Palladium and gold nanoparticles are used in the chemical sector for their ability to promote hydrogenation reactions and the formation of bonds between carbon atoms. Another very important function of palladium and gold in nanoparticles is the facilitation of the oxidative decomposition processes of the dioxin produced by the combustion of waste incinerators.

The agricultural sector has seen, in recent years, a significant increase in the use of nanomaterials due to the high versatility of the substances used in nanometric dimensions. The fields of application in which nanotechnologies and nanomaterials are used in agriculture are many, including plant protection, soil and water treatment, and diagnostics (Iavicoli et al. 2017).

The automotive industry is one of the main sectors for the use of nanomaterials. In fact, metallic nanoparticles enter the composition of every component, mechanical, electrical and coating of the car. The use of nanofluids in the composition of the brakes increases resistance and reduces their wear; copper and aluminum oxides reduce brake friction (Kao et al. 2007). The addition of silicon dioxide nanoparticles, carbon black and multi-walled carbon nanotubes in the rubber compound increases the mechanical tensile strength and reduces tire wear (Giftson et al. 2014); it allows the mixture to acquire particular chemical-physical characteristics and allows to create specific profiles for the rubbers. Silver nanoparticles make up the filters that clean the air entering the passenger compartment from the outside, while titanium dioxide nanofibers, together

with platinum nanoparticles, reduce emissions from the car's exhaust systems (Asmatulu et al. 2013)

Engineered nanoparticle exposure: non exhaustive examples

Although an internationally joined consensus on the strategies to evaluate the environmental exposure to nanomaterials has not been reached, different studies are currently available in literature that attempted to investigate such exposure in variable occupational contexts.

Few-Layers Graphene (FLG) and engineered amorphous silica nanoparticles (SiO_2NPs) are the object of two case studies of the research project 'NanoKey' that integrated the exposure assessment through personal measurements and sampling in the workplace, with the biomonitoring of exposed workers (Boccuni et al. 2020). Values of particle number concentration and lung deposited surface area within the FLG production resulted higher than the background far field (FF), even if comparable to the near field (NF). Concerning the particle dimensions, these resulted higher during the production than the particle dimensions found at the NF background but always lower than the FF values. During the SiO_2NPs production, the personal breathing zone values showed levels of particle number concentration and lung deposited surface area were higher than the background with a confrmed presence of rare silica nanoparticles.

The personal exposure to multi-walled carbon nanotubes during their synthesis and handling in a commercial production facility was assessed by Kuijpers et al. (2016). Exposure levels of multi-walled carbon nanotubes observed in the production area during the full scale synthesis of multi-walled carbon nanotubes were comparable to levels observed during further handling of these nanomaterials. In the research and development area and the office, exposure levels of MWCNTs were significantly lower. Maintenance of the reactor, and powder conditioning were associated with higher exposure levels in the production area, whereas increased exposure levels in the research and development area were related to handling of multi-walled carbon nanotubes powder. Industrial packaging and bagging activities often released large nanomaterial particle agglomerates into the air. Physical and chemical synthesis were associated with potential releases of airborne engineered nanoparticles in smaller sizes. No significant release was observed in comparison to background levels during the experimental-scale production of nanofibers, pyrolysis production of TiO2, chemical vapor deposition growth of CNTs and MWC-NTs, and synthesis of Ag by mixing

sodium citrate with silver nitrate (Ding et al. 2017). In contrast, one study showed significant at-source releases from the chemical vapor deposition production of CNTs at sizes below 100 nm (probably carbonaceous by-products) and from 7 to 200 nm; using higher injection temperatures released more particles of reduced diameters (from 20–200 to 7–90 nm) (Ding et al. 2017). Synthesis of TiO2 generated noticeable particle concentrations with a bimodal distribution (<30 nm and 70–100 nm), whereas induced-coupled-plasma production of Ag resulted in significant releasesin the 20–30 nm range. A study of our group employed the personal monitoring to assess the exposure of workers employed in the laboratories of one of the most important centers dedicated to the research and development of nanoscale engineered materials in Italy (Iavicoli et al. 2018). In this center, the production and / or use of nanomaterials was carried out for the sole purpose of research and generally consisted in the use of relatively small quantities of nanoscale materials. However, some work phases, including the opening of the reactors for loading and unloading of materials and for ordinary cleaning and maintenance activities, could pose a risk to the involved workers. Personal monitoring was performed at the following sampling sites: the workplace monitoring in the following sampling sites: (i) the graphene synthesis facility, (ii) the semiconductor nano- wires laboratory, (iii) the Transmission Electron Microscopy (TEM) facility, (iv) the lyophilization laboratory and (v) the NP synthesis laboratory. Sampling was carried out using direct reading instruments (DiscMini; Partector; Personal Ultrafine Particle Counter (PUFP)) and personal samplers that made it possible to evaluate both the particle count and the surface area of the nanoparticles. In the aforementioned research laboratories, the particle numbers and surface area concentrations measured during workplace monitoring were found to be quite low. The only conditions in which the levels of nanoparticles showed an increase, although limited, compared to the background levels were during the cleaning process of the different parts of the reactor used for the synthesis of graphene. Very low concentrations were also determined in the semiconductor nanowire synthesis laboratory, except for higher levels measured within the glove box. Comparable results were determined in the transmission microscopic analysis laboratory, where the instrumentation used failed to detect any increase in measured concentrations, thus suggesting that occupational exposure to nanoparticles during work activities carried out in this laboratory was very unlikely.

Nanomaterial exposure evaluation: biological monitoring

Given the increasing exposure of workers to both incidental and engineered nanoparticles, a careful analysis of their exposure through biological monitoring is of first importance. In fact, it represents an essential step to complement data obtained through the environmental analyses for a more comprehensive evaluation of the exposure. It consists in the search for biological indicators in tissues, organs and systems. These indicators should be sensitive and as specific as possible for the substances to which workers are exposed in working environment. It is able to provide information on previous exposure and indications, if a dose-response relationship is defined, on the possible toxic effects secondary to the absorption of a given substance (Manno et al. 2010; Schulte et al. 2012). Biological monitoring can function as a complementary means for the assessment of exposure which takes into account interindividual variability in absorption, recent and past exposure, individual workload (Manno et al. 2010).

Incidental nanomaterials in welding

Engineered nanomaterials

There are currently no examples of the application of biological monitoring as an integral part of the routine exposure assessment of workers involved in the synthesis, production, or use of nanomaterials. However, there is a growing body of toxicological research that has shown how some nanomaterials can be detected in biological matrices of animals, including blood, plasma, urine and feces. Following the exposure to metal-based nanomaterials or metal oxides, both by inhalation and intratracheal exposure (Balasubramanian e coll. 2013; He X et al. 2010; Sundarraj et al. 2017; Sung et al. 2009; Yu et al. 2007), the elemental metal content has been determined in the blood, although, generally, in very small quantities. A positive dose-response relationship was demonstrated between the concentration of inhaled silver nanoparticles and the metal content in the blood of the treated mice (Sung et al. 2009). Size-dependent biodistribution was demonstrated for gold nanoparticles, since those with a smaller size (7 nm) resulted in a higher metal concentration in the blood than their larger 20 nm diameter counterparts (Balasubramanian ecoll .2013). The duration of exposure may influence the interpretation of the biomonitoring data. In fact, silver in blood was detected after 15 days of treatment with silver nanoparticles, but not after an exposure period shorter than 5 days. Detectable metal concentrations were also determined in

urine samples collected from animals treated with metal or metal oxide nanomaterials via the respiratory or skin route (Balasubramanian et al. 2013; Gulson et al. 2010, 2012; Sundarraj et al. 2017).

Exhaled air condensate could represent a promising matrix for biological monitoring of human exposure, as demonstrated by the increase in Ti levels in exhaled and post-shift samples collected from workers exposed to TiO2 nanoparticles in a production facility compared to unexposed controls (Pelclova e coll. 2015, 2016b). Silver, Indium and Molibdenum content, in blood and urine samples, have been determined in workers exposed to such metals at the nanoscale (Lee et al. 2012, 2015; Liu et al. 2012).

Incidental nanoparticles

Several studies have focused on the biological monitoring of the exposure to metals during welding operations. Unfortunately, although we can argue that such activities may cause an exposure to incidental nanoparticles, the lack of specificity of the biomarkers employed (the metal content in different biological matrices) prevent us to extrapolate definite conclusions on the assessment of incidental nanoparticle exposure. A Polish investigation (Stanislawska et al 2020), conducted on 67 welders and 52 subjects not exposed to welding fumes, showed that the former had significantly higher concentrations of Ni in urine and Cr in plasma and red blood cells than the group of control, suggesting how such matrices could be useful for the evaluation of the internal dose in subjects exposed to these metals. On the contrary, no difference emerged between the exposed and the controls regarding the Mn levels in serum and blood. Regarding changes related to the work shift, the same authors highlighted how the biological concentrations of Cr and Ni increased significantly after the work shift to indicate how these biological indicators could be useful biomarkers of current exposure.

These results are in line with those previously reported by Ellingsen et al. (2017) who reported significantly higher urinary and serum levels of Cr, Molybdenum, Vanadium and Tungsten in welders (70) than in unexposed controls (74). The greatest difference was evident in the urinary matrix, with the concentrations of Cr and Tungsten in the urine of the welders 10 and 4.5 times higher than the levels found in the control group. A recent study (Santonen et al. 2022), carried out as part of the European project Human Biomonitoring for Europe (HBM4EU), was aimed at evaluating occupational exposure to Cr (VI) by biological monitoring on the urinary, blood and in the exhaled breath condensate of 399 exposed workers: chrome plating, painting, welding and

mechanical treatment workers, compared with 203 subjects not exposed to Cr. Conversely to the results obtained by Stanislawska et al. (2020), such work demonstrated no significant difference in Cr concentrations in red blood cells between the welders and the control group. In the same study, the analysis of workers by job task showed that welders had significantly lower concentrations of Cr in red blood cells than plating workers. In contrast, the welders had significantly higher plasma Cr concentrations than the control group. This finding suggests that in the choice of biomarkers to be used for the assessment of exposure, the evaluation of the activities carried out and of the working methods adopted is of extreme importance to define the most appropriate biological monitoring protocols for the context of exposure considered. Another biological matrix that has been studied for evaluating the exposure of welders is the exhaled breath condenstae. The concentrations of Cr and Ni were determined in the exhaled breath condensate and urine of 100 stainless steel welders, before (T0) and after the Friday shift (T1) and before the Monday morning shift (T2). Cr concentrations were significantly lower before the Monday shift than the measurements taken before and after the Friday shift. Ni concentrations were found to be below the limit of determination (LOD), with no significant differences between T0, T1, T2. The same study determined different results for the same metals in the urinary matrix, demonstrating a different trend in the concentrations of the metals in relation to the matrix examined. Hulo and coll. (2014) showed how the levels of Mn and Ni in the exhaled breath condensate of welders (n.17) were significantly higher than those determined in unexposed subjects (n. 16). In particular, with regard to the possible correlation between different biomarkers, the levels of Mn and Ni in the exhaled breath condensate were related to those determined in the urine ($p = 0.41$ and $p = 0.15$, respectively).

A recent Swedish study (Ljungkvist et al. 2022) investigated the presence of particles, even in ultrafine size, in the condensate of exposed workers. The tool used is based on the particle count and the inertial impact of the particles on filters. The impactor present in the instrument is able to sample particles with a diameter between 0.5 and 5 μm. In short, the subjects studied exhaled through a double valve system, which allowed them to inhale ambient air and exhale it into the device by opening a second valve. Sampling was conducted on 19 welders before and after the work shift. At the same time, environmental monitoring of exposure to Cr, Ni and Mn was carried out. Although this last sampling showed a clear exposure to the three metals during the welding processes

(data also confirmed by the biological monitoring conducted on blood and urine), the authors could not demonstrate any significant difference in the content of metal particles in the exhaled air, highlighting a lack of correlation between biological and environmental monitoring. However, the possible presence of background metal particles in the biological matrix examined and a contamination in the sampling phase cannot be excluded and require further investigation.

The evaluation of the possible application of biological indicators requires the verification of their correlation with the levels of environmental exposure. A positive correlation was found between the environmental concentrations of Cr, in particular Cr in the inhalable fraction of the particulate, as well as Cr (III) and Cr (VI) in the same fraction and the metal concentrations in the urinary matrix (Stanislawska et al. . 2020). No correlation was found for the concentrations of the metal in the urine and its levels in the respirable fraction of the airborne particulate. A weaker correlation was determined between the Ni in the two fractions of the particulate matter and the Ni in the urine. Ellingsen et al. (2017) demonstrated a significant correlation between determinations in biological monitoring and metal concentrations in ambient air, particularly if environmental monitoring was performed only one day after biological monitoring, compared to environmental monitoring performed two days afterwords. These results are in line with those obtained on 12 healthy subjects, exposed to increasing concentrations of welding fumes for six hours, who showed an increase directly related to the exposure concentration (0.1 and 2.5 mg / m3) of Ni and Cr levels in the urine (Gube et al. 2013). Stanislawska et al. (2020) demonstrated the lack of a significant correlation between airborne Ni and urinary metal concentrations, while a positive correlation was determined between concentrations of total Cr, Cr (III) and Cr (VI) in inhalable particulate and those of the metal in the urine and serum. No significant correlation was found between the Cr in the inhalable fraction and the levels of the metal determined in the red blood cells.

Aim of the study

Concerning the exposure to both ultrafine particles and engineered nanoparticles in occupational settings, only preliminary and fragmented data are available. In general, the elemental metal content of metal-based nanomaterials have been used in biological monitoring analyses. As concerns engineered nanoparticles, Silver, Indium and Molibdenum content, in blood and urine samples, have been determined in workers exposed to such metals at the nanoscale (Lee et al. 2012, 2015; Liu et al. 2012). The presence of ≥ 100 nm sized TiO_2-crystals in the exhaled breath condensate (EBC) samples collected from TiO_2-NP production workers could be determined in one study carried out by Pelclova and co-workers in 2015. Regarding ultrafine particle exposure, in airport workers, exposed to incidental ultrafine particles generated by jet engines (mean particle size of 17.7 nm), particles, of around 500 nm, primarily composed of Chromium, Cadmium and Aluminum were detected in the EBC (Marie-Desvergne et al. 2016).

In this scenario, aims of the present research were to assess, via biological monitoring, the exposure of workers through an innovative analytical protocol using the Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS). In particular, our scope was to assess:

- The number concentration and size of metal-oxide based ultrafine particles, Chromium(III) oxide (Cr_2O_3), Manganese(II,III) oxide (Mn_3O_4) and nickel oxide (NiO) NPs in different biological matrices (EBC, plasma and urine) of welders of two Italian Companies (A and B);
- The number concentration and size of engineered nanoparticles in blood and urine of researchers employed in a lab where metal based-NPs were synthetised (Company C) and in workers of a chemical facility where nano-precious metals were used for the production of catalytic converters (Company D).

Results obtained from this investigation are expected to inform te definition of adequate strategies to assess both ultrafine particles and engineered nanoparticle exposure in different occupational settings. Overall, this may provide interesting guidance to future investigations aimed to gain insight into risk assessment and management procedures in nano-related occupations.

Materials and Methods

Investigated populations and samples collection

Eighteen male welders were enrolled from two Italian mechanical engineering companies (Company A and B). Fifteen administrative workers were included as unexposed controls. Table 1 describes the welding techniques, electrodes and alloys used by each worker. Iron (Fe), Chromium (Cr), Nickel (Ni), Manganese (Mn) and Silicium (Si) were main metal constituents of the alloy used, the 304L, a widely employed "18-8" chromium-nickel austenitic stainless steel Two samples of EBC and urine were collected from each welder. The first one at pre-shift on the 1st day of the working week and the second at post-shift on the 5th day of the working week. Blood was collected from each welder at post-shift on the 5th day of the working week. For the control group only one sample of EBC, blood and urine were collected (time not specified).

Table 1. Welder technique, alloys and electrodes

Company	Welder	Technique	Alloys (metal composition*)	Electrodes (metal composition*)
A	**1W**	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	2W	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	3W	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	4W	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	5W	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	6W	TIG	304L (Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
B	**1W**	MMA	625 (Cr. Ni. Fe. Nb. Ta. Ti. Mn)	Cr. Ni. Fe. Nb+Ta. Ti. Mn
	2W	MMA	309L (Cr. Ni. Fe. Si. Mn)	Cr. Ni. Fe. Si. Mn
	3W	SAW	Duplex (Cr. Ni. Mo. Mn. Si)	Cr. Ni. Mo. Mn. Si
	4W	MIG	Duplex (Cr. Ni. Mo. Mn. Si)	Cr. Ni. Mo. Mn. Si
	5W	MIG	Duplex (Cr. Ni. Mo. Mn. Si)	Cr. Ni. Mo. Mn. Si
	6W	TIG	308 (Fe. Cr. Ni. Mn. Si)	Cr. Ni. Mo. Mn. Si
	7W	MIG	AISI 316/308 (Fe. Cr. Ni. Mo. Mn. Si)	Fe. Cr. Ni. Mo. Mn. Si
	8W	SAW	NiCro 625 (Ni. Co. Cr. Mo. Nb+Ta)	Cr. Mo. Mn. Nb. Fe. Si
	9W	GMA	ER2209 (Cr. Ni. Mo. Mn. Cu)	Cr. Ni. Mo. Mn. Cu
	10W	MIG	Superduplex S32750 (Cr. Ni. Mo. Mn. W. Cu)	Cr. Ni. Mo. Mn. W. Cu
	11W	SAW	AISI 309/316 (Fe. Cr. Ni. Mo. Mn. Si)	Cu
	12W	SAW	AISI 309LMO/316L (Fe. Cr. Ni. Mo. Mn. Si)	Mn. Si. Cu. Ni. Mo. Ti. V. Nb. Al

TIG: Tungsten Inert Gas welding (manual); MMA: Manual Metal Arc welding; SAW: Submerged Arc welding; MIG: Manual Metal-Arc Inert Gas welding; GMA– Gas Metal Arc welding

* decreasing order in percent by weight

As regards the engineered NP exposure, NP researchers (n. 3) used mainly NPs of Gold (Au), Silver (Ag), Indium (In), Palladium (Pd) and Titanium (Ti), while in the Company B, workers (n. 3) used Iridium (Ir), Pd and Platinum (Pt)-NPs to produce automotive catalysts. Therefore, the size and NP number concentrations of such metal-NPs were investigated in urine samples collected from these workers at the beginning and at the end of the shift of 4 workweek days (1^{st}-4^{th} day) and in blood samples collected each worker at the end of the working week (4^{th} day). Unexposed controls (n. 2) provided samples at the same time points of exposed workers.

Samples preparation

Samples of the EBC weret alected with the TurboDECCS (Medivac, Parma Italy). Previously reported procedures were followed (Santonen et al., 2022). EBC sampling took ca. 15 minutes to collect a volume of ca. 1-2 mL from each subject. Approximately 6 mL of blood weret alected, and blood withdrawal was carried out using metal free containers (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and metal free needles (Becton Dickinson Labware) to reduce the risk of metal contamination. Plasma has been then separated by centrifugation of blood samples for 5 minutes at 2700 g (Santonen et al., 2022). EBC samples were stored at 4°C, while plasma and urine samples at -20 °C until the SP-ICP-MS analysis.

Urine from exposed employees were collected in high density 100 mL polyethylene bottles (Kartell, Milan, Italy), previously decontaminated with 10% ultrapure HNO_3 (Normatom, Leuven, Belgium).

Urine samples were thawed at room temperature and shaken before use. Then one mL of urine was diluted 1:10 with ultrapure deionized water (Milli-Q Element, Bedford, MA, USA).

Approximately 6 mL of blood was collected from each worker a. The blood withdrawal was carried out using metal free containers (K-EDTA vacutainer BD tubes; Becton Dickinson Labware, Franklin Lakes, NJ, USA) and metal free needles (Becton Dickinson Labware) to reduce the risk of metal contamination. Biological samples were immediately stored at -20 ° C until analysis. Controls provided samples at the same time points of exposed workers. Blood samples were thawed at room temperature and shaken before use. One mL of blood was subjected to alkaline extraction with the addition of

3.5 mL of 25% v/v tetramethylammonium hydroxide (TMAH, Sigma-Aldrich); the sample was then sonicated for 1 hour in an ice-cooled water bath and left for 24 hours at room temperature. At full extraction, 0.5 mL of a 0.1% v/v Triton-X solution (Alfa Aesar, Ward Hill, MA, USA) was added. Plasma has been then separated by centrifugation of blood samples for 5 minutes at 2700 g (Santonen et al., 2019; Santonen et al., 2022).

SP-ICP-MS analysis of incidental and engineered nanoparticles

To detect incidental and engineered nanoparticles, samples were analysed by the iCAP-Q ICP-MS in Single Particle (SP) mode (Thermo Fisher, Bremen, Germany) using the kinetic energy discrimination (KED) mode with helium as thet alision cell gas. The instrumental and method parameters used are reported in Table 2. To calculate the sensitivity of the ICP-MS system, single-element stock solutions were used (CPAChem, C.P.A. Ltd., Stara Zagora, Bulgaria) at the analytical concentration of 1 µg/L.

Table 2. Instrumental parameters for SP-ICP-MS data acquisition of metal-oxide-NPs

Parameter	Value
Instrument	iCAP-Q (Thermo Fisher)
Nebulizer	Concentric
Spray Chamber	Cyclonic. quartz
Cones	Pt sampler. Pt skimmer
RF Power	1450 W
Collision cell gas	4.8 mL/min of He
Sample uptake rate	0.35 mL/min
Dwell time	5 msec
Sampling Time	60 sec
Nebulization efficiency (%)	4%
Acquisition mode	Q-Cell in KEDs
Isotopes	^{52}Cr. ^{55}Mn. ^{60}Ni
Metal mass fraction	0.68 Cr; 0.72 Mn; 0.79 Ni
Density	5.22 g/cm^3 Cr_2O_3; 4.86 g/cm^3 Mn_3O_4; 6.67 g/cm^3 NiO

In the welding exposure setting, the Au NP reference standard (60 nm) was used to evaluate the transport efficiency of the ICP-MS sample introduction system (Sigma-Aldrich, St. Louis, USA). In the welding exposure contexts, the isotopes of ^{52}Cr, ^{55}Mn

and ^{60}Ni, the dwell time of 5 msec, the analysis time of 30 sec per sample were used for quantification.

In the engineered particle exposure contexts, the isotopes ^{107}Ag, ^{197}Au, ^{115}In, ^{193}Ir, ^{106}Pd, ^{195}Pt and ^{47}Ti were used for quantification; a dwell time of 5 msec and an analysis time of 60 sec per sample were applied. For metal-oxides as In_2O_3 and TiO_2, the mass fraction by weight was used to convert the metal mass measured by SP-ICP-MS in the metal-oxide mass. The nanoparticle concentrations were determined based on a molecular weight calculation and assuming that all the metal found came from its oxides. Then, the density of the metal and the assumption of the spherical shape of particles were used to convert the measured mass into the particle diameter. Mean particle diameter (in nm) and particle number concentration (as particles/mL) were generated by the Thermo Scientific™ Qtegra software.

Results

Human biomonitoring results in welders

Exhaled breath condensate

Table 3 reports the size (±SD) and particle concentration (particles/mL) of Cr_2O_3 incidental NPs found in the EBC of welders at both A and B Companies**.** A median of 33994 particles/mL at the pre-shift at the beginning of the workweek and 127358 particles/mL at the post-shift end of the work week could be detected in the EBC samples of welders at Company A. Ar regards the size of incidental NPs determined at these two time points, dimension ranges of 50.2-66.9 nm and 43.6-67.9 nm could be respectively) In the EBC samples of welders at Company B, a median particle concentration of 14829 particles/mL of Cr_2O_3 NPs were found at the pre-shift and 40018 particles/mL at the post-shift, with size ranges between 50.3-65.5 nm and 51.6-65.4 nm, respectively at the two time points. A significantly higher (p-value < 0.001) particle number concentration of Cr_2O_3 NPs in the EBC samples at the post-shift end of the week (median, 64645 particles/mL) was determined compared to pre-shift beginning of week (median, 15836 particles/mL). Size of Cr_2O_3 NPs in EBC samples was comparable between the post-shift end of the week (median, 55 nm) and the beginning of the pre-shift beginning of week (median, 58 nm). Regarding controls, no particles of Cr_2O_3, were observed at both Companies. NiO-NPs were found in the EBC of workers at Company A only (**Table 3).** NiO NPs were below the LoD in EBC

samples of unexposed control and pre-shift, while particles were detected in 5/6 welders in post-shift samples with a median of 22000 particles/mL and a size range of 59.3-108 nm**.** No Mn_3O_4 NPs were found in EBC samples of welders employed at both Companies.

Table 3. Size and number particle concentration of Cr_2O_3 and NiO NPs in EBC and plasma of welders

Cr_2O_3					
	EBC				
Company *	**Welder t**	**Size (nm) Pre-shift**	**Size (nm) Post-shift**	**Particle/mL Pre-shift**	**Particle/mL Post-shift**
A	1W	50.9±12.2	43.6±9.19	41975	259865
	2W	56.7±10.6	67.9±13.7	59307	67202
	3W	66.9±13.0	60.2±17.0	29493	187513
	4W	50.2±9.47	48.5±11.3	12078	62088
	5W	61.7±16.4	60.5±14.8	10980	210806
	6W	54.6±14.2	67.8±15.8	38494	51731
Median		**55.7**	**60.3**	**33994**	**127358**
Min-Max		**50.2-66.9**	**43.6-67.9**	**10980-59307**	**51731-259865**
B	1W	50.3±19.9	51.6±10.5	16300	51465
	2W	62.8±15.4	53.1±10.1	8784	21429
	3W	56.6±18.6	52.6±11.5	8928	21045
	4W	55.1±14.2	58.7±18.6	7465	23077
	5W	51.6±12.7	51.8±9.78	12444	28571
	6W	61.5±18.7	51.6±14.9	86664	229852
	7W	57.5±14.0	59.4±18.6	15372	104516
	8W	57.9±16.1	52.9±14.8	16653	103946
	9W	65.5±25.0	65.4±21.3	14286	22527
	10W	60.8±21.2	54.7±16.6	41026	156102
	11W	58.9±18.7	64.5±23.8	172161	172527
	12W	62.1±18.0	55.6±14.3	8212	23513
Median		**58.4**	**53.9**	**14829**	**40018**
Min-Max		**50.3-65.5**	**51.6-65.4**	**7465-172161**	**21045-229852**
NiO					
Company **	**Welders**	**Size (nm) Pre-shift**	**Size (nm) Post-shift**	**Particle/mL Pre-shift**	**Particle/mL Post-shift**
A	1W	nd	59.3±7.86	nd	27268
	2W	nd	64.8±11.1	nd	7509
	3W	nd	63.6±10.5	nd	6081
	4W	nd	108±3.94	nd	22000
	5W	nd	73.0±10.8	nd	67857
	6W	nd	nd	nd	nd
Median		**nd**	**64.8**	**nd**	**22000**
Min-Max			**59.3-108**		**6081-67857**

*No Cr_2O_3 and NiO NPs detected in unexposed controls at both Companies

** No NiO NPs detected in welders from Company B and in unexposed controls at both Companies

Plasma

In plasma, the Cr_2O_3 incidental NPs showed a median of 10376 particles/mL at post-shift in the size range between 30.5-47.3 nm in welders at Company A, and a median of 7762 particles/mL in the range 31.8-51.9 nm in welders at Company B (Table 4).

Table 4. Size and number particle concentration of Cr_2O_3 and NiO NPs in plasma of welders

Cr_2O_3			
	Plasma		
Company *	**Weldert**	**Size (nm) Post-shift**	**Particle/mL Post-shift**
A	1W	32.9±5.31	25254
	2W	37.7±3.01	17413
	3W	30.5±2.57	2547
	4W	45.9±4.93	23139
	5W	46.6±4.12	3338
	6W	47.3±2.48	2373
Median		**41.8**	**10376**
Min-Max		**30.5-47.3**	**2373-25254**
B	1W	51.9±7.03	3846
	2W	50.6±7.13	3477
	3W	47.5±6.90	2745
	4W	37.2±2.44	3840
	5W	32.1±5.50	2895
	6W	38.2±2.73	12444
	7W	48.9±2.15	10806
	8W	43.5±4.82	7977
	9W	48.5±2.58	8601
	10W	44.3±5.09	7762
	11W	31.8±8.20	28915
	12W	nd	nd
Median		**44.3**	**7762**
Min-Max		**31.8-51.9**	**nd-28915**
		NiO	
Company **	**Welders**	**Size (nm) Post-shift**	**Particle/mL Post-shift**
A	1W	37.1±1.90	8248
	2W	nd	nd
	3W	nd	nd
	4W	37.4±1.45	6227
	5W	42.7±1.58	15934
	6W	nd	nd
Median		**37.4**	**8248**
Min-Max		**37.1-42.7**	**6227-15934**

*No Cr_2O_3 and NiO NPs detected in unexposed controls at both Companies
** No NiO NPs detected in welders from Company B and in unexposed controls at both Companies

In this matrix, median of 8248 particles/mL and size between 37.1-42.7 nm of NiO NPs were found in 3/6 welders. No Mn_3O_4 NPs were detected in plasma of the whole cohort of welders and controls as well.

Urine

Nanosized particles of Cr_2O_3, NiO and Mn_3O_4 were not determined in urine of welders involved in both Companies, nor in controls.

Analyses of NPs concentration and size according to the biological matrices

A sub-analysis of the particle number concentration retrieved in EBC and in plasma showed a significantly higher particle number concentration (p-value < 0.001) of Cr_2O_3 incidental nanoparticles in the EBC (median, 64645 particles/mL) samples of welders compared to plasma (median, 7762 particles/mL). The diameter of Cr_2O_3 incidental NPs in the EBC samples collected at the post-shift end of the week (median, 55 nm) was higher (p-value < 0.001) than in plasma (median, 44 nm) at the post-shift end of the week.

No NiO incidental NPs were found in EBC samples collected at the pre-shift beginning of week, while differences (p-value < 0.001) were found between concentration (median, 22000 vs. 8240 particles/mL) and size (median, 65 vs. 37 nm) of NiO incidental NPs in EBC samples respect to plasma samples at the post-shift end of the week. Finally, a positive correlation between concentration of Cr_2O_3 NPs in EBC samples at the pre- shift and those collected at the post-shift end of week was obtained with a Spearman ρ of 0.692 ($p = 0.001$); while only a minor correlation was obtained between EBC samples and plasma samples at the post-shift end of the week ($\rho = 0.441$; $p = 0.067$).

Human biomonitoring results in workers exposed to engineered NPs

Urine

Concerning the biological monitoring results obtained in engineered NP exposed workers (Table 5), only one worker (n. 3) from Company C showed Ag NPs in urine both at pre- and post-shift with mean diameter of ca. 29 nm and mean particle number concentration of 19180 particles/mL at the pre-shift and 13955 particles/mL at the post-shift. No NPs of Au, In, Ir, Pd, Pt and Ti were found in any exposed worker of either Company C or Company D. In controls, NPs of Ag were found with diameter of ca. 29 nm and mean number of particles of 7680 particles/mL pre-shift and 6630 particles/mL post-shift for one subject (no. 7) and 12215 particles/mL pre-shift and 10470 particles/mL post-shift for another subject (no.8).

Table 5. Analysis of metal NPs in urine of workers from Company A (no. 1-3), Company B (no. 4-6) and controls (no. 7-8), at pre-shift (1st-4th day, mean) and post-shift (1st-4th day, mean)

Metal NPs	Workers no. (Company)	Diameter±SD (nm)		Number of particles±SD (particel/mL)	
		pre	post	pre	post
Ag	1-2 (A)	nd	nd	nd	nd
	3 (A)	29.2±2.9	29.4±2.5	19180±9921	13955±4608
Au, In, Pt, Ti	1-3 (A)	nd	nd	nd	nd
Ir, Pd, Pt	4-6 (B)	nd	nd	nd	nd
Metal NPs	**Controls no.**	**Diameter±SD (nm)**		**Number of particles±SD (particel/mL)**	
		pre	post	pre	post
Ag	7	28.9±1.9	28.4±2.5	7680±12327	6630±4087
	8	28.6±2.1	28.2±2.6	12215±4916	10470±396
Au, In, Ir, Pd, Pt, Ti	7-8	nd	nd	nd	nd

Blood

The results of the analysis of metal NPs in blood of workers from Company A, B and controls, at the end of working week (4th day), are reported in **Table 6**. The Au NPs were observed in all the workers of Company A and in all controls with a comparable diameter of 15 nm and number of particles ranging from 62794 to 251177 particles for

workers and from 10989 to 95838 particles/mL for controls. In addition, Ti NPs were detected both in workers and controls, with a size between 82 nm and 90 nm and number of particles between 10989 particles/mL and 95838 particles/mL. The NPs of In were detected only in two worker from Company A (no. 2 and 3) and not in the control group. The size measured was ca. 38 nm and the number of particles was 14020 particles/mL (no. 2) and 6722 particles/mL (no.3). No NPs were detected in blood of workers from Company B.

Table 4. Analysis of metal NPs in blood of workers from Company A (no. 1-3), Company B (no. 4-6) and controls (No. 7-8), at the end of working week (4th day)

Metals NPs	Workers no. (Company)	Diameter±SD (nm)	Number of particles±SD (particel/mL)
Ag	1-3 (A)	nd	nd
Au	1 (A)	15.3±2.3	251177
	2 (A)	15.5±2.5	62794
	3 (A)	15.1±2.2	65934
In	1 (A)	nd	nd
	2 (A)	37.9±1.6	14020
	3 (A)	37.6±1.3	6722
Pt	1-3 (A)	nd	nd
Ti	1 (A)	82.4±12.5	49092
	2 (A)	83.4±13.3	16709
	3 (A)	86.8±14.8	17315
Ir, Pd, Pt	4-6 (B)	nd	nd
Metals NPs	**Controls no.**	**Diameter±SD (nm)**	**Number of particles±SD (particel/mL)**
Ag	7-8	nd	nd
Au	7	15.1±1.5	10989
	8	15.3±2.2	95838
In, Ir, Pd, Pt	7-8	nd	nd
Ti	7	84.5±12.0	16245
	8	90.4±16.4	14334

Discussion

The increasing likelihood for occupational exposure to both incidental and engineered NPs, together with the lack of joined strategies to evaluate such exposure through environmental and biological monitoring require great scientific efforts to develop suitable analytical methodologies for the assessment of the internal doses, sensitive and specific for NP exposure.

In fact, human biological monitoring provides a valid and complementary tool with respect to environmental one, to determine the effective internal doses experienced by workers in different occupational settings. In this context, this study aimed to assess the applicability of an innovative analytical method, based on the SP-ICP-MS, able to determine the size and concentration of incidental and engineered NPs. Indeed, this methodology was applied in the stainless-steel welding occupational scenario as well as during the synthesis or handling of NPs, measuring the single particles separately from larger ones.

In the welding occupational settings, the specific Cr_2O_3, Mn_3O_4 and NiO incidental NPs were selected because Cr, Mn and Ni were the major constituents of the alloys and electrodes used by welders working at the two Italian Companies. In addition, these metals were those most commonly found in aerosols generated during metal inert gas welding of mild and stainless steel and linked with severe health outcomes (Mei et al. 2018; Soares and Soares, 2021). Different biological matrices have been employed to assess the systemic levels of incidental nanoparticles in workers (plasma and urine), as well as the dose of inhaled nanparticles in the lungs (EBC). Urine and plasma have been employed to assess the exposure of nano-researchers and employees of a catalytic converters facility. Also in this case, the metals choosen to be measured reflected those most frequently handled and potentially resleased into the workplace: Au, Ag, In, Ti, Pt, Pd, Ir.

The SP ICP-MS methodology proved to be reliable in quantifying incidental and engineered nanoparticles in all the investigated matrices due to i) its ability to simultaneously detect the concentration of particles (particles/mL) and their size (nm); ii) the straightforward sample preparation without pre-concentration steps; and iii) the high instrumental sensitivity and precision; iv) the speed of execution of the analysis (30 sec) and consequently the low volume of samples required.

As a confirm of the method reliability, this technique was able to detect quantifible levels of Cr_2O_3 NPs and NiO NPs in the EBC and plasma samples of exposed welders, while was not able to determine particles in controls. This latter finding suggests that the background level of incidental particles in the workplaces was not relevant. In addition, the significant increase detected in the EBC samples collected from welders at the post-shift at the end of the working week compared to the pre-shift samples collected at the beginning of the week support the role of occupational exposure in affecting the internal doses determined. Particularly, the presence of Cr_2O_3 NPs in EBC samples collected at pre-shift could reflect the chronic exposure of workers due to their history of welding operations; while the increase in NPs found in post-shift samples may be associated to the exposure during the working week. The analysis of NPs in EBC samples of occupationally exposed subjects, and particularly in welders, is an innovative aspect of this work, as these data have been rarely reported in previous published papers (Pelclova et al. 2015; Marie-Desvergne et al., 2016). Concerning NiO NPs, a lower number of particles with respect to Cr_2O_3 NPs were detected in EBC samples of welders at Company A (5 workers out of 6), while workers at Company B did not present detectable NiO particles (**Table 5**).

As regards the plasma samples, the present study demonstrated a lower amount of Cr_2O_3 and NiO NPs in welders respect to their levels in EBC samples, with ca. 10% of Cr_2O_3 NPs translocated from the lungs to the plasma. Regarding urine, no NPs could be detected in any of the workers. This may be dependent by the fact that particle glomerular filtration is highly dependent on their size. Particles with a diameter < 6 nm are typically filtered and excreted via the renal system, while those > 8 nm are not normally capable of glomerular filtration and are eliminated via the hepatobiliary system (Longmire et al. 2008).

Also in conditions of engineered NP exposure, the SP-ICP-MS resulted a good methodology to assess internal levesl of exposure in workers. In Company C, Ag (mean 28 nm) was detected in urine in only 1 out of 3 exposed workers. Interestingly, all the two subjects enrolled as controls had comparable or at least one order of magnitude lower levels of Ag particle number concentration compared to those determined in the exposed worker. This may suggest a possible extra-professional source of exposure. Silver-NPs, in fact, are widely used in consumer products and in the food industry. This may cause a general living exposure responsible for the detection of the NP counts determined in both the worker and the controls. No Ag-NPs could be determined in

blood of exposed workers and controls. Conversely, in the same company, no Au-NPs could be found in urine samples collected from both exposed employees and unexposed subjects. However, when this NPs were measured in blood, comparable levels of particle number concentration could be determined in workers and controls. Considering the limited possibility for a general living exposure, an occupational source of contamination in the Company for both exposed employees and controls can be argued that may affect also the administrative staff, not directly involved in tasks in contact with NPs.

Detectable levels of titanium-NPs (mean 84 nm) were determined in blood of exposed and unexposed subjects, while this metal was not demonstrated in urine samples. The widespread use of Ti-NPs in consumer products for daily use may be responsible for a possible general living exposure to the metal even at the nanoscale. The only determinable concentrations of In (mean diameter 38 nm) were found in blood of occupationally exposed subjects, while no levels could be determined in urine samples of this same group, as well as in urine and blood collected from controls. This result supports the specificity of the In level in blood as a biomarker of internal dose in case of occupational exposure. For all the other metals, such as Pt in both companies and Ir and Pd in Company B, no determinable particle number concentration could be determined in both the biological matrices investigated in workers and controls.

In general, the low levels measured, or the lack of determinability of metals in the urine of occupationally exposed subjects can support the effectiveness of the collective and individual preventive and protective measures adopted to control the exposure. Additionally, the lack of any increase in the NP number concentration in urine samples collected before and after the workshift can further confirm the role of extraprofessional sources of exposure in affecting the biological monitoring results. Also in Company B, engaged in the production of nano-enabled catalytic converters, including prototypes, urinary concentrations of Pt, Pd and Ir were not determinable, confirming the importance and effectiveness of the preventive measures adopted by the company.

Although these preliminary results seem interesting, some limits of our study need to be considered for a correct interpretation of the results. First of all, the limited number of workers enrolled in our study that require to be enlarged in future investigations. Additionally, considering the multitude of engineered nanomaterial applications and the various occupational contexts where incidental particles can be produced it seems important to plan adequate and specifically focused strategies for

biological monitoring that may vary according to the specific NPs and work processes applied. Additionally, it is essential to stress the relevance to perform biological monitoring using diverse biological matrices taking into account the possibility for incidental or engineered particles to differently distribute in specific sites of the organism, in relation to the qualitative and quantitative aspects of the exposure and to the diverse physico-chemical properties should be also deeply explored in future studies. Moreover, the influencing role of the biomolecular interaction that different particles can have in the biological fluids should be also carefully considered for a suitable interpretation of human biological monitoring results.

Conclusions

Overall, our findings support the possibility to more deeply investigate the application of the SP-ICP-MS methodology to evaluate the exposure to incidental or engineered NPs in diverse occupational settings. Unfortunately, the preliminary nature of our data and the limited number of subjects involved do not allow us to extrapolate definitive conclusions on the interpretation of the results obtained. Therefore, further studies should be conducted in order to adequately assess the exposure of workers involved in the synthesis, production, and use of NPs or exposed to incidentally produced ones in order to extrapolate information that can adequately inform risk assessment and management strategies.

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