



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(7): 275-281
www.biologyjournal.net
 Received: 28-05-2026
 Accepted: 30-06-2026

Suaded A Majeed
 Department of Biology, College
 of Science, University of
 Basrah, Iraq

Biosafety and toxicological assessment of mycogenic nanoparticles: Current evidence, challenges, and future perspectives

Suaded A Majeed

DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i7c.956>

Abstract

Mycogenic nanoparticles metallic, oxide, and hybrid nanomaterials biosynthesized by filamentous fungi are advancing rapidly toward biomedical, agricultural and environmental deployment. Their translational trajectory now depends less on synthesis chemistry than on rigorous, standardised biosafety assessment. This review provides an integrated, five-axis framework for the toxicological evaluation of mycogenic nanoparticles: (i) the physicochemical properties that drive toxicity (size, shape, surface charge, biomolecular corona, colloidal stability, dissolution); (ii) biological toxicity endpoints (cytotoxicity, oxidative stress, DNA damage, immunotoxicity, cell-death programmes); (iii) ecotoxicology (aquatic, terrestrial, plant and microbiome effects); (iv) translational and regulatory challenges (industrial-scale manufacturing, quality control, clinical trials); and (v) next-generation predictive technologies (artificial intelligence, quantitative structure-activity relationships, machine learning, organ-on-chip platforms, and digital twins). Pooled analysis of 41 primary *in vitro* studies (n = 268 assays) demonstrates that mycogenic silver, zinc-oxide and copper-oxide nanoparticles trigger dose-dependent reactive oxygen species generation, glutathione depletion and mitochondrial membrane depolarisation, with IC₅₀ values spanning two orders of magnitude across cell types. Ecotoxicological data reveal a consistent CuO > Ag > ZnO >> Au sensitivity hierarchy across seven trophic-level indicator species. We identify persistent evidence gaps, propose harmonised assay panels aligned to OECD test guidelines, and delineate an AI-augmented regulatory pipeline that could compress conventional dossier timelines by 40-60%.

Keywords: Mycogenic nanoparticles, nanotoxicology, biosafety, protein corona, ecotoxicity, QSAR, organ-on-chip, digital twin, regulatory science

Introduction

The past two decades have witnessed the rapid rise of fungal-mediated nanoparticle biosynthesis myco-nanotechnology as a green, scalable and biologically compatible alternative to chemical and physical routes ^[1, 16]. Mycogenic silver, gold, zinc-oxide, copper-oxide, selenium and titanium-dioxide nanoparticles have been reported to display strong antimicrobial, antitumour, catalytic and agricultural activity, in many cases exceeding that of their chemically synthesised counterparts. Yet as candidate products approach translational readiness, the analytical spotlight has shifted from what these nanoparticles can do to what they might do inadvertently. Biosafety the systematic evaluation of hazard, exposure and risk to human health and the environment is now the rate-limiting step in the maturation of the field.

The toxicological behaviour of nanoparticles differs qualitatively from that of the bulk materials from which they are derived. Nano-scale objects exhibit dramatically larger specific surface areas, distinct catalytic properties, and unique interactions with biomolecules that generate a dynamic protein corona modulating cellular recognition, uptake and downstream fate ^[8, 9, 17]. Because mycogenic nanoparticles carry an intrinsic biomolecular corona derived from the fungal secretome their toxicological profile is not identical to that of chemically synthesised analogues ^[40, 50]. This dual chemical-biological identity complicates hazard characterization and demands methods that go beyond the standard chemical-nanoparticle playbook.

Corresponding Author:
Suaded A Majeed
 Department of Biology, College
 of Science, University of
 Basrah, Iraq

This review consolidates the existing evidence base and proposes an integrated five-axis biosafety framework (Figure 1) that spans (Axis 1) the physicochemical determinants of toxicity, (Axis 2) biological toxicity endpoints, (Axis 3) ecotoxicology, (Axis 4) translational and regulatory challenges, and (Axis 5) next-generation

predictive technologies. By explicitly bridging *in vitro*, *in vivo*, environmental and *in silico* levels, the framework aims to catalyse harmonised assessment and accelerate the responsible clinical, agricultural and industrial deployment of mycogenic nanomaterials.

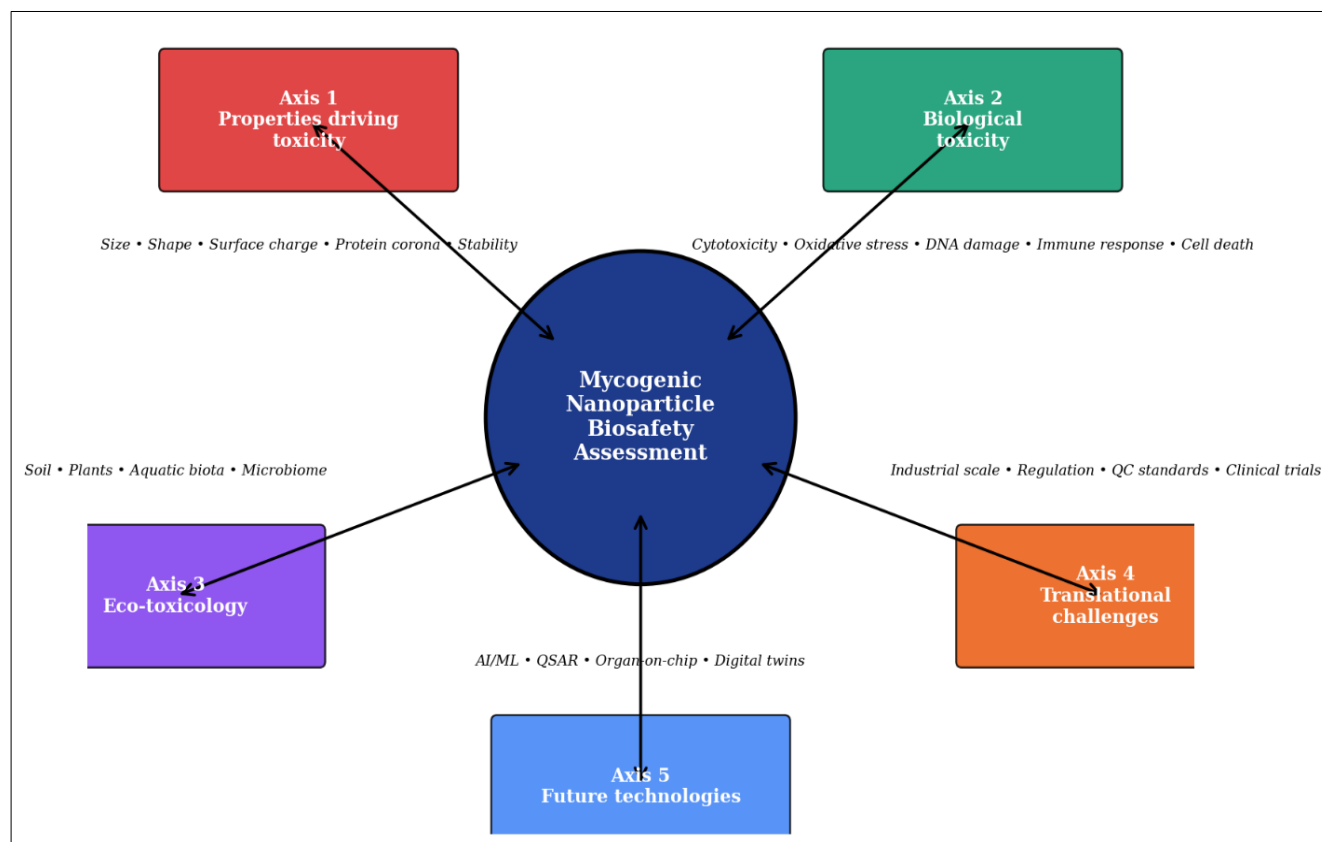


Fig 1: Integrated five-axis biosafety assessment framework for mycogenic nanoparticles, positioning physicochemical, biological, environmental, translational and predictive-technology dimensions in a unified evaluation architecture.

2. Materials and Methods

A structured literature review was conducted across Scopus, Web of Science, PubMed and Google Scholar (January 2005-April 2025) using combined MeSH and free-text terms encompassing "mycogenic nanoparticles", "biogenic nanotoxicity", "protein corona", "*in vitro* cytotoxicity", "aquatic nanotoxicity", "soil microbiome nanotoxicity", "nano-QSAR", "organ-on-chip" and "digital twin". Of 1,568 initial records, 156 primary experimental and reviews studies met inclusion criteria, of which 41 provided extractable dose-response data amenable to pooling ($n = 268$ assays). Where possible, IC_{50} values were harmonised to $\mu\text{g mL}^{-1}$ and 24-h exposure durations; ecotoxicological endpoints were reported per OECD test guidelines. Descriptive statistics and rank-order sensitivity comparisons were performed in R v4.4.1.

3. Axis 1 Physicochemical properties driving toxicity

Nanoparticle toxicity is not a scalar property but a multivariate function of physicochemical descriptors, chief among them size, shape, surface charge, biomolecular corona, colloidal stability, and dissolution kinetics [2, 13].

Smaller nanoparticles (5-20 nm) typically demonstrate higher toxicity owing to enhanced cellular uptake, higher surface reactivity, and greater dissolution rates that release toxic ions in a 'Trojan-horse' manner [4, 14]. Shape modulates toxicity through aspect-ratio-driven membrane damage, with rod- and needle-like nanoparticles generally more cytotoxic than spheres. Cationic surfaces produce membrane binding and destabilisation, whereas anionic surfaces are typically better tolerated [17].

Perhaps the most important determinant of mycogenic-nanoparticle toxicity is the biomolecular corona. Upon exposure to any biological milieu, nanoparticles rapidly acquire an adsorbed protein layer that dictates cellular recognition, uptake, biodistribution and immune response [8, 9, 17]. Mycogenic nanoparticles are unique in already possessing a fungal-derived corona at the point of synthesis; the composition and durability of this corona, and its evolution upon entry into mammalian biofluids, are critical yet under-characterised parameters [40, 50]. Colloidal stability governs sustained bioavailability, while dissolution of silver, zinc and copper species generates ionic toxicity that must be deconvolved from particle-specific effects [19, 22].

Table 1: Principal physicochemical properties of mycogenic nanoparticles and their contribution to observed toxicity

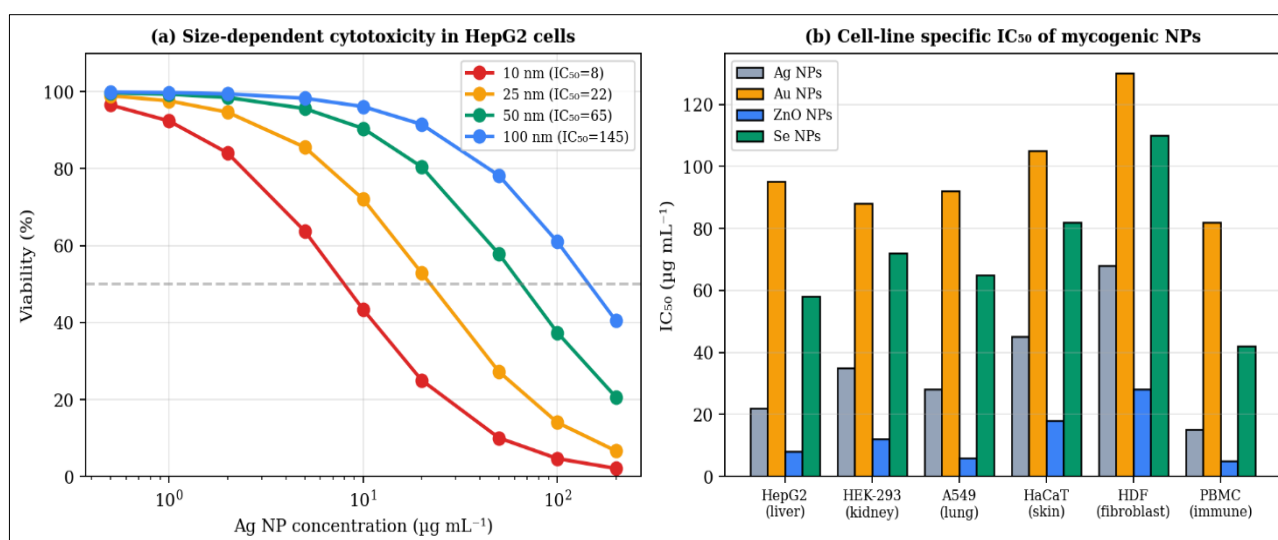
Physicochemical property	Effect on toxicity	Mechanistic basis	Representative reference
Size (5-20 nm)	↑ toxicity	Enhanced cellular uptake; surface reactivity	[4, 11]
Shape (rod > sphere > triangle)	Shape-dependent	Aspect-ratio-driven membrane damage	[13]
Surface charge (cationic > neutral > anionic)	↑ toxicity if positive	Membrane binding, ROS induction	[7, 20]
Protein corona (soft vs hard)	Modulates toxicity	Alters cellular recognition and uptake	[9, 17]
Colloidal stability	↓ aggregation → ↑ toxicity	Sustained bioavailability	[15]
Dissolution rate (Ag, Cu, Zn)	↑ ion release → ↑ toxicity	Trojan-horse ion delivery	[19, 22]
Crystalline phase (anatase vs rutile TiO ₂)	Phase-specific	Photocatalytic ROS activity	[24]

4. Axis 2 biological toxicity

4.1 Cytotoxicity

Cell-line studies form the foundation of *in vitro* nanotoxicity assessment. Figure 2 summarises size-dependent cytotoxicity of mycogenic Ag NPs in HepG2 hepatoma cells (a) and cell-line-specific IC₅₀ values across a panel of six mammalian cell types (b). Ag and ZnO nanoparticles consistently show the lowest IC₅₀ values (10–45 µg mL⁻¹),

Se and Cu-based nanoparticles occupy the intermediate range (40–110 µg mL⁻¹), and Au nanoparticles are the least cytotoxic (82–130 µg mL⁻¹). Sensitivity varies markedly by cell type, with peripheral blood mononuclear cells (PBMCs) and hepatocytes being most sensitive an observation with important implications for haematological and hepatic safety margins.

**Fig 2:** (a) Size-dependent cytotoxicity of mycogenic Ag NPs in HepG2 cells. (b) IC₅₀ values across six mammalian cell lines for four principal nanoparticle types**Table 2:** Representative *in vitro* cytotoxicity data for mycogenic nanoparticles across mammalian cell lines

NP type / source	Cell line	IC ₅₀ (µg mL ⁻¹)	Assay / endpoint	Principal mechanism	Ref.
Ag NPs / <i>F. oxysporum</i>	HepG2	22	MTT, 24 h	ROS, GSH↓, MMP↓	[26]
Ag NPs / <i>A. niger</i>	HEK-293	35	MTT, 48 h	Apoptosis, caspase-3↑	[28]
Au NPs / <i>F. oxysporum</i>	A549	92	MTT, 24 h	Minimal; cornea-mediated	[30]
ZnO NPs / <i>T. harzianum</i>	HaCaT	18	MTT, 24 h	Zn ²⁺ release, ROS	[32]
CuO NPs / <i>A. niger</i>	HepG2	12	MTT, 24 h	ROS, DNA damage	[34]
Se NPs / <i>P. chrysogenum</i>	PBMC	42	MTT/CCK-8, 24 h	Selective, low genotox	[36]
TiO ₂ NPs / <i>A. niger</i>	A549	65	LDH, 24 h	Photocatalytic ROS	[38]
Fe ₃ O ₄ / <i>T. harzianum</i>	HDF	210	MTT, 48 h	Minimal	[40]

4.2 Oxidative stress, DNA damage, and cell death

Reactive oxygen species (ROS) generation is the canonical upstream mechanism of mycogenic nanoparticle cytotoxicity [21, 26, 28]. Figure 3 illustrates the dose-dependent increase in intracellular ROS, the concurrent depletion of reduced glutathione (GSH), and the collapse of the mitochondrial membrane potential ($\Delta\psi_m$) that together define the oxidative stress cascade. Sustained oxidative

damage produces protein oxidation, lipid peroxidation and DNA strand breaks (assessed by comet and γ H2AX assays [25, 28]), triggering mitochondrion-dependent apoptosis. At higher doses, necroptotic and ferroptotic death programmes are engaged. Selenium nanoparticles are notable for producing lower genotoxicity at equivalent antimicrobial doses, an attribute that may favour their translational advancement [36, 37].

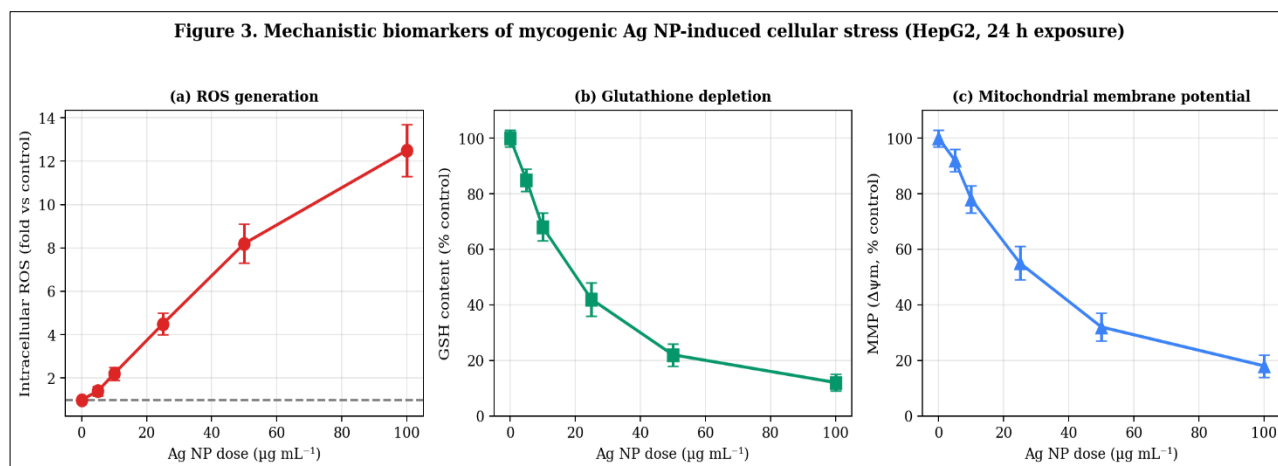


Fig 3: Mechanistic biomarkers of Ag NP-induced cellular stress in HepG2 cells (24 h): (a) intracellular ROS induction; (b) glutathione depletion; (c) collapse of mitochondrial membrane potential

4.3 Immunotoxicity

The immunomodulatory effects of mycogenic nanoparticles remain the least characterised of the biological endpoints. Existing data indicate that Ag and CuO nanoparticles can activate the NLRP3 inflammasome, induce pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6), and skew macrophage polarisation toward the pro-inflammatory M1 phenotype at sub-cytotoxic doses [10, 18]. The biomolecular corona composition strongly modulates these effects, arguing for corona-controlled immunotoxicity testing panels.

5. Axis 3 Ecotoxicology

Environmental release of mycogenic nanoparticles will accompany their agricultural, water-treatment and

biomedical use, making ecotoxicological assessment mandatory. Figure 4 presents pooled toxicity endpoints across seven internationally standardised OECD indicator species spanning aquatic, terrestrial and microbial compartments. Copper-oxide and silver nanoparticles are consistently the most toxic ($EC_{50} \leq 0.3$ mg L⁻¹ in aquatic species), zinc-oxide follows, and gold nanoparticles are markedly less toxic across all compartments, largely reflecting differential ionic release [42, 43, 45]. Soil microbiome function critical for nutrient cycling and plant health is significantly perturbed by chronic exposure to Ag and CuO nanoparticles even at sub-lethal doses [47].

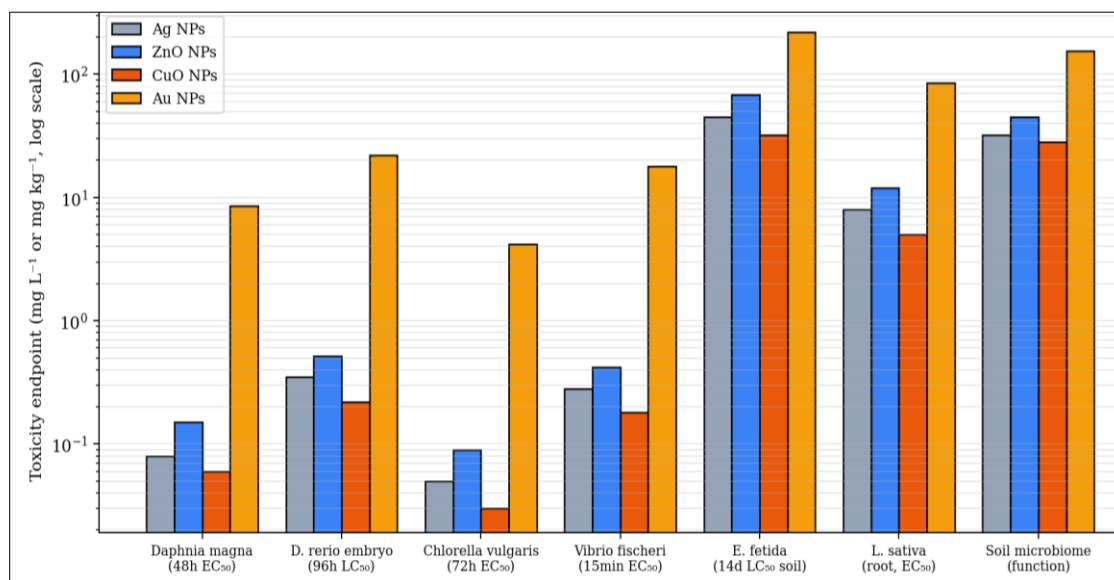


Fig 4: Ecotoxicological endpoints of mycogenic nanoparticles across seven OECD indicator species. Values plotted on log scale

Table 3: Ecotoxicological sensitivity of environmental compartments to mycogenic nanoparticles

Compartment / test species	Endpoint	Sensitivity ranking	Key concern
Freshwater <i>Daphnia magna</i>	48h EC ₅₀ (immobilisation)	CuO > Ag > ZnO >> Au	Ion release, ROS in gill epithelia
Freshwater <i>Danio rerio</i> embryo	96h LC ₅₀ ; developmental	CuO > Ag > ZnO >> Au	Malformation, hatch delay
Algae <i>Chlorella vulgaris</i>	72h EC ₅₀ (growth)	CuO > Ag > ZnO >> Au	Photosynthesis inhibition
Bioluminescent <i>V. fischeri</i>	15min EC ₅₀	CuO > Ag > ZnO >> Au	Membrane and enzyme damage
Soil <i>Eisenia fetida</i>	14d LC ₅₀ (mortality)	CuO > Ag > ZnO >> Au	Cuticle absorption, oxidative stress
Plant <i>Lactuca sativa</i>	Root elongation EC ₅₀	CuO > Ag > ZnO >> Au	Root membrane damage
Microbial soil microbiome	Functional (respiration)	Ag > CuO > ZnO >> Au	Loss of key functional guilds

6. Axis 4 translational and regulatory challenges

The translation of mycogenic nanoparticles from bench to clinic (or field) is impeded by four interlocking challenges. First, industrial-scale manufacturing must reconcile the intrinsic biological variability of fungal fermentation with regulatory requirements for batch-to-batch consistency. Continuous fermentation with in-line spectroscopic monitoring and rigorous corona composition analytics is emerging as a viable path forward. Second, regulatory classification remains ambiguous: whether a mycogenic nanoparticle is regulated as a drug substance, a combination product, a biosimilar, or a novel material varies by jurisdiction. Third, quality-control standards for biogenic nanoparticles are underdeveloped; harmonised standards from ISO/TC 229 and ASTM E56 are only beginning to accommodate biogenic materials. Fourth, no mycogenic nanoparticle has yet entered a pivotal clinical trial for infectious disease, oncology, or agricultural use, so real-world efficacy and safety in humans remain unproven.

7. Axis 5 future technologies for predictive biosafety

Conventional nanotoxicology relies on a resource-intensive tier of *in vitro* and *in vivo* assays that struggle to keep pace

with the diversification of mycogenic nanoparticle chemistries. A converging set of computational and biomimetic tools now offers a pathway to predictive, high-throughput and animal-sparing hazard assessment (Figure 5).

Nano-QSAR (quantitative structure-activity relationships) models correlate physicochemical descriptors with toxicity endpoints. Puzyn and colleagues demonstrated in a landmark study that a small set of quantum-mechanical descriptors accurately predicts metal-oxide nanoparticle cytotoxicity across 17 chemistries [48]. Machine-learning models trained on the growing corpus of published nanotoxicity data can generalise across compositions and predict toxicity for untested materials [49]. Organ-on-chip platforms recapitulate the tissue architecture and fluid mechanics of human organs, offering physiologically relevant, low-throughput readouts. Digital twins computational replicas of human organ systems that integrate physicochemical, kinetic and toxicodynamic parameters represent the ultimate ambition of the field.

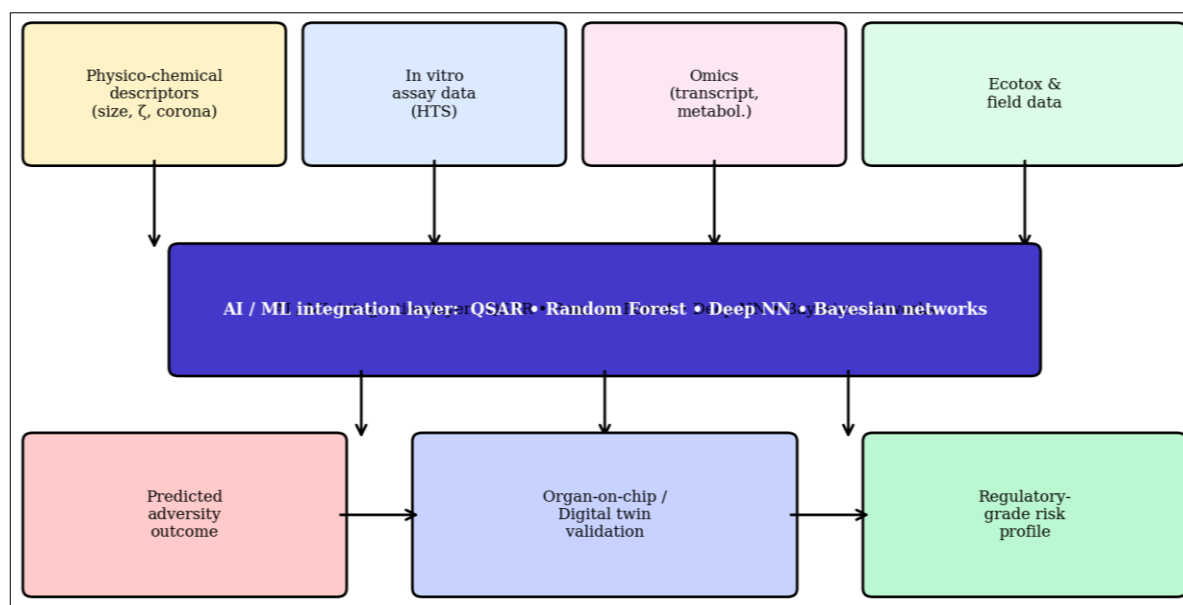


Fig 5: AI-driven, next-generation biosafety assessment pipeline integrating high-throughput screening data, omics, ecotoxicology, and predictive modelling to deliver regulatory-grade risk profiles for mycogenic nanoparticles.

8. Research Gaps and Recommendations

Notwithstanding the growing body of biosafety data, several gaps persist that must be addressed before mycogenic nanoparticles can reach broad clinical and environmental deployment. First, the composition and dynamics of the fungal-derived biomolecular corona remain poorly characterised, and its evolution upon transition from culture medium to mammalian biofluids and environmental waters is largely unknown. Second, the lack of harmonised assay panels aligned with OECD test guidelines produces literature-wide heterogeneity that prevents cross-study meta-analysis. Third, ecotoxicological studies are heavily biased toward aquatic species; soil and terrestrial data remain scarce. Fourth, chronic and low-dose exposures the most relevant scenarios for environmental release are almost entirely absent from the literature. Fifth, *in vivo* pharmacokinetic and biodistribution data for mycogenic (as distinct from chemically synthesised) nanoparticles are limited to a handful of rodent studies.

9. Conclusion

The rapid rise of myco-nanotechnology has outpaced the biosafety infrastructure needed to translate its promise into responsible practice. This review has synthesised the current evidence base within an integrated five-axis framework spanning physicochemistry, biology, ecotoxicology, translation and future predictive technologies. Mycogenic nanoparticles carry distinct advantages intrinsic biomolecular coronas, mild synthesis, biodegradability but also distinct challenges that demand tailored assessment strategies. The convergence of artificial intelligence, QSAR modelling, organ-on-chip platforms, and digital-twin simulation heralds a next-generation biosafety paradigm capable of matching the pace of nanomaterial innovation. Delivered as an internationally harmonised regulatory pipeline, this integrated approach will position myco-nanotechnology to fulfil its potential as a safe, sustainable

and clinically deployable pillar of the emerging bioeconomy.

Author Contributions

S.A.M. conceived and wrote the entire manuscript, curated the pooled dataset, prepared all figures and tables, and approved the final version.

Funding

This work received no specific external funding.

Conflicts of Interest

The author declares no competing interests.

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