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Review Article

One-Health Nanotoxicology at the Nano–Bio Interface: Cross-Taxa Multimodal Biomarkers of Nanoparticle and Metal Exposure and Toxicity Enzymatic, Mirna/Omics, Histopathological, Behavioral, and Edna Evidence

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Abstract

Nanoparticles and metals increasingly intersect with biological systems, demanding biomarkers that are mechanistically informative, field-deployable, and interpretable across species. Framed within a One-Health perspective, this literature review synthesizes evidence on the nano–bio interface from molecular to ecosystem scales. We first situate exposure pathways, environmental fate, and bioavailability highlighting agglomeration, protein corona dynamics, dissolution/redox cycling, and uptake routes that condition internal doses. We then map mechanistic cascades (oxidative stress, immune modulation, genotoxic/epigenetic regulation, tissue injury/repair) onto Adverse Outcome Pathways (AOPs) to clarify where biomarker families read out along the continuum from initiating events to organismal and population effects. Assay domains are examined comparatively: enzymatic/biochemical markers (e.g., CAT, SOD, GPx, LPO), miRNA and broader omics (transcriptomic/proteomic/metabolomic pathways), histopathology and digital pathology, behavioral/physiological endpoints, and environmental DNA (eDNA) biosurveillance. Cross-taxa synthesis spans aquatic vertebrates and invertebrates, amphibians/reptiles, and birds/mammals, distilling concordance/discordance patterns among endpoints and contexts. For multimodal inference, we review Weight-of-Evidence, multivariate panel construction (PCA/PLS/clustering), and probabilistic/Bayesian fusion with attention to calibration and uncertainty. Quality and standards are emphasized (controls, effect sizes, MIQE/FAIR/GLP elements) alongside nano/metal-specific interferences and key confounders (life stage, genotype, co-stressors, matrix effects, particle traits/metal speciation). Applications span aquaculture health, wildlife conservation, environmental compliance, and translational/clinical monitoring. We identify critical gaps—chronic low-dose and mixture exposures, under-studied taxa/ecosystems, longitudinal field realism—and propose minimal core panels for lab screening and field deployment, plus a staged roadmap for method harmonization, reference materials, and open data resources. Collectively, the review outlines a path to robust, cross-taxa biomarker architectures that strengthen nanotoxicology inference and One-Health decision-making.

Keywords: One Health; metal nanoparticles; nano–bio interface; Adverse Outcome Pathways (AOPs); oxidative stress biomarkers; environmental DNA (eDNA).

1. INTRODUCTION AND SCOPE

Engineered metal and metal-oxide nanoparticles (MNPs) including Ag, Au, ZnO, TiO_2, Fe_3O_4, CuO, and mixed-metal nanostructures are now integrated into medicine (drug delivery, imaging, antimicrobial coatings), agriculture (nano-fertilizers, pesticides), consumer products (textiles, cosmetics), and industrial processes. Their expanding use has increased human and animal exposure through occupational contact, medical administration, food chains, and environmental release (Zhang *et al.*, 2022; Ma *et al.*, 2024). After release, these particles rarely stay "pristine." They transform through aggregation, oxidation/reduction, sulfidation, and most importantly partial dissolution into metal ions, which can drive biological effects as strongly as (or more strongly than) the particle core (Soares & Solé, 2021; Wang *et al.*, 2024). These transformations begin at the nano–bio interface, the dynamic boundary where nanomaterial surfaces meet biomolecules and cells, shaping corona formation, uptake, distribution, and toxicity (Pulido-Reyes *et al.*, 2017; Acet *et al.*, 2024).

A One-Health framing is essential because human health, animal health, and ecosystem health are tightly linked. Metal-based nanoparticles designed for biomedical benefit may enter wastewater, alter microbial communities, bioaccumulate in aquatic organisms, and re-enter humans through diet or water—creating connected exposure loops across sectors (Wang *et al.*, 2024; Zhang *et al.*, 2025). Interface processes control hazard across species: proteins, lipids, polysaccharides, and natural organic matter rapidly adsorb to nanoparticle surfaces to form a "corona" that redefines biological identity and transport, and corona composition differs among human plasma, fish mucus, plant root exudates, or soil pore water (Acet *et al.*, 2024; Pinals & Rotello, 2020). In parallel, dissolution and metal speciation couple biomedical and ecological risks because ion release and redox/valence shifts depend on pH, salinity, ligands, and organic matter, which vary widely between oceans, freshwater ponds, soils, and intracellular compartments (Soares & Solé, 2021; Amin *et al.*, 2025). Finally, real-world exposure pathways are connected: aquatic systems receive wastewater and runoff, making algae, invertebrates, and fish key sentinels for risks that may later manifest in terrestrial animals and humans via food webs and shared environments (Čaloudová *et al.*, 2021; Kerin *et al.*, 2023).

Accordingly, this article synthesizes evidence from 2015–2025 to clarify how metal-based nanoparticles and their dissolved ionic forms behave at nano–bio interfaces and how these behaviors translate into cross-taxa outcomes relevant to One Health. The objectives are to explain how size, shape, surface coating/charge, and corona formation govern recognition and uptake across taxa and media; to determine when ions versus particulate cores dominate toxicity and how speciation/valence transitions alter bioavailability; to map shared mechanistic pathways such as oxidative stress, membrane injury, immune dysregulation, microbiome disturbance, and genotoxicity; to show how biological and environmental matrices reshape these effects; and to identify safer-by-design levers (coatings, alloying, dissolution control, greener synthesis) and risk metrics that reduce One-Health hazards without sacrificing function (Pulido-Reyes *et al.*, 2017; Soares & Solé, 2021; Zhang *et al.*, 2022; Xiao *et al.*, 2024; Wang *et al.*, 2024).

The scope covers representative taxa and contexts where MNPs are most active and interconnected: aquatic biota (algae/cyanobacteria, invertebrates, fish) exposed in water columns, sediments, and biofilms with clear trophic-transfer relevance; terrestrial biota (soil microbes/fungi, plants, terrestrial invertebrates, mammals) exposed via soils, aerosols, and food chains; biomedical systems (human cell lines, epithelial and immune barriers, microbiome-linked studies) where corona and dissolution govern both therapy and safety; aquaculture/livestock settings where nanoparticles enter via feeds, therapeutics, and water treatments; and environmental compartments (freshwater/marine waters, soils, wastewater pathways) that connect the above domains over time (Čaloudová *et al.*, 2021; Khan *et al.*, 2024; Wang *et al.*, 2024).

Terminology is used consistently. "Nanomaterials/nanoparticles" refer to engineered materials with at least one dimension ~1–100 nm, including aggregates/agglomerates that retain nanoscale surface reactivity; this review prioritizes metal and metal-oxide nanoparticles, referencing other classes only for contrast (Zhang *et al.*, 2022; Ma *et al.*, 2024). "Surface coating" denotes organic or inorganic shells (e.g., citrate, PVP, PEG, silica, sulfidation layers) that modify charge, steric stabilization, and dissolution kinetics (Zhang *et al.*, 2022; Xiao *et al.*, 2024). "Dissolution" denotes partial conversion of particles into solvated ions, controlled by size, crystal facets/defects, coating permeability, and matrix chemistry; acidic conditions or strong ligands often enhance ZnO/CuO ion release, whereas salinity or sulfidation can suppress free-ion activity via aggregation or insoluble surface phases (Soares & Solé, 2021; Amin *et al.*, 2025). "Metal speciation/valence" includes oxidation state (e.g., Fe^{2+}/Fe^{3+}, Cu^{+}/Cu^{2+}), free versus complexed ions, and nano-surface redox transformations that govern transport and reactivity; hence, particle–ion co-exposure is treated as a coupled system (Soares & Solé, 2021; Wang *et al.*, 2024). "Exposure matrices" are grouped into biological matrices (serum/plasma, mucus, gut fluids, plant exudates, intracellular milieus), environmental matrices (variable-chemistry waters, sediments, soils, wastewater), and agri-feed matrices that precondition particles before uptake (Khan *et al.*, 2024; Wang *et al.*, 2024). The review emphasizes chronic and sub-lethal, realistically conditioned exposures, and de-emphasizes studies lacking adequate nanomaterial characterization (Pulido-Reyes *et al.*, 2017; Zhang *et al.*, 2022).

2. Conceptual Framework: Nano–Bio Interface & One-Health

This review uses a single integrated framework in which the nano–bio interface acts as the main translation layer that converts metal nanoparticle and metal-ion properties into biological and ecosystem outcomes. When metal or metal-oxide nanoparticles enter real media such as blood or serum, gut fluid, fish mucus, plant exudates, soil pore water, or wastewater, they rapidly adsorb biomolecules and natural organic matter, producing a dynamic surface coating often termed the protein corona or eco-corona (Sun *et al.*, 2024; Sarimov *et al.*, 2025; Samal *et al.*, 2025). The corona changes with time and depends on particle size, surface curvature, charge, coating chemistry, and the surrounding matrix. It therefore becomes the effective biological identity that governs stability, transport, cellular recognition, and uptake across organisms and environments (Ahmadi *et al.*, 2025; Sun *et al.*, 2024).

Mechanistically, the framework follows a causal cascade that begins with physicochemical traits and ends with ecosystem outcomes. Core composition (for example Ag, ZnO, CuO, Fe-oxides), particle size and shape, crystallinity, surface ligands, and redox or dissolution potential determine how quickly nanoparticles aggregate, age, oxidize or sulfidize, and dissolve into ions (Hedberg *et al.*, 2019; Chakraborty *et al.*, 2025; Islam *et al.*, 2025). Dissolution matters for One-Health reasoning because released ions such as Ag^{+}, Zn^{2+}, and Cu^{2+} can be more mobile and sometimes more toxic than the particle core, and their bioavailability depends on speciation and valence that shift with pH, salinity, and ligand chemistry in different habitats and inside cells (Hedberg *et al.*, 2019; Islam *et al.*, 2025). The corona and these transformations then control transport and barrier interactions, including whether particles remain suspended or settle, and whether they cross gills, gut epithelium, skin, placental-like barriers, or plant roots (Havelikar *et al.*, 2024; Djanaguiraman *et al.*, 2024).

At the cellular scale, corona-mediated recognition determines uptake routes (often endocytosis), intracellular trafficking, and local ion release in acidic compartments such as lysosomes (Stanco *et al.*, 2024; Havelikar *et al.*, 2024). These proximal processes consistently converge on a conserved set of mechanisms across taxa: reactive oxygen species generation and oxidative stress, inflammation and immune signaling changes, mitochondrial dysfunction, membrane injury, and downstream genotoxic or proteotoxic damage (Bengalli *et al.*, 2025; Kose *et al.*, 2023; Sleiman *et al.*, 2024). Under chronic or repeated exposure, these cellular perturbations scale upward to tissue-level injury and dysfunction (for example intestinal barrier weakening, gill or liver pathology, altered immune capacity) and then to organismal effects such as impaired growth, reproduction, behavior, and disease resistance (Kose *et al.*, 2023; Stanco *et al.*, 2024; Havelikar *et al.*, 2024). When exposure is widespread or trophically transferred, organism-level effects accumulate into population declines and community shifts, especially in aquatic systems that receive and redistribute large fractions of released metal nanomaterials (Islam *et al.*, 2025; Havelikar *et al.*, 2024).

Adverse Outcome Pathways (AOPs) are used as the organizing scaffold to make these cross-scale links explicit and comparable. An AOP connects a molecular initiating event at the nano–bio interface to measurable key events, and finally to an adverse outcome at organism or population level (Bengalli *et al.*, 2025; Kose *et al.*, 2023). For metal nanomaterials, oxidative stress, inflammation, and cytotoxicity repeatedly emerge as hub key events that can lead to barrier disruption, reproductive and developmental toxicity, neuro-immune effects, or altered survival (Bengalli *et al.*, 2025; Sleiman *et al.*, 2024; Stanco *et al.*, 2024). Aligning evidence through AOPs allows biomarker panels to be placed at shared key events rather than being restricted to a single species. For instance, antioxidant imbalance, pro-inflammatory cytokines, mitochondrial-injury markers, and DNA-damage signals can be tracked in human cells, fish tissues, soil invertebrates, or plants to situate each taxon along the same causal chain (Kose *et al.*, 2023; Djanaguiraman *et al.*, 2024). This AOP alignment also supports safer-by-design decisions by identifying interface properties, such as coatings that slow dissolution or reduce corona-driven uptake, that interrupt early key events before irreversible outcomes develop (Hedberg *et al.*, 2019; Bengalli *et al.*, 2025).

One-Health integration is layered on top of the AOP-anchored cascade. One Health is formally defined as an integrated approach that balances and optimizes the linked health of people, animals, and ecosystems within shared environments (Centers for Disease Control and Prevention [CDC], 2025; World Health Organization [WHO], 2025). In this review, One-Health integration means interpreting each key event and adverse outcome simultaneously as a human-health indicator (barrier integrity, immune and oxidative-stress biomarkers in human-relevant models), an animal-health indicator (parallel stress and fitness effects in aquatic and terrestrial fauna, including aquaculture species), and an environmental indicator (microbial function, primary productivity, trophic transfer, and community-level change). Such co-interpretation is necessary because metal nanoparticles and their ions move through connected exposure networks; medical, agricultural, and industrial sources feed environmental compartments, which then feed animals and humans through water, soil, and food chains (Hill *et al.*, 2024; Open Research Europe, 2025).

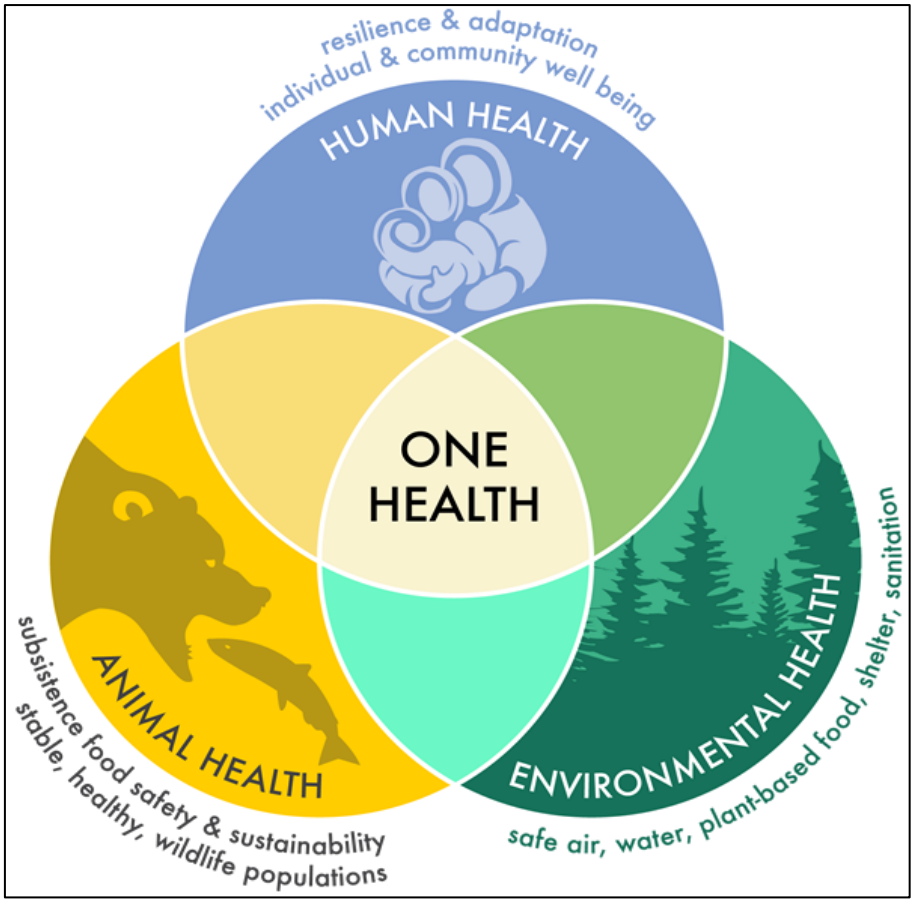

Figure 1: One-Health Triad Linking Human, Animal, and Environmental Health

This Venn-style One-Health diagram visualizes the integration core of our conceptual framework: health outcomes cannot be separated across humans, animals, and ecosystems. It supports the cross-scale logic where nano–bio interface events in one domain (e.g., aquatic biota) propagate through exposure networks to others (e.g., food-web transfer to humans). In our AOP-anchored cascade, this figure represents the final integrative layer that aligns biomarkers and adverse outcomes across all three compartments.

3. Exposure Pathways, Environmental Fate, and Bioavailability

Metal and metal-oxide nanoparticles enter One-Health systems through several overlapping source streams. Industrial manufacturing and downstream processing release nanoparticles to air and wastewater via abrasion, high-temperature processes, and effluent discharges, making inhalation and occupational contact major human exposure routes (Bocca *et al.*, 2023; El-Kalliny *et al.*, 2023). In medicine, these nanoparticles are intentionally administered in diagnostics, imaging, drug delivery, wound dressings, and antimicrobial coatings; after use, they can be excreted or washed into municipal wastewater, linking clinical benefit to environmental loading (Ma *et al.*, 2024; Wang *et al.*, 2024). Consumer products such as cosmetics, textiles, and surface sprays contribute chronic low-dose release through washing, wear, and disposal, and these releases often converge in aquatic sinks (Tran *et al.*, 2024; Wang *et al.*, 2024). Agriculture adds a further layer: nano-enabled fertilizers, pesticides, and food-packaging materials introduce particles to soils and crops, while sludge-amended fields can re-introduce particles originally sourced from urban wastewater (Devra *et al.*, 2022; Afzal *et al.*, 2024). Aquaculture and veterinary contexts are increasingly important because nanoparticles are used in feeds, disease control, and water treatments; these applications place particles directly into ponds and coastal systems where farmed species and non-target biota co-experience exposure (Khan *et al.*, 2024; Tran *et al.*, 2024).

After release, environmental fate is governed less by the pristine particle than by transformation processes occurring in real matrices. Aggregation and agglomeration often begin within minutes to hours as ionic strength and divalent cations screen surface charges, shifting particles from stable colloids to settled phases in sediments or soils (Amde *et al.*, 2017; Arienzo *et al.*, 2022). Simultaneously, biomolecules and natural organic matter form eco-coronas that can either stabilize particles against aggregation or promote hetero-aggregation with clays and biofilms; corona composition also changes with season, salinity, and biological activity, so fate is dynamic rather than fixed (Khort *et al.*, 2022; Chakraborty, 2022). Dissolution into metal ions is

another central driver of fate and hazard. Metal-oxide particles such as ZnO and CuO can dissolve rapidly in acidic or ligand-rich waters and even faster inside organisms, whereas coatings, sulfidation, or aging can slow dissolution and reduce free-ion activity (Amde *et al.,* 2017; Avramescu *et al.,* 2022). Redox cycling further modifies both particles and ions, especially for transition-metal systems where surface oxidation states shift with oxygen availability, light, or microbial mediation; these shifts alter speciation and reactivity, sometimes converting a relatively inert surface into a catalytic ROS-producing interface (Amde *et al.,* 2017; Wang *et al.,* 2024). Together, aggregation, corona dynamics, dissolution, and redox aging determine where particles accumulate, how long they persist, and whether exposure occurs mainly as particles, ions, or mixed forms (Arienzo *et al.,* 2022; Tran *et al.,* 2024).

Uptake routes mirror these transformation outcomes and vary by taxon and habitat. In aquatic organisms, gills and skin are major portals for waterborne particles and ions; their large surface area and thin epithelia allow both direct adsorption and endocytic uptake, particularly for smaller or corona-stabilized nanoparticles (Amde *et al.,* 2017; Wang *et al.,* 2024). Dietary uptake through the gut is equally important, especially when particles settle into sediments, bind to biofilms, or accumulate in prey; gut lumen chemistry can promote further dissolution, and species-specific gut anatomy changes the effective dose reaching epithelial cells (van der Zande *et al.,* 2020; Wang *et al.,* 2024). In terrestrial animals and humans, ingestion and inhalation dominate. Inhalation is the primary occupational pathway for airborne engineered nanoparticles, with deposition in the respiratory tract followed by macrophage interaction, translocation across alveolar barriers, and potential entry to systemic circulation (Bocca *et al.,* 2023; El-Kalliny *et al.,* 2023). Ingestion occurs via contaminated water, food-chain transfer, or intentional intake through nano-enabled products; intestinal barrier models show that mucus interactions and tight-junction status modulate particle crossing and local ion release (Ritarossi *et al.,* 2025; van der Zande *et al.,* 2020). Plants primarily take up metal nanoparticles through roots from soil pore water or through foliage when particles deposit from aerosols or sprays; root exudates and rhizosphere microbes can reshape aggregation and dissolution before uptake (Afzal *et al.,* 2024; Djanaguiraman *et al.,* 2024). Once internalized, intracellular trafficking usually proceeds via endosomes and lysosomes, with acidic lysosomal conditions often accelerating dissolution and producing high local ion concentrations that drive oxidative stress and organelle injury (Avramescu *et al.,* 2022; Havelikar *et al.,* 2024).

Bioavailability, defined here as the fraction of particle-derived metal that can interact with biological targets, is context-dependent rather than intrinsic. Across taxa, smaller primary size and higher surface area generally increase bioavailability by enhancing both membrane contact and dissolution rates, but this effect can be reversed if strong aggregation limits transport to target surfaces (Amde *et al.,* 2017; Khort *et al.,* 2022). Surface coatings can reduce bioavailability by sterically blocking interactions and slowing dissolution, or increase it by stabilizing dispersions and promoting receptor-mediated uptake through corona reshaping (Khort *et al.,* 2022; Havelikar *et al.,* 2024). Matrix chemistry is equally decisive: low pH, high chloride or sulfide, and abundant organic ligands shift speciation toward complexes that either suppress or amplify free-ion exposure, depending on metal type (Avramescu *et al.,* 2022; Amde *et al.,* 2017). Habitat-specific factors create predictable patterns. Estuarine and marine salinity often accelerates aggregation and sulfidation, reducing waterborne particle bioavailability but increasing sediment-associated exposure. Freshwaters with high dissolved organic matter may stabilize colloids and prolong gill exposure. Soils rich in clays and humics tend to immobilize particles yet can enhance plant root exposure through rhizosphere dissolution gradients (Arienzo *et al.,* 2022; Tran *et al.,* 2024; Afzal *et al.,* 2024). Biological traits also matter, including barrier thickness, mucus composition, feeding strategy, and microbiome activity, which together control whether exposure is dominated by particles, ions, or their combination (van der Zande *et al.,* 2020; Wang *et al.,* 2024). In One-Health terms, these determinants explain why a nanoparticle appearing safe in a controlled biomedical context may become more bioavailable and hazardous after environmental transformation, or why a particle that seems inert in seawater can still generate ionic pulses inside organisms after ingestion (Tran *et al.,* 2024; Havelikar *et al.,* 2024).

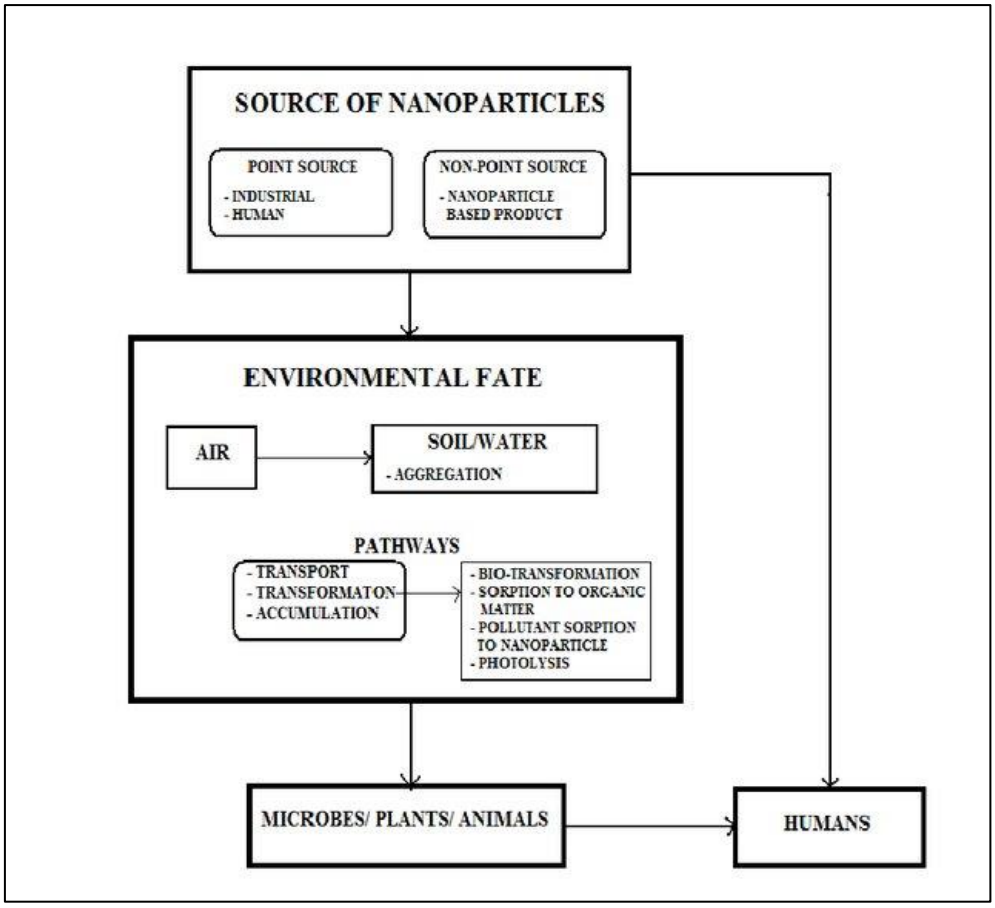

Figure 2: Sources, Environmental Fate, and Exposure Pathways of Metal Nanoparticles Across One-Health Systems

This schematic summarizes how nanoparticles released from point sources (industrial/medical) and non-point sources (consumer/agro-aquaculture products) move into air, soil, and water, then undergo transport and transformation. It highlights key fate processes central to bioavailability, including aggregation in soils/waters, inter-media transport, and trophic transfer to biota and ultimately humans. In your framework, this figure anchors the "exposure–fate–uptake" part of the cascade that precedes nano–bio interface interactions and AOP key events.

4. Biomarker Families and Assay Methodologies

Biomarkers are the practical readouts that locate organisms along the adverse outcome pathway chain described earlier. Because metal nanoparticles generate mixed particle–ion exposures and their effects depend strongly on medium chemistry and organism traits, robust One-Health inference needs converging evidence from biochemical, molecular, histological, behavioral, and ecosystem-surveillance layers rather than a single marker family (Damasco *et al.*, 2020; Bengalli *et al.*, 2025).

Enzymatic and biochemical markers are the most established early-warning tools in metal-nanoparticle research. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and related systems (GST, GR, reduced/oxidized glutathione pools) are widely assessed because nanoparticle surfaces and released metal ions frequently induce reactive oxygen species formation and redox imbalance across taxa (Do *et al.*, 2024; Correia *et al.*, 2025). Lipid peroxidation indices such as malondialdehyde (MDA) or TBARS, along with protein carbonyls, complement enzyme data by indicating membrane and macromolecular damage (Do *et al.*, 2024; Batir-Marin *et al.*, 2025). These assays are typically colorimetric or fluorometric and must be normalized to total protein, tissue mass, or cell number, with exposure time clearly reported to enable cross-study comparison (Do *et al.*, 2024). …Therefore, matched medium controls and nanoparticle-only blanks are necessary to separate true biology from assay artifacts (Correia *et al.*, 2025; Do *et al.*, 2024). Recent studies combining Ag nanoparticle exposure with oxidative-stress panels in *Drosophila* and mammalian cell lines illustrate this pattern, showing coordinated shifts in CAT, SOD, GPx, glutathione status, and lipid peroxidation under carefully controlled assay conditions (Rafique *et al.*, 2025a, 2025b). Interpretability requires caution: baseline enzyme activities differ among tissues, developmental stages, diets, and temperature or salinity regimes; additionally, some nanoparticles can interfere

optically or catalytically with assay reagents. Therefore, matched medium controls and nanoparticle-only blanks are necessary to separate true biology from assay artifacts (Correia *et al.,* 2025; Do *et al.,* 2024).

MicroRNA markers and broader omics approaches extend the biochemical layer by revealing upstream regulation and pathway architecture. MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate stress, immune, endocrine, and metabolic responses; they are increasingly used as sensitive indicators in fish and other models because their expression often shifts before visible pathology appears (Raza *et al.,* 2022; Bhat *et al.,* 2024). Platforms include targeted RT-qPCR panels for known stress-linked miRNAs as well as discovery small-RNA sequencing, both requiring stable reference miRNAs or spike-in controls and careful correction for extraction and batch effects (Raza *et al.,* 2022). Transcriptomics (RNA-Seq), proteomics (LC-MS/MS), and metabolomics or lipidomics (LC-MS, GC-MS, NMR) have become central "nanotoxicomics" tools for metal nanoparticles, enabling unbiased detection of perturbed pathways such as oxidative stress, apoptosis, metal-handling proteins, immune signaling, and altered bioenergetics (Shin *et al.,* 2018; Abdelkader *et al.,* 2023; González-Vega *et al.,* 2025). Multi-omics integration strengthens causal inference by linking gene regulation to protein effectors and metabolic end-states, but demands stricter controls: randomized processing, technical replicates, negative extraction controls, and explicit batch correction are required so pathway signals are not misattributed to matrix-driven artifacts (Hayes *et al.,* 2024; Abdelkader *et al.,* 2023). Omics results are most interpretable when mapped onto adverse outcome pathway key events, which allows candidate biomarkers to be compared and reused across species and settings (Bengalli *et al.,* 2025; González-Vega *et al.,* 2025).

Histopathology and histochemistry provide the tissue-level bridge between molecular key events and organismal outcomes. In metal-nanoparticle exposures, common lesions include epithelial lifting and lamellar fusion in gills, hepatocyte vacuolation or necrosis in liver, renal tubular degeneration, and inflammatory infiltrates, typically coinciding with oxidative-stress biomarkers (Rana *et al.,* 2024; Damasco *et al.,* 2020). Standard workflows use semi-quantitative lesion scoring on blinded slides to reduce observer bias, and include attention to fixation shrinkage, sectioning chatter, staining variability, and nanoparticle pigments or precipitates that can mimic disease (Rana *et al.,* 2024). Digital pathology is emerging as a reliability upgrade: whole-slide imaging plus automated or AI-assisted quantification can measure lesion area, cell density, or fibrosis markers more reproducibly than manual scoring, but only when training data are diverse and inter-rater calibration is documented (Rana *et al.,* 2024).

Behavioral and physiological endpoints translate tissue injury into fitness-relevant outcomes. Sublethal changes such as reduced swimming performance, altered feeding, impaired predator avoidance, disrupted reproduction or social behavior, and shifts in anxiety-like or learning responses are repeatedly observed for metal nanomaterials and often occur at concentrations below those causing mortality (Ford *et al.,* 2021; Ferreira *et al.,* 2023). These endpoints are ecologically meaningful because they directly affect survival and reproductive success, creating a clear link to population-level consequences in the One-Health cascade (Ford *et al.,* 2021). Reliable behavioral assays require standardized photoperiod, temperature, arena geometry, and observer blinding; they should be interpreted alongside internal dose or bioaccumulation measures, since weak behavioral effects can also reflect low bioavailability rather than true safety (Ford *et al.,* 2021; Ferreira *et al.,* 2023).

Environmental DNA (eDNA) and biosurveillance biomarkers extend monitoring beyond individuals to populations and communities. eDNA assays detect DNA fragments shed into water or soil, enabling non-invasive tracking of biodiversity change, invasive species spread, and community turnover under nanoparticle stress regimes (Johnsen *et al.,* 2020; Çevik *et al.,* 2025). Targeted qPCR or ddPCR quantify specific taxa, while metabarcoding or shotgun sequencing can profile entire communities; all designs require strict contamination control using field blanks, filtration blanks, extraction negatives, and no-template PCR controls (Johnsen *et al.,* 2020; Yoon *et al.,* 2025). Because eDNA transport and decay are affected by temperature, UV, microbes, and hydrodynamics, abundance inference is strongest when paired with occupancy or state-space models that include detection probability and decay, rather than treating non-detection as true absence (Johnsen *et al.,* 2020; Chang *et al.,* 2025). Within One Health, eDNA provides the ecosystem-scale early-warning layer that can be aligned with organismal and cellular biomarkers to detect community disruption before irreversible ecological change occurs (Çevik *et al.,* 2025).

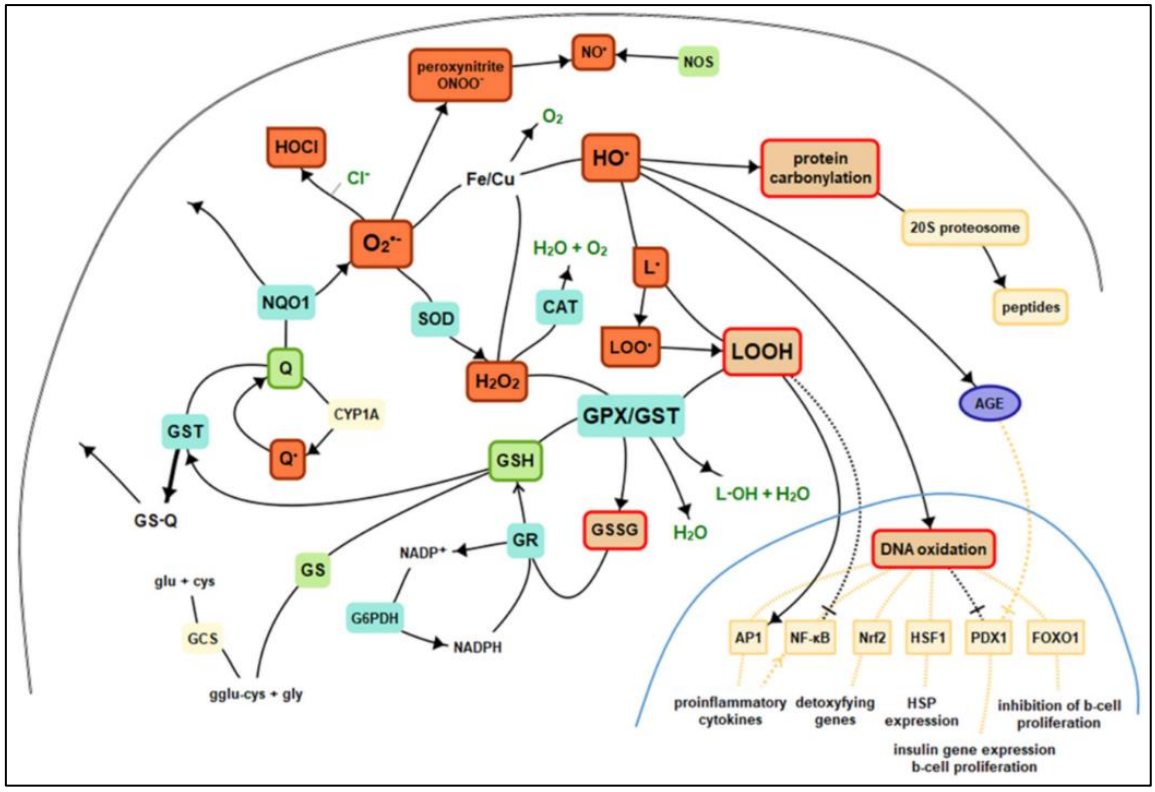

Figure 3: Oxidative-Stress Biomarker Network Centered on CAT, SOD, and GPx

This diagram maps the core enzymatic and biochemical biomarkers used in your framework, showing how superoxide and hydrogen peroxide are detoxified by SOD, CAT, and GPx/GST, with downstream lipid and protein oxidation as injury readouts. It visually supports why CAT, SOD, GPx, GSH/GSSG balance, and lipid-peroxidation markers are treated as early AOP key-event indicators across taxa. In your One-Health lens, the same network underpins biomarker interpretation in human cells, fish, soil biota, and plants exposed to metal nanoparticles and their ions.

5. Cross-Taxa Evidence Synthesis

Evidence from 2015 to 2025 shows that metal and metal-oxide nanoparticles produce a partly shared, partly taxon-specific toxicity signature, shaped by nano–bio interface transformations and ion release. Aquatic vertebrates, especially teleost fish, remain the most ecologically and translationally informative vertebrate models because they integrate real environmental exposure routes with vertebrate organ systems comparable to mammals. In fish, gills and gut are primary portals, while liver, kidney, brain, and gonads are frequent accumulation and injury targets. Oxidative-stress panels (CAT, SOD, GPx, glutathione systems, lipid peroxidation), together with hematology, serum biochemistry, and organ histopathology, consistently detect early key events that map onto adverse outcome pathways for Ag, ZnO, CuO, and other metal nanomaterials (Kose *et al.*, 2023; Zeid *et al.*, 2025; Zahran *et al.*, 2025). Strengths of fish panels include multi-organ resolution and linkage to fitness endpoints such as growth, reproduction, and behavior, but limits remain: baseline enzyme activities vary with temperature, salinity, diet, and life stage, and nanoparticle dissolution can make it hard to separate particle-specific from ion-driven effects without parallel ion controls and speciation measurements (Kose *et al.*, 2023; Formicki *et al.*, 2025).

Aquatic invertebrates, led by Daphnia species, provide high-throughput and high-sensitivity screening that complements fish studies. Their short generation time and clonal reproduction allow robust dose–response mapping for survival, molting, reproduction, and swimming behavior, often at concentrations lower than those that produce clear vertebrate pathology (Willems *et al.*, 2024). Daphnia are also valuable for mechanistic work on eco-coronas and particle aging because they are exposed directly in water columns where aggregation, corona dynamics, and dissolution control internal dose (Chakraborty, 2022). These invertebrate assays are a strong early-warning layer for ecosystems, but they can over-represent waterborne pathways and under-capture longer-term organ remodeling that vertebrates reveal (Willems *et al.*, 2024).

Model insects such as Drosophila melanogaster extend cross-taxa synthesis into a genetically tractable whole-organism system positioned between ecotoxicology and biomedicine. Drosophila studies show that Ag and CuO nanoparticles can disrupt intestinal barriers, alter hemocyte immunity, and induce DNA damage, effects that often track with oxidative-

stress shifts but may appear even when classic enzyme panels are modest. This highlights the value of combining biochemical and genotoxic readouts (Alaraby *et al.*, 2019; Tagorti *et al.*, 2025). The throughput of Drosophila, plus conserved stress and immune pathways, makes it a practical bridge for identifying mechanisms likely to generalize to vertebrates, while still reflecting organism-level exposure complexity (Alaraby *et al.*, 2019).

Beyond these core models, amphibians and reptiles provide insight into developmental and skin-barrier sensitivities that are not fully captured by fish or invertebrates. Amphibian nanomaterial ecotoxicology repeatedly reports oxidative stress, developmental delay, and immune disruption in larvae, with strong dependence on water chemistry and nanoparticle dissolution, making amphibians useful sentinels for wetland and agricultural runoff scenarios (Galdiero *et al.*, 2019). Birds and mammals, including rodent and human-relevant occupational or biomedical contexts, supply translational anchors. Cross-species comparisons indicate that innate immune perturbation, oxidative stress, and neurobehavioral deficits occur in both fish and mammals after metal-nanoparticle exposure, supporting One-Health continuity, while also emphasizing route differences such as inhalation dominance in mammals (Swartzwelter *et al.*, 2021; Vojnits *et al.*, 2025).

Across taxa and environments, several concordance patterns are stable. First, oxidative-stress and inflammation biomarkers are the most reproducible shared signature from invertebrates to vertebrates, consistent with metal-ion redox cycling and surface-catalyzed ROS generation at the nano–bio interface. Second, barrier tissues are common early targets: gills and gut in fish and Daphnia, intestinal epithelium in Drosophila, and airway or gut barriers in mammals, indicating that transport and local dissolution at interfaces are universal drivers. At the same time, discordance arises from differences in exposure matrices and physiology. Invertebrates can show sharp life-history or behavioral impacts at low external concentrations because their internal dose tracks waterborne speciation closely, whereas fish may show stronger organ-specific remodeling and histopathology under longer exposures. Genetic models can reveal molecular or genotoxic key events that precede overt biochemical collapse, so relying on enzyme panels alone may under-estimate risk in some taxa. Overall, the cross-taxa evidence supports a tiered biomarker strategy that begins with high-throughput invertebrate screens, validates mechanisms in fish, and then checks human or mammalian relevance for One-Health risk framing.

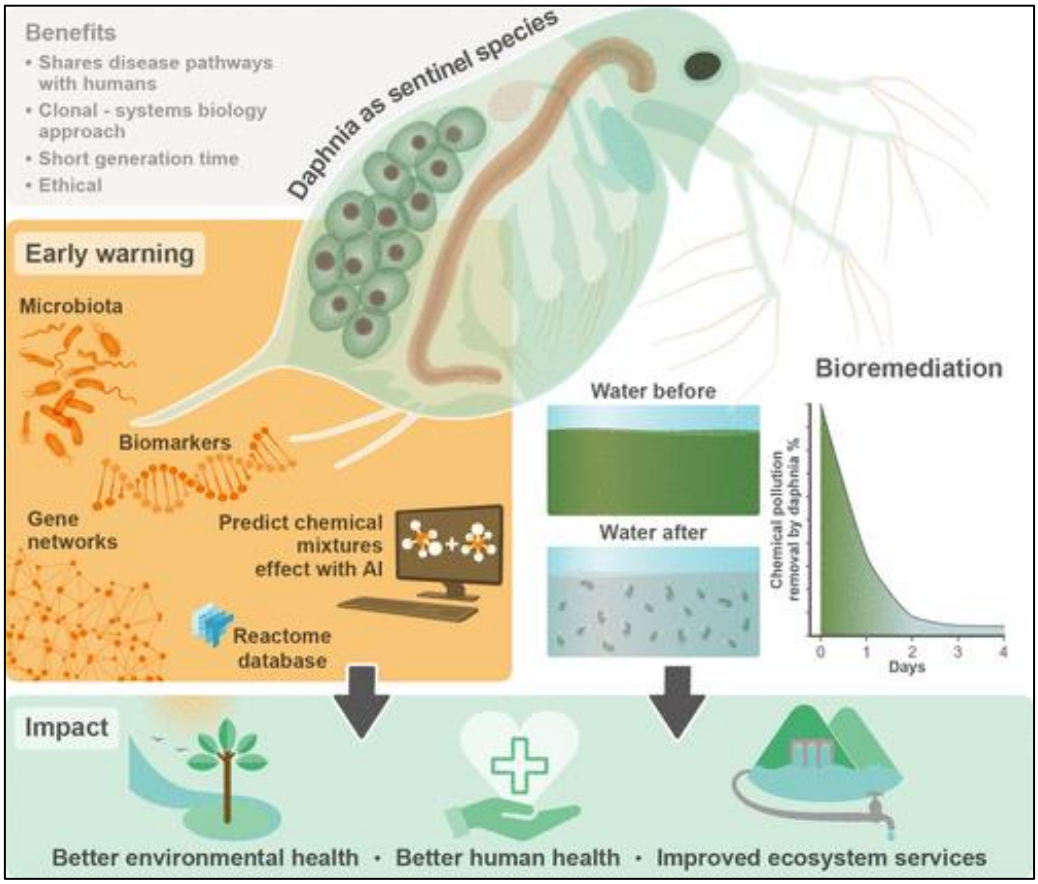

Figure 4: Daphnia as a One-Health Sentinel for Cross-Taxa Nanoparticle Risk

This figure illustrates why Daphnia are central high-throughput sentinels in cross-taxa synthesis, linking environmental exposure to biological key events. It highlights biomarker and microbiome readouts in aquatic invertebrates that warn of ecosystem stress before population collapse. In the One-Health cascade, such early signals guide where to deploy deeper vertebrate panels in fish and mammalian models. The image supports your tiered approach to aligning biomarkers across species and habitats.

6. Multimodal Integration and Inference

Multimodal datasets in nano–bio One-Health studies typically combine biochemical enzymes, omics layers, histopathology, behavior, and environmental surveillance signals. To interpret such heterogeneous evidence without over-weighting any single endpoint, structured Weight-of-Evidence (WoE) approaches are recommended. WoE frameworks start by defining the review question, pre-specifying lines of evidence, evaluating study quality, and then integrating consistency, relevance, and biological plausibility into a transparent narrative conclusion (Martin *et al.*, 2018; OECD, 2019; Parsai *et al.*, 2021). In nanomaterial risk contexts, this is particularly important because particle aging, dissolution, and matrix effects generate strong between-study variability; grading evidence by reliability and coherence helps distinguish robust mechanistic signals from context-driven noise (Suter *et al.*, 2020; Parsai *et al.*, 2021).

Quantitative fusion strengthens WoE by treating biomarker families as multivariate panels rather than isolated tests. Principal component analysis (PCA), partial least squares (PLS), and unsupervised clustering are widely used to compress correlated biomarkers into a smaller number of latent stress gradients, often revealing exposure patterns that single markers miss (Ghisi *et al.*, 2017; Vieira *et al.*, 2019). Composite indices such as the Integrated Biomarker Response (IBR) or PCA-derived indices further translate multi-endpoint panels into a single standardized score, enabling comparisons across treatments, sites, seasons, or taxa (Pires *et al.*, 2021; Willems *et al.*, 2024). These indices are not substitutes for mechanism; they are summary layers that should be traced back to contributing biomarkers during adverse outcome pathway mapping and One-Health interpretation (Ghisi *et al.*, 2017; Pires *et al.*, 2021).

Because nano-impact datasets differ in scale, error structure, and missingness, probabilistic inference is increasingly adopted. Bayesian hierarchical models allow enzymes, histology, and behavior to be modeled jointly while accounting for study-to-study heterogeneity and varying measurement precision, producing posterior effect sizes with credible intervals rather than single point estimates (Ring *et al.*, 2023; Rehms *et al.*, 2024). Bayesian networks are also being used to encode causal or semi-causal relations among exposure, transformation, bioavailability, biomarkers, and adverse outcomes, and to propagate uncertainty to risk predictions under field-realistic mixtures (Mentzel *et al.*, 2024; Furxhi *et al.*, 2025). In One-Health synthesis, these frameworks keep uncertainty explicit, clarifying where evidence is strong, weak, or sensitive to assumptions about particle speciation and interface transformation.

For lab-to-field portability, panel design must prioritize endpoints that are both mechanistically anchored and technically robust across taxa and matrices. Portable panels commonly combine a core oxidative-stress and inflammation suite with at least one higher-order endpoint (histopathology or behavior) and an internal-dose surrogate (bioaccumulation or ion-release proxy), then calibrate fused scores against controlled gradients or reference sites (Ghisi *et al.*, 2017; U.S. EPA, 2024). Harmonization is key: shared normalization rules, assay-interference controls for nanoparticles, and standardized reporting of dispersion and dissolution conditions reduce between-study variance and improve comparability for meta-analysis and Bayesian updating (Martin *et al.*, 2018; OECD, 2019). Practically, this supports tiered multimodal panels that begin with high-throughput screens and add mechanistic depth only where uncertainty remains, yielding interpretable field indicators aligned with One-Health risk needs (Parsai *et al.*, 2021; Willems *et al.*, 2024).

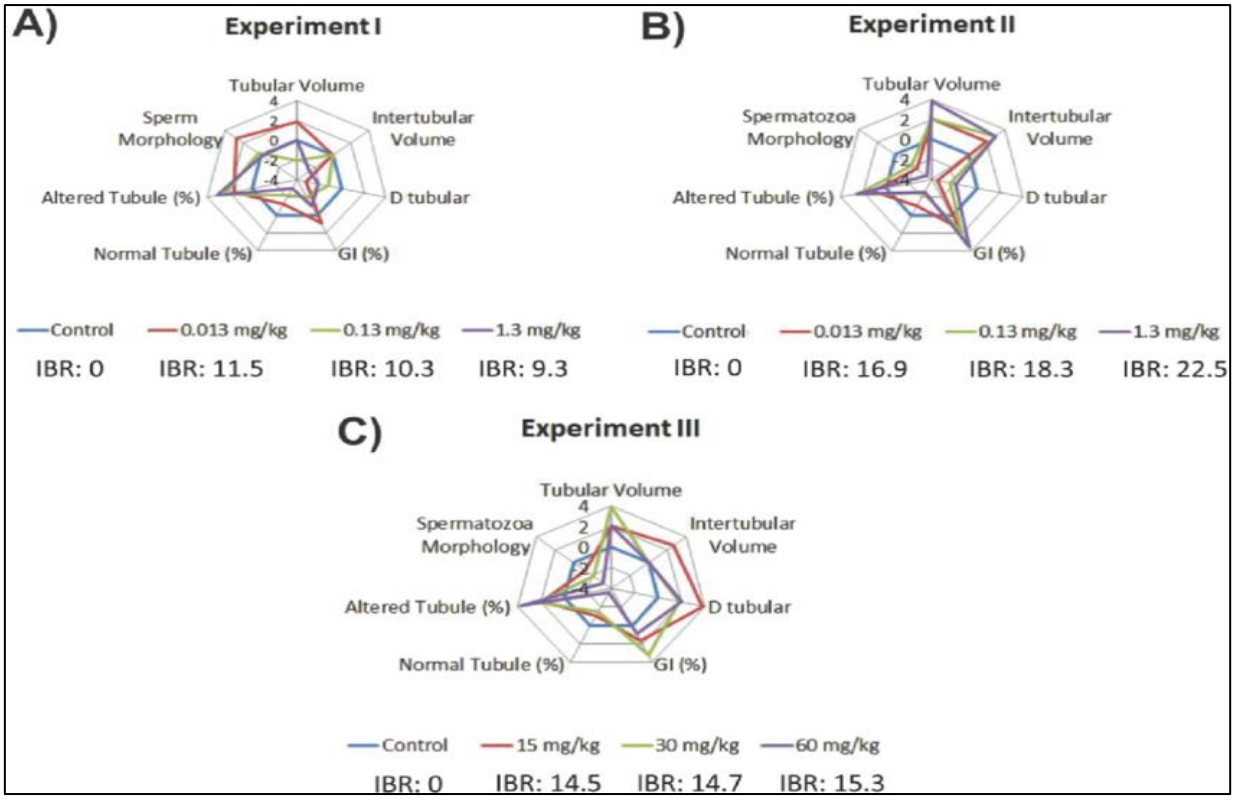

Figure 5: Integrated Biomarker Response Star Plot as a Multimodal Fusion Output

This radar-style IBR star plot compresses many biomarkers into one stress signature and a single index score. Each axis is a biomarker family, so the polygon shape reflects multivariate effects rather than a single marker. Paired with PCA/PLS, it enables clear cross-site and cross-taxa comparison and supports a portable lab-to-field indicator in your One-Health workflow.

7. Quality Assurance, Reporting Standards, and Confounders

High-quality nano–bio interface evidence depends as much on study design and reporting as on biological novelty. Over the last decade, community standards have converged on the idea that nanomaterial studies must report a minimum, structured set of variables so results are reproducible, comparable across taxa, and reusable for One-Health risk synthesis. The most widely adopted checklist in bio–nano research is MIRIBEL, which specifies three required domains: rigorous material characterization, complete biological model and exposure description, and transparent reporting of outcomes and statistics (Faria *et al.*, 2018; Ammar *et al.*, 2024). For corona-focused experiments, MINBE extends this logic by requiring detailed reporting of protein source, incubation conditions, separation methods, and corona quantification, since small procedural differences can flip biological interpretation (Chetwynd *et al.*, 2019). Together, these standards make it harder for nano-specific confounders to hide behind incomplete methods sections (Halamoda-Kenzaoui *et al.*, 2022; Sharifi *et al.*, 2022).

Minimum reporting in One-Health nanotoxicology therefore includes, at a bare minimum, clear negative and positive controls, exposure-matched ion controls where dissolution is expected, adequate biological and technical replicates, and effect sizes with uncertainty intervals rather than only p values (Faria *et al.*, 2018; Forest, 2022). Preregistered analysis plans or at least pre-specified primary endpoints are increasingly encouraged for high-dimensional panels to reduce selective reporting, especially in omics-heavy studies (Ammar *et al.*, 2024). Nanomaterials must be characterized not only "as supplied" but also "as exposed" in the relevant matrix, including primary size and shape, agglomeration state, surface chemistry or coating, zeta potential, dissolution or ion release kinetics, and any redox-active transformations during exposure (Halamoda-Kenzaoui *et al.*, 2022; Sharifi *et al.*, 2022; SCCS, 2020). Without these data, cross-study disagreements are often impossible to resolve, and meta-analytic integration becomes unreliable (Robinson *et al.*, 2016; Faria *et al.*, 2018).

Platform-specific standards are essential when biomarkers cross into molecular and sequence-based domains. For qPCR and miRNA assays, MIQE 2.0 and related MIQE guidance require full disclosure of RNA quality, primer or probe sequences, amplification efficiency, normalization strategy, and negative controls, because minor deviations can generate large, non-biological fold changes (Bustin *et al.*, 2024; Bustin *et al.*, 2025). For environmental DNA, metabarcoding, or transcriptomics, FAIR-aligned reporting plus MIxS-style

metadata capture sampling location, timing, filtration or extraction protocols, library prep, sequencing settings, and bioinformatic pipelines, enabling later reanalysis and cross-site occupancy inference (Takahashi *et al.,* 2024; Eloe-Fadrosh *et al.,* 2024; Kim *et al.,* 2025). Even when full GLP is not feasible in academic work, GLP elements such as traceable sample IDs, calibration logs, blinded scoring, and documented deviations strongly improve credibility and portability (Forest, 2022; Pathak *et al.,* 2025).

Nano- and metal-specific assay interferences are a recurring quality threat, especially for optical or enzyme-based biomarkers. Metal nanoparticles can adsorb proteins and dyes, scatter or absorb light in colorimetric assays, quench fluorescence, catalyze redox reactions in assay mixtures, or continue releasing ions during the assay itself, producing false positives or masked toxicity (Andraos *et al.,* 2020; Martin *et al.,* 2024). Mitigation strategies include nanoparticle-only blanks, matrix-matched calibration curves, alternative readout wavelengths, separation of particles before measurement where justified, and parallel analytical confirmation of ion release and particle stability across time (Andraos *et al.,* 2020; Martin *et al.,* 2024). Reporting these checks is now considered part of minimum good practice because interference risk differs by particle type, coating, and medium (Faria *et al.,* 2018; Halamoda-Kenzaoui *et al.,* 2022).

Biological and environmental modifiers act as confounders or effect amplifiers and must be explicitly designed for or controlled. Life stage and size strongly alter uptake and antioxidant baselines in fish, amphibians, and invertebrates; sex and genotype influence immune and endocrine sensitivity; and co-stressors such as temperature, pH, salinity, hypoxia, UV, and food availability can shift aggregation, dissolution, and organismal tolerance simultaneously (Forest, 2022; SCCS, 2020; Pathak *et al.,* 2025). Matrix effects are equally important: natural organic matter, proteins, and competing ions reshape coronas, change surface charge, and regulate metal speciation, often explaining why laboratory toxicity does not translate linearly to field conditions (Chetwynd *et al.,* 2019; Sharifi *et al.,* 2022). The practical takeaway for One-Health synthesis is simple: studies that do not report particle state in the exposure matrix, key covariates in organisms, and co-stressor context cannot be graded as high-confidence evidence, no matter how sophisticated the biomarker panel appears (Faria *et al.,* 2018; Ammar *et al.,* 2024).

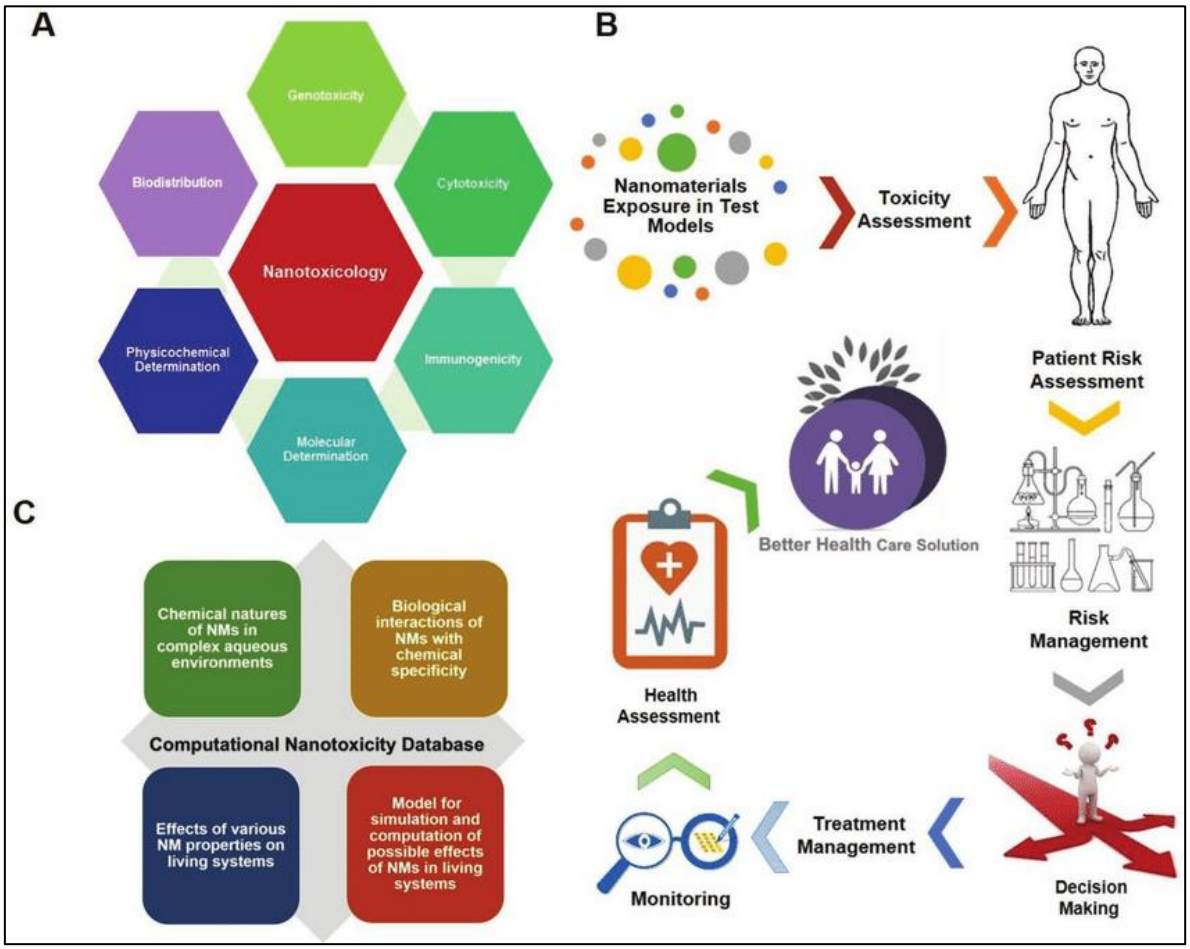

Figure 6: Essential Reporting Elements and Confounder Controls for One-Health Nanotoxicology

This figure summarizes the core experimental information that must be captured for reliable nanotoxicology, spanning nanomaterial characterization, exposure conditions, and biological endpoints. It visually reinforces minimum reporting logic underlying MIRIBEL and related checklists, highlighting the need to document particle properties in real matrices. The schematic also emphasizes that quality controls and standardized metadata enable integration across laboratory, field, and biomedical contexts. In your article, it supports the argument that transparent reporting is the gatekeeper for trustworthy cross-taxa and One-Health inference.

8. Applications, Knowledge Gaps, and Research Roadmap

One-Health nanotoxicology of metal nanoparticles is moving from proof-of-concept biomarkers toward decision-support tools that can operate across aquaculture, wildlife, ecosystems, and clinical or occupational settings. In aquaculture, nanoparticle exposure comes from feeds, additives, antifouling coatings, and contaminated inflows, so biomarker panels are increasingly used for early disease and stress surveillance before growth or mortality losses occur (Oliveira *et al.,* 2024; Khan *et al.,* 2024). In wildlife conservation, the same panels help diagnose sublethal contamination in sentinel species and interpret community-level changes alongside eDNA monitoring, enabling rapid assessment of habitat quality and recovery after pollution events (Wang *et al.,* 2024; Rocco *et al.,* 2025). For environmental compliance, regulators are beginning to align engineered nanomaterial testing with adverse outcome pathway logic and standardized evidence integration, so that laboratory panels can inform risk categorization and site-specific management (Saarimäki *et al.,* 2023; Cassee *et al.,* 2024). Translationally, biomedical and occupational studies use comparable oxidative stress, inflammation, genotoxicity, and barrier-tissue endpoints to evaluate inhalation, dermal, or gastrointestinal exposures, helping map shared key events across humans and non-human biota (Saarimäki *et al.,* 2023; Rocco *et al.,* 2025).

Despite this progress, several critical gaps still limit practice-ready One-Health inference. First, most datasets remain short-term and high-dose, whereas real environments produce chronic, low-dose, mixed-stressor exposures that alter corona dynamics, dissolution, and organism tolerance over time (Wang *et al.,* 2024; Cassee *et al.,* 2024). Second, mixture effects are under-resolved: metal nanoparticles often co-occur with dissolved metals, pesticides, antibiotics, or microplastics, yet factorial or longitudinal mixture designs are rare, making it hard to predict non-additive outcomes (Rocco *et al.,* 2025). Third, taxonomic and habitat coverage is uneven. Teleost fish and Daphnia dominate aquatic evidence, while amphibians, reptiles, pollinators, soil mesofauna, and many tropical ecosystems remain under-studied even though they can be uniquely sensitive to ion speciation and thermal or moisture stress (Wang *et al.,* 2024; Saarimäki *et al.,* 2023). Finally, field realism is still limited. Few studies track the same populations across seasons with parallel measurements of particle state in the matrix, internal dose, and multimodal biomarkers, so lab-to-field transferability remains uncertain (Cassee *et al.,* 2024).

A practical way forward is to recommend minimal core biomarker panels that are expandable but comparable across contexts. For laboratory screening, a compact core should include oxidative stress enzymes (CAT, SOD, GPx or GST, GSH redox balance) plus one damage marker (lipid peroxidation or DNA damage), alongside basic viability or reproduction endpoints in a high-throughput model such as Daphnia or Drosophila (El-Agri *et al.,* 2022; Saarimäki *et al.,* 2023). For vertebrate mechanistic validation, the same oxidative core should be paired with organ histopathology (gill, liver, kidney) and at least one functional endpoint such as swimming performance or feeding, because barrier injury and metabolic disruption are conserved cross-taxa signals (Oliveira *et al.,* 2024; Zahran *et al.,* 2025). For field deployment, the panel should prioritize robustness and portability: oxidative core enzymes from a sentinel species, a mucus or immune indicator, a simplified lesion score from key organs, and an exposure metric capturing particle stability and ion release in situ, with eDNA biodiversity readouts when community effects are a concern (Oliveira *et al.,* 2024; Mortensen *et al.,* 2025).

Building on these panels, a One-Health roadmap can be staged in three horizons. In the near term, priority should go to method harmonization and reporting completeness for metal nanoparticles, including routine in-matrix characterization, dissolution kinetics, and assay-interference controls, so datasets become comparable across labs and species (Cassee *et al.,* 2024). New reference materials representing realistic aged or eco-corona-coated particles are also needed to benchmark bioavailability and biomarker sensitivity under shared protocols (Cassee *et al.,* 2024). In the midterm, research should expand chronic and mixture designs, integrate multi-omics into adverse outcome pathways for multiple taxa, and link biomarker changes to population-level fitness and ecosystem services (Saarimäki *et al.,* 2023; Rocco *et al.,* 2025). In the long term, open nanosafety infrastructures are essential. FAIR adverse outcome pathway resources, nanoinformatics platforms, and shared biomarker databases should enable Bayesian updating across studies and rapid translation to regulation and clinical guidance (Mortensen *et al.,* 2025; Zouraris *et al.,* 2025). The closing perspective is that One-Health readiness will not come from inventing new biomarkers alone, but from aligning minimal portable panels with standardized exposure characterization, long-horizon evidence, and open data ecosystems that keep uncertainty explicit and usable

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