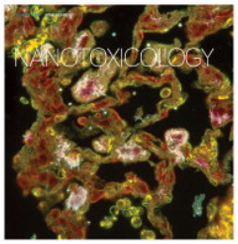

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REVIEW ARTICLE

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Inflammation related to inhalation of nano and micron sized iron oxides: a systematic review

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ABSTRACT

Inhalation exposure to iron oxide occurs in many workplaces and respirable aerosols occur during thermal processes (e.g. welding, casting) or during abrasion of iron and steel products (e.g. cutting, grinding, machining, polishing, sanding) or during handling of iron oxide pigments. There is limited evidence of adverse effects in humans specifically linked to inhalation of iron oxides. This contrasts to oxides of other metals used to alloy or for coating of steel and iron of which several have been classified as being hazardous by international and national agencies. Such metal oxides are often present in the air at workplaces. In general, iron oxides might therefore be regarded as low-toxicity, low-solubility (LTLS) particles, and are often considered to be nontoxic even if very high and prolonged inhalation exposures might result in diseases. In animal studies, such exposures lead to cancer, fibrosis and other diseases. Our hypothesis was that pulmonary-workplace exposure during manufacture and handling of SPION preparations might be harmful. We therefore conducted a systematic review of the relevant literature to understand how iron oxides deposited in the lung are related to acute and subchronic pulmonary inflammation. We included one human and several in vivo animal studies published up to February 2023. We found 25 relevant studies that were useful for deriving occupational exposure limits (OEL) for iron oxides based on an inflammatory reaction. Our review of the scientific literature indicates that lowering of health-based occupational exposure limits might be considered.

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1. Introduction

Exposure by inhalation of respirable dusts of iron oxides is frequent in many occupations, including welding, foundry work, iron and steel manufacture and iron-ore mining. Iron oxide may become aerosolized by thermal processes (welding, casting) or during abrasion (e.g. cutting, grinding, machining, polishing, sanding) of iron and steel products. Also, iron oxides are some of the most used pigments, e.g. black magnetite, red hematite and yellow goethite, and inhalable dusts may be generated in some work processes. Epidemiological studies have indicated that industry workers with high exposure of iron oxides have higher incidence of lung disease, including lung fibrosis and siderosis, asthma, chronic obstructive pulmonary and cardiovascular disease (ECHA 2022; Kornberg et al. 2017; Quanjel et al. 2015; Riccelli et al. 2020; Sjögren et al. 2022). However, in most cases the studied workers were exposed to other hazardous exposures, and it is difficult to separate the effects of iron oxide of from that of other co-exposures (ECHA 2022; Kornberg et al. 2017; Morgan, Bell, and Jones 2020; Pease, Rücker, and Birk 2016; Stokinger 1984).

Exposure of workers to iron oxide particles during production and usage of nanoscale iron oxide for (bio)medical and scientific research purposes is an emerging issue. Associated health effects are related to respirable dusts, i.e. with aerosol diameter less than about five micrometer. At the nanoscale, some iron oxide nanoparticles possess paramagnetic or superparamagnetic properties that are used in biomedical applications. Recently, superparamagnetic

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iron oxide nanoparticles (SPIONs) have been proposed as contrast agent in nuclear magnetic resonance (NMR) in vitro diagnostics (e.g. COVID tests), diagnostic imaging (e.g. contrast agent), for targeting drug delivery and hyperthermia therapy by the aid of an external magnetic field for medical treatment applications (Geppert and Himly 2021; Valdiglesias et al. 2016). Nanosized iron oxides have also been explored for dietary treatment of anemia or as iron supplement, and for parenteral administration when oral administration is ineffective. However, in rare occasions intravenous administration of iron medicines caused serious allergic reactions (EMA 2013; FDA 2018; Gomollón et al. 2014).

There is a growing number of IONP-based products used in products and for manufacturing. More recently, IONPs have been produced for biomedical and clinical applications, and there is not only an increasing need to continuously evaluate the safety for patients intentionally exposed to iron-based medical treatments and diagnostics, but there is also a need to assess the occupational risks resulting from unintentional exposure to iron oxide. As an inhalable dust, iron oxides might be classified as low-toxicity low-solubility particles (Driscoll 1996; Hartman and MAK Commission 2016; Monteiller et al. 2007; Pott and Roller 2005; Pott et al. 1987), sometimes referred to as biodurable- (Hartman and MAK Commission 2016) or nuisance dusts (OSHA 2021). Several investigations indicate that the pulmonary toxicity of low-toxicity low-solubility particles dusts is proportional to the total surface area of the material deposited in the alveolar region of the lung rather than mass (Cosnier et al. 2021; Driscoll 1996; Donaldson et al. 2013; Henderson et al. 1995; Hirano et al. 1994; Morimoto et al. 2016; Oberdorster 1996; Osier and Oberdörster 1997)

Because iron is an essential element necessary for many enzymatic and physiological processes its occurrence and speciation in the mammalian organism is under strict metabolic control during intestinal uptake, in circulation, in different tissues, during recycling and excretion. Biochemical and physiological mechanisms control the cell and systemic availability preventing deficiency and overload (Camaschella, Nai, and Silvestri 2020; Gao et al. 2019; Saito 2014). However, humans are probably less adapted to deposition of large amounts of iron species in the lung. If the antioxidative pathways become overloaded due to excessive exposure, iron oxide particles can accumulate in the lungs where they have the potential to induce oxidative stress and inflammation. A reason why iron metabolism is so intricate is that iron is one of the transitional metals that catalyze the Fenton reaction (Fenton 1894; Møller and Wallin 1998) and uncontrolled iron ion redox reactions produce harmful reactive oxygen species. Iron may, thus, propagate disease directly by producing reactive oxygen species in tissues. However, reactive oxygen species and other adverse effects may also be produced in an inflammatory response (Knaapen et al. 2004). Inflammation is a complex process involving several cell types and released cytokines/chemokines. Persistent pulmonary inflammation is thought to be driving processes leading to lung cancer, fibrosis, and other chronic diseases (Balkwill and Mantovani 2001; Knaapen et al. 2004; Virchow, Chance, and Goodsir 1860). However, there is still challenges to with associating toxicological outcomes with inflammation (Villeneuve et al. 2018).

In spite of being an essential element, excess exposure to iron oxides has been related to disease, for example, genetically determined deficiencies in iron oxide metabolism may lead to accumulation of iron in tissues, hemochromatosis (Abbaspour, Hurrell, and Kelishadi 2014; Lieu et al. 2001). This has been proposed to cause cirrhosis, hepatomas, diabetes, cardiomyopathy in carriers of one of a few defective genes (OMIM 2023). The condition can be alleviated by an iron-restricted diet. Occupational exposure to iron oxides has been proposed to pulmonary disease including lung cancer (Toyokuni 2009).

Recently the WHO, International Agency for Research on Cancer (IARC 2018) stated that welding fumes are carcinogenic to humans (Group 1). This was based on sufficient evidence for increased lung cancer incidence in exposed humans. However, there was insufficient evidence in animal experiments. Although welding fumes contain many different chemicals, it is noteworthy that epidemiological data failed to distinguish the risk of stainless-steel welding, rich in carcinogenic nickel, chromium and vanadium, from other welding processes containing much less of these transition metals. ECHA's Committee for Risk Assessment (RAC) has provided an opinion on occupational exposure limits for fumes from welding and related processes (ECHA 2022), but the recommendations have not, at this time, been adopted by the EU commission.

It is also important that iron species occur in foods, urban air pollution, volcanic ashes and soils. Inhalation exposure might therefore occur, e.g. in urban air pollution and as long-distance transport of dusts from volcanoes or wind erosion of desert sands.

2. Methods

In this review we chose to use infiltration of proinflammatory cells into the lung lining fluid as a sensitive and relevant biomarker for adverse effects. The hallmark of pulmonary inflammation is the recruitment of polymorphonuclear neutrophiles (PMN) and this is a key event in the adverse outcome pathways of inhaled particles in the development of lung diseases (Bos et al. 2019; Halappanavar et al. 2023). The standard for determining infiltration of inflammatory cells is by microscopic visual identification and counting of cells in bronchoalveolar lavage (BAL) fluid or in induced sputum. Especially, PMN in BAL is a valid and sensitive endpoint in inhalation studies with poorly soluble particles, and usually it is more sensitive than measurement of cytokines and macrophages in BAL (Boots et al. 2021; Halappanavar et al. 2020; Poland et al. 2013; Pauluhn 2011; Schulte, Kuempel, and Drew 2018). It is a frequently reported response in rodent in vivo studies (Boots et al. 2021) and sometimes reported for humans (Lay et al. 1999).

Here we review the relevant literature to understand how iron oxides deposited in the lung are related to acute pulmonary inflammation. We surveyed the literature on inflammation related to lung deposition of a variety of iron oxide polymorphs to calculate the no observed adverse effect concentration (NOAECs) and lowest observed adverse effect concentration (LOAECs) for the acute inflammatory response by pulmonary neutrophilia which we believe is a relevant and sensitive endpoint: This was for understanding the dose-response relationships that can be related to workplace exposure during manufacturing and handling of iron oxide nanoparticle preparations.

3. Literature search strategy

A systematic literature search was performed using Medline, Embase and Web of science to identify relevant studies. The following search terms were used: ferric oxide OR iron oxide OR ferrosoferric oxide OR magnetite OR maghemite OR SPION*))) AND TS=((inhalation* OR inhaled OR instillation* OR instilled OR aspiration* OR intratracheal OR intra-tracheal)). Additional searches with search terms with chemical names, polymorphs, color index pigment names or food additive-names did not result in any additional relevant hits. A variety of iron oxide species and crystalline phases were included. After removing duplicates, the search identified 1109 unique references (updated by February 2023). A two-tiered approach was followed for the systematic screening of literature between a first-line reader and an unbiased referee of the selected articles.

After screening titles and abstracts, 75 articles were read in full length and assessed for eligibility to determine dose response of infiltration of neutrophil cells by counting lung lavage cells. From the screening of the reference lists and associated archives five additional relevant reports were included. We excluded studies:

1. Poorly described or irrelevant experimental protocols
2. Reports with effects registered after three days after last exposure, because of experimental inconsistances in longer follow up studies and uncertainties of the time course of neutrophila after this time.
3. No data on neutrophil numbers in lavage
4. Animals were exposed to other chemicals
5. The chemical identity was uncertain

A total of 25 articles met the selection criteria and were included in the review.

4. Evaluation criteria

Only studies on acute pulmonary inflammation, measured as increased polymorphonuclear leukocytes (PMNs) in bronchioalveolar lavage fluid (BALF), were included. This sensitive determinant of acute inflammation is thought to be an important driver of pathological effects (Villeneuve et al. 2018). Pulmonary exposure after inhalation, oropharyngeal aspiration, or bolus intratracheal instillation were considered. It has been suggested that the inflammatory effects are proportional to the dose deposited in the alveolar region whether delivered by inhalation or intratracheal instillation (Baisch et al. 2014; Costa et al. 2006; Gaté et al. 2019; Morimoto et al. 2012, 2015, 2016). (In this review, we used the calculated deposited dose as described by the authors. For studies with administration by instillation and aspiration we assumed that the 50% of a corresponding aerosol would have been deposited in the alveolar region of the lung. This approximation is fair if the aerodynamic diameter is less than one micrometer.) At large doses the response is saturated and in our experience 0.6–1 million neutrophils (or close 100% of the cells) can maximally be extracted from a mouse (unpublished data). We

assumed that insoluble materials, that remain in the lung for long times, may cause the same effects whether they are administrated by inhalation or bolus exposure (unlike chemicals that are metabolized or distributed rapidly beyond the lung). Data for determining the persistence of duration of infiltration of neutrophils were insufficient for determining inflammation at different time-points, therefore only studies with post-exposure time of less than 3 days (3h to 3 days) were included (even if daily exposures were repeated many times).

5. Estimation of no observed adverse effect concentration (NOAEC) and lowest observed adverse effect concentration (LOAEC)

To apply to risk assessment and risk management in the workplace, it is important to determine the safe exposure level in the workplace. This means determining when adverse effects are under a threshold-dose or one that causes minimal harm. In this, it is necessary to understand how the health effects are related to the dose. For most agents, effects occur above a certain threshold dose. This point of departure (POD) in the animal experiments is the starting point for extrapolation by use of Assessment/Uncertainty Factors to derive the reference human dose (e.g. Derived No Effect Level (DNEL) or Occupational Exposure Limit (OEL)) that is then used for the evaluation of the human risk the agent might exert (ECHA 2016).

Historically, the NOAEC concentration approach has been the standard for estimating the POD. It is defined as the highest dose at which no (adverse) effects were observed in the test animals (U.S. EPA, 1987). For those endpoints that show a dose-related change in effect, the lowest dose that statistically significantly differs from the negative control is the LOAEC (Lowest Observed Adverse Effect Concentration). For each endpoint, the dose below the LOAEC is the endpoint-specific NOAEC (Bos et al. 2019; ECHA 2012, 2016).

NOAEC and LOAEC were calculated for individual studies. If the NOAEC/LOAEC was not explicitly reported in the publication, the NOAEC was reported as the highest dose given that did not result in a statistically significant increase in PMN (count or percentage) from the control group. Similarly, the LOAEC was reported as the lowest dose group that did result in a statistically significant increase in PMN (count or percentage) from the control group. The resulting animal NOAEC/LOAEC was further adjusted to estimate a human-worker equivalent dose.

In inhalation studies, the exposure ranged from 3–6 hours per day in rodents, which differs from that for worker (assumed 8 hours per day). Therefore, a concentration-time inverse correction (time scaling) must be applied.

For instillation studies, the NOAEC in rats/mice (in mg/rodent or mg/kg bw/day was converted to an inhalation corrected NOAEC

(mg/m^3) for 8 hours workday as Equation (1):

$$NOAEC\ worker\ 8h\left(\frac{mg}{m3}\right) = \frac{NOAEC\ rat\ /\ mice\ (mg)}{minute\ volume\left(\frac{l}{\min}\right) * \left(\frac{8\left(\frac{h}{\min}\right) * 60\left(\frac{\min}{h}\right)}{1000\left(\frac{l}{m3}\right)}\right) * deposition\ rate} \quad (1)$$

For inhalation, we assumed that 50% of the dose was deposited in the respiratory tract adding a factor of 0.5 as deposition rate when no other information was available. For doses reported as mg/kg, default weight of a rat (250 g) was used (ECHA 2012). Standard respiratory volume (minute volume) for a rat (250 g) is 0.2 L/min/rat (ECHA 2012) were used providing an inverse correction factor of 0.048 (see Equation (2)):

$$\begin{aligned} NOAEC\ worker\ 8h\left(\frac{mg}{m3}\right) &= \frac{NOAEC\ rat\ (mg)}{0.2\left(\frac{l}{\min}\right) * \left(\frac{8\left(\frac{h}{\min}\right) * 60\left(\frac{\min}{h}\right)}{1000\left(\frac{l}{m3}\right)}\right) * 0.5} \\ &= \frac{NOAEC\ rat\ (mg)}{0.048\ m3} \end{aligned} \quad (2)$$

For mice we used standard values of 25 g (which is typical for young C57Bl/6 and other common strains used in toxicological tests) and a minute volume of 0.05 l/min (BAuA 2022), providing an inverse correction factor of 0.012:

NOAEC

$$NOAEC\ worker\ 8h\left(\frac{mg}{m3}\right) = \frac{NOAEC\ mouse\ (mg)}{0.05\left(\frac{l}{min}\right) * \left(\frac{8\left(\frac{h}{min}\right) * 60\left(\frac{min}{h}\right)}{1000\left(\frac{l}{m3}\right)}\right) * 0.5} = \frac{NOAEC\ mouse\ (mg)}{0.012\,m3} \quad (3)$$

6. Results and discussion

6.1. Inhalation

By applying the methodology and criteria presented above, the Boolean search identified 7 relevant inhalation studies, as summarized in Table 1. Except for the study from Pettibone et al. in mice, all studies were performed in rats. This included 4 acute (Guo et al. 2021; Présumé et al. 2015; Zhou et al. 2003), 2 sub-acute (Pettibone et al. 2008; Srinivas et al. 2012) 3 sub-chronic studies (Pauluhn 2011, 2012; Sutunkova et al. 2016) and one study ranging from 4 to 39 weeks (NTP 2020). The daily exposure time ranged from 3 to 6 hours per day, which differs from that of a worker (assumed 8 hours per day). For all studies a concentration-time correction was applied to correct the NOAEC/LOAEC in rodents to the exposure duration for workers (8 h) (Table 1) as is the standard for setting exposure limits.

In the three studies with acute exposure, no effect of γ-Fe_2O_3 (Zhou et al. 2003) Fe_3O_4 or mixed iron oxides (Guo et al. 2021) or 24 nm Fe_2O_3 (Présumé et al. 2015) were observed.

At a concentration of 0.5 mg/m^3 for 3 hours a day for 3 days, Guo et al. (2021) found no effect of Fe_3O_4 (19 nm) or FeO mix on BALF neutrophils in rats 24 hours after last exposure. Similarly, inhalation exposure of 0.09 mg/m^3 Fe_2O_3 (72 nm) for 6h/d x 3 days in whole body chambers did not affect BAL neutrophil numbers in rats (Zhou et al. 2003). No effect on PMN numbers was detected in C57Bl mice after 1 d after 1 and 4 days of 3 h inhalation of 330 µg/m^3 spark generated iron oxide (8 h Equivalent NOAEC 123 µg/m^3) (Présumé et al. 2015). In these three acute studies the tested doses might have been below detection threshold or the time span was too short to detect any infiltration of PMNs.

At a much higher concentration, Srinivas et al. (2012), exposed Wistar rats to Fe_3O_4 (15–20 nm) with nose only inhalation (4h exposure) for up to two

Table 1. Inhalation studies; key parameters and references.

Reference	Species	Agent, size	Dosing regimen (exposure method. Frequency, doses)	Corrected NOAEC (8 hr, mg/m^3)	Corrected LOAEC (8 hr, mg/m^3)
Zhou et al. (2003)	Sprague-Dawley rat	ϒ-Fe_2O_3, 72 nm (20–140 nm)	Inhalation, acute 6h/d x 3d 57 and 90 µg/m^3	0.07	
Guo et al. (2021)	Sprague-Dawley Rat	Fe_3O_4, 19 ± 16 nm	Inhalation, acute 3 h/d*3 d 47.6 and 487 µg/m^3	0.18	
		FeOx-mixture	Inhalation, acute 3 h/d*3 d 507.8 µg/m^3	0.19	
Présumé et al. (2015)	C57Bl/6	24 nm Fe_2O_3	Inhalation, acute 1 d after 1 or 4 days (3 h a day)	0.12	
Pettibone et al. (2008)	male C57BL/6 mice	Fe core + γ-Fe_2O_3+ Fe_3O_4, 25 nm	Inhalation, subacute (4h/d*5d/w *2 w) 3.55 mg/m3		1.8
Srinivas et al. (2012)	Wistar Rat	Fe_3O_4, 15–20 nm	Inhalation, subacute 4h x 1, 2 or 14d 640 mg/m^3		320
Pauluhn and Wiemann (2011)	Wistar Rat	Magnetite Fe_3O_4, 15–20 nm	6h/d*5d/w*4w inhalation, 33 or 99 mg/m^3, PMN increased shortly after both doses		44
Pauluhn (2012)	Wistar rat	Fe_2O_3, 1.3 µm	Inhalation, subchronic 6h/d*5d/w*13w 4.7,16.6 and 52.1 mg/m^3	3.8	12
Sutunkova et al. (2016)	outbred rats	Fe_2O_3, 14 nm	Inhalation, subchronic 4h/d*5d/w for 3 months 1 mg/m^3		0.75
National Toxicology Program, report 91	Sprague-Dawley	Specular hematitite, (95% Fe_2O_3)	Inhalation 4w-subchronic 6h/d*5d/w 15, 30 and 60 mg/m^3 *4 w	22.5	45
			16, 26 and 39 w		11

weeks at the only tested dose of 640 mg/m^3. In addition to a 5-fold increase in PMN in BAL, they observed increased levels of pro-inflammatory cytokines, as well as increased levels of LDH as an indicator of membrane damage and acute cytotoxicity. However, the excessive dose assessed in this study is much greater than effective doses in other studies, and also the OELs for iron oxide (ECHA 2022) and the value of this study for regulatory purposes is therefore limited.

Pettibone et al. exposed C57BL/6 mice by whole body inhalation of 3.55 mg/m^3 nebulized iron core with Fe_3O_4 and Fe_2O_3 nanoparticles (25 nm) for 4 h/day, 5 days/week for 2 weeks (Pettibone et al. 2008). There was statistically significant neutrophilia one hour after last inhalation exposure. A LOAEC of 1.8 mg/m^3 was derived.

Two inhalation studies (Pauluhn 2012 and NTP 2020) reported both a NOAEC and a LOAEC. In the Pauluhn sub-chronic 13-weeks inhalation study Wistar rats were exposed to iron oxide dust (Fe_3O_4, 1.3 um) for 6 h/day, 5 days a week for 13 weeks at measured concentrations of 0, 4.7, 16.6, and 52.1 mg/m^3. Of several endpoints, the most sensitive appeared to be percent of PMN in BAL. No effect on PMN numbers was detected at 4.7 mg/m^3 and the lowest effective dose was 16.6 mg/m^3. Then with correction for a human daily 8 working hours we derived the NOAEC to be 3.8 mg/m^3 and a LOAEC 13.2 mg/m^3.

It was reported by the National Toxicology Programme (NTP 2020) of Sprague-Dawley rats that were exposed by inhalation to 700–800 nm of 15, 30 and 60 mg/m^3 to specular hematite (95% Fe_2O_3) 6 hours per day 5 days a week. BAL was collected from the right lung lobe male rats per exposure group at 4, 16, 26, and 39 weeks. At 4 weeks a NOAEC 30 mg/m^3 and LOAEC 60 mg/m^3 (human NOAEC, 22.5 and LOAEC 45 mg/m^3) were derived. However, after 16 weeks and later a LOAEC of 30 mg/m^3 (human LOAEC, 22.5 mg/m^3) was derived.

Sutunkova et al. (2016) detected significant infiltration of PMN one day in rats after inhalation exposure of three months inhalation exposure of 1 mg/m^3 of 14 nm Fe_2O_3, providing a calculated LOAEC of 0.75 mg/m^3.

With acute iron oxide exposures, no effects were detected, perhaps because the concentrations were below threshold. The derived NOAECs from the acute studies were in rather narrow range from 0.07 to 0.2 mg/m^3. For subacute and subchronic studies NOAECs ranged from 4–22 mg/m^3 (this range was restricted to 4–11 mg/m^3 however if longer exposures were considered).

The weighted LOAEC values was between of 0.75–12 mg/m^3. Two studies seemed to adhere to OECD test guidelines (Pauluhn 2012 and NTP 2020) with limits of benchmark doses between 3.8 mg/m^3 and of 12 mg/m^3.

6.1.1. Instillation studies

By search methodology and criteria presented in Methods section, we identified 17 bolus exposure studies. In most studies intratracheal instillation was used and just oropharyngeal aspiration was used. The studies and their analysis are summarized in Table 2. Interestingly, one study was in human volunteers (Lay et al. 1999); eight studies were in rats and seven in mice. Iron oxide materials were mostly experimentally synthetized or provided as laboratory chemical reagents. Only a few studies were using industrial iron oxide pigments designed for paints. In most studies, instillation was performed only once. For all studies, the NOAEC in rat/mice (in mg/rodent or mg/kg bw/day was converted to an inhalation corrected NOAEC (mg/m^3) for 8 hours workday.

In the single study in humans, bronchoscopy and intrapulmonary instillation were used to assess the response of the lung to an instilled burden of respirable-micron-sized iron oxide particles, 5 mg Fe_2O_3 particles (median diameter 2600 nm, geometric st. dev. 1300 nm) were instilled in a subsegment of lingula lobe of the lung in 34 volunteers (Lay et al.1999). The same subsegment was lavaged on day 1, 2, 4, 28 and 91. In the subsample of 10 persons the number of neutrophils were increased on day 1 but not in a subsample on day 2 or thereafter. This part of the lung was calculated to represent 1.6 m^2 of the total of 112 m^2 of the human lung. With an assumed respiratory volume of 10 m^3/8 hours (ECHA 2012) and a 50% deposition rate LOAEC will be 35 mg/m^3.

In the same study, it was reported of male Fischer rats that were instilled with three doses of the same Fe_2O_3 (Lay et al. 1999). Significant PMN numbers were increased at the highest dose (4.83 mg/rat), but not at the lower tested doses (0.16 and 1.61 mg/rat). The calculated human equivalent NOAEC was 3.3 mg/m^{3}and the LOAEC 34 mg/m^3.

Following to the outcome of Lay et al. (1999), NOAEC and a LOAEC after bolus exposure could be extracted in subsequent five studies. Beck-Speier et al. (2009) instilled 500 or 1500 nm Fe_2O_3 particles in male Wistar-Kyoto rats. The number of neutrophils in BALF in rats increased one day after instillation of 4 mg/kg 1 500 nm but not 500 nm Fe_2O_3

Table 2. Instillation and aspiration studies with key parameters identified and their references.

Reference	Species	Agent, size	Dosing regimen (exposure method. frequency, doses)	Corrected NOAEC (8 hr, mg/m3)	Corrected LOAEC (8 hr, mg/m3)
Antonini et al. (1996)	Male CD/VAF rats	0.9 µm γ-Fe_2O_3	Instillation 1 mg/rat PMN increased 1 and 7 d days after instillation of		5.2
Wesselius et al. (1996)	Sprague-Dawley rat	0.1 µm Fe2O3	Instillation 12.5 (1) mg/rat		260
Lay et al. (1999)	Fischer 344 Rats	Fe_2O_3, 2.6 µm	Instillation (1) 0.16, 1.61 and 4.83 mg/rat	3.3	34
Lay et al. (1999)	Humans	Fe_2O_3, 2.6 µm	Intrapulmonary Instillation of 5 mg/person into a subsegment of the lingula lobe		35
Zhu et al. (2008)	Sprague Dawley rats	Fe_2O_3, 22 or 280 nm	instillation (1) 0.8 and 20 mg/kg		4.2
Beck-Speier et al. (2009)	Wistar Kyoto	Fe_2O_3, 0.5 µm	Instillation (1) 1.3 and 4 mg/kg	21	
		Fe_2O_3, 1.5 µm	Instillation (1) 1.3 and 4 mg/kg	6.8	21
Demokritou et al. (2010)	Male Sprague-Dawley rat	Fe_2O_3, 1.6 µm (aggregates of 2 nm)	Instillation (1) 1 mg/kg		5.2
Katsnelson et al. (2012)	outbred rats, f.	Fe_3O_4, 10,50,1000nm	Instillation (1) 2 mg/rat		42
Sutunkova et al. (2016)	outbred rats	Fe_2O_3, 14 nm	Instillation (1) 0.3 mg/rat		6.2
Ban et al. (2012)	Balb/c mice, f.	Fe_2O_3, 35, 147 nm	Instillation (1, 4) 0.25, 0.375 and 0.5 mg/mouse		21
Gustafsson et al. (2015)	Balb/c mice, f.	α-Fe_2O_3, 30 nm	Instillation (1) 1.25 and 2.5 and 5 mg/kg	2.60	5.2
Billing et al. (2020)	C57BL/6 mice, f.	Fe_3O_4, or $CoFe_2O_4$ with an iron to cobalt ratio 5:1, 3:1, 1:3, Co_3O_4, of <40 nm in diameter	Instillation, 54 or 162 Fe_3O_4 µg/mouse 1d: 3d:		4.5
		Fe_3O_4 (3 d)		4.5	14
Hadrup et al. (2020)	C57BL/6 mice, f.	α-Fe_2O_3 particle, 20–60 nm	Instillation (1) 0.7, 2.1, 6.4 mg/kg	3.6	11
		α-Fe_2O_3 rods, 40–150*250–600 nm	Instillation (1) 0.7, 2.1, 6.4 mg/kg	3.6	11
Falcone et al. (2018)	A/J mice	Fe_2O_3 600 ± 200 nm	Oroparhyngeal aspiration (5) of 1 or 2 mg/kg mouse on day 0, 7, 28 and 94 d. PMN increase in lavage on d 1, 7 but not 28 and 94 d		83
Cho et al. (2009)	BALB/c, m.	36 nm Cy5.5-SPIONs	Intratracheal instillation. PMN increase after 1 d with 1.8 and 5.4 but not with 0.6 mg/kg	1.2	3.8
Présumé et al. (2016)	C57Bl/6 m.	20–25 nm Fe_2O_3 or Fe_3O	Oropharyngeal aspiration of 5 or 50 µg/mouse, PMN one day after a single, or two or four weekly aspirations	4.2	13.5

(Beck-Speier et al. 2009). The NOAEC for 500 nm Fe_2O_3 was then 21 mg/m^3 and 6.6 mg/m^3 for 1500 nm mg/m^3 Fe_2O_3. The LOAEC for the 1500 nm Fe_2O_3 was 21 mg/m^3.

In a study with female BALB/C mice 1.25, 2.5 and 5 mg/kg 30 nm α-Fe_2O_3, after one day neutrophilia was detected with 2.5 and 5 mg/kg but not with 1.25 mg/kg (Gustafsson et al. 2015). These results gave an adjusted NOAEC of 2.6 mg/m^3 and LOAEC of 5.2 mg/m^3.

Fe_3O_4 (magnetite)was tested together with cobalt ferrite materials in ratios 5:1, 3:1, 1:3, 0:1 Co_3O_4/Fe_3O_4 w/w (Billing et al. 2020). In cobalt ferrite Co and and Fe are replaceable in the same crystal

structure which also is paramagnetic. For all treatment groups, there was a strong neutrophil influx, except for low dose pure magnetite nanoparticles which reached base level 3 days after exposure. A LOAEC for the magnetite of 4.5 mg/m^3 was derived for day 1.

When 14, 43 and 128 µg Fe_2O_3 rods and spherical particles were instilled in female C57/Bl6 mice neutrophilia was observed only one day after 128 µg Fe_2O_3 rods and only after 28 days with the 128 µg spheres (Hadrup et al. 2020). The resulting NOAEC for spherical Fe_2O_3 was then 3.6 mg/m^{3}and and the NOAEC 11 mg/m^3.

Negatively charged polymer coated, 36 nm Cy5.5-conjugated thermally cross-linked superparamagnetic iron oxide nanoparticles were instilled intratracheally into the lungs of male BALB/c mice at 0.6, 1.8 and 5.4 mg/kg. PMN numbers were increased 1 day after instillation with 1.8 and 5.4 mg/kg but not with 0.6 mg/kg (Cho et al. 2009). A NOAEC of 2 mg/m^3 and LOAEC 6 mg/m^3 were derived.

In one study only NOAEC was reported, where 5 and 50 µg 20–25 nm Fe_2O_3 or Fe_3O_4 were administered by oropharyngeal aspiration in male C57Bl/6 mice. One day after 1, 2 or 4 repeated exposures there was no change in the number of neutrophil numbers in BALF (Présumé et al. 2016). A NOAEC of 4.2 mg/m^3 was derived.

In eight rodent studies, only a LOAEC was reported (Antonini et al. 1996; Ban et al. 2012; Demokritou et al. 2010; Falcone et al. 2018; Katsnelson et al. 2012; Sutunkova et al. 2016; Wesselius et al. 1996; Zhu et al. 2008).

Antonini et al. (1996) instilled 1 mg 20–25 nm γ-Fe2O3 iron oxide (4 mg/kg rat) in male CD/VAF rats and PMN numbers were increased 1 and 7 days after instillation. A LOAEC of 5.2 mg/m^3 was derived.

Zhu et al. (2008) instilled 0.8 mg/kg bw and 20 mg/kg 22 or 280 nm Fe_2O_3 in rats. Neutrophilia in BALF was detected after all doses and for both sizes after 1, 7 and 30 days except 0.8 mg/kg after 30 days. A LOAEC of 4.2 mg/m^3 was derived.

Wesselius et al. (1996) instilled a very high dose of 50 mg/kg of 100 nm Fe_2O_3 50 mg/kg of 1in female Sprague Dawley rats. Instillation of iron oxide increased neutrophil numbers only on day 1. By a dose of 50 mg/kg, and 250 g/rat the LOAEC was then 260 mg/m^3.

Katsnelson et al. (2012) instilled 2 mg of 10, 50 or 1000 nm Fe_3O_4 in female rats (8 mg/kg) and analyzed the BALF cell composition on the next day. Neutrophilia was detected for all sizes (50 nm > 10 nm > 1000 nm). The calculated LOAEC was then 42 mg/m^3.

Sutunkova et al. (2016) instilled rats with 0.3 mg 14 nm Fe_2O_3 per rat and detected influx of PMN after 24 hours. The calculated LOAEC was then 6.2 mg/m^3.

Ban et al. (2012) instilled 250, 375 and 500 µg of 35 nm or 147 nm Fe_2O_3 per female mouse for 4 times. After two days (of the last dose) the fraction of PMN in BALF was increased at all doses. The PMN fractions were greater for the 35 nm than for the 147 nm particles. The calculated LOAEC was then 21 mg/m^3.

Falcone et al. (2018) gave 1 and 2 mg 600 nm Fe_2O_3 to A/J mice by oropharyngeal aspiration. With repeated aspiration exposure on day 1, 7 and 28 mice were lavaged after 1, 7, 28 and 94 days. PMN numbers were not increased on day 28 and 94. The calculated human LOAEC (a day after first aspiration one day and a day after a second aspiration on day seven) was 83 mg/m^3.

Demokritou et al. (2010) instilled 1 mg γ-Fe_2O_3/kg rat. The number of neutrophiles were increased in lavages one day after instillation. A LOAEC of 5.2 mg/m^3 was derived.

In summary, instillation of a wide variety Fe_2O_3 and one Fe_3O_4 (Katsnelson et al. 2012) particles were instilled in mice and rats. LOAECs ranged from 4.2 to 260 mg/m^3 with several LOAECs ranging from 5 to 10 mg/m^3. NOAEC ranged from 2.6 to 21 mg/m^3. The NOAEC of 21 mg/m^3 for 0.5 µm Fe_2O_3 was obtained in the same study with 1.5 µm Fe_2O_3 for which a NOAEC of 6.8 mg/m^3 was calculated.

Interestingly, data on neutrophil infiltration in human subjects was calculated to give a LOAEC of 35 mg/m^3 (Lay et al. 1999).

6.1.2. Inhalation vs. instillation

In two inhalation studies performed according in principle by OECD guidelines both NOAEC and LOAEC could be derived (NTP 2020; Pauluhn 2012). The NOAEC values after four weeks of daily inhalation were 4 or 22.5 mg/m^3. The corresponding derived lowest LOAEC values from the same two studies were 11 and 12 mg/m^3. From the other inhalation studies LOAEC values ranged from 0.5 to over 300 mg/m^3.

Most data from instillation experiments were more consistent than inhalation data and a weight of NOAEC values between of 2.5–3 and the lowest LOAEC were 4–5 mg/m^3.

6.1.3. Chemical identity and speciation

We chose to include a wide variety of iron species and crystalline phases. In many of the publications

it was not possible to deduce the crystalline phase. Most studies were on trivalent (Fe(III) iron oxides but we included also data on mixed oxidation state Fe_3O_4 and even with particles reported to consist of a core of metallic iron and a mixture of oxides in the shell of the particles in oxidized iron particles a gradient of magnetite and γ-Fe_2O_3 (maghemite) toward the surface (Pettibone et al. 2008). In the other inhalation study aerosols of iron oxide NPs generated online by a spark generator with pure iron (Sutunkova et al. 2016). Rats were exposed by nose-only inhalation 4 h a day, 5 days a week. In one study, Cho et al. (2009), instilled polymer coated, Cy5.5-conjugated thermally cross-linked superparamagnetic iron oxide nanoparticles with an iron oxide content of 20%, We chose also to include a comprehensive study of specular hematite although it was only 95% pure (NTP 2020). Data did not reveal any clear indications that one oxidation state or one crystalline phase or even a complex chemical composition was more potent than the other, but perhaps too little data were available and more research is needed for this conclusion,

6.1.4. Size and shape

The size of the particles reported in the 25 studies varied greatly and the techniques of determining primary particle size and agglomeration and aggregation were also different. A few studies were on parallel examinations of particles of different sizes. Zhu instilled 22 nm and 280 nm Fe_2O_3 in Sprague Dawley rats. Neutrophilia was similar for the two differently sized Fe_2O_3 particles after 1, 7 and 30 days after an intratracheal instillation (Zhu et al. 2008). Hadrup et al. examined two sizes of Fe_2O_3, 80 nm rods and 72 nm spherical ones. Hadrup and coworkers found larger numbers of neutrophils for rods on day one than for the spherical particles but this was reversed after day 28 (Hadrup et al. 2020). In the study of Ban et al., 35 nm nano-Fe_2O_3 were more inflammatory than sub-micron (147 nm) at 250, 375 or 500 µg/mouse (Ban et al. 2012). Neutrophils represented 5–15% of BAL cells in mice treated with submicron iron particles and 15–40% in mice treated with nano-iron particles (Ban et al. 2012). Katsnelson et al. instilled three sizes of Fe_3O_4 (10, 50 and 1000 nm) in rats. After one day neutrophilia was greater for 50 nm, than for the 10 nm which was greater than for the 1000 nm Fe_3O_4 (Katsnelson et al. 2012).

Much of the instrumentation for determination of nanoparticle size and agglomeration was developed and came into wide-spread use first during the last 10–15 years. Therefore, there might inaccuracies in particle size and agglomeration state.

Perhaps like with ZnO particles, inflammation seemed unrelated to to particle size. The toxicity of ZnO seems to be driven by dissolution to $Zn2^+$ (Cho et al. 2011; Donaldson et al. 2013; Yeh et al. 2012) and ZnO is quite soluble in tissues and especially in lysosomal fluid. ZnO-induced neutrophil influx and blood levels of C-reactive protein for micro- and nanozised ZnO particles were not associated with particle size and hence deposited particle surface area, but were more strongly related to the deposited mass in human volunteers (Monsé et al. 2018, 2021).

In contrast to zinc oxides, iron oxides are slowly dissolved in mammalian tissues (Jacobsen et al. 2015; Sohal et al. 2018; Sutunkova et al. 2016) but the dissolution rates probably vary between different iron species and crystalline phases.

We conclude that the available data indicate that the size of iron oxide nanoparticles is not related to pulmonary neutrophilia. This is also true for a few solulble particles like ZnO nanoparticles. This is in contrast to insoluble TiO_2 and carbon black for which the literature indicates a strong relationship between size (and surface area) (Cosnier et al. 2021; Tran et al. 2000).

For some particulate materials, long fibers (more than 5–15 µm long) are significantly more toxic than spherical ones. In one study there was an indication that Fe_2O_3 rods were more inflammogenic than spheres (Hadrup et al. 2020). However, there are too few comparative studies to determine whether elongated iron oxide particles are more inflammogenic than short or spherical ones.

6.1.5. Exposure and resolution times

In many studies neutrophil infiltration was transient and decreased rapidly on day one after instillation or inhalation exposure (e.g. Lay et al. 1999). However, in some instances, PMN numbers were reported to be increased after exposure at times well beyond the 1–3 days we set as inclusion criterium, for example, it should be noted that at 90 days after a single instillation, a NOAEC of 2.08 mg/m^3 and LOAEC of 4.17 mg/m^3 could be calculated with a single instillation of 10 nm Fe_2O_3 particles in ICR mice (Park et al. 2015). At the highest dose tested (2 mg/kg) the particles were still present in the lung 90 days post-exposure and infiltration of neutrophils in the lung, as well increased LDH, was detected. Also, on day one after inhalation of specular hematite for four weeks a NOAEC of 22.5 mg/m^3 was detected,

whereas after 16, 26 and 39 weeks of the same exposure the LOAEC was of 11.2 mg/m^3.

Eight studies reported only a LOAEC, i.e. some of these studies investigated only one single dose. Only for two of the inhalation studies, the LOAEC (1.78 mg/m^3) was lower than the bench mark dose (BMD) span. In one of the studies, metallic iron core particles were nebulized. The particles were oxidized at the surface with a gradient of Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite). In the other inhalation study, aerosols of iron oxide NPs were generated online by a spark generator with pure iron (Sutunkova et al. 2016). Rats were exposed by nose-only inhalation 4 h a day, 5 days a week. The LOAEC was calculated based on the effect twenty-four days after three months of inhalation to 0.75 mg/m^3. It was stated that effects were 'quite similar' after six and 12 months of exposure. Considering the Sutunkova study a NOAEC of 0.75 mg/m^3 should be considered as the POD.

Finally, to evaluate the risks, an exposure estimate should be either experimentally measured or retrieved from the literature, and further compared to the selected POD. This was indeed performed by Cazzagon et al. (2022), where the authors assessed exposure scenarios for each life cycle stage using data from literature, inputs from industries and results of a questionnaire distributed to healthcare professionals. Then, exposure concentrations were evaluated either from predictive exposure models or monitoring campaigns designed specifically for the study. The probabilistic risk assessment revealed negligible risks for workers along the life cycle of superparamagnetic iron oxide (magnetite) nanoparticles used as contrast agent for the diagnosis of tumor cells in all exposure scenarios, except for one scenario when risk was considered acceptable only after the adoption of specific risk management measures (Cazzagon et al. 2022).

The POD can be anywhere between zero and the detectable effect size. However, in practice, this point is typically overlooked, and the NOAEC is simply considered as a dose where the effect has been shown to be zero. This is a major disadvantage of the approach, since in some cases the detectable effect size is not negligible, and biologically significant effects cannot be excluded. The detectable effect size of a study depends on the number of used test animals, which also influences the value of the NOAEC. In practice the NOAEC tends to be higher when fewer animals are used, which is controversial, since less data points would add uncertainty, which should normally be reflected by a conservative approach (Hoffman and Hammonds 1994). Furthermore, the NOAEC can only be one of the applied doses in a study, which implies that the NOAEC strongly depends on the choice of dose concentrations and number of animals per dose. Therefore, by changing the study design the value of the NOAEC is likely to change as well. The uncertainty in each NOAEC value may be large, but it cannot be assessed, which is another disadvantage of the approach (Hoffman and Hammonds 1994).

Given the disadvantages of the NOAEC approach, an alternative Benchmark Dose (BMD) method for deriving a POD has been proposed by Crump (1984). The BMD is defined as a dose level that is associated with a pre-defined change in response (i.e. benchmark response) compared with the control (Crump 1984). The BMD is estimated from toxicity data by fitting a dose-response model or a mathematical function to the observations. To take the uncertainties arising from experimental errors into account, the lower confidence limit of the BMD (i.e. the BMDL) is normally used as the POD. However, also the BMD method is strongly dependent on study design and number of data points. Both BMD and LOAEC/NOAEC determination is hampered by too few available data in the low dose area.

7. Conclusion

This review provides evidence of concordance in POD values even though data come from a large panel of iron chemical test protocols and test systems. It was our hypothesis that neutrophil influx correlated with the dose deposited in the alveolar-region of the lung after inhalation and bolus exposure were similar. In regulatory toxicology, bolus exposure by intratracheal instillation and aspiration are usually not given the same attention as inhalation by OECD guideline inhalation studies. We suggest that PMN infiltration caused by materials deposited in the lung and remain over time may cause the similar effects whether they are administrated by inhalation over time or if administered by bolus exposure. This is important because delivered doses are much better controlled by bolus administration and the costs and work efforts are less. We wanted to review all lung deposition data and bolus exposures are more often reported. Inhalation studies are many times more time consuming and expensive. Following the OECD guidelines may however mean that important mechanistic aspects are missed. We do recognize that there also important

aspects that have to be considered when translating bolus to inhalation exposure. One such is that the deposited solid material should be relatively stable in the lung and the critical effects should be related to deposition and effects in the deep lung. Still PODs we derived from bolus exposure are similar to those derived from inhalation studies even if duration of exposure and experimental design vary considerably. Of seventeen studies, both NOAEC and LOEL could be retrieved for six studies. For these six studies the range between the NOAECs and the LOAECs overlapped the BMD range between 3.9 and 7.3 mg/m^3. Of the seventeen studies, three studies reported only a NOAEC and all these were lower than the BMD range. Eight studies reported only a LOAEC, i.e. some of these investigated only a single dose. Only for two of the inhalation studies, the LOAEC (1.78 mg/m^3) was lower than the BMD span. Twenty-four hours after three months of inhalation the LOAEC was calculated to 0.75 mg/m^3. It was stated that effects were 'quite similar' after six and 12 months of exposure. Considering the Sutunkova study and all other studies a NOAEC of 0.75 mg/m^3 should be considered as the POD.

In summary, from the evaluation of the literature that the point of departure for the effect of infiltration of PMN into the lung appears to range from a singular study from 0.75 mg/m^3 (Sutunkova et al. 2016) and several other studies a weight of the span of POD may be in the range of 3–5 mg/m^3. It is also important that infiltration of PMN was detected even after a single instillation of Fe_2O_3 in human lung at the calculated LOAEC of 35 mg/m^3. This suggests that risk estimates and safe levels of exposure in the workplace might need to be revised. There is a need for more research in mice and rats with different time points and other pathological effects.

Superparamagnetic iron oxide nanoparticles (SPIONs) become superparamagnetic when exposed to an external magnetic field. This is utilized in medical and clinical applications, including diagnostic imaging, drug delivery and hyperthermia therapy. However, as the use and manufacture of IONPs expand there is a concern of the potential adverse health effects induced by these materials. Occupational exposure is of particular concern, as workers may be exposed for longer time periods and at higher exposure concentrations of IONPs compared to end-users. This review was focused on inflammation determined by neutrophil infiltration determined by cellular composition in bronchiolar lavage fluid and was motivated within the EU 2020 project Safe-N-MedTech by the increasing adoption of iron oxides nanoparticles in medical applications. Theoretically, SPIONs may be released into air during production and use workers may be exposed. Under the conditions described above exposures were estimated to be very low, less or much less than 100 µg/m^3 because it was handled in very small amounts in aqueous suspensions and we could not identify any process were it was likely to be aerosolized. We concluded that there was no risk was for workers engaged in production or use of the product (to be published, final report, EU Horizon 2020 project: Safety Testing in the Life-cycle of nanotechnology Nanotechnology-enabled Medical Technologies for Health.)

We therefore searched the literature on the inflammatory potential of different iron oxide species or determine at what air concentrations there is risk of health effects in exposed workers.

Although there is little evidence from epidemiological studies that iron oxide-inhalation poses any health risk to humans at current OELs this might be due to that these studies focussed on other agents (Kornberg et al. 2017; IARC 2018; Stokinger 1984). This raises the need for more research.

We were also interested in whether there were indications that the inflammatory effect was different after bolus deposition in the lung compared to inhalation exposure. There was no indication that IONPs pulmonary toxicity was different between in vivo inhalation and instillation studies, although the data may be too few for this conclusion and other endpoints should be studied as well. As proposed by Boots et al. (2021) it seems like the dose of poorly solubility particles with low toxicity produces the same short term inflammatory effect after bolus delivery as after inhalation. However, in contrast to some other more well studied materials, inflammation induced by iron oxides there seem to be not correlated to surface area. In one study inflammation was greater for larger particles (Beck-Speier et al. 2009) or in other the effect particle size was inconsistent (Ban et al. 2012; Hadrup et al. 2020; Katsnelson et al. 2012; Zhu et al. 2008). It might be speculated that mice would be more sensitive to iron oxides than rats. However, our review did not provide evidence for that.

From the evaluation of the literature, we conclude that the POD for the effect of infiltration of PMN into the lung appears to several studies range between 3–5 mg/m^3. A singular inhalation study indicates a POD of 0.75 mg/m^3 (Sutunkova et al. 2016). In a singular study with instillation of Fe_2O_3

in human lung at the calculated LOAEC of 35 mg/m^3 (No NOAEC was determined.) We evaluated only a single sensitive and robust endpoint, neutrophilia, as determinant of lung inflammation. We do recognize that the studies reviewed here vary greatly in design and quality. If data on other more relevant endpoints such as chronic disease are available, these should be given greater weight. Iron oxide exposures are common and many people world-wide are exposed. More data are needed. Especially epidemiological studies of workers with mixed exposures and with careful source apportionment and more rodent testing is needed. We think that a careful revisiting of risk estimates and safe levels of exposure to iron species in the workplace is motivated.

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author, H.W.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Norwegian, National Institute of Occupational Health or the Danish, National Center for the Working Environment.

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