Toxicology and Applied Pharmacology 299 (2016) 112–124

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Biomarkers of susceptibility: State of the art and implications for occupational exposure to engineered nanomaterials

Ivo Iavicoli [a,*], Veruscka Leso [b], Paul A. Schulte [c]

[a] Department of Public Health, Division of Occupational Medicine, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy
[b] Institute of Public Health, Section of Occupational Medicine, Catholic University of the Sacred Heart, Largo Francesco Vito 1, 00168 Rome, Italy
[c] National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 4676 Columbia Parkway, MS C-14, Cincinnati, OH 45226, USA

ARTICLE INFO

Article history:
Received 10 October 2015
Revised 7 December 2015
Accepted 21 December 2015
Available online 24 December 2015

Keywords:
Nanomaterial susceptibility factors
Occupational biomonitoring
Genetic variances
Toxicogenomic information
Epigenetic alterations
Nanomaterial risk assessment

ABSTRACT

Rapid advances and applications in nanotechnology are expected to result in increasing occupational exposure to nano-sized materials whose health impacts are still not completely understood. Scientific efforts are required to identify hazards from nanomaterials and define risks and precautionary management strategies for exposed workers. In this scenario, the definition of susceptible populations, which may be at increased risk of adverse effects may be important for risk assessment and management. The aim of this review is to critically examine available literature to provide a comprehensive overview on susceptibility aspects potentially affecting heterogeneous responses to nanomaterials workplace exposure. Genetic, genotoxic and epigenetic alterations induced by nanomaterials in experimental studies were assessed with respect to their possible function as determinants of susceptibility. Additionally, the role of host factors, i.e. age, gender, and pathological conditions, potentially affecting nanomaterial toxicokinetic and health impacts, were also analysed.
Overall, this review provides useful information to obtain insights into the nanomaterial mode of action in order to identify potentially sensitive, specific susceptibility biomarkers to be validated in occupational settings and addressed in risk assessment processes. The findings of this review are also important to guide future research into a deeper characterization of nanomaterial susceptibility in order to define adequate risk communication strategies. Ultimately, identification and use of susceptibility factors in workplace settings has both scientific and ethical issues that need addressing.

1. Introduction

Rapid advances in nanotechnology worldwide are leading to a massive production and application of engineered nanomaterials in consumer products. As a consequence, an increasing number of workers are expected to become exposed to nanomaterials, while the potential health and safety impacts are still unknown (Iavicoli et al., 2014; Schulte et al., 2014). Therefore, efforts to actively anticipate potential hazards of nanomaterials and to define risks and preventive needs for exposed workers have become necessary (Schulte and Trout, 2011; Trout and Schulte, 2010). In this context, precautionary risk management may be enhanced by defining susceptible populations which develop adverse effects from nanomaterial exposure due to the lack of capacity, beyond the limits of human variability, to tolerate or respond effectively to these potential exogenous toxicants (Manno et al., 2010). Moreover, the need to define susceptible populations to nanomaterials, has been motivated by recent epidemiologic findings reporting that ultrafine particles can contribute to adverse respiratory and cardiovascular effects resulting in morbidity and mortality, particularly, in susceptible parts of the population (Oberdörster et al., 2005; Penttinen et al., 2001; Peters et al., 1997a, 1997b; von Klot et al., 2002).

Evidence indicates that inherited and acquired genetic susceptibility, epigenetic modifications as well as alterations in physiological structures and functions induced by age, pathological conditions, and lifestyle factors, may lead to different phenotypic expressions from xenobiotic exposures. Particularly, inherited genetic susceptibility may play a role in influencing the individual response to exogenous exposures in a complex "gene–environment" interaction (Hunter, 2005). Therefore, understanding which genetic polymorphisms, genotoxic changes, epigenetic profiles and host factors may affect

Abbreviations: Ag, silver; AhR, aryl-hydrocarbon-receptor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATM, ataxia telangiectasia mutated protein kinase; ATR, ataxia telangiectasia and Rad3-related protein kinase; Au, gold; CdTe-QDs, cadmium telluride quantum dots; $CoFe_2O_4$, cobalt ferrite; CYP450, cytochrome P450; Ercc-2, excision repair cross-complementing rodent repair deficiency complementation group 2; Fe_2O_3, iron(III) oxide; Fe_3O_4, iron(II,III) oxide; GST, glutathione transferase; IC50, half maximal inhibitory concentration; MW-CNTs, multi-walled carbon nanotubes; NP, nanoparticle; OGG1, 8-OhdG-DNA glycosylase 1; PARP-1, poly (ADP-ribose)polymerases-1; PEG, polyethylene glycol; ROS, reactive oxygen species; Si, silica; SiO_2, silicon dioxide; SW-CNTs, single walled-carbon nanotubes; TiO_2, titanium dioxide; Xpa, xeroderma pigmentosum group A protein complex.

* Corresponding author.
E-mail addresses: ivo.iavicoli@unina.it (I. Iavicoli), veruscka@email.it (V. Leso), pas4@cdc.gov (P.A. Schulte).

http://dx.doi.org/10.1016/j.taap.2015.12.018
0041-008X/

the toxicokinetic and dynamic nanoparticle (NP) modelling, appear essential to get insights into the still not understood NP exposure/disease continuum and to identify susceptibility biomarkers indicative of an elevated sensitivity to NP effects. This seems an even more challenging issue considering that the same great variability in NP physicochemical properties, i.e. in terms of size, chemical composition and surface area, that make them so attractive for a variety of product applications may also prove complex and changeable exposure scenarios, potentially influencing individual response to NP toxicity. Therefore, the aim of this review was to critically assess experimental studies addressing susceptibility aspects, potentially affecting the health impact of NP exposure, in order to identify possible susceptibility biomarkers to be further studied and validated in occupational populations exposed to nanomaterials. These biological indicators may be useful to provide quantitative estimates of a population variability to be employed into an adequate occupational NP risk assessment and consequently in the plan of specific or implemented workplace preventive and protective measures. This information could also possibly be used in deriving occupational exposure limits. Overall, this information may give stimulus to innovative research intended to contribute to a more comprehensive, effective assessment and management of potential NP risks in occupational settings.

2. Materials and methods

A bibliographic search of scientific databases including PubMed, ISI web of Science and Scopus was conducted to identify experimental studies addressing susceptibility aspects potentially affecting individual responses to nanomaterial exposure published up to September 2015. We carried out a preliminary search for the terms "nanomaterials" to assess the exposure context, and "susceptibility factors" as the outcome of the research, combined with the operator "AND". The authors, independently examined all titles and abstracts retrieved and selected articles that met the inclusion criteria. These included peer-reviewed *in vitro*, *in vivo* and human studies published in English and exploring aspects potentially affecting the health impact of engineered nanomaterial exposure. Exclusion criteria were applied for studies not focusing on the topic of research. The preliminary search retrieved 45 references through PubMed, 8 results through ISI web of Science and 9 via Scopus database. Out of these, after the exclusion of studies that did not met the inclusion criteria and removal of duplicates, only 3 were considered suitable for our scope by title and abstract screening. Therefore, we extended our research including the following keywords as free terms in the electronic search: "nanomaterial exposure", "nanoparticle exposure", which were individually combined with the operator "AND" with the terms related to the major subject of "factors involved in susceptibility to adverse health effects" such as "genotype", "genetic polymorphisms", "metabolic enzymes", "CYP450", "DNA repair systems", "epigenetic*", "age", "gender", "pathological conditions", "susceptible population". All full texts of the papers considered valuable for the aim of our review were obtained and a critical evaluation performed. The citation pool of relevant publications identified in the literature search was further supplemented through the manual assessment of the reference list accompanying published papers for other potentially eligible articles. Overall, our search retrieved a total of 69 publications for review.

3. Results

The following paragraphs will present a critical review of the available literature to provide a comprehensive view on the NP susceptibility issue with a specific focus on those aspects that emerged as potentially influencing the individual variability to tolerate or respond to such xenobiotics.

3.1. Inherited genetic variability and nanomaterials

Inherited genetic variabilities, including polymorphisms, that may affect individual susceptibility to NP exposure are still unknown. Genotype is responsible for recognition and responses to xenobiotics and, consequently, relative susceptibility to induced health effects. To date, information on heritable genome alterations able to influence the individual susceptibility to adverse health effects resulting from NP exposure are not directly available. Particularly, genetic polymorphisms that can alter the activities of enzymes involved in xenobiotic activation/detoxification reactions have not been investigated, although they may be prime candidates for identifying susceptibility biomarkers due to their capability to cause diverse responses to chemical insults. Additionally, the role of genetic variants in genes involved in DNA damage repair pathways, as determinants of susceptibility to nanomaterial insults, has not been explored. However, this topic merits wider investigation in order to define variants useful as potential biomarkers of NP susceptibility. This seems important considering that an affected capacity to repair the DNA damage may be associated with a variable risk of disease due to genome instability directly contributing to human pathologies and tumorigenesis (Tuteja and Tuteja, 2001). This lack of information is probably due to the limited knowledge regarding the NP toxicokinetic and dynamic behaviour, and particularly on the role of the above mentioned enzymes in NP metabolism as well as on their protective action against potential NP induced genotoxic effects. Moreover, the multitude of still unexplored pathways potentially involved in NP adverse effects, as well as the lack of information concerning the presence of physiological factors that may offset the effects of potential genetic variants currently prevent reaching definite conclusions regarding possible genetic susceptibility factors.

3.2. Nanomaterials and metabolic pathways

Alterations induced by nanomaterials in biological systems, generally involved in xenobiotic metabolism, may affect the individual susceptibility to adverse health effects. In this context, available toxicogenomic data, concerning gene, protein, and metabolite expression changes induced by NPs in pathways responsible for the metabolism of the vast majority of exogenous substances existing in occupational and general living environments may provide advantageous information. This may be helpful to understand NP modes of action and to explicate core biological processes affected by nanomaterials or possibly involved in their toxico-dynamic behaviour to identify potential parameters of individual susceptibility. In this context, it should be taken into account that most of the studies in this review did not compare the susceptibility to nanomaterials with that to particles characterized by the same chemical composition but larger size since these investigations were more generally conducted to probe mechanism and identify response. Moreover, from the perspective of a possible "drug–drug" interactions, it is worth noting that metabolic alterations induced by nanomaterials may result in antagonistic, synergistic and additive "mixture" of effects, modifying toxicities induced by co-exposed substances and thus disease susceptibilities. The next section focuses on the alterations induced by NP exposure in the expression and functionality of metabolic enzymes. These changes may provide data to guide the future identification of potential NP susceptibility factors. Finally, it is important to recognize that the metabolism of xenobiotics is a complex process and while individual factors may be identified multiple factors and systems may be required to affect susceptibility.

3.2.1. Nanomaterial induced alterations on phase I and II metabolic enzymes

Several *in vitro* and *in vivo* studies demonstrated that NPs were able to induce alterations in biotransformation phase I and II enzymatic pathways. In humans, in fact, biotransformation of xenobiotics occurs by a two stage process involving the functional group oxidation, exerted

Table 1
Nanoparticle induced alterations in phase I and phase II metabolic enzymes.

Outcomes	Type of Nanoparticles	Physicochemical properties	Experimental protocol	Results	References
In vitro studies					
Enzymatic functionality	Ag-NPs	Average diameter: 15 nm	Microsomes isolated from insect cells transfected with human CYP450s (Baculosomes) incubated with 40 μl of NP (50 ppm) solution	Inhibition (%): CYP1A2 (95.0 ± 0.8), CYP2C9 (83.5 ± 2.4), CYP2C19 (97.8 ± 3.2) and CYP3A4 (98.7 ± 0.5).	Sereemaspun et al., 2008
	Ag-NPs	–	Human liver microsomes incubated with an Ag-NP solution at concentrations of 0–70 μM	Maximum inhibition (IC_{50}) for CYP2C9 (9.3 ± 0.2 μM), CYP2C19 (6.4 ± 0.1 μM), and CYP3A4 (8.0 ± 0.3 μM) activities. Intermediate inhibition for: CYP2D6 and CYP2E1; minimum for CYP1A2, CYP2A6, and CYP2B6.	Lamb et al., 2010
	Ag-NPs	Average diameter: 12.42 ± 2.48 nm	Microsomes isolated from insect cells transfected with human CYP450s (Baculosomes) incubated with 40 μl of 10–80 μM of NPs	Inhibition (IC_{50}): CYP3A4 (13.52 ± 0.01 μM); CYP2C9 (26.46 ± 0.01 μM); CYP2C19 (14.31 ± 0.01 μM); CYP1A1 (43.51 ± 0.03 μM).	Warisnoicharoen et al., 2011
	Ag-NPs	Average size: <100 nm. SSA: 5 m^2/g	Liver microsomes from untreated rats were incubated with NPs at a concentration of 0–100 μg/ml for 2 min	Inhibition (IC_{50}): CYP2C (28 μg/mL) and CYP2D (23 μg/mL). Weak inhibition of CYP1A and CYP2E1 (23–25% at the highest concentration). No inhibition of CYP3A.	Kulthong et al., 2012
	SiO_2-Ag (1%)-NPs	Size: ~nm; SSA: ~100 m^2/g	Huh7 cells exposed to 0.001–0.1 mg/ml NPs (+/− CYP1A1 inducer BaP)	NPs; co-exposure NPs + BaP did not affect CYP1A; pre-treatment with NPs for 24 h + BaP reduced induction of CYP1A.	Christen and Fent, 2012
	Au-NPs	Average diameter: 9 nm	Microsomes isolated from insect cells transfected with human CYP450s (Baculosomes) incubated with 40 μl of Au-NP (44 ppm) solution	Inhibition (%): CYP1A2 (6.3 ± 2.6), CYP2C9 (28.5 ± 2.9), CYP2C19 (32.0 ± 5.4) and CYP3A4 (26.0 ± 5.9).	Sereemaspun et al., 2008
	Au-NPs	Hydrodynamic diameter: 40.5 ± 0.1 nm	5 pmol of rat CYP2B1 incubated with 100 or 500 ng pentachlorobiphenyl and 10 or 200 nmol Au-NPs for 30 min	The highest NP concentration decreased CYP mediated pentachlorobiphenyl biotransformation.	Lu et al., 2013
	Tannic acid stabilized Au-NPs	Size range: 5–100 nm	Human liver microsomes incubated with 100–300 μM NPs	Inhibition (IC_{50}):CYP2D6 (61.04 μM); CYP3A4 (68.3 μM), CYP2C9 (103.4 μM), CYP2C19 (137.1 μM), CYP3A4 (203.9 μM) by 5 nm NPs. CYP1A2 was the less susceptible isoform. Minimal effects with 100 nm NPs.	Ye et al., 2014
	Carboxyl polystyrene (CPS) latex NPs	Size: 20–500 nm	Microsomes isolated from insect cells transfected with human CYP450s (Baculosomes), normal liver cells and rat hepatoma cell line H4-II-F-C3 exposed to 50–200 μg/ml NPs (+/− cimetidine)	Inhibition (Baculosomes): 20 and 60 nm NPs dose-dependently inhibited CYP3A4 > CYP2C9 > CYP2D6 > CYP1A2. Inhibition (normal liver extracts): CYP3A4 (~65% with 20 nm NPs); 20 and 60 nm NPs increased the cimetidine inhibitory activity.	Fröhlich et al., 2010
Enzymatic gene and mRNA expression	TiO_2-NPs	Crystalline structure: rutile and anatase, size: 30–205 nm	Human lung fibroblast WI-38 cells exposed to 50 and 200 μg/ml NPs for 24 and 48 h	Up-regulation of CYP1A1gene at both doses and time points. Up-regulation of GSTM3 (4-fold) and GSTA4 (8-fold) mRNA expression	Periasamy et al., 2014
	SWCNTs	Purity >90%, diameter 1.36–1.42 nm, length 1–5 μm	NHBE, A549, HepG2, MCF-7 cells exposed to 0.00001–0.1 mg/ml CNTs for 24 h	Gene expression: CYP1A1, CYP19A1, CYP1B1, CYP2S1, GSTM3 down-regulation; GSTA4 up-regulation in NHBE and A549 cells. mRNA expression: CYP1A1 and CYP1B1 dose-dependent inhibition in NHBE, and A549 cells. Basal and TCDD induced mRNA: reduced CYP1A1 and CYP1B1 expression in both conditions of treatment in A549, HepG2, MCF-7 cells.	Hitoshi et al., 2012
	Carbon-NPs	Size: <50 nm	Human mesenchimal stem cells exposed to 50–100 μg/ml for 24 h	Gene expression: 4-fold up-regulation of CYP1A1 at both concentrations; GSTM3 was down-regulated by 50 μg/ml and 2-fold up-regulated by 100 μg/ml compared to the lowest dose.	Alshatwi et al., 2013
	Graphene nanoplatelets	High purity (99.9%) single-layer graphene oxide and carboxyl graphene	Topminnow fish (*Poeciliopsis lucida*) hepatoma cell line (PLHC-1) exposed to 4 and 16 μg/ml nanoplatelets with AhR agonists for 24 h	CYP1A mRNA expression: increased by nnaoplatelest. (Functionality induced by AhR agonists alone was increased by NPs).	Lammel et al., 2015
In vivo studies					
Enzymatic functionality	PEG-coated Au-NPs	Average size: 4, 13 and 100 nm	BALB/C mice (9 per group) were exposed via a single tail vein injection to 3.04×10^{13}, 8.80×10^{11}, 2.04×10^{9}	Up-regulation of CYP1A1 at day 7 by 4 nm NPs; up-regulation CYP2B at day 7 by 4 and 13 nm NPs, and at 24 h by	Cho et al., 2010

Table 1 (*continued*)

Outcomes	Type of Nanoparticles	Physicochemical properties	Experimental protocol	Results	References
			of 4, 13 and 100 nm NPs, respectively. Mice were euthanized 30 min- 6 months post administration	13 nm NPs.	
	Ag-NPs	Average size: <100 nm. SSA: 5 m^2/g	Sprague-Dawleys rats (5 per group) were orally administered with NP suspensions at doses of 0–1000 mg/kg for 2 weeks	No significant changes for CYP1A1, CYP2C2, CYP2D, CYP2E1, CYP3A up to the highest tested dose.	Kulthong et al., 2012
	Ag-nano-colloid formulation	Elemental Ag-NPs with a mean size of 32.8 nm	Human volunteers orally ingested for 1–14 days the Ag colloidal formulation at a dose of 480 mcg/day for the selected 32 ppm concentrations. CYP450 activity probes, i.e. caffeine, losartan, omeprazole, dextromethorphan, midazolam and chlorzoxazone were administered at day 1 and 14.	The parent to metabolite probe ratios for single dose or 14 day multiple dose active Ag-NP formulation did not demonstrate significant inhibition/induction of the CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.	Munger et al., 2015
Enzymatic gene, and mRNA expression	TiO_2-NPs	Crystalline structure: 100% anatase; average size: 7 nm	CD-1 ICR mice (20 per group) were given intragastric administration of NPs (0–50 mg/kg) every other day for 60 days	Liver mRNA expression: CYP1A1 increased 1.67 and 2.2 fold after 10 and 50 mg/kg NPs, respectively.	Cui et al., 2010
	TiO_2-NPs	Crystalline structure: anatase; average size: 5–6 nm	CD-1 ICR mice (20 per group) were given intratracheal instillation of NPs (0–10 mg/kg) every day for 90 days	Lung mRNA expression: CYP1A increased in a dose dependent manner	Sun et al., 2012
	Au-NPs	Average size: 20 nm	Wistar rats (6 per group) were exposed to 0.01 mg/kg NPs via a single tail-vein injection and sacrificed 1 day, 1 week, 1 and 2 months post-administration	Liver gene expression (2 months post exposure): 28 fold up-regulation of CYP1A1; ~2.2–2.4 up-regulation of CYP4A10, CYP4A22 and CYP3A13; 6.8 down-regulation of CYP2C40.	Balasubramanian et al., 2010
	Pristine-; Acid oxided- (O-); Tween-80-dispersed (T-) MW-CNTs	Pristine MW-CNTs: diameter 10–20 nm, length 5–50 μm	Kunming mice (10 per group) were exposed to 10 and 60 mg/kg CNTs by intravenous injection for 15 and 60 days	Liver gene expression: O-MW-CNTs and T-MW-CNTs (60 mg/kg) induced down-regulation of CYP2C50 and up-regulation of CYP2B19. GSTA2 was down-regulated by T- MW-CNTs (60 mg/kg).	Ji et al., 2009
Enzymatic protein expression	Si-NPs and Cd coated Si-NPs	Cd/Si-NPs: size range: 20–80 nm; SSA: ~200 m^2/g; Si-NPs: size range: 60–90 nm; SSA: ~240 m^2/g;	Sprague-Dawleys rats (6 per group) were intratracheally instilled with 1 mg/rat Cd/Si-NPs and 600 μg/rat Si-NPs and euthanized 1–30 days post administration	Pulmonary CYP450: immunoreactivity was enhanced by Cd/Si-NPs, in a time-dependent manner (24 h < 7 days <30 days) at bronchiolar and alveolar level. Si-NPs did not influence CYP450 epitope immunolabeling.	Coccini et al., 2013

A549 cells, human lung carcinoma cells; Ag-NPs, silver nanoparticles; Au-NPs, gold nanoparticles; BaP, benzo(a)pyrene; CYP, cytochrome P; GST, glutathione-S-transferase; HepG2 cells, human hepatic carcinoma cells; Huh7 cells, human hepatoma cells; IC_{50}, half maximal inhibitory concentration; MCF-7 cells, human breast carcinoma cells; PEG, poly(ethylene)glycol; MW-CNT, multi walled carbon nanotube; SSA, specific surface area; NHBE cells, primary normal human bronchial epithelial cells; Si-NP, silica nanoparticle; TiO_2-NPs, titanium dioxide nanoparticles.

by phase I enzymes, belonging to the cytochrome P450 (CYP450) family, and the subsequent conjugation with, amongst others, glucuronic acid, gluthatione and sulfate groups depending on phase II enzymes, i.e. glutathione transferase (GST) (Gay et al., 2010). Variations in the CYP450 metabolism may be caused by genetic variants, gender differences, age and external induction/inhibition of isoenzymes (Ingelman-Sundberg et al., 2007).

The CYP450 enzymes were vulnerable to NP exposure in cellular and animal models (Table 1). In fact, several *in vitro* studies reported the ability of different metal, metal oxide as well as carbon based and polymeric NPs to acutely (after up to 24 h of treatment) or sub-acutely (after up to 48 h of treatment) affect the gene (Alshatwi et al., 2013; Hitoshi et al., 2012; Periasamy et al., 2014) and mRNA expression (Hitoshi et al., 2012; Lammel et al., 2015) as well as the functionality (Christen and Fent, 2012; Fröhlich et al., 2010; Kulthong et al., 2012; Lu et al., 2013; Lamb et al., 2010; Sereemaspun et al., 2008; Warisnoicharoen et al., 2011; Ye et al., 2014) of CYP metabolic enzymes, although with different results maybe in relation to the type and physico-chemical characteristics of the NPs investigated. Moreover, induction or inhibition of CYP450 gene (Balasubramanian et al., 2010; Ji et al., 2009), mRNA (Cui et al., 2010; Sun et al., 2012) and protein expression (Coccini et al., 2013), as well as alterations in its metabolic functionality (Cho et al., 2010; Kulthong et al., 2012) were also reported in *in vivo* studies under various conditions of exposure involving a series of different NPs (Table 1). Conversely, a first human assessment of CYP450 enzyme activity failed to detect significant alterations after NP systemic exposure (Munger et al., 2015) (Table 1). Additionally, as previously mentioned, in phase II enzymes, GST is a family of detoxification enzymes that catalyzes the conjugation of glutathione to a wide variety of endogenous and exogenous compounds, such as therapeutic drugs, environmental toxicants and products of oxidative stress. GST polymorphisms may be disease modifying, determining a significant and biologically relevant impact on pathogenic susceptibility (Hayes et al., 2005). NPs demonstrated the ability to affect the expression of different isoforms of GST. Interestingly, a significant up-regulation in the mRNA expression level of GSTM3 (4-fold) and GSTA4 (8-fold) in WI-38 cells treated with titanium dioxide (TiO_2)-NPs was reported (Periasamy et al., 2015). Comparably, Hitoshi et al., 2012 found that these two isoforms were significantly up-regulated in NHBE cells. Conversely, an *in vivo* experiment showed a down-regulation of GSTA2 gene expression following Tween-80 dispersed multi-walled carbon nanotubes (MW-CNTs), while no changes were detected after acid oxide-MW-CNT exposure (Ji et al., 2009) (Table 1).

3.2.2. Susceptibility and phase I and II metabolic enzymes

It is well known that NPs are not a homogeneous group of substances (Luyts et al., 2013). In this context, it may be assumed that their different characterization may be responsible for a diverse reactivity with the enzymatic systems, potentially determining a variable susceptibility to adverse effects. However, the diverseness in the influences of NPs on CYP450s is still poorly understood, particularly as regard the action of NP physico-chemical features in modulating such interactions. Chemical composition seemed to play a key-role in the NP-enzyme relationship. As an example, silver (Ag)-NPs *in vitro* were demonstrated to exert a three-fold greater inhibitory effect on four CYP450 isoenzymes compared to gold (Au)-NPs with a similar size (Sereemaspun et al., 2008). Additionally, *in vivo*, no alterations in the pulmonary CYP450 expression of rats instilled with silica (Si)-NPs were detected compared to the increase induced by Cd containing-Si-NPs (Coccini et al., 2013). However, the finding that enzymatic changes could be observed after treatment with a variety of NPs which differed in several physico-chemical features other then chemical composition, do not allow exclusion of the role of other NP parameters in determining enzymatic alterations. In this regard, nano-scale size should be stressed as a potential modifier of the NP effects on CYP450s. Fröhlich et al. (2010), in fact, showed that the inhibition potency of carboxyl polystyrene NPs increased with the decrease of particle size. These results closely accorded with those obtained by Ye et al. (2014) which found that the smallest, 5 nm, Au-NPs showed a more pronounced, dose-dependent inhibitory effect on some CYP450 isoforms compared to the minimal changes exerted by 100 nm NPs. Comparably, Cho et al. (2010) reported that 4 and 13 nm Au-NPs transiently activated CYP450 enzymes in mouse liver tissues, while larger 100 nm NPs failed to induce such alterations. Additionally, the different time-dependent alterations in CYP450 function, as reported in liver microsome models (Ye et al., 2014), may be an interesting topic of future research aimed to understand the clinical pharmacokinetic or toxicological consequences of the NP-CYP450 interactions, considering also that, in occupational settings, repeated as well as long-term exposures are quite common. The physico-chemical properties of NPs may be responsible for driving different types of NP-CYP450 interactions, i.e. directly affecting CYP gene expression changes (Hitoshi et al., 2012) and physical enzyme conformation, thus leading to perturbations in the stereo-selective enzymatic metabolism or indirectly inducing enzymatic micro-environment alterations (Fröhlich et al., 2010; Lamb et al., 2010; Lu et al., 2013; Ye et al., 2014). The hydrophobicity, surface charge, the larger curvature of smaller NPs as well as the surface capping agents at the NP-enzyme interface have all emerged as features potentially affecting NP-enzyme and NP-enzymatic microenvironment interactions *in vitro* (Ye et al., 2014).

In turn, also the unique features of the CYP450 isoenzymes, i.e. structural diversity, heterogeneity, and plasticity as well as the different active site cavity volumes and drug substrate specificity should be considered to understand the NP-induced effects on the enzymatic functions. In fact, not all the CYP450s showed an equal sensitivity to NP exposure as demonstrated by the variable alterations induced, as well as by the half maximal inhibitory concentrations (IC_{50}) necessary to affect different enzyme isoforms (Fröhlich et al., 2010; Lamb et al., 2010; Sereemaspun et al., 2008; Ye et al., 2014). However, the complex interplay between the impressive range of chemical modifications that CYP enzymes and nano-xenobiotics may accomplish is still not understood and needs to be deeply investigated.

Additionally, common biological alterations exerted by NP exposure in treated cells and animal models, i.e. the generation of inflammatory reactions closely related to oxidative stress, should be carefully viewed as possible triggering mechanisms of CYP450 alterations. The formation of reactive oxygen species (ROS), in fact, as reported with different NP types (Periasamy et al., 2014; Christen and Fent, 2012; Cui et al., 2010; Sun et al., 2012), could damage cell membranes, thereby facilitating NP cellular internalization, possible cytotoxic effects and changes in the synthesis and functionality of the CYP family. However, these same biological processes may exert different effects according also to the intrinsic or acquired capacity of an organism to activate specific defence mechanisms. Redox homeostasis of cells, in fact, is ensured by their complex endogenous antioxidant defence system (Pisoschi and Pop, 2015). Beside this defence, metallic-NP detoxification systems, based on the metallothionein protein expression, may be another protective mechanism against NP toxicity (Kaewamatawong et al., 2014; Zhang et al., 2015). Therefore, diverse sensitivity to specific NPs may be related to the diverse ability of cells to activate such mechanisms. This capacity may be related to the cell lineage analysed, differences in primary versus transformed cells, diverse species investigated as well as to the conditioning insults previously undergone by cells and organisms. In this context, also understanding how cells sense ROS and transduce these stimuli into downstream biological responses is still a major challenge. ROS can provoke reversible and irreversible modifications into proteins involved in diverse signalling pathways. These post-translational modifications may lead to oxidative damage and/or trigger structural alterations of target proteins, therefore affecting cellular processes and sensitivity to NP effects (Ge et al., 2011). Unfortunately, the limited number of available studies, as well as the different outcomes investigated, prevents the extrapolation of specific information and requires further investigation.

The great variety of affected CYP450s and their unique substrate spectrum, raise concerns regarding the possibility that the NP induced alterations may affect the pharmaco-toxico-kinetic modelling of other co-exposed substances in "drug–drug" interactions. This seems important considering that nanomaterial workers may be treated with different pharmacological agents or may be occupationally co-exposed to other chemical substances whose metabolism may be quantitatively or qualitatively affected by NPs, therefore resulting in altered therapeutic or toxicological effects. In this scenario, Hitoshi et al. (2012) demonstrated the persistence of the inhibitory effect of single walled-carbon nanotubes (SW-CNTs) on CYP1A1 and CYP1B1 mRNA expression in cellular models even following the cell treatment with a strong CYP450 inducer, tetrachlorodibenzo-p-dioxin. In human hepatoma (Huh7) cells, silicon dioxide (SiO_2)–1% Ag-NP pre-exposure, reduced the CYP1A-induction caused by benzo(a)pyrene (Christen and Fent, 2012). In human CYP450-expressing baculosomes and microsomes from normal animal liver, carboxyl polystyrene latex NPs decreased CYP450 activity enhancing the own effects of cimetidine, a known inhibitor of CYP3A4 (Fröhlich et al., 2010). The existence of combination effects between NPs and environmental pollutants has been also recently reported by Lammel et al. (2015) with graphene nanoplatelets in fish hepatoma cells. This type of nanomaterials, in fact, showed a potentiating effect on the inductive action exerted by several aryl-hydrocarbon-receptor (AhR) agonists on CYP1A mRNA expression and functionality. The authors argued that the graphene dependent potentiation on CYP1A could be explained by the nanoplatelet-induced structural damage or destabilization of the plasma membrane which may facilitate the passive diffusion of AhR agonists and the CYP1A induction. As previously mentioned, this seems an intriguing topic of future investigation, because in this preliminary phase of knowledge, it is important not to disregard that the inflammatory and oxidative stress reactions caused by NP phagocytosis or endocytosis, irrespective of their specific physico-chemical properties, may directly determine perturbations in enzymatic pathways. These inflammatory effects may influence the NP interactions with other contaminants already existing in the occupational settings, provoking an enhancement of the toxicity that needs to be carefully considered in risk assessment procedures (Lammel et al., 2015).

Concerning phase II enzymes, although the limited number of studies prevents drawing definite conclusions, understanding the differential effects exerted by various NPs, under different conditions of exposure, on diverse enzyme isoforms, and the possible triggering mechanisms i.e. inflammatory or oxidative stress reactions, may be

important to define the NP toxicokinetic and dynamic profile as well as the individual variability in capacity to metabolize co-exposed substances therefore determining potential disease susceptibilities.

3.3. Nanomaterials and DNA repair systems: alterations and susceptibility

Various *in vitro* studies demonstrated that different types of NPs up- or down-regulated specific DNA repair systems as a response to the NP induced oxidative stress or to a direct double strand break DNA damage (Asharani et al., 2012; Hwang Do et al., 2012; Lan et al., 2014; Mei et al., 2012). Initiation of DNA repair response was observed in U251 brain cancer cells treated with Ag-NPs as demonstrated by increased levels of the ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein kinases, which act as sensors of double strand breaks thus activating downstream targets for DNA repair (Asharani et al., 2012). When Ag-NPs were used to treat mouse lymphoma cells, genes such as the xeroderma pigmentosum group A protein complex (Xpa) and excision repair cross-complementing rodent repair deficiency, complementation group 2 (Ercc-2), related to DNA repair were significantly down regulated, potentially resulting in increased levels of gene mutations and chromosomal alterations (Mei et al., 2012). TiO_2-NPs induced a wide range of repair pathway activation in human A549 cells, including severe DNA double strand break repair, and the up-regulation of 8-OhdG-DNA glycosylase 1 (OGG1), a specific system for repairing NP induced oxidative DNA damage (Lan et al., 2014). However, no OGG1 up-regulation, was evident in the same human cell types when carbon black NPs, SW-CNTs and Fullerenes C_{60} were used for treatment, suggesting that some mechanisms, other than those related to oxidative stress damage may contribute to the severe DNA damage in cells as well as to the activation of specific repairing systems. Additionally these data point out the importance to understand the NP genotoxic mechanisms of action, eventually influenced by their physico-chemical properties to define potential susceptibility features (Lan et al., 2014). In line with this consideration, the NP chemical form appeared important in influencing the expression profiles of DNA repairing genes (Hwang do et al., 2012). In fact, a significant up-regulation of specific genes involved in DNA damage repair was demonstrated in *in vitro* and *ex vivo* experiments carried out with silica-free magnetic-core cobalt ferrite ($CoFe_2O_4$)-NPs, while silica coated counterparts showed a gene expression pattern similar to the untreated controls (Hwang do et al., 2012).

Interestingly, in *in vivo* results, rats treated with carbon NPs showed an increased expression of the DNA- apurinic or apyrimidinic endonuclease 1 (APE-1), a multifunctional protein that possesses both DNA repair and redox regulating activity, compared to controls (Wessels et al., 2011). Additionally, magnetic-core $CoFe_2O_4$-NPs were reported to enhance the expression of the protein kinase ATM repairing pathways in liver of treated mice (Hwang Do et al., 2012) (Table 2).

These data support the idea that different NPs may affect variable DNA defense systems leading to distinct susceptibility to genotoxic effects that should be clearly defined considering the relevance DNA damage may have for a variety of diseases. Moreover, as previously addressed, the role of the oxidative stress reactions induced by NP exposure, and that of the antioxidant defence patterns, should be carefully evaluated as one of the possible influencing factors in determining the genotoxic potentials of these xenobiotics as well as specific pathways of enzymatic responses.

Table 2
Nanoparticle induced alterations in DNA repair systems.

Outcomes	Types of Nanoparticles	Physicochemical properties	Experimental protocol	Results	References
Gene expression of DNA repair systems	Ag-NPs	Size range: 6–20 nm	Normal human lung fibroblasts (IMR90) and human brain cancer cells (U251) treated with 400 μg/ml NPs for 48 h	IMR-90: down-regulation of genes involved in base excision repair (Mbd4, Ape1, OGG1, Mutyh), mismatch repair (Mutyh, Abl1, Pms1, MSH2), double strand break repair pathways (ATM). U251: up-regulation of ATM, ATR, XRCC5 and Xpa genes; down-regulation of Ape1, OGG1, Mbd4, MSH2.	AshaRani et al., 2012
	Ag-NPs	Size range: <4–12 nm	L5178Y/Tk$^{+/-}$ mouse lymphoma cell line exposed to 5 μg/ml NPs for 4 h	Xpa and Ercc-2 genes were significantly down regulated.	Mei et al., 2012
	Silica coated $CoFe_2O_4$ magnetic fluorescence NPs; magnetic core NPs (only $CoFe_2O_4$)	Magnetic core NP size: 32.6 ± 4.5 nm	Human hepatoma Hep3B cells exposed to 100 μg NPs for 24 h. Liver tissues isolated from BALB/C mice (3 per group) 24 h after administration of 500 μg of NP suspension via the tail vein	XRCC2 (Hep3B) and ATM, Rad23a and Rad50 (liver tissues) were up-regulated by silica free magnetic core NPs, compared to untreated and silica coated $CoFe_2O_4$ magnetic fluorescence NP treated cells and tissues.	Hwang Do et al., 2012
	TiO_2-NPs, Carbon black-NPs; SW-CNTs; C60 fullerenes	SSA: TiO_2 anatase 274.2; Carbon black-NPs: 110.6; SW-CNTs: 342.9; C60 fullerenes: 0.2 m^2/g	Human lung epithelial A549 cells exposed to NPs for 0.5–24 h	TiO_2-NPs: double strand repair system and OGG1 up-regulation. No OGG1 up-regulation was induced by the other carbon based NPs. C_{60} fullerenes induced NHEJ repairing pathway.	Lan et al., 2014
mRNA expression of DNA repair systems	Carbon NPs	Average size: 55.3 ± 1.80–58.8 ± 1.65 nm	C57BL/6J mice (12 per group) and Fischer F344 rats (6 per group) were exposed by nose-inhalation to NPs (141–152 μg/m^3) for 4 h on one day and for 4 h on 3 days	DNA based excision repair genes OGG1, Ape1, XRCC1, DNA POL β were not changed in mouse lungs; DNA endonuclease Ape1 was increased in rat lungs compared to controls.	Wessels et al., 2011

Abl, a proto-oncoprotein belonging to the Src family of non-receptor tyrosine kinases; Ag-NPs, silver nanoparticles; Ape1, DNA-apurinic or apyrimidinic endonuclease 1; ATM, Ataxia Telangiectasia Mutated protein kinase; ATR, ATM and Rad3-related protein kinase; $CoFe_2O_4$, cobalt ferrite; DNA POL β, DNA polymerase-β; Ercc-2, Excision repair cross-complementing rodent repair deficiency, complementation group 2; Mutyh, DNA glycosylase; NHEJ, non-homologous end-joining; CYP450, cytochrome P450; Mbd4, methyl-CpG-binding domain protein 4; MSH2, MutS homologues 2; NP, nanoparticle; OGG1, 8-OhdG-DNA glycosylase 1; Pms1, post-meiotic segregation protein; SSA, specific surface area; SW-CNT, single walled-carbon nanotube; TiO_2-NPs, titanium dioxide nanoparticles; XRCC1, X-ray cross complementing group-1; XRCC2, X-ray cross complementing group-2; XRCC5, X-ray cross complementing group-5; Xpa, Xeroderma pigmentosum, group A protein complex.

3.4. Epigenetics and nanomaterials

A number of studies highlighted the ability of certain nano-sized compounds to induce epigenetic effects, such as DNA methylation changes, histone modifications, as well as specific alterations in post-transcriptional regulator molecules, i.e. miRNA expression. The human genome mapping provides an invaluable and long-awaited glimpse into the relationship between genotype and phenotype. However, to gain perspective on the mechanistic basis of NP-susceptibility, there is need to assess additional factors that cannot be explained only by the genome sequence. In this scenario, epigenetic factors contribute to heritable changes in gene expression occurring without changes in DNA sequence. The issue of how NP induced epigenetic processes, including DNA methylation, histone tail modifications, and non-coding microRNA expression may significantly modulate cellular behaviour in response to this chemical insult is an emerging topic (Stoccoro et al., 2013; Kim et al., 2012). Particularly, SiO_2-NPs, but not SiO_2 microparticles, were reported to induce a global reduction in genomic DNA methylation while increased the methylation of poly (ADP-ribose)polymerases-1 (PARP-1) promoter, therefore causing a decrease in PARP gene expression (Gong et al., 2010, 2012). DNA methylation appears to be an important controlling factor in gene expression, particularly when found in the CpG islands in promoter regions (Stirzaker et al., 2004). In general, loss of DNA methylation may lead to gene activation, whereas inactive genes were often methylated (Jaenisch and Bird, 2003). These early epigenetic dysregulations may mediate, in whole or in part, the long term consequences as well as the susceptibility to SiO_2-NP toxicity. PARP-1, in fact, is a pivotal gene involved in DNA repair processes and therefore its reduced expression may predispose to genomic instability.

Moreover, as regards modifications induced on histones, global hypoacetylation of histones was detected in human breast carcinoma cells treated with cadmium telluride quantum dots (CdTe-QDs) (Choi et al., 2008). Conformational changes in histone proteins may either facilitate or depress the access of transcriptional machinery to the promoter region of some genes, leading to gene silencing or activation, respectively. Histone deacethylation was related to a more condensed chromatin state and transcriptional repression as Choi et al. (2008) demonstrated for anti-apoptotic genes, thereby promoting cellular death. Therefore, the described effects of NPs on chromatin structure point towards possible intermediate processes that NP exposure may imprint on the gene expression patterns therefore affecting the susceptibility to long term consequences.

MicroRNAs have been investigated in an attempt to identify fine, regulator molecules in NP induced toxicity (Eom et al., 2014; Li et al., 2011a, 2011b; Ng et al., 2011). MicroRNAs in fact, can regulate the flux of genetic information by repressing gene expression at the post-transcriptional level thus potentially affecting a wide variety of cellular processes (He and Hannon, 2004). The changes in the microRNA expression profiling induced by exposure to iron(III) oxide (Fe_2O_3)-NPs, CdTe-QDs and MW-CNTs were demonstrated to globally alter the mRNA and protein output of NIH/3T3 treated cells, subsequently affecting many key biological processes (Li et al., 2011a, 2011b). The diversity and abundance of microRNA targets offer an enormous level of combinatorial possibilities, and suggests that microRNAs and their targets appear to form a complex biological regulatory network (Eom et al., 2014; Ng et al., 2011). In this context, concurrent analysis of altered microRNAs and mRNAs target pairs is a powerful approach to explore the direct response of the genome to the NP toxicant exposure. Au-NPs induced the up-regulation of microRNA155 and a concomitant inhibition of PROS1 gene which codes for Protein S involved in controlling blood clotting thus leading to coagulopathic disorders (Ng et al., 2011). Moreover, microRNA expression profiling of human Jurkat T cells resulted in 63 differentially expressed microRNAs upon exposure to Ag-NPs (Eom et al., 2014). Particularly, the decreased expression of has-microRNA-219-5p was negatively correlated to the mRNA expression of metallothionein 1F and TRIB3 proteins. These findings may indicate a possible NP epigenetic effect on metal homeostasis and cellular signalling pathways in which these two proteins are involved, respectively (Eom et al., 2014).

Even more challenging may be to understand how such alterations may affect the NP-related disease risk in more advanced biological organisms. In this context, the inhalation exposure of C57BL/6BomTac mice to TiO_2-NPs, induced pulmonary up-regulations of microRNA449a, microRNA1, and microRNA135b, which have been implicated in inflammation and immune response processes (Halappanavar et al., 2011). However, further characterization of microRNA responsive genes and their role in pulmonary adverse effects need to be performed to determine the biological relevance of such epigenetic modifications. Interestingly, a model involving trans-placental exposure of mice demonstrated that Au-NPs were able to exert epigenetic effects in fetal tissues. In fact, repeated NP intra-peritoneal exposure in pregnant dams determined a significant up-regulation of microRNA let-7a and microRNA-183 expression both in fetus lungs and livers while failed to induce adverse effects on adult dams (Balansky et al., 2013) (Table 3). Importantly, the effects that NP induced epigenetic modifications may have on a number of biological processes, may be an issue of future investigation to define possible mechanisms of specific susceptibility to NP toxicity.

3.5. Other susceptibility factors

In order to define populations susceptible to NP effects, a series of factors require further investigation. Life stage in which NP exposure may occur seems a critical aspect in determining susceptibility to nanomaterial induced adverse health effects, particularly as concerns elderly and youth. Aging is a complex physiological process characterized by the decline of cellular and organic functions which may predispose elderly to certain metabolic, cardiovascular, respiratory, and neurological diseases which may be aggravated by NP exposures or in turn may increase the susceptibility to NP adverse effects (Li et al., 2014). Considering the aging of the active working population, the appropriate management of occupational risks in balance with emerging age-related functional limitations appears a challenging issue. Age has been reported to modify the susceptibility of rat liver mitochondria to iron(II,III) oxide (Fe_3O_4)-NPs which impaired all complexes of the mitochondrial respiration chain in middle aged animals (18 month-old), but not in young rats (3 month-old) (Baratli et al., 2013, 2014). Several mechanisms might explain these results, like increased fragility of older mitochondria and an excessive iron accumulation considered a feature of the aging process that becomes a potential causative factor of age-related mitochondrial dysfunction under conditions of cellular stress (Baratli et al., 2014). Unlike young and adult rats, old animals were more sensitive to cardiovascular and respiratory alterations induced by inhalation of SiO_2-NPs (Chen et al., 2008). The difference in toxicologic sensitivity between old, adult, and young rats may be due to the higher respiration volume of old animals compared to adult or young rats which could mean a higher uptake of SiO_2-NPs and more severe health effects. Interestingly, the youngest (9–10 weeks) and the oldest (30–35 weeks) age groups of rats treated with copper, Ag-, or aluminium-NPs showed the greatest metal NP induced neurotoxicity, as compared to the middle age group, although the precise mechanisms behind this age-related effect were not defined (Sharma et al., 2013). Also in the case of TiO_2-NP oral exposure, young rats (3 weeks) were reported to be more susceptible to liver and heart injuries and to non-allergic mast cell activation in stomach tissues compared to adult animals (8 weeks) (Wang et al., 2013). Elderly and youth hypersensitivity to NP exposure determined in experimental investigations, was also supported by the association between ultrafine particle exposure and several asthma-related outcomes reported in pediatric and elderly populations (Benor et al., 2015; Evans et al., 2014; Peters et al., 2011). Extrapolated to a workplace setting, these findings may mean that both elderly workers and young trainees may be more vulnerable to NP

Table 3
Nanoparticle induced epigenetic alterations.

Types of Nanoparticles	Physicochemical properties	Experimental protocol	Results	Reference
In vitro studies				
SiO_2-NPs	Primary size: 15 nm	HaCaT cells exposed to 0–10 μg/ml NPs for 24 h	Global DNA methylation level decreased with increased NP dose. The mRNA and protein expression of methyltransferases (DNMT1 and DNMT3) dose dependently decreased.	Gong et al., 2010
SiO_2-NPs	Primary size: 15 nm	HaCaT cells exposed to 0–10 μg/ml NPs for 24 h	PARP1 mRNA and protein expression decreased in a dose dependent manner while there was an increase in the level of PARP-1 methylation in treated cells compared to controls.	Gong et al., 2012
CdTe-QDs	–	MCF-7 cells exposed to 5 μg/ml QDs for 24 h	Nucleous undergoes chromatic condensation and hypoacetylation of histone 3 after treatment. Western blot analysis revealed global hypoacetylation at <5 μg/ml QDs which was linked to decreased transcription of anti-apoptotic genes, i.e. cIAP-1, GPx, Hsp70.	Choi et al., 2008
CdTe-QDs; Fe_2O_3-NPs, MW-CNTs	Size: 1–3 nm CdTe-QDs; 3–9 nm Fe_2O_3-NPs, <50–400 nm in length MW-CNTs	NIH/3T3 cells treated with 100 μg/ml Fe_2O_3-NPs; 100 μg/ml MW-CNTs and 30 μg/ml CdTe-QDs for 24 h	Expression of microRNA was widely dysregulated after NP exposure. By affecting the output of targeted mRNAs, microRNAs widely regulated the KEGG pathways and GO biological processes in NP treated cells.	Li et al., 2011a
CdTe-QDs	Size: 1–3 nm CdTe-QDs	NIH/3T3 cells treated with 30 μg/ml CdTe-QDs for 24 h	Expression of microRNA was globally altered by NP exposure in a dose dependent manner.	Li et al., 2011b
Au-NPs	Diameter: 20 nm	MRC5 cells treated with NPs at a final concentration of 1 nM for 48 or 72 h	Up-regulation of non coding microRNA155 in treated cells compared to controls. MicroRNA155 could regulate the expression of PROS1.	Ng et al., 2011
Ag-NPs	Size: <100 nm	Human Jurkat T cells exposed to 0.2 mg/ml NPs for 24 h	The expression of 63 microRNAs was altered by Ag-NPs (has-miR-1238 and has-miR-938 were most decreased). The expression of microRNA, has-miRNA-219-5p was negatively correlated with those of mRNA for MT1F and TRIB3.	Eom et al., 2014
In vivo studies				
TiO_2-NPs	Crystalline form: rutile; average size 20.6 ± 0.3 nm; SSA: 107.7 m^2/g	Female C57BL/6BomTac mice (8 per group) treated via inhalation for 1 h daily to 42.4 ± 2.9 mg/m^3 surface coated NPs for 11 days	The lung expression of 55 microRNAs was altered by Ag-NPs. Up-regulation of microR1, microR449a and microT135b was detected.	Halappanavar et al., 2011
Au-NPs	Average size: 40 and 100 nm	Pregnant mice treated with a single intraperitoneal injection of 3.3 mg/kg on days 10, 12, 14 and 17 of gestation	MicroRNA expression was significantly affected only by 100 nm NPs in fetal lungs (28 microRNAs) and livers (5 microRNAs). Let-7a and microRNA-183 were the only microRNAs up-regulated in both tissues.	Balansky et al., 2013

Ag-NPs, silver nanoparticles; cIAP-1, inhibitor of apoptosis; Fe_2O_3-NPs, iron oxide nanoparticles; GPx, glutathione peroxidise; HaCaT cells, Human epidermal keratinocyte cell line; Hsp70, Heat shock protein 70; MCF-7 cells, human breast carcinoma cells; MRC5 cells, human fetal fibroblasts; MW-CNT, muti walled carbon nanotube; NIH/3T3 cells, mouse embryonic fibroblast *cell* line; NP, nanoparticle; PARP1, poly (ADP-ribose) polymerases-1; CdTe-QDs, cadmium telluride quantum dots; SiO_2, silicon dioxide; TiO_2-NPs, titanium dioxide nanoparticles.

adverse effects and this should be carefully considered in risk assessment and management processes.

NP exposure during pregnancy should be also considered with caution in terms of susceptibility to adverse effects both for the women health as well as for possible trans-generational effects. In fact, NP-induced toxicity may be amplified in the pregnant population due to the neuroendocrine and cell-mediated immunity changes that occur during pregnancy (Li et al., 2014). Moreover, concerning the possible adverse effects of prenatal exposure, recent research demonstrated that various types of NPs could cross the placental barrier and enter the fetus with an increased NP-materno-fetal transfer in case of intrauterine inflammation (Qi et al., 2014; Tian et al., 2013). Decreased gestational success rate (Yamashita et al., 2011), fetal malformations, retarded neonatal development as well as toxicity to the nervous, renal and reproductive systems in offsprings (Ema et al., 2010; Noori et al., 2011; Shimizu et al., 2009; Umezawa et al., 2011) were reported after various NP exposure during pregnancy, i.e. TiO_2-, Fe_3O_4-NPs and CNTs. This seems a critical issue to face while evaluating occupational NP risks for women of childbearing age employed in the nanomaterial sectors, and requires the adoption of a precautionary management approach before all of the evidence concerning prenatal susceptibility to NP exposure is completed.

Gender differences may also determine variability in responses to NP exposure. This seems an important issue in pharmacokinetic and pharmacodynamic research since men and women differ in many aspects of vulnerability to xenobiotics, which mainly involve substance absorption and metabolism, as well as expression and inducibility of CYP450s. Moreover, lifestyle, psychosocial and hormonal factors may all modify the kinetics and responsiveness to external substances of male and female subjects (Gochfeld, 2007). In this regard, gender related differences were reported in NP biokinetic profile of Ag-NPs that showed longer half-lives of elimination in female mice (Xue et al.,

2012) as well as in Ag and Au-NP organ distribution, with greater metal accumulation in kidneys of female compared to male animals (Kim et al., 2008, 2009; Sung et al., 2009, 2011; Xue et al., 2012). Interestingly, in line with NP accumulation data, obvious kidney damage was evident in females treated with polyethylene glycol (PEG)-coated Au-NPs while male animals showed more severe alterations in blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels as biomarkers of hepatic function (Chen et al., 2013). However, the exact functional meaning of the gender-related differential accumulations and the causal anatomically or hormonally based mechanisms underlining different health effects are poorly understood and should be investigated to define susceptibility factors which may deserve occupational health attention.

Pathological conditions, such as cardiovascular disorders, which may disable physiological functionalities, therefore resulting in susceptibility to NP effects should be evaluated to comprehensively assess the risk of toxicity after NP exposure. SW-CNTs (Li et al., 2007) and MW-CNTs (Xu et al., 2012) as well as nickel hydroxide-NPs (Kang et al., 2011) were able to accelerate the atherosclerosis progression in aortas of *in vivo* susceptible models. More recently, MW-CNTs were reported to induce a transient decrease in blood pressure, a long-term reduction in the heart rate as well as structural changes in large arteries in spontaneously hypertensive rats compared to healthy animals (Chen et al., 2015). These results are in line with epidemiological findings that have implicated particulate air pollution, and specifically its ultrafine size fraction, as an important contributor to morbidity and mortality from cardiovascular causes (Peters et al., 2011). It is likely that high ultrafine particle exposure may lead to systemic inflammation and oxidative stress responses thereby promoting the progression of atherosclerosis, and precipitate acute cardiovascular responses ranging from increased blood pressure to myocardial infarction (Delfino et al., 2005). Induced inflammation and oxidative stress may add to the burden of known life style risk factors for cardiovascular disease such as diet, tobacco smoke and stress. Additionally, several categories of individuals within the general population may be at higher risk for air pollution–mediated cardiovascular morbidity, such as people with pre-existing cardiovascular disease, people with diabetes, and elderly individuals (Simkhovich et al., 2008).

Additionally, concerning respiratory disorders, exposure to different types of NPs was able to exacerbate pre-existing inflammatory conditions of the respiratory tract, as reported in animal models of lipopolysaccharide-induced respiratory disease (Cesta et al., 2010; Inoue, 2011) or to aggravate allergen induced airway hyper-reactivity (Hussain et al., 2011; Inoue et al., 2009, 2010) as well as to exacerbate inflammation, mucous cell metaplasia and fibrosis in mice asthma (Glista-Baker et al., 2014; Ryman-Rasmussen et al., 2008) and cystic fibrosis models (Geiser et al., 2014). Taken together, NP exposure may synergistically facilitate pathological inflammatory conditions in the lung via both innate and adaptive immunological abnormalities. As also addressed for cardiovascular susceptibility, associations between respiratory disease exacerbation and ultrafine particle exposure in general living environments have been reported for asthmatic subjects (Peters et al., 1997b). The carbon core of the particles was responsible for the decline in lung function (McCreanor et al., 2007). Overall, these results highlight the importance to provide an outlook on the potential to apply general environmental findings to workplace settings, where employees are exposed to engineered nanomaterials. This seems an even more important issue considering recent evidence demonstrating that CNTs from anthropogenic sources might be an important component of the airborne particulate matter, as demonstrated by the detection of these nano-sized materials inside lung cells of asthmatic children (Kolosnjaj-Tabi et al., 2015).

Finally, also liver disorders could be aggravated by NP exposure. In animal hepatitis models, in fact, liver damage was exacerbated by Au-nanorod (Bartneck et al., 2012) and PEG-coated Au-NP exposures (Hwang et al., 2012) as indicated by the increase in necrotic hepatocytes and in serum ALT and AST levels compared to controls.

Overall, these findings support the need to investigate the widest possible spectrum of conditions susceptible to be aggravated by NP exposure. Priority for research should be given to those conditions of exposure pointed out as potentially predisposing to a greater susceptibility to nanomaterial induced adverse effects. Among those, the elderly exposure, whose relevance relies also on the aging of the active workforce and the prenatal exposure, considering the evidence of possible trans-generational effects following in utero exposures to NPs, also in terms of potential epigenetic modifications, should be carefully evaluated. Moreover, pathological conditions, such as cardiovascular or respiratory disorders, reported to be associated with or exacerbated by ultrafine particles exposure in epidemiological data, should be also deeply investigated for their susceptibility to intentionally produced nanomaterials. All this information may be important to plan, even in this preliminary phase of investigation, appropriate preventive and protective measures as well as adequate health surveillance programs.

4. Discussion

Gene–environment interactions, in specific occupational NP exposure settings, may contribute to a wide and still not fully understood range of possible outcomes, from early biological alterations to disease development and progression, potentially affected by other factors of individual susceptibility. In this context, the conventional health risk assessment paradigm for chemical exposures could include data on genetic differences, epigenetic modifications, metabolic and DNA repair system alterations, as well as on other life stage or pathological conditions of potential susceptibility, thus providing the opportunity to understand, as well as to better quantify, inter-individual variability in NP response. This challenging approach assumes that both gene networks involved in physiological response and the extent of exposure may be identified in workers and integrated in a comprehensive model tailored to individual subjects and specific subpopulations or in an exposure standard that protects all workers including the most susceptible. Beyond establishing a protective occupational exposure limit, tailoring exposure limits to specific defined subpopulations is complex and controversial, and may result in negative impacts on workers in the form of discrimination and prejudice (Schulte, 2006; NIOSH, 2010). However, to effectively incorporate genetic and epigenetic in occupational risk assessment and standards requires the existence of such data. Molecular epidemiologic studies in worker populations exposed to NPs and animal studies of the NP can generate such data (Schulte et al., 2015). In this regard, scientific efforts should be focused to define, through innovative and standardised molecular epidemiological methods (Yao and Costa, 2013), those genes and pathways potentially involved in NP susceptibility and to put these findings in perspective. In fact, not all the identified genetic perturbations or effect modifying pathways, will result in significant effects on exposed individuals. Therefore, to characterize susceptibility risks with NP exposure will require population data and some indication of the attributable fraction of dysfunction due to the effect modifiers as well as the exposure (Witte et al., 2014). In this scenario, appropriate parameters for a correct evaluation/characterization of NP occupational exposures and suitable indicators for biological monitoring dosimetry should be defined in order to predict possible health risks across a population. Human molecular epidemiology, in this regard, may offer the opportunity to overcome the intrinsic difficulties found in extrapolating dosimetry and toxicokinetic data from studies performed with cellular and animal models.

Moreover, in this complex task, toxicogenomic *in vitro* and *in vivo* investigations may provide complementary and helpful information, leading to better understand NP mode of action, and to extrapolate possible biomarkers of susceptibility to be investigated and validated in the real workplace settings, under strictly controlled and ethically acceptable conditions (Nebert et al., 2013). However, caution should be addressed in extrapolating data from these studies, since they may be

not always representative of the real human conditions. Therefore, human molecular epidemiology and clinical data, should be pursued, as providing advantages in interpretation and reliability of information.

NP induced alterations in gene expression profiling of enzymatic systems involved in detoxification or activation of external xenobiotics, or in repair DNA damages, may act as promising biomarkers able to assess exposure to NPs, to define the underlying mechanisms of action, to stratify possible differential effects of occupational exposures, and to identify susceptible populations.

Moreover, further investigation is necessary to define the role that physico-chemical properties may have in NP biological reactivity, the possible mechanisms underlying the interaction of NPs with metabolic and DNA damage repair systems, the NP ability to induce epigenetic process and possible health consequences, as well as the influencing role of inter- and intra-individual variabilities in NP susceptibility. In this regard, a list of physico-chemical characteristics may be important to understand the biological activity as well as the toxico-kinetic and dynamic properties of NPs. Particle size and size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, chemistry, and charge as well as porosity were suggested as key characteristics (Luyts et al., 2013; Oberdörster et al., 2005). Additionally, phenomena occurring during the contact between NPs and cellular media or biological fluids need consideration (Fubini et al., 2010). However, the complexity of the interplay between NP properties, biological systems and individual susceptibility factors, prevent a definite nanomaterial categorization for assessing potential health risks.

In this scenario, the attempt to explain NP susceptibility from a mechanistic perspective should not underestimate the individual capacity to tolerate NP insults through a variety of defence systems. Different abilities to activate mechanisms that can reduce uptake of NPs into the cells and pathways of endogenous anti-oxidative defence, together with the protective effects of exogenous antioxidants provided by dietary intake, may all influence the individual susceptibility to the impact of NPs. Moreover, also the diverseness in oxidative post-translational modifications induced by ROS in proteins involved in cellular signalling pathways may be viewed as a possible factor influencing NP susceptibility. Overall, these issues underline the importance to undertake global gene expression analysis, or to employ other omic approaches to reach a more comprehensive mechanistic and predictive toxicological approach to understand nanomaterial susceptibility.

Concerning the selective influence of NPs on CYP450 activities, future studies should be aimed at clarifying if they are potentially determined by a direct disruption of the enzyme structure or by the alterations in the enzymatic microenvironments, as well as the role played by dose, NP physico-chemical properties and length of exposure in variable NP-CYP450 interactions. These interactions should be investigated to define specific susceptibility biomarkers. Toward this end, future research should clarify how different types of NPs may affect a still unexplored variety of metabolic pathways and specifically, which may be the "level" of their action. Genetic perturbations, toxicogenomic alterations in mRNA and enzymatic protein expression, also affected by epigenetic changes, as well as the stereo-selective modifications of enzymatic metabolic activity. These may all act as determinants of different profiles of susceptibility to complex nanomaterial workplace exposures. Investigation of possible susceptibility differences between materials at the nano-level and those with the same composition, but larger size may provide ulterior support to understand peculiar susceptibility factors for NPs. This information may in turn guide the synthesis of NPs "safe by design" and the identification of "sustainable" NP conditions of workplace exposure.

Additionally, from the perspective of "multiple xenobiotic interactions", an in depth investigation of the NP effects on specific isoforms of CYP450s, may be important. The purpose of this line of investigation is to anticipate how NP exposure, affecting enzymes responsible for the clearance of particular chemical substrates, may result in undesirable and potentially dangerous internal doses of co-exposed pharmacological or industrial substances. From an occupational health perspective, this seems an important issue to adequately contextualise the concept of "nanomaterial susceptibility" into more realistic workplace exposure scenarios, where multiple chemical exposures may occur and where an adequate evaluation of susceptibility to adverse health effects should consider the complex interplay between substances.

Additionally, considering that inter-individual differences in the epigenetic state may also affect susceptibility to xenobiotics and the associated risk of disease, epigenetic research may provide novel insights into the variable relationship between genome and work environment as well as into the potential mechanisms of susceptibility to NP toxicity. In this context, it appears important to plan additional research to define application of novel technologies to establish reliable epigenetic screening, "nanoepigenetics", to predict toxicity, susceptibility to adverse effects thus providing guidance for creating safe and more biocompatible nanomaterials (Stoccoro et al., 2013).

Susceptibility investigation should also take in consideration other individual conditions, such as age, gender, health status, pregnancy, lifestyle factors i.e. diet, smoking habit and physical exercise but also pharmacological therapies, perceived stress as well as past environmental and occupational exposure history, that may all affect gene expression, host metabolism and physiology, thus potentially increasing susceptibility to NP adverse health effects. Overall, these considerations point out a critical topic of discussion concerning the possibility that sources of differences in NP response may be due both to inter and intra individual variabilities related to a series of conditions and habits people may experience in certain periods of their lives.

Ultimately, the general criteria for biomarker selection apply also to nanomaterial biomarkers, and include the evaluation of their validity and relevance for protection of the health of concerned workers with due regard to their sensitivity and specificity, the interpretation, communication and management of the results, the risk-benefit dilemma and also the challenging issue of the informed consent (Manno et al., 2014). Special attention should be given also to the ethical aspects related to susceptibility biomarkers. The use of these indicators, in fact, should not result in discrimination or reduction of job opportunities for the workers involved.

5. Conclusions

Identifying populations susceptible to adverse effects from nanomaterial exposure may be important in risk management. This review attempts to draw on information, from *in vitro*, *in vivo* and human studies, to identify potential nanomaterial susceptibility factors and possible susceptibility biomarkers to be validated in occupational contexts. Although definitive conclusions cannot be extrapolated from the reviewed studies, some interesting aspects can be pointed out, which may help guide future research on occupational risk assessment. Heritable genome alterations able to influence the individual susceptibility to adverse health effects resulting from NP exposure are not directly available. Toxicogenomic data demonstrated the ability of NPs to exert a stimulating or inhibitory action on the gene expression or functionality of phase I and phase II enzymatic pathways involved in the metabolism of the vast majority of environmental xenobiotics. However, the role of the NP physico-chemical properties in affecting such different effects is still poorly understood. As a response to the oxidative stress reactions and genotoxic effects induced by NP exposure, a number of DNA repairing pathways were up- or down- regulated, although the effects of such changes on DNA damage and disease susceptibility need to be deeply investigated. Nanomaterial exposure induced epigenetic modifications which resulted in alterations in gene expression patterns, also at post-transcriptional level, leading to changes in a series of cellular processes. Life stage in which nanomaterial exposure may occur seem to influence susceptibility to adverse health effects. Prenatal exposure should be viewed with caution considering that these xenobiotics may cross the placental barrier inducing toxic effects in fetuses. Pathological

conditions, such as cardiovascular and respiratory diseases, that may be exacerbated by nanomaterial exposure, require specific attention as potential conditions of hyper-susceptibility.

From what herein detailed some important issues can be extrapolated which need future scientific efforts:

1) The role that physico-chemical properties may play in determining NP biological alterations should be strongly investigated. To this aim, suitable NP characterization should be performed as an essential tool to understand the *in vitro* and *in vivo* toxicological impact of these xenobiotics as well as to obtain a correct interpretation of the results. This may allow the identification of specific biomarkers of susceptibility for variable conditions of exposure and provide information supporting future design and production of safer nanomaterials;
2) *In vitro* studies should be performed with the aim to clarify the NP molecular mechanisms of action. These investigations may provide the basic information concerning which biological systems may be primarily affected by NP exposure and which defence mechanisms cells may activate against NP insults. Overall, these data may be important to define pathways, whose inherited or acquired alterations may be responsible for different NP susceptibility profiles;
3) *In vivo* investigations should be performed to define the toxicokinetic and -dynamic behaviour of NPs. This research may identify those metabolic pathways potentially involved in the kinetic modelling of NPs and particularly in their bio-transformation whose inherited or acquired genetic/genomic variances may affect susceptibility to NPs;
4) The genotoxic potential of NPs and susceptibility to adverse health effects due to the level of activation of the DNA damage repair systems should be strongly elucidated;
5) Further investigation should be focused on deeply defining epigenetic processes induced by NP exposure and biological pathways affected by epigenetic modifications in order to understand potential pathological implications;
6) Aging, cardiovascular and respiratory diseases should be investigated as potential hyper-susceptibility conditions to NP adverse effects. Useful information to guide such research may be extrapolated from previous epidemiological data obtained with ambient ultrafine particle exposure.

Overall, before biomarkers of susceptibility are considered in biological monitoring plans in occupational settings, they should be carefully evaluated in terms of sensitivity and specificity, interpretation, communication and management of the results, as well as from an ethical perspective to not discriminate or reduce job opportunities (Schulte and Hauser, 2012) and the quality of life for involved workers. Meanwhile, susceptibility biomarker data may be useful in risk assessment and risk management efforts.

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of their respective organizations.

Transparency document

The Transparency document associated with this article can be found, in the online version.

References

Alshatwi, A.A., Periasamy, V.S., Subash-Babu, P., Alsaif, M.A., Alwarthan, A.A., Lei, K.A., 2013. CYP1A and POR gene mediated mitochondrial membrane damage induced by carbon nanoparticle in human mesenchymal stem cells. Environ. Toxicol. Pharmacol. 36, 215–222.

Asharani, P., Sethu, S., Lim, H.K., Balaji, G., Valiyaveettil, S., Hande, M.P., 2012. Differential regulation of intracellular factors mediating cell cycle, DNA repair and inflammation following exposure to silver nanoparticles in human cells. Genome Integr. 3, 2.

Balansky, R., Longobardi, M., Ganchev, G., Iltcheva, M., Nedyalkov, N., Atanasov, P., Toshkova, R., De Flora, S., Izzotti, A., 2013. Transplacental clastogenic and epigenetic effects of gold nanoparticles in mice. Mutat. Res. 751–752, 42–48.

Balasubramanian, S.K., Jittiwat, J., Manikandan, J., Ong, C.N., Yu, L.E., Ong, W.Y., 2010. Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials 31, 2034–2042.

Baratli, Y., Charles, A.L., Wolff, V., Ben Tahar, L., Smiri, L., Bouitbir, J., Zoll, J., Piquard, F., Tebourbi, O., Sakly, M., Abdelmelek, H., Geny, B., 2013. Impact of iron oxide nanoparticles on brain, heart, lung, liver and kidneys mitochondrial respiratory chain complexes activities and coupling. Toxicol. in Vitro 27, 2142–2148.

Baratli, Y., Charles, A.L., Wolff, V., Ben Tahar, L., Smiri, L., Bouitbir, J., Zoll, J., Sakly, M., Auger, C., Vogel, T., Abdelmelek, H., Tebourbi, O., Geny, B., 2014. Age modulates Fe_3O_4 nanoparticles liver toxicity: dose-dependent decrease in mitochondrial respiratory chain complexes activities and coupling in middle-aged as compared to young rats. Biomed. Res. Int. 2014, 474081.

Bartneck, M., Ritz, T., Keul, H.A., Wambach, M., Bornemann, J., Gbureck, U., Ehling, J., Lammers, T., Heymann, F., Gassler, N., Lüdde, T., Trautwein, C., Groll, J., Tacke, F., 2012. Peptide-functionalized gold nanorods increase liver injury in hepatitis. ACS Nano 6, 8767–8777.

Benor, S., Alcalay, Y., Domany, K.A., Gut, G., Soferman, R., Kivity, S., et al., 2015. Ultrafine particle content in exhaled breath condensate in airways of asthmatic children. Breath Res. 9, 026001.

Cesta, M.F., Ryman-Rasmussen, J.P., Wallace, D.G., Masinde, T., Hurlburt, G., Taylor, A.J., Bonner, J.C., 2010. Bacterial lipopolysaccharide enhances PDGF signaling and pulmonary fibrosis in rats exposed to carbon nanotubes. Am. J. Respir. Cell Mol. Biol. 43, 142–151.

Chen, J., Wang, H., Long, W., Shen, X., Wu, D., Song, S.S., Sun, Y.M., Liu, P.X., Fan, S., Fan, F., Zhang, X.D., 2013. Sex differences in the toxicity of polyethylene glycol-coated gold nanoparticles in mice. Int. J. Nanomedicine 8, 2409–2419.

Chen, R., Zhang, L., Ge, C., Tseng, M.T., Bai, R., Qu, Y., Beer, C., Autrup, H., Chen, C., 2015. Subchronic toxicity and cardiovascular responses in spontaneously hypertensive rats after exposure to multiwalled carbon nanotubes by intratracheal instillation. Chem. Res. Toxicol. 28, 440–450.

Chen, Z., Meng, H., Xing, G., Yuan, H., Zhao, F., Liu, R., Chang, X., Gao, X., Wang, T., Jia, G., Ye, C., Chai, Z., Zhao, Y., 2008. Age-related differences in pulmonary and cardiovascular responses to SiO_2 nanoparticle inhalation: nanotoxicity has susceptible population. Environ. Sci. Technol. 42, 8985–8992.

Cho, W.S., Cho, M., Jeong, J., Choi, M., Han, B.S., Shin, H.S., Hong, J., Chung, B.H., Jeong, J., Cho, M.H., 2010. Size-dependent tissue kinetics of PEG-coated gold nanoparticles. Toxicol. Appl. Pharmacol. 45, 116–123.

Choi, A.O., Brown, S.E., Szyf, M., Maysinger, D., 2008. Quantum dot-induced epigenetic and genotoxic changes in human breast cancer cells. J. Mol. Med. (Berl) 86, 291–302.

Christen, V., Fent, K., 2012. Silica nanoparticles and silver-doped silica nanoparticles induce endoplasmatic reticulum stress response and alter cytochrome P4501A activity. Chemosphere 87, 423–434.

Coccini, T., Barni, S., Vaccarone, R., Mustarelli, P., Manzo, L., Roda, E., 2013. Pulmonary toxicity of instilled cadmium-doped silica nanoparticles during acute and subacute stages in rats. Histol. Histopathol. 28, 195–209.

Cui, Y., Gong, X., Duan, Y., Li, N., Hu, R., Liu, H., Hong, M., Zhou, M., Wang, L., Wang, H., Hong, F., 2010. Hepatocyte apoptosis and its molecular mechanisms in mice caused by titanium dioxide nanoparticles. J. Hazard. Mater. 183, 874–880.

Delfino, R.J., Sioutas, C., Malik, S., 2005. Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. Environ. Health Perspect. 113, 934–946.

Ema, M., Kobayashi, N., Naya, M., Hanai, S., Nakanishi, J., 2010. Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod. Toxicol. 30, 343–352.

Eom, H.J., Chatterjee, N., Lee, J., Choi, J., 2014. Integrated mRNA and micro RNA profiling reveals epigenetic mechanism of differential sensitivity of Jurkat T cells to AgNPs and Ag ions. Toxicol. Lett. 229, 311–318.

Evans, K., Halterman, J.S., Hopke, P.K., Fagnano, M., Rich, D.Q., 2014. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. Environ. Res. 129, 11–19.

Fröhlich, E., Kueznik, T., Samberger, C., Roblegg, E., Wrighton, C., Pieber, T.R., 2010. Size-dependent effects of nanoparticles on the activity of cytochrome P450 isoenzymes. Toxicol. Appl. Pharmacol. 242, 326–332.

Fubini, B., Ghiazza, M., Fenoglio, I., 2010. Physico-chemical features of engineered nanoparticles relevant to their toxicity. Nanotoxicology 4, 347–363.

Gay, S.C., Roberts, A.G., Halpert, J.R., 2010. Structural features of cytochromes P450 and ligands that affect drug metabolism as revealed by X-ray crystallography and NMR. Future Med. Chem. 2, 1451–1468.

Ge, Y., Bruno, M., Wallace, K., Winnik, W., Prasad, R.Y., 2011. Proteome profiling reveals potential toxicity and detoxification pathways following exposure of BEAS-2B cells to engineered nanoparticle titanium dioxide. Proteomics 11, 2406–2422.

Geiser, M., Stoeger, T., Casaulta, M., Chen, S., Semmler-Behnke, M., Bolle, I., Takenaka, S., Kreyling, W.G., Schulz, H., 2014. Biokinetics of nanoparticles and susceptibility to particulate exposure in a murine model of cystic fibrosis. Part. Fibre Toxicol. 11, 19.

Glista-Baker, E.E., Taylor, A.J., Sayers, B.C., Thompson, E.A., Bonner, J.C., 2014. Nickel nanoparticles cause exaggerated lung and airway remodeling in mice lacking the T-box transcription factor, TBX21 (T-bet). Part. Fibre Toxicol. 11, 7.

Gochfeld, M., 2007. Framework for gender differences in human and animal toxicology. Environ. Res. 104, 4–21.

Gong, C., Tao, G., Yang, L., Liu, J., Liu, Q., Li, W., Zhuang, Z., 2012. Methylation of PARP-1 promoter involved in the regulation of nano-SiO_2-induced decrease of PARP-1 mRNA expression. Toxicol. Lett. 209, 264–269.

Gong, C., Tao, G., Yang, L., Liu, J., Liu, Q., Zhuang, Z., 2010. SiO(2) nanoparticles induce global genomic hypomethylation in HaCaT cells. Biochem. Biophys. Res. Commun. 397, 397–400.
Halappanavar, S., Jackson, P., Williams, A., Jensen, K.A., Hougaard, K.S., Vogel, U., Yauk, C.L., Wallin, H., 2011. Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. Environ. Mol. Mutagen. 52, 425–439.
Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51–88.
He, L., Hannon, G.J., 2004. MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531.
Hitoshi, K., Katoh, M., Suzuki, T., Ando, Y., Nadai, M., 2012. Changes in expression of drug-metabolizing enzymes by single-walled carbon nanotubes in human respiratory tract cells. Drug Metab. Dispos. 40, 579–587.
Hunter, D.J., 2005. Gene–environment interactions in human diseases. Nat. Rev. Genet. 6, 287–298.
Hussain, S., Vanoirbeek, J.A., Luyts, K., De Vooght, V., Verbeken, E., Thomassen, L.C., Martens, J.A., Dinsdale, D., Boland, S., Marano, F., Nemery, B., Hoet, P.H., 2011. Lung exposure to nanoparticles modulates an asthmatic response in a mouse model. Eur. Respir. J. 37, 299–309.
Hwang do, W., Lee, D.S., Kim, S., 2012. Gene expression profiles for genotoxic effects of silica-free and silica-coated cobalt ferrite nanoparticles. J. Nucl. Med. 53, 106–112.
Hwang, J.H., Kim, S.J., Kim, Y.H., Noh, J.R., Gang, G.T., Chung, B.H., Song, N.W., Lee, C.H., 2012. Susceptibility to gold nanoparticle-induced hepatotoxicity is enhanced in a mouse model of nonalcoholic steatohepatitis. Toxicology 294, 27–35.
Iavicoli, I., Leso, V., Manno, M., Schulte, P.A., 2014. Biomarkers of nanomaterial exposure and effect: current status. J. Nanoparticle Res. 16, 2302.
Ingelman-Sundberg, M., Sim, S.C., Gomez, A., Rodriguez-Antona, C., 2007. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol. Ther. 116, 496–526.
Inoue, K., 2011. Promoting effects of nanoparticles/materials on sensitive lung inflammatory diseases. Environ. Health Prev. Med. 16, 139–143.
Inoue, K., Koike, E., Yanagisawa, R., Hirano, S., Nishikawa, M., Takano, H., 2009. Effects of multi-walled carbon nanotubes on a murine allergic airway inflammation model. Toxicol. Appl. Pharmacol. 237, 306–316.
Inoue, K., Yanagisawa, R., Koike, E., Nishikawa, M., Takano, H., 2010. Repeated pulmonary exposure to single-walled carbon nanotubes exacerbates allergic inflammation of the airway: Possible role of oxidative stress. Free Radic. Biol. Med. 48, 924–934.
Jaenisch, R., Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat. Genet. 33 (Suppl), 245–254.
Ji, Z., Zhang, D., Li, L., Shen, X., Deng, X., Dong, L., Wu, M., Liu, Y., 2009. The hepatotoxicity of multi-walled carbon nanotubes in mice. Nanotechnology 20, 445101.
Kaewamatawong, T., Banlunara, W., Maneewattanapinyo, P., Thammachareon, C., Ekgasit, S., 2014. Acute and subacute pulmonary toxicity caused by a single intratracheal instillation of colloidal silver nanoparticles in mice: pathobiological changes and metallothionein responses. J. Environ. Pathol. Toxicol. Oncol. 33, 59–68.
Kang, G.S., Gillespie, P.A., Gunnison, A., Moreira, A.L., Tchou-Wong, K.M., Chen, L.C., 2011. Long-term inhalation exposure to nickel nanoparticles exacerbated atherosclerosis in a susceptible mouse model. Environ. Health Perspect. 119, 176–181.
Kim, M., Bae, M., Na, H., Yang, M., 2012. Environmental toxicants-induced epigenetic alterations and their reversers. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 30, 323–367.
Kim, W.Y., Kim, J., Park, J.D., Ryu, H.Y., Yu, I.J., 2009. Histological study of gender differences in accumulation of silver nanoparticles in kidneys of Fischer 344 rats. J. Toxicol Environ. Health A 72, 1279–1284.
Kim, Y.S., Kim, J.S., Cho, H.S., Rha, D.S., Kim, J.M., Park, J.D., Choi, B.S., Lim, R., Chang, H.K., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S., Yu, I.J., 2008. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague–Dawley rats. Inhal. Toxicol. 20, 575–583.
Kolosnjaj-Tabi, J., Just, J., Hartman, K.B., Laoudi, Y., Boudjemaa, S., Alloyeau, D., et al., 2015. Anthropogenic carbon nanotubes found in the airways of Parisian children. EBioMedicine http://dx.doi.org/10.1016/j.ebiom.2015.10.012.
Kulthong, K., Maniratanachote, R., Kobayashi, Y., Fukami, T., Yokoi, T., 2012. Effects of silver nanoparticles on rat hepatic cytochrome P450 enzyme activity. Xenobiotica 42, 854–862.
Lamb, J.G., Hathaway, L.B., Munger, M.A., Raucy, J.L., Franklin, M.R., 2010. Nanosilver particle effects on drug metabolism in vitro. Drug Metab. Dispos. 38, 2246–2251.
Lammel, T., Boisseaux, P., Navas, J.M., 2015. Potentiating effect of graphene nanomaterials on aromatic environmental pollutant-induced cytochrome P450 1A expression in the topminnow fish hepatoma cell line PLHC-1. Environ. Toxicol. 30, 1192–1204.
Lan, J., Gou, N., Gao, C., He, M., Gu, A.Z., 2014. Comparative and mechanistic genotoxicity assessment of nanomaterials via a quantitative toxicogenomics approach across multiple species. Environ. Sci. Technol. 48, 12937–12945.
Li, S., Wang, H., Qi, Y., Tu, J., Bai, Y., Tian, T., Huang, N., Wang, Y., Xiong, F., Lu, Z., Xiao, Z., 2011a. Assessment of nanomaterial cytotoxicity with SOLiD sequencing-based microRNA expression profiling. Biomaterials 32, 9021–9030.
Li, S., Wang, Y., Wang, H., Bai, Y., Liang, G., Wang, Y., Huang, N., Xiao, Z., 2011b. MicroRNAs as participants in cytotoxicity of CdTe quantum dots in NIH/3T3 cells. Biomaterials 32, 3807–3814.
Li, Y., Zhang, Y., Yan, B., 2014. Nanotoxicity overview: nano-threat to susceptible populations. Int. J. Mol. Sci. 15, 3671–3697.
Li, Z., Hulderman, T., Salmen, R., Chapman, R., Leonard, S.S., Young, S.H., Shvedova, A., Luster, M.I., Simeonova, P.P., 2007. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ. Health Perspect. 115, 377–382.
Lu, Z., Ma, G., Veinot, J.G., Wong, C.S., 2013. Disruption of biomolecule function by nanoparticles: how do gold nanoparticles affect phase I biotransformation of persistent organic pollutants? Chemosphere 93, 123–132.
Luyts, K., Napierska, D., Nemery, B., Hoet, P.H., 2013. How physico-chemical characteristics of nanoparticles cause their toxicity: complex and unresolved interrelations. Environ. Sci. Processes Impacts 15, 23–38.
Manno, M., Sito, F., Licciardi, L., 2014. Ethics in biomonitoring for occupational health. Toxicol. Lett. 231, 111–121.
Manno, M., Viau, C. in collaboration with, Cocker, J., Colosio, C., Lowry, L., Mutti, A., Nordberg, M., Wang, S., 2010. Biomonitoring for occupational health risk assessment (BOHRA). Toxicol. Lett. 192, 3–16.
McCreanor, J., Cullinan, P., Nieuwenhuijsen, M.J., Stewart-Evans, J., Malliarou, E., Jarup, L., et al., 2007. Respiratory effects of exposure to diesel traffic in persons with asthma. N. Engl. J. Med. 357, 2348–2458.
Mei, N., Zhang, Y., Chen, Y., Guo, X., Ding, W., Ali, S.F., Biris, A.S., Rice, P., Moore, M.M., Chen, T., 2012. Silver nanoparticle-induced mutations and oxidative stress in mouse lymphoma cells. Environ. Mol. Mutagen. 53, 409–419.
Munger, M.A., Hadlock, G., Stoddard, G., Slawson, M.H., Wilkins, D.G., Cox, N., Rollins, D., 2015. Assessing orally bioavailable commercial silver nanoparticle product on human cytochrome P450 enzyme activity. Nanotoxicology 9, 474–481.
Nebert, D.W., Zhang, G., Vesell, E.S., 2013. Genetic risk prediction: individualized variability in susceptibility to toxicants. Annu. Rev. Pharmacol. Toxicol. 53, 355–375.
Ng, C.T., Dheen, S.T., Yip, W.C., Ong, C.N., Bay, B.H., Lanry Yung, L.Y., 2011. The induction of epigenetic regulation of PROS1 gene in lung fibroblasts by gold nanoparticles and implications for potential lung injury. Biomaterials 32, 7609–7615.
NIOSH, 2010. Genetics in the workplace. Department of Health and Human Services. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, OH (DHHS (NIOSH) Publication No. 2010–101.).
Noori, A., Parivar, K., Modaresi, M., Messripour, M., Yousefi, M.H., Amiri, G.R., 2011. Effect of magnetic iron oxide nanoparticles on pregnancy and testicular development of mice. Afr. J. Biotechnol. 10, 1221–1227.
Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113, 823–839.
Penttinen, P., Timonen, K.L., Tiittanen, P., Mirme, A., Ruuskanen, J., Pekkanen, J., 2001. Ultrafine particles in urban air and respiratory health among adult asthmatics. Eur. Respir. J. 17, 428–435.
Periasamy, V.S., Athinarayanan, J., Al-Hadi, A.M., Juhaimi, F.A., Alshatwi, A.A., 2015. Effects of titanium dioxide nanoparticles isolated from confectionery products on the metabolic stress pathway in human lung fibroblast cells. Arch. Environ. Contam. Toxicol. 68, 521–533.
Peters, A., Döring, A., Wichmann, H.E., Koenig, W., 1997a. Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349, 1582–1587.
Peters, A., Rückerl, R., Cyrys, J., 2011. Lessons from air pollution epidemiology for studies of engineered nanomaterials. J. Occup. Environ. Med. 53 (6 Suppl), S8–S13.
Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J., Heyder, J., 1997b. Respiratory effects are associated with the number of ultra-fine particles. Am. J. Respir. Crit. Care Med. 155, 1376–1383.
Pisoschi, A.M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: a review. Eur. J. Med. Chem. 97, 55–74.
Qi, W., Bi, J., Zhang, X., Wang, J., Wang, J., Liu, P., Li, Z., Wu, W., 2014. Damaging effects of multi-walled carbon nanotubes on pregnant mice with different pregnancy times. Sci. Report. 4, 4352.
Ryman-Rasmussen, J.P., Tewksbury, E.W., Moss, O.R., Cesta, M.F., Wong, B.A., Bonner, J.C., 2008. Inhaled multiwalled carbon nanotubes potentiate airway fibrosis in murine allergic asthma. Am. J. Respir. Cell Mol. Biol. 40, 349–358.
Schulte, P.A., 2006. Framework for considering genetics in the workplace. Med. Lav. 92, 339–347.
Schulte, P.A., Geraci, C.L., Murashov, V., Kuempel, E.D., Zumwalde, R.D., Castranova, V., Hoover, M.D., Hodson, L., Martinez, K.F., 2014. Occupational safety and health criteria for responsible development of nanotechnology. J. Nanoparticle Res. 16, 1–17.
Schulte, P.A., Hauser, J.E., 2012. The use of biomarkers in occupational research, practice, and policy. Toxicol. Lett. 213, 91–99.
Schulte, P.A., Trout, D.B., 2011. Nanomaterials and worker health: medical surveillance, exposure registries, and epidemiologic research. J. Occup. Environ. Med. 53 (6 Suppl.), S3–S7.
Schulte, P.A., Whittaker, C., Curran, C.P., 2015. Considerations for using genetic and epigenetic information in occupational health risk assessment and standard setting. J. Occup. Environ. Hyg. 12 (1 Suppl.), S69–S81.
Sereemaspun, A., Hongpiticharoen, P., Rojanathanes, R., Maneewattanapinyo, P., Ekgasit, S., Warisnoicharoen, W., 2008. Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: a preliminary to nanogenomics. Int. J. Pharmacol. 4, 492–495.
Sharma, A., Muresanu, D.F., Patnaikm, R., Sharma, H.S., 2013. Size- and age-dependent neurotoxicity of engineered metal nanoparticles in rats. Mol. Neurobiol. 48, 386–396.
Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M., Takeda, K., 2009. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. Part. Fibre Toxicol. 6, 20.
Simkhovich, B.Z., Kleinman, M.T., Kloner, R.A., 2008. Air pollution and cardiovascular injury epidemiology, toxicology, and mechanisms. J. Am. Coll. Cardiol. 52, 719–726.
Stirzaker, C., Song, J.Z., Davidson, B., Clark, S.J., 2004. Transcriptional gene silencing promotes DNA hypermethylation through a sequential change in chromatin modifications in cancer cells. Cancer Res. 64, 3871–3877.
Stoccoro, A., Karlsson, H.L., Coppedè, F., Migliore, L., 2013. Epigenetic effects of nano-sized materials. Toxicology 313, 3–14.
Sun, Q., Tan, D., Ze, Y., Sang, X., Liu, X., Gui, S., Cheng, Z., Cheng, J., Hu, R., Gao, G., Liu, G., Zhu, M., Zhao, X., Sheng, L., Wang, L., Tang, M., Hong, F., 2012. Pulmotoxicological

effects caused by long-term titanium dioxide nanoparticles exposure in mice. J. Hazard. Mater. 235-236, 47–53.
Sung, J.H., Ji, J.H., Park, J.D., Song, M.Y., Song, K.S., Ryu, H.R., Yoon, J.U., Jeon, K.S., Jeong, J., Han, B.S., Chung, Y.H., Chang, H.K., Lee, J.H., Kim, D.W., Kelman, B.J., Yu, I.J., 2011. Subchronic inhalation toxicity of gold nanoparticles. Part. Fibre Toxicol. 8, 16.
Sung, J.H., Ji, J.H., Park, J.D., Yoon, J.U., Kim, D.S., Jeon, K.S., Song, M.Y., Jeong, J., Han, B.S., Han, J.H., Chung, Y.H., Chang, H.K., Lee, J.H., Cho, M.H., Kelman, B.J., Yu, I.J., 2009. Subchronic inhalation toxicity of silver nanoparticles. Toxicol. Sci. 108, 452–461.
Tian, X., Zhu, M., Du, L., Wang, J., Fan, Z., Liu, J., Zhao, Y., Nie, G., 2013. Intrauterine inflammation increases materno-fetal transfer of gold nanoparticles in a size-dependent manner in murine pregnancy. Small 9, 2432–2439.
Trout, D.B., Schulte, P.A., 2010. Medical surveillance, exposure registries, and epidemiologic research for workers exposed to nanomaterials. Toxicology 269, 128–135.
Tuteja, N., Tuteja, R., 2001. Unraveling DNA repair in human: molecular mechanisms and consequences of repair defect. Crit. Rev. Biochem. Mol. Biol. 36, 261–290.
Umezawa, M., Kudo, S., Yanagita, S., Shinkai, Y., Niki, R., Oyabu, T., Takeda, K., Ihara, T., Sugamata, M., 2011. Maternal exposure to carbon black nanoparticle increases collagen type VIII expression in the kidney of offspring. J. Toxicol. Sci. 36, 461–468.
von Klot, S., Wölke, G., Tuch, T., Heinrich, J., Dockery, D.W., Schwartz, J., et al., 2002. Increased asthma medication use in association with ambient fine and ultrafine particles. Eur. Respir. J. 20, 691–702.
Wang, Y., Chen, Z., Ba, T., Pu, J., Chen, T., Song, Y., Gu, Y., Qian, Q., Xu, Y., Xiang, K., Wang, H., Jia, G., 2013. Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. Small 9, 1742–1752.
Warisnoicharoen, W., Hongpiticharoen, P., Lawanprasert, S., 2011. Alteration in enzymatic function of human cytochrome P450 by silver nanoparticles. Res. J. Environ. Toxicol. 5, 58–64.
Wessels, A., Van Berlo, D., Boots, A.W., Gerloff, K., Scherbart, A.M., Cassee, F.R., Gerlofs-Nijland, M.E., Van Schooten, F.J., Albrecht, C., Schins, R.P., 2011. Oxidative stress and DNA damage responses in rat and mouse lung to inhaled carbon nanoparticles. Nanotoxicology 5, 66–78.
Witte, J.S., Visscher, P.M., Wray, N.R., 2014. The contribution of genetic variants depends on the ruler. Nat. Rev. Genet. 15, 765–776.
Xu, Y.Y., Yang, J., Shen, T., Zhou, F., Xia, Y., Fu, J.Y., Meng, J., Zhang, J., Zheng, Y.F., Yang, J., Xu, L.H., Zhu, X.Q., 2012. Intravenous administration of multi-walled carbon nanotubes affects the formation of atherosclerosis in Sprague–Dawley rats. J. Occup. Health 54, 361–369.
Xue, Y., Zhang, S., Huang, Y., Zhang, T., Liu, X., Hu, Y., Zhang, Z., Tang, M., 2012. Acute toxic effects and gender-related biokinetics of silver nanoparticles following an intravenous injection in mice. J. Appl. Toxicol. 32, 890–899.
Yamashita, K., Yoshioka, Y., Higashisaka, K., Mimura, K., Morishita, Y., Nozaki, M., Yoshida, T., Ogura, T., Nabeshi, H., Nagano, K., Abe, Y., Kamada, H., Monobe, Y., Imazawa, T., Aoshima, H., Shishido, K., Kawai, Y., Mayumi, T., Tsunoda, S., Itoh, N., Yoshikawa, T., Yanagihara, I., Saito, S., Tsutsumi, Y., 2011. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat. Nanotechnol. 6, 321–328.
Yao, Y., Costa, M., 2013. Genetic and epigenetic effects of nanoparticles. J. Mol. Genet. Med. 7, 86.
Ye, M., Tang, L., Luo, M., Zhou, J., Guo, B., Liu, Y., Chen, B., 2014. Size- and time-dependent alteration in metabolic activities of human hepatic cytochrome P450 isozymes by gold nanoparticles via microsomal coincubations. Nanoscale Res. Lett. 9, 642.
Zhang, H., Wang, X., Wang, M., Li, L., Chang, C.H., Ji, Z., et al., 2015. Mammalian cells exhibit a range of sensitivities to silver nanoparticles that are partially explicable by variations in antioxidant defense and metallothionein expression. Small 11, 3797–3805.