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Iron Oxide Nanoparticle Genotoxicity, ROS Production, and Epigenetic Alterations: A Comprehensive Review

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Abstract— *This review provides an overview of the current insights into the genotoxic effects of iron oxide nanoparticles (IONPs), the mechanisms of reactive oxygen species (ROS) production, and the subsequent epigenetic alterations. With the increasing utilization of IONPs in biomedical and industrial sectors, it is imperative to address the potential negative impacts associated with their use. This article consolidates research findings regarding the pathways through which IONPs can cause DNA damage, the generation of ROS via Fenton reactions and alternative mechanisms, and the modifications to the epigenetic landscape, including changes in DNA methylation, histone modifications, and the expression of non-coding RNAs. Furthermore, we explore the physicochemical characteristics that affect IONP toxicity, methodologies for assessing toxicity, regulatory implications, and prospective avenues for future research. A thorough understanding of these mechanisms is essential for the advancement of safer IONP-based technologies while minimizing risks to human health and environmental safety.*

Keywords— ***Iron oxide nanoparticles, genotoxicity, reactive oxygen species, epigenetic alterations, nanotoxicology***

I. INTRODUCTION

The swift progress in nanotechnology has facilitated the creation and utilization of a diverse array of nanomaterials across various sectors, with iron oxide nanoparticles (IONPs) standing out as exceptionally adaptable substances characterized by distinctive magnetic, catalytic, and optical attributes. Composed mainly of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), IONPs have been extensively employed in the field of biomedicine, serving purposes such as enhancing magnetic resonance imaging (MRI) contrast, enabling targeted drug delivery, providing hyperthermia treatment for cancer, facilitating biosensing, and contributing to environmental remediation efforts [1].

However, alongside their promising applications, the potential toxicity of IONPs has emerged as a significant concern warranting thorough investigation. The diminutive size and elevated surface area-to-volume ratio of these nanoparticles impart unique characteristics that may result in unforeseen interactions with biological systems [2]. Notably, the genotoxic effects of IONPs are of particular concern, as they may arise from direct interactions with DNA or indirectly through the production of reactive oxygen species (ROS) and the ensuing oxidative stress (3).

Moreover, recent findings indicate that IONPs may provoke epigenetic modifications, such as changes in DNA methylation patterns, histone alterations, and variations in non-coding RNA expression, which could lead to enduring biological consequences even in the absence of direct genetic damage (4). These epigenetic alterations may hold significant implications for comprehensively understanding the toxicological profile of IONPs and for the development of safer nanomaterials.

This review seeks to consolidate existing knowledge regarding the genotoxicity of IONPs, the mechanisms underlying ROS generation, and the nature of epigenetic modifications, emphasizing the interconnectedness of these phenomena. Additionally, we will explore the physicochemical properties that affect IONP toxicity, methodologies for toxicity evaluation, regulatory considerations, and prospective avenues for future research.

II. IRON OXIDE NANOPARTICLES: CHARACTERISTICS AND UTILIZATIONS

2.1 Varieties and Physicochemical Characteristics

Iron oxide nanoparticles (IONPs) are predominantly found in several crystalline forms, with magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) being the most extensively researched and utilized in biomedical fields due to their distinctive magnetic properties and relative compatibility with biological systems (5). Magnetite comprises both Fe^{2+} and Fe^{3+} ions, whereas maghemite is composed exclusively of Fe^{3+} ions accompanied by cation vacancies, leading to variations in their magnetic characteristics and reactivity [6].

The physicochemical characteristics of IONPs, such as dimensions, morphology, surface charge, crystallinity, and surface modifications, play a critical role in their interactions with biological systems and potential toxicity [7]. The size of the particles is particularly significant, as it influences cellular uptake mechanisms, biodistribution, and degradation rates. IONPs typically have diameters ranging from 1 to 100 nm, with smaller particles often exhibiting improved cellular penetration but potentially increased reactivity and toxicity (8)

Surface characteristics are essential in determining the behavior of IONPs within biological contexts. Uncoated IONPs are prone to aggregation under physiological conditions due to their elevated surface energy, which restricts their stability and functional efficacy [9]. Consequently, a variety of surface modification techniques and coating strategies have been devised to improve colloidal stability, biocompatibility, and functionality, including the application of polymers (such as polyethylene glycol, dextran, and chitosan), surfactants, proteins, and inorganic materials (like silica and gold) [10].

2.2 Biomedical and Industrial Applications

The distinctive characteristics of iron oxide nanoparticles (IONPs) have facilitated their extensive use across multiple domains. In the realm of biomedicine, superparamagnetic iron oxide nanoparticles (SPIONs) have been the subject of considerable research for both diagnostic and therapeutic purposes [11]. As agents for magnetic resonance imaging (MRI), SPIONs improve image contrast by modifying the magnetic relaxation times of adjacent water protons, which enhances the visualization of tissues and pathological states (12). Several SPION-based contrast agents, including Feridex® and Resovist®, have received clinical approval; however, some have been withdrawn from the market due to commercial factors or safety issues [1].

In the context of cancer treatment, IONPs are utilized for targeted drug delivery, employing magnetic guidance to direct therapeutic agents to tumor locations, thereby reducing systemic toxicity (13). Furthermore, magnetic hyperthermia, a technique in which IONPs produce heat when subjected to alternating magnetic fields, has demonstrated potential for tumor ablation, either as a standalone treatment or in conjunction with traditional therapies[14]. Outside of biomedicine, IONPs are applied in environmental remediation efforts aimed at extracting heavy metals and organic contaminants from water through adsorption and catalytic degradation methods (15). In industrial applications, IONPs are utilized as catalysts, pigments, and integral components in sensors, data storage devices, and ferrofluids (5). Despite the advantageous applications of IONPs, their increasing production and use raise significant concerns regarding potential

environmental release and human exposure, highlighting the need for thorough safety evaluations and a comprehensive understanding of their toxicological profiles (16).

III. MECHANISMS OF GENOTOXICITY

3.1 Direct DNA Interaction and Damage

Iron oxide nanoparticles (IONPs) can elicit genotoxic effects through direct interactions with DNA or other cellular components that are crucial for maintaining genomic stability. While the nuclear membrane generally serves as a barrier to nanoparticle entry, research has indicated that smaller IONPs possess the capability to infiltrate the nucleus, particularly during mitosis when the nuclear envelope temporarily disintegrates (3). Once inside the nucleus, IONPs may interact with DNA via electrostatic forces, potentially leading to structural alterations, unwinding of the double helix, or crosslinking events that can hinder vital cellular functions such as DNA replication and transcription [17].

In addition, IONPs can disrupt the function of nuclear proteins that play essential roles in DNA repair, replication, and transcription processes. For example, they may interfere with topoisomerases, which are enzymes necessary for alleviating torsional stress during DNA replication, resulting in stalled replication forks and the potential occurrence of double-strand breaks (DSBs) [18]. Furthermore, interactions with DNA repair proteins may impair the cell's capacity to correct DNA damage, thereby increasing genomic instability [19].

3.2 Indirect Mechanisms of Genotoxicity

In addition to direct interactions, indirect mechanisms play a significant role in genotoxicity, particularly through the production of reactive oxygen species (ROS) and the resulting oxidative stress. These pathways are recognized as key contributors to DNA damage induced by iron oxide nanoparticles (IONPs) (20). The release of iron ions from IONPs can engage in Fenton and Haber-Weiss reactions, leading to the formation of highly reactive hydroxyl radicals (•OH) that can cause various types of DNA damage, such as strand in base structure, and crosslinking between DNA and proteins [21].

The oxidative stress stemming from excessive ROS can also impact cellular components that re crucial for maintaining genomic integrity. For example, oxidative damage to tochondrialNA may impair mitochondrial function, which in turn can elevate ROS production, establishing a self-reinforcing cycle of oxidative stress and damage [22]. Furthermore, products of lipid peroxidation, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), have the potential to form adducts with DNA, thereby introducing mutagenic lesions (23).

3.3 Chromosomal Aberrations and Micronuclei Formation

Exposure to iron oxide nanoparticles (IONPs) has been linked to a range of chromosomal abnormalities, which encompass both structural alterations (such as breaks, gaps, translocations, and rings) and numerical changes (including aneuploidy and polyploidy) [24]. These chromosomal anomalies may arise from DNA strand breaks induced by IONPs or from disruptions to the mitotic spindle apparatus, which can hinder the correct alignment and segregation of chromosomes during cell division [25].

The formation of micronuclei, recognized as a biomarker for chromosomal damage, has been reliably documented following exposure to IONPs across various cell types [26]. Micronuclei are formed from acentric fragments of chromosomes or entire chromosomes that do not integrate into the daughter nuclei during cell division, resulting in the emergence of distinct small nuclear structures within the cytoplasm. Consequently, the micronucleus assay serves as a highly sensitive measure of genotoxicity induced by IONPs, indicating both clastogenic (chromosome breakage) and aneugenic (chromosome loss) effects [17].

3.4 Effects on the Cell Cycle

Iron oxide nanoparticles (IONPs) have the potential to disrupt the normal progression of the cell cycle, which may lead to genomic instability. Research has shown that IONPs can induce cell cycle arrest, particularly at the G0/G1 and G2/M checkpoints, likely as a cellular response to DNA damage [24][27]. Extended periods of cell cycle arrest can result in abnormal mitotic processes, such as the formation of multipolar spindles, the presence of lagging chromosomes, and failures in cytokinesis, ultimately leading to the

development of multinucleated cells or cells exhibiting abnormal DNA content [9].

Moreover, IONPs may disrupt the expression and functionality of proteins that regulate the cell cycle, including cyclins, cyclin-dependent kinases (CDKs), and checkpoint proteins like p53, p21, and CHK1/2[28]. The dysregulation of these proteins can hinder the cell's capacity to accurately evaluate and respond to DNA damage, potentially facilitating the transmission of genetic abnormalities to daughter cells [29].

IV. REACTIVE OXYGEN SPECIES (ROS) GENERATION

4.1 Mechanisms of ROS Production Induced by Iron Oxide Nanoparticles

The production of reactive oxygen species (ROS) is widely acknowledged as a fundamental mechanism contributing to the toxicity of iron oxide nanoparticles (IONPs)[30]. Multiple pathways are involved in the ROS generation induced by IONPs, functioning concurrently and possibly in a synergistic manner. The inherent characteristics of IONPs, especially their redox-active surfaces, facilitate electron transfer reactions that can lead to the direct formation of superoxide radicals ($O_2\bullet^-$) through interactions with molecular oxygen [31]. Furthermore, the high surface area-to-volume ratio of IONPs enhances their catalytic activity, thereby promoting reactions that generate ROS at the nanoparticle interface [21].

The internalization of IONPs by cells via endocytosis results in their accumulation within lysosomes, where the acidic conditions (pH 4.5-5.0) can expedite the dissolution of the nanoparticles, releasing iron ions that engage in ROS-generating processes (32). This destabilization of lysosomes may further exacerbate oxidative stress by releasing hydrolytic enzymes into the cytosol, which can potentially harm cellular structures (33).

4.2 Fenton/Haber-Weiss Reactions

The Fenton and Haber-Weiss reactions are pivotal pathways through which iron ions originating from iron oxide nanoparticles (IONPs) produce highly reactive hydroxyl radicals (•OH) (34). In the Fenton reaction, ferrous iron (Fe^{2+}) interacts with hydrogen peroxide (H_2O_2), resulting in the formation of hydroxyl radicals and ferric iron (Fe^{3+}):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$$

Ferric iron can then be reduced back to ferrous iron by cellular reductants or through its reaction with superoxide radicals in the Haber-Weiss reaction:

$$Fe^{3+} + O_2\bullet^- \rightarrow Fe^{2+} + O_2$$

These reactions create a cyclical mechanism that perpetually produces hydroxyl radicals, which are recognized as some of the most powerful oxidizing agents in biological systems, capable of interacting with nearly all cellular constituents, including DNA, proteins, and lipids (23). The extreme reactivity and lack of selectivity of hydroxyl radicals play a crucial role in the oxidative damage induced by IONPs and the resulting genotoxic effects (20).

4.3 Mitochondrial Dysfunction

Mitochondria serve as both targets and origins of oxidative stress induced by iron oxide nanoparticles (IONPs)[35]. These nanoparticles can accumulate in proximity to mitochondria, leading to disruptions in the electron transport chain (ETC) either through direct interactions or oxidative damage. This disruption results in electron leakage and an increase in superoxide generation (36). Notably, the inhibition of ETC complexes I, II, and III due to IONPs has been documented, which adversely affects mitochondrial functionality and ATP production [37].

Exposure to IONPs has been associated with mitochondrial membrane depolarization across various cell types, likely due to the activation of mitochondrial permeability transition pores (MPTP) as a response to oxidative stress (38). This depolarization can trigger the release of cytochrome c, thereby initiating caspase-dependent apoptotic pathways [27]. Furthermore, mitochondrial DNA (mtDNA) is particularly susceptible to oxidative damage due to its lack of protective histones and limited repair capabilities compared to nuclear DNA, which further intensifies mitochondrial dysfunction and reactive oxygen species (ROS) production in a self-perpetuating cycle [22].

4.4 Antioxidant Depletion

Cellular antioxidant systems, which encompass both enzymatic components such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-

enzymatic elements like glutathione and vitamins C and E, play a crucial role in maintaining redox homeostasis by neutralizing reactive oxygen species (ROS)[39]. Nevertheless, exposure to iron oxide nanoparticles (IONP) can surpass the capacity of these protective mechanisms, resulting in their depletion or inactivation [40].

Research has indicated a decrease in glutathione (GSH) levels and modifications in the activity of antioxidant enzymes following exposure to IONP, highlighting the cellular challenges in mitigating oxidative stress [27][21]. Notably, the impact on antioxidant systems tends to vary based on dosage, duration, and cell type, often characterized by an initial compensatory increase in activity that is subsequently followed by depletion with prolonged exposure (5).

4.5 Relationship Between ROS and Genotoxic Effects

The association between reactive oxygen species (ROS) generated by iron oxide nanoparticles (IONP) and genotoxic effects is well-documented, with oxidative stress serving as a mediator for various types of DNA damage (20). ROS, especially hydroxyl radicals, have the capacity to directly interact with DNA, resulting in the formation of oxidized bases (such as 8-oxo-7,8-dihydroguanine), abasic sites, single-strand breaks (SSBs), and double-strand breaks (DSBs) [17].

Moreover, the lipid peroxidation induced by ROS leads to the production of reactive aldehydes, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which can create mutagenic DNA adducts (23). The oxidation of proteins may impair DNA repair processes, transcription factors, and elements of the mitotic apparatus, thereby further enhancing genomic instability [41].

The ongoing oxidative stress associated with IONP exposure may also trigger inflammatory responses through the activation of redox-sensitive transcription factors such as NF-κB. This activation results in the secretion of pro-inflammatory cytokines and additional ROS production by inflammatory cells, establishing a self-reinforcing cycle of oxidative damage [35]. Such a chronic inflammatory environment can intensify genotoxicity through ROS and DNA-damaging cytokines released by inflammatory cells [42].

V. EPIGENETIC MODIFICATIONS

5.1 Alterations in DNA Methylation

Recent studies indicate that iron oxide nanoparticles (IONPs) may induce modifications in DNA methylation patterns, which could serve as a mechanism for enduring biological effects (4). DNA methylation, characterized by the addition of methyl groups to cytosine residues within CpG dinucleotides, is essential for the regulation of gene expression, maintenance of genomic stability, and facilitation of cellular differentiation [43]. Exposure to IONPs has been linked to both global and specific alterations in methylation across various cell types and experimental models [44].

Research has shown that IONPs can lead to hypomethylation of repetitive elements, including LINE-1 and Alu sequences, which represent a significant fraction of the human genome. The demethylation of these elements may promote genomic instability by increasing transposition and recombination events (45). Additionally, instances of hypermethylation in the promoters of tumor suppressor genes following exposure to IONPs have been documented, potentially resulting in the silencing of genes that are critical for DNA repair, cell cycle control, and apoptosis [30].

From a mechanistic perspective, oxidative stress induced by IONPs may influence DNA methylation through several pathways: (1) oxidative damage to DNA can impede the binding of DNA methyltransferases (DNMTs) to their target sites; (2) reactive oxygen species (ROS) can convert 5-methylcytosine into 5-hydroxymethylcytosine, which is not recognized by maintenance DNMTs, leading to passive demethylation during DNA replication; and (3) oxidative stress may modulate the activity and expression of DNMTs and demethylating enzymes, such as ten-eleven translocation (TET) proteins (46).

5.2 Histone Modifications

Histones, which are integral protein components of chromatin, are subject to a variety of post-translational modifications (PTMs) that significantly affect chromatin architecture and its accessibility, thus

playing a crucial role in the regulation of gene expression [47]. These modifications encompass acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation, which together form the "histone code" that directs patterns of gene expression [48].

Research has demonstrated that iron oxide nanoparticles (IONPs) can induce modifications in histone patterns, likely through mechanisms mediated by oxidative stress [49]. For example, reactive oxygen species (ROS) may inhibit histone deacetylases (HDACs), resulting in elevated levels of histone acetylation and potentially leading to dysregulated gene expression (50). Furthermore, oxidative damage to histones can modify their interactions with DNA and other nuclear proteins, thereby influencing chromatin dynamics (51).

Investigations have indicated that IONPs can cause significant changes in histone acetylation (notably H3K9ac and H3K27ac) and methylation (including H3K4me3, H3K9me3, and H3K27me3) marks that are associated with genes related to oxidative stress responses, DNA repair mechanisms, inflammation, and apoptosis (50). These modifications may endure even after the cessation of IONP exposure, potentially leading to long-lasting biological consequences (4).

5.3 Alterations in Non-coding RNA Expression

Non-coding RNAs (ncRNAs), which encompass microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), are pivotal in the regulation of gene expression through a variety of mechanisms and are integral to essential cellular functions such as development, differentiation, and responses to stress [52]. The exposure to iron oxide nanoparticles (IONPs) has been linked to significant changes in the expression of numerous ncRNAs, which may play a role in toxicological effects [53].

MicroRNAs, which are small non-coding RNAs that modulate gene expression post-transcriptionally by promoting mRNA degradation or inhibiting translation, have been the focus of extensive research regarding IONP exposure [44]. Notably, several miRNAs that are implicated in oxidative stress responses, inflammation, apoptosis, and DNA repair show altered expression levels following treatment with IONPs [54]. For example, the upregulation of miR-21, known to target tumor suppressor genes and antioxidant enzymes, has been documented after IONP exposure, potentially leading to increased oxidative stress and genomic instability [55].

Long non-coding RNAs, which are characterized by their length of over 200 nucleotides, also exhibit changes in expression following IONP exposure. These ncRNAs regulate gene expression through various mechanisms, including chromatin remodeling, transcriptional control, and post-transcriptional modifications (56). Such alterations may influence multiple cellular pathways, particularly those related to stress responses, inflammation, and the repair of DNA damage [53].

5.4 Transgenerational Effects

Perhaps most concerning are reports suggesting potential transgenerational effects of IONP-induced epigenetic modifications (4). While research in this area remains limited, some studies indicate that epigenetic changes resulting from parental IONP exposure may be transmitted to offspring, potentially affecting their development, susceptibility to disease, and response to environmental stressors [44].

Mechanisms of epigenetic inheritance may involve incomplete erasure of epigenetic marks during gametogenesis and early embryonic development, allowing some IONP-induced modifications to persist across generations [57]. Additionally, IONP exposure during critical periods of development, such as embryogenesis or gametogenesis, may induce more stable epigenetic alterations with potential transgenerational consequences [58]. These findings underscore the importance of comprehensive toxicological assessments that consider not only immediate genotoxic and cytotoxic effects but also potential long-term and transgenerational impacts mediated through epigenetic mechanisms (4).

VI. FACTORS AFFECTING TOXICITY

6.1 Dimensions, Morphology, and Surface Characteristics

The physicochemical attributes of iron oxide nanoparticles (IONPs) play a pivotal role in determining their toxicological behavior, with dimensions, morphology, and surface characteristics identified as key factors [7]. The size of the particles influences mechanisms of cellular uptake, distribution

within the body, and elimination processes. Smaller IONPs (less than 50 nm) typically show increased cellular internalization, yet they may also present heightened reactivity and toxicity due to their greater surface area relative to volume (8).

The morphology of IONPs further impacts their interactions with biological systems, influencing the efficiency of cellular uptake and the localization within cells. For example, rod-shaped IONPs may have distinct uptake dynamics and levels of cytotoxicity compared to their spherical counterparts, likely due to variations in the energetics of membrane wrapping during the endocytic process [59]. Additionally, the presence of sharp edges or irregular surfaces can exert mechanical stress on cellular membranes, which may contribute to their toxic effects (60). Surface characteristics, such as charge, hydrophobicity, and the presence of functional groups or coatings, significantly influence the interactions between IONPs and cells, as well as the resulting biological responses [9]. IONPs with a positive charge tend to exhibit improved cellular uptake due to electrostatic attractions with the negatively charged cell membrane; however, they may also demonstrate greater cytotoxicity compared to neutral or negatively charged particles [61]. Surface modifications, including the application of polymers (such as PEG, dextran, or chitosan), proteins, or lipids, can enhance biocompatibility, decrease aggregation, and alter cellular interactions, potentially reducing toxicity [10].

6.2 Dose-Response Relationships

The toxicity of iron oxide nanoparticles (IONPs) generally follows a dose-dependent pattern; however, some studies have identified non-linear relationships that complicate the assessment of associated risks (16). At lower doses, IONPs may trigger adaptive biological responses, such as the activation of antioxidant systems and enhancement of DNA repair mechanisms, which could provide a protective effect against subsequent exposures, a phenomenon known as hormesis {62}. In contrast, elevated doses can surpass the capacity of cellular defense systems, resulting in oxidative stress, DNA damage, and ultimately, cell death [27]. It is crucial to note that conventional mass-based dosing metrics may not adequately reflect the toxicity of IONPs, as smaller particles with larger surface areas can produce more significant biological effects per unit mass [63]. Therefore, alternative dosing metrics—such as particle number, surface area, and surface reactivity—should be considered, as they may provide a more accurate correlation with toxicological outcomes and enhance the rigor of risk assessments (64).

6.3 Cell/Tissue-Specific Responses

The biological impacts of iron oxide nanoparticles (IONPs) exhibit significant variability among different cell types and tissues, which can be attributed to variations in endocytic mechanisms, antioxidant capacities, rates of proliferation, and metabolic functions (16). For example, macrophages, known for their strong phagocytic capabilities, are likely to internalize IONPs more effectively than epithelial cells, which may lead to heightened oxidative stress and cytotoxic effects (3). Likewise, cells that divide rapidly may show increased vulnerability to IONP-induced genotoxicity, a consequence of their elevated rates of DNA synthesis and mitosis [17].

Furthermore, tissue-specific responses can be influenced by factors such as exposure conditions, patterns of nanoparticle accumulation, and the characteristics of the local microenvironment (5). The blood-brain barrier, for instance, generally serves to limit the entry of nanoparticles into the central nervous system, which may reduce the risk of neurotoxicity following systemic exposure (65). Nevertheless, IONPs that possess particular surface modifications or are of smaller dimensions may circumvent this barrier, thereby raising concerns about potential neurological repercussions (66).

6.4 Routes and Duration of Exposure

The pathways through which exposure occurs play a crucial role in determining the biodistribution, clearance, and toxicological effects of iron oxide nanoparticles (IONPs) [67]. Inhalation primarily impacts the respiratory system, with the possibility of systemic distribution following the translocation of particles across the alveolar-capillary membrane (68). In contrast, ingestion generally leads to gastrointestinal exposure, characterized by limited absorption but potential localized effects on the intestinal epithelium and gut microbiota [69]. When administered intravenously, IONPs are rapidly distributed throughout the system, with a tendency to accumulate in organs of the reticuloendothelial

system, such as the liver, spleen, and lymph nodes(70).

The duration of exposure also plays a significant role in shaping toxicological responses. Acute exposures can overwhelm cellular defense mechanisms, resulting in immediate cytotoxic effects, whereas chronic low-dose exposures may elicit adaptive responses or cumulative effects due to progressive particle accumulation and prolonged oxidative stress (5). Furthermore, repeated exposures may lead to either sensitization or tolerance, which complicates the evaluation of toxicological impacts [71].

VII. EXPERIMENTAL APPROACHES AND CHALLENGES

7.1 In Vitro Assessment Methods

In vitro methodologies serve as essential instruments for exploring the mechanisms of toxicity associated with iron oxide nanoparticles (IONPs) in a controlled environment. These methods present several advantages, including ethical considerations, cost efficiency, and reproducibility. A variety of cellular models have been utilized, such as immortalized cell lines, primary cells, co-cultures, and three-dimensional (3D) organoid systems, each with its own set of benefits and drawbacks (72)

Standard cytotoxicity assays, including MTT, LDH release, and neutral red uptake, yield valuable information regarding cell viability and membrane integrity following exposure to IONPs [73]. Nonetheless, these assays may be influenced by nanoparticle interactions through optical, chemical, or catalytic pathways, which could result in misleading positive or negative outcomes (71). Consequently, it is crucial to employ multiple complementary assays alongside appropriate controls to ensure reliable toxicity evaluations. Genotoxicity assessments typically utilize methods such as the comet assay to detect DNA strand breaks, the micronucleus test to identify chromosomal damage, and the Ames test to evaluate mutagenicity (3). Furthermore, advanced techniques like γ-H2AX immunostaining for detecting DNA double-strand breaks, the TUNEL assay for identifying apoptotic DNA fragmentation, and chromosomal aberration analysis offer a comprehensive understanding of genetic damage [17].

Assessments of oxidative stress involve measuring reactive oxygen species (ROS) levels using fluorescent probes (e.g., DCFH-DA, DHE), quantifying lipid peroxidation products (such as MDA and 4-HNE), and evaluating the status of the antioxidant system (including GSH levels and the activities of superoxide dismutase and catalase) [30]. Epigenetic analyses incorporate techniques such as bisulfite sequencing for examining DNA methylation, chromatin immunoprecipitation (ChIP) for assessing histone modifications, and RNA sequencing for profiling non-coding RNA expression (4).

7.2 In Vivo Models

In vitro studies yield valuable mechanistic insights; however, in vivo models provide a more holistic understanding of the toxicokinetics, biodistribution, and systemic effects of iron oxide nanoparticles (IONPs) within living organisms [74]. A variety of animal models, such as rodents, zebrafish, and Drosophila, have been utilized to evaluate the toxicity of IONPs across diverse exposure routes, dosages, and durations (66).

Assessments of in vivo genotoxicity generally involve the examination of different tissues for indicators of DNA damage, including comet assays, chromosomal abnormalities, and the formation of micronuclei [27]. Studies on biodistribution frequently employ methodologies such as magnetic resonance imaging (MRI), inductively coupled plasma mass spectrometry (ICP-MS), and histological analysis to elucidate patterns of IONP accumulation and identify potential target organs (70). Functional evaluations, which encompass biochemical parameter analysis, hematological assessments, histopathological examinations, and behavioral tests, provide a thorough understanding of systemic toxicity and organ-specific effects [75]. Long-term investigations are essential for exploring potential chronic effects, carcinogenicity, and transgenerational consequences, although such research remains relatively sparse concerning IONPs (4).

7.3 Analytical Techniques

Advanced analytical methodologies are essential for the characterization of iron oxide nanoparticles (IONPs) and the elucidation of their toxicity mechanisms [7]. Techniques such as transmission electron microscopy (TEM) and scanning electron

microscopy (SEM) yield comprehensive data regarding the size, morphology, and aggregation of the particles. Additionally, dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) provide valuable insights into the hydrodynamic size distributions of these nanoparticles in solution [9].

Surface characterization methods, including X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy (FTIR), and zeta potential measurements, deliver critical information about surface chemistry, functional groups, and charge, all of which significantly affect biological interactions (8). Furthermore, advanced mass spectrometry techniques, such as time-of-flight secondary ion mass spectrometry (ToF-SIMS) and matrix-assisted laser desorption/ionization (MALDI), facilitate in-depth analysis of protein corona formation and the interactions between biomolecules and nanoparticles.

Emerging methodologies, such as single-cell analysis, high-content screening, and various omics approaches (including transcriptomics, proteomics, metabolomics, and epigenomics), provide extensive insights into the molecular responses elicited by IONP exposure, potentially uncovering novel toxicity pathways and biomarkers [7]. The integration of these sophisticated analytical techniques with conventional toxicological evaluations significantly enhances our comprehension of the biological effects induced by IONPs and the mechanisms underlying these effects.

7.4 Standardization Issues

Despite notable progress in the field, nanotoxicology research continues to encounter significant obstacles related to standardization and reproducibility. Variability in the methods used for nanoparticle synthesis, characterization techniques, and experimental conditions hinders the ability to compare results across different studies and complicates the interpretation of findings [76]. Moreover, insufficient characterization of critical physicochemical properties, especially under conditions that mimic real-world exposure, diminishes the understanding of underlying mechanisms and impairs risk assessment efforts [63].

Dosimetry presents further complications, as conventional mass-based dose metrics may not adequately reflect the biological effects of nanomaterials. Alternative metrics, such as particle number, surface area, and surface reactivity, may provide a more accurate correlation with toxicological effects; however, they currently lack standardized measurement protocols [63]. Additionally, the effective doses that reach cells or tissues can differ from nominal doses due to factors such as nanoparticle aggregation, sedimentation, dissolution, and the formation of a protein corona, which complicates dose-response evaluations [77]. In response to these challenges, various international initiatives are working to develop standardized protocols for the characterization, dispersion, and toxicity assessment of nanomaterials. These efforts involve organizations such as the Organization for Economic Cooperation and Development (OECD), the International Organization for Standardization (ISO), and the National Cancer Institute's Nanotechnology Characterization Laboratory (NCL) [76]. The establishment of these standards, along with thorough reporting of experimental methodologies, is crucial for the advancement of nanotoxicology research and for facilitating informed risk assessments and regulatory decisions (72).

VIII. REGULATORY CONSIDERATIONS AND RISK ASSESSMENT

8.1 Current Regulatory Frameworks

The regulatory environment governing nanomaterials, particularly iron oxide nanoparticles (IONPs), is in a state of continuous development, reflecting advancements in scientific knowledge and improved risk characterization [78]. Presently, most regions regulate nanomaterials, including IONPs, under the established frameworks for traditional chemicals, albeit with certain modifications and specific guidance tailored to nanomaterials.

In the European Union, the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation includes provisions for nanomaterials, with recent updates mandating specific data requirements for the registration of these materials, such as comprehensive physicochemical characterization and toxicity evaluations (Rauscher et al., 2019). In a similar vein, the United States Food and Drug Administration (FDA) employs existing regulatory structures for products containing nanomaterials, taking into account the distinct

characteristics of nanomaterials during their safety evaluation [79].

Global organizations, such as the OECD and ISO, have established guidelines and technical standards for the safety assessment of nanomaterials; however, achieving complete harmonization across different jurisdictions remains an ongoing challenge [76]. For example, the OECD Working Party on Manufactured Nanomaterials (WPMN) has undertaken a comprehensive testing initiative to assess the relevance of current testing protocols for nanomaterials and to formulate specific guidance where necessary [80].

8.2 Challenges in Nanoparticle Risk Assessment

Despite advancements in regulatory frameworks, the assessment of risks associated with nanoparticles continues to encounter significant obstacles due to the distinctive characteristics of nanomaterials and existing knowledge deficiencies regarding their interactions within biological systems and the environment [81]. Conventional risk assessment methodologies, which are primarily tailored for traditional chemicals, may not sufficiently encompass the specific considerations pertinent to nanomaterials, thereby necessitating the development of modified approaches.

The evaluation of exposure presents unique difficulties, particularly due to the constraints of detection and characterization techniques for nanomaterials, especially within intricate environmental contexts [82]. Furthermore, the transformations that nanoparticles undergo—such as aggregation, dissolution, and surface modification—in both environmental and biological settings can significantly influence their toxicological profiles, thereby complicating the understanding of exposure-response dynamics [83].

Hazard characterization is similarly challenged, facing issues such as potential interference of nanoparticles with standard toxicity testing methods, variability in dosimetry practices, and the complexities involved in extrapolating findings from acute to chronic exposure scenarios. Additionally, the heterogeneity of nanomaterials, even within specific groups like iron oxide nanoparticles (IONPs), may hinder the ability to draw broad conclusions, necessitating assessments on a case-by-case basis [63]. Risk characterization must synthesize these intricate exposure and hazard factors, while also addressing uncertainties that stem from knowledge gaps and methodological constraints [81]. In light of these challenges, innovative risk assessment frameworks have been developed, which incorporate tiered strategies, high-throughput screening, computational modeling, and adverse outcome pathways to improve both efficiency and mechanistic insight.

8.3 Safety Guidelines

While comprehensive regulatory frameworks continue to develop, various guidelines and best practices have emerged to promote the safe handling, use, and disposal of nanomaterials, including IONPs [84]. These guidelines typically emphasize thorough physicochemical characterization, comprehensive toxicity assessment, and precautionary measures to minimize exposure and environmental release [81].

For occupational settings, guidelines recommend engineering controls (e.g., ventilation systems, closed processes), personal protective equipment, and workplace monitoring to reduce worker exposure . Similarly, guidelines for laboratory handling emphasize containment measures, waste management protocols, and worker training to minimize risk [85]. For biomedical applications, safety guidelines focus on thorough preclinical testing, including comprehensive physicochemical characterization, sterility assurance, endotoxin testing, and biocompatibility assessment following regulatory standards such as ISO 10993 for medical devices. Additionally, guidelines for environmental risk mitigation address potential release pathways, transformation processes, and ecotoxicological considerations throughout the product lifecycle [82]. While these guidelines provide valuable direction, they continue to evolve as scientific understanding advances and regulatory frameworks develop. Furthermore, implementation challenges persist, particularly for small enterprises with limited resources and expertise in nanotechnology safety assessment [78].

IX. FUTURE PERSPECTIVES

9.1 Research Gaps and Priorities

Despite significant advances in understanding IONP toxicity, several knowledge gaps persist, necessitating

targeted research efforts [7]. Long-term effects of chronic low-dose exposure remain inadequately characterized, particularly regarding potential carcinogenicity, neurodevelopmental impacts, and transgenerational effects mediated through epigenetic mechanisms . Similarly, interactions between IONPs and vulnerable populations, including those with pre-existing conditions, developing organisms, and the elderly, require further investigation. Mechanistic understanding would benefit from elucidating the complex interplay between direct and indirect genotoxicity pathways, clarifying the role of specific ROS species and oxidative DNA lesions, and characterizing the relationship between IONP-induced epigenetic modifications and functional outcomes [9]. Additionally, the contribution of IONP-protein corona interactions to biocompatibility, cellular uptake, and toxicity warrants deeper exploration, considering the dynamic nature of corona formation in complex biological environments. Environmental behavior and ecotoxicological impacts represent another priority area, including IONP transformation processes (aggregation, dissolution, surface modification), bioaccumulation potential, and effects on various ecological receptors and ecosystem functions [83]. Such research would enhance environmental risk assessment and inform sustainable nanomaterial design and application [86].

9.2 Novel Assessment Strategies

Addressing these research gaps necessitates innovative assessment strategies that enhance mechanistic understanding while improving efficiency and predictive capacity [87]. High-throughput screening approaches, utilizing automated systems and miniaturized assays, enable rapid evaluation of multiple endpoints across various nanoparticles, concentrations, and cell types, facilitating comprehensive toxicity profiling and structure-activity relationship development [88].

Advanced in vitro models, including co-cultures, three-dimensional organoids, and microfluidic "organ-on-a-chip" systems, better recapitulate physiological conditions and complex cell-cell interactions, potentially enhancing the predictive value of preclinical assessments [89]. Similarly, zebrafish embryos and Caenorhabditis elegans represent valuable alternative in vivo models, offering advantages in terms of rapid development, optical transparency, and ethical considerations . Omics approaches, including transcriptomics, proteomics, metabolomics, and epigenomics, provide comprehensive molecular insights into cellular responses to IONP exposure, potentially identifying novel biomarkers, toxicity pathways, and susceptibility factors [7]. Integration of these multi-omics data through systems biology approaches enhances mechanistic understanding and supports adverse outcome pathway (AOP) development, linking molecular initiating events to adverse outcomes at the organism level [79]. Computational approaches, including quantitative structure-activity relationship (QSAR) modeling, physiologically based pharmacokinetic (PBPK) modeling, and machine learning algorithms, complement experimental studies by predicting toxicological outcomes, extrapolating across species and exposure scenarios, and prioritizing nanomaterials for experimental testing . These approaches, collectively termed "in silico nanotoxicology," enhance efficiency and reduce animal testing while providing mechanistic insights [90].

9.3 Safer-by-Design Approaches

The acknowledgment of potential toxicity associated with iron oxide nanoparticles (IONPs) has led to an increased focus on safer-by-design methodologies. These methodologies prioritize safety considerations throughout the entire development process of nanomaterials, rather than depending solely on risk assessments conducted after development [91]. The objective of these approaches is to preserve or enhance advantageous properties while simultaneously minimizing possible adverse effects through deliberate design alterations.

One of the primary strategies for implementing safer-by-design principles in IONPs involves surface modifications. Various coatings have been shown to diminish the generation of reactive oxygen species (ROS), cellular uptake, and cytotoxicity, all while retaining essential functional characteristics [10]. For example, polymer coatings such as polyethylene glycol (PEG) form a hydrophilic layer that reduces protein adsorption and cellular recognition, which may lead to decreased uptake and related toxicity . Likewise, natural polymer coatings, including chitosan and dextran, improve biocompatibility and offer functional advantages such as colloidal stability

and targeted delivery [6]. Modifications to the core composition, such as doping with elements like zinc, manganese, or gadolinium, can modify magnetic properties while potentially decreasing the release of iron ions and the associated generation of ROS. Furthermore, controlling the size and shape of the particles can enhance functional performance while alleviating toxicological issues, as evidenced by research indicating reduced genotoxicity linked to specific morphologies and size ranges [7].

Green synthesis methods, which employ biological systems (such as plants and microorganisms) or environmentally benign reagents, present additional safer-by-design options. These methods can minimize the use of toxic precursors and harmful byproducts while potentially enhancing biocompatibility through the use of natural capping agents. Such approaches are consistent with sustainability principles and may effectively address safety concerns [92].

X. CONCLUSION

Iron oxide nanoparticles (IONPs) are exceptional materials with a wide range of applications in biomedicine, environmental remediation, and various industrial fields. Nonetheless, the potential adverse effects associated with these nanoparticles, particularly their genotoxicity linked to reactive oxygen species (ROS) generation and epigenetic alterations, underscore the need for thorough safety evaluations and risk management strategies.

This review has consolidated existing knowledge regarding the mechanisms of IONP-induced genotoxicity, emphasizing both direct interactions with DNA and cellular components, as well as indirect pathways that involve ROS production through Fenton reactions, mitochondrial dysfunction, and depletion of antioxidants. Furthermore, emerging data on IONP-induced epigenetic changes, such as modifications in DNA methylation, histone alterations, and variations in non-coding RNA expression, indicate potential long-term and possibly transgenerational effects that require further exploration.

The toxicological characteristics of IONPs are heavily influenced by their physicochemical properties, including size, shape, surface features, and core composition, which presents opportunities for designing safer alternatives that retain functionality while minimizing risks. Additionally, the conditions of exposure—such as dosage, duration, and route—significantly affect biological responses, complicating risk assessments but also offering pathways for risk reduction through controlled exposure. Despite notable advancements in research, there remain significant gaps in understanding chronic effects, identifying vulnerable populations, elucidating complex interactions between nanomaterials and biomolecules, and assessing ecotoxicological consequences. Addressing these gaps necessitates interdisciplinary strategies that incorporate advanced experimental models, omics technologies, computational approaches, and standardized protocols to improve mechanistic insights and predictive capabilities. As the applications of IONPs continue to grow, it is increasingly crucial to balance technological advancements with safety considerations.

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