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REVIEW

# Biomarkers of nanomaterial exposure and effect: current status

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**Abstract** Recent advances in nanotechnology have induced a widespread production and application of nanomaterials. As a consequence, an increasing number of workers are expected to undergo exposure to these xenobiotics, while the possible hazards to their health remain not being completely understood. In this context, biological monitoring may play a key role not only to identify potential hazards from and to evaluate occupational exposure to nanomaterials, but also to detect their early biological effects to better assess and manage risks of exposure in respect of the health of workers. Therefore, the aim of this review is to provide a critical evaluation of potential biomarkers of nanomaterial exposure and effect investigated in human and animal studies. Concerning exposure biomarkers, internal dose of metallic or metal oxide nanoparticle exposure may be assessed measuring the elemental metallic content in blood or urine or other biological materials, whereas specific molecules may be carefully evaluated in target tissues as possible biomarkers of biologically effective dose. Oxidative stress biomarkers, such as 8-hydroxy-deoxy-guanosine, genotoxicity biomarkers, and inflammatory response indicators may also be useful, although not specific, as biomarkers of nanomaterial early adverse health effects. Finally, potential biomarkers from "omic" technologies appear to be quite innovative and greatly relevant, although mechanistic, ethical, and practical issues should all be resolved before their routine application in occupational settings could be implemented. Although all these findings are interesting, they point out the need for further research to identify and possibly validate sensitive and specific biomarkers of exposure and effect, suitable for future use in occupational biomonitoring programs. A valuable contribution may derive from the studies investigating the biological behavior of nanomaterials and the factors influencing their toxicokinetics and reactivity. In this context, the application of the most recent advances in analytical chemistry and biochemistry to the biological monitoring of nanomaterial exposure may be also useful to detect and define patterns and mechanisms of early nanospecific biochemical alterations.

**Keywords** Nanotechnology · Nanomaterials · Workplace · Biological monitoring · Biomarkers of exposure · Biomarkers of effect · Health effects · Risk assessment · Risk management

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## Introduction

Advances in nanotechnology over the past few decades have offered the opportunity to produce novel engineered nanomaterials, with distinctive and technologically interesting physicochemical properties. These achievements allowed nanomaterial applications in structural engineering, electronics, optics, consumer products, energy production and storage, soil and water remediation, as well as in medicine for therapeutic or diagnostic purposes (Biskos and Schmidt-Ott 2012). As a consequence of the widespread nanomaterial incorporation in products, an increasing number of workers are expected to become exposed to these materials throughout the product life cycles.

However, despite the increasing likelihood of an occupational exposure, our understanding of the health and safety aspects of nanomaterials is still in a developing phase. This lack of information has raised scientific issues and regulatory concerns regarding the health of occupationally exposed subjects. Efforts to actively anticipate hazard identification from nanomaterials and to define preventive needs for the workers have become absolutely necessary to assure adequate occupational health and safety standards (Schulte and Trout 2011; Trout and Schulte 2010).

In this context, a key role for prevention may be played by biological monitoring. This has been defined as "the repeated, controlled measurement of chemical or biochemical markers in fluids, tissues or other accessible samples from subjects exposed or exposed in the past or to be exposed to chemical, physical or biological risk factors in the workplace" (Manno et al. 2010). Thus, biological markers may contribute to identifying potential hazards of nanomaterials and understanding their modes of action, as well as the mechanistic steps along the exposure–disease continuum. Moreover, in occupational settings, biomarkers may be useful in evaluating exposure and early biological effects in workers, thus supporting a more adequate assessment and management of risk in respect of nanomaterial exposure and effect (Schulte and Hauser 2012).

Although beneficial for the occupational health practice, biological monitoring data from nanomaterial exposed workers are seriously lacking. Moreover, the inherent heterogeneity in nanomaterial physicochemical parameters, which can influence the toxicokinetic and toxicological behaviors of these materials, as well as the different methodological designs of the in vivo studies, may contribute to the complex challenge to identify and validate suitable biomarkers of nanomaterial exposure and effect.

The present review aims to provide a comprehensive evaluation of the potential biomarkers of nanomaterial exposure and effect currently available as investigated in human and animal studies. It critically evaluates the feasibility of such biomarkers in the workplace, taking also into consideration the enormous recent progresses in analytical chemistry and biochemistry, which will probably make it possible in the near future to utilize various biological monitoring protocols both for epidemiologic research and for occupational health practice. Finally, this overview may provide a background on biological markers that can contribute to the overall assessment and management of risks derived from nanomaterial exposure in occupational settings.

## Biomarkers of exposure and effect

The National Academy of Sciences (USA) has defined biomarkers as an alteration in cellular or biochemical components, processes, structures, or functions, which is measurable in a biological system or sample (Manno et al. 2010). Biomarkers are traditionally classified as biomarkers of exposure, effect, and susceptibility. In the present review, special attention will be paid to the first two categories considering their relevance for and applicability to the occupational health practice.

## Biomarkers of exposure

A biomarker of exposure is a chemical or its metabolite or the product of an interaction between a chemical and some target molecule or macromolecule, which is measured in a compartment or a fluid of an organism. In occupational health practice, biomarkers of exposure play a highly relevant role since they make it possible to assess exposure by all routes, taking also into account the inter-individual variabilities in absorption, metabolism, and excretion; and the

individual workload, as well as the recent versus past exposure.

These biomarkers allow obtaining more informative and accurate assessment of occupational exposure compared with environmental investigations, which may result in considerable under or overestimation of the actual internal dose and may be biased by several practical technical difficulties (Manno et al. 2010). Moreover, when the dose–response relationship is defined, the biomarker of exposure does not only indicate the dose actually adsorbed, but provides also a reasonably accurate quantitative estimate of the occupational risks at the group and/or individual level. Examples of these valuable indicators in the "non-nano" occupational exposure settings include, among others, lead in blood, cadmium in blood and urine, and mercury in urine (Jakubowski 2012; Jakubowski and Trzcinka-Ochocka 2005). Therefore, the definition of such kinds of biomarkers is one of the most intriguing aims of nanotoxicological research. In order to recommend a suitable biological monitoring strategy for the assessment of exposure, the toxicokinetics of nanomaterials should be known in some detail. This seems a quite challenging exercise, because both the intrinsic and the extrinsically acquired nanoparticle (NP) properties, as well as the exposure media and the route of entry appear to be able to influence NP kinetics. As regards NP adsorption, both the uptake by the respiratory system and the cutaneous penetration may realistically occur in workplaces. Gastrointestinal adsorption may also result from the mucociliary clearance of inhaled NPs, in case of accidental events or when proper standards of personal and industrial hygiene are not met.

The following text will provide a summary of the current understanding about potential internal and/or biologically effective dose biomarkers, measured in accessible biological matrices such as blood, urine, and feces, which deserve consideration for the biological monitoring of nanomaterial exposure in workplaces.

## Internal dose biomarkers

In order to extrapolate data useful to define potential biological indicators of exposure to NPs, we reviewed human and animal studies focused on the evaluation of nanomaterial internal dose in blood, plasma, urine, and feces due to exposure via the respiratory, cutaneous, and gastrointestinal systems.

### *Blood and plasma*

As regards inhalation, the measurement of the elemental metal content in blood has turned out to be a good biological marker to assess exposure to metal NPs, such as Ag (Takenaka et al. 2001; Sung et al. 2009)- and Au-NPs after acute to sub-chronic treatment (Takenaka et al. 2006; Yu et al. 2007; Balasubramanian et al. 2013) (Table 1). This measurement resembles the elemental metal determination performed to evaluate the internal dose of non-nano metallic compounds, i.e., cadmium, chromium, lead, mercury, and nickel (Jakubowski and Trzcinka-Ochocka 2005). This biomarker seemed to reflect the external exposure levels very well, as confirmed by the positive dose–response relationship found between the concentrations of the inhaled Ag-NPs and the Ag content in blood (Sung et al. 2009). Internal doses of nanomaterials, such as the total amount adsorbed by the body following external exposure, was also evaluated in a study performed on two workers exposed to Ag-NPs by measuring Ag concentrations in blood (Lee et al. 2012a). Unfortunately, the limited number of workers, the lack of adequate measurements of the NP concentrations in the workplace environment, and the absence of other studies for comparison do not allow one to obtain suitable information on NP biological monitoring from this study.

Some intrinsic NP features, as well as the specific conditions of exposure, appear to be able to affect biomonitoring results and, therefore, should be taken into consideration in-depth while interpreting the data. NP size, for instance, has been demonstrated to influence the metal-NP distribution and the subsequent metal content detected in blood, as assessed by the greater Au concentration observed in rats treated with 7-nm-sized Au-NPs compared to those of rats exposed to 20-nm NPs (Balasubramanian et al. 2013). The importance of particle size in biological monitoring was confirmed when the blood recoveries of other types of NPs, such as fluorescent carboxyl-coated polystyrene spheres (Sarlo et al. 2009) and radiolabeled NPs, were investigated (Choi et al. 2010; He et al. 2010; Kreyling et al. 2009). Unfortunately, however, analytic tracing techniques based on NP labeling, although useful for gaining a deeper

**Table 1** Possible biomarkers of exposure (in blood or plasma) for different types of NPs

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Metal nanoparticles | | | | | | |
| Ag-NPs | Ag-NPs were compact, spherical, and electron dense with diameters of 4–10 nm | 16 female Fischer 344 rats were exposed (133 μg/m$^3$; 3 × 10$^6$ particles/cm$^3$) via inhalation, for 6 h | Ag was measured 30 min, 2 h and 1, 4, and 7 days after exposure | Ag concentrations (ng/g): 8.9 ± 6.2 (day 0); 6.2 ± 0.8 (day 1); 2.9 ± 1.5 (day 4); 1.0 ± 0.2 (day 7); Ag controls: <0.6 ± 0.1 | Ag in blood | Takenaka et al. (2001) |
| | Geometric mean diameter: 18–19 nm; total number concentration: 6.64 × 10$^5$ to 2.85 × 10$^6$ particles/cm$^3$; surface area: 1.08 × 10$^9$ to 6.61 × 10$^9$ nm$^2$/cm$^3$ | Male and female Sprague–Dawley rats (Slc:SD) were exposed (6 h/day, 5 days/week, for 13 weeks), via inhalation, to low, middle, and high dose (49–513 μg/m$^3$, 0.6 × 10$^6$ to 3.0 × 10$^6$ particles/cm$^3$, 1.0 × 10$^9$ to 5.0 × 10$^9$ nm$^2$/cm$^3$) | Ag was determined in blood at the end of the exposure period (13 weeks) | Ag concentrations in male and female rats (ng/g): 0.68 ± 0.08–0.85 ± 0.14 (low dose); 1.82 ± 0.20–2.10 ± 0.22 (middle dose); 4.31 ± 0.37–6.86 ± 0.6 (high dose); 0.09 ± 0.02–0.05 ± 0.01 (control) | | Sung et al. (2009) |
| | Diameters of Ag-NPs ranged from 20 to 30 nm | Ag-NPs collected through personal sampling (159–350 min) on mixed cellulose ester filters. Workers were exposed to Ag concentrations of 0.35 and 1.35 μg/m$^3$ | Ag was detected in blood samples collected at the end of the work shift | Ag concentrations in the 2 blood samples were: 0.034 and 0.03 μg/dl | | Lee et al. (2012a, b) |
| | Ag-NPs had diameters ranging from 52.7 to 70.9 nm (average 60 nm) | 40 male and female Sprague–Dawley rats were treated with 28-day repeated oral administration at low, middle and high dose: 30–1,000 mg/kg/day | Ag was determined in blood at the end of the exposure period | Ag concentrations in male and female rats (mg/g): 0.18 ± 0.0 and 0.16 ± 0.0 (low dose); 0.43 ± 0.1 and 0.44 ± 0.1 (middle dose); 0.80 ± 0.2 and 0.70 ± 0.3 (high dose); 0.01 ± 0.01 and 0.00 ± 0.00 (control) | | Kim et al. (2008) |
| | Citrate-coated Ag-NP's diameter: 7.9 ± 0.95 nm; mean particle surface area: 7.53 × 10$^2$ nm$^2$/particle; mean particle mass 2 × 10$^{-17}$ g; mean particle volume:1.9 × 10$^3$ nm$^3$ | Male SD rats were treated with a single oral administration of either 1 or 10 mg/kg of Ag-NPs | Ag was determined in blood at 10 min and at 1, 2, 4, 8, 24, 48, and 96 h after treatment | Oral administration of 1 mg/kg: Elevated Ag concentrations from 2 to 24 h, no detection after 48 h. Oral administration of 10 mg/kg: Peak concentration at 8 h | | Park et al. (2011) |
| | Ag-NPs and PVP-coated Ag-NPs; Average particle core size: 17.7 ± 3.3 (Ag-NPs) and 12.1 ± 8 nm (PVP-coated Ag-NPs) | Male Sprague–Dawley rats were exposed daily for 28 days by oral gavage to 90 mg/kg body weight | Ag was determined in blood weekly until day 28 and on days 36 and 84 | The ratio between the Ag concentrations in blood (μg/kg) and the daily exposure dose (mg/kg) was ~2 | | Van der Zande et al. (2012) |
| | Ag-NPs were produced in the presence of PVP; Hydrodynamic size: 14 ± 2–50 ± 9 nm | Female Wistar Hannover Galas rats were exposed twice a day (12.6 mg/kg) for 28 days by oral gavage | Ag was determined 24 h after the end of treatment | Ag concentration: ~0.1 μg/g | Ag in plasma | Loeschner et al. (2011) |

**Table 1** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Au-NPs | Individual Au-NPs were spherical and electron dense with diameters of 5–8 nm | 16 male Wistar-Kyoto rats were exposed, via inhalation, for 6 h (88 μg/m$^3$, 4 × 10$^6$ particles/cm$^3$ | Au in blood was measured on days 0, 1, 4, and 7 after exposure | Au concentrations(μg/l): 0.69 ± 0.42 (day 0); 0.73 ± 0.29 (day 1); 0.41 ± 0.12 (day 4); 0.40 ± 0.13 (day 7); controls: 0.1 ± 0.07 | Au in blood | Takenaka et al. (2006) |
| | Median size of 76–79 nm and number concentrations of 2 × 10$^6$/cm$^3$ | Male Wistar rats were exposed via inhalation for 6 h (5 and 15 consecutive days) to 2 × 10$^6$/cm$^3$ | Au in blood was measured at the end of exposure | Au concentrations: 4,930 ± 1,611 ng/g (5 days exposure) and 18,747 ± 0.923 ng/g (15 days exposure); controls: 5,830 ± 3,020 ng/g | | Yu et al. (2007) |
| | Au-NPs were either in solid form (1.4 nm) or in aqueous suspension (18 nm) | Intratracheal instillation in female Wistar-Kyoto rats of 26.5 μg/50 ml (1.4 nm) and 2.7 μg/50 ml (18 nm) | NP content in blood was evaluated 24 h after treatment | Au concentrations: <0.1 % (18 nm) and 0.6 ± 0.2 % (1.4 nm) of the instilled dose | | Semmler-Behnke et al. (2008) |
| | Diameters: 7 and 20 nm; number concentrations: 1.44 × 10$^6$ and 1.27 × 10$^6$ particles/cm$^3$ | Inhalation exposure (6 h/day; 5 days/week for 3 weeks) of 21 male Wistar rats. 7 nm NPs: 0.086 ± 0.007–0.912 ± 0.07: 20 nm NPs: 0.053 ± 0.005–0.565 ± 0.05 mg/m$^3$ | Au was detected in blood 2 days after the end of treatment | Au concentrations (ng/g): 5.6 ± 1.1 (7 nm); <0.18 (limit of detection) (20 nm); <0.18 (limit of detection) (controls) | | Balasubramanian et al. (2013) |
| | Diameters: 4, 10, 28 and 58 nm | BALB/c mice were exposed for 7 days to 2 × 10$^5$ ng Au/g water in drinking water | Au was measured in blood 12 h after end of exposure | Au concentrations (ng/g): 6.83 ± 0.69 (4 nm); 0.77 ± 0.07 (10 nm); 0.47 ± 0.20 (28 nm); −0.09 ± 0.10 ng/g (58 nm) | | Hillyer and Albrecht (2001) |
| $^{192}$Ir-NPs | Diameters (nm):15–20 and 70–80; Number concentrations (n/cm$^3$): 3 and 5 × 10$^7$; mass concentrations (mg/m$^3$): 0.2 and 6 | Twelve male WKY/Kyo@Rj rats were ventilated during 1 h via an endotracheal tube | $^{192}$Ir fraction was detected 24 h after inhalation | Fractional 24 h retention was ~0.03 for 20-nm-sized NPs and 0.001 for 80-nm-sized NPs | Ir in blood | Kreyling et al. (2009) |
| Metal oxide nanoparticles | | | | | | |
| Eu-doped-$Gd_2O_3$-NPs | NPs are approximately spherical, and the majority had a diameter of 80–100 nm | Oropharyngeal aspiration in NIH Swiss mice of 40 μl of 0.160 ± 0.02 g/l Eu/Gd suspended in water | Eu doped-$Gd_2O_3$-NPs levels were measured 24 h after exposure | Eu-$Gd_2O_3$-NP mass recovery: 1.3 ± 0.8 ng (<0.1 % of delivered dose) | Eu and Gd in blood | Abid et al. (2013) |
| ZnO-NPs | ZnO-NPs were spherical shaped with diameters of 20 and 70 nm | 18 male and female Sprague–Dawley rats were treated with a single dose of 50–2,000 mg/kg of ZnO-NPs by oral gavage | Zn was detected in plasma at 0.5–96 h post exposure | 20 nm NPs: 50 mg/kg dose increased Zn level at 0.5–2.0 h; Peak levels with 300 and 2,000 mg/kg doses at 6–24 h; similar results with 70 nm NPs | Zn in plasma | Baek et al. (2012) |

**Table 1** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| ZnO-NPs and $TiO_2$-NPs | ZnO-NPs and $TiO_2$-NPs: primary size: 89.3 ± 44.7 and 26.4 ± 6.1 nm; hydrodynamic size: 201.8 ± 17.2 and 37.8 ± 0.4 nm; surface area: 60 ± 10 and 50 ± 15 $m^2/g$ | Male and female Sprague–Dawley rats were exposed (oral administration) for 13 weeks (7 days/week) to $TiO_2$-NPs (260.4–1,041.5 mg/kg/day) and ZnO-NPs (134.2–536.8 mg/kg/day) | Ti and Zn levels were determined 24 h after the end of treatment | Zn concentrations (μg/g) in male and female rats: ~1.7–4.8 and ~1–4.3; Ti concentrations (μg/g) in male and female rats: ~0.38–0.45 and ~0.48–0.52 | Zn and Ti in blood | Cho et al. (2013) |
| Sunscreen containing $^{68}$ZnO-NPs | Diameter: 19 nm | Formulations were applied on the back skin of male and female human volunteers twice day (4.3 mg/$cm^2$) for a 5-day summer period | $^{68}$Zn was measured 8 days before administration, after the last application and 6 days after the end of the trial | $^{68}Zn/^{64}Zn$: increased with exposure and continued to increase post exposure. $^{68}$Zn tracer: 6–31 μg $\Delta^{68}$Zn % increase: 0.42 for post-exposure, 0.23 on the last day | Zn in blood | Gulson et al. (2010) |
| | Diameter: 30 nm | Formulations were applied on the back skin of male and female human volunteers twice a day (2 mg/$cm^2$) for a 5-day winter period | $^{68}$Zn was measured prior to application, and at regular intervals up to 50 days post trial | $^{68}Zn/^{64}Zn$: increased over time with the highest value at 14 days from the first blood sampling $^{68}$Zn tracer: 12–35 μg | | Gulson et al. (2012) |
| $^{59}Fe_2O_3$-NPs | Particle size: 22 ± 5 nm; Hydrodynamic diameter: 144 ± 36 nm; Surface area: 53.27 $m^2/g$ | 48 male Sprague–Dawley rats instilled with 40 μl of $^{59}Fe_2O_3$ NPs suspension solution (4 mg/rat) | $^{59}$Fe was determined at 10–60 min, 2–8 h and 1–50 days | $^{59}$Fe levels (ng/ml): 54.7 ± 16.5 (10 min); 6.05 ± 1.95 (7 days); ~1.3 (50 days) | Fe in blood | Zhu et al. (2009) |
| $^{141}CeO_2$-NPs | Particle size: 6.6 ± 0.9 nm; hydrodynamic mean diameter: 12.8 ± 2.2 nm; Surface area: 86.85 $m^2/g$ | 42 male Wistar rats were exposed to $^{141}CeO_2$-NPs via intratracheal instillation (0.1 ml of a solution of 2 mg/ml $^{141}CeO_2$-NP in water) | NPs were detected at 10 and 30 min, 1, 2, 4, 6 and 24 h and, 7 and 28 days after exposure | $^{141}$Ce concentrations: ~0.0001 μg/g (10 min); ~0.0006 μg/g (6 h); ~0.00025 (24 h); ~0.00062 (7 days); ~0.0023 (28 days) | Ce in blood | He et al. (2010) |
| Other nanoparticles | | | | | | |
| Polystyrene latex spheres | Surface carboxylated 20 nm and 100 nm polystyrene latex spheres | Single and repeated (10) pharyngeal aspiration in female F344 rats. 20 nm NPs: $1 \times 10^{14}$, 44 μg/rat and $4 \times 10^{14}$, 176 μg/rat; 100 nm NPs: $5 \times 10^{12}$, 276 μg/rat and $2.2 \times 10^{13}$, 1200 μg/rat | Polystyrene latex spheres were measured 1 h and 1, 7, 28, 60, 90 and 120 days after exposure | Single treatment: only 20-nm spheres detected on days 1 and 7; Repeated treatment: 20-nm spheres detected on days 1 and 7; 100-nm spheres detected from days 7 to 120 | Number of polystyrene latex spheres in blood | Sarlo et al. (2009) |

**Table 1** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Inorganic/organic hybrid NPs: CdSe (ZnCdS); CdTe (ZnS); silica/CdSe (ZnS); organic NPs: HAS; mPEG20k; PS-PAA | Hydrodynamic diameter of inorganic/organic hybrid NPs: 5–320 nm Hydrodynamic diameter of organic NPs:7–270 nm | NPs were administered to male Sprague–Dawley rats at the dose of 10 pmol/g through intrapulmonary instillation | Fluorescent and $^{99m}$Tc-conjugated NPs were used to evaluate blood levels during 1 h after treatment | 5- and 27-nm-sized hybrid NPs were detected in blood after a short time (<20 min) at concentrations inversely proportional to diameter | Fluorescent and $^{99m}$Tc-conjugated NPs in blood | Choi et al. (2010) |

understanding of the NP kinetics for the development of suitable biomonitoring strategies, are not directly applicable to the occupational health practice.

Metal recovery in blood could be also affected by the different durations of treatment (Yu et al. 2007). Significant Au accumulation in blood of rats exposed to Au-NPs was detected after 15 days of exposure but not after a shorter 5-day period, suggesting that this biomarker may better reflect the body burden of the element as influenced by homeostatic processes, than the current degree of exposure. These results may also be influenced by the half-life of NPs in blood, itself affected by their dissolution rate in biological material. When, investigating $Fe_2O_3$-NP kinetics, Zhu et al. (2009) revealed a long 22.8 day elimination half-life of $^{59}$Fe in blood, implying that a persistent NP circulation occurred. This may be due to a slow release of NPs from the lung, as well as to their limited dissolution in ions, which are characterized by a shorter half-life. These results are particularly important to select the time when samples should be taken, based on the duration of exposure they represent, as well as to decide how often sampling should be repeated for a suitable biological monitoring strategy.

Concerning the percentage of the total amount of Au measured in blood after NP inhalation, a value of <1 % was calculated (Takenaka et al. 2006), as was also confirmed when labeled NPs were recovered in the same matrix (Semmler-Behnke et al. 2008; Abid et al. 2013). For the occupational health practice, this small value supports the need to refine the current analytic techniques, in order to overcome the difficulties to quantify trace metals in biological media.

At present, data regarding cutaneous exposure to NPs in workplaces are not available. However, the results of two preliminary studies demonstrated that small amounts of Zn from the stable isotope $^{68}$ZnO-NPs in sunscreen were detectable in blood after application on healthy human skin (Gulson et al. 2010, 2012) (Table 1). Although obtained through a mode of exposure rather unrealistic for the occupational scenario, these findings should still be viewed with careful attention considering the relevance of the dermal route of exposure particularly for workers handling NPs in liquid media.

As regards the metallic or metal oxide NP exposure via the oral route, it could be evaluated by measuring the levels of the elemental metal or metal oxide in blood (Table 1). In fact, when different types of NPs, such as Ag-NPs (Hillyer and Albrecht 2001, Kim et al.

2008; Loeschner et al. 2011; Park et al. 2011; van der Zande et al. 2012), ZnO-NPs (Baek et al. 2012; Cho et al. 2013), $TiO_2$-NPs (Cho et al. 2013), $Al_2O_3$-NPs (Balasubramanyam et al. 2009), and $MnO_2$-NPs (Singh et al. 2013a) were orally administered to animals, increased contents of these potential biomarkers were detected in blood or plasma compared with controls. In several cases, such an increase was in a manner directly dependent on the administered dose (Kim et al. 2008; Park et al. 2011; Balasubramanyam et al. 2009; Baek et al. 2012; Cho et al. 2013; Singh et al. 2013a). However, it is important to be aware that these dose–concentration relationships were observed under unrealistically high doses of treatment and that the time–concentration curves determined with Ag (Park et al. 2011)- and ZnO-NPs (Baek et al. 2012) were also highly dependent on the administered dose. Various parameters, therefore, could influence the kinetics of NP adsorption and consequently the timing of bloodstream peaks, thus affecting the most effective time-point to measure metal concentrations in blood.

Furthermore, the relevance of solubility (Cho et al. 2013) and surface functionalization (Park et al. 2011) as key features in determining NP fate and outcomes in biological systems and consequently their biological monitoring results has already been demonstrated. In fact, while comparing oral ZnO- and $TiO_2$-NP treatments, the blood Zn concentration was almost tenfold higher than the Ti levels, possibly due to their different NP biopersistences in biological fluids (Cho et al. 2013). Moreover, Park et al. (2011) found a low level of Ag in blood after oral administration of citrate-coated Ag-NPs because of their low bioavailability, related maybe to the hydrophilic coating of the NPs which partially prevented their gastrointestinal adsorption. All these physicochemical properties should be taken into account in-depth while assessing an appropriate biomarker of exposure in blood.

*Urine*

Urine has been considered as an excellent biological material of choice to be employed in biomonitoring investigations. It is easy to obtain in sufficient amounts, even under routine conditions, and without unacceptable discomfort or health risks for the workers. *In vivo* experiments demonstrated the renal system as an effective excretion apparatus for different types of NPs (Kreyling et al. 2002; Semmler-Behnke et al. 2008; Cho et al. 2009; Lee et al. 2012b; Lozano et al. 2012; Yamago et al. 1995). In the occupational biomonitoring study carried out by Lee et al. (2012a), detectable concentrations of Ag were determined in the urine from a worker after exposure to Ag-NPs. However, from the analysis of these results, the same critical issues, detailed above for blood data, could be pointed out for urine. The urinary detection of the elemental metal content after metallic Au- or metal oxide $^{59}Fe_2O_3$-NP exposure via the respiratory tract has been evaluated as a possible biomarker of exposure (Zhu et al. 2009; Balasubramanian et al. 2013) (Table 2). Interestingly, the kinetic curve of $^{59}Fe$ in urine showed a temporal trend, with a peak during the first week post-exposure and a slow decrease thereafter, comparable to that found for $^{59}Fe$ in blood (Zhu et al. 2009). This good correlation between $^{59}Fe$ counts in the two matrices suggests a suitable role for the urinary metal as a current exposure indicator. Conversely, little or no amount of Au was detected in urine after a sub-chronic Au-NP inhalation, even as a significant Au rise could be detected in blood, and a clear Au deposition was present in kidney tissue (Balasubramanian et al. 2013). Inhaled Au-NPs were able to reach the blood but not able to pass the kidney filtration barrier. This is an interesting finding, suggesting that the chemical composition of NPs could influence the recovery of the metal in urine and, therefore, limit the possibility to monitor the internal dose through this matrix. In a speculative manner, as has been viewed for cadmium (Klotz et al. 2013; Prozialeck and Edwards 2010), Au metal accumulation could be detected in kidney tissues (Balasubramanian et al. 2013). Unfortunately, a long-term release of Au in urine remains to be verified. Moreover, the surface charge, acquired by inhaled Au-NPs while being adsorbed and translocated into the organism, should be taken into account as possibly affecting kidney filtration (Balasubramanian et al. 2013; Semmler et al. 2004). Future research should focus on these aspects to define whether and under which conditions urinary Au can be indicative of the cumulative Au-NP long-term exposure.

Urine was demonstrated to be a suitable matrix to determine dermal exposure to $^{68}ZnO$-NPs (Gulson et al. 2010, 2012), as $^{68}Zn$ values in urine showed, in fact, a significant increase after exposure; however, it is not clear whether $^{68}Zn$ was in the form of ZnO-NPs or of ionic Zn, a relevant detail for the causation of toxic effects, as described below (Table 2).

**Table 2** Possible biomarkers of exposure (in urine) for different types of NPs

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Metal nanoparticles | | | | | | |
| Radiolabeled $^{192}$Ir-NPs | Count median diameters: 15 and 80 nm; number concentrations:$3 \times 10^7$ and $0.5 \times 10^7$ n/cm$^3$; mass concentrations: 0.2 and 6 mg/m$^3$ | Male WKY/NCrl BR rats were exposed to 0.2 (15-nm NPs) and 6.0 (80-nm NPs) mg/cm$^3$ via endotracheal intubation; 18 rats were treated through intratracheal instillation (10 kBq, 25 μg) | Ir was detected 6 h and 7 days post-exposure | An Ir fraction of $0.02 \pm 0.003$ and 0.001 was excreted via the urine in the inhalation and instillation study, respectively | Ir in urine | Kreyling et al. (2002) |
| Au-NPs | Diameters: 7 and 20 nm; Number concentrations: $1.44 \times 10^6$ and $1.27 \times 10^6$ particles/cm$^3$ | Inhalation exposure (6 h/day; 5 days/week for 3 weeks) of 21 male Wistar rats. 7-nm NPs:$0.086 \pm 0.007$–$0.912 \pm 0.07$: 20-nm NPs: $0.053 \pm 0.005$–$0.565 \pm 0.05$ mg/m$^3$ | Au measured at 6 time points (1–15 days) | Au concentrations (ng/g): <0.18–$0.3 \pm 0.1$ (7 nm); <0.18–$0.2 \pm 0.2$ (20 nm); $0.3 \pm 0.4$ (controls) | Au in urine | Balasubramanian et al. (2013) |
| Ag-NPs | Citrate-coated Ag-NPs diameter: $7.9 \pm 0.95$ nm; mean particle surface area: $7.53 \times 10^2$ nm$^2$/particle; mean particle mass $2 \times 10^{-17}$ g; mean particle volume:$1.9 \times 10^3$ nm$^3$ | Male SD rats were treated with a single oral administration of either 1 or 10 mg/kg of Ag-NPs | Ag was determined in the urine at 24 h after exposure | Oral administration of 1 and 10 mg/kg: $0.003 \pm 0.005$ and $0.042 \pm 0.031$ μg/ml | Ag in urine | Park et al. (2011) |
| | Ag-NPs were produced in presence of PVP; Hydrodynamic size: $14 \pm 2$–$50 \pm 9$ nm | Female Wistar Hannover Galas rats were exposed twice a day (12.6 mg/kg) for 28 days by oral gavage | Ag was determined in urine collected at the 3rd week of treatment | Ag (% of 24 h intake): $0.10 \pm 0.05$ μg ($0.005 \pm 0.003$ %) | | Loeschner et al. (2011) |
| Metal oxide nanoparticles | | | | | | |
| $^{59}Fe_2O_3$-NPs | Particle size: $22 \pm 5$ nm; Hydrodynamic diameter: $144 \pm 36$ nm; Surface area: 53.27 m$^2$/g | 48 male Sprague–Dawley rats instilled with 40 μl of $^{59}Fe_2O_3$ NPs suspension solution (4 mg/rat) | $^{59}$Fe measured at 4–12 h and 1–50 days post exposure | Peak level: $0.81 \pm 0.50$ μg/ml (day 3); 0.7 % of the dose excreted up to 50 day; total $^{59}$Fe excreted: 26.8 μg | Fe in urine | Zhu et al. (2009) |
| $Fe_2O_3$-NPs | Size: $29.75 \pm 1.87$ nm; surface area: 38.02 m$^2$/g | Albino Wistar female rats were exposed to 500, 100 and 2,000 mg/kg of NPs by oral gavage | Fe measured in urine collected from 0 to 72 h after treatment | Fe concentrations (μg/g): ~80–50 (500 mg/kg); ~170–75 (1,000 mg/kg); ~345–115 (2,000 mg/kg); ~10 μg/g (controls) | | Singh et al. (2013b) |

**Table 2** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| ZnO-NPs | ZnO-NPs were spherically shaped with diameters of 20 and 70 nm | 18 male and female Sprague–Dawley rats were treated with a single dose of 50–2,000 mg/kg of ZnO-NPs by oral gavage | Zn was detected in 4–10 h and 1–14 days post oral administration | 20-nm Zn levels in male–female rats (50, 300, 2,000 mg/g): 0.07, 0.28, 1.18–0.05, 0.19, 0.67 mg; 70-nm Zn levels in male and female rats (50, 300, 2,000 mg/g): 0.09, 0.26, 1.20–0.07, 0.19, 0.71 mg | Zn in urine | Baek et al. (2012) |
| Sunscreen containing $^{68}$ZnO-NPs | Diameter: 19 nm | Formulations were applied on the back skin of male and female human volunteers twice day (4.3 mg/cm$^2$) for a 5-day summer period | $^{68}$Zn was measured 8 days before administration, after the last application and 6 days after the end of the trial | $\Delta^{68}$Zn %: values peaked at the end of the exposure phase (day 5) | | Gulson et al. (2010) |
| | Diameter: 30 nm | Formulations were applied on the back skin of male and female human volunteers twice a day (2 mg/cm$^2$) for a 5-day winter period | $^{68}$Zn measured in urine of the morning and then at least 3 times each treatment day. Urine samples were collected for up to 50 days post-trial | $^{68}$Zn/$^{64}$Zn abundance: variations across individuals with the maximum value at day 5. Increase in female was 6 times greater than male | | Gulson et al. (2012) |
| ZnO-NPs and $TiO_2$-NPs | ZnO-NPs and $TiO_2$-NPs: primary size: 89.3 ± 44.7 and 26.4 ± 6.1 nm; hydrodynamic size: 201.8 ± 17.2 and 37.8 ± 0.4 nm; surface area: 60 ± 10 and 50 ± 15 m$^2$/g | Male and female Sprague–Dawley rats were exposed (oral administration) for 13 weeks (7 days/week) to $TiO_2$-NPs (260.4–1,041.5 mg/kg/day) and ZnO-NPs (134.2–536.8 mg/kg/day) | Ti and Zn concentrations were determined 24 h after treatment | Zn concentrations (μg/g) in male and female rats: ~0.4–13 and ~0.8–4.0; Ti concentrations (μg/g) in male and female rats: ~0.65–0.55 and ~0.5–0.75 | Zn and Ti in urine | Cho et al. (2013) |

As regards the oral route of exposure, metal urinary excretion was demonstrated to be a suitable biological marker of Ag (Park et al. 2011; Loeschner et al. 2011)-, ZnO (Baek et al. 2012; Cho et al. 2013), $MnO_2$ (Singh et al. 2013a)-, and $Fe_2O_3$-NP exposures (Singh et al. 2013b) (Table 2). In comparison, when $Al_2O_3$-NPs were administered via oral gavage to rats, a significant increase of the metal oxide content in urine was detected (Balasubramanyam et al. 2009). A positive dose–response trend was reported in different cases (Cho et al. 2013; Singh et al. 2013a, b). Conversely, no significant increase in Ti content could be detected in urine after the oral administration of $TiO_2$-NPs compared with controls (Cho et al. 2013).

The comparable dissolution properties of both ZnO- and Ag-NPs into ions have been speculated to contribute to their quantitatively similar excretion pathways, which resulted to be <1.5 % for both NP types (Loeschner et al. 2011, Baek et al. 2012). Orally administered ZnO-NPs are expected to readily dissolve under gastric pH conditions to form ions (Baek et al. 2012). This information appears to be important also in consideration of the role of dissolved $Zn^{+2}$ ions in the NP-induced toxicity (Brunner et al. 2006; Song et al. 2010; Xia et al. 2008). Moreover, NP size seemed to be inversely responsible for the rapid urinary clearance of ZnO-NPs (Baek et al. 2012), thus affecting the adequate timing of biological monitoring samplings. Therefore, the dissolution coefficient and other physicochemical properties of NPs in biological fluids should be thoroughly investigated and possibly quantified, while aiming to assess occupational risks from experimental exposure data.

### *Feces*

Macrophage-mediated mucociliary escalation followed by fecal excretion is a pathway for clearing the inhaled NPs from the body (Semmler-Behnke et al. 2007). This pathway has been demonstrated to occur for different labeled NPs (Kreyling et al. 2002; Sarlo et al. 2009; Abid et al. 2013; He et al. 2010). Although it is rather difficult to routinely employ feces as a suitable biological matrix for occupational biomonitoring, on the account of the aforementioned clearance mechanism, the measurement of the elemental metal content in feces could still be useful to evaluate the recent/current exposure to metal-NPs, as reported for Au-NPs (Balasubramanian et al. 2013) and $^{59}Fe_2O_3$-NPs (Zhu et al. 2009) (Table 3). In addition, the peculiar patterns of fecal excretion after exposure to differently sized Au-NPs, in terms of quantity recovered (greater after 20-nm- compared to 7-nm-sized Au-NPs) and excretion timing (rapidly decreasing *vs* relatively stable during the 7 days after 20- and 7-nm-sized Au-NPs, respectively) support the idea that the primary size of inhaled NPs could affect their toxicokinetics, thus modulating the results of the biological monitoring.

When $^{14}C$-labeled water-soluble fullerenes (Yamago et al. 1995), $CeO_2$-NPs (Hirst et al. 2013), or silicon carbide (SiC)-NPs (Lozano et al. 2012), were administered to rats via the oral route, NPs were mostly not absorbed by the gastrointestinal tract and therefore excreted via the feces. Elimination of NPs could also be assessed through a significant dose-dependent increase in the content of the elemental metal in feces, as detected after acute-to-sub-chronic exposure to metal or metal oxide NPs, such as Ag-NPs (Park et al. 2011; Loeschner et al. 2011; van der Zande et al. 2012), $TiO_2$-NPs (Cho et al. 2013), ZnO-NPs (Baek et al. 2012; Cho et al. 2013), $MnO_2$-NPs (Singh et al. 2013a), $Fe_2O_3$-NPs (Singh et al. 2013b), and mesoporous silica-NPs (Fu et al. 2013) (Table 3). These measurements in feces may be interpreted, overall, as a tentative indirect method to assess the effective bioavailability of the administered NPs.

### Biologically effective dose

The so-called biologically effective dose biomarkers constitute an important subclass of the exposure biomarkers. They represent the products of the interaction between a reactive chemical or a metabolite and a target molecule, and are known also as adducts. As far as we know, no studies are available as to the potential use of NP-derived adducts as biologically effective dose biomarkers in vivo in humans or animals. Research in this area is very much necessary, as biologically effective dose biomarkers may help one to explain the shape of the dose–response curve, thus making risk assessment more accurate.

## Biomarkers of effect

A biomarker of effect is a measurable biochemical, structural, functional, behavioral, or any other kind of

**Table 3** Possible biomarkers of exposure (in feces) for different types of NPs

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Metal nanoparticles | | | | | | |
| Radiolabeled $^{192}$Ir-NPs | Count median diameters: 15 and 80 nm; number concentration:3 × $10^7$ and 0.5 × $10^7$ n/cm$^3$; mass concentration: 0.2 and 6 mg/m$^3$ | Male WKY/NCrl BR rats were exposed to 0.2 (15-nm NPs) and 6.0 (80-nm NPs) mg/cm$^3$ via endotracheal intubation; 18 rats were treated through intratracheal instillation (10 kBq, 25 μg) | Ir was detected 6 h–7 days post-exposure | An Ir fraction of 0.47 ± 0.06 (15 nm), 0.36 ± 0.07 (80 nm) and of 0.35 was cleared by feces in the inhalation and instillation study, respectively | Ir in feces | Kreyling et al. (2002) |
| Au-NPs | Diameters: 7 and 20 nm; Number concentrations: 1.44 × $10^6$ and 1.27 × $10^6$ particles/cm$^3$ | Inhalation exposure (6 h/day; 5 days/week for 3 weeks) of 21 male Wistar rats. 7-nm NPs:0.086 ± 0.007–0.912 ± 0.07: 20-nm NPs: 0.053 ± 0.005–0.565 ± 0.05 mg/m$^3$ | Au measured at 6 time points (1–15 days) | Au concentrations (ng/g): 4.4 ± 1.7–25.7 ± 31.3 (7 nm); 35.4 ± 35.9–164.0 ± 92.6 (20 nm); <0.18 (limit of detection) (controls) | Au in feces | Balasubramanian et al. (2013) |
| Ag-NPs | Citrate-coated Ag-NPs - Diameter: 7.9 ± 0.95 nm; mean particle surface area: 7.53 × $10^2$ nm$^2$/particle; mean particle mass 2 × $10^{-17}$ g; mean particle volume:1.9 × $10^3$ nm$^3$ | Male SD rats were treated with a single oral administration of either 1 or 10 mg/kg of Ag-NPs | Ag was determined in the feces at 24 h after exposure | Oral administration of 1 and 10 mg/kg: 377.6 ± 173.8 and 1,663.1 ± 522.2 μg/g | Ag in feces | Park et al. (2011) |
| | Ag-NPs were produced in presence of PVP; Hydrodynamic size: 14 ± 2–50 ± 9 nm | Female Wistar Hannover Galas rats were exposed twice a day (12.6 mg/kg) for 28 days by oral gavage | Ag was determined in urine collected at the 3rd week of treatment | Ag concentration (% of 24 h intake): 1,190 ± 430 μg (63 ± 23 %) | | Loeschner et al. (2011) |
| | Ag-NPs and PVP-coated Ag-NPs; Average particle core size: 17.7 ± 3.3 (Ag-NPs) and 12.1 ± 8 nm (PVP-coated Ag-NPs) | Male Sprague–Dawley rats were exposed daily for 28-days by oral gavage to 90 mg/kg body weight | Ag was determined weekly up to day 28 and on days 36 and 84 | Ag: ~1.0 × $10^4$ ratio between the concentration in feces and the daily exposure dose | | Van der Zande et al. (2012) |

**Table 3** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Metal oxide nanoparticles | | | | | | |
| $^{59}Fe_2O_3$-NPs | Particle size: 22 ± 5 nm; Hydrodynamic diameter: 144 ± 36 nm; Surface area: 53.27 $m^2/g$ | 48 male Sprague–Dawley rats instilled with 40 µl of $^{59}Fe_2O_3$ NPs suspension solution (4 mg/rat) | $^{59}Fe$ measured at 4–12 h and 1–50 days post exposure | Peak level: 504 ± 143 µg/ml (day 1); 73.4 % of the dose excreted up to 50 day; total $^{59}Fe$ excreted: 2,935 µg | Fe in feces | Zhu et al. (2009) |
| $Fe_2O_3$-NPs | Size: 29.75 ± 1.87 nm; Surface area: 38.02 $m^2/g$ | Albino Wistar female rats were exposed to 500, 100 and 2,000 mg/kg of NPs by oral gavage | Fe measured in urine collected from 0 to 72 h after treatment | Fe concentrations (µg/g): ~5,000–2,000 (500 mg/kg); ~13,500–6,000 (1,000 mg/kg); ~34,000–19,000 (2,000 mg/kg); no detectable in controls | | Singh et al. (2013b) |
| $^{141}CeO_2$-NPs | Particle size: 6.6 ± 0.9 nm; Hydrodynamic mean diameter: 12.8 ± 2.2 nm; Surface area: 86.85 $m^2/g$ | 42 male Wistar rats were exposed to $^{141}CeO_2$-NPs via intratracheal instillation (0.1 ml of a solution of 2 mg/ml $^{141}CeO_2$-NP in water) | NPs were detected from 6 h to 28 days after exposure | $^{141}CeO_2$: 35–45 µg/g Calculated clearance rate: from 1.00 to 0.98 µg/day at 4–7 days post-exposure. Daily content in feces was >0.8 µg/day | Ce in feces | He et al. (2010) |
| CeO-NPs | Diameter of individual NPs: 3–5 nm | 36 CD-1 mice were exposed to 2 or 5 doses of 0.5 mg/kg of NPs by oral gavage | Clearance was measured 1 week after exposure | 98 % clearance through feces | | Hirst et al. (2013) |
| Eu doped-$Gd_2O_3$-NPs | NPs were nearly spherical, and the majority had a diameter of 80–100 nm | Oropharyngeal aspiration in NIH Swiss mice of 40 µl of 0.160 ± 0.02 g/l Eu/Gd suspended in water | Eu doped-$Gd_2O_3$-NPs levels were measured 24 h after exposure | Eu-$Gd_2O_3$-NP mass recovery: 1,306 ± 301 ng (20.4 % of delivered dose) | Eu and Gd in feces | Abid et al. (2013) |
| ZnO-NPs | ZnO-NPs were spherically shaped with diameters of 20 and 70 nm | 18 male and female Sprague–Dawley rats were treated with a single dose of 50–2,000 mg/kg of ZnO-NPs by oral gavage | Zn was detected in 4–10 h and 1–14 days post oral administration | 20-nm Zn levels in male–female rats (50, 300, 2,000 mg/g): 3.59, 28.27, 227.21–2.52, 21.24, 201.57 mg; 70-nm Zn levels in male and female rats (50, 300, 2,000 mg/g): 3.52, 26.67, 230.04–2.69, 22.76, 202.01 mg | Zn in feces | Baek et al. (2012) |

**Table 3** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of exposure | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| ZnO-NPs and $TiO_2$-NPs | ZnO-NPs and $TiO_2$-NPs: primary size: 89.3 ± 44.7 and 26.4 ± 6.1 nm; hydrodynamic size: 201.8 ± 17.2 and 37.8 ± 0.4 nm; surface area: 60 ± 10 and 50 ± 15 $m^2/g$ | Male and female Sprague–Dawley rats were exposed (oral administration) for 13 weeks (7 days/week) to $TiO_2$-NPs (260.4–1,041.5 mg/kg/day) and ZnO-NPs (134.2–536.8 mg/kg/day) | Ti and Zn concentrations were determined 24 h after treatment | Zn concentrations (μg/g) in male and female rats: ~100–2,500 and ~100–1,400; Ti concentrations (μg/g) in male and female rats: ~100–7,500 and ~100–7,000 | Zn and Ti in feces | Cho et al. (2013) |
| Other nanoparticles | | | | | | |
| Polystyrene latex spheres | Surface carboxylated 20 nm and 100 nm polystyrene latex spheres | Repeated (10) pharyngeal aspiration in female F344 rats. 20-nm NPs: $4 \times 10^{14}$, 176 μg/rat; 100-nm NPs: $2.2 \times 10^{13}$, 1,200 μg/rat | Polystyrene latex spheres were detected in after 1–120 days post exposure | Spheres (20 nm) detected on days 1 and 7 (0.5–>5 %); Spheres (100 nm) detected up to 120 days (0.5–>5 %) | Number of polystyrene latex spheres in feces | Sarlo et al. (2009) |
| Silicon carbide NPs | Average diameter: 36.2 ± 8.6 nm; Surface area: 30 $m^2/g$; | Female Sprague-Dawley rats were used for acute (0.5–600 mg/kg) and subacute (0.5 and 50 mg/kg) oral toxicity studies | Si levels measured in feces collected 1–3, and 1–28 days for acute and subacute exposure, respectively | Acute exposure-control: from 5,912 ± 263 (day 1) to 5,943 ± 293 (day 3); Treated rats: from 12,673 ± 331 (day 1) to 7,242 ± 288 (day 3); Subacute exposure-Control: from 8,720 ± 499 (day 1) to 7,561 ± 441 (day 28); Treated rats: from 15,574 ± 598 (day 1) to 20,106 ± 627 (day 28) | Si in feces | Lozano et al. (2012) |
| Mesoporous silica NPs | Average diameter: 110 nm; hydrodynamic diameter: 165 nm | Female ICR-mice were treated with oral administration of 500, 100 and 2,000 mg/kg of NPs | Si content was measured 24 h and 7 days after oral administration | Si levels (mg/kg): ~700 mg/kg (7 days); ~950 mg/kg (24 h); : ~600 mg/kg (controls) | | Fu et al. (2013) |

alteration in an organism that, according to its magnitude, can be associated with an established or potential health impairment or disease. A subclass of biomarkers of effect is represented by biomarkers of early disease, i.e., tests which are more closely indicative of a subclinical effect or even an early, reversible clinical response (Manno et al. 2010). Molecular biomarkers, obtained from biological samples, such as blood, urine, and tissues, constitute an objective indicator for correlating exposure with various physiological or pathological conditions or with variations of the disease state (Casado et al. 2008; Ferté et al. 2010). The use of biomarkers not only enables the early detection of a current disease or clinical condition, but also allows predictions to be made on the risk of acquiring the disease in the future. Therefore, biomarkers of effect for nanomaterials could be invaluable in predicting potential toxicity and establishing strategies for the development of safe nanomaterials' production and use (Higashisaka et al. 2011, 2012).

## Blood

Damage caused by NPs may elicit the activation of oxidative stress and/or inflammatory response. Indeed, human and animal studies aimed at evaluating early effects of NP exposure focused on the evaluation of oxidative insult markers and antioxidant enzyme activities, as well as on the expression of acute phase proteins or changes in lymphocyte subset distribution as potential early responses to NP toxicity, possibly applicable as potential early biomarkers of NP effects (Table 4).

As regards systemic evaluation of the oxidative stress and the anti-oxidant status induced by NP exposure, a recent occupational monitoring study demonstrated a lower anti-oxidant enzymatic activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) in plasma of workers engaged in the manufacture and/or application of nanomaterials compared with controls (Liou et al. 2012). Reductions in anti-oxidant defense were recently confirmed in experimental animals treated with silica NPs (Du et al. 2013; Liu and Sun 2013), multiwalled CNTs (Reddy et al. 2011) and Ag-NPs (Genter et al. 2012). A dose-dependent elevation of ROS and malondyaldehyde (MDA) levels in serum was also observed in these studies (Du et al. 2013; Reddy et al. 2011; Tiwari et al. 2011).

A number of potential biomarkers of inflammatory response were observed in human studies by Liou et al. (2012), who observed significantly higher fibrinogen and interleukin (IL)-6 levels in blood of exposed workers compared with controls. Moreover, human volunteers exposed to ultrafine particles from wood smoke, showed increased values of serum amyloid A (SAA), factor VIII, factor VIII/von Willebrand factor ratio and C reactive protein (CRP) compared with the clean air exposed controls (Barregard et al. 2006). The induction of an inflammatory response characterized by a significant dose- dependent rise in blood concentrations of pro-inflammatory cytokines, i.e., tumor necrosis factor (TNF)-$\alpha$, IL-1, IL-6 and interferon-$\gamma$, was demonstrated to occur in animals exposed acutely to sub-acutely to different types of metallic or metal oxide-NPs such as $TiO_2$ (Park et al. 2009)-, $CeO_2$ (Srinivas et al. 2011)-, $Fe_3O_4$ (Park et al. 2010a; Chen et al. 2010; Srinivas et al. 2012)-, Ag (Park et al. 2010b)-, and silica-NPs (Downs et al. 2012; Du et al. 2013; Lu et al. 2011), as well as with carbon-based NPs (Erdely et al. 2009). Interestingly, in some cases, cytokines became more sensitive, compared with regular blood biochemical and hematological parameters, toward NP-induced toxicity, supporting their potential role as early biomarkers of effect (Srinivas et al. 2011, 2012).

Acute phase proteins in blood could also be considered as interesting biomarkers of the NP-induced inflammatory response, as supported by a series of in vivo studies demonstrating an early increased expression of these proteins after treatment with silica-NPs (Higashisaka et al. 2011, 2012; Liu and Sun 2013), $CeO_2$-NPs (Nalabotu et al. 2011), CNTs (Erdely et al. 2009), as well as printex 90 carbon-based NPs (Bourdon et al. 2012). Particularly, haptoglobin, SAA, CRP and hemopexin have been identified as possible biomarkers of an acute phase reaction induced by silica NPs, being positively related to the administered dose and inversely dependent on the particle size (Higashisaka et al. 2011, 2012). Comparably, a positive dose-dependent increase in CRP levels, was determined by silica NPs, to a greater extent for the smallest particles (Du et al. 2013). However, although highly sensitive, these inflammatory biomarkers appear to be poorly specific for NP-induced damage as they are possibly influenced by other, nonoccupational factors. In this regard, the validity of these biomarkers may be

**Table 4** Possible biomarkers of effect for several types of NPs in different biological matrices

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of effect | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Biomarkers of effect in blood | | | | | | |
| Ag-NPs | Size: 25 nm | Male C57BL/6 J mice were exposed by intranasal instillation (single treatment) to 100 or 500 mg/kg of NPs | Reduced GSH level was measured at 1 and 7 days post instillation | Significant increase of GSH after 7 days | GSH | Genter et al. (2012) |
| | Sizes: 22, 42, 71, and 323 nm | Male and female ICR mice were exposed for 28 consecutive days to 0.25–1 mg/kg (cytokines) and to 1 mg/kg for 14 days (lymphocyte subsets and CD4 +/CD8 +) by oral administration | Cytokine levels, lymphocyte distribution and $CD^{4+}$/$CD^{8+}$ were assessed at the end of exposure | IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-β increased in dose dependent manner; Increased distribution of B and NK cells; reduced $CD^{4+}$/$CD^{8+}$ | Different types of cytokines; Lymphocyte subsets; $CD^{4+}$/$CD^{8+}$ | Park et al. (2010b) |
| | Sizes: 15–40 nm; shape: rounded | Wistar rats were injected intravenously at 5-day intervals with 4–40 mg/kg | ROS level was measured in serum at 1–5 weeks | Dose-dependent increase of ROS | ROS | Tiwari et al. (2011) |
| $CeO_2$-NPs | Size: 10.14 ± 0.76 nm | Male Sprague–Dawley rats were exposed to 1–7 mg/kg by intratracheal instillation | Serum proteins were measured at 28 days post exposure | Increase of acute phase proteins | Acute phase proteins | Nalabotu et al. (2011) |
| | Size: 55 nm; Surface area: 30–50 $m^2$/g; Crystallinity: cubic | 72 male and female Wistar rats were exposed (inhalation exposure) for 4 h to 641 mg/$m^3$ | Cytokine levels were assessed at 24, 48 h and 14 days post exposure | IL-1β, TNF-α, and IL-6 levels were elevated in 24 h and 14 days period in rats (M and F) | Pro-inflammatory cytokines | Srinivas et al. (2011) |
| $Fe_3O_4$-NPs | Size: 5.3 ± 3.6 nm | ICR male mice were exposed to 250, 500 μg/kg, and 1 mg/kg by intratracheal instillation | Cytokine levels, $CD^{4+}$/$CD^{8+}$ and lymphocyte distribution were assessed at 1–28 days post exposure | During 1–28 days period, proinflammatory cytokines, IL2, IL-12; Th2 cytokines; and TGF-β levels were elevated; Increased distribution of T cells; Increased $CD^{4+}$/$CD^{8+}$ | Different types of cytokines; Lymphocyte subsets; $CD^{4+}$/$CD^{8+}$ | Park et al. (2010a) |
| | Size: 48.18 ± 7.54 nm; Surface area: ≥40 $m^2$/g; Crystallinity: tetrahedral | 72 male and female Wistar rats were exposed (inhalation exposure) for 4 h to 640 mg/$m^3$ | Cytokine levels were assessed at 24, 48 h and 14 days post exposure | IL-1β, TNF-α, and IL-6 levels were elevated in 24 h and 14 days period in rats (M and F) | Pro-inflammatory cytokines | Srinivas et al. (2012) |
| | Sizes: 20.3–95.5 nm | 40 ICR mice were exposed to 300–1,200 mg/kg by single gastric perfusion | Lymphocyte subsets were investigated at 14 days post exposure | CD4 and CD8 subsets were increased; Increased $CD^{4+}$/$CD^{8+}$ | Lymphocyte subsets; $CD^{4+}$/$CD^{8+}$ | Wang et al. (2011) |
| | Size: 20 nm | Female and male ICR mice were exposed to 5.14–51.4 mg/kg by intravenous injection | Cytokine levels were assessed at 72 h post exposure | Low dose significantly increased the levels of IFN-γ, IL-2, IL-10, and IL-4 | Th1 and Th2 cytokines | Chen et al. (2010) |
| $TiO_2$-NPs | Size: ~20 nm; surface area: 50 $m^2$/g | ICR mice were exposed to 5–50 mg/kg (cytokines) and to 20 mg/kg (lymphocyte distribution and CD4 +/CD8 +) by single intratracheal instillation | Cytokine levels were assessed at 1–14 days post exposure; lymphocyte distribution and $CD^{4+}$/$CD^{8+}$ was evaluated 14 days post exposure | Significant increase of TNF-α, IL-6, IL-10, and IL-12 levels. Alterations of lymphocyte distribution; reduced $CD^{4+}$/$CD^{8+}$ | Th1 and Th2 cytokines; Lymphocyte subsets; $CD^{4+}$/$CD^{8+}$ | Park et al. (2009) |
| | Size: 5–6 nm | CD-1(ICR) mice were exposed, by intragastric administration (90 days) to 2.5–10 mg/kg | Lymphocyte subsets and $CD^{4+}$/$CD^{8+}$ were investigated at the end of exposure period | CD3, CD4, CD8, and B cell, natural killer cell subsets, were significantly decreased; reduced $CD^{4+}$/$CD^{8+}$ | Lymphocyte subsets; $CD^{4+}$/$CD^{8+}$ | Sang et al. (2012) |

**Table 4** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of effect | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Silica NPs | Sizes: 92.30 ± 7.42, 60.80 ± 4.36, 27.26 ± 4.92 | Male Wistar rats were exposed for 16 times through intratracheal instillations to 2, 5 and 10 mg/kg of NPs | Biochemical analyses were carried out in serum at the end of exposure | Dose-dependent increase of cytokines, CRP, ROS, and MDA; and significant decrease of SOD and GSH-px | ROS, MDA, SOD, and GSH-px | Du et al. (2013) |
| | Sizes: 30, 70, 300 and 1,000 nm; 70-nm NPs were functionalized with carboxyl and amino groups | Female BALB/c mice were exposed to 0.05–0.8 mg by intravenous injection (70–1,000 nm); and to 0.5 mg by intranasal exposure (30 and 70 nm) | Acute phase protein levels were measured at 6, 24 h, 3, and 7 days after intravenous treatment and 24 h after intranasal exposure | Acute phase proteins may be useful biomarkers for predicting the risk from exposure to silica NPs through various routes of exposure | Haptoglobin, CRP, and SAA | Higashisaka et al. (2011) |
| | Sizes: 30, 70, and 300 nm | Male BALB/c mice were exposed to 10 (30 nm), 10–40 (70 nm), and 10–200 (300 nm) mg/kg by intravenous injection | Cytokine levels were assessed at 3 h post exposure | Dose-dependent increase of of TNF-α and IL-6 levels | TNF-α and IL-6 | Lu et al. (2011) |
| | Sizes: 15 and 55 nm; surface area: 200 and 50 $m^2/g$ | Male Wistar rats were exposed to 25–50 (15 nm); and 25–125 (55 nm) mg/kg by 3 consecutive intravenous injections | Cytokine levels were assessed at the end of exposure | Significant increase of TNF-α and IL-6 levels | TNF-α and IL-6 | Downs et al. (2012) |
| | Sizes: 70, 300, and 1,000 nm; 70-nm NPs were functionalized with carboxyl and amino groups | Female BALB/c mice were exposed to 0.8 mg (70–1,000 nm); and 0.05–0.8 mg (70 nm) by intravenous injection and to 0.5 mg by intranasal exposure (30 and 70 nm) | Hemopexin levels were measured at 6–24-h post exposure (70–1,000 nm) and 24-h post exposure (only 70 nm) | The levels of hemopexin in the plasma increased, in dose-dependent manner, as the silica particle size decreased | Hemopexin | Higashisaka et al. (2012) |
| SWCNTs and MWCNTs | SWCNTs: 0.8–1.2 nm in diameter and 0.1–1 μm in length; MWCNTs: (∼ 80 nm in diameter and 10–20 μm in length | C57BL/6 mice were exposed by pharyngeal aspiration to 40 μg of SWCNTs and MWCNTs | Cytokine and acute phase protein levels were assessed at 4 h post exposure | SWCNT and MWCNT exposure increased IL-6, total and active PAI-1 levels. Exposure to MWCNT also increased CXCL1 IL-5, CCL11, and CCL22 | Different types of cytokines | Erdely et al. (2009) |
| MWCNTs | Sizes: 90–150 and 60–80 nm; surface area: 197 and 252 $m^2/g$ | Male Wistar albino rats were exposed through intratracheal instillations to single dose of 0.2, 1, and 5 mg/kg of MWCNT | Total antioxidant status and lipid peroxidation products were measured in serum at 24 h and 3 months post instillation | Significant dose-dependent reduction of GSH, catalase, and SOD; and increase of MDA levels | Glutathione, catalase, SOD, and MDA | Reddy et al. (2011) |
| CB-NPs | Size: 14 nm; surface area: 295–338 $m^2/g$ | Female C57BL/6 mice were exposed to 0.162 mg by intratracheal instillation | SAA levels were measured at 1, 3, and 28 days post exposure | Significant increase of SAA levels | SAA | Bourdon et al. (2012) |
| NPs in wood smoke | Mass concentrations: 240–280 $\mu g/m^3$; number concentrations: 95,000–180,000/$cm^3$ | 13 subjects were exposed to wood smoke two 4-h sessions, 1 wk apart. About half of the particles were ultrafine | Biochemical analyses were carried out in blood samples collected before and after the experiment | Increase of: SAA, factor VIII, factor VIII/vonWillebrand ratio, and CC16 | SAA, factor VIII, and CC16 | Barregard et al. (2006) |

**Table 4** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of effect | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Type and time of analysis | | | |
| Different types of engineered NPs | Sizes: from 20 to 100 nm | 227 workers who handled nanomaterials were recruited in this study. Control banding from the Nanotool Risk Level Matrix was used to categorize the exposure risk levels | Antioxidant enzyme activities, inflammation, oxidative damage, cardiovascular, and genotoxicity biomarkers were measured | SOD and GSH-px was significantly lower than in control workers, while fibrinogen, ICAM, and IL6 were significantly higher in exposed workers; No changes in CC16 levels | SOD, GSH-px, fibrinogen, ICAM, and IL6; | Liou et al. (2012) |
| Biomarkers of effect in urine | | | | | | |
| Ag-NPs | Geometric mean diameter: 18–19 nm; total number concentration: $6.64 \times 10^5$ to $2.85 \times 10^6$ particles/cm$^3$; surface area: $1.08 \times 10^9$ to $6.61 \times 10^9$ nm$^2$/cm$^3$ | Male and female Sprague–Dawley rats (Slc:SD) were exposed (6 h/day, 5 days/week, for 13 weeks), via inhalation, to low, middle and high dose (49–513 μg/m$^3$, $0.6 \times 10^6$ to $3.0 \times 10^6$ particles/cm$^3$, $1.0 \times 10^9$ to $5.0 \times 10^9$ nm$^2$/cm$^3$) | 8-iso-PGF2α was determined in urine at the end of the exposure period (13 weeks) | Significant increase of 8-iso-PGF2α was detected in the urine from male rats exposed to 513 μg/m$^3$ | Ag in blood | Sung et al. (2009) |
| NPs in wood smoke | Mass concentrations: 240–280 μg/m$^3$; number concentrations:95,000–180,000/cm$^3$ | 13 subjects were exposed to wood smoke in two 4-h sessions, 1 week apart. About half of the particles were ultrafine | Biochemical analyses were carried out in urine samples collected before and after the exposure | Urinary levels of 8-iso-PGF2α were significantly increased in the morning after exposure | 8-iso-PGF2α | Barregard et al. (2006) |
| Biomarkers of effect in exhaled air and exhaled breath condensate | | | | | | |
| NPs in wood smoke | Mass concentrations: 240–280 μg/m$^3$; number concentrations:95.000–180.000/cm$^3$ | 13 subjects were exposed to wood smoke in two 4-h sessions, 1 week apart. About half of the particles were ultrafine | Breath samples were taken before and at various intervals after exposure to measure NO and MDA | Increase of NO was detected 3 h after exposure compared with those subjects exposed to clean air; Increase of MDA levels immediately after exposure | NO and MDA | Barregard et al. (2008) |
| Different types of engineered NPs | Sizes: from 20 to 100 nm | 227 workers who handled nanomaterials were recruited in this study. Control banding from the Nanotool Risk Level Matrix was used to categorize the exposure risk levels | Nuclear factor-κB transcription factor activation in exhaled breath condensate and exhaled breath NO | Nuclear factor-κB transcription factor activation in exhaled breath condensate and exhaled breath NO, did not differ between exposed workers and controls | NF-κB (?), NO (?) | Liou et al. (2012) |

enhanced by utilizing them in combination (Higashisaka et al. 2011).

Interestingly, as an additional approach to determine early biomarkers of nanomaterial immune system effect, various changes in lymphocyte subpopulations were assessed in experimental animals, such as lymphocyte distribution (Park et al. 2009; Sang et al. 2012), T-cell percentage (Park et al. 2010a), or B-cell proportion (Park et al. 2010b). However, the limited number of studies available, as well as the nonhomogeneous results obtained, prevents definite conclusion from being drawn on their practical use for human biological monitoring. The $CD^{4+}/CD^{8+}$ T-cell ratio may also be considered as a possible biomarker of effect, since ratio differences in NP exposed *vs* nonexposed animals were reported. An increased ratio after $Fe_3O_4$-NP exposure (Park et al. 2010a; Wang et al. 2011) and reduced ones following $TiO_2$ (Park et al. 2009; Sang et al. 2012)- and Ag-NP treatments, were reported (Park et al. 2010b). These findings may be due to differences in type of metal-NPs employed, route of exposure used, and dosage administered. Indeed, future investigation is necessary to clarify whether this ratio may be a suitable early marker of NP toxicity and, possibly, to define any specific trends related to the various NPs studied.

Another potential biomarker of effect, i.e., the serum level of Clara cell pneumoprotein (CC16), was investigated as being possibly more specifically representative of the pneumotoxic effects of nanomaterials (Barregard et al. 2008, Liou et al. 2012). In fact, CC16 levels increased after ultrafine particle exposure to wood smoke in healthy volunteers (Barregard et al. 2008), while no differences were detected in workers exposed to engineered nanomaterials (Liou et al. 2012). Therefore, potential biomarkers of early respiratory damage need further investigations.

### Urine

Limited information is currently available on possible biomarkers of effect in urine (Table 4). In human volunteers exposed to ultrafine particles in wood smoke urinary levels of 8-iso-PGF2α, a measure of oxidative stress, showed a significant increase (Barregard et al. 2006). Kidney function was evaluated by the means of the urinary protein levels (Sung et al. 2009). A significant increase in this biological parameter was detected in male rats sub-chronically exposed to Ag-NPs. The relevance of urinary biomarkers of effect relies on the fact that urine is not simply an excretory medium, but also a product of kidney function (Apostoli 2002). Therefore, a concurrent evaluation of both NP excretion and renal impairment should always be performed.

### Exhaled air and exhaled breath condensate

Inflammatory biomarkers, monitored in exhaled breath condensate collected from NP exposed workers (Liou et al. 2012) or from healthy volunteers exposed to incidental NPs (Barregard et al. 2008), showed conflicting results in terms of breath nitric oxide, nuclear transcription factor-kB activation and MDA concentrations (Table 4). Although these are just potential biomarkers, still in a preliminary phase of knowledge, they could be useful to assess the respiratory health status of exposed subjects and, eventually, to clarify the local pulmonary target tissue alterations induced by nanomaterials. So, further research appears to be necessary to verify the applicability of these promising biomarkers in occupational health practice.

### 8-Hydroxy-deoxy-guanosine

DNA is one of the biologically most significant targets of NP-induced oxidative attack. Once it is damaged by reactive oxygen species (ROS), numerous oxidative DNA damage by-products are formed, such as 8-hydroxy-deoxy-guanosine (8-OH-dG) (Donaldson et al. 2005; Dalle-Donne et al. 2006; Khatri et al. 2013). Urinary 8-OH-dG concentration has been investigated as a potential biomarker of oxidative stress in response to exposure to incidental NPs emitted from photocopiers, and it was found significantly increased as compared with background levels (Khatri et al. 2013). Conversely, in workers handling nanomaterials in 14 plants in Taiwan, 8-OH-dG urinary and plasma levels did not show significant differences compared with controls (Liou et al. 2012).However, not only in human studies, but also in animal experiments, conflicting results were reported (Song et al. 2012; Downs et al. 2012) and, importantly, the positive findings were obtained at high doses and via intravenous and intraperitoneal injection, two quite unrealistic exposures for the

workplace settings. Therefore result interpretation deserves great caution.

## Genotoxicity biomarkers

A particular type of biomarkers are those of genotoxicity. This type of biomarkers is used extensively in experimental animals for toxicity testing or in humans to assess the effects of occupational and environmental exposure to genotoxic and carcinogenic chemicals in an effort to predict the risk of disease, or to monitor the effectiveness of preventive procedures. A recent study performed on workers exposed to engineered nanomaterials failed to reveal significant differences in markers of genotoxicity compared with unexposed controls (Liou et al. 2012).

Biomarkers of genotoxicity have been also investigated in various experimental studies on NPs, including in vitro and in vivo models, although with non univocal results (Table 5). Due to their small size and high surface area, coupled with other physicochemical features, such as metal contamination and charged surfaces, NPs may well have unpredictable genotoxic properties in vitro. Increased DNA strand breaks and micronucleus (MN) frequency were found in human peripheral blood cells treated with metal or metal oxide-NPs (Colognato et al. 2008; Di Bucchianico et al. 2013; Flower et al. 2012; Ghosh et al. 2010, 2012, 2013; Kang et al. 2008, 2011; Paino et al. 2012; Tavares et al. 2014), carbon-based NPs (Cveticanin et al. 2010; Tavares et al. 2014), dendrimers (Ziemba et al. 2012) or incidental-NPs (Dwivedi et al. 2012). Genotoxic effects generally showed a dose-dependent relationship (Colognato et al. 2008; Cveticanin et al. 2010; Dwivedi et al. 2012; Kang et al. 2008, 2011; Di Bucchianico et al. 2013). Moreover, as regards the role of NP physicochemical features in their genotoxic potential, the crystalline form of $TiO_2$-NPs was demonstrated to differently affect the frequency of binucleated micronucleated cells (BMNCs), that was increased by anatase and rutile but not by their mixture (Tavares et al. 2014). Concerning the role of NP dimension and morphology, a genotoxic potential was evident whatever the size and shape of CuO-NPs (Di Bucchianico et al. 2013). Moreover, surface amide functionalization did not affect MN induction caused by noncoated CNTs (Cveticanin et al. 2010).

As concerns in vivo investigations, a significant increase in DNA strand breaks and MN formation was detected in the peripheral blood cells of $TiO_2$-NP treated animals, as compared with controls (Trouiller et al. 2009; Song et al. 2012). However, the genotoxic effects of $TiO_2$-NPs were not confirmed in two other studies (Lindberg et al. 2012, Sadiq et al. 2012). These conflicting results may be explained, again, by the differences in terms of $TiO_2$ crystalline structure, route of exposure and administered dose. Other types of metallic or metal oxide NPs, such as CuO-, $Fe_2O_3$-, $Fe_3O_4$, $MnO_2$-, $Al_2O_3$-, Ag- and silica NPs, were reported to induce a dose-dependent genotoxic damage in peripheral blood cells (Balasubramanyam et al. 2009; Downs et al. 2012; Singh et al. 2013a; Song et al. 2012, Tiwari et al. 2011). Interestingly, the peak induction of micronucleated reticulocytes (MNRETs) after CuO-, $Fe_2O_3$-, $Fe_3O_4$, and Ag-NP exposure appeared 48 h after the administration and declined to the control level at 72 h thereafter (Song et al. 2012). These findings support the idea that MNRETs could originate from lesions induced only during a short time after administration and underline the importance to properly plan the time-periods of blood sampling while evaluating NP genotoxic action through biological monitoring. In line with the above mentioned "unpredictable" genotoxic role of nanosized particles, $Fe_2O_3$-NPs (Singh et al. 2013b) and Au-NPs (Downs et al. 2012) failed to induce DNA damage in peripheral blood cells.

Overall, these findings underline the distinct need to focus future research on specific associations between genotoxic responses and nanomaterial physicochemical features, in order to identify clearer genotoxicologic trends and to clarify their mechanisms of action. It should be possible to extrapolate similar risks in respect of nanomaterials with similar characteristics, and thus to adopt more effective measures to protect the health and safety of the exposed workers.

## Biomarkers from "omic" techniques

"Omic" techniques are increasingly used in an effort to develop novel biomarkers of exposure, to screen for new, yet unknown, toxicologic effects, and to investigate the mechanisms of chemical toxicity. Toxicogenomics, defined as the gene-expression profiling, and

**Table 5** Possible biomarkers of genotoxicity for different types of NPs

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of genotoxicity | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Genotoxicity test | | | |
| Biomarkers of genotoxicity | | | | | | |
| Ag-NPs | Sizes: 40–60 nm | Human peripheral blood cells were treated with 50 and 100 μg/ml for 5 min and 3 h | Comet assay | Statistically significant increase in DNA fragmentation after 5 min and 3 h treatment with 50 and 100 μg/ml | % Tail DNA fragmentation | Flower et al. (2012) |
| | Sizes: 75–130 nm, average size: ~125 nm; surface area: 50 $m^2$/g | Human peripheral blood lymphocytes were treated with 0–200 μg/ml for 3 h | Comet assay | DNA fragmentation was higher than controls at all concentrations with statistically significant responses at 25, 50 and 200 μg/ml | % Tail DNA fragmentation | Ghosh et al. (2012) |
| | Sizes: 15–40 nm | Blood samples were collected from Wistar rats exposed by intravenous injection (for 32 days at intervals of 5 days) to 4–40 mg/kg | Comet assay | A dose-dependent DNA damage in blood cells was observed | % Tail DNA fragmentation | Tiwari et al. (2011) |
| Au-NPs | Au-NPs-PAMAM: size 7.2 ± 2.7 nm; Au-NPs-citrate: size 7.3 ± 1.2 nm | Human peripheral blood mononuclear and HepG2 cells were treated with 1 and 50 μM for 3 h | DNA Damage index obtained by comet visual score | Damage index was statistically significant for Au-NPs-citrate and Au-NPs-PAMAM in HepG2 at 50 and 1–50 μM and for Au-NPs-PAMAM in PBMC at 50 μM | DNA damage index | Paino et al. (2012) |
| Au-NPs and silica NPs | Au-NPs size: 2- and 20-nm silica NPs Sizes: 15 and 55 nm; surface area: 200 and 50 $m^2$/g | White blood cells and reticulocytes were collected from male Wistar rats treated with 3 intravenous injections of 6 μg/animal of Au-NPs and of 25–125 mg/kg of silica NPs | Frequency of MNRETs and Comet assay | Au-NPs: No increase in the DNA damage of white blood cells and in the percentage of circulating MNRETs; Silica NPs: ~1.5-fold increase in DNA damage of white blood cells and 1.8-fold increase in the percentage of circulating MNRETs (15 nm); approximately twofold increase in the circulating percentage of MNRETs (55 nm) | % Tail DNA fragmentation and frequency of MNRETs | Downs et al. (2012) |

**Table 5** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of genotoxicity | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Genotoxicity test | | | |
| $Al_2O_3$-NPs | Sizes: 30 and 40 nm | Peripheral blood cells were collected, after 4–72 h, from female Wistar rats treated, by oral gavage, with 500–2,000 mg/kg of NPs | Frequency of MN cells and % Tail DNA fragmentation | Dose-dependent increase in DNA damage and MN frequency in peripheral blood cells | Frequency of MN cells and % Tail DNA fragmentation | Balasubramanyam et al. (2009) |
| $^{60}Co$-NPs | Sizes: from 100 to 500 nm; median value 246 nm | Human peripheral blood leukocytes were treated with $10^{-5}$ to $10^{-4}$ M for 2 h | Frequency of MN cells and % Tail DNA fragmentation | $^{60}Co$-NPs changed statistically significant the frequency of BNMN ($2 \times 10^{-5}$ M) and induced a statistically dose-dependent increase in the percentage of tail DNA fragmentation | Frequency of MN cells and % Tail DNA fragmentation | Colognato et al. (2008) |
| CuO-NPs | Sizes and surface area: powder 10–100 nm and not available; spheres $7 \pm 1$ nm and 60 $m^2/g$; rods $7 \pm 1 \times 40 \pm 10$ nm and 51 $m^2/g$; spindles $1{,}200 \pm 250 \times 270 \pm 50 \times 30 \pm 10$ nm and 18 $m^2/g$ | RAW 264.7 cells were treated with 0.1–10 μg/ml for 2 and 24 h | Cytokinesis block micronucleus and Comet assays | Independently of size/shape, was observed a dose-dependent increase in DNA fragmentation and in MN frequency | Frequency of MN cells and % Tail DNA fragmentation | Di Bucchianico et al. (2013) |
| $Fe_2O_3$-NPs | Size: $29.75 \pm 1.87$; surface area: 38.02 $m^2/g$ | Peripheral blood cells were collected (at 6–72 h post exposure) from Albino Wistar female rats treated with a single oral dose of NPs (500–2,000 mg/kg) | Comet assay and percentage of micronucleated polychromatic erythrocytes | No significant increase in the DNA damage of peripheral leukocytes and in micronucleated polychromatic erythrocytes | % Tail DNA fragmentation and percentage of circulating micronucleated polychromatic erythrocytes | Singh et al. (2013b) |
| $MnO_2$-NPs | Size: <30 nm; surface area 52.21 $m^2/g$ | Peripheral blood leukocytes were collected from Albino Wistar male and female rats treated, for 28 days by oral route, with 30–1,000 mg/kg/day | Comet assay | Significant increase in the DNA damage in peripheral blood leukocytes | % Tail DNA fragmentation | Singh et al. (2013a) |

**Table 5** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of genotoxicity | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Genotoxicity test | | | |
| $TiO_2$-NPs | Size: ~30 nm; surface area: 50 $m^2/g$ | Human peripheral blood lymphocytes were treated with 20–100 μg/ml for 6–24 h | Cytokinesis block micronucleus and Comet assays | Dose- and time-dependent increase of $TiO_2$-NPs on DNA fragmentation and micronucleus frequency | Frequency of MN cells and % Tail DNA fragmentation | Kang et al. (2008) |
| | Size: ~100 nm | Human peripheral blood lymphocytes were treated with 0–2 mM for 3 h | Comet assay | $TiO_2$-NPs were found to be genotoxic at a low dose (0.25 mM) followed by a decrease in extent of DNA damage | % Tail DNA fragmentation | Ghosh et al. (2010) |
| | Size: 20 nm; surface area: 48.08 $m^2/g$ | Human peripheral blood lymphocytes were treated with 1 and 5 μg/ml for 24 h | Cytokinesis block micronucleus and Comet assays | Dose- and time-dependent increase of DNA fragmentation and slight increase of the prevalence of MN; UVA irradiation enhanced these effects | Frequency of MN cells and % Tail DNA fragmentation | Kang et al. (2011) |
| | Sizes: 35–56 nm | Human peripheral blood lymphocytes were treated with 0–100 μg/ml for 3 h | Comet assay | DNA fragmentation was statistically significant at 25 μg/ml | % Tail DNA fragmentation | Ghosh et al. (2013) |
| | Crystal structure:mixture of 75 % anatase and 25 % rutile; primary particle size was 21 nm; specific surface area of 50 ± 15 $m^2/g$ | Peripheral blood was collected from C57Bl/6J$p^{un}/p^{un}$ mice exposed to 60–600 μg/ml of NPs in drinking water for 5 days | Frequency of MN cells and % Tail DNA fragmentation | Significant increase in DNA fragmentation in white peripheral blood cells and increase in MN formation in peripheral blood erythrocytes | Frequency of MN cells and % Tail DNA fragmentation | Trouiller et al. (2009) |
| | Sizes: 10–60 nm; average size: 21 nm; surface area: 61 $m^2/g$ | Peripheral blood erythrocytes were collected from Male C57BL/6J mice exposed by inhalation to (4 h/day during 5 consecutive days) 0.8–28.5 mg/$m^3$ | Frequency of micronuclei in peripheral blood erythrocytes | No increase of micronuclei frequency in peripheral polychromatic erythrocytes | Frequency of micronuclei in peripheral polychromatic erythrocytes | Lindberg et al. (2012) |
| | Size: 12.1 ± 3.2 nm; $TiO_2$-NPs have a slight ellipsoidal shape | Peripheral reticulocytes and erythrocytes were collected from male B6C3F1 mice exposed, by intravenous injection, to 0–50 mg/kg/day (for 3 consecutive days) | Pig-A assay and frequency of MNRETs | No increase in the frequency of MNRETs or in the mutant frequencies of the endogenous phosphatidylinositol glycan complementation group A gene in reticulocytes and erythrocytes was observed | Frequency of MNRETs and the mutant frequencies of the Pig-A gene | Sadiq et al. (2012) |

**Table 5** continued

| Type of NPs | Physicochemical properties | Experimental protocol | | Results | Proposed biomarkers of genotoxicity | References |
|---|---|---|---|---|---|---|
| | | Characteristics of exposure | Genotoxicity test | | | |
| $TiO_2$-NPs; MWCNTs | Sizes and surface area: anatase 20.8 ± 1.6–33.0 ± 1.5 nm and 90 $m^2$/g; hydrophobic rutile 21.9 ± 1.4–37.9 ± 1.6 nm and 60 $m^2$/g; hydrophilic rutile 19.0 ± 1.5–25.8 ± 1.4 and 60 $m^2$/g; Rutile-anatase 20.0 ± 1.3–29.6 ± 1.3 nm and 61 $m^2$/g; MWCNTs thickness from 10.7 to 69.4 nm, length from 368.7 to 4,423.6 nm, surface area from 24 to 300 $m^2$/g; | Human peripheral blood lymphocytes were treated with 0–250 μg/ml (TiO2-NPs and MWCNTs) for 24 h | Frequency of MN cells | Increased frequencies of BMNCs at doses of 125 (Anatase), 5 and 45 (hydrophobic rutile), 15 and 45 (hydrophilic rutile) μg/ml. No effects with rutile-anatase; MWCNTs (10.7, 11.1, and 69.4 nm) induced a significant increase of frequencies of BMNCs, however, no dose–effect relationship was found | Frequency of MN cells | Tavares et al. (2014) |
| CuO-NPs, $TiO_2$-NPs, $Fe_2O_3$-NPs, $Fe_3O_4$-NPs and Ag-NPs | Size and surface area of CuO-NPs, $TiO_2$-NPs, $Fe_2O_3$-NPs, $Fe_3O_4$-NPs, and Ag-NPs: 27.2–95.3 nm and 10–35 $m^2$/g, 19.7–101 nm and 15–77 $m^2$/g, 60–100 nm and 15 $m^2$/g, 80 nm, and 38 $m^2$/g and <100 nm and 5 $m^2$/g | Peripheral blood was collected (0–72 h) from female ICR mice exposed to 0–3 mg/kg by intra peritoneal injection | Frequency of micronucleated reticulocytes (MNRETs) | Increases of frequencies of MNRETs in all NPs treated groups | Frequency of MNRETs | Song et al. (2012) |
| CNTs | SWCNTs, MWCNTs and amide functionalized purified SWCNTs: MWCNTs had outer and inner diameters of 20–40 nm and a length of 1–5 μm | Human peripheral blood lymphocytes were treated with 0.25–150 μl/5 ml of total cell culture volume of different CNTs | Frequency of MN cells | Different types of CNTs induced a dose-dependent increase in MN formation | Frequency of MN cells | Cveticanin et al. (2010) |
| Poly(propylene imine) dendrimers | Unmodified 4th generation PPI dendrimers (PPI-g4) and PPI dendrimers with ~40 % (PPI-g4-OS) and ~90 % (PPI-g4-DS) of the surface amino groups substituted with maltotriose residues | Human peripheral blood mononuclear cells were treated with 0.05–5 mg/ml for 1 h | Comet assay | PPI-g4 showed the highest level of genotoxicity (0.5 mg/ml); functionalized PPI dendrimers were less genotoxic | % Tail DNA fragmentation | Ziemba et al. (2012) |
| Incidental NPs present in coal fly ash | Sizes: 11–25 nm; average size 14 nm; presence of heavy metals (Fe, Ni, Cu and Cr); shape predominantly spherical | Human peripheral blood mononuclear cells were treated with 3–2,400 ppm for 3 and 24 h | Cytokinesis block micronucleus and Comet assays | Significant concentration-dependent DNA damage and dose-dependent increase in the total number of BMNCs | Frequency of MN cells and % Tail DNA fragmentation | Dwivedi et al. (2012) |

metabolomics or proteomics, which measure the complete profile of small endogenous molecules or proteins, respectively, have all been proposed as innovative techniques aimed at identifying some consistent patterns of biochemical changes that may assist in the identification of early, innovative biomarkers of effect (Bourdon et al. 2013; Nicholson et al. 2002).

Although promising, limited data are currently available on the application of toxicogenomic analyses to accessible biological matrix such as blood or peripheral blood cells. Preliminary findings demonstrated that Au-NP-olygonucleotide complexes (Kim et al. 2012) and single- or multiwalled CNTs (Erdely et al. 2009) were able to activate genes involved in immune, inflammatory and oxidative stress responses in in vitro and in vivo models, respectively. As an additional mechanism of gene expression regulation (Lee et al. 1993), the blood micro-RNA profile of rats treated with Au-NPs showed significant changes, that could represent potential innovative acute and subchronic biomarkers of NP exposure and/or effect (Chew et al. 2012). Several studies investigated the suitability of metabolomics, a systematic approach for studying biochemical profiles, as a rapid in vivo screening for nanotoxicity with the aim to identify target organ toxicity and toxicological mechanisms trough the measurement of a wide range of molecules in body fluids (Bu et al. 2010; Lei et al. 2008). The results of metabolic analysis performed in serum and urine of rats treated with Cu (Lei et al. 2008)- and $TiO_2$-NPs (Bu et al. 2010; Tang et al. 2010, 2011) not only provided additional evidence for the hepatotoxicity and nephrotoxicity of these NPs as detected by conventional clinical chemistry, but revealed also useful information on the mechanism underlying their toxic effects. In fact, the observation in these studies of significant changes in the concentration of a large number of metabolites demonstrated the occurrence of dysfunctions in renal glomerular filtration and tubular reabsorption, as well as disturbances in the energy and amino-acid metabolism. Other studies reported the ability of Ag-NPs (Hadrup et al. 2012), $Fe_2O_3$-NPs (Feng et al. 2010), silica-NPs (Lu et al. 2011; Parveen et al. 2012), carbon$^{14}$-labeled C60 fullerenes (Sumner et al. 2010) and CNTs (Lin et al. 2013) to affect several physiological functions, as assessed by the alterations found in serum and urine metabolic pathways. Interestingly, metabolomic analysis was demonstrated to be more sensitive, than histopathological examination and clinical chemistry, in recognizing early events during silica NP-induced toxicity (Lu et al. 2011).

Proteomic investigation has also been employed to identify candidate protein biomarkers for the evaluation of the NP exposure-induced early effects. In this regard, Higashisaka et al. (2011, 2012) identified increased plasma levels of haptoglobin, hemopexin and alpha-1-acid glycoprotein 1 as possible early inflammatory biomarkers of silica NP exposure in vivo. The suitability of these proteins as biological indicators of effect was confirmed by traditional biochemistry, thus supporting the effectiveness of proteomic analysis in detecting NP toxicity.

Overall, the great variability in terms of innovative techniques employed, parameters investigated, as well as results obtained, requires further investigation to define both the relevance and the data and the appropriate methodologies to possibly use biomarkers from "omic" technologies in future occupational biomonitoring programs.

## Discussion

The ideal biomarkers of exposure should be specific and sensitive for assessing occupational nanomaterial exposure by all routes and at low levels, to complement more effectively the information obtained by workplace environmental monitoring (Manno et al. 2010). Besides, biomarkers of effect should be able to provide strong mechanistic insight on the molecular and biochemical bases of the disease, reflect early adverse health effects, have clinical relevance, and be easy to use (Li and Nel 2011). At present, nanomaterial biomarkers that are able to meet all these criteria, and hence, can be practically employed for occupational biological monitoring, are not available. Using a stepwise approach, however, we provided here a comprehensive critical evaluation of the human and experimental studies on the potential biomarkers that may be eventually validated and used in the assessment of nanomaterial exposure and effects in occupational setting conditions. Although the limited number of studies available and their methodological differences prevented the conclusive identification of suitable indicators of exposure or specific health outcomes for use in validated occupational biological monitoring programs, the following interesting points have

emerged for discussion, which deserve future attention and investigation.

## Biomarkers of exposure: strengths and criticisms

As a possible internal dose biomarker for metallic or metal oxide NP exposure, the simple measurement of the elemental metal content in different biological matrices should be taken into consideration as an accessible and easily available indicator. However, this relatively easy measurement—be it in blood, urine, or feces—requires an accurate knowledge of the toxicokinetic and toxicodynamic properties of the nanoxenobiotics involved, for its correct interpretation. Therefore, NP characterization is strongly required, considering that features such as physicochemical form, NP size and surface functionalization can all affect NP biological behavior and the interpretation of biomonitoring results. Biological monitoring may provide information which is more closely representative of the worker's real exposure level, thus overcoming the weaknesses of environmental data due to the current lack of standardized personal monitoring strategies (Methner et al. 2010). Moreover, biomonitoring can provide information on the features that nanomaterials can progressively acquire while coming into contact with complex biological environments or because of the adsorption of biomolecules on their surface (Monopoli et al. 2012). The positive dose–response trend reported in several studies demonstrated the relatively good sensitivity of the metal content in biological fluids in respect to the exposure doses, although these were frequently unrealistically high (Sung et al. 2009; Kim et al. 2008; Baek et al. 2012; Cho et al. 2013). Therefore, known dose–response relationships should be verified in future investigations by adopting more realistic, low-concentration, and long-term exposures, similar to those generally found in workplaces.

The evaluation of the biologically effective dose through the search for and measurement of NP–DNA or NP–protein adducts, seems to be of great relevance because of the "multifunctional role" of this kind of biomarkers. In fact, they can behave not only as indicators of exposure, their concentrations depending on the exposure levels, but also as biomarkers of effect and susceptibility, since they can indicate a damage to a target molecule and reflect the degree of metabolic activation and/or DNA repair (Manno et al. 2010).

## Biomarkers of effect: strengths and criticisms

The effects of nanomaterials on health, particularly those of long-term and low doses, are to a large extent unknown, and currently there is no report of a definite human disease that is caused by nanomaterial exposure (Bergamaschi 2012). Therefore, the selection of candidate biomarkers of effect is an even more challenging task. It is further complicated by the nonspecificity of the most frequently investigated biomarkers, which are generally focused on the early detection of systemic oxidative and inflammatory responses, and therefore may possibly be affected by other, nonoccupational risk factors (Li et al. 2008; Oberdorster 2001; Valavanidis et al. 2008). Importantly, since the main route of entry of nanomaterials in occupational settings is the respiratory tract, it may be relevant to define local dose and biomarkers of effect specifically with reference to this site of first contact. In this context, exhaled breath biomarkers may attract considerable attention, although practical and scientific limitations still prevent their use in routine occupational biomonitoring.

## Biological monitoring programs

As in the case of traditional chemicals, i.e., metals, such as lead and mercury, or solvents, the definition of standardized biological monitoring protocols should be viewed as a beneficial tool for the assessment and management of risks to health from nanomaterial exposure in workplaces. These programs may first provide an evaluation of the current exposure as well as an indication of the appropriateness of safe work procedures and controls while handling nanomaterials. Biomonitoring may also be a relevant component of the occupational health surveillance and a means to monitor extraordinary exposures and health effects after accidental events. The information extrapolated from biological monitoring may then lead to the adoption and/or implementation of preventive and protective measures for the health and safety of workers.

Importantly, an insight into the relationship between internal doses and effects, as well as into the NP mechanisms of action, may be useful to define and standardize biological monitoring protocols, in terms of sample collection, data reporting, and biomarker validation. To this aim, a combined employment of

indicators of exposure and effect may be useful in defining specific biomarker profiles for different types of metal-, metal oxide- or carbon-based nanomaterials, thus making at the same time some the prevalently used research-oriented indicators to be more practical and useful (Kharitonov and Barnes 2006). This may be the case of biomarkers from "omic" techniques. These methods, in fact, are able to identify early NP biological responses, although their routine use is still prevented by both theoretical and practical issues, such as uncertain toxicological significance, high cost, and complex analytic requirements (Sheehan 2007).

For several nanomaterials, the accumulating evidence suggests a genotoxic potential (Singh et al. 2009; Magaye and Zhao 2012). In occupational settings, genotoxic indicators are generally intended as group markers for the biological assessment of risk of carcinogens from workplace exposure (Valverde and Rojas 2009). They are generally sensitive but not specific, and in some cases, difficult to interpret correctly. Importantly, ethical considerations relative to the possible adverse impact on the worker's status of employment and/or quality of life, should be always taken into account while using these biomarkers to assess the workers' fitness to his/her job as, for instance, in the case of workers exposed to antiblastics or other genotoxic carcinogens (Manno et al. 2010).

## Proposals for CNT and $TiO_2$-NP biological monitoring

The National Institute for Occupational Safety and Health (NIOSH) recently reported that respiratory exposure to CNTs and carbon nanofibers could cause adverse pulmonary effects including inflammation, granulomas, and pulmonary fibrosis (NIOSH 2010; Castranova et al. 2013; Shvedova et al. 2009). Moreover, pulmonary exposure to multiwalled CNTs has been shown to result in their migration to subpleural tissue and intrapleural space (Mercer et al. 2010; Stapleton et al. 2012). They could also be detected in the lavage of the pleural space (Porter et al. 2013) or in the diaphragm and even in systemic organ sections (Mercer et al. 2013)—an interesting observation while considering their proposed roles in the promotion of lung tumors in exposed animals (Sargent et al. 2013). In addition, multiwalled CNTs were detected in small amounts in systemic organs including the brain (Mercer et al. 2013).

Therefore, considering the relevance that the pulmonary responses observed in short-term and subchronic studies in animals may have for occupationally exposed subjects, NIOSH recommended that, to minimize the potential health risks associated with occupational exposure, environmental concentrations of CNTs and carbon nanofibers be kept below the exposure limit of 1 $\mu g/m^3$ of respirable elemental carbon as an 8-h time-weighted average (TWA) (NIOSH 2010).

In this context, biological monitoring data could be used effectively to verify the efficacy of the exposure limit in protecting the health of the workers. However, given that CNTs have not till now been demonstrated in blood and that they are hydrophilic, so that they may move out of blood rapidly to systemic tissues, biomarkers of early effect could be more useful than biomarkers of exposure. Among those, alterations of inflammatory mediators, such as cytokines and acute-phase proteins (Erdely et al. 2009), as well as biomarkers of oxidative stress and antioxidant-activity depletion should be viewed with great attention (Reddy et al. 2011).

Comparable assumptions could be made for inhaled $TiO_2$-NPs, an agent that NIOSH considers to be a potential occupational carcinogen, thus recommending an exposure limit of 0.3 $mg/m^3$ for ultrafine (including engineered nanoscale) $TiO_2$ as a TWA concentration for up to 10 h/day during a 40-h workweek (NIOSH 2011). Until now, a proposal for a $TiO_2$-NP biomonitoring plan is rendered complex by the lack of information given the limited number of studies investigating possible indicators in accessible biological matrices. This knowledge gap should be urgently overcome, considering the exponential rise in the industrial use of these NPs and the increasing likelihood of quantitatively significant occupational-exposure scenarios. In this perspective, to better assess $TiO_2$-NP occupational exposure, future investigations should be focused primarily on the measurement of Ti content in blood and urine, as only preliminary data are currently available (Cho et al. 2013). Moreover, as regards $TiO_2$-NP effects, the concurrent assessment of alterations in cytokine levels, i.e., TNF-$\alpha$ and IL-6 as markers of systemic inflammation, as well as in the lymphocyte subsets and $CD^{4+}/CD^{8+}$ ratios, should be carefully considered for tentative use in controlled human biomonitoring studies (Park et al. 2009; Sang et al. 2012).

## Conclusions

Biomarkers of exposure to and effect from nanomaterials to be used in occupational health practice should be chosen primarily for their validity and relevance for protection of the health of the workers, with due regard not only to their sensitivity, specificity, and predictive value but also to their ethical sustainability. In this perspective, far more research on NPs is required as a prerequisite for the definition of standardized biological monitoring protocols to be routinely employed in occupational settings. Both toxicokinetic and toxicodynamic studies on specific nanomaterials are needed to identify suitable biological markers of exposure and to pin-point specific target organs wherein early biological effects may occur. A multiple biomarker approach may be useful to overcome the low specificity of single nanomaterial insult indicators, by integrating all information available on metabolism, dose–response and temporal-response kinetics, biological relevance, and positive predictive value. This approach may importantly lead to a more rational selection of appropriate biomarkers and to a more effective interpretation and management of biological monitoring data. Moreover, while planning studies on animal models, researchers should adopt more realistic levels and conditions of exposure, similar to those generally found in workplace settings. Also, they should consider and, if necessary, perform appropriate characterizations of the nanomaterial physicochemical properties which may heavily affect biological monitoring results. Finally, it may be important to take advantage of the latest, the most sensitive techniques, as they can be of assistance in detecting unknown hazards from nanomaterial exposure, leading to the identification of new and possibly more appropriate biomarkers. In conclusion, information on nanomaterial biological monitoring, currently extrapolated from either human or experimental studies, as well as from traditional or innovative technique-based toxicological studies, is still incomplete, hard to interpret, and sometimes inconsistent in terms of occupational risk assessment. Therefore, it is very much necessary to identify suitable biomarkers of exposure to and effect from nanomaterials to be used in routine occupational health practice for the assessment and management of risk of exposure undergone by workers. Future scientific efforts should be primarily focused to test the currently available potential indicators of exposure and effect, mostly developed in mechanism-based animal models, through their validation in the real workplace settings under strictly controlled and ethically acceptable conditions.

## Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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