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Life-Cycle Assessment and Toxicity Profiling of Green Nanomaterials: Bridging Sustainability and Biomedical Safety

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ABSTRACT: The production of green nanomaterials may utilize renewables as feedstocks, biological reducing agents, aqueous processing, or minimize the use of hazardous reagents, which makes them potentially useful for drug delivery, imaging, diagnostics, tissue engineering, antimicrobial systems, and regenerative medicine. But green synthesis does not necessarily ensure life-cycle sustainability or biomedical safety. This review combines the concepts of life-cycle assessment (LCA), physicochemical characterization, exposure analysis, and toxicity profiling in order to facilitate safe-and-sustainable-by-design development. LCA establishes the functional unit, system boundary, lists material and energy flows, and quantifies burdens related to the acquisition, synthesis, purification, formulation, use, release, recycling, and disposal of feedstock. Toxicity profiling includes the study of size, morphology, composition, crystallinity, surface chemistry, aggregation, dissolution, protein-corona formation, delivered dose, cellular uptake, biodistribution, persistence, and clearance. These endpoints are related to cytotoxicity, oxidative stress, genotoxicity, inflammation, hemocompatibility, immunotoxicity, organ toxicity, reproductive effects and ecotoxicity. Integration is important since reducing energy and/or solvent consumption can affect purity, stability, dose, effectiveness, or toxicity; and safer surface modifications can add to processing requirements.

The use of multi-criteria decision analysis to identify trade-offs, combined with a framework that compares functionally equivalent alternatives, quantifies uncertainty and applies mechanistically relevant models, should be used. The challenges are the

incompleteness of the inventory, the lack of consistency in how the dose is measured, biological batch variability, assay interference, lack of long-term studies and inadequate end-of-life transformation data. Quality-by-design manufacturing, harmonized reporting, reference materials, high throughput and new approach methodologies, toxicokinetic modeling, and regulatory alignment are some of the items needed for future translation. When connected to toxicity evidence, LCA can avoid burden shifting and facilitate the development of green nanomaterials to make them good for both the environment and society, and effective for clinical applications.

1. INTRODUCTION

Green nanomaterials encompass the different types of nanoscale materials (metals, metal oxides, polymers, lipids, carbon-based structures, silica, composites and biologically-derived systems) manufactured and/or modified with methods designed to minimize the use of hazardous substances, energy usage, waste generation, or reliance on non-renewable resources. They are biomedical relevant due to their tunable size, tunable surface area, tunable optical and magnetic properties, tunable cargo capacities, and tunable surface functionalization enabling drug delivery, imaging, diagnostics, antimicrobial activities, tissue engineering and regenerative applications. However, the term 'green' is frequently used only for the synthesis process, and important considerations such as cultivation or mining of the raw materials, preparation of the extracts, recovery of solvents, purification, sterilisation, cold-chain, release during use and treatment at end-of-life are not always taken into account. (3) LCA offers a structured approach to defining the functional unit and the system boundary, compiling input and emissions inventories, transforming inventories to impact categories, and interpreting the uncertainty within the product system. (4)A complementary question is addressed in toxicity profiling – how physicochemical identity, route of exposure, dose received, transformation, persistence and host biology will lead to adverse outcomes. Safe-and-sustainable-by-design approaches attempt to incorporate these aspects as early as possible in the process of composition, morphology, coatings, manufacturing, and application, when redesign is cheap. The integrated framework used in this review that connects raw-material sourcing, green synthesis, downstream processing, biomedical use, and end-of-life management with environmental assessment, hazard evaluation, characterisation of exposure, and performance is shown in Figure 1. The purpose of this review is to explain how to apply appropriate LCA methods, summarize the nano-specific requirements for toxicity and toxicokinetic aspects, pinpoint decision criteria for weighing sustainability with biomedical functionality and establish priorities for standardized evidence generation. Social and operational considerations, such as supply chain resilience, worker protection, affordability, access, and healthcare system management of the disposal of nanoparticles, should also be acknowledged. The core idea is that it is important to measure sustainability, safety, and clinical performance in relation to the same functional purpose – otherwise, comparisons can result in the selection of low-burden materials that deliver minimal efficacy and/or seemingly safe materials that bear the burden of production to energy, water, ecosystems, workers or waste systems. (7)

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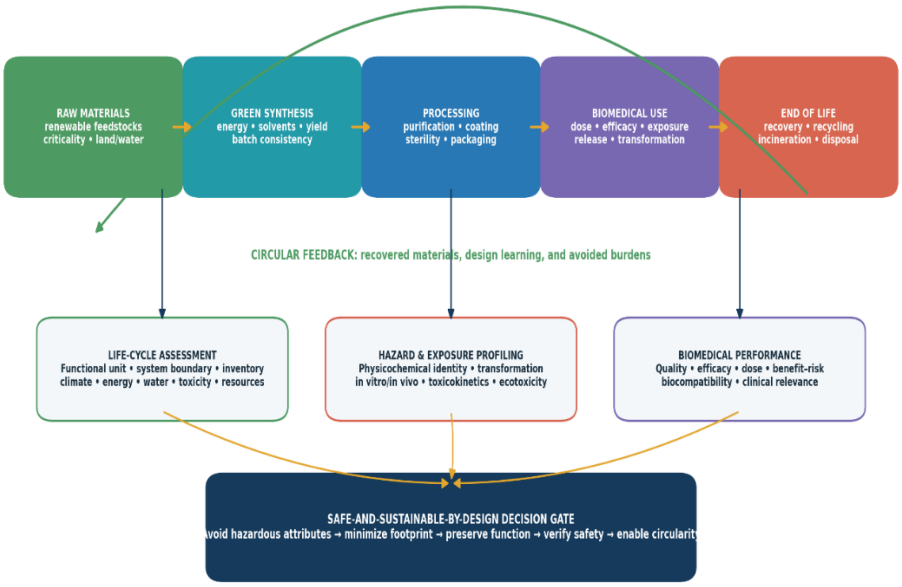

Figure 1. Integrated life-cycle and safe-and-sustainable-by-design framework for green nanomaterials, from raw-material sourcing to biomedical use and end-of-life management.

2. LIFE-CYCLE ASSESSMENT OF GREEN NANOMATERIALS

A realistic LCA starts with defining the decision context, the intended audience, the functional unit, the reference flow, the system boundary, the allocation rules, and the comparator. Biomedical nanomaterials: The therapeutic effect can often rely on the delivered dose, the length of time the dose is released, the sensitivity of the imaging, the antimicrobial effect, or the service life of a device; in such cases, the mass of the mass equivalent product must not be used as the functional unit. (9) The inventory should include feedstock cultivation or extraction, the production of precursors and solvents, electricity and heat, water, biological media, purification, centrifugation, drying, coating, sterilization, packaging, transport, losses of yield, rejected batches, and treatment of waste. (10) Scale is important because the impacts from lab sonication, freeze drying, high-purity reagents, and low yields can overwhelm the impacts from industrial recovery, continuous processing, or process intensification, respectively. The characterization factors for nanoparticle emissions are not fully available, but are included in the impact assessment of a typical scenario, which typically takes into account climate change, cumulative energy demand, water use, acidification, eutrophication, particulate formation, resource depletion, human toxicity, and freshwater or terrestrial ecotoxicity. Table 1 is structured to present life-cycle stages, inventory parameters, environmental indicators, assessment tools and sustainability questions. Release from coatings, implants, diagnostics or drug carriers, dissolution, aggregation, corona formation, ageing and transfer to wastewater, sludge, soil, sediment and/or incineration residues should be taken into account when using-phase modelling. End-of-life scenarios might be recovery, recycling, biodegradation, sterilisation, hazardous waste processing, incineration or landfill; sensitivity analyses can be carried out when pathways are not known in reality. Prospective assessments should simulate learning curves, anticipated industrial production volumes, regional electricity mixes, solvent recovery and other clinical pathways to look at the results of the commercialization of an early laboratory protocol. Interpretation should not be limited to a single environmental score, as illustrated in Figure 1, but should also focus on identifying hotspots, trade-offs, data quality and potential for burden shifting. Distinguishing robust conclusions from assumptions. (13) Scenario analysis, Monte Carlo uncertainty, parameter sensitivity, and contribution analysis. Reproducibility and meaningful comparison require transparent inventories, geographically and temporally appropriate datasets, and reporting of cut-offs. (14)

Table 1. Life-cycle stages, inventory parameters, environmental-impact indicators, assessment methods, and sustainability considerations for green nanomaterials.

Life-cycle stage	Inventory parameters	Impact indicators	Assessment methods	Key sustainability question
Raw materials	Feedstock origin, land, water, fertilizer, mining, precursor production	Climate, water scarcity, land use, resource criticality	Supplier data, databases, mass balance	Is the renewable or mineral input genuinely lower burden?
Synthesis	Energy, heat, mixing, solvents, biological media, yield	Energy demand, emissions, water use, occupational exposure	Unit-process inventory, hotspot analysis	Does green chemistry reduce total rather than local burden?
Purification/for mulation	Washing, centrifugation, filtration, drying, coating, sterilization	Energy, solvent loss, waste, rejected batches	Process modeling, sensitivity analysis	Do downstream steps dominate the footprint?
Use phase	Clinical dose, service life, release, transformation, efficacy	Patient exposure, environmental release, avoided burden	Scenario and exposure modeling	Is performance sufficient at the assessed functional unit?
End of life	Recovery, recycling, degradation, incineration, landfill	Persistence, ecotoxicity, resource recovery	Scenario, uncertainty and circularity analysis	Can release be prevented and value recovered?

3. PHYSICOCHEMICAL PROPERTIES, EXPOSURE, AND TOXICITY PROFILING

The first step in toxicity profiling involves the material to be tested, since nominally identical green nanomaterials can vary in primary size, size distribution, morphology, aspect ratio, composition, crystallinity, defects, surface chemistry, charge, porosity, and residual precursors, endotoxin, and biological capping agents. Characterization should be carried out in the stock dispersion and in the appropriate exposure media, where the ionic strength, protein, lipids, pH, and NOM can vary the agglomeration, dissolution, sedimentation, corona formation, and delivered dose. These dynamic properties are connected to various pathways of occupational, dermal, oral, inhalation, injection, implantation and environmental exposure, followed by membrane interactions, cellular trafficking, lysosomal processing, oxidative stress, inflammation, barrier crossing, organ distribution, clearance, persistence, and outcome. Delivered or administered concentration should be differentiated from cellular or tissue dose, particularly in an in vitro system where cells are submerged, in which agglomeration and sedimentation lead to varying exposure. Table 2 is a comparison of cell-based assays, organoids, organ-on-chip, ex vivo models, animals, ecotoxicity tests and computational approaches along with endpoints and limitations. Biological endpoints measured in the core include cytotoxicity, oxidative stress, genotoxicity, hemolysis, coagulation, complement activation, immunotoxicity, irritation, sensitization, organ toxicity, reproductive effects and local tissue response. Particle-only controls and orthogonal methods are needed for (18) assay

interference due to light absorption, fluorescent, catalytic, protein binding or adsorption of indicator molecules. Toxicokinetic studies should estimate the proportion of absorbed, distributed, metabolised or transformed and excreted, using validated analytical techniques, where possible, to separate the particulate from the dissolved species. To interpret the results of biodistribution to liver, spleen, kidney, lung, lymphatic tissues or target lesions, clearance and recovery should be taken into account, not just the concentration measured. (21) Environmentally realistic aging, dissolving, sediment interaction, trophic transfer and organism-specific exposure should be included in ecological profiling. Study designs should include positive, negative, vehicle, precursor, extract and endotoxin controls and preregistered acceptance criteria and blinded analysis if possible. A combination of physicochemical identity, dosimetry, mechanistic biomarkers, histopathology, and functional outcomes is more informative in a weight-of-evidence design than any single viability or radical scavenging assay.

Figure 2. Relationship between nanomaterial physicochemical properties, exposure pathways, biological interactions, and toxicity outcomes.

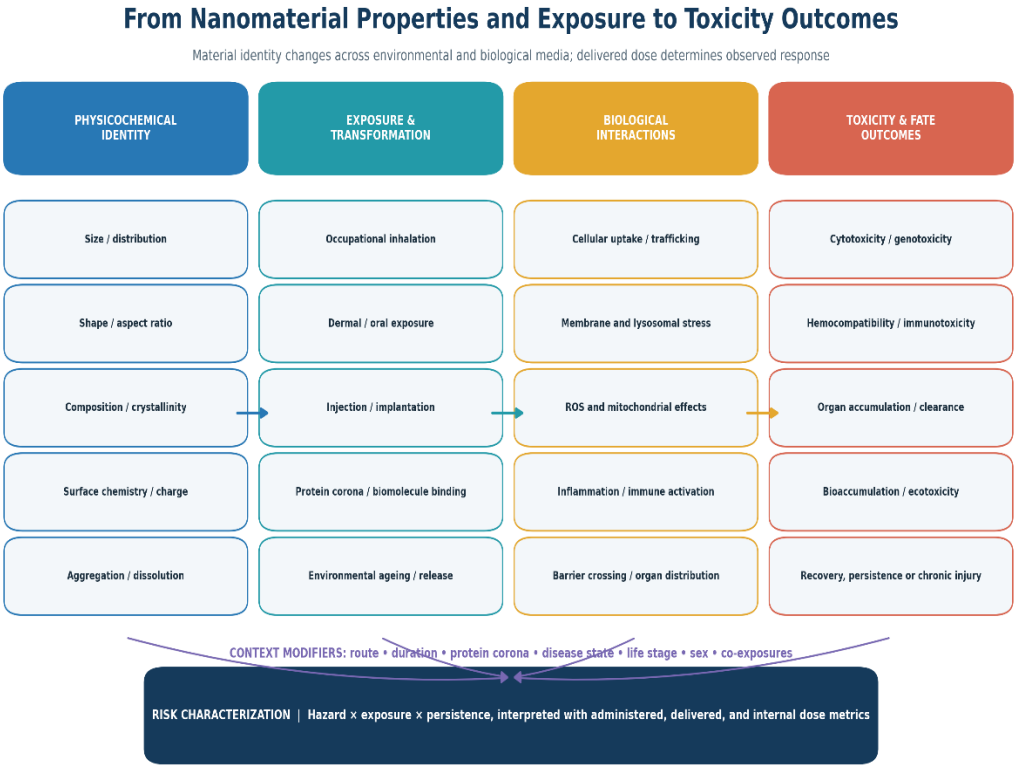

Table 2. Toxicity-testing models, endpoints, biomarkers, exposure conditions, advantages, and limitations.

Testing approach	Models	Representative endpoints	Advantages	Limitations/controls
In vitro	Primary cells, immune cells, blood, relevant cell lines	Viability, ROS, DNA damage, cytokines, hemolysis, complement	Rapid, mechanistic, high-throughput	Dosimetry and assay interference; limited systemic context
Advanced in vitro	3D tissues, organoids, barrier	Transport, tissue injury, inflammation, functional response	Human relevance and dynamic exposure	Complexity, validation and

Testing approach	Models	Representative endpoints	Advantages	Limitations/controls
	and organ-on-chip systems			limited chronic modeling
Ex vivo	Blood, perfused organs, tissue slices	Compatibility, uptake, metabolism, local injury	Preserves tissue architecture	Short viability and donor variability
In vivo	Rodent or justified non-rodent models	Toxicokinetics, organ toxicity, immunity, reproduction, recovery	Systemic integration and repeated-dose evidence	Species translation, ethics, cost and dose relevance
Ecotoxicity	Algae, daphnids, fish, soil and sediment organisms	Growth, reproduction, mortality, bioaccumulation	Environmental relevance	Transformation, settling and exposure maintenance
In silico	PBPK, fate models, QSAR/read-across, machine learning	Internal dose, hazard ranking, scenario prediction	Prioritization and reduced testing	Dependent on data quality and applicability domain

4. INTEGRATING SUSTAINABILITY WITH BIOMEDICAL SAFETY

The first step to integration is to recognize that the process of biological or plant-mediated synthesis does not necessarily carry the burden of sustainability requirements of conventional synthesis; renewable inputs can carry agricultural land, irrigation, fertilizer, extraction requirements, as well as requirements for preservation and batch-variability, and downstream purification or sterility requirements may be greater. Safe-and-sustainable-by-design thus considers not only hazard reduction, but also environmental impact, circularity, functionality and socioeconomic aspects in the selection of materials and in the design development of processes. The selection of alternatives should be performed by providing normalized criteria for feedstock criticality, energy, energy consumption, water, emissions, waste, worker exposure, physicochemical hazard, biomedical safety, effectiveness, stability, manufacturability, and end-of-life options, and by selecting candidate nanomaterials with a minimum performance threshold. Table 3 is an integrated assessment matrix and proposed decision questions. By defining weights, outranking or using Pareto analysis, multi-criteria decision analysis can make value judgments explicit, and uncertainty ranges eliminate the possibility of making small score differences definitive rankings. The framework should incorporate stage gates: eliminate unacceptable hazards, verify performance, identify environmental hotspots, model exposure, test plausible transformations and re-evaluate the design following coating, formulation, sterilisation, scale-up and route changes. (26) The intended use will have an impact on regulatory relevance as medicinal products, medical devices, biologics, cosmetics, food contact materials and environmental products will have different evidentiary pathways. Chemistry, manufacturing and controls data; batch specification; impurity and endotoxin limits; stability; validated potency; biocompatibility; pharmacokinetics; dose justification; and benefit–risk assessment are examples of biomedical translation requirements. Reporting should be comparable and cover source identity, synthesis parameters, yield, purification, complete characterization, dispersion preparation, exposure media, the administered and delivered dose, assay controls, raw data accessibility and uncertainty. Participation by stakeholders can help to define what levels of uncertainty are acceptable in light of the clinical benefits that can be gained, and what thresholds in the environment, work place, or patient safety must not be overcome. All evidence does not need to be combined into one

score for integration. The more defensible decision record keeps environmental, safety, performance, and data-quality dimensions separate; specifies non-negotiable thresholds; and captures the rationale for a trade-off for a particular clinical benefit and patient population.

Table 3. Integrated sustainability–safety assessment matrix for selecting green nanomaterials for biomedical applications.

Decision domain	Core indicators	Minimum evidence	Decision criterion	Design response
Functionality	Potency, targeting, stability, release, service life	Benchmark against current alternative	Meets prespecified clinical performance	Reformulate or reject if threshold is not met
Environmental footprint	Climate, energy, water, resources, emissions, waste	Cradle-to-grave inventory and sensitivity	No material burden shifting across stages	Change feedstock, process, recovery or scale
Human health safety	Hazard, exposure, dose, toxicokinetics, biocompatibility	Tiered nano-relevant testing with controls	Acceptable margin for intended route and population	Modify size, coating, impurity or dose
Ecological safety	Release, persistence, dissolution, ecotoxicity	Relevant ageing and fate scenarios	No unacceptable persistent or ecosystem hazard	Contain, recover, redesign or restrict use
Circularity/manufacture	Yield, recovery, recyclability, batch consistency	Pilot-scale mass balance and quality attributes	Scalable and reproducible with manageable waste	Process intensification and closed-loop recovery
Evidence quality	Completeness, uncertainty, transparency, reproducibility	Standard reporting and accessible data	Decision robust to plausible uncertainty	Generate targeted data before progression

5. CHALLENGES, FUTURE PERSPECTIVES, AND CONCLUSION

Challenges to progress are the limited datasets for inventories at the nanoscale, uncertainty in emission factors, inconsistencies in functional units, variability of biological source materials, poor interlaboratory reproducibility, and a lack of chronic and end-of-life evidence. Scale-up can have an effect on mixing, heat and mass transfer, particle size, surface corona, impurity profiles, yield and worker exposure, and results in the laboratory cannot be extrapolated to the pilot. Harmonized reference materials, minimum reporting standards are needed as well as validated dispersion and dosimetry procedures and interoperable databases are needed to correlate material identity with environmental and biological results. High throughput screening, transcriptomics, proteomics, metabolomics, imaging, adverse outcome pathways, organoids and organ-on-chip models can provide improved mechanistic resolution and help to decrease reliance on poorly informative animal studies. Machine learning or quantitative structure–activity or property–activity models may be used to prioritize designs, but require curated, negative as well as positive data, uncertainty estimates, and applicability domain testing. The formulation, dose, route and scale of production should be reflected in the toxicokinetic and toxic exposure models, and prospective LCA of the toxic effects should be used in conjunction with process optimization. The policy should encourage joint consultation on its provisions, sustainability statements,

protection of workers, environmental accountability, and purchasing policies which encourage verified and not assumed greenness. Finally, green nanomaterials can make a contribution to biomedical care without imposing an unpalatable burden only if function, life cycle impacts, exposure, and toxicity are considered together with uncertainty. The most realistic route from a lab promising material to demonstrating reproducible clinically useful and environmentally responsible materials is an iterative safe and sustainable by design process. (35)

Declarations

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Conflict of Interest: The authors declare that they have no conflict of interest.

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