



Review Article

Stratified Safety Evaluation Framework for Natural Products Based on New Approach Methodologies in Integrative Medicine: A Narrative Review



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Received: July 02, 2026 | Revised: August 24, 2026 | Accepted: September 15, 2026 | Published online: September 28, 2026

Abstract

The widespread assumption that “natural” equals “safe” has been repeatedly challenged by adverse events associated with medicinal plant-derived products. Unlike synthetic pharmaceuticals, these natural products present distinct safety concerns because of their compositional complexity, inherent variability, and batch-to-batch inconsistency. Consequently, a one-size-fits-all approach to toxicological assessment is insufficient. In this narrative review, we propose a pragmatic “classify-then-evaluate” framework that stratifies natural products into three operational categories according to compositional complexity and degree of chemical characterization—TCM compound formulae, active fractions/extracts, and purified single compounds. This framework enables safety evaluations to be tailored to product complexity by integrating conventional toxicological assays with emerging new approach methodologies. By linking chemical characterization, hazard identification, quality control, and risk assessment within a tiered system, this framework bridges the gap between evidence generation and regulatory decision-making, offering a scientifically grounded and proportionate pathway for evaluating the safety of natural products used in integrative medicine, with a primary focus on herbal and TCM-related products. The review does not aim to provide a comprehensive systematic evidence map; further validation of the proposed framework is required

Introduction

Natural products (NPs) constitute one of the most prolific sources of therapeutic agents in human history, encompassing a remarkably diverse spectrum of bioactive substances derived from plants, animals, marine organisms, fungi, and microorganisms.^{1,2} The World Health Organization (WHO) reports that more than 80% of the world’s population in over 170 Member States uses some form of traditional medicine.¹ In pharmaceutical development, NPs have contributed substantially to modern drug discovery and development, underscoring their enduring significance in modern

medicine.^{3,4} In this review, “natural products” is used as the broader scientific term, whereas “herbal medicines,” “botanical products,” and “TCM products” are used only in their corresponding medicinal or regulatory contexts.

Within this expansive domain, for the purposes of this review, NPs are grouped into three categories that differ in their compositional complexity and developmental trajectories in drug discovery. First, traditional Chinese medicine (TCM) compound formulae represent multi-component preparations guided by traditional medical theories such as the “Jun-Chen-Zuo-Shi” (monarch-minister-assistant-courier) principle, a traditional role-based formulation framework in which individual herbs are assigned primary, supportive, toxicity-mitigating, or harmonizing roles within a formula.^{5,6} This framework expresses traditional formulation logic rather than a validated one-to-one biomedical mechanism. These formulae embody a holistic therapeutic philosophy where efficacy and safety emerge from the integrated action of numerous chemical constituents. Second, active fractions and extracts occupy an intermediate position, comprising partially purified preparations with relatively defined but still complex chemical profiles, where manufacturing processes significantly

Keywords: Natural products; Safety evaluation; New approach methodologies; Traditional Chinese medicine; Hepatotoxicity; Herb–drug interactions; Regulatory science.

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How to cite this article: Cao M, Duan X, Li J, Zhou Z, Jin H. Stratified Safety Evaluation Framework for Natural Products Based on New Approach Methodologies in Integrative Medicine: A Narrative Review. *Future Integr Med* 2026;5(3):139–164. DOI: 10.14218/FIM.2026.00017.

influence the final composition.^{7,8} Third, single bioactive compounds, such as artemisinin, paclitaxel, and berberine, are structurally defined single compounds that most closely approximate the conventional pharmaceutical paradigm.^{9,10}

Beyond compositional complexity, the distinction between NPs and synthetic drugs extends to several fundamental dimensions, including quality consistency (batch-to-batch variation versus consistent synthesis), clinical use context (experience-based prescribing versus target-driven development), and the underlying philosophy of safety evaluation.^{11,12} For synthetic drugs, the approach follows a “known structure → predicted toxicity endpoints” logic, whereas for NPs, particularly complex preparations, the paradigm shifts to “complex system → discovery of unknown risks”.^{13,14} These distinctions necessitate fundamentally different approaches to safety evaluation.

Nevertheless, the public perception that natural products are inherently safe represents a dangerous misconception that has contributed to numerous adverse events reported in different geographic settings.^{15,16} For example, aristolochic acid-associated nephropathy has been documented in multiple geographic settings and exemplifies the serious consequences of inadequate safety vigilance.^{17,18} Similarly, safety concerns have been reported for pyrrolizidine alkaloid-containing herbs, *Polygonum multiflorum* (He Shou Wu), and *Tripterygium wilfordii* (Lei Gong Teng).¹⁹⁻²¹ Regulatory responses to other herbal products, including Ephedra- and kava-containing products, have likewise varied according to country and product category. Epidemiological studies from Chinese mainland indicate that herb-induced liver injury (HILI) represents an important contributor to drug-induced liver injury (DILI).²²

These incidents underscore a critical reality: different categories of NPs face distinct safety challenges that cannot be addressed through a one-size-fits-all evaluation approach.^{23,24} TCM formulae present challenges related to multi-component interactions and processing-dependent toxicity profiles²⁵; active fractions raise concerns about toxic component enrichment during extraction²⁶; and even pure single compounds may exhibit unexpected toxicities through multi-target pharmacology or gut microbiota-mediated biotransformation.²⁷

In light of these considerations, we adopt a classification-based perspective to critically examine current approaches to the safety evaluation of medicinal plant-derived natural products and related preparations. New approach methodologies (NAMs) are methods that can provide information on chemical hazard and risk assessment without the use of intact animals. We focus on how NAMs can be appropriately integrated with conventional toxicological assessment according to product complexity and degree of chemical characterization. In this narrative review, we therefore propose a pragmatic “classify-then-evaluate” framework linking product classification, compositional characterization, risk identification, toxicity testing, quality control, pharmacovigilance, and regulatory considerations.

In this review, we focus primarily on herbal and TCM-related natural products, including TCM compound formulae, active fractions/extracts, and purified single compounds, while using selected non-herbal natural products only when they illustrate broadly relevant toxicological principles. Therefore, this narrative review aims to synthesize concepts and propose a pragmatic

“classify-then-evaluate” framework that stratifies natural products into three operational categories based on their degree of chemical characterization—TCM compound formulae, active fractions/extracts, and purified single compounds—and links product classification to proportionate, fit-for-purpose safety evaluation by integrating conventional toxicological assessment with NAMs, rather than to provide a comprehensive systematic evidence map.

Modern methodologies: from conventional approaches to NAMs

Conventional toxicological approaches and their limitations for natural products

In vivo studies

Traditional animal-based toxicological studies have historically formed an important component of NP safety assessment, with acute and repeated-dose studies in rodents commonly used to characterize systemic toxicity in herbal preparations.²⁸⁻³¹ Such studies can provide whole-organism toxicity information, including toxicokinetic and systemic effects that cannot be fully replicated *in vitro*.

However, conventional animal studies face significant limitations when applied to NPs. Species differences can limit translation of toxicity findings to humans, and this issue may be particularly relevant to multi-component preparations.³² The enormous number of constituents in TCM formulae makes it practically impossible to attribute observed toxicity to specific components through conventional dose-response studies.³³ Furthermore, the ethical imperative to reduce, refine, and replace animal use (3Rs principle) has gained substantial momentum. In the United States, the FDA Modernization Act 2.0 (2022) amended the statutory definition of a “nonclinical test” so that specified *in vitro*, *in silico*, *in chemico*, and other non-animal approaches may be used as nonclinical evidence; this change does not establish a universal removal of animal studies from all drug-development programs.^{34,35}

Classical *in vitro* approaches

Traditional cell-based systems, including immortalized cell lines (liver: HepG2, kidney: HK-2, and heart: H9c2) and primary cell cultures, have served as first-tier screening tools for decades.^{36,37} While offering advantages of cost-effectiveness and throughput, these systems suffer from limited physiological relevance—two-dimensional monocultures cannot recapitulate the complex multicellular architecture, mechanical forces, and metabolic zonation present in intact organs.^{38,39} For NPs specifically, the absence of metabolic competence in many cell lines means that bioactivation-dependent toxicities may be missed entirely.⁴⁰

NAMs

New approach methodologies (NAMs) refer to a broad range of technologies, methodologies, approaches, or combinations thereof that provide information relevant to chemical hazard and risk assessment while reducing, refining, or replacing traditional animal testing.^{41,42} Regulatory usage of the term NAM is not fully uniform; some definitions restrict NAMs to non-animal methods. Non-mammalian model organisms, including zebrafish, *Caenorhabditis elegans* and *Drosophila melanogaster*, have increasingly

been incorporated into NAM-based testing strategies. Although these organisms are not NAMs per se, assays developed using these models can contribute to NAM frameworks by providing mechanistic, high-throughput and animal-sparing information for chemical hazard assessment. For NP safety evaluation, NAMs offer unique advantages in addressing the multi-component challenge while improving human relevance and predictive accuracy. The FDA's 2026 draft guidance, General Considerations for the Use of New Approach Methodologies in Drug Development, is a non-binding draft that provides general, fit-for-purpose validation and reporting recommendations when NAM data are used in drug-development submissions; it does not establish product-specific acceptance for botanicals or complex mixtures.⁴³

Alternative *in vivo* models

Zebrafish

Zebrafish (*Danio rerio*) have emerged as a powerful vertebrate model bridging the gap between cell-based assays and mammalian studies.^{44,45} Their optical transparency during development, rapid reproduction cycle, genetic tractability, and conservation of major organ systems between zebrafish and humans make them particularly valuable for NP toxicity screening.⁴⁶ High-throughput zebrafish platforms can evaluate developmental toxicity, hepatotoxicity, cardiotoxicity, and neurotoxicity of complex NP mixtures simultaneously, with studies demonstrating concordance with mammalian outcomes for selected endpoints.⁴⁷ Recent advances include higher-throughput phenotypic screening approaches and multi-organ toxicity applications.^{48,49} Recent applications include zebrafish-based evaluation of the cardiotoxicity of TCM-derived compounds such as periplocin and bufalin,⁵⁰ and identification of hepatotoxic constituents in *Polygonum multiflorum* extracts.⁵¹

Caenorhabditis elegans

The roundworm *Caenorhabditis elegans* offers complementary advantages including a fully sequenced genome, invariant cell lineage, short lifespan enabling rapid aging studies, and fully mapped neuronal connectivity.⁵² For NP evaluation, *C. elegans* provides a tractable whole-organism model for rapid toxicity and mixture-effect assessment.⁵³

Drosophila

The fruit fly *Drosophila melanogaster* model provides sophisticated genetic tools for investigating toxicity susceptibility in defined genetic backgrounds, with applications in studying oxidative stress responses to plant-derived compounds.^{54,55}

Advanced *in vitro* models

Organoid technology

Organoids—three-dimensional, self-organizing structures derived from stem cells or tissue progenitors—represent one of the most significant advances in toxicological modeling.⁵⁶ Hepatic organoids recapitulate key features of liver architecture including bile canaliculi formation, metabolic zonation, and CYP450 expression profiles, enabling assessment of metabolism-dependent hepatotoxicity.^{56,57} Kidney organoids containing proximal tubule-like structures express organic anion transporters (OAT1/3), making them particularly relevant for evaluating nephrotoxic NPs such as aristolochic acid.^{58,59} Intestinal organoids model the first

barrier encountered by orally administered NPs, incorporating both epithelial and immune cell components.⁶⁰

For NP evaluation, organoids offer the critical advantage of maintaining long-term cultures, which can support repeated-exposure experimental designs.⁶¹ Patient-derived organoids further enable investigation of inter-individual variability in NP metabolism and susceptibility, a crucial consideration given the pharmacogenomic diversity in populations using traditional medicines.^{62,63}

Organ-on-a-chip (OoC) systems

Microfluidic organ-on-a-chip systems integrate living cells within microfabricated devices that recapitulate tissue-level physiology through controlled mechanical forces, fluid flow, and multicellular interactions.^{64,65} Liver-on-a-chip platforms incorporating hepatocytes, stellate cells, Kupffer cells, and sinusoidal endothelial cells under physiological flow conditions demonstrate superior prediction of drug-induced liver injury compared to static cultures.^{32,66} Heart-on-a-chip devices measuring contractility, electrophysiology, and calcium transients enable real-time assessment of cardiotoxicity from aconitine and other NP-derived cardiotoxins.⁶⁷⁻⁷⁰

Multi-organ-on-a-chip systems connecting liver, kidney, heart, and intestine compartments are particularly useful for NP evaluation, as they enable the study of metabolite-mediated inter-organ toxicity—a phenomenon highly relevant to complex NP preparations where hepatic metabolism may generate nephrotoxic or cardiotoxic metabolites.^{71,72} A liver-on-a-chip study demonstrated hepatotoxic effects of aristolochic acid, whereas its nephrotoxicity is well established in other models.^{17,18,73}

3D bioprinting

Three-dimensional bioprinting technologies enable fabrication of tissue constructs with precisely controlled spatial architecture, incorporating multiple cell types, extracellular matrix components, and vascular networks.^{74,75} Bioprinted liver models with physiologically relevant lobular organization have shown improved prediction of idiosyncratic hepatotoxicity compared to conventional models.⁷⁶ For NP evaluation, bioprinted multi-tissue platforms offer the potential for personalized toxicity assessment, where patient-specific cells can be incorporated to predict individual susceptibility to NP-induced adverse effects.^{77,78}

Computational toxicology and systems biology

Quantitative structure-activity relationship (QSAR) and machine learning

Computational approaches have become indispensable for predicting toxicity of NP single compounds, leveraging structural features to estimate hazard potential without experimental testing.^{79,80} Traditional QSAR models have been supplemented by machine learning algorithms (random forests, support vector machines, deep neural networks) trained on large toxicity databases, showing useful predictive performance for specific endpoints such as hERG channel inhibition and Ames mutagenicity.^{81,82} Recent advances in graph neural networks and transformer architectures have further improved predictive performance for complex molecular scaffolds characteristic of NPs.⁸³

For NP-specific applications, computational approaches have been used to screen hepatotoxic ingredients in TCMs,⁸⁴ while

toxicological evidence has been synthesized for alkaloid-related safety risks,⁸⁵ and computational tools can predict specific chemical toxicities such as skin sensitization.⁸⁶ The integration of physiologically based pharmacokinetic (PBPK) modeling with toxicity prediction enables estimation of human-relevant internal exposure and margin of safety calculations.⁸⁷

Network pharmacology and network toxicology

Network-based approaches are uniquely suited to NP safety evaluation because they embrace, rather than simplify, the multi-component and multi-target nature of complex preparations.^{88,89} Network toxicology constructs target-pathway-toxicity networks that identify convergent mechanisms through which multiple NP constituents may synergistically or antagonistically affect safety-critical pathways.⁹⁰ This approach has been used to investigate hepatotoxic mechanisms of specific *Polygonum multiflorum* components and to explore efficacy-toxicity mechanisms in *Fritillaria thunbergii*.^{91,92}

Molecular docking, molecular dynamics simulations, and free energy perturbation calculations complement network approaches by providing atomistic insights into NP-target interactions.^{93,94} Artificial intelligence (AI)-driven computational platforms are increasingly being applied across phytochemical screening and toxicity prediction.⁹⁵⁻⁹⁷

Multi-omics integration

Omics technologies, including genomics, proteomics, metabolomics, and spatial multi-omics, have emerged as essential tools for elucidating the toxicity mechanisms of natural products. These high-throughput, high-content approaches can systematically reveal the biological pathways and key targets underlying toxic effects at the molecular level, compensating for the limitations of traditional toxicity endpoints, such as cell viability or death, in mechanistic interpretation.^{69,91,98-101} For instance, in hepatotoxicity studies, metabolomic analysis combined with a liver organoid-on-a-chip system demonstrated that cadmium exposure induced hepatic dysfunction in a dose-dependent manner, whereas selenium supplementation alleviated toxicity at specific concentrations; however, high concentrations of selenium paradoxically diminished this protective effect by inducing oxidative stress and disrupting metabolic pathways, thereby revealing the complexity of dose-effect relationships.⁹⁸ Similarly, by integrating metabolomics with a 3D myocardial chip, researchers provided evidence that processing of Tiebangchui may reduce cardiotoxicity through esterification of diterpenoid alkaloids: the crude drug caused increased lactate dehydrogenase release, calcium overload, reactive oxygen species accumulation, and tricarboxylic acid cycle dysregulation, whereas the processed product significantly ameliorated these pathological changes.⁶⁹

Spatial multi-omics technologies further enhance the spatial resolution of mechanistic toxicity studies, enabling the delineation of heterogeneous toxicity response distributions at the tissue or even subcellular level, and providing new perspectives for understanding the effects of natural products on organ development, the nervous system, or the tumor microenvironment.⁹⁹ Furthermore, emerging omics approaches are being used to investigate gut-microbiota and host-microbe interactions relevant to immune and inflammatory responses.¹⁰⁰ Complementary organoid and organ-on-a-chip approaches can model host-microbiome interactions in

complex physiological environments, including the vaginal and pulmonary systems.¹⁰¹

NAMs application strategy

A central proposition of the framework presented in this review is that the applicability of NAMs differs across the three product categories according to compositional complexity and degree of chemical characterization. For pure single compounds, NAMs may be most readily applied within the proposed framework, including computational prediction, organoid-based toxicity profiling, and OoC systems. Whether a NAM can reduce or replace an animal study remains context-specific and depends on fit-for-purpose validation and acceptance for the intended regulatory use. For active fractions, NAMs can serve as screening and mechanistic tools that complement conventional studies. For TCM formulae, NAMs currently function primarily as supplementary methods for screening, mechanistic investigation, and interaction prioritization. This tiered application represents a proposed framework based on differences in compositional complexity and degree of chemical characterization, rather than an established regulatory classification. Figure 1 summarizes the proposed integration of these NAM domains across product categories.

Stratified safety evaluation strategies for natural products

TCM compound formulae

Safety evaluation characteristics

TCM compound formulae operate under the theoretical framework of traditional Chinese medicine, in which the “Jun-Chen-Zuo-Shi” configuration principle guides herb combinations.¹⁰² Within this traditional framework, “assistant” and “courier” herbs are assigned roles that may include mitigating adverse effects of “monarch” components. “Pei Wu Jian Du” (toxicity reduction through herbal compatibility) refers to the traditional idea that combining selected herbs may lessen adverse effects associated with one component. It does not denote a universal biomedical mechanism. Proposed modern explanations—such as altered exposure or biotransformation of toxic constituents and pharmacological antagonism—have been suggested in selected formula- or herb-pair-specific studies; they require direct experimental verification in each relevant preparation and should not be generalized.¹⁰²⁻¹⁰⁴ Safety evaluation of TCM formulae may include acute/repeated-dose toxicity and core safety pharmacology assessment (cardiovascular, respiratory, and central nervous systems), depending on the applicable pathway and product characteristics.¹⁰⁵⁻¹⁰⁷

Under National Medical Products Administration (NMPA) pathways, human-use experience and traditional-use evidence may inform evidence requirements for certain TCM categories; however, any reduction or modification of requirements is product- and pathway-specific.^{106,107} This historical evidence, however, has limitations: traditional use may not capture rare idiosyncratic reactions, and modern preparation methods may alter toxicity profiles compared to traditional decoctions.¹⁰⁸ Jurisdiction-specific regulatory pathways, including those applicable to certain classical TCM prescriptions in China, may take documented historical use into account when determining evidence requirements; however, the applicable requirements depend on the

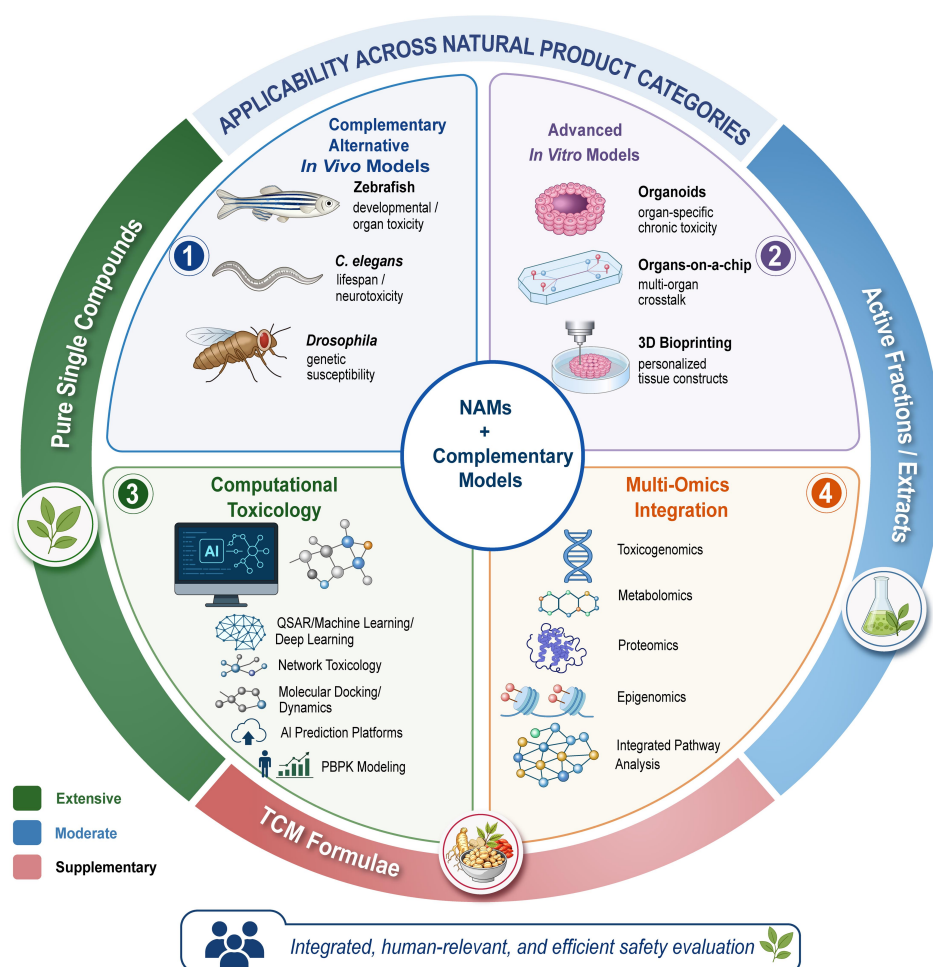


Fig. 1. Integrated framework of NAMs and complementary alternative models for natural product safety evaluation. The hub-and-spoke diagram illustrates four major NAMs domains: (1) complementary alternative *in vivo* models (zebrafish for high-throughput developmental/organ toxicity screening, *C. elegans* for lifespan/neurotoxicity, *Drosophila* for genetic susceptibility studies); (2) advanced *in vitro* models (organoids for organ-specific chronic toxicity, organ-on-a-chip systems for dynamic multi-organ crosstalk modeling, 3D bioprinting for personalized tissue constructs); (3) computational toxicology (QSAR/machine learning/deep learning for toxicity prediction, network toxicology for multi-target mapping, molecular docking/dynamics, AI prediction platforms, PBPK modeling); (4) multi-omics integration (toxicogenomics, metabolomics, proteomics, epigenomics, and integrated pathway analysis). The outer ring visualizes the authors' proposed relative research applicability of NAMs across the three product categories (figure labels: extensive, moderate, and supplementary). These labels do not represent jurisdiction-specific regulatory acceptance or a formal regulatory classification. 3D, three-dimensional; AI, artificial intelligence; NAMs, new approach methodologies; PBPK, physiologically based pharmacokinetic; QSAR, quantitative structure–activity relationship; TCM, traditional Chinese medicine.

specific product, manufacturing process, intended use, and supporting quality and safety data.

Unique safety concerns for TCM formulae include traditionally defined incompatible herb combinations, such as the 'Eighteen Incompatible Medicaments' and 'Nineteen Mutual Antagonisms', two traditional lists of herb combinations historically advised against because they were considered to increase toxicity or otherwise produce undesirable interactions; processing (Pao Zhi), the traditional pretreatment of crude medicinal materials using defined methods to alter their properties before clinical use; and decoction-dependent changes in toxic constituent extraction.^{103,104,109,110} These are traditional safety or processing concepts rather than

single biomedical mechanisms, and any mechanistic interpretation must be evaluated for the specific herb pair or processing procedure. One illustrative pair traditionally listed among the "Eighteen Incompatible Medicaments" (EIM) is *Aconitum carmichaelii* Debeaux (Chuanwu, CW) combined with *Pinellia ternata* (Thunb.) Makino (Banxia, BX). Available experimental observations summarized in the literature suggest pair-dependent changes in aconite-alkaloid composition and biotransformation, but whether these changes account for the traditional incompatibility rule remains under investigation. Such findings should not be generalized beyond the specific herb pair, dose ratio, processing method, and experimental context studied.¹⁰⁴

NAMs application space

Although the application of NAMs to TCM formulae remains limited by compositional complexity, emerging applications include cell-based screening of hepatotoxicity and CYP3A4 induction for herbal components,¹¹¹ zebrafish-based developmental toxicity screening of formula components,¹¹² *in silico* polypharmacology approaches for mapping multi-component–multi-target relationships in natural products.¹¹³

Active fractions and extracts

Safety evaluation characteristics

Active fractions have an intermediate level of complexity, with relatively simplified composition compared to whole formulae but retaining multi-component character.¹¹⁴ The manufacturing process critically influences safety profiles, as extraction solvents, separation conditions, and purification steps may selectively enrich or deplete toxic constituents.¹¹⁵ Published reviews of herbal-product regulation indicate that safety expectations are case-specific and vary with product classification, intended use, exposure, and jurisdiction; repeated-dose, genotoxicity, and reproductive/developmental assessments may be required depending on the applicable pathway.¹¹⁶

The concentration effect poses a particular safety concern—active fraction preparation may inadvertently concentrate toxic minor components to pharmacologically relevant levels.¹¹⁵ Spectrum-effect relationships linking chemical fingerprints to toxicological endpoints provide crucial tools for identifying responsible constituents.^{117,118} Batch consistency directly impacts safety reliability, requiring robust quality control measures.¹¹⁹

NAMs application space

NAMs offer substantial value for active fractions, including cell-based screening and disposition characterization to prioritize components,^{111,120} metabolomics-guided biomarker discovery for toxicity monitoring,¹²¹ and organoid-based toxicity studies in human-relevant systems.^{57,58}

Purified single compounds

Safety evaluation characteristics

Purified NP compounds, with defined molecular structures, most closely approximate the synthetic drug evaluation paradigm.^{122,123} The nonclinical package is determined by the applicable development pathway and product-specific safety questions; depending on context, it may include acute or repeated-dose toxicity, safety pharmacology, genotoxicity, reproductive/developmental toxicity, and carcinogenicity assessment. While purified NP compounds can be evaluated using established approaches for small-molecule therapeutics, compound-specific considerations may remain important, including potential multi-target pharmacological activity, gut microbiota-mediated biotransformation,¹²⁴ and structural features such as chirality and stereochemistry that may influence pharmacokinetics, metabolism, or *in vivo* activity.

NAMs application space

Purified single compounds may represent the most readily applicable category for NAMs within the proposed framework, for which computational prediction, organoid-based screening, OoC systems, and integrated omics approaches may complement

conventional testing and, where fit for purpose and accepted for the intended use, potentially reduce reliance on selected animal studies.^{43,57,66,79-83} The FDA's 2026 draft guidance provides non-binding, fit-for-purpose validation recommendations for NAM data used in drug development; it does not establish blanket regulatory acceptance for purified NP single compounds.⁴³

Stratified evaluation framework

The stratified framework recognizes that evaluation rigor should be proportional to both the potential risk and the feasibility of characterization. Within this proposed hierarchy, TCM formulae are treated as the most compositionally complex category, with NAMs mainly serving complementary roles; active fractions occupy an intermediate position; and pure single compounds offer greater opportunities for applying NAMs, although any reduction in animal studies remains context-specific and dependent on validation and the applicable regulatory pathway. Table 1 presents the stratified framework.

The framework proceeds through seven linked steps: (1) product classification, distinguishing complex formulae, active fractions/extracts, and purified single compounds; (2) compositional characterization, including chemical fingerprinting, marker quantification, batch comparison, and impurity/adulterant screening; (3) risk identification, integrating known toxic constituents, organ-specific signals, exposure route, duration, vulnerable populations, and herb–drug interaction potential; (4) NAMs selection, matching computational models, alternative organisms, organoids, organs-on-a-chip, 3D systems, PBPK modeling, and omics tools to the product's chemical definition and expected mechanism; (5) conventional toxicity testing, retained where systemic exposure, reproductive/developmental toxicity, chronic toxicity, or mixture uncertainty cannot be adequately resolved by NAMs; (6) quality-control-linked risk mitigation, translating toxic markers, spectrum–effect relationships, process analytical technology, and metabolomic batch profiling into release criteria; and (7) clinical pharmacovigilance and regulatory decision-making, using clinical monitoring, Roussel Uclaf Causality Assessment Method (RUCAM)-based causality assessment, spontaneous reporting, real-world data, and jurisdiction-specific requirements to update risk management throughout the product life cycle.

Within this stratified framework, the subsequent sections are organized according to their functional roles in safety evaluation. Organ-specific toxicity and herb–drug interactions inform hazard identification and endpoint prioritization; quality control supports compositional consistency and the interpretation of toxicity data; pharmacovigilance provides clinical and post-marketing safety feedback; and regulatory considerations translate the accumulated evidence into product-category-specific decision-making. NAMs, multi-omics, and computational approaches serve as enabling tools selected according to product complexity and the identified safety question.

Specific toxicities and mechanisms of natural products

The following toxicity domains are presented not as an exhaustive catalogue, but to illustrate how identified hazards guide endpoint selection and the choice of appropriate NAMs and conventional testing within the stratified framework. Figure 2 summarizes representative mechanisms of organ-specific toxicity.

Table 1. Proposed stratified safety evaluation framework for the three operational product categories considered in this review

Parameter	TCM compound formulae	Active fractions/extracts	Pure single compounds	Synthetic drugs (comparison)	Key distinction
Compositional complexity	Extremely high (numerous constituents)	Moderate (tens to hundreds of components)	Single compound, defined structure	Single compound, fully characterized	Complexity decreases left to right
Quality consistency	Low-moderate (batch variation, matrix effects)	Moderate (process-dependent)	High (structural confirmation)	Very high (synthesis reproducibility)	Consistency increases from left to right
Illustrative regulatory evaluation considerations	Case-specific; acute/repeated-dose toxicity + safety pharmacology may be required; historical use may be considered when justified	Case-specific; repeated-dose, genotoxicity, and reproductive/developmental studies may be considered as applicable	Product/pathway-specific; ICH principles may inform the nonclinical package	Product/pathway-specific; ICH principles commonly inform the nonclinical package	Requirements are jurisdiction-, product-, route-, exposure-, and question-specific
NAMs applicability	Proposed role: primarily complementary—interaction screening, network toxicology, mechanistic support	Proposed role: intermediate—component screening, omics-guided biomarkers, mechanistic studies	Proposed role: greater opportunity for computational, organoid, OoC, and AI-based approaches	Context-specific integration where methods are fit for purpose and accepted for the intended use	These are proposed relative roles, not regulatory classifications; applicability depends on validation and context of use
Special safety concerns	Traditionally defined herb-pair concerns (including EIM); Pao Zhi-dependent changes; pair-, formula-, and processing-specific interactions and HDIs	Toxic-component enrichment; process-dependent composition; batch consistency	Compound-specific multi-target effects; microbiota-mediated biotransformation; stereochemical effects	Off-target effects; reactive metabolites	Risk considerations are product- and compound-specific
Clinical evidence base	Extensive historical use (centuries); limited modern controlled safety data	Growing clinical evidence; moderate pharmacovigilance data	Established clinical pharmacology; standard adverse drug reaction (ADR) reporting	More standardized clinical safety data	Historical use provides safety context but does not establish comprehensive safety

The three natural-product categories shown here are operational categories proposed for stratified safety evaluation rather than a universal taxonomic classification of natural products. Regulatory and toxicological requirements are jurisdiction- and product-specific and may vary according to intended use, claims, route of administration, dose and exposure duration, formulation, degree of chemical characterization, and available historical, nonclinical, and clinical evidence. Accordingly, the relative NAMs applicability shown here represents the proposed framework of this review rather than an established regulatory classification. AI, artificial intelligence; EIM, Eighteen Incompatible Medicaments; HDI, herb–drug interaction; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; NAMs, new approach methodologies; OoC, organ-on-a-chip; TCM, traditional Chinese medicine.

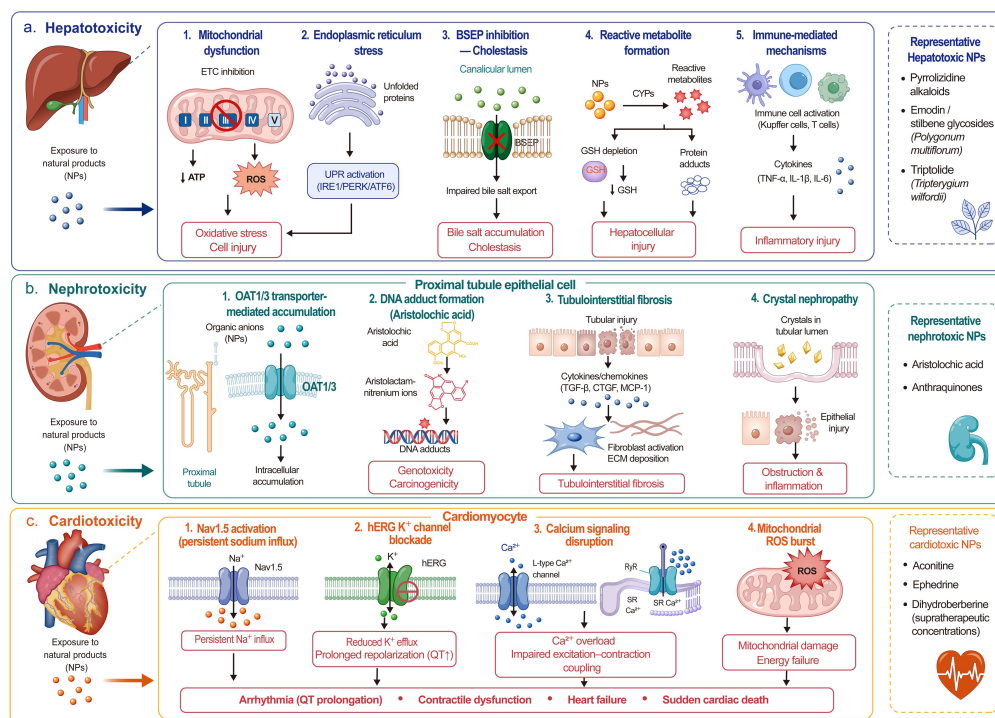


Fig. 2. Molecular mechanisms of organ-specific toxicity induced by natural products. (a) Hepatotoxicity mechanisms including mitochondrial dysfunction (ETC inhibition, ROS generation), endoplasmic reticulum stress (UPR activation), bile salt export pump (BSEP) inhibition leading to cholestasis, reactive metabolite formation (GSH depletion, protein adducts), and immune-mediated mechanisms. Representative hepatotoxic NPs: pyrrolizidine alkaloids, emodin/stilbene glycosides from *Polygonum multiflorum*, triptolide from *Tripterygium wilfordii*. (b) Nephrotoxicity mechanisms centered on OAT1/3 transporter-mediated tubular accumulation, DNA adduct formation (aristolactam-nitrenium ions), tubulointerstitial fibrosis, and crystal nephropathy. Representative nephrotoxic NPs: aristolochic acid, anthraquinones. (c) Cardiotoxicity mechanisms including persistent voltage-gated sodium channel (Nav1.5) activation, hERG potassium channel blockade, calcium signaling disruption, and mitochondrial ROS burst. Representative cardiotoxic NPs: aconitine, ephedrine, dihydroberberine. ATF6, activating transcription factor 6; ATP, adenosine triphosphate; CTGF, connective tissue growth factor; CYPs, cytochrome P450 enzymes; DNA, deoxyribonucleic acid; ECM, extracellular matrix; ETC, electron transport chain; GSH, reduced glutathione; hERG, human ether-à-go-go-related gene; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IRE1, inositol-requiring enzyme 1; MCP-1, monocyte chemoattractant protein-1; NPs, natural products; OAT1/3, organic anion transporters 1 and 3; PERK, protein kinase R-like endoplasmic reticulum kinase; QT, electrocardiographic QT interval; ROS, reactive oxygen species; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; UPR, unfolded protein response.

Organ-specific toxicity

Hepatotoxicity

The liver is a frequently reported target organ of toxicity associated with herbal and natural-product preparations, reflecting its central role in xenobiotic metabolism and first-pass exposure.^{125,126} The reported burden of HILI varies among countries and study populations. In Chinese mainland, herbal and traditional medicine products have been reported as important contributors to DILI, whereas prospective studies from Republic of Korea have reported country-specific incidence estimates for HILI.^{127,128} Differences in study design, case ascertainment, and patterns of herbal-product use should be considered when comparing these findings across regions.

Pyrrolizidine alkaloid (PA)-containing plants (*Senecio*, *Crotalaria*, *Heliotropium* species) cause hepatic sinusoidal obstruction syndrome through CYP3A4-mediated bioactivation to reactive dehydro-PA metabolites that crosslink proteins and

DNA.^{129,130} *Polygonum multiflorum* hepatotoxicity involves multiple mechanisms including mitochondrial dysfunction, endoplasmic reticulum stress, and immune-mediated idiosyncratic injury, with anthraquinone and stilbene glycoside components implicated.^{131,132} *Tripterygium wilfordii* (Thunder God Vine) hepatotoxicity has been linked to triptolide-mediated inhibition of XPB/TFIIH, triggering apoptosis in hepatocytes.¹³³ Patients with pre-existing viral hepatitis warrant particular attention when evaluating TCM-associated hepatotoxicity. In patients with chronic hepatitis B (CHB), TCM-related liver injury may clinically resemble an acute exacerbation of the underlying viral hepatitis, making causal attribution particularly challenging.¹³⁴ A prospective study of CHB patients hospitalized with liver dysfunction found that 7 of 45 patients (15.6%) had liver injury attributable to TCM use, with several experiencing severe clinical outcomes.¹³⁵ More broadly, hepatotoxicity associated with herbal medicines and herb–drug interactions has been reported in patients with viral hepatitis-related liver disease.¹³⁴ Therefore, assessment

of suspected TCM-associated liver injury in this population should carefully consider the temporal relationship with TCM exposure, activity of the underlying viral infection, concomitant medications including antiviral therapy, potential herb–drug interactions, and alternative causes of hepatic decompensation.

Mechanistically, NP-induced hepatotoxicity encompasses mitochondrial dysfunction (electron transport chain inhibition, uncoupling), endoplasmic reticulum stress (unfolded protein response activation), bile salt export pump (BSEP) inhibition (cholestatic injury), reactive metabolite formation (glutathione depletion, protein adducts), and immune-mediated mechanisms (hapten formation, danger signal release).¹³⁶⁻¹³⁹

Nephrotoxicity

Aristolochic acid (AA) nephropathy represents the paradigmatic NP nephrotoxicity, affecting tens of thousands of patients worldwide through contaminated herbal preparations.^{17,18} AAs are taken up by renal proximal tubular cells through organic anion transporters, particularly OAT1 and OAT3. After uptake, aristolochic acid I (AAI) undergoes nitroreductive bioactivation—most efficiently by NQO1, with CYP1A2 also contributing under some conditions—to reactive aristolactam/nitrenium intermediates that form characteristic DNA adducts (including dA-AAI and dG-AAI).^{17,18,140-142} These adducts drive both acute tubular necrosis and chronic tubulointerstitial fibrosis, with documented progression to upper urinary tract urothelial carcinoma.^{141,142} AA-associated mutational signatures have been reported in liver cancers and other hepatic malignancies.^{143,144} Kidney organoid models have recently enabled mechanistic studies of drug-induced tubular injury in human-derived systems.⁵⁸

Other nephrotoxic herbal exposures include anthraquinone-containing preparations and *Tripterygium* preparations.¹⁴⁵

Cardiotoxicity

Aconitine and related C19-diterpenoid alkaloids from *Aconitum* species cause cardiac arrhythmias through persistent activation of voltage-gated sodium channels (Nav1.5), prolonging the action potential and triggering ventricular tachycardia and fibrillation.¹⁴⁶ For *Aconitum* specifically, chemical and toxicity analyses of defined processed preparations have shown lower levels of toxic diester diterpenoid alkaloids; this processing-associated change may reduce, but does not eliminate, cardiotoxic risk.¹⁴⁷ This detoxification pattern is herb- and processing-specific and should not be generalized to Pao Zhi as a whole. A biomimetic 3D anisotropic heart-on-a-chip model has been employed to evaluate aconitine cardiotoxicity, with significant elevations in extracellular lactate dehydrogenase (LDH) levels indicating myocardial injury.⁶⁹ Ephedrine/pseudoephedrine stimulate sympathetic activation, increasing heart rate, blood pressure, and myocardial oxygen demand, with clinical reports of myocardial infarction, arrhythmias, and fatal cardiovascular events.¹⁴⁸⁻¹⁵⁰

Additional cardiotoxic mechanisms include hERG potassium channel blockade (dihydroberberine), calcium signaling disruption (digitalis glycosides), and mitochondrial reactive oxygen species generation (doxorubicin, originally derived from *Streptomyces*).¹⁵¹⁻¹⁵³

Other organ toxicities

Examples of NP-related neurotoxicity include arecoline-induced

neuronal injury and vincristine-induced peripheral neuropathy.^{154,155} Domoic acid exposure has also been associated with cardiotoxic effects in animal studies.¹⁵⁶

Herb-drug interactions (HDIs)

Evidence supporting HDIs spans multiple levels, ranging from clinically documented pharmacokinetic interactions to mechanistic findings from *in vitro* and animal studies and predictions generated by computational approaches. These different levels of evidence should be distinguished when evaluating the clinical relevance of a potential HDI.

CYP450-mediated interactions

The pregnane X receptor (PXR)–cytochrome P450 (CYP)–P-glycoprotein (P-gp) axis represents an important mechanistic pathway through which herbal constituents may alter the disposition of concomitantly administered drugs. PXR regulates the expression of major drug-metabolizing enzymes and transporters, including CYP3A4, CYP2C9, and P-gp.^{157,158} Experimental studies have shown that specific herbal constituents, such as alisol B 23-acetate, can activate PXR and thereby potentially modify the expression of downstream drug-metabolizing enzymes and transporters.¹⁵⁹ *In vitro* studies have also demonstrated that constituents of *Schisandra chinensis* and standardized medicinal plant extracts can inhibit CYP3A4/3A5 and/or CYP2D6 activity.^{160,161} These findings provide mechanistic evidence for potential HDIs but, in isolation, should not be interpreted as proof of clinically meaningful interactions.

In contrast, human pharmacokinetic and clinical interaction evidence has been reported for selected plant-derived products. Grapefruit is a well-documented example: its furanocoumarins inhibit intestinal CYP3A4 and can increase systemic exposure to susceptible orally administered drugs, including selected calcium-channel blockers, statins, and immunosuppressants.¹⁶²⁻¹⁶⁴ Goldenseal also has clinically relevant interaction potential; its effects on CYP-mediated drug metabolism have been characterized using clinical pharmacokinetic observations together with *in vitro* experiments and physiologically based pharmacokinetic modeling.¹⁶⁵ These human-supported examples should therefore be distinguished from interaction signals derived primarily from experimental or computational systems.

St. John's wort (*Hypericum perforatum*) provides a well-characterized example in which mechanistic evidence is supported by direct human pharmacokinetic data. In healthy volunteers, 14 days of St. John's wort administration reduced digoxin exposure by approximately 18%, increased duodenal P-glycoprotein/MDR1 and CYP3A4 expression by approximately 1.4- and 1.5-fold, respectively, and increased hepatic CYP3A4 activity by approximately 1.4-fold.¹⁶⁶ In a separate clinical pharmacokinetic study, St. John's wort reduced indinavir area under the concentration-time curve by 57% and the extrapolated 8-h trough concentration by 81%.¹⁶⁷ Together, these findings demonstrate that St. John's wort-mediated enzyme and transporter induction can produce clinically meaningful reductions in systemic exposure to susceptible co-administered drugs.

Transporter-mediated interactions

Drug transporters constitute another important mechanism underlying potential HDIs.¹⁶⁸ Experimental evidence indicates that

herbal constituents such as curcumin, piperine, and quercetin can modulate P-gp activity and may thereby alter the disposition of transporter substrates.¹⁶⁹ In addition, studies using humanized mice have demonstrated the involvement of organic anion-transporting polypeptides OATP1B1 and OATP1B3 in the disposition of glycyrrhizin and associated interaction potential.¹⁷⁰ However, such transporter-mediated findings are derived largely from mechanistic, *in vitro*, or animal studies and should be regarded as potential or preclinical interaction signals until their magnitude and clinical relevance are confirmed in appropriately designed human pharmacokinetic studies.

Breast cancer resistance protein (BCRP) represents another transporter capable of influencing drug disposition. Preclinical studies demonstrate that changes in BCRP activity can substantially affect drug distribution¹⁷¹; however, such findings establish the pharmacokinetic importance of the transporter rather than, by themselves, demonstrating a clinically established herb–drug interaction. Consequently, extrapolation of BCRP-related experimental findings to clinical HDI risk requires specific evidence that a herbal constituent produces a relevant change in transporter activity at clinically achievable exposures.

Special considerations for complex preparations

Prediction of HDIs is particularly challenging for complex herbal and TCM preparations because multiple constituents may simultaneously modulate drug-metabolizing enzymes, transporters, and pharmacodynamic pathways, potentially resulting in additive, synergistic, or antagonistic interactions. Computational approaches have therefore been explored to prioritize potential interaction signals in complex TCM systems. Graph-based models have been developed for interaction prediction,¹⁷² while network pharmacology provides a framework for mapping multi-component–multi-target relationships.¹⁷³ Nevertheless, these approaches remain primarily predictive and hypothesis-generating. Their outputs should not be regarded as clinically established HDIs without subsequent validation using appropriately designed experimental, pharmacokinetic, and clinical studies. Representative products, mechanisms, and interaction examples are summarized in Table 2.^{17,18,70,129–133,137,141,142,147,150,162–164,166,167,174–192}

Quality control, clinical safety, and regulatory oversight

These components represent the implementation and feedback layers of the stratified evaluation framework, linking product quality, clinical safety signals, and regulatory requirements with the preceding hazard-assessment process.

Clinical safety and pharmacovigilance

Clinical trial safety monitoring

Safety monitoring in NP clinical trials requires adapted approaches reflecting their unique characteristics. The RUCAM provides structured causality assessment for suspected liver injury.^{193,194} For herbal medicines and other multi-component natural product preparations, causality assessment also requires careful exposure characterization and exclusion of alternative causes.³³ Standardized adverse drug reaction (ADR) documentation, including onset timing relative to NP administration, dechal-

lenge/rechallenge information, and concomitant medication assessment, is essential.^{195–197}

Post-marketing pharmacovigilance

Spontaneous reporting systems including the FDA Adverse Event Reporting System (FAERS), WHO Vigibase, and China's National ADR Monitoring System provide critical post-marketing safety surveillance.^{198–201} However, underreporting of NP-related adverse events is well-documented.^{202,203}

Real-world data and evidence

Real-world data (RWD) from electronic health records, insurance claims databases, and prescription registries offer emerging opportunities for NP safety signal detection and TCM-specific data-mining applications.^{204–206} Machine-learning approaches applied to RWD have been explored for covariate assessment and adverse-event risk prediction in other drug settings, suggesting methodological relevance to NP pharmacovigilance.^{207,208}

Regulatory frameworks: a comparative perspective

Regulatory approaches to NP safety evaluation vary substantially across jurisdictions, reflecting different cultural relationships with traditional medicine and pharmaceutical regulation philosophies.^{209,210} Key recent developments include the U.S. FDA Modernization Act 2.0 (2022), which amended the statutory definition of a “nonclinical test” to allow specified non-animal approaches without creating a universal exemption from animal studies^{34,35}; in 2023, the European Medicines Agency's Committee on Herbal Medicinal Products (HMPC) planned or advanced revisions to several herbal-medicine guidance documents, including nonclinical genotoxicity guidance²¹¹; NMPA released revised regulatory and technical guidelines for ancient classical Chinese medicinal formula preparations in the same year¹⁰⁶; the WHO Global Traditional Medicine Strategy 2025–2034 emphasizes evidence generation, safety, quality, and appropriate regulatory mechanisms for traditional medicine.²¹²

The U.S. FDA regulates botanical drugs under specific guidance while dietary supplements fall under DSHEA with limited pre-market safety requirements.^{213,214} The EMA maintains Traditional Herbal Medicinal Product (THMP) registrations requiring demonstration of traditional use alongside safety data.²¹⁵ China's NMPA has established category-specific pathways for TCM drugs, including innovative TCM drugs and ancient-classical-formula preparations.^{106,107} Japan's Pharmaceuticals and Medical Devices Agency (PMDA) regulates Kampo medicines; established approval standards apply to certain OTC Kampo formulations.²¹⁶ International initiatives through ICH and the WHO Traditional Medicine Strategy may support methodological convergence, but do not establish automatic mutual recognition of safety data. Table 3 provides a simplified comparative overview.^{34,35,43,106,107,209,211–219}

Quality control and its impact on safety

The inseparable relationship between quality control and safety assessment in NPs requires integrated consideration. Chemical fingerprinting using HPLC, LC-MS, and NMR provides batch-to-batch consistency verification critical for safety reliability.^{220,221} Spectrum-effect relationship analysis correlates chromatographic profiles with biological/toxicological endpoints, enabling identification of toxic markers.¹¹⁷ Process analytical technology

Table 2. Representative herbal and plant-derived products/constituents illustrating major safety risks and herb–drug interactions

Natural product/source	Toxic component(s)	Primary target organ	Key molecular mechanism/pathway	Research models used	Clinical significance	References
<i>Aristolochia</i> spp.	Aristolochic acid I/II	Kidney	Nitroreductive bioactivation and DNA-adduct formation → tubular injury/fibrosis; aristolochic-acid mutational signatures are linked to urothelial carcinogenesis	Mechanistic <i>in vitro/in vivo</i> studies; chemoproteomics/metabolomics; human tumor-genomic evidence	Aristolochic acid nephropathy; association with urothelial cancer	17,18,141,142,174,175
<i>Polygonum multiflorum</i>	Stilbene-glycoside metabolites; anthraquinone-related constituents (candidate contributors)	Liver	Mitochondrial/ER stress and immune-mediated idiosyncratic injury have been reported; constituent-specific bioactivation may contribute	Mechanistic <i>in vitro/in vivo</i> studies; translational studies and recent reviews	HILI has been reported; culpable constituents and mechanisms may vary with preparation and exposure	131,132,176,177
<i>Tripterygium wilfordii</i>	Triptolide (representative diterpenoid)	Liver; other organs reported	XPB/TFIIH interaction and multiple cell-death/mitochondrial pathways described in experimental studies	Mechanistic studies and reviews, largely preclinical	Multi-organ toxicities have been reported; hepatotoxicity is a recognized concern	133,137,178,179
<i>Aconitum</i> spp.	Aconitine, mesaconitine, hypaconitine	Heart	Persistent voltage-gated sodium-channel activation/dysregulation → prolonged action potentials and ventricular arrhythmias	Zebrafish; microfluidic cardiac model; human poisoning series	Clinical poisoning can involve severe, sometimes fatal arrhythmias; processing can reduce diester diterpenoid alkaloid content/toxicity but does not eliminate risk	70,147,180,181
<i>Ephedra sinica</i>	Ephedrine, pseudoephedrine	Heart, CNS	Sympathomimetic α/β -adrenergic effects → increased HR/BP and myocardial oxygen demand	Clinical case reports, including direct Ephedra/Ma Huang exposure; pharmacology/toxicology literature	Cardiovascular adverse effects, including arrhythmias, have been reported; current U.S. FDA Import Alert 54-13 provides for detention without physical examination of dietary supplements and bulk dietary	150,182,183

Table 2. Representative herbal and plant-derived products/constituents illustrating major safety risks and herb–drug interactions (*continued*)

Natural product/source	Toxic component(s)	Primary target organ	Key molecular mechanism/pathway	Research models used	Clinical significance	References
					ingredients containing ephedrine alkaloids	
Pyrrolizidine alkaloid plants (<i>Senecio</i> , <i>Crotalaria</i>)	Pyrrolizidine alkaloids (PAs)	Liver	CYP-mediated bioactivation to reactive dehydro-PAs → protein/DNA adduct formation → hepatic injury/SOS	Primary hepatocytes; other <i>in vitro/in vivo</i> models	Hepatotoxicity, including sinusoidal obstruction syndrome, has been reported	129,130,184
<i>Hypericum perforatum</i> (St. John's wort)	Hyperforin	Systemic (HDI)	PXR-related CYP3A4 induction and P-gp modulation → reduced exposure to susceptible co-administered drugs	Mechanistic/computational and animal evidence; direct human PK/intestinal-biopsy and clinical interaction studies	Human studies showed reduced digoxin and indinavir exposure; magnitude and clinical relevance are substrate- and exposure-dependent	166,167,185,186
<i>Citrus × paradisi</i> (Grapefruit)	Furanocoumarins (bergamottin, DHB)	Systemic (HDI)	Intestinal CYP3A4 inhibition by grapefruit furanocoumarins → increased exposure to susceptible oral CYP3A4 substrates	Direct human PK study; clinical interaction reviews; PBPK and mechanistic CYP3A4 modeling	Clinically documented increases in exposure have been reported for selected CYP3A4 substrates; magnitude is drug- and exposure-dependent	162-164,187,188
<i>Piper nigrum</i> (Black pepper)	Piperine	Systemic (HDI)	Preclinical evidence suggests P-gp/CYP3A4 modulation that may alter absorption or clearance of co-administered drugs	Preclinical/mechanistic and pharmacokinetic literature; clinical curcumin–piperine co-administration trials not designed to establish a PK HDI	Bioenhancing effects have been proposed/reported, but the magnitude and clinical significance of piperine-mediated HDIs remain uncertain	189,190
<i>Digitalis purpurea</i>	Digoxin	Heart	Na ⁺ /K ⁺ -ATPase inhibition → altered Na ⁺ /Ca ²⁺ handling and increased intracellular Ca ²⁺	Clinical TDM methods; mechanistic cardiac studies	Digoxin has a narrow therapeutic window; TDM can support dose management in appropriate clinical settings	191,192

Selection rationale: The examples were selected to illustrate reported organ toxicities, clinically documented or mechanistically informative herb–drug

interactions, regulatory relevance, and diverse experimental approaches. They are representative examples rather than an exhaustive list or a ranking of the highest-risk natural products. Evidence-level note: the references in each row collectively support the specific mechanistic, model, clinical, or regulatory statements to which they relate; preclinical or computational findings are not treated as established clinical HDIs without human evidence. BP, blood pressure; CNS, central nervous system; CYP, cytochrome P450; DHB, 6',7'-dihydroxybergamottin; DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; FDA, U.S. Food and Drug Administration; HDI, herb–drug interaction; HILI, herb-induced liver injury; HR, heart rate; OAT, organic anion transporter; PA, pyrrolizidine alkaloid; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein; PK, pharmacokinetic; PXR, pregnane X receptor; SOS, sinusoidal obstruction syndrome; TDM, therapeutic drug monitoring; TFIIH, transcription factor IIH; XPB, xeroderma pigmentosum group B.

(PAT) enables real-time monitoring of critical quality attributes during manufacturing, preventing inadvertent generation of toxic impurities or degradation products.²²²

Multi-component quantitative analysis using reference standards enables simultaneous monitoring of both efficacy and safety markers, with recent advances in untargeted metabolomics offering comprehensive compositional surveillance.^{2,223} Recent AI-assisted near-infrared spectroscopy models have been used for rapid, non-destructive quality control of TCM products, including discrimination of expired versus normal samples and prediction of key active-compound contents.²²⁴

Importantly, these quality-control tools can directly support safety-related decision-making. Chemical fingerprinting and spectrum–effect relationship analysis can help link compositional variation with biological or toxicological endpoints and identify potential safety-related markers.^{117,118,220,221} PAT, metabolomic profiling, and data-driven quality models may further support detection of abnormal compositional or process-related patterns and inform additional testing or process-adjustment decisions.^{222–224} Within the proposed framework, post-marketing safety signals identified through pharmacovigilance systems could also be linked back to batch-specific analytical and manufacturing data to support root-cause investigation and refinement of subsequent quality-control measures.^{198–203}

Challenges in standardizing safety evaluation of multi-component natural products

Despite significant advances in the field of NP safety evaluation, several formidable challenges persist. The extreme compositional complexity of TCM formulae—potentially containing thousands of chemical entities—means that no single toxicity endpoint can adequately represent overall safety.¹³ Meanwhile, NAMs lack standardized validation protocols specifically designed for multi-component NP preparations, which complicates reproducibility, interpretation, and regulatory use.^{225–227} Disparities among international regulatory frameworks create barriers to cross-jurisdictional comparison and reuse of safety data.²²⁸ Furthermore, the fundamental tension between TCM's individualized “pattern differentiation” prescribing—the practice of classifying a patient's constellation of symptoms and signs into a traditional pattern and tailoring treatment accordingly—and standardized safety evaluation methodologies remains unresolved.^{229,230} To bridge this gap, some scholars have proposed the development of “TCM Regulatory Science” (TCMRS) as an interdisciplinary solution, aiming to integrate modern scientific methods with TCM theory to address critical issues concerning safety, efficacy, and quality control.²¹⁰

The related concept of “Bian Zheng Yong Yao” (pattern-differentiated medication) refers to selecting medicines according

to the individual's identified TCM pattern rather than applying a fixed prescription to all patients. This traditional decision framework does not currently map to a single validated biomedical mechanism; efforts to standardize and scientifically characterize TCM patterns remain ongoing.^{230,231} Finally, greater convergence in NP safety evaluation will require clearer study designs, endpoint definitions, quality standards, and pharmacovigilance practices.^{209,232,233}

At the same time, NAMs performance for multi-component and poorly characterized NP mixtures remains uncertain. Matrix effects can alter solubility, permeability, protein binding, and bioavailability; unknown constituents or degradation products may drive toxicity despite low abundance; metabolism-dependent toxicity may be missed in systems with insufficient metabolic competence; batch variation can shift the toxicological profile; and inter-laboratory reproducibility remains challenging when extract preparation, exposure normalization, and endpoint selection are not standardized. Therefore, NAMs should be selected and interpreted according to chemical characterization, exposure relevance, and fit-for-purpose validation rather than assumed to be universally interchangeable with conventional toxicology.

Taken together, future progress should prioritize the validation of NAMs for complex and incompletely characterized preparations, improved methodological standardization and inter-laboratory reproducibility, and stronger integration of mechanistic evidence with quality control, clinical pharmacovigilance, and regulatory decision-making. Addressing these challenges will be essential for translating the proposed stratified evaluation framework from a conceptual model into a more practical and evidence-based approach to natural product safety assessment.

Future directions

Emerging computational and personalized approaches represent potentially important future directions for natural-product safety assessment. AI- and machine-learning-based integration of multi-omics, chemical, and literature data may support hypothesis generation, hazard prioritization, and virtual screening of complex constituent interactions.^{234–237} Digital twins and virtual clinical trials could, in the longer term, provide complementary tools for exploring exposure–response relationships and inter-individual variability,^{238–240} while pharmacogenomics and patient-derived cellular models may help characterize susceptibility to selected toxicities.^{241,242} However, these applications remain largely investigational in the context of natural-product safety evaluation. Their incorporation into routine safety assessment or regulatory decision-making will require prospective validation, standardized and representative datasets, external reproducibility, uncertainty quantification, and demonstration of added predictive value over established approaches. These challenges are particularly

Table 3. Comparative global regulatory frameworks for natural product safety evaluation

Jurisdiction	Regulatory agency	Product categories	Pre-market safety requirements	Regulatory position on NAMs	Post-market surveillance	Key legislation/guidance
United States	FDA	Botanical drugs (IND/NDA); dietary supplements (DSHEA/FD&C Act)	Botanical drugs: drug-development safety package is case-specific under IND/NDA Dietary supplements: generally no FDA pre-market approval; a 75-day new dietary ingredient notification is required when applicable	Case-specific. FDA Modernization Act 2.0 permits specified non-animal approaches to qualify as “nonclinical tests”; the 2025 FDA Roadmap describes a phased strategy to reduce animal testing in selected preclinical safety contexts; and the 2026 draft NAM guidance provides non-binding, fit-for-purpose validation recommendations. Applicability remains product-, endpoint-, and context-specific; no blanket acceptance is stated for botanicals or complex mixtures	FAERS/MedWatch for drugs; dietary-supplement adverse-event reporting and FDA post-market oversight	FD&C Act; DSHEA (1994); Botanical Drug Development Guidance (2016); FDA Modernization Act 2.0 (2022); FDA Roadmap (2025); FDA Draft NAM Guidance (2026) ^{34,35,43,213,214}
European Union	EMA/HMPC and national competent authorities	Herbal medicinal products (traditional-use registration or well-established use); food supplements regulated separately under EU food law	Traditional-use registration: evidence of at least 30 years of medicinal use, including at least 15 years in the EU, plus quality and safety information; well-established-use applications rely on bibliographic evidence under the applicable legal pathway	Case-specific. EMA/HMPC guidance is consistent with 3Rs principles and endpoint-specific requirements; whether an alternative method is acceptable depends on the applicable EU/ICH/OECD framework, product, endpoint, and regulatory question. No blanket HMPC acceptance is	EudraVigilance and national pharmacovigilance systems; applicable periodic safety reporting requirements	Directive 2001/83/EC as amended by Directive 2004/24/EC; EMA/HMPC guidance and Q&A documents ^{211,215}

Table 3. Comparative global regulatory frameworks for natural product safety evaluation (*continued*)

Jurisdiction	Regulatory agency	Product categories	Pre-market safety requirements	Regulatory position on NAMs	Post-market surveillance	Key legislation/guidance
				stated for complex herbal mixtures.		
China	NMPA	TCM drugs, including innovative TCM drugs and ancient-classical-formula preparations; health foods are regulated separately	Category-specific. Human-use experience may contribute to the evidence package for certain pathways; ancient-classical-formula preparations may use a simplified pathway but still require the safety, quality, and other evidence specified for that category	Case-specific. The 2023 Special Provisions establish category-specific TCM registration pathways and allow human-use evidence to inform specified contexts, but they do not establish blanket NAM acceptance for complex TCM mixtures. Any alternative approach remains product- and pathway-specific	National ADR monitoring and product-specific post-marketing risk management/re-evaluation requirements	Drug Administration Law; Traditional Chinese Medicine Law; NMPA TCM Registration Classification and Dossier Requirements (2020); Special Provisions on TCM Registration (2023) ^{106,107}
Japan	MHLW/PMDA	Prescription/OTC Kampo medicines and crude-drug preparations; foods regulated separately	OTC Kampo products conforming to established approval standards may be reviewed using those standards; nonconforming or new products require case-specific supporting data	Case-specific. Official Kampo approval standards do not constitute a blanket acceptance of NAMs; alternative approaches depend on the applicable product and regulatory guidance	JADER and PMDA/MHLW safety communications	Pharmaceuticals and Medical Devices Act; Japanese Pharmacopoeia; MHLW Approval Standards for OTC Kampo Formulations ²¹⁶
Republic of Korea	MFDS	Herbal medicinal products; health functional foods are regulated under a separate framework	Requirements depend on regulatory classification and intended use; safety evidence is evaluated according to the applicable pharmaceutical or health-functional-food regulatory framework	Case-specific. No blanket herbal-specific NAM acceptance is established; applicability of alternative methods depends on the regulatory endpoint and relevant MFDS/OECD guidance	KAERS-based adverse event reporting and applicable post-marketing safety requirements	Pharmaceutical Affairs Act; Health Functional Foods Act; applicable MFDS herbal-medicine approval guidance; OECD-aligned alternative test method guidance; KAERS pharmacovigilance system ²¹⁷⁻²¹⁹

Table 3. Comparative global regulatory frameworks for natural product safety evaluation (continued)

Jurisdiction	Regulatory agency	Product categories	Pre-market safety requirements	Regulatory position on NAMs	Post-market surveillance	Key legislation/guidance
WHO (international)	WHO (normative guidance; not a marketing-authorization authority)	Traditional, complementary and integrative medicine guidance for Member States	Not applicable as a supranational marketing-authorization requirement; WHO provides guidance for national quality, safety, evidence, regulation, and pharmacovigilance systems	WHO supports evidence-based innovation and strengthened regulatory systems but does not confer jurisdictional “acceptance” of NAMs for specific products	WHO Programme for International Drug Monitoring/VigiBase supports global pharmacovigilance	WHO Global Traditional Medicine Strategy 2025–2034; WHO global report on traditional and complementary medicine 2019 ^{209,212}

This table provides a simplified comparative overview and is not a substitute for jurisdiction-specific regulatory guidance. Pre-market requirements, regulatory use or acceptability of NAMs, and post-market surveillance pathways may vary depending on product type, intended use, claims, regulatory classification, endpoint, and context of use. ADR, adverse drug reaction; DSHEA, Dietary Supplement Health and Education Act; EMA, European Medicines Agency; EU, European Union; FAERS, FDA Adverse Event Reporting System; FD&C Act, Federal Food, Drug, and Cosmetic Act; FDA, U.S. Food and Drug Administration; HMPC, Committee on Herbal Medicinal Products; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; IND, investigational new drug; JADER, Japanese Adverse Drug Event Report database; KAERS, Korea Adverse Event Reporting System; MFDS, Ministry of Food and Drug Safety; MHLW, Ministry of Health, Labour and Welfare; NAMs, new approach methodologies; NDA, new drug application; NMPA, National Medical Products Administration; OECD, Organisation for Economic Co-operation and Development; OTC, over-the-counter; PMDA, Pharmaceuticals and Medical Devices Agency; TCM, traditional Chinese medicine; WHO, World Health Organization.

important for complex herbal and TCM preparations, for which variable composition and incompletely characterized constituents may further limit model transferability.

Limitations

This review has several limitations. First, it is a narrative and framework-oriented review, so the evidence was selected to illustrate major concepts rather than through a systematic search, formal inclusion criteria, or evidence grading. Second, NAMs regulation is evolving rapidly, and regulatory positions may change as validation experience accumulates. Third, the heterogeneity of NPs means that conclusions drawn from herbal or TCM-related products may not apply equally to marine, fungal, microbial, dietary supplement, or purified NP drug development contexts. Fourth, NAMs remain insufficiently validated for many complex mixtures, particularly where matrix effects, unknown constituents, metabolism-dependent toxicity, batch variation, and chronic exposure are central to risk. Finally, several regulatory and pharmacovigilance examples are jurisdiction-specific and should not be generalized globally without local context. Future work should prioritize fit-for-purpose validation of NAMs for defined NP categories, reference materials for complex mixtures, exposure-normalized testing strategies, and interoperable pharmacovigilance systems. AI-driven toxicity prediction, generative AI, digital twins, pharmacogenomics, and virtual clinical trials are promising but should be regarded as emerging research directions rather than mature stand-alone regulatory tools for complex NP evaluation. Their regulatory utility will depend on transparent model validation, uncertainty quantification, data

quality, and prospective performance testing.

Conclusions

The safety evaluation of natural products in integrative medicine requires a classification-aware approach that accounts for differences in compositional complexity, degree of chemical characterization, clinical context, and regulatory requirements. Accordingly, this review proposes a “classify-then-evaluate” framework in which safety-assessment strategies are tailored to three operational product categories: TCM compound formulae, herbal extracts/active fractions, and purified single compounds. Within this proposed framework, NAMs may be more readily applied to well-characterized single compounds; whether they can reduce or replace selected animal-based tests remains fit-for-purpose, product-, endpoint-, and jurisdiction-specific. For active fractions and complex TCM formulae, they currently serve mainly as complementary tools for screening, mechanistic investigation, and evidence integration. Conventional toxicology, quality control, pharmacovigilance, and regulatory assessment therefore remain important components of the overall safety-evaluation process.

Important limitations remain, particularly for complex, poorly characterized preparations. Matrix effects, unknown or variable constituents, batch-to-batch variation, metabolism-dependent toxicity, exposure uncertainty, and limited inter-laboratory reproducibility can restrict the predictive performance and regulatory applicability of current NAMs. Their regulatory use and acceptability remain context-specific and continue to evolve; neither should be assumed to be uniform across product categories

or jurisdictions. Emerging technologies such as artificial intelligence, multi-omics, advanced cellular systems, and digital twins may further strengthen natural product safety assessment, but their roles in complex-product evaluation and regulatory decision-making remain largely developmental and require further validation. Future progress will therefore depend on rigorous validation, transparent integration of multiple evidence streams, and continued coordination among toxicological science, quality control, pharmacovigilance, and regulatory science.

Acknowledgments

AI-based tools were used only at a preliminary stage as visual references during figure preparation. All figures included in the manuscript were subsequently manually redrawn, reconstructed, and finalized by the authors using Adobe Illustrator. No AI-generated image was directly incorporated into the figures presented in the manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 82474169) and the Research Project of Clinical Toxicology Transformation from the Chinese Society of Toxicology (No. CST2021CT101).

Conflict of interest

Dr. Hongtao Jin has been an Associate Editor of *Future Integrative Medicine* since November 2021. Dr. Hongtao Jin is the chairman of Beijing Union-Genius Pharmaceutical Technology Development Co., Ltd. The authors have no other conflicts of interest to declare.

Author contributions

Writing – review and editing (MC, XD, JL, ZZ, HJ), writing – original draft (MC), validation (MC), data curation (MC, XD, JL, ZZ, HJ), investigation (MC, XD, JL, ZZ, HJ), conceptualization (MC, XD, JL, ZZ, HJ), methodology (HJ), supervision (HJ), resources (HJ), and funding acquisition (HJ). All authors have approved the final version and publication of the manuscript.

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