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Review

A Scoping Review of Magnetic Iron Oxide Toxicity Across Animal Models: Mechanistic Insights, Particle Size Effects, and Implications for Air Pollution Biomonitoring

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Abstract

Iron oxide particles (magnetite Fe_3O_4, hematite α-Fe_2O_3, and maghemite γ-Fe_2O_3) are prevalent constituents of atmospheric particulate matter (PM) and have gained increasing attention due to potential health implications. This scoping review provides a broad mapping of published in vivo and in vitro studies addressing the biological and toxicological effects of iron oxide particles across particle size fractions (PM10, PM2.5, PM1.0, and nanoscale) and exposure routes, including inhalation, intranasal instillation, and intravenous administration. As a scoping review, no formal risk-of-bias appraisal was conducted; however, studies were selected through predefined eligibility criteria and a structured screening workflow. Iron oxide exposure is consistently associated with oxidative stress and inflammatory responses, while mitochondrial dysfunction, genotoxicity, and neurological effects are frequently reported depending on particle characteristics and exposure context. Among studies with explicit crystalline phase identification, magnetite is most frequently associated with higher biological reactivity, whereas hematite and maghemite display more variable and context-dependent responses. Limited human evidence aligns with experimental findings, identifying magnetite-rich nanoparticles in neural and cardiovascular tissues alongside markers of oxidative and mitochondrial damage. Overall, this scoping review highlights dominant research trends, mechanistic pathways, and key knowledge gaps regarding iron oxide-containing PM, emphasizing the need for integrative approaches linking atmospheric particle characterization with toxicological research.

Keywords: iron oxide nanoparticles; magnetite; particulate matter (PM1.0, PM2.5, PM10); oxidative stress; mitochondrial dysfunction; air pollution biomonitoring; scoping review

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1. Introduction

Iron oxides are among the most extensively investigated transition metal oxides due to their wide range of biomedical, environmental, and technological applications. However, the rapid expansion of their use has raised increasing concern regarding their potential environmental and human health implications. Iron oxides are naturally occurring minerals composed of iron and oxygen, widely distributed across the Earth's surface and present in multiple crystalline structures. They exhibit diverse physicochemical properties, including optical and thermal stability, superparamagnetic behavior, high chemical reactivity, biodegradability, biocompatibility, and environmental abundance. These properties are strongly influenced by particle size, morphology, and specific surface area, which collectively govern their biological and environmental behavior [1,2].

The most common crystalline phases of iron oxides include hematite (α-Fe_2O_3), the thermodynamically most stable and most abundant form in the biosphere, typically exhibiting a reddish coloration at the nanoscale, and maghemite (γ-Fe_2O_3), a metastable iron oxide characterized by ferrimagnetic properties and a brown appearance [2,3]. Magnetite (Fe_3O_4), commonly referred to as black iron oxide, displays the strongest magnetic response among transition metal oxides due to its mixed-valence structure [4]. The physicochemical characteristics of iron oxides (IOs) can vary substantially depending on particle size, shape, origin, and surface functionalization. In this review, particular attention is given to particulate matter fractions PM1.0, PM2.5, and PM10, as distinct biological responses have been reported even within the same size category [5,6]. Such variability has been associated with adverse effects on human health, impacts on other organisms, and the incorporation of iron oxide particles into food webs through multiple exposure pathways [7,8].

Atmospheric particulate matter is commonly classified into coarse particles (PM10), with aerodynamic diameters between 2.5 and 10 μm, including dust, pollen, and mold; fine particles (PM2.5), with diameters smaller than 2.5 μm, typically associated with combustion processes and organic compounds; and ultrafine particles or nanoparticles (PM1.0), ranging approximately from 1 to 100 nm, predominantly generated by coal combustion, metallic emissions, and high-temperature industrial processes [9]. Compared with PM2.5 and PM10, PM1.0 particles exhibit a markedly higher surface-area-to-volume ratio, increased surface free energy, enhanced surface charge density, and elevated chemical reactivity, all of which amplify their biological interactions and toxic potential [10,11].

Iron oxide nanoparticles originate from both natural and anthropogenic sources and are continuously introduced into the atmosphere, aquatic systems, and terrestrial ecosystems. Once deposited, they may enter food chains through ingestion or absorption by a wide range of organisms [12]. In urban environments, their abundance has increased substantially due to anthropogenic activities such as fossil fuel combustion, brake and tire wear, steel manufacturing, coal burning, dust storms, wildfires, volcanic activity, vehicular traffic, and other globally distributed industrial processes [13,14]. This widespread environmental presence has stimulated a growing number of experimental studies examining the biological effects of iron oxide particles under diverse conditions, including variations in particle size, surface properties, exposure routes, and biological models [15,16]. Additional investigations have addressed differences in dose, exposure duration, and particle transformation processes, particularly in environmental and biomedical contexts [17,18]. Other studies have emphasized how experimental design and application frameworks influence toxicological interpretation and cross-study comparability [19,20]. Collectively, this body of evidence underscores the complexity of assessing iron oxide particle toxicity across heterogeneous scenarios [21].

Several studies suggest that iron oxide particles within the PM1.0 fraction may pose a higher biological risk due to their small size, enhanced cellular penetration capac-

ity, and elevated reactivity. In addition, the crystalline phase—magnetite, hematite, or maghemite—has been reported to influence the magnitude and nature of observed biological responses. Nevertheless, the reported outcomes remain highly heterogeneous, reflecting differences in experimental design, exposure pathway, dose, biological model, and physicochemical particle characteristics.

In this context, the present scoping review aims to map and synthesize the breadth of available experimental evidence on the biological effects associated with exposure to iron oxide particles across animal and cellular models. As a scoping review, this study did not include formal risk-of-bias assessment; however, studies were selected through a structured screening process based on predefined eligibility criteria. The review organizes existing evidence according to particle size, crystalline phase, exposure route, biological model, and exposure context, with the objective of identifying dominant research trends, recurring mechanistic pathways, and key knowledge gaps relevant to air pollution biomonitoring and environmental health research.

2. Materials and Methods

This scoping review examined experimental studies reporting biological effects associated with exposure to iron oxide particles, including magnetite (Fe_3O_4), hematite (α-Fe_2O_3), and maghemite (γ-Fe_2O_3). The review encompassed studies conducted in animal-related biological models, including in vivo, in vitro, and ex vivo systems, and considered a wide range of exposure routes, such as inhalation, intratracheal instillation, oral, dermal, intravenous, intraperitoneal, as well as aquatic and environmentally relevant exposure scenarios.

For organizational and comparative purposes, iron oxide particles were grouped according to particulate matter size fractions (PM1.0, PM2.5, and PM10) when these were explicitly reported or could be numerically derived from the quantitative size measurements provided in each study. For size-to-PM classification, particle fractions were categorized as PM10 (2.5–10 μm), PM2.5 (<2.5 μm), PM1.0 (<1 μm), or nanoscale (<100 nm), based on the quantitative size metric explicitly reported in each study. Nanoparticles reported within the approximate 1–100 nm range were classified within the nanoscale fraction for comparative purposes. When multiple size metrics were available, priority was given to aerodynamic diameter when reported; otherwise, primary particle size measured by electron microscopy was used. Hydrodynamic diameter measured by dynamic light scattering (DLS) was considered only when no primary particle size was available and was interpreted with caution because of potential agglomeration effects.

The review focused on original experimental research articles published in English. No lower temporal restriction was applied in order to capture the full scope of the available evidence. The literature search covered publications from database inception to 15 January 2026. The final search was conducted on 15 January 2026. The earliest eligible study identified and included in the analysis was published in 1925, and the most recent eligible study was published in 2026, defining a temporal range of 1925–2026. Earlier publications were retained when relevant for historical context, foundational methodological contributions, or long-term occupational and chronic exposure frameworks.

The literature search was conducted across multiple bibliographic databases, including PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect, SpringerLink, and Wiley Online Library. Google Scholar was used as a complementary source to identify additional relevant literature not indexed in the major databases. Search terms were combined using keywords related to iron oxide particles and biological effects, including “iron oxide nanoparticles”, “magnetite”, “hematite”, “maghemite”, “toxicity”, “damage”, and “biological model”.

A structured screening process was applied to enhance methodological transparency. After removal of duplicate records, studies were screened by title and abstract, followed by full-text eligibility assessment according to predefined inclusion criteria. Studies focusing exclusively on non-oxide iron materials (e.g., elemental iron or soluble iron salts), non-biological systems, or purely therapeutic and clinical applications (such as magnetic hyperthermia, drug delivery, or imaging) were excluded, as they fell outside the environmental and toxicological scope of this review. Consistent with the scoping review design, no formal quantitative risk-of-bias scoring or quality-grading system was implemented.

The initial database search retrieved 223 records. After removal of 70 duplicate entries, 153 records were screened based on title and abstract. Following title and abstract screening, 67 records were excluded according to the predefined eligibility criteria. After full-text evaluation, 86 studies met the inclusion criteria and were incorporated into the qualitative synthesis. The study selection process followed PRISMA-ScR guidance for scoping reviews, and the corresponding checklist and supplementary information are provided in the Supplementary Materials.

From each included study, relevant descriptive variables were extracted, including iron oxide phase, particle size or size range, concentration or dose, exposure route, exposure duration, biological model, and reported biological effects. Among the 86 included studies, those that explicitly reported the crystalline phase of the iron oxide particles were considered for phase-specific comparisons. Studies lacking explicit phase identification were included in the overall qualitative synthesis but were not incorporated into phase-ranking interpretations. This approach ensured that comparative statements regarding magnetite, hematite, or maghemite were based solely on phase-resolved evidence. The extracted information was organized descriptively according to particle type, size fraction, and biological model, and summarized in comparative tables to facilitate visualization of research trends, commonly reported outcomes, and knowledge gaps. Data organization and management were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). This scoping review followed a qualitative and descriptive synthesis approach rather than a quantitative systematic methodology. Accordingly, no meta-analysis or statistical aggregation was conducted. Findings were synthesized narratively to reflect the diversity of experimental designs, exposure conditions, and reported outcomes across studies. Emphasis was placed on identifying recurring biological responses, mechanistic patterns, and areas of consistency or divergence within the literature. Methodological heterogeneity and the frequent use of laboratory-engineered particles were recognized as important factors influencing comparability across studies, highlighting the need for greater standardization and more environmentally representative experimental designs in future research.

A structured search strategy was developed for each database, with syntax adjustments applied as required. The complete database-specific search strings are provided in Supplementary File S2 to ensure reproducibility.

3. Results

3.1. Iron Oxides and Biological Damage Across Animal Models

Historically, numerous studies have investigated the biological effects of iron oxide particles without explicitly specifying the crystalline phase involved. Although the specific iron oxide type can often be inferred from the chemical formulation or source material, such assumptions were intentionally avoided in the present review. Instead, results are presented and discussed by differentiating exposure route, particle size, and biological model, irrespective of incomplete phase identification. A summary of the analyzed data is provided in Tables 1 and 2.

3.1.1. Rats

Rats represent one of the most widely used animal models in experimental sciences. In this context, toxic damage induced by 10 nm iron oxide nanoparticles administered intravenously at a dose of 50 mg/kg body weight has been evaluated in Sprague–Dawley rats. Magnetic resonance imaging (MRI) was performed one hour post-administration, and animals were sacrificed 72 h after exposure. The results demonstrated that 10 nm nanoparticles penetrated brain tissue, which was associated with a significant reduction in T_2 relaxation values. Particle retention within brain tissue was observed, indicating translocation from cerebral vasculature to the brain ventricles. These findings were accompanied by a significant reduction in striatal dopamine levels and its metabolites, as well as neuropathological alterations involving neuronal cell bodies, dopaminergic terminals, and cerebral vasculature [22].

In a complementary experimental study, transgenic TgF344-AD rats and wild-type (WT) rats of both sexes were exposed from approximately postnatal day 28 to traffic-related air pollution (TRAP) or filtered air (FA) for extended periods, with evaluations conducted at 3, 6, 10, and 15 months of age. TRAP exposure was carried out continuously in real time using air drawn directly from a traffic tunnel with mixed light- and heavy-duty vehicle flow, whereas control animals were exposed to purified ambient air. The deposition of refractory particles in the brain was assessed using hyperspectral imaging, together with behavioral, biochemical, and histopathological analyses. Animals exposed to TRAP exhibited higher brain particle burdens, accompanied by behavioral alterations and neuropathological features consistent with Alzheimer's disease phenotypes, including β-amyloid accumulation, increased phosphorylated tau (PHF1), and glial activation. These effects were shown to be dependent on age, sex, and genotype [23].

Beyond central nervous system involvement, recent experimental evidence has demonstrated that iron oxide nanoparticles may also induce significant systemic toxicity. Subchronic oral exposure to Fe_2O_3 nanoparticles (20–40 nm) in male Sprague–Dawley rats produced dose- and time-dependent endocrine disruption, characterized by significant reductions in circulating T_3 and T_4 levels together with elevated TSH concentrations, indicating hypothyroid alterations. Concomitantly, marked renal dysfunction was observed, evidenced by significant increases in serum creatinine and urea levels, suggesting impaired glomerular filtration and compromised renal function [24].

Similarly, controlled intraperitoneal administration of magnetic iron oxide nanoparticles for 28 consecutive days induced dose-dependent hepatotoxicity and systemic immunomodulation. Significant elevations in ALT, AST, and ALP activities, together with increased total leukocyte counts and alterations in lipid profiles, were reported. Histopathological examination revealed progressive hepatic damage ranging from vascular congestion and sinusoidal dilation at lower doses to hepatocellular necrosis, fibrosis, portal inflammation, and severe lobular architectural disruption at higher doses. These findings underscore the capacity of repeated iron oxide nanoparticle exposure to elicit multi-organ toxicity extending beyond neural tissues [25].

3.1.2. Cellular Models

Multiple in vitro studies have investigated the neurobiological effects of iron and iron oxide nanoparticles (Fe-NPs/IONs) using neuronal and endothelial cellular models. In human SH-SY5Y neuronal cells, 24 h exposure to spherical Fe-NPs of 10 and 30 nm at concentrations ranging from 2.5 to 10 μg/mL was associated with marked dopaminergic dysfunction, evidenced by significant reductions in intracellular dopamine content (−68% for 10 nm and −52% for 30 nm at 10 μg/mL). Increased α-synuclein expression and activation of oxidative stress-related pathways, including c-Abl/phospho-c-Abl signaling,

were reported. Dose-dependent increases in reactive oxygen species, mitochondrial dysfunction, inhibition of cell proliferation, and activation of pro-apoptotic pathways were also observed. In parallel, an in vitro blood–brain barrier model based on rat brain microvascular endothelial cells (rBMVECs) showed that 24 h exposure to Fe-NPs induced increased ROS production and functional impairment of barrier integrity, as demonstrated by enhanced fluorescein efflux and altered transendothelial electrical resistance (TEER) values [22].

Subsequent studies using human primary fibroblasts (HF) and human glioblastoma cells (U251) evaluated the cytotoxic, genotoxic, and oxidative effects of bovine serum albumin-coated iron oxide nanoparticles with sizes of 85 ± 10 nm, 36 ± 6 nm, and 38 ± 6 nm following direct exposure for 24 and 48 h. No cytotoxic effects were detected after 24 h; however, at 48 h, reduced cell viability, increased ROS generation, and DNA fragmentation assessed by comet assay were reported, reaching up to 23% at the highest dose. In contrast, PEG-coated nanoparticles of 38 ± 6 nm did not induce detectable toxic effects [26]. Finally, murine N13 microglial cells were employed to assess the cytotoxicity of PEG-coated iron oxide nanoparticles with different sizes and morphologies, including spherical particles of 3 and 14 nm and cubic particles of 19–20 nm. Following 24 h exposure to concentrations ranging from 0.1 to 100 μg/mL, no significant reduction in cell viability was observed in murine in vitro models [27].

3.1.3. Humans

Bourgkard et al. evaluated the association between occupational exposure to iron oxides and lung cancer mortality in workers from a carbon steel manufacturing plant with at least one year of employment. The longitudinal follow-up conducted between 1968 and 1998 included 17,701 individuals. Despite inhaled dust containing 10–50% iron oxides, no significant association with lung cancer risk was observed. In contrast, a significant increase in bladder cancer mortality was identified in relation to oil mist exposure, with a clear dose–response relationship reported [28].

3.1.4. Mice

Mouse models have been extensively used to investigate the systemic and organ-specific effects of iron-containing particulate matter. In a murine model, Marchini et al. evaluated cardiovascular effects following acute exposure to residual oil fly ash (ROFA), a complex environmental particulate material rich in metals, including iron. Particles with a mean aerodynamic diameter of 2.06 ± 1.57 μm were administered intranasally to female Swiss mice at a single dose of 1.0 mg/kg body weight. A significant reduction in cardiac oxygen consumption was observed at 1 and 3 h post-exposure, accompanied by mitochondrial alterations, including impaired state 3 and state 4 respiration, inhibition of respiratory chain complex II, depolarization of mitochondrial membrane potential, and a marked decrease in ATP production [29]. Subsequent investigations expanded the analysis to systemic and pulmonary responses. Increased lipid peroxidation, evidenced by elevated TBARS levels, was observed primarily at 1 and 3 h post-exposure, while protein oxidation increased from 3 h onward, reaching approximately 16% at 5 h. These effects were accompanied by systemic redox imbalance, characterized by decreased GSH, increased GSSG, and reduced vitamin C levels. Temporal modulation of SOD activity and sustained increases in pro-inflammatory cytokines (TNF-α and IL-6), together with polymorphonuclear leukocyte activation, were reported following particulate matter exposure [30].

In another study, Radu et al. evaluated pulmonary oxidative and inflammatory responses in male CD-1 mice (12–14 weeks old) following exposure to iron oxide nanoparticles encapsulated in phospholipid polymeric micelles (DSPE-PEG). The average particle

size of the micelle-encapsulated iron oxide nanoparticles (IONPs-PM) was approximately 21.5 nm. Nanoparticles were administered intravenously via the tail vein at doses of 5 and 15 mg Fe/kg body weight, and biological parameters were assessed at 2, 3, 7, and 14 days post-exposure. Alterations were observed in the activities of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR), together with a sustained depletion of reduced glutathione (GSH). Increased levels of malondialdehyde (MDA) and protein carbonyls were detected, along with a reduction in lactate dehydrogenase (LDH) activity, particularly at days 3 and 7, followed by partial recovery by day 14. In addition, pro-apoptotic signaling was evidenced by increased Bax expression, active caspase-3, and TNF-α, accompanied by decreased Bcl-2 levels, with more pronounced effects at the higher dose. Histopathological analysis revealed dose-dependent pulmonary lesions, including inflammatory cell infiltration, thickening of alveolar walls, and collapse of terminal bronchioles [31].

Gestational exposure to iron oxide nanoparticles has been evaluated in relation to developmental and reproductive outcomes. In pregnant CD-1 mice, a single intraperitoneal administration of Fe_2O_3 nanoparticles coated with polyethyleneimine (positively charged, ~28 nm) or polyacrylic acid (negatively charged, ~30 nm) was performed on gestational days 8, 9, or 10 at doses of 10 or 100 mg/kg. No significant maternal or fetal effects were observed at the lower dose. In contrast, the higher dose induced surface charge- and timing-dependent toxicity, with a significant increase in fetal loss, particularly following exposure to positively charged nanoparticles on gestational day 10, where resorption rates approached 42%. Moreover, offspring exposed in utero exhibited persistent reproductive alterations in adulthood, including endometrial hyperplasia in females and germ cell loss in male testes. Increased fetal loss was also observed in second-generation crosses, even in the absence of further nanoparticle exposure [32]. In contrast, Caro et al. investigated the in vivo behavior and acute toxicity of PEGylated iron oxide nanoparticles in Balb/c mice. Cubic nanoparticles with an approximate size of 20 nm were administered intravenously via the tail vein at a dose of 5 mg Fe/kg. Magnetic resonance imaging revealed rapid hepatic uptake and early systemic signal enhancement, indicating effective circulation of the contrast agent. Histological analyses showed no detectable alterations in liver or kidney tissues, and body weight remained stable throughout the observation period. No acute toxicity was detected following intravenous administration of these PEGylated nanoparticles [27].

Finally, Dos Santos et al. evaluated the toxicological effects of industrial iron oxide-rich particulate matter originating from an iron ore pelletizing process, without specification of crystalline phase or particle morphology. Environmental characterization identified predominance of PM10 and PM2.5 fractions, with coarse particles being more abundant. Wild-type (WT) mice and genetically modified mice with reduced vesicular acetylcholine transporter expression (VAChT KD) were exposed under three environmental conditions: vivarium (control), an indoor pelletizing facility, and an urban area located 3.21 miles from the industrial source. Exposure occurred via passive inhalation of contaminated ambient air for approximately two weeks during both summer and winter seasons, under real atmospheric pollution conditions. Measured PM10 concentrations ranged from approximately 33 to 164 $\mu g/m^3$, depending on location and season. Pulmonary inflammation was more pronounced in cholinergic-deficient animals compared with wild-type controls following exposure to iron-rich particulate matter [33].

3.1.5. Fish

Among aquatic vertebrate models, zebrafish (*Danio rerio*) has been increasingly employed to assess nanoparticle toxicity; few studies have systematically evaluated in vivo

effects of iron oxide nanoparticles in this model. In the study considered here, zebrafish embryos were exposed to PEGylated magnetic iron oxide nanoparticles and manganese ferrite nanoparticles with sizes ranging from 3 to 20 nm at concentrations of 0.01, 0.1, 1, 10, and 100 μg/mL for 24, 48, 72, and 144 h post-fertilization (hpf). Exposure to iron oxide nanoparticles did not induce mortality, malformations, or alterations in hatching rates at any tested concentration, with survival remaining close to 100% up to 6 days post-fertilization. In contrast, manganese-based nanoparticles exhibited clear dose-dependent toxicity, with high mortality observed at 100 μg/mL after 6 days post-fertilization. All nanoparticle types were associated with accelerated hatching at 48 hpf [27].

3.1.6. Amphibians

Evidence regarding iron oxide nanoparticle exposure in amphibian models remains scarce. In tadpoles of *Duttaphrynus melanostictus*, commercially available iron oxide nanoparticles (IONPs) with reported sizes of 20–40 nm and unspecified morphology were evaluated in an aquatic system at concentrations of 5, 10, and 50 mg/L over a 30-day exposure period. Exposure resulted in increased iron bioaccumulation in blood, liver, and kidney tissues, accompanied by hematological alterations affecting red blood cell count, hematocrit, hemoglobin, and erythrocyte indices, as well as lymphocyte levels. In addition, elevated oxidative stress biomarkers and marked reductions in antioxidant enzyme activity were observed, with SOD activity decreasing by approximately 3.6-fold and CAT activity by 6.7-fold relative to controls. Morphological abnormalities were reported at concentrations of 10 and 50 mg/L, whereas no morphological effects were observed at 5 mg/L [34].

Table 1. Biological effects of iron oxide-containing particulate matter across animal models.

Authors	Biological Model	Exposure Route/Particle Type	Particle Type	Particle Size	Main Cellular Effects Observed
Marchini et al., 2013 [29]	Female Swiss mouse	Intranasal instillation (1 mg/kg)	Residual oil fly ash (ROFA)	PM2.5 (2.06 ± 1.57 μm; aerodynamic diameter)	Reduced cardiac O_2 consumption; mitochondrial dysfunction; decreased ATP production
Marchini et al., 2014 [30]	Female Swiss mouse	Intranasal instillation (1 mg/kg)	Residual oil fly ash (ROFA)	PM2.5 (2.06 ± 1.57 μm; aerodynamic diameter)	Systemic oxidative damage; redox imbalance (↓ GSH, ↑ GSSG); systemic inflammation (↑ TNF-α, ↑ IL-6)
Imam et al., 2015 [22]	Sprague–Dawley rat	Intravenous injection (50 mg/kg)	Iron oxide nanoparticles (IONPs)	10.8 ± 0.2 nm (ultrafine, <100 nm)	Brain penetration and retention; decreased T_2 relaxation values; reduced striatal dopamine; neuronal, dopaminergic, and cerebrovascular damage
Radu et al., 2015 [31]	Male CD-1 mouse	Intravenous injection (5 and 15 mg/kg)	Magnetite nanoparticles	21.8 nm (ultrafine, <100 nm)	Pulmonary oxidative stress; early apoptotic activation; dose-dependent lung injury (high dose > low dose)
Di Bona et al., 2015 [32]	Pregnant CD-1 mouse	Intraperitoneal injection (10 and 100 mg/kg)	Iron oxide nanoparticles (IONPs)	28–30 nm (ultrafine, <100 nm)	Fetal loss; surface charge-dependent reproductive toxicity; transgenerational effects

Table 1. *Cont.*

Authors	Biological Model	Exposure Route/Particle Type	Particle Type	Particle Size	Main Cellular Effects Observed
Caro et al., 2019 [27]	Balb/c mouse	Intravenous injection (5 mg Fe/kg)	Ferrite nanoparticles	~20.3 ± 1.6 nm (ultrafine, <100 nm)	Rapid hepatic uptake without histopathological damage; absence of acute systemic toxicity
Patten et al., 2021 [23]	TgF344-AD and WT rats (♂/♀)	Chronic inhalation (15.6 ± 3.7 μg/m^3)	Traffic-related air pollution (TRAP)	33 ± 4 nm (reported ultrafine-dominant fraction)	Increased cerebral particle burden; behavioral impairments; Alzheimer-like phenotype (↑ β-amyloid, ↑ phosphorylated tau, glial activation), dependent on age, sex, and genotype
Dos Santos et al., 2024 [33]	WT and VAChT KD mice	Passive environmental inhalation	Iron-rich PM (mining-derived metallic dust)	PM2.5 and PM10 (aerodynamic diameter ≤2.5 μm and ≤10 μm)	Pulmonary inflammation; airway hyperresponsiveness; oxidative stress; exacerbated effects under cholinergic deficiency
Akhtar et al., 2025 [25]	Sprague–Dawley rat	Intraperitoneal administration (75, 150, and 300 mg/kg)	Iron oxide nanoparticles	1–100 nm (nanoparticle range; ultrafine fraction)	Dyslipidemia (↑ cholesterol, triglycerides, LDL); elevated ALT, AST, and ALP at medium–high doses; increased total leukocyte counts; dose-dependent hepatic damage: congestion and sinusoidal dilation at low dose, necrosis, fibrosis, portal inflammation, and lobular disorganization at high dose.

The studies summarized in Table 1 report biological effects associated with iron oxide exposure across different biological models, exposure routes, and particle physicochemical properties. In murine models, systemic administration (intravenous or intraperitoneal) was associated with brain, pulmonary, and hepatic biodistribution, accompanied by oxidative, inflammatory, and apoptotic alterations. Inhalation or intranasal exposure was associated with dose- and time-dependent pulmonary, cardiovascular, and neurological responses. Reported outcomes ranged from the absence of detectable histopathological effects in PEGylated nanoparticles developed for biomedical applications to systemic oxidative damage, mitochondrial dysfunction, neurodegenerative alterations, and reproductive effects observed under environmental or occupational exposure conditions. Variability in responses was reported in relation to organismal physiological status, genetic background, and regulatory system integrity, including cholinergic signaling.

Table 2. In vitro cellular effects of iron oxide nanoparticles.

Authors	Cell Model	Exposure Conditions	Particle Type	Particle Size	Main Cellular Effects Observed
Imam et al., 2015 [22]	Human neuronal cells (SH-SY5Y)	Direct exposure (10–30 nm; 2.5–10 μg/mL, 24 h)	Iron oxide nanoparticles	Not specified (reported as nanoparticles; presumably <100 nm)	Dopaminergic dysfunction; increased ROS production; mitochondrial impairment; activation of pro-apoptotic pathways

Table 2. *Cont.*

Authors	Cell Model	Exposure Conditions	Particle Type	Particle Size	Main Cellular Effects Observed
Imam et al., 2015 [22]	Rat brain microvascular endothelial cells (rBMVECs)	Direct exposure (24 h)	Iron oxide nanoparticles	10–30 nm (ultrafine fraction, <100 nm)	Increased ROS levels; reduced transendothelial electrical resistance (TEER); increased permeability; functional impairment of the blood–brain barrier
Abakumov et al., 2018 [26]	Human fibroblasts (HF) and glioblastoma cells (U251)	Direct exposure (24–48 h)	Magnetic iron oxide nanoparticles	36–85 nm (ultrafine fraction, <100 nm)	Size- and time-dependent cytotoxicity and genotoxicity; increased ROS generation; DNA fragmentation
Caro et al., 2019 [27]	Murine microglial cells (N13)	Direct exposure (0.1–100 µg/mL, 24 h)	Magnetic ferrite nanoparticles	3.5 ± 0.6 nm and 20.3 ± 1.6 nm (ultrafine fraction, <100 nm)	No detectable cytotoxicity; preserved cell viability; high biocompatibility

In vitro studies report cell type-specific responses following exposure to iron and iron oxide nanoparticles, varying according to particle size, surface properties, and exposure conditions. In human neuronal models (SH-SY5Y), exposure was associated with dopaminergic dysfunction, increased oxidative stress, mitochondrial impairment, and activation of pro-apoptotic pathways. In blood–brain barrier endothelial models (rBMVECs), nanoparticle exposure was associated with increased reactive oxygen species production, reduced transendothelial electrical resistance, and increased permeability. Cytotoxic and genotoxic effects were reported in human fibroblasts and glioblastoma cells, with outcomes varying according to particle size, surface coating, and exposure duration. PEGylated formulations were associated with reduced cytotoxic effects in these models. In murine N13 microglial cells, no acute cytotoxicity was observed under the tested conditions.

3.2. Magnetite and Damage Induced in Biological Models

Magnetite (Fe_3O_4) is one of the most common iron oxides, occurring naturally and as a result of anthropogenic activities. It is widely distributed in igneous rocks, soils, sediments, and industrial emissions. Due to its mixed Fe^{2+}/Fe^{3+} structure, magnetite exhibits high electrical conductivity and magnetic properties, which support its extensive technological, biomedical, and environmental applications. In biological and ecotoxicological contexts, magnetite has attracted increasing attention because of its potential to induce adverse effects. In this section, toxicological evidence obtained across different biological models is summarized, with emphasis on particle size, dose, exposure route, and observed biological effects (Tables 3 and 4).

3.2.1. Rats

One of the earliest studies evaluating pulmonary toxicity of magnetite was conducted by Pauluhn using *Rattus norvegicus* exposed to magnetite particles by nose-only inhalation. Pigment-grade Fe_3O_4 particles (300–600 nm) were administered over 4 weeks, followed by post-exposure observation periods of up to 6 months, at concentrations ranging from 10.1 to 95.8 mg/m^3. Pulmonary particle accumulation increased with inhaled concentration, and lung clearance was reduced above ~30 mg/m^3. Inflammatory responses, characterized by increased neutrophils, lactate dehydrogenase (LDH), and total protein in bronchoalveolar lavage, were reported mainly at concentrations ≥50 mg/m^3. An inflammatory threshold near 1 mg of retained particles per lung was reported [35].

In a subsequent inhalation study, Pauluhn evaluated pigment-sized magnetite (Fe_3O_4; Ferroxide® black 88P) in Wistar rats following OECD TG#413 guidelines. Animals were exposed for 13 weeks at concentrations of 0, 4.7, 16.6, and 52.1 mg/m^3. Neutrophilia in bronchoalveolar lavage was identified as the most sensitive endpoint, together with inflammatory changes in the upper and lower respiratory tract. Increased lung and lung-associated lymph node weights, collagen fiber accumulation, and particle translocation to lymph nodes were observed at higher doses. An empirical NOAEL of 4.7 mg/m^3 (BMCL 4.4 mg/m^3) was established, and a chronic occupational exposure limit of 2 mg/m^3 (alveolar fraction) was proposed [36].

Using intratracheal instillation, Szalay et al. reported that spherical magnetite nanoparticles (<50 nm) administered to Wistar rats at doses of 1 and 5 mg/kg induced reduced body-weight gain and focal interstitial inflammation, with mild pulmonary fibrosis detected after 30 days, while no relevant histopathological alterations were observed in other organs [37]. Similarly, Tada et al. demonstrated that single intratracheal instillation of smaller magnetite nanoparticles (5–15 nm) in Fischer 344 rats induced dose-dependent pulmonary inflammation, granuloma formation, macrophage accumulation, and particle deposition in paratracheal lymph nodes [18].

Systemic exposure studies also evaluated tissue retention of magnetite nanoparticles. Jarockyte et al. reported slow, dose-dependent clearance of oleate-coated superparamagnetic Fe_3O_4 nanoparticles following intramuscular injection in Wistar rats, with incomplete elimination observed at higher doses even after two months [38]. In addition, Matusiak et al. demonstrated that intravenous administration of low doses of PEG-coated iron oxide nanoparticles induced transient but significant alterations in hepatic elemental composition, including persistent changes in Ca, Cu, and Zn levels, despite the absence of marked systemic toxicity [39].

3.2.2. Cell Models

In vitro studies have evaluated cellular responses to magnetite particles of different sizes. In human lung epithelial A549 cells, Könczöl et al. showed that bulk, respirable, alveolar, and nanoscale magnetite particles induced oxidative stress, mitochondrial membrane potential alterations, DNA damage, and micronucleus formation. Sustained activation of JNK signaling and modulation of NF-κB pathways were also reported. Partial attenuation of these effects was observed in the presence of antioxidants [40]. Ramesh et al. reported that ultrafine magnetite nanoparticles (~9 nm) induced dose-dependent inhibition of proliferation, oxidative stress, antioxidant depletion (GSH and SOD), caspase activation, and apoptosis in rat pulmonary epithelial cells, with pronounced effects at higher concentrations [41]. Similarly, Ahamed et al. demonstrated dose-dependent cytotoxicity, oxidative stress, DNA damage, and apoptosis in human epithelial cell lines (A431 and A549) following exposure to ~25 nm magnetite nanoparticles [42].

Rafieepour performed a comparative evaluation of hematite (α-Fe_2O_3) and magnetite (Fe_3O_4) nanoparticles (<40 nm) in human A549 lung cells, examining concentration- and time-dependent cytotoxic responses. Nanoparticles were dispersed in serum-free DMEM and characterized by distinct morphologies under TEM, with hematite forming nanocylinders and magnetite nanospherical structures [43]. Following 24 and 72 h exposure to concentrations ranging from 10 to 250 μg/mL, both phases induced mitochondrial dysfunction, increased ROS production, reduced intracellular GSH levels, and decreased mitochondrial membrane potential. Greater cytotoxicity and apoptosis–necrosis rates were observed in hematite-exposed cells compared to magnetite under equivalent conditions [43].

Könczöl et al. observed increased superoxide generation, antioxidant imbalance, and p21-mediated cell cycle arrest in A549 and H1299 cells exposed to micro- and nanoscale

magnetite particles, in the absence of caspase-3/7 activation [44]. In contrast, oleate-coated magnetite nanoparticles showed high biocompatibility in NIH3T3 fibroblasts, with limited cytotoxicity and vesicular accumulation reported only after prolonged exposure [38]. In hepatocytes, Gokduman et al. demonstrated that magnetite nanoparticle toxicity was dependent on both concentration and exposure pattern, with cumulative dosing resulting in reduced viability, impaired hepatic function, increased ROS generation, and progressive cell death [45]. Wu et al. further showed that ultrasmall Fe_3O_4 nanoparticles (<5 nm) induced enhanced cytotoxicity and hydroxyl radical (·OH) generation, nuclear penetration, and reduced viability in MCF-7 cells, whereas larger particles exhibited lower toxicity [20].

3.2.3. Humans

Kirschvink et al. identified nanometric magnetite crystals in postmortem human brain tissue using SQUID magnetometry and electron microscopy techniques. The morphology and organization of the particles were consistent with an endogenous biomineralization origin [46]. In contrast, Maher et al. identified exogenous iron-rich nanoparticles in cardiac mitochondria of individuals residing in highly polluted urban environments. These particles were associated with severe mitochondrial damage and oxidative stress [14].

Table 3. Toxicological effects of magnetite nanoparticles in rat and mouse models.

Authors	Biological Model	Route of Administration	Damage Induced/Observed Effects
Pauluhn, 2009 [35]	*Rattus norvegicus*	Nose-only inhalation	Dose-dependent pulmonary accumulation; reduced pulmonary clearance; lung inflammation (↑ LDH, proteins, PMNs in BAL); cellular infiltration; prolonged particle retention
Pauluhn, 2012 [36]	Wistar rat	Nose-only inhalation, subchronic	Pulmonary inflammation; ↑ neutrophils in BAL; histopathological changes in the respiratory tract; ↑ lung and lung-associated lymph node weights; increased septal collagen; NOAEL 4.7 mg/m^3
Szalay et al., 2012 [37]	Male Wistar rat	Single intratracheal instillation	Focal pulmonary inflammation; mild pulmonary fibrosis; reduced body-weight gain; decreased relative weights of lung, liver, and kidney
Tada et al., 2012 [18]	Fischer 344 rat	Single intratracheal instillation	Increased lung weight; accumulation in alveolar macrophages; inflammatory infiltration; multinucleated cells; pulmonary granulomas; reactive epithelial changes
Jarockyte et al., 2016 [38]	Wistar rat	Intramuscular injection	Dose-dependent tissue retention; slow clearance; persistence of nanoparticles at high doses
Matusiak et al., 2017 [39]	Male Wistar rat	Intravenous injection	Transient increase in liver mass; elevated hepatic Fe levels; persistent alterations in Ca, Cu, and Zn; no significant changes in body weight

Table 3. *Cont.*

Authors	Biological Model	Route of Administration	Damage Induced/Observed Effects
Totsuka et al., 2014 [47]	*Mus musculus* (ICR and gpt delta)	Intratracheal instillation (single and repeated)	Pulmonary DNA damage; ↑ oxidative adducts (8-oxodG, HεdG/HεdC); mutagenesis; pulmonary inflammation; granuloma formation
Orel et al., 2015 [48]	Male C57BL/6 mouse	Intravenous injection	Mitochondrial alterations in tumor cells; ↑ oxidative stress; ↓ ATP production; inhibition of tumor growth (controlled therapeutic application)
Wu et al., 2022 [20]	Male ICR mouse	Intravenous injection	Size-dependent acute and lethal toxicity; cardiotoxicity; ↑ ROS and ·OH; elevated ALT/AST; systemic tissue damage

3.2.4. Mice

In murine models, Totsuka et al. demonstrated that intratracheal instillation of magnetite nanoparticles induced dose-dependent pulmonary DNA damage, oxidative stress markers, inflammation, and increased mutation frequency [47]. In contrast, Orel et al. evaluated magnetite-based nanocomplexes in a therapeutic context and reported enhanced antitumor efficacy of doxorubicin when combined with magnetite nanoparticles and electromagnetic irradiation [48,49]. However, Wu et al. showed that intravenous administration of ultrasmall magnetite nanoparticles (<5 nm) induced acute lethality, severe oxidative stress, and cardiac injury at high doses, with increased hydroxyl radical (·OH) generation observed under these conditions [20].

Table 4. In vitro toxicological studies of magnetite (Fe_3O_4) nanoparticles.

Authors	Cell Model	Exposure Type	Induced Damage/Observed Effects
Könczöl et al., 2011 [40]	Human pulmonary epithelial cells (A549)	Direct exposure in culture	Oxidative stress (↑ ROS), mitochondrial membrane potential disruption, DNA damage (Comet assay), micronucleus formation, sustained JNK activation and NF-κB modulation; ROS-dependent genotoxicity
Ramesh et al., 2012 [41]	Rat pulmonary epithelial cells (RL-65)	Direct exposure in culture	Dose-dependent inhibition of proliferation, ↑ ROS and lipid peroxidation, ↓ GSH and SOD, activation of caspases-3/-8, DNA fragmentation, and apoptosis
Ahamed et al., 2013 [42]	Human epithelial cells A431 (skin) and A549 (lung)	Direct exposure in culture	Dose-dependent cytotoxicity, ↑ LDH, ↑ ROS and MDA, ↓ GSH, DNA damage, and caspase-3/-9-mediated apoptosis

Table 4. *Cont.*

Authors	Cell Model	Exposure Type	Induced Damage/Observed Effects
Könczöl et al., 2013 [44]	Human cells A549 and H1299	Direct exposure in culture	Size-dependent superoxide radical generation, ↓ GSH, ↑ CAT activity, cell-cycle alteration (↑ sub-G1 population), p21 activation without apoptosis induction
Jarockyte et al., 2016 [38]	Mouse embryonic fibroblasts (NIH3T3)	Direct exposure in culture	Perinuclear endocytic internalization, minimal cytotoxicity, mild morphological changes; relatively high biocompatibility
Gokduman et al., 2018 [45]	Primary hepatocytes from Lewis rats	Direct exposure in culture (single and cumulative dosing)	Reduced viability, ↑ ROS, impaired hepatic functions (albumin and urea production), time- and accumulation-dependent increase in cell death
Wu et al., 2022 [20]	MCF-7 cells (human breast carcinoma)	Direct exposure in culture	Size-dependent toxicity (<5 nm), ·OH generation, ↑ ROS, marked reduction in viability, nuclear entry of ultrasmall nanoparticles, and acute cellular damage
Rafieepour et al., 2025 [43]	Human lung A549 cells	Direct in vitro exposure	Magnetite exposure induced increased ROS (notably after 24 h), reduced SDH activity and mitochondrial membrane potential (MMP), decreased GSH levels at 72 h, and elevated apoptosis/necrosis rates accompanied by morphological alterations.

3.2.5. Aquatic and Invertebrate Models

In aquatic models, magnetite nanoparticles impaired sperm motility and induced oxidative stress in rainbow trout (*Oncorhynchus mykiss*) spermatozoa, with alterations reported in membrane integrity and antioxidant status following exposure [50]. In zebrafish (*Danio rerio*), embryotoxicity outcomes varied according to concentration and exposure conditions. Low concentrations were associated with negligible developmental alterations, whereas higher doses or specific exposure routes resulted in increased oxidative stress biomarkers, DNA damage, apoptotic responses, and developmental abnormalities during early life stages [51]. Alterations in hatching rate, larval survival, and morphological endpoints were also reported under elevated exposure scenarios [51].

In invertebrate models, multiple biological endpoints were affected by magnetite exposure. In *Caenorhabditis elegans*, reproductive toxicity, reduced brood size, neurobehavioral alterations, oxidative imbalance, and DNA damage were documented following nanoparticle exposure [52,53]. In dipteran insects, developmental delay, decreased survival rates, and oxidative stress responses were reported after magnetite nanoparticle treatment [54]. In mollusks, exposure induced lipid peroxidation, protein carbonylation, genotoxic effects, and alterations in antioxidant enzyme activity, indicating measurable biochemical and cellular responses across different tissues [51]. These effects were generally influenced by particle concentration, exposure duration, and developmental stage.

3.3. Maghemite and Adverse Effects Induced by Exposure in Living Organisms

Toxicological, cytotoxic, and genotoxic evaluations of maghemite (γ-Fe_2O_3) have frequently involved surface-functionalized or coated nanoparticles, particularly in studies assessing their biological interactions and biocompatibility profiles. Experimental investigations have examined these formulations across different in vitro and in vivo models, evaluating endpoints such as cell viability, oxidative stress markers, inflammatory responses, DNA damage, and tissue distribution. The evidence across organisms is summarized below, with comparative data presented in Tables 5 and 6.

3.3.1. Rats

In Wistar rats, in vivo biocompatibility of ~10 nm spherical γ-Fe_2O_3 nanoparticles functionalized with 3,4-dihydroxyphenethylamine (fluorescent labeling; no protective biocompatible shell) was assessed following a single intravenous dose (0.8 mg/kg). Rapid systemic clearance was reported (≈40% removed from circulation at 24 h; ≈75% at 72 h), mainly via urinary excretion. However, leukocytosis (~50% increase) and inflammatory infiltration in the lung, liver, and kidney were detected histologically [55].

Kim et al. (2026) evaluated the subacute inhalation toxicity of spherical γ-Fe_2O_3 nanoparticles in male Sprague–Dawley rats using a nose-only exposure system for 28 days (6 h/day, 5 days/week) at concentrations of 0.56, 1.63, and 4.92 mg/m^3, followed by 7- and 28-day recovery periods [56]. The nanoparticles exhibited a primary size of 16.89 ± 7.68 nm, high purity (99.01%), a negative zeta potential (−19.5 mV), and low solubility in artificial lysosomal fluid (~1.09%). The generated aerosol displayed a mass median aerodynamic diameter (MMAD) of 1.52–1.75 µm, within the range associated with alveolar deposition. No significant clinical, hematological, or histopathological alterations were observed, even at the highest exposure level. Biphasic pulmonary clearance kinetics (T½ ≈ 3.6–7 days), approximately 25% iron retention after 28 days, and intracellular biotransformation within alveolar macrophages were documented. Systemic translocation occurred predominantly in ionic form toward the liver and spleen, whereas intact particles remained largely confined to the lungs and lung-associated lymph nodes [56].

3.3.2. Cell Models

In human dermal fibroblasts, DMSA-coated γ-Fe_2O_3 nanoparticles (~6 nm) were tested across 10^{-6}–10^{-1} g/L and 2–48 h. Only a mild viability decrease was observed at lower concentrations (10^{-6}–10^{-3} g/L) in early exposures, while no genotoxicity was detected by the Comet assay. Aggregation of nanoparticles was observed at higher concentrations, coinciding with reduced effective cell–particle interaction and increased5 mitochondrial activity. Longer-term and cross-model evaluations were described as necessary within the study design [16]. In murine NIH/3T3 fibroblasts, sub-10 nm γ-Fe_2O_3 formulations showed near-complete viability after 24 h (typically 1–10 mM), whereas cellular uptake depended strongly on coating-driven colloidal stability: citrate-coated particles destabilized in serum-containing medium, formed aggregates, sedimented, and yielded high apparent uptake (~250 pg Fe/cell), whereas PAA2K-coated particles remained colloidally stable and showed markedly lower uptake (<30 pg/cell). Differences in uptake were associated with coating type and colloidal stability under the tested conditions [55].

In human endothelial cells (HUVEC; Ea.hy 926), ~10 nm γ-Fe_2O_3 nanoparticles functionalized with 3,4-dihydroxyphenethylamine were efficiently internalized and localized to cytoplasm and membrane-bound organelles without nuclear entry. Reduced viability at 48–72 h, increased cytotoxicity from 24 h, and elevated ROS were reported, whereas caspase-3 activation was not detected. Cell death under the tested conditions was characterized by increased cytotoxicity without caspase-3 activation and showed limited dose

dependence [57]. In bovine spermatozoa, DMSA-coated γ-Fe_2O_3 nanoparticles (5.3–8.1 nm) tested for 4 h (0.015–0.06 mg Fe/mL) did not affect motility, membrane integrity, acrosomal reaction, or ultrastructure. Electron microscopy revealed agglomerates near the sperm head without evidence of cytoplasmic internalization. No acute alterations were detected in the evaluated reproductive parameters under the tested conditions [58,59].

3.3.3. Humans

Postmortem human brain tissue analyses identified iron-oxide nanoparticles, with crystallographic data indicating a substantial fraction consistent with maghemite, especially among smaller particles (predominantly 10–70 nm) frequently organized in clusters of ~50–100 particles. The absence of geological contaminants and the presence of morphological similarities to biogenic iron oxides were reported [46]. In cardiac tissue from young individuals chronically exposed to severe urban air pollution, exogenous iron-rich nanoparticles—mainly magnetite and maghemite (15–40 nm, rounded)—were identified inside myocardial mitochondria, accompanied by marked mitochondrial structural disruption and increased oxidative/ER stress markers. Translocation of inhaled Fe-rich nanoparticles to cardiac tissue was reported in the study [14].

3.3.4. Mice

In BALB/cJ mice, polyacrylic-acid-coated γ-Fe_2O_3 nanoparticles (~6.8 nm) administered intravenously (10 mg/kg; 100 μL) accumulated rapidly in the liver (within 1 h), followed by the kidney and spleen, remaining detectable up to 96 h. Renal function (glomerular filtration and acid–base balance) was not altered, whereas a transient reduction in mean arterial pressure (12–24 h) and reversible mesenteric artery contractility changes were observed, without evidence of renal injury [57,60].

In pregnant ICR mice, oral exposure to spherical γ-Fe_2O_3 nanoparticles (20–30 nm) during early gestation (1–100 mg/kg/day for 7 days; evaluation on day 15) resulted in developmental toxicity at the highest dose, including increased fetal resorption/embryonic death and reduced placental weight. Placental histology showed structural disruption, alongside reduced *Crat* expression, decreased mitochondrial ATP synthesis, and oxidative stress alterations. Reduced mitochondrial ATP synthesis and oxidative stress alterations were reported at the highest exposure level [50].

3.3.5. Fish

In *Poecilia reticulata*, chronic aqueous exposure (21 days) to environmentally relevant citrate-functionalized γ-Fe_2O_3 nanoparticles (~3.97 ± 0.85 nm; 0.3 mg Fe L^{-1}; n ≈ 200) induced progressive DNA damage and time-dependent increases in erythrocytic nuclear abnormalities (e.g., micronuclei and other nuclear morphologies), with significant genotoxic and mutagenic effects under low but sustained exposure [61]. Complementary work reported enlarged and denser melanomacrophage centers (MMCs) and hepatic immune activation (MMC aggregates, steatosis, exudates, hemorrhagic foci) [62].

In zebrafish embryos, developmental effects were reported following aqueous exposure, including delayed hatching without major mortality in one study [63], whereas another study documented substantial developmental toxicity (≈30% mortality at 100 μg/L by 120 hpf), reduced hatching, impaired growth and heart rate, behavioral alterations, early apoptosis, mitochondrial dysfunction (ΔΨm loss, ATP reduction), and oxidative stress (↑ MDA; ↓ CAT/SOD). The authors described the concurrent occurrence of mitochondrial impairment and oxidative stress under these exposure conditions [50].

In rainbow trout (*Oncorhynchus mykiss*), exposure to γ-Fe_2O_3 (1–25 mg/L, 10 days) induced severe histopathological damage in kidney, liver, and gills, together with iron accumulation (notably in liver) and oxidative stress responses at higher aqueous concentra-

tions [63]. The authors described these alterations under the tested exposure levels. In male *P. reticulata*, chronic exposure (0.3–3.0 μg L^{-1}; 14–21 days) did not alter sexual behavior but induced testicular histopathology and spermatogenesis disruption [64]. Conversely, in *Oreochromis niloticus*, acute exposure (24–96 h; 0–100 mg/L) produced transient bioaccumulation in blood, gills, hepatopancreas, muscle, and digestive tract tissues without relevant genotoxicity (micronucleus/Comet assay) or major histological alterations. Tissue iron concentrations declined during the recovery period under the evaluated conditions [52].

3.3.6. Primates

In non-human primates (*Cebus* spp.), intravenous administration of DMSA-coated γ-Fe_2O_3 nanoparticles (~8 nm; 0.5 mg Fe/kg) produced preferential distribution to lungs, liver, and kidneys, with progressive reduction in agglomerates by 30–90 days. Particles were detected mainly in alveolar macrophages, hepatocytes, and renal proximal tubules, while severe lesions, necrosis, and hemorrhage were not observed; only mild long-term hepatic alterations were reported. Hematological, biochemical, behavioral, and body-weight parameters remained within physiological ranges, and progressive reduction in particle agglomerates was observed over time in this primate model [65].

3.3.7. Mollusks

In *Mytilus galloprovincialis*, exposure to γ-Fe_2O_3 nanoparticles (including zeolite-incorporated formulations) produced measurable oxidative stress and cellular damage endpoints. Reported alterations included increased reactive oxygen species (ROS) production, elevated TBARS levels, enhanced protein carbonylation, DNA damage, accumulation of ubiquitin conjugates, and shifts in pro-/antioxidant balance (PAB). Comparative analyses within the study indicated stronger biochemical and cellular responses in organisms exposed to zeolite-incorporated nanoparticles relative to non-incorporated formulations under the same experimental conditions. Differences in oxidative and proteotoxic markers were documented across exposure treatments, reflecting variability in biological responses depending on nanoparticle formulation and exposure parameters.

Across the evaluated aquatic and invertebrate models, γ-Fe_2O_3 nanoparticles were associated with endpoints including oxidative imbalance, DNA damage, histopathological alterations, developmental effects, immune activation, and tissue-specific bioaccumulation. Reported outcomes varied according to particle size, surface modification, concentration, exposure duration, and biological model. Both mild and pronounced responses were described across studies, depending on the experimental design and exposure conditions.

Table 5. Biological effects of maghemite (γ-Fe_2O_3) nanoparticles in mammalian models.

Authors	Biological Model	Route of Administration	Induced Damage/Observed Effects
Hanini et al., 2011 [55]	Wistar rats	Single intravenous injection	~50% increase in leukocyte count; inflammatory infiltration and cellular damage in lung, liver, and kidney; no changes in body weight or severe clinical signs
Iversen et al., 2013 [59]	BALB/cJ mice	Intravenous injection	Significant but transient decrease in mean arterial pressure (12–24 h) and reversible reduction in arterial contractility; no renal damage

Table 5. *Cont.*

Authors	Biological Model	Route of Administration	Induced Damage/Observed Effects
Huang et al., 2019 [50]	Pregnant ICR female mice	Oral (gastrointestinal) exposure	At high doses: fetal resorption and embryonic death, reduced placental weight, placental histological alterations, decreased *Crat* expression, reduced mitochondrial ATP production, and oxidative stress-related alterations
Monge-Fuentes et al., 2011 [65]	Primate (*Cebus* spp.)	Intravenous injection	Mild long-term hepatic alterations; particle presence in alveolar macrophages, hepatocytes, and renal tubules; no necrosis, hemorrhage, or hematological or behavioral alterations
Kim et al., 2026 [56]	Male Sprague–Dawley rats	Nose-only inhalation system	Subacute exposure to γ-Fe_2O_3 did not produce observable toxicity; however, it resulted in pulmonary persistence and intracellular transformation relevant to prolonged exposure scenarios.

Table 5 summarizes in vivo studies assessing the biological effects of maghemite (γ-Fe_2O_3) nanoparticles in mammalian models, considering exposure route and associated outcomes. In Wistar rats, single intravenous administration induced a subclinical systemic inflammatory response, evidenced by leukocytosis and inflammatory infiltration in peripheral organs without severe clinical manifestations. In BALB/cJ mice, intravenous exposure resulted in acute, mild, and reversible cardiovascular changes, with no structural renal damage reported. By contrast, oral exposure during gestation in ICR females revealed embryotoxic effects at high doses, characterized by placental alterations, oxidative stress, and mitochondrial metabolic changes. In non-human primates (*Cebus* spp.), preferential biodistribution to lungs, liver, and kidneys was observed, with mild long-term hepatic changes and no severe structural lesions reported.

Table 6 compiles in vitro studies evaluating the biological responses to maghemite nanoparticles across different cellular models. In fibroblasts and spermatozoa, DMSA-coated formulations were generally associated with minimal or no detectable alterations under the tested exposure conditions. In human endothelial cells, exposure was associated with reduced viability, increased reactive oxygen species production, and non-apoptotic patterns of cell death. Reported responses varied according to cell type, nanoparticle coating, concentration, and exposure duration across the evaluated models.

Table 6. In vitro studies on cytotoxicity and biocompatibility of maghemite (γ-Fe_2O_3) nanoparticles in cellular models.

Authors	Biological Model (Cells)	Exposure Type	Induced Damage/Observed Effects
Auffan et al., 2006 [16]	Human dermal fibroblasts	In vitro exposure	Mild reduction in cell viability at 10^{-6}–10^{-3} g/L (2–24 h); absence of genotoxicity (Comet assay); increased mitochondrial activity at higher concentrations; no detectable genetic damage

Table 6. *Cont.*

Authors	Biological Model (Cells)	Exposure Type	Induced Damage/Observed Effects
Safi et al., 2010 [57]	Murine fibroblasts (NIH/3T3)	In vitro exposure	No cytotoxic effects observed (≈100% viability); cellular uptake dependent on surface coating: high uptake with citrate (~250 pg Fe/cell) and low uptake with PAA2K (<30 pg/cell); no associated cellular damage
Hanini et al., 2011 [55]	Human endothelial cells (HUVEC, Ea.hy 926)	In vitro exposure	Significant reduction in viability at 48–72 h; increased cytotoxicity from 24 h; elevated ROS production; absence of caspase-3 activation, suggesting predominantly necrotic cell death
Caldeira et al., 2018 [58]	Bovine spermatozoa	In vitro exposure	No alterations in motility, membrane integrity, acrosomal reaction, or ultrastructure; presence of extracellular aggregates without internalization; absence of toxic effects

3.4. Hematite and Its Biological Effects Following Exposure

This section presents experimental studies evaluating the biological effects of hematite exposure across multiple models. A summary of the reported findings is provided in Table 5.

3.4.1. Rats

Early in vivo evidence was provided by Garry et al., who evaluated the genotoxic effects of hematite (Fe_2O_3) administered alone or combined with benzo[a]pyrene (B[a]P) in adult male Sprague–Dawley rats via endotracheal instillation (3.75 mg/kg). While hematite alone did not induce detectable DNA damage, B[a]P—particularly when adsorbed onto hematite particles—produced significant genotoxic effects in pulmonary cells, lymphocytes, and hepatocytes. Genotoxic effects were more pronounced when B[a]P was adsorbed onto hematite particles [66].

Subsequent studies in Wistar rats demonstrated that repeated intravenous exposure to hematite nanoparticles (30–35 nm) induced dose- and time-dependent hematological alterations and systemic oxidative stress, including leukocytosis, lipid peroxidation, and reduced antioxidant defenses, without detectable DNA damage in blood cells [67]. Intranasal exposure studies further revealed organ-specific proteomic alterations in brain, liver, and lung. Proteomic alterations were reported in oxidative, inflammatory, and neurochemical pathways [64,68]. In contrast, orally administered green-synthesized hematite nanoparticles produced no systemic toxicity at low doses (≤10 mg/kg), although histopathological alterations were observed at higher doses [69].

3.4.2. Cells

In vitro studies have evaluated the cytotoxic and genotoxic responses to hematite particles across different cellular models. Early work using alveolar macrophages demonstrated efficient phagocytosis of respirable hematite with minimal cytotoxicity, except at high particle burdens [70]. Similarly, hematite alone did not induce genotoxicity in isolated rat cells, whereas B[a]P adsorbed onto hematite significantly amplified DNA damage. Greater DNA damage was observed when B[a]P was adsorbed onto hematite particles [15].

Comparative studies using human pulmonary cell lines showed that nanometric hematite exhibited greater cytotoxic and oxidative potential than micrometric particles

at high concentrations. Aggregation and protein corona formation were reported to influence these responses [71]. Other investigations reported minimal acute toxicity across epithelial and macrophage models [71]. In contrast, studies using intestinal and placental barrier systems demonstrated size-dependent disruption of epithelial integrity without overt cell death [72,73]. Additional studies demonstrated that morphology, crystallinity, and surface defects were associated with differences in cellular uptake and ROS generation [74,75], whereas most cellular systems tolerated hematite exposure at moderate concentrations [62,76–79].

3.4.3. Amphibians

Embryotoxicity assessments using *Xenopus laevis* (FETAX protocol) indicated low acute toxicity of hematite nanoparticles, with developmental effects observed only at very high concentrations under the tested conditions [77].

3.4.4. Humans

Available human data include occupational exposure studies and retrospective cohort analyses. Occupational exposure studies reported pulmonary fibrosis and iron deposition following chronic inhalation of respirable hematite dust in mining settings [76,80]. However, large retrospective cohort analyses of hematite miners did not demonstrate increased lung cancer mortality under the evaluated industrial conditions [81].

3.4.5. Mice

Murine models demonstrated that intranasally administered hematite particles accessed the central nervous system via olfactory and trigeminal pathways, resulting in iron accumulation and neuronal damage in specific brain regions [82]. In contrast, stereotactic hippocampal administration of nanorhombohedral hematite showed efficient microglial uptake without evidence of inflammatory activation under the tested conditions [76]. Oral exposure to biosynthesized hematite nanoparticles did not induce acute systemic toxicity [83].

3.4.6. Fish

In aquatic models, hematite nanoparticles were associated with limited developmental effects in zebrafish embryos under the tested exposure conditions [50], whereas rainbow trout exposed to higher concentrations showed dose-dependent histopathological alterations and oxidative stress responses reported at higher concentrations [63].

3.4.7. Guinea Pigs

Classic inhalation and instillation studies in guinea pigs demonstrated mild to moderate pulmonary inflammation following exposure to hematite dust [84,85]. Persistent biochemical and mitochondrial alterations were also reported in lung tissue in the absence of overt fibrosis [86].

3.4.8. Crustaceans

Finally, biosynthesized hematite nanoparticles showed low acute toxicity in *Artemia salina*, with mild oxidative and neurochemical responses observed at high concentrations under the tested conditions [83].

Table 7 summarizes key studies employing animal models to assess the toxicological effects of hematite (Fe_2O_3) particles and nanoparticles under different exposure routes. The reported evidence spans early guinea pig inhalation studies of respirable dust to rat and mouse models evaluating biodistribution, systemic toxicity, neurological effects, hematological alterations, and organ-specific molecular responses. Reported outcomes

varied according to exposure route, particle size, and experimental design, including persistent inflammatory and metabolic alterations in some models and low acute toxicity in others. Several studies described systemic iron mobilization together with mitochondrial, genotoxic, and neurotoxic endpoints under the evaluated exposure conditions.

Table 7. Biological effects of hematite (Fe_2O_3) exposure in animal models according to the route of administration.

Author (Year)	Biological Model	Route of Administration	Induced Damage/Observed Effects
Carleton (1927) [84]	Guinea pig (*Cavia porcellus*)	Inhalation of fine dust in a closed chamber	Mild–moderate pulmonary inflammatory response (alveolar epithelial proliferation, transient bronchitis); dust migration to subpleural regions and lymph nodes; progressive pulmonary clearance without fibrosis; limited systemic iron accumulation, mainly in spleen
Jaiswal et al. (1979) [85]	Guinea pig (*Cavia porcellus*)	Intratracheal instillation	Persistent pulmonary metabolic alterations: increased fructose-1,6-bisphosphatase, decreased G6PD, marked reduction in mitochondrial succinate dehydrogenase and carbonic anhydrase; mitochondrial dysfunction without overt fibrosis
Das et al. (1983) [86]	Guinea pig (*Cavia porcellus*)	Intratracheal instillation	Systemic iron mobilization to liver, spleen, bone marrow, kidney, and heart; pulmonary mitochondrial accumulation; increased cytochrome c oxidase and altered mitochondrial permeability, without marked pulmonary fibrosis
Wang et al. (2024) [8]	Male CD-ICR mouse	Intranasal instillation	Brain iron accumulation in olfactory bulb and brainstem; increased Fe(III); neuronal degeneration in hippocampal CA3; evidence supporting transport via olfactory and trigeminal pathways
Lewis et al. (2016) [76]	MacGreen transgenic mouse	Bilateral stereotactic injection into dorsal hippocampus	Uptake by hippocampal microglia without inflammatory activation or apparent structural alterations at 24 h
Rajendran et al. (2021) [83]	Swiss albino mouse (*Mus musculus*)	Oral gavage administration	No mortality or acute toxicity; hematological, biochemical, and histopathological parameters largely normal, with a slight increase in bilirubin
Garry et al. (2004) [15]	Male Sprague–Dawley rat	Endotracheal instillation	Hematite alone showed no genotoxicity; B[a]P and B[a]P-coated hematite induced significant DNA damage, indicating enhancement under co-exposure
Gaharwar & Paulraj (2015) [67]	Male Wistar rat	Intravenous injection	Dose- and time-dependent hematological and inflammatory alterations; systemic oxidative stress without detectable genotoxic damage

Table 8 compiles major in vitro studies using cellular models to evaluate the biological effects of hematite (Fe_2O_3) particles and nanoparticles across respiratory, intestinal, placental, renal, and neural systems. Reported responses included low cytotoxicity in multiple cell types under acute exposure conditions, as well as size- and concentration-related variations

in oxidative stress, cellular internalization, epithelial barrier integrity, and mitochondrial function. Smaller nanoparticles and particles with distinct surface characteristics were associated with increased cellular uptake and reactive oxygen species generation in several models, even in the absence of immediate cell death. In addition, some studies reported increased genotoxic responses when hematite particles were combined with co-occurring contaminants under the tested experimental conditions.

Table 8. In vitro studies in cellular models exposed to hematite (Fe_2O_3) particles and nanoparticles.

Author (Year)	Biological Model	Exposure Type	Induced Damage/Observed Effects
Warshawsky et al. (1994) [70]	Alveolar macrophages	Direct in vitro exposure	Efficient particle phagocytosis; preserved viability at most concentrations; moderate viability reduction (~67%) only at the highest dose
Garry et al. (2003) [66]	Alveolar macrophages, pulmonary cells, hepatocytes, and peripheral lymphocytes from Sprague–Dawley rats	In vitro exposure	Hematite alone: no genotoxicity; B[a]P alone: DNA damage; B[a]P adsorbed onto Fe_2O_3: marked enhancement of genotoxic damage in a cell type-dependent manner, demonstrating a vector effect of hematite
Bhattacharya et al. (2012) [71]	Human lung cells (BEAS-2B and IMR-90)	In vitro exposure	Intracellular internalization; reduced viability and increased ROS at $\geq$50 μg/mL, more pronounced for nanoparticles; toxicity strongly modulated by agglomeration and biomolecule interactions
Freyria et al. (2012) [72]	Murine alveolar macrophages (MH-S) and human pulmonary epithelial cells (A549)	In vitro exposure	No cytotoxicity, apoptosis/necrosis, DNA damage, or nitric oxide production; low acute toxicity under the evaluated conditions
Kalive et al. (2012) [73]	Human intestinal epithelial cells (Caco-2)	Single exposure	Size-dependent disruption of epithelial barrier integrity; 17 nm particles reduced TEER, altered cell junctions and epithelial architecture without inducing early cell death
Faust et al. (2014) [74]	Human placental epithelial cells (BeWo b30)	In vitro exposure	Progressive and irreversible TEER reduction with 50 and 78 nm particles; increased ROS, tight junction disruption, and apoptotic-related alterations
Cardillo et al. (2016) [75]	Canine renal epithelial cells (MDCK)	In vitro exposure	Morphology-dependent internalization; elongated particles showed higher uptake; significant ROS increase observed only for particles with specific surface characteristics
Lewis et al. (2016) [76]	Primary microglia from MacGreen mice and N9 microglial cell line	In vitro exposure	Size-dependent internalization; overall low cytotoxicity, with significant effects only at the highest concentration and longer exposure times
Lee et al. (2020) [62]	Human HeLa cells	In vitro exposure	Minimal effects at $\leq$50 μg/mL; reduced viability, oxidative stress, and cell death observed at 100 μg/mL

4. Discussion

Studies addressing the biological effects induced by exposure to iron oxide particles consistently demonstrate that toxicological outcomes are critically governed by physico-chemical and experimental parameters, including particle size and shape, surface coating,

applied concentration, exposure duration, and route of administration. Collectively, these variables determine the magnitude of observed damage, biocompatibility, cellular internalization efficiency, and the molecular mechanisms activated across biological models.

4.1. Cellular Models

Magnetite has been the most extensively evaluated iron oxide in vitro, using human and rodent cell lines such as pulmonary epithelial cells (A549, H1299), tumor cells (A431, MCF-7), murine fibroblasts (NIH/3T3), and primary rat hepatocytes. Across these models, reduced cell viability and oxidative stress have been consistently reported [20,38]. Mitochondrial dysfunction and DNA damage were further documented in independent experimental systems [40,41], together with micronucleus formation and activation of stress-related signaling pathways, including JNK, TNFR, and p21 [42,43]. Caspase-mediated apoptosis (caspases-3, -7, -8, and -9) has also been described under specific exposure conditions [44,45].

A strong size dependency was observed, with nanoparticles <10 nm exhibiting markedly higher toxicity, particularly under oxidative conditions such as hydrogen peroxide co-exposure [20]. Higher concentrations and prolonged exposures further exacerbated these effects, including cell-cycle alterations and depletion of antioxidant defenses such as GSH [2,41]. Surface coatings were shown to play a dual role, either attenuating toxicity by limiting direct cell–particle interactions [38] or enhancing cellular damage when coatings such as citrate promoted uptake and reactivity [20].

Hematite has been investigated across a broader spectrum of cellular systems, including macrophages, lymphocytes, pulmonary, intestinal, placental, renal, embryonic, and neural cells. Reported effects ranged from efficient phagocytosis and intracellular accumulation [70,72] to epithelial barrier disruption and oxidative stress [62,79]. Mitochondrial impairment, apoptosis, and gene expression changes have also been documented in different experimental models [15,50], with additional evidence supporting diverse cellular responses depending on exposure context [70,79].

Under acute exposure conditions ($\leq$24 h) and low-to-moderate concentrations, particularly in resilient cells such as alveolar macrophages, hematite generally exhibited low cytotoxicity [70,72]. In contrast, higher concentrations ($\geq$50 μg/mL) and longer exposures resulted in increased ROS production, mitochondrial damage, and reduced viability [62,79]. Importantly, hematite acted as an effective particulate carrier for benzo[a]pyrene, significantly amplifying genotoxic and cytotoxic responses through adsorption-mediated co-exposure [15].

Compared with magnetite and hematite, maghemite has been evaluated in fewer cellular models, mainly human dermal fibroblasts, murine fibroblasts (NIH/3T3), human endothelial cells (HUVEC), and bovine spermatozoa. Observed effects generally included mild reductions in viability and oxidative stress responses [18,55], together with ROS generation and necrotic cell death under specific exposure conditions [56,58]. These findings may partly reflect the more limited experimental coverage. Nevertheless, surface functionalization emerged as a key determinant, with some coatings reducing toxicity by stabilizing particles, while others enhanced uptake and damage [57].

Recent comparative evidence further supports phase-dependent differences in cytotoxicity. Direct exposure studies in human pulmonary A549 cells demonstrated that although both magnetite and hematite induce oxidative stress and mitochondrial dysfunction, hematite may elicit more pronounced apoptosis–necrosis responses at higher concentrations, reinforcing the importance of crystalline phase in modulating cellular outcomes [43]. Overall, in vitro evidence indicates that magnetite exhibits the highest cytotoxic potential among iron oxides, followed by hematite, whereas maghemite appears compara-

tively less harmful. However, these trends are strongly modulated by surface chemistry, particle size, morphology, and concentration, underscoring the need for standardized experimental frameworks and deeper mechanistic investigations to improve cellular-level risk assessment.

4.2. Rat Models

Rattus norvegicus has been widely employed to investigate iron oxide toxicity, enabling evaluation of organ accumulation, inflammatory responses, genotoxicity, and systemic effects under controlled variations in size, coating, exposure route, dose, and duration. Across studies, magnetite consistently induced the most severe effects, particularly following inhalation and intratracheal exposure. Subchronic inhalation resulted in persistent pulmonary accumulation, bronchiolar hyperplasia, inflammatory cell infiltration, enzymatic release (LDH, NAG), and structural lung damage at moderate concentrations ($\geq$3 mg/m^3) [35,36]. These findings were corroborated by reports of pulmonary granulomas and epithelial remodeling [18,37], as well as mild fibrosis, weight loss, hepatorenal involvement, and prolonged particle retention, indicating slow clearance and systemic distribution [38].

Maghemite exposure has been primarily investigated due to its biomedical relevance. In contrast to magnetite, oral administration emerged as the most deleterious route, despite the larger particle size (PM1.0 range), likely due to higher applied doses. Severe outcomes included fetal resorption, embryonic lethality, placental structural damage, and compromised mitochondrial energy metabolism during development [50,59].

Hematite exhibited an intermediate and highly context-dependent toxicity profile. Endotracheal administration of respirable particles did not induce intrinsic genotoxicity, but markedly enhanced benzo[a]pyrene toxicity through adsorption mechanisms [66]. Intravenous exposure mainly triggered oxidative stress and immunohematological alterations without DNA damage [67], whereas intranasal exposure produced the most pronounced effects, including neurotoxicity, inflammation, oxidative stress, neurotransmitter disruption, and organ-specific proteomic alterations [68].

Recent subacute studies have also refined the understanding of exposure kinetics and systemic involvement. Controlled intraperitoneal administration of iron oxide nanoparticles has demonstrated dose-dependent dyslipidemia, hepatic enzyme elevation, leukocytosis, and progressive histopathological liver damage, supporting systemic immunometabolic disruption under repeated exposure conditions [25]. In parallel, controlled nose-only inhalation of γ-Fe_2O_3 nanoparticles did not produce overt clinical toxicity but revealed biphasic pulmonary clearance, partial iron retention, and intracellular biotransformation within alveolar macrophages, highlighting the relevance of persistence mechanisms even in the absence of acute pathology [56].

Collectively, these findings indicate that although all iron oxides can induce adverse effects in rats, magnetite is consistently the most toxic, particularly via inhalation and intratracheal routes. Maghemite shows route-specific toxicity dominated by oral exposure, while hematite responses are more dependent on experimental context, with intranasal exposure posing the greatest risk due to neurotoxic outcomes.

4.3. Mouse Models

Consistent with rat studies, mouse models (*Mus musculus*) identified magnetite as the most toxic iron oxide, with severity strongly influenced by particle size, coating, dose, and exposure route. Toxicity increased with decreasing particle size and absence of surface coatings [20,47], with intravenous exposure producing the most severe systemic effects and intranasal exposure associated with genotoxicity and neurotoxicity [48].

Maghemite studies in mice revealed more pronounced systemic and developmental toxicity, likely reflecting specific experimental designs. Intravenous exposure resulted in organ accumulation, transient hypotension, and vascular effects [59], while oral exposure was associated with fetal resorption, embryonic death, and placental histopathology, contrasting sharply with magnetite outcomes.

For hematite, intranasal exposure again emerged as the most hazardous route, inducing neurotoxic effects and neuronal morphological alterations. In contrast, direct stereotactic injection into the brain did not elicit damage, likely due to differences in particle size (PM1.0 vs. PM2.5) and exposure context [76,82]. Overall, magnetite remains the most toxic iron oxide in murine systems, particularly in the ultrafine fraction and without surface coatings.

4.4. Human Evidence

Human data remain limited and methodologically constrained. Nevertheless, available evidence indicates marked differences among iron oxides. Magnetite is the only oxide with documented chronic environmental inhalation data, showing translocation to cardiac tissue and mitochondria, accompanied by severe structural damage, albeit in very small sample sizes [14]. Direct toxicity of maghemite in humans has not yet been clearly established, as available studies did not isolate this phase independently [46]. Hematite has been evaluated primarily in occupational contexts, where chronic exposure produced progressive inflammatory responses without definitive conclusions regarding carcinogenic risk [80,81]. Taken together, existing human evidence supports magnetite as the most biologically aggressive iron oxide, while emphasizing the need for more controlled and systematic investigations.

4.5. Knowledge Gaps and Current Limitations

Despite substantial experimental evidence on the biological activity of iron oxide particles, several critical knowledge gaps and methodological limitations persist. First, many experimental particles lack representativeness of real atmospheric particulate matter. Commercially synthesized or surface-engineered nanoparticles often differ from environmental particles in aggregation state, surface chemistry, and coexistence with other contaminants, limiting direct extrapolation to real-world air pollution scenarios. Second, particle size classification remains insufficiently standardized. While PM2.5 and PM10 are commonly reported, the PM1.0 fraction—characterized by higher reactivity, deeper tissue penetration, and greater toxicological relevance—remains underexplored, despite its increasing prevalence in urban and traffic-related environments.

Third, contradictory findings regarding magnetite toxicity, surface coating effects, and apparent biocompatibility of some formulations highlight the complex, non-linear interactions among size, aggregation, dose, exposure duration, and biological context. Fourth, most studies rely on acute or short-term exposures at relatively high concentrations, whereas data on chronic low-dose exposure—more representative of environmental and occupational conditions—remain scarce, particularly for inhalation scenarios where cumulative effects are likely underestimated. Although recent controlled inhalation studies have begun to characterize pulmonary clearance and intracellular biotransformation dynamics, long-term cumulative and environmentally realistic exposure data remain insufficient.

Finally, insufficient integration exists between atmospheric sciences and experimental toxicology. Few studies directly link detailed physicochemical and magnetic characterization of iron-rich particles with biological responses within unified experimental frameworks, limiting translation to risk assessment and air quality management. Overall, these gaps underscore the need for multidisciplinary approaches that integrate realistic atmospheric

particle characterization with biologically relevant models, promote experimental standardization, and prioritize environmentally relevant exposure scenarios to improve assessment of iron oxide-associated health risks.

In addition, as a scoping review, this study did not include a formal standardized risk-of-bias appraisal or quality grading of individual studies. Consequently, while the synthesis allows identification of recurring mechanistic patterns and frequently reported biological responses, it does not provide hierarchical weighting of evidence strength across iron oxide phases, exposure routes, or biological models. Comparative statements presented herein should therefore be interpreted as trends emerging from a heterogeneous experimental literature rather than as definitive quantitative rankings. Future systematic reviews incorporating standardized quality appraisal tools would further strengthen confidence in comparative environmental health interpretations.

5. Conclusions

By mapping experimental evidence across a wide range of biological models and exposure scenarios, this scoping review indicates that iron oxide particles represent a biologically active and mechanistically complex class of environmental contaminants whose toxicological behavior varies substantially according to crystalline phase, particle size, and exposure conditions. Rather than exhibiting uniform effects, the reported biological responses arise from the interaction between key physicochemical properties—particularly particle size, surface chemistry, aggregation state, and magnetic characteristics—and biological variables such as exposure route, dose, duration, and organismal susceptibility.

Across the reviewed experimental literature, and particularly among studies with explicit crystalline phase identification, magnetite (Fe_3O_4) is most frequently reported as being associated with higher biological reactivity, especially within ultrafine and nanoscale fractions. Its mixed-valence structure, redox activity, and high cellular internalization capacity are recurrently linked to oxidative stress, mitochondrial dysfunction, inflammatory activation, genotoxic responses, and, in some models, systemic effects. These outcomes are most commonly reported following inhalation-related exposure routes, which are considered environmentally relevant to air pollution scenarios. Evidence from animal and limited human studies suggests that magnetite-rich particles may translocate beyond the respiratory system, reaching cardiovascular and neural tissues, although the extent and long-term health significance of such processes remain incompletely characterized.

Hematite (α-Fe_2O_3) exhibits a heterogeneous toxicity profile across studies with phase-resolved characterization. While often reported as having relatively low intrinsic cytotoxicity under acute exposure conditions, hematite frequently acts as a particulate carrier capable of enhancing the biological effects of co-occurring contaminants, such as polycyclic aromatic hydrocarbons. Nanoscale hematite particles have also been associated with epithelial barrier disruption, mitochondrial alterations, and neuroinflammatory responses under specific exposure pathways, underscoring the importance of particle size and route of exposure in shaping biological outcomes.

Maghemite (γ-Fe_2O_3) is generally reported as exhibiting lower apparent toxicity across the reviewed experimental contexts when explicitly characterized. However, the mapped literature also documents adverse outcomes—including developmental, reproductive, vascular, and oxidative stress-related effects—under high-dose, prolonged, or gestational exposure scenarios. These findings indicate that maghemite cannot be universally regarded as biologically inert and that surface functionalization, colloidal stability, and exposure timing are key modifiers of its biological impact.

Collectively, oxidative stress and mitochondrial dysfunction emerge as the most consistently reported mechanistic pathways across iron oxide particle types within the

heterogeneous experimental literature reviewed, often accompanied by inflammatory signaling, barrier impairment, and genotoxic effects. Importantly, the reviewed studies reveal substantial variability between experimental designs employing acute high-dose exposures and the chronic low-dose conditions characteristic of environmental and occupational settings, which remain comparatively underrepresented in the literature.

From an environmental health perspective, this scoping review highlights a persistent disconnect between atmospheric science and toxicological research. Many experimental investigations rely on synthetic or highly controlled nanoparticles that may not adequately reflect the physicochemical complexity of iron-rich particulate matter present in real-world air pollution, limiting the direct translation of toxicological findings to environmental risk assessment. In this context, magnetic biomonitoring emerges from the mapped evidence as a promising approach for linking particle composition, source attribution, and spatial distribution of iron oxide-rich particulate matter with biological relevance. When integrated with toxicological data, magnetic techniques can strengthen exposure assessment frameworks, particularly in urban environments dominated by traffic-related emissions, supporting air pollution biomonitoring strategies aimed at improving environmental and human health.

Overall, the mapped evidence underscores the need for standardized particle characterization, environmentally realistic exposure models, and multidisciplinary research strategies that integrate atmospheric monitoring with mechanistic toxicology. This scoping review provides a structured framework for interpreting iron oxide toxicity evidence in the context of air pollution biomonitoring. These insights may guide future experimental design, exposure assessment, and interdisciplinary research to better understand the role of iron-rich particles in air pollution-related health effects and to support more informed environmental monitoring and public health decision-making. Recent experimental findings further reinforce that exposure kinetics and particle persistence may be as relevant as intrinsic cytotoxicity in shaping long-term biological outcomes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos17030290/s1, Supplementary File S1. PRISMA-ScR Checklist; Supplementary File S2. Detailed Database-Specific Search Strategies; Supplementary File S3. Data Extraction Template and Variable Definitions.

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