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Review

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Human inhalation exposure to iron oxide particles

Abstract: In the past decade, many studies have been conducted to determine the health effects induced by exposure to engineered nanomaterials (NMs). Specifically for exposure via inhalation, numerous in vitro and animal in vivo inhalation toxicity studies on several types of NMs have been published. However, these results are not easily extrapolated to judge the effects of inhaling NMs in humans, and few published studies on the human response to inhalation of NMs exist. Given the emergence of more industries utilizing iron oxide nanoparticles as well as more nanomedicine applications of superparamagnetic iron oxide nanoparticles (SPIONs), this review presents an overview of the inhalation studies that have been conducted in humans on iron oxides. Both occupational exposure studies on complex iron oxide dusts and fumes, as well as human clinical studies on aerosolized, micron-size iron oxide particles are discussed. Iron oxide particles have not been described to elicit acute inhalation response nor promote lung disease after chronic exposure. The few human clinical studies comparing inhalation of fine and ultrafine metal oxide particles report no acute changes in the health parameters measured. Taken together existing evidence suggests that controlled human exposure to iron oxide nanoparticles, such as SPIONs, could be conducted safely.

Keywords: human; inhalation; iron oxide; nanoparticle; occupational health.

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Introduction

Nanotechnology is one of the few material technologies that researchers have proactively examined for human health effects in parallel with its development. However, given the complexity of many engineered nanomaterials (NMs), which are often multi-component structures versus pure materials, researchers have faced challenges in measuring toxicokinetic parameters and interpreting data to determine mechanisms of action. Although much knowledge on the toxicity of NMs has been gained in the past decade, the nanotoxicology research community remains hesitant to answer the public's question: Are NMs toxic or not? As researchers continue to study this question, human exposures to NMs are occurring through consumer products and occupational exposure. Workers manufacturing and handling NMs will likely be the first subpopulation to exhibit any potential chronic effects due to often daily exposures at the workplace. Inhalation is of significant concern since inhaled particulates are known to induce various respiratory conditions. In addition, compared to dermal and oral exposures, inhalation is more likely to result in a systemic effect [1–3]. Due to their small size, NMs can deposit in the lower, gas exchange region of the lungs. Therefore, exposure to high concentrations of airborne NMs could lead to physiologic effects. Particle deposition and biokinetics of NMs in the lungs have been reviewed in depth in recent articles [4, 5].

Although numerous in vitro and animal in vivo inhalation toxicity studies on several types of NMs have been published, these results are not easily extrapolated to judge the effects in humans. Currently, there are few published studies on the human response to inhalation of NMs. Many safety and ethical concerns restrict the possibility of conducting controlled exposure studies in humans. In addition, how should it be determined which NMs should advance to human testing? At the preclinical level, researchers are presented with a daunting number of NMs to test due to varying chemical composition, size, shape, surface characteristics, and dispersion. This only names a few of the major parameters that can be manipulated, and the number will increase as our ability to control matter at the nanoscale continues to become more sophisticated. To date, nanotoxicology research has focused on NMs with high production levels categorized by chemical composition. In addition, the Organization for Economic Cooperation and Development (OECD) identified fourteen priority NMs for evaluation [6]. These are carbon black, C_{60}, single and multi-walled carbon nanotubes, Ag, Au, Fe, TiO_2, Al_2O_3, CeO_2, ZnO, SiO_2, dendrimers, and nanoclays.

Of these, pharmaceutical formulations of Ag, Au, and Fe nanoparticles plus dendrimers have undergone human clinical trials for intravenous (IV) administration [7].

Only iron nanoparticles, specifically superparamagnetic iron oxide nanoparticles (SPIONs), have been approved by both the US Food and Drug Administration and the European Medicines Agency for IV medical use. To the authors' knowledge, no controlled human inhalation exposure study has been conducted using SPIONs. However, micron-sized bare and radiolabeled iron oxide particles have been used as routine tracer aerosols for magnetopneumography, lung function, and particle clearance measurements. In addition, occupational exposure studies on complex iron oxide dusts and fumes produced during industrial processes have been conducted. The potential hazards associated with inhalation of particulates, including nanoparticles, have been discussed extensively in the literature, and the consequences of exposure include the onset of lung disease and systemic effects due to particle translocation [8–10]. This review presents an overview of the inhalation studies that have been conducted in humans on iron oxides particles, with some discussion of in vivo animal inhalation studies using iron nanoparticles, to guide future studies on human inhalation of iron oxide nanoparticles.

Occupational inhalation exposure to iron oxide particles

Reports on the human health effects due to inhalation of iron oxides date back to 1867 with Zenker suggesting a link between lung fibrosis and iron oxide exposure [11]. The X-ray shadows often observed in iron oxide exposed workers were suggested by Collis in 1923 not to be signs of fibrosis but visualization of retained iron oxide particles in the lungs [12]. More recently, a case study corroborates this retention theory by demonstrating a significant recovery of particles from the lungs as well as a reduction in computerized tomography (CT) contrast in the lungs of a welder after undergoing bronchial alveolar lavage [13]. These reports suggest that inhalation of iron oxide, despite particle retention in the lungs, results in little to no gross adverse health effects. However, an increased incidence of lung disease is associated with workers in occupations involving exposure to iron oxide dusts or fumes. The industries of most concern for human exposure to inhaled iron oxide particles include four distinct and historically relevant groups: iron welders, iron foundry workers, iron and steel manufacturers, and iron ore miners. Epidemiological evidence on exposed cohorts from these four groups indicates higher risk of lung fibrosis, siderosis, and silicosis. In addition, iron oxide exposure is suspected to lead to an increased risk of lung cancer for workers in these industries [14, 15]. However, these studies contain several methodological drawbacks, due in part to their retrospective nature, that do not directly correlate iron oxide exposure with the observed health effects, perpetuating the uncertainty of a causal relationship.

In response, industrial hygienists included iron oxides when crafting the first list of Threshold Limit Values (TLV), the exposure level that is deemed acceptable over a working lifetime [16]. The limit has changed over time from the original 1949 TLV of 15 mg/m^3 (total particulates) to 10 mg/m^3 (total particulates) in 1964 to 5 mg/m^3 (respirable fraction) in 2006 [17]. While the International Agency for Research on Cancer (IARC) classifies iron and steel founding exposures as Group 1 substances, which are considered carcinogenic to humans, hematite and ferric oxides are listed as Group 3 substances, which are considered not classifiable as a carcinogen to humans [18]. Few human epidemiological studies specifically investigate the risk of cancer in relation to iron oxide exposure. Occupational cohort study results are difficult to interpret due to potential confounding with multiple exposures, namely to other potential and proven carcinogens such as PAHs, silica, and formaldehyde [19–21]. Additionally, failure to report on the use of personal protective equipment (PPE), namely respiratory protective devices, makes it impossible to accurately discern differences between workplace concentration of iron oxide and personal exposure, specifically, the inhaled dose. The following studies summarized in Table 1 on workers in steel factories show inconsistent results and have shared a similar concomitant exposure problem. Bourgkard et al. investigated a cohort of 16,742 males and 959 females employed for at least 1 year in a French carbon steel-producing factory between 1959 and 1997 [14]. Overall, no correlation was determined between iron oxide exposure and mortality from lung cancer relative risk adjusted with asbestos, silica, and polycyclic aromatic hydrocarbon (PAH) exposures (RR=0.80; 95% CI, 0.55–1.17). However, this retrospective study suffers from an incomplete exposure assessment for iron oxides. Characterization of iron oxide exposure was mostly qualitative with the mineralogy and particle size not reported. The exposure assessment was primarily based on a job exposure matrix and historical air monitoring measurements performed in the factory. Only total dust concentrations, 30% of which were above 10 mg/m^3, were collected from workplace air measurements. The authors noted that the percentage of iron in total dust ranged from 10% to 50%, and reported quartiles of total iron ranging from 0.18, 0.32 and 0.85 mg/m^3 for a 10% total iron content to 0.89, 1.61 and

Table 1 List of epidemiologic studies involving human occupational inhalation of iron oxides.

Type of study	# Volunteers	Occupational setting	Exposure	Endpoint	Exposure assessment	PPE	Results	Adjusted for	Conclusion	Year	Reference
Cross-sectional	14 (mean age=43)	Rouge polish manufacturing plant	Pure iron oxide (Fe_2O_3) dust, average length=10 years	Lung fibrosis; cancer	The exposure concentration in all parts of the plant ranged between 10 and 15 mg/m^3 (20–25 particles/cm^3, with 30% in the submicron size range) to 500 and 700 mg/m^3 (>3000 particles/cm^3)	NR	<50% (38 out of 113) had chest X-rays opacities or shadows, further examination of 14 workers revealed no lung function changes	NR	Exposure did not correlate with lung fibrosis or cancer. Pure iron oxide is not fibrogenic in the lung	1973	[11]
Cross-sectional	42 (mean age=46)	Shipyard arc welding	Fe_3O_4 (based on literature, not workplace exposure measurements), exposure time ranged from 1 to 40 years	Amount of welding fume lung contaminants; lung retention and clearance rate	Welding fumes containing 25–70% iron oxide (90% Fe_3O_4) particles with mass median diameter of 0.5 µm and concentrations ranging from 2 to 400 mg/m^3	NR	Average alveolar deposition rate ~20–40 mg/year, alveolar retention after 5 years of continuous exposure ~200 mg, clearance rate ranged from 10 to 20%/year	NR	The results were compared with post mortem studies of coal miners. Welder particle retention rate seemed lower but clearance rate was similar	1978	[29]
Dynamic cross-sectional, historical cohort study	4288 (M), 609 (F) working 1968–1991	Steel factory (production of stainless and alloyed steel)	"Iron and iron oxides" (used interchangeably), no airborne exposure level measurements, exposure duration at least 1 year	Overall mortality; lung cancer mortality	Job exposure matrix (JEM). Lack of exposure measurements	NR	No lung cancer excess for exposure to metals and/or their compounds (iron) (OR 0.94, CI 0.48±1.86)	PAHs, silica and smoking	No relationship between lung cancer and exposure to iron, chromium, nickel and/or their compounds	2000	[15]
Prospective cohort study	16,742 (M), 959 (F) working 1959–1997	Carbon steel-producing factory	"Iron oxides", no airborne exposure level measurements, exposure duration at least 1 year	Lung cancer mortality	Factory-specific job exposure matrix (JEM) validated with atmospheric measurements. Total dust atmospheric measurements in the factory showed a large exposure gradient: 10% of individual measurements	NR	For subjects exposed at an intensity level >2, RR 0.80 (95% CI 0.55–1.17). No dose-response relationships were observed in the Poisson regression models with the highest exposure level in work history (RR per added	Asbestos, silica, and PAH	No relationship between exposure to iron oxides and lung cancer mortality	2009	[14]

(**Table 1** Continued)

Type of study	# Volunteers	Occupational setting	Exposure	Endpoint	Exposure assessment	PPE	Results	Adjusted for	Conclusion	Year	Reference
					and 30% of area measurements were >10 mg/m^3		level 0.98, 95% CI 0.87 to 1.10)				
Workplace exposure assessment	N/A	Seven facilities (small, medium, large manufacturers, and end users of nanoscale particles)	Exposure assessment surveys were conducted at <0.1 µm diameter metal oxides, including iron oxides	Exposure	Half and full shift sampling based on direct reading, mass based area, and personal aerosol sampling. Majority of the particles were agglomerated, with the predominant particle size range 0.1–1 µm	NR	Iron oxide is expressed as titanium equivalent for comparison purposes, thus not providing an actual mass for pure iron oxide particles	NR	The greatest potential for exposure to workers occurred during the handling process, levels were well below established limits	2011	[35]

NR, not reported; M, male; F, female.

4.24 mg/m^3 for a 50% total iron content. However, these levels are estimated from sporadic measurements leaving uncertainty in the actual dust composition. Moulin et al. conducted a dynamic, cross-sectional, cohort study of 4288 male and 609 female workers employed for at least 1 year in a French steel factory between 1968 and 1991 [15]. Overall, the authors did not find any excess risk of lung cancer in relation to exposure to iron oxides (Odds Ratio adjusted for asbestos, PAHs, silica and smoking <0.50). However, the authors note that the job exposure matrix showed simultaneous exposures to some chemicals and dusts may have occurred, thus making it difficult to distinguish the individual effects of pure iron oxide to the risk of lung cancer mortality. Also, similar to the Bourgkard study, the exposure assessment of iron oxide fails to accurately assess true worker exposure. Due to the lack of exposure measurements, speciation of the metals at the workplace was not considered, indicating that iron oxide exposure estimates may have been inaccurate. In addition to inadequate exposure assessment, many studies such as the ones previously described have focused on occupational tasks that result in iron oxide exposure and their relationship to carcinogenicity instead of directly linking health effects to iron oxide exposure [22, 23].

Iron welders utilize an industrial process that releases small, solid particles into the air creating a plume, known as welding fume. The contents of these fumes are complex and depend on the components of the base metal, coatings, filler materials, and temperatures used in the welding process [24, 25]. Iron represents the predominant component of welding fumes, containing 80–95 wt% iron, and this relates to the fact that most welding fumes are generated from mild steel or carbon steel materials [26]. In regards to specific welding processes, iron and steel arc welding, including gas metal arc welding (GMAW) and shielded metal arc welding (SMAW), are known to produce iron containing fumes [25, 26]. A characterization of welding fumes conducted by Jenkins determined the presence of magnetite (Fe_3O_4) in the GMAW process and $MnFe_2O_4$ in the SMAW process [25]. A more recent welding fume characterization study assessed the components of arc welding fume and found three crystalline phases of iron: Fe^0, FeO and Fe_3O_4 [27]. It is important to note that characterization of welding fumes has confirmed the presence of nanosized iron oxide particles, providing likely evidence for occupational exposure to inhaled particles of this size [25, 28]. Kalliomaki et al. studied three welder cohorts (2 years, 5 years, 13 years continuous exposure) who were exposed to welding fumes containing 25–70% iron oxide (90% Fe_3O_4) particles with mass median diameter of 0.5 µm and concentrations ranging from 2 to

400 mg/m^3 [29]. The methods used to characterize the dust content and the use of personal protective equipment (PPE) by the workers were not reported. From these workers, they report that a constant lung contamination was reached in <9 years with a balance between particle retention and clearance reached between 5 and 9 years of continuous exposure. The average amount retained for 5–30 years exposure was determined to be approximately 200 mg by converting measurements collected using a SQUID magnetometer. Therefore particle clearance was calculated to be approximately 23% of the deposited dose per year. Interestingly, more recent occupational studies of welders overlook iron oxide, focusing on other workplace hazards such as asbestos, hexavalent chromium, and manganese [30]. The lung effects including carcinogenicity of welding fumes have been reviewed in depth and current evidence points to co-exposure with known carcinogens (i.e., asbestos, Cr, Mg, Ni) as a possible explanation for elevated cancer risk [26, 31].

It is evident that the lack of accurate exposure assessment and the inability to differentiate complex, occupational exposure scenarios, which may or may not involve use of PPE, makes the relationship between pure iron oxide exposure and related health effects difficult to ascertain from epidemiological studies. While semi-quantitative exposure estimates (such as job exposure matrixes) are often used in occupational cohort studies when exposure measurements are not always documented, this lack of quantitative exposure levels to iron oxide weakens any potential study conclusions [32]. Few occupational exposures involve pure iron oxide dusts or fumes. Teculescu and Albu reported the pulmonary function of male workers exposed to pure iron oxide (Fe_2O_3) dust in a plant manufacturing rouge polish [11]. The exposure concentration in all parts of the plant were above 10 mg/m^3, with the lowest at 10–15 mg/m^3 or 20–25 particles/cm^3 with 30% of particles in the submicron size range measured. The highest concentration reported was 500 and 700 mg/m^3 or more than 3000 particles/cm^3. The methods used to characterize the dust content and the use of PPE by the workers were not reported. Less than half of the examined workers (38 out of 113) had opacities or shadows in their chest X-rays, and further examination of 14 workers with an average exposure duration of 10 years revealed no lung function changes to suggest lung fibrosis. A more recent review of occupational lung diseases corroborates their conclusion stating that siderosis, or iron oxide accumulation in lung macrophages, does not lead to fibrosis or impairment in lung function and adds that the X-ray abnormalities observed are reversible [33].

It is important to note that occupational exposures to iron oxide particles are not limited to metal workers, miners, and iron oxide manufacturers. Iron oxides have become increasingly important as a pigment due to their pure hue, consistent properties, and tinting strength. Single-component forms are mainly produced with red (hematite, Fe_2O_3, 70% Fe), yellow (limonite/goethite, FeO(OH), 63% Fe), orange (lepidocrocite, γ-FeO(OH), 63% Fe) and black (magnetite, Fe_3O_4, 72% Fe) colors. Its use is highest in the construction and coatings industries, with uses also in ceramics, paint, ink, rubber, plastics, and cosmetics [34]. There are many other applications including: (a) additives in fertilizers; (b) catalysts; (c) fluid tracers; (d) magnetic materials; (e) water purification adsorbers; and (f) biomedical imaging and therapeutic agents. Therefore, a new group of workers potentially exposed to iron oxide particles include producers and users of nanoscale iron oxide for medical, scientific, or industrial purposes. However, the novel applications of iron oxide nanoparticles have not yet given rise to epidemiological studies of these uniquely exposed occupational groups. The limited number of workers directly exposed to NMs in such occupational settings further hinders such research [35].

A recent study from Curwin and Bertke presents exposure data for various metal oxides (including iron oxide) in seven companies that produce or utilize nanoscale metal oxides [35]. Half and full shift sampling based on direct reading and mass based area and personal aerosol sampling was employed to measure metal oxide exposure. Overall, the authors found that medium-sized facilities had higher particle number and particle surface area concentrations in the air, followed by small facilities for all particle sizes measured. Production processes had the highest particle number concentrations, particularly for the smaller particles when compared with handling processes. However, the authors note that the greatest potential for exposure to all workers in the study occurred during the handling process. The majority of the particles were agglomerated, with the predominant particle size being between 100 and 1000 nm (measured by TEM). The authors concluded that exposure levels were well below established and proposed limits in the US. Unfortunately because the predominant metal analyzed was titanium dioxide, other metal oxides, including iron oxide, are expressed as titanium equivalents for comparison purposes, thus not providing an actual mass for pure iron oxide particles. This study pointed out that the number of employees specifically involved in producing and handling the metal oxide nanoparticles in each facility was minimal, with usually only one or two employees involved, highlighting the difficulties of modern day epidemiological studies of workers exposed to iron oxide nanoparticles. Overall, it should be noted that characterizing

nanoparticle exposure in the workplace is challenging given the lack of standard methods for assessing exposure scenarios. Despite this fact, this study provided salient information on occupational exposures to metal oxide nanoparticles and highlighted the importance of accurate characterization methods in the workplace.

Controlled human volunteer inhalation studies

Epidemiological studies involve assessing the health effects from chronic exposure to aerosol mixtures. While more representative of real world scenarios, they are limited with regard to identification of (a) biomarkers of exposure, (b) dose-response relationships, and (c) individual substances responsible for measured effects. Therefore, controlled human volunteer studies, which comprise clinical studies, can fill this knowledge gap by contributing human exposure data where many exposure parameters are defined. Surprisingly to the authors' knowledge, there are no standardized methods of conducting controlled human inhalation exposures. While there are discussions in the literature about the benefit of controlled human inhalation studies, no standard such as those published by OECD (403, 412, 413) and the International Organization for Standardization (ISO) (10801, 10808) for conducting controlled animal inhalation studies exist for human clinical studies [36, 37].

Since the 1950s, iron oxide (Fe_2O_3 or Fe_3O_4) particles have been studied in human volunteer inhalation experiments. The iron oxide particles served as a tracer aerosol utilizing either the particles' inherent magnetic properties or radiolabeling (^{59}Fe, ^{198}Au, ^{111}In, or ^{99m}Tc) for detection and measurement. The primary aim of these earlier studies was to understand human lung physiology as well as particle deposition, clearance rate, and clearance mechanism(s) in the lungs of healthy volunteers and patients with lung disease. The human experimental data generated from these studies was used to develop a model of the human respiratory tract which is discussed extensively in the International Commission on Radiological Protection (ICRP) Publication 66 [38]. For the purposes of this review, these experiments were examined for toxicological endpoints. Overall, none of the reviewed studies reported acute toxicity or adverse effects due to inhalation of iron oxide aerosols. The reviewed studies spanned over 30 years and included over 475 volunteers. All of these studies employed micron-sized iron oxide particles with physical diameters ranging from 1 to 6 μm and about half used Fe_2O_3 and half Fe_3O_4. Exposure durations ranged from <1 min to 30 min with multiple exposures conducted in some cases. A summary of the human inhalation studies reviewed is presented in Table 2.

Besides inherent toxicity, a substance can also elicit adverse effects if it is persistent or bioaccumulative. The epidemiological studies presented in the previous section suggest iron oxides exhibit both of these qualities since X-ray shadows resulting from iron oxide retained in the lungs were reported for exposed workers. Interestingly, the ICRP clearance model for particles is based in large part on measurements of iron oxide particle clearance in human volunteers [38]. Two phases of clearance, a fast phase on the order of days representing mucociliary clearance in the tracheobronchial region and a slow phase on the order of years representing macrophage clearance in the alveolar region were defined. Studying ^{59}Fe labeled iron oxide dust, Albert and Arnett found that particle clearance rate was dependent on size [39]. When the same dose of 100 μCi was inhaled, clearance of ~47% was measured after 2.4 h for particles with diameters of 1.4–2.3 μm while ~87% of larger particles with diameters of 3.5–4.3 μm cleared after 2 h. Note that in this study and for about half of all studies reviewed, the methods used to characterize the aerosol content were not reported. This size dependent clearance was further investigated by Stahlhofen et al. using particles with aerodynamic equivalent diameters of 1, 2, 3, and 6 μm [40]. Their results, which gave the fraction of particles quickly cleared as ~75% for 6 μm particles compared to ~40% for 1–2 μm particles, corroborate the observations of Albert and Arnett. A later study by Stahlhofen et al. examined Fe_3O_4 particles with an average aerodynamic equivalent diameter of 1.3 μm and reported a slow phase clearance half-life of ~110 days [41]. More than 1 year post exposure, particle retention in the lungs was also detected, approximately 15% of the initial measured signal, without any associated health effects reported.

Not only has iron oxide been administered by aerosol inhalation, healthy human volunteers have also undergone intrapulmonary instillation, which involves instilling a solution of particles directly into the lungs. Lay et al. investigated clearance of instilled Fe_2O_3 particles with average physical diameter of 2.5 μm by conducting bronchioalveolar lavage (BAL) to harvest alveolar macrophages and to determine the number of particles recovered [42, 43]. Clearance after instillation was also found to be similar to inhalation, with a measured fast phase clearance half-life of 0.5 days and a calculated slow phase clearance half-life of 110.1 days. Uneven distribution of particles in alveolar macrophages, some containing 0–1 particle and some loaded with >70 particles, was

Table 2 List of iron oxide human inhalation studies.

NP Type	Surface label	Method	Diameter, μm	Number conc., particles/cm³	Mass conc., mg/m³	Exposure conditions	# Volunteers	Assays performed	Year	Reference
Fe_2O_3	^{198}Au	Spinning disc atomizer (Technical machine co.)	2.04 (MMD by microscope with graticule), GSD=1.08	2.5 (measured by Timbrell aerosol spectrometer)	0.033	5 min, 15 breaths/min	19 (16 M, 3 F, mean age=29±9)	Pulmonary function test, γ camera analysis	1971	[46]
Fe_2O_3	^{99m}Tc	Spinning disc aerosol generator	1.7–4.7 (MMD by microscope with filar micrometer)	1–13	0.1	1–1.5 min	7 (age range=23–41)	γ counting, spirometry	1976	[94]
Fe_3O_4	Bare	Dautrebande generator	1.1 (MMAD by unreported method), GSD=1.41	2.9×10^4	60	30–45 min, mouth only inhalation exposure	41 [15 healthy, 14 smokers, 8 CF (ages <35); 4 emphysema (age range=58–71)]	Magneto-pneumography	1988	[95]
Fe_2O_3	^{198}Au, ^{99m}Tc	Spinning disc aerosol generator	3.5, 6 (by unreported method)	NR	NR	2 min (^{198}Au tagged Fe_2O_3, before exposure), 2 min (^{99m}Tc-Fe_2O_3, after exposure)	10 M (mean age=32±9)	Respiratory mechanics (forced expiratory volume, forced vital capacity, mid-maximal expiratory flow rate, peak expiratory flow rate), scintillation detector	1989	[96]
Fe_2O_3	^{99m}Tc	Spinning top aerosol generator	6 (MMAD by light microscope analysis) GSD <1.14	NR	NR	10 min, 30 breaths/min, 2 times	12 (7 M, 5 F, mean age=29)	γ camera analysis	1990	[97]
Fe_2O_3	^{198}Au, ^{111}In	Spinning top aerosol generator	1, 2, 3, 6 (AED)	NR	NR	50 cm³ bolus, 250 cm³/s	1 M	Body counter (γ ray analysis)	1990	[40]
Fe_2O_3	^{99m}Tc	Spinning top aerosol generator	5 (MMAD by unreported method), GSD=1.25	NR (30 μCi)	NR (30 μCi)	5 min, 30 breaths/min, 3 times	10 (5 M, 5 F, mean age=33)	γ camera analysis	1992	[47]
Fe_3O_4	Bare	Spinning top aerosol generator	1.3 (by sedimentation cell)	10^{10} (10^8 particles deposited)	50 (0.5 mg deposited)	30 min (30 breaths), 250 cm³/s, 1 L tital volume	1	Magneto-pneumography	1992	[41]
Fe_2O_3	^{99m}Tc	Spinning top aerosol generator, Pari bolus delivery system	3.5 (MMAD by unreported method)	NR (10–15 μCi)	NR (10–15 μCi)	15–20 min, 10–20 boluses of 40 mL, mouth only inhalation exposure	16 (10 M, 6 F, age range=20–43)	γ camera analysis, atomic dead space, effective air space dimensions	1998	[98]

(**Table 2** Continued)

NP Type	Surface label	Method	Diameter, μm	Number conc., particles/cm³	Mass conc., mg/m³	Exposure conditions	# Volunteers	Assays performed	Year	Reference
Fe_2O_3	Bare	Spinning disc aerosol generator	2.6 (CMD by unreported method), GSD=1.3	(3×10^7 particles/mL instilled – counted by hemocytometer)	(8.3 mg instilled)	Instillation	30 (24 M, 6 F, mean age=25.5±4.3)	BAL, microscopy	1998	[43]
Fe_2O_3	^{99m}Tc	Spinning top aerosol generator, Pari bolus delivery system	3.5 (MMAD by unreported method)	NR (10–15 μCi)	NR (10–15 μCi)	15–20 min, 10–20 boluses of 40 mL, mouth only inhalation exposure	11 (age range=20–43)	γ camera analysis, atomic dead space, effective air space dimensions	1999	[99]
Fe_2O_3	Bare	Spinning disc aerosol generator	2.6 (CMD by SEM), GSD=1.3	(3×10^7 particles/mL instilled)	(8.3 mg instilled)	Intrapulmonary instillation	34 (27 M, 7 F, mean age=25.8±4.3)	Bronchoscopy, BAL	1999	[42]
Fe_2O_3	^{99m}Tc	NR	5 (MMAD by unreported method), GSD <1.2	NR (20–30 μCi)	NR (20–30 μCi)	15 breaths/min	22 (11 M, mean age=26±6; 3 M CF, 7 F CF, mean age=31±8)	γ camera analysis, regional deposition, regional ventilation	2001	[50]
Fe_2O_3	Bare	Neidle & Barab synthesis, ultrasonic nebulizer (DeVilbis Ultra-Neb 99)	1.5 (MMAD by Anderson cascade impactor), GSD=2.1	2.4×10^3 ($1.8–3.1\times10^3$)	12.7±0.5 mg/m³ (9.4–16.3 mg/m³)	30 min, 2 times, mouth only inhalation	16 (8 M, 8 F, age range=18–34)	Pulmonary clearance halftime, pulmonary function test, respiratory epithelial permeability	2001	[45]
Fe_3O_4	Bare	Spinning top aerosol generator	2.9 (AED by sedimentation cell), 1.35 (GMD by EM), GSD <1.1	$1.6–1.8\times10^3$	60–67	40 min (40 breaths), 250 cm³/s, 1 L tidal volume	39 Healthy (19 with age <39, 20 with mean age=53±7), 15 COB (9 M, 6 F, mean age=60±8), 12 IPF (5 M, 7 F, mean age=49±15), 15 SAR (5 M, 10 F, mean age=48±14)	Plethysmography, spirometry, lung function test, magneto-pneumography (30 min, 2 d, 1 week & 1,5,9 month)	2001	[53]
Fe_3O_4	Bare	Spinning top aerosol generator	2.9 (AED by sedimentation cell), 1.35 (GMD by EM), GSD <1.1	NR	NR	2 breaths	17 Healthy (mean age=54±7), 12 IPF (mean age=49±15)	Magneto-pneumography	2001	[55]
Fe_3O_4	^{99m}Tc	Spinning top aerosol generator	2, 3, 4 (AED by convection free sedimentation cell)	NR	NR	1000–2000 cm³, 200 cm³/s, mouth only inhalation	10 (7 M, 3 F, mean age=54)	Pulmonary function test, γ camera analysis	2003	[51]
Fe_3O_4	Bare	Spinning top aerosol generator	4.2 (AED by sedimentation cell), 1.9 (GMD by EM)	NR	NR	100 cm³ bolus w/ breath hold, 250 cm³/s,	13 (mean age=37±11)	Body plethysmography and spirometry, lung function test, magneto-pneumography	2004	[100]

(**Table 2** Continued)

NP Type	Surface label	Method	Diameter, μm	Number conc., particles/cm³	Mass conc., mg/m³	Exposure conditions	# Volunteers	Assays performed	Year	Reference
Fe_3O_4	Bare	Spinning top aerosol generator	2.9 (AED by sedimentation cell), 1.35 (GMD by EM), GSD <1.1	1.6–1.8×10³	60–67	40 min (30–40 breaths), 250 cm³/s, 1 L tidal volume	17 Healthy (12 M, 5 F), 18 COB (10 M, 8 F), 12 IPF (5 M, 7 F), 15 SAR (5 M, 10 F)	Body plethysmography and spirometry, lung function test, magneto-pneumography	2006	[52]
Fe_3O_4	Bare	Spinning top aerosol generator	4.2 (AED by sedimentation cell), 1.9 (GMD by EM)	NR	NR	100 cm³ bolus	7 (PCD, mean age=35±12)	Pulmonary function test, physiological dead space, magneto-pneumography	2006	[54]
Fe_3O_4	^{99m}Tc	Spinning top aerosol generator	3.52±0.17 (AED by sedimentation cell), 1.59±0.80 (GMD by EM)	NR	NR	7–9 breaths, 250 cm³/s	14 Healthy (13 M, 1 F, mean age=36±10), 10 BHR (5 M, 5 F, mean age=51±13), 23 COPD (17 M, 6 F, mean age=63±8)	Pulmonary function test, Fowler dead space, histamine challenge, γ ray spectrometry	2008	[56]

NR, not reported; M, male; F, female.
[a]Value calculated using aerodynamic diameter and a reference value particle density of 3 g/cm³ [38].

suggested to indicate intracellular overload and release of particles which are rephagocytized by other macrophages. It is important to note that instillation is known to result in different lung deposition of particles compared to inhalation [44]. At the instilled concentration (3×10^8 particles or 3.2 particles per alveolar macrophage), an acute inflammatory response was observed one day post-instillation with reactive oxygen species generation leading to measurable lipid peroxidation and cell injury [42]. In a follow-up inhalation study with healthy volunteers exposed to ~12 mg/m³ of Fe_2O_3 particles with aerodynamic diameter of 1.5 μm, no signs of inflammation or altered pulmonary function were detecting using non-invasive techniques [45]. In addition, using another tracer aerosol, technetium labeled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA), clearance half-lives were similar for air and iron oxide exposed volunteers, approximately 50–200 min post-inhalation. These results suggest that short-term iron oxide particle inhalation does not alter normal lung function.

No adverse effects were reported in studies using iron oxide particles to measure particle deposition and clearance differences between non-smokers and smokers as well as patients with lung disease. Both radiolabeled Fe_2O_3 and Fe_3O_4 particles as well as magnetite (Fe_3O_4) particles served as the tracer aerosol. Deposition patterns for ^{198}Au labeled Fe_2O_3 particles with an average physical diameter of 2 μm were found to be similar between smokers and non-smokers by Lourenco et al.; however, clearance was significantly slower in the first hour after exposure in smokers [46]. This was not the case for asymptomatic smokers as Bennett et al. reported their mucociliary clearance of ^{99m}Tc labeled Fe_2O_3 as comparable to healthy volunteers over time [47]. Although fast phase clearance was slower in smokers vs. non-smokers, Cohen et al. found the impairment of clearance was even more pronounced around 1 year post-exposure with smokers retaining ~50% of the administered particles while non-smokers retaining ~10% [48]. In the Cohen study and predominantly in more recent studies, retained particles were measured by magnetopneumography which uses the inherent magnetic property of Fe_3O_4 for detection, making it better suited for monitoring over longer time periods compared to radioactive methods. Magnetometry, which includes magnetopneumography, and human studies using this method were recently reviewed by Aizawa and Kudo [49]. Magnetic relaxation was found to be delayed following different types of chemical exposures.

In addition to smokers, the lung function of cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), emphysema, bronchial hyper-responsiveness (BHR), sarcoidosis (SAR), idiopathic pulmonary fibrosis

(IPF), chronic obstructive bronchitis (COB), and primary cilia dyskinesia (PCD) patients were also studied with iron oxide particles [50–56]. The primary objective of the studies by Brown, Meyer and Scheuch was to determine the influence of controlled breathing on particle deposition in order to optimize the delivery of therapeutic aerosols in lung disease patients. In comparison, the studies by Moller et al. focused on alveolar clearance kinetics and differences in slow phase clearance between healthy and diseased patients. Interestingly, iron oxides were still chosen as the tracer aerosol in these more recent studies despite evidence of long-term particle retention. We suspect that the risks associated with lung retention of iron oxide particles do not outweigh their utility in lung function studies.

All of the before mentioned studies on iron oxide particles were conducted using particles with average diameters in the micron size range. The authors are not aware of any published human inhalation studies using iron oxide nanoparticles. However, several controlled human inhalation studies have exposed volunteers to other ultrafine particles [57–66]. In addition, many human inhalation studies have been conducted using Technegas or ^{99m}Tc labeled ultrafine carbon particles; however, this literature is beyond the scope of this review. There are two published human inhalation studies comparing the effects of nanosized and submicron sized metal oxide particles. Kuschner et al. conducted a study comparing the physiological response to inhaling ultrafine and fine magnesium oxide particles in healthy and former smoker, male and female volunteers [61]. Six volunteers were exposed for 15–45 min to MgO generated by a furnace system at a median concentration of 133 mg/m^3, which consisted primarily of particles <1.8 μm in aerodynamic diameter, determined by micro-orifice uniform deposit impactor analysis. No significant differences in pulmonary function, hematology and bronchoscopy/bronchoalveolar lavage were measured after 18–20 h post-exposure.

Beckett et al. conducted a study comparing the physiological response to inhaling ultrafine and fine zinc oxide particles in healthy male and female volunteers [59]. Twelve volunteers were exposed for 2 h to ZnO generated by an electric arc discharge system brought to a concentration of 500 μg/m^3, which consisted of either 4.6×10^7 particles for the ultrafine (~40 nm in aerodynamic diameter) particles or 1.9×10^5 particles for the fine (~300 nm in aerodynamic diameter) particles. Particle concentration and size were determined by a condensation particle counter and an electrostatic classifier respectively. Several effects of metal fume fever were monitored, but no significant changes in these parameters were measured. The concentration was ultimately deemed below the level where acute systemic effects occur. In earlier papers, the same research group and another reported observing symptoms of metal fume fever in healthy volunteers exposed to 4.5, 5 and 33 mg/m^3 ZnO dust, which are concentrations above the 2 mg/m^3 TLV for ZnO [67–71]. In these studies, the ZnO particles had average diameters of 300 nm (Fine and Gordon) and 170 nm (Kuschner), the latter having primary particle sizes of 8–40 nm. These studies suggest that controlled human exposure to some metal oxide nanoparticles can be conducted safely and that current threshold limit values can serve as reference concentrations for study design.

Animal inhalation studies on iron oxide nanoparticles

Although to date no human inhalation clinical studies have been conducted using iron oxide nanoparticles, there are beagle dog and several rodent inhalation studies reported in the literature. Of over 30 papers reviewed, 43% of the studies report exposure via instillation while 57% of the studies report generating an aerosol for exposure via inhalation. Focusing on the inhalation studies, iron oxide particle concentrations ranged from 2×10^3–2×10^9 particles/cm^3 or 0.04–640 mg/m^3. Most of the authors (90%) reported concentration in terms of mass, and for reference, particle concentrations were calculated based on the reference density of 3 g/cm^3 in Table 3. Particle sizes ranged from the nanoscale (0.01 μm) to comparable particle sizes found in the previously discussed human studies (1.5 μm). No acute toxicity was reported in the studies that tested micron sized particles [72–74]. While oxidative stress and inflammation were reported in some nanoparticle inhalation studies, these experiments involved multiple exposures or very high (orders of magnitude above the current TLV) exposure concentrations [75–77]. Beyond acute toxicity, a comprehensive carcinogenicity evaluation of several types of iron oxides was conducted in rats by Steinhoff et al. [78]. The shortest dimension of the seven iron oxide particles tested ranged from 0.03 to 1 μm. An instilled dose of 1530 mg/kg resulted in tumor induction in 1–2% of exposed rats; however, the tumors were attributed to non-specific stress effects rather than specific carcinogenic effects of the other iron oxide formulations. Therefore based on these findings, iron oxides are not considered to be carcinogenic.

Although a few studies reported oxidative stress and inflammation responses to iron oxide nanoparticle inhalation, the effects occurred without associated acute

Table 3 List of iron oxide animal inhalation studies.

NP Type	Surface character	Method	Diameter, μm	Number conc., particles/cm³	Mass conc., mg/m³	Route of admin.	Species	# Animals	Exposure conditions	Assays performed	Year	Reference
Fe_2O_3	Bare	Dust feed aparatus	<1 (88%)	1500–2000, 2500–3000, 5000	2.3–7.8[a]	Inhalation	Rats	12 (NR)	3 h/d, 140 d	Radiography, histology	1947	[101]
Fe_2O_3	^{59}Fe	D30 jet aspirator with 10 ψ air pressure	0.090 (CMD by EM), GSD=1.8	2.6×10^{7} [a]	30	Nose only aerosol exposure	Beagle dogs	6 (F)	1 h nose only exposure w/ post-exposure evaluation up to 6 month	Scintillation, Texas well counter	1962	[102]
Fe_2O_3	^{59}Fe	Lauterbach generator from solution with 30 ψ pressurized air	0.068 (CMD by EM), GSD 1.62	$0.6–1.8\times10^{9}$ [a]	300–900	Nose only aerosol exposure	Rochester rats	45 (M)	45 min nose only exposure w/ post-exposure evaluation up to 30 d	Texas well counter, histology	1966	[103]
Iron oxide	Bare	0.3–3 mg Fe deposited 1 d post-exposure, short term exposure t1/2=1 d, t1/2=33 d (rat), 70 d (man)	0.3 (MAD by EM), GSD=1.8	1.6×10^{7} [a]	700	Inhalation	Albino rats	NR (M)	16, 30, 235 min inhalation w/ post-exposure evaluation up to 10 d	NAA	1974	[104]
Fe_2O_3	Bare	Iron pentacarbonyl combustion	0.1 (d) 0.1–3 (l) (SEM)	$1.1–1.3\times10^{8}$ [a]	170–200	Whole body exposure	CD-1 mice	NR	3 h, up to 14 months	Histology by light and electron microscopy	1975	[105]
Fe_2O_3	Bare	Iron pentacarbonyl combustion	0.005 (primary particle), 0.15 (MMAD), GSD=2.2	5.7×10^{7} [a]	300	Inhalation	CD-1 mice	10 (M)	3 h, w/ post exposure evaluation up to 7 d	Histology by light and electron microscopy	1979	[106]
Fe_3O_4	^{59}Fe	Aerosol generation method referenced (can't access)	1.5 (GSD 1.8, by unknown method)	2.9×10^{3} [a]	15.4±4.5	Nose only aerosol exposure	Fischer 344 rats	320 (M)	2 h nose only exposure	NaI detector	1984	[74]
γ- Fe_2O_3	Bare	Aerosol diluted straight from combustion generator	0.155 (TEM), 0.73 (MMAD by concentric aerosol spectrometer, 1.26 GSD)	$3.3–4.9\times10^{5}$	200–300	Nose only aerosol exposure	New Zealand white rabbits	50 (M)	0.5–1 h nose only exposure	Magnetometry	1984	[107]

(**Table 3** Continued)

NP Type	Surface character	Method	Diameter, µm	Number conc., particles/cm³	Mass conc., mg/m³	Route of admin.	Species	# Animals	Exposure conditions	Assays performed	Year	Reference
γ- Fe_2O_3	Bare	Laminar diffusion flame system	0.072 (0.020–0.140, by TEM)	0.97–1.5×10⁵ [a]	0.057, 0.090	Whole body exposure	Sprague-Dawley rats	24 (M)	6 h/d for 3 d	BAL, LDH, TNB, CDNB, IL-1b, TNF-α, ferritin	2003	[77]
γ- Fe_2O_3	Bare	Collison nebulizer	0.025±0.002 (TEM); 0.2 (GSD 1.3, SMPS)	3–6×10⁵ [a]	3.55, 7.62	Whole body exposure	C57Bl/6 mice	63 (M)	4 h/d, 5 d/week for 2 weeks w/ post-exposure evaluation up to 3 weeks	BAL, LDH, histopathology, LCP-MS	2008	[75]
Fe_3O_4 (FeREX)	Dextran	LC Star jet nebulizers operated with Devilbiss PulmoAide compressors	0.01 (manufact.), 5.6±0.8 [MMAD by Aerosizer (TSI) aerosol particle spectrometer, GSD 1.3±0.03]	510[a]	140	Nose only aerosol exposure	BALB/c mice	12 (F)	2 h	MRI	2008	[81]
$CoFe_2O_4$	RITC-TEOS-PVP	TSI single jet atomizer	0.009 (TEM, core); 0.049 (GSD 1.8, by SMPS), 0.051 (GSD 1.7, by SMPS)	0.8–1.6×10⁶	0.16–0.32	Nose only aerosol exposure	Slc:ICR mice	30 (M/F)	4 h/d, 5 d/week for 4 weeks	SMPS, confocal, MRI	2008	[79]
Fe_3O_4 (Ferroxide Black ^{88}P)	Bare	Wright Dust Feeder	1.5 (MMAD, GSD 2.1 by cascade impactor)	0.19–1.8×10⁴ [a]	10.1±1.44, 19.7±3.27, 45.61±6.77, and 95.84±17.6	Nose only aerosol exposure	Wistar rats strain Bor:WISW (SPF-Cpb)	72 (M, 6 per group)	6 h/d, 5 d/week for 4 weeks w/ post-exposure evaluation up to 6 month	BAL, lung weight, AAS	2009	[108]
$CoFe_2O_4$	RITC-TEOS-PVP	TSI single jet atomizer	0.009 (TEM, core); 0.049 (GSD 1.8, by SMPS), 0.051 (GSD 1.7, by SMPS)	0.8–1.6×10⁶	0.16–0.32	Nose only aerosol exposure	Slc:ICR mice	30 (M/F)	4 h/d, 5 d/week for 4 weeks	Hematology, histology, weight & behavior change	2009	[80]
Fe_2O_3	Bare	Diffusion flame system	0.072 (0.045–0.110, by TEM)	4.2×10⁵ [a]	0.25	Whole body exposure	Sprague-Dawley rats	32 (NR)	6 h/d for 3 d	BAL, LDH, GSH, FRAP, ferritin, IL-1β, TNF-α	2010	[109]

(**Table 3** Continued)

NP Type	Surface character	Method	Diameter, µm	Number conc., particles/cm³	Mass conc., mg/m³	Route of admin.	Species	# Animals	Exposure conditions	Assays performed	Year	Reference
Fe_3O_4	Oleic acid	Ultrasonic atomizer (nominal frequency 1.7 MHz, purchased online from Mainland-Mart.com)	0.022±0.002, 0.1±0.013, 0.198±0.031 (VMD by DLS); 0.61±1.8–1.07±2.03 (MMAD±GSD by cascade impactor)	2×10^{6} [a]	384±30	Inhalation	CD-1 mice	9 (F)	5 min	HPLC	2010	[82]
Fe_3O_4 (Ferroxide Black ^{88}P)	Bare	Wright Dust feeder	1.4 (MMAD, GSD=2 by cascade impactor)	$0.7–2.3\times10^{4}$ [a]	30, 100	Nose only aerosol exposure	Wistar rats	136 (M, 68 per group)	6 h/d, 5 d/week for 4 weeks w/ post-exposure evaluation up to 3 month	BAL, AAS, TBARS, qPCR for heme oxigenase-1 & ferritin mRNA, 8-oxoguanidine IHC, respiratory function testing	2011	[73]
Fe_3O_4 (Ferroxide Black ^{88}P)	Bare	Wright Dust feeder	1.3–1.5 (MMAD, GSD 1.9–2.2, by cascade impactor)	$0.2–2.3\times10^{4}$ [a]	10, 20, 50, 100	Nose only aerosol exposure	Wistar rats strain HsdCpb:WU	75 (M/F, 5 per group)	6 h/d, 5 d/week for 13 weeks	BAL, AAS, hematology, urinalysis, organ weights, histopathology	2011	[72]
Fe_2O_3	Bare	Flame spray pyrolysis	0.05 (FMPS), GSD=1.6	$2–3\times10^{5}$	0.04–0.06[a]	Whole body exposure	Sprague-Dawley rats	NR (M)	5 h	In vivo chemi-luminescence	2012	[110]
Fe_3O_4	Bare	Wright Dust feeder	0.015–0.02 (manufact.), 0.048±0.007 (SEM), 0.651 (DLS), 2.25 (SMPS), GSD=2.56	3.6×10^{4} [a]	640	Nose only aerosol exposure	Wistar rats	72 (M/F)	4 h	Hematology, BAL, IL-1b, TNF-α, IL-6, TBAR, GSH, SOD, catalase	2012	[76]

[a]Value calculated using aerodynamic diameter and a reference value particle density of 3 g/cm³ [38].

toxicity, morbidity, or mortality. Pettibone et al. exposed mice 4 h per day for 2 weeks to γ-Fe_2O_3 nanoparticle concentrations as high as 7.6 mg/m^3 and found increased cell counts in BAL fluid, which returned to baseline 3 weeks post exposure, with no acute toxicity or signs of pathology [75]. In a study by Zhou et al., rats exposed 6 h per day for 3 days to 90 μg/m^3 of γ-Fe_2O_3 nanoparticles presented with mild respiratory effects measured by BAL (i.e., induction of ferritin, increased lavage protein, elevated oxidative stress and inflammatory markers) but no significant cytotoxicity [77]. Also testing rats, Srinivas et al. reported elevated oxidative stress and inflammation markers after a single 4 h exposure to 640 mg/m^3 Fe_3O_4 nanoparticles but no morbidity, mortality or changes in blood biochemistry, despite using a concentration that is over 100 times higher than the current TLV for iron oxides [76]. The observed oxidative stress and inflammation could be due to free iron released from the particles. Although iron oxides are relatively insoluble in aqueous conditions, Beck-Speier et al. reported that Fe_2O_3 particles can dissolve in the acidic lysosomal environment after phagocytosis by alveolar macrophages [27]. However, they also report that the intracellular free iron may suppress particle induced inflammation since the level of inflammatory marker IL-6 was not significantly elevated.

In addition to particle dissolution, impurity of the stock solution can also contribute free iron. Lay et al. measured an acute inflammatory response in human volunteers instilled with Fe_2O_3 particles synthesized in their laboratory [42]. Additional testing in rats led the authors to attribute the observed inflammatory response to free iron present in the laboratory made particles compared to commercially available Fe_2O_3 particles from Alfa Chemicals or Sigma Chemicals. One should note that in both human and animal inhalation studies an analysis of the purity of the iron oxide particles is rarely reported. This becomes more important when testing complex NMs since multiple synthesis steps increase the number of possible impurities. Only four animal studies were found that tested the inhalation of surface modified iron oxide nanoparticles [79–82]. The coatings included dextran, oleic acid, and fluorescent-labeled silica. No acute toxicity or pulmonary effects were reported for mice exposed to aerosols containing these surface modified iron oxide nanoparticles for durations ranging from 5 min to 4 weeks. For the fluorescent-labeled iron oxide nanoparticles which were inhaled 4 h per day, 5 days per week for 4 weeks, systemic effects were reported with particles found not only in the lungs but also in the liver, spleen, brain and testes [79]. In addition, decreased body weight, increased white blood cell counts and extramedullary hematopoiesis were observed [80]. As the latter two conditions suggest an immune response, this also raises questions on the purity of the test particles. Since many regulatory authorities require that materials tested in controlled human inhalation studies be produced under good manufacturing practice (GMP) conditions, NMs that advance to human testing will be quality controlled, with impurities identified and within acceptable levels.

Respiratory medicine applications of SPIONs

While no inhalation clinical studies have been conducted using iron oxide nanoparticles, SPIONs have undergone extensive preclinical and clinical studies, which has resulted in their regulatory approval for medical IV and oral administration [83–86]. These include SPION formulations in two size ranges (USPIO: <50 nm and SPIO: >50 nm) and with several surface modifications (aminosilane, citrate, dextran, polyethylene glycol-starch, polyglucose sorbitol carboxymethyl ether, siloxane, and sulphonated styrene–divinylbenzene copolymer) [87]. The indications include magnetic resonance imaging contrast agent for liver and gastrointestinal cancers (dextran and silicone coated SPIONs), and treatment of iron deficiency anemia (modified dextran coated SPIONs).

However, SPIONs have yet to be administered by inhalation in humans. One of the most promising applications of SPION aerosols is targeted imaging and treatment of lung disease. Dames et al. showed theoretically by computer-aided simulation, and for the first time experimentally in mice, that targeted delivery of SPIONs in the lungs can be achieved with a directed magnetic gradient field [88]. A SPION aerosol was generated using an ultrasonic nebulizer, which outputs droplets of 2.5–4 μm in diameter. The SPIONs were delivered to mice via intratracheal intubation and an eight-fold increase in SPION lung deposition was found in the presence of a magnetic field, measured by magnetorelaxometry and qualitatively confirmed by histology. More recently, this group demonstrated experimentally in mice that an increase in SPION deposition can be achieved using more realistic exposure routes of nose-only and whole body inhalation [89]. The aerosol was generated using both ultrasonic and jet nebulizers, which output micron sized droplets. A two-fold increase in SPION deposition in the presence of a magnet was measured by magnetorelaxometry and pDNA quantification.

Although Dames et al. determined dry powder formulations of SPIONs will not undergo magnetic direction,

Upadhyay et al. formulated drug and SPION containing lipid microparticles for dry powder inhaler based lung delivery [90]. Cascade impactor measurements showed that 30% of the inhaler-generated aerosol consisted of particles with a diameter <2.5 μm, which would deposit deeper into the lungs. Magnetic mobility testing resulted in 100% recovery at a magnet distance of 5 mm and ~5% recovery at a magnet distance of 2 cm. Since a 0.2 T magnet was employed, higher recovery at longer distances may be achieved with a stronger magnet. For reference, a 1.3 T magnet was used in sheep for SPION mediated drug delivery for treating inflammatory joint disease [91]. However, a major issue with magnetic direction is that the strength of the magnetic field decreases with distance to the fourth power compared to optical methods where light radiation decreases with the distance squared. Therefore, despite encouraging results in rodents, clinical use of SPIONs in human respiratory medicine is still in the horizon. Since no human clinical trials testing inhalation of SPIONs have been conducted, the determinants of pulmonary deposition and kinetics of SPIONs after inhalation can only be extrapolated from in vitro and animal in vivo studies.

Discussion

Both human epidemiological and clinical studies contribute to our understanding of the physiological effects of inhaling particles, and neither type of study alone gives a clear picture on the relationship between exposure and health effects. While occupational cohort studies provide data on real workplace conditions, they are often limited due to incomplete exposure assessment, exposures to complex mixtures, and potential confounding from multiple exposures. In clinical studies, investigators have control over the exposure conditions; however, these studies are limited to assessing short-term effects. When examined together, the two study types provide complementary information that present a better understanding of the health effects from exposure.

With their extensive use in industry as well as a multitude of emerging applications, this review has focused on iron oxide particles since iron oxide nanoparticles, such as SPIONs, are a strong candidate for controlled human inhalation studies. No adverse effects were reported in all of the reviewed clinical studies using iron oxide tracer aerosols. Although most were designed to determine deposition and clearance of particles in the lungs versus toxicological endpoints, the few that did assess biomarkers of exposure did not report acute effects from inhalation [42, 43, 45]. Acute effects are also not associated with workplace exposure to dusts and fumes primarily composed of iron oxide. While increased risk of developing lung disease has been correlated with iron oxide exposure, co-exposure to other known carcinogens present in the dusts and fumes as well as smoking confound the relationship. Those that examined workers exposed to, at times, very high concentrations of pure Fe_2O_3 dust did not indicate acute toxicity but rather asymptomatic particle retention in the lungs [11].

Taking into account the findings from human inhalation studies on micron sized iron oxide particles and other ultrafine particles as well as animal inhalation studies on iron oxide nanoparticles, do we have enough data to extrapolate the consequences of iron oxide nanoparticle inhalation in humans? While past studies allow researchers to formulate more precise hypotheses, these new hypotheses still need to be confirmed experimentally. For example, based on animal studies demonstrating persistent inflammation after TiO_2 nanoparticle exposure, many countries have adopted lower occupational exposure limits (OEL) for ultrafine TiO_2 compared to fine TiO_2 particles. However, a recent study on a TiO_2 nanoparticle production plant reported exposure concentrations up to 30 mg/m^3, which is significantly higher than European Union OELs for inert dust [92, 93]. Elevated oxidative stress biomarkers were measured in exposed workers, although it is unclear whether this was due to the high exposure concentration or specifically nanoparticle inhalation. Despite 40 years between the 1970s health effects study on Fe_2O_3 pigment factory workers and the 2010s study on TiO_2 nanoparticle production plant workers, assessments of workplace air still reveal instances where conditions are above established OELs and unfortunately PPE use continues to be unreported [11, 93]. This example illustrates that weight of evidence analysis for respiratory effects may not always be strong enough to support regulatory enforcement.

While human clinical studies should be maintained at a minimum, they will remain a requisite in some risk assessments until accepted and validated model systems for human inhalation and resulting effects exist. Direct correlation studies comparing human in vivo and human in vitro measurements could pave the way for developing modern in vitro techniques to potentially replace acute inhalation testing. However, approval of these human studies faces similar challenges. In order to gain the most knowledge from controlled human inhalation experiments, consistency in reporting the physiochemical characterization of NMs along with the exposure parameters is essential. Inadvertently, the type of study heavily influences which

parameters are measured and reported, resulting in some publications missing the aerosol particle concentration. The lack of a standard method of conducting controlled human inhalation exposures further supports the need for consistently detailed documentation of the experimental conditions.

Nanotechnology is raising new questions and provoking additional oversight in regards to human health research. Although controlled human exposure studies play an important role alongside epidemiological, animal in vivo, and in vitro studies when investigating human health effects, the criteria to justify human testing of NMs remains unclear. The rapid pace of development, matched with various uncertainties produce additional hurdles to overcome. It is essential to recognize that nanotoxicology researchers are testing a wide range of NMs, from simple and passive NMs to complex and active/interactive NMs. Many of these more sophisticated nanostructures are not ready and may never reach the stage of human testing. Nevertheless, the wide scope of nanotechnology should not block the onset of testing some NMs in controlled human inhalation studies.

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