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Critical Review

# Exploiting Omic Data to Advance Predictive Ecotoxicology

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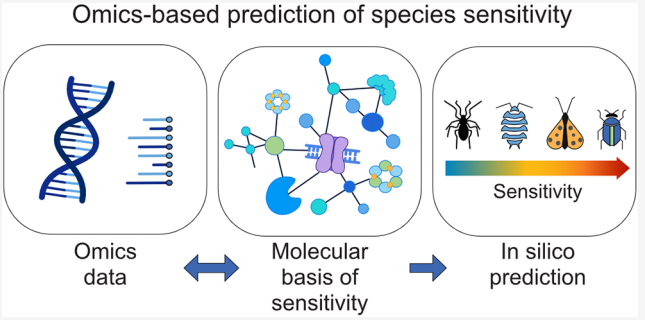
**ABSTRACT:** Predicting species-specific chemical sensitivity using in silico approaches has the potential to transform environmental risk assessment, conservation, and biomonitoring, while reducing, and ultimately replacing, animal testing. Genomic and transcriptomic data capture extensive sensitivity-relevant variation, including differences in molecular targets, xenobiotic metabolism, and damage mitigation pathways. Large-scale sequencing initiatives therefore offer an unprecedented opportunity to address ecotoxicology's "too many species" problem. Although existing omic-based predictive tools provide proof of concept, they have so far been applied to a narrow set of relatively straightforward prediction scenarios. To achieve broader applicability, current and future tools must be firmly grounded in the diverse molecular mechanisms underlying differential chemical responses. Here, we critically evaluate the emerging field of predicting species sensitivity using molecular variation inferred from omic data. We analyze the strengths and limitations of current omic-based approaches and identify major sequence and ecotoxicological data gaps, as well as critical bioinformatic challenges. We then review the current knowledge of how molecular biology underlies differential chemical sensitivity, outlining research paths to allow the next generation of sensitivity prediction tools to exploit ever expanding omic data.




**KEYWORDS:** *species sensitivity, genomics, transcriptomics, in silico risk assessment, toxicodynamics, toxicokinetics*

## 1. THE NEED FOR SPECIES SENSITIVITY PREDICTION

The chemical industry delivers substantial economic and societal benefits, but the large-scale production and use of chemicals also impose environmental costs. Chemical manufacture and postuse release can adversely affect species and communities, placing pressure on ecosystem functions and services.[1] Assessing the environmental toxicity of chemicals is particularly challenging given the scale of chemical use, with over 22,000 high-production chemicals (>1 tonne) registered with ECHA by the end of 2022.[2]

To address the vast number of chemicals and potentially exposed species, environmental "safe" concentrations are conventionally derived from toxicity tests on a very limited set of organisms, typically representing only a few vertebrate, invertebrate, and plant taxa. For example, under EU REACH regulations, minimum requirements for industrial chemicals include testing only a primary producer (e.g., algae), a primary consumer (e.g., a cladoceran), and a secondary consumer (e.g., a fish). Extrapolating toxicity data from this small group of 'representative' species to all species is highly uncertain and increasingly questioned.[3] Current hazard assessment attempts to manage this uncertainty through the application of relatively arbitrary safety factors, commonly dividing toxicity end points such as LCx, ECx, or NOEC values by 10, 100, or 1000. Improving our ability to extrapolate toxicity from a limited set of tested organisms to the broader diversity of species remains one of the most significant challenges in ecotoxicology and environmental hazard assessment.[4,5]

A key step toward addressing this challenge is the development of approaches to predict species-specific chemical sensitivity (that are regulation-aligned, validated, reproducible, and quantitative). Achieving this would offer several important benefits:

### 1.1. Informing Risk Assessment for Untestable Species

Sensitivity prediction could shift hazard assessment from generic, safety factor–based approaches toward substance-specific estimates tailored to individual species, particularly benefiting vulnerable taxa that are poorly represented by standard test organisms.

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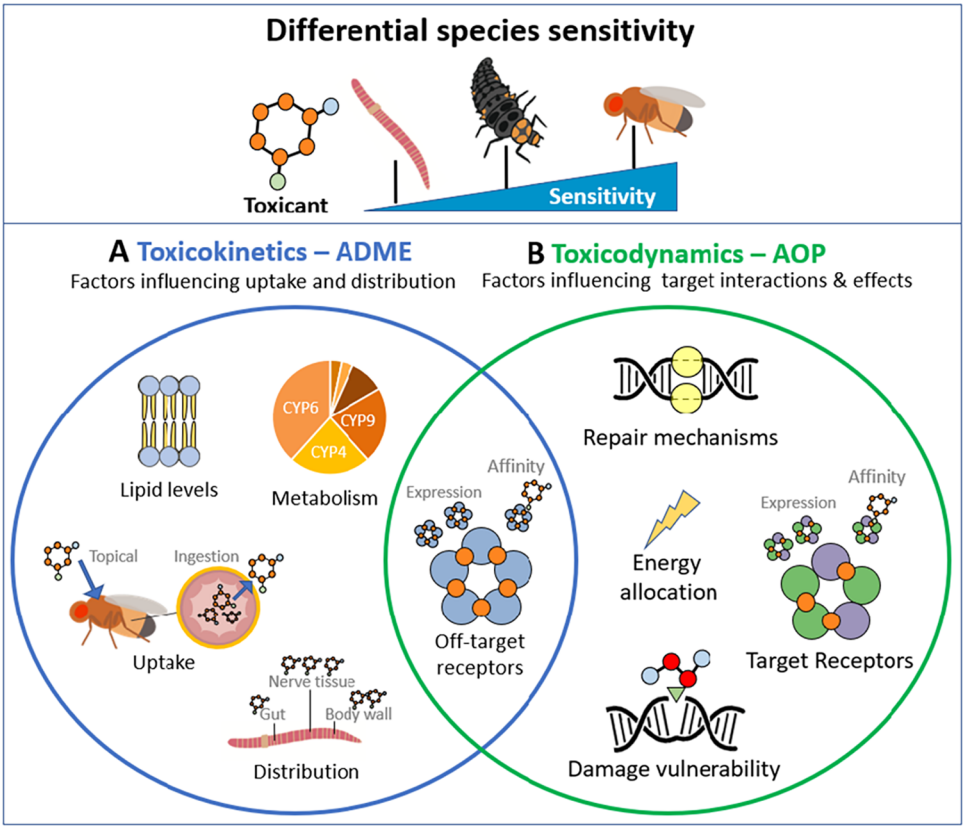


**Figure 1.** A heuristic framework of toxicokinetic (TK) and toxicodynamic (TD) factors influencing species sensitivity to chemicals. (A) TK factors affecting absorption, distribution, metabolism, and excretion (ADME), including differences in uptake routes, distribution, and phenotypic traits (e.g., lipid content) that influence internal exposure. Omics can inform variation in metabolic capacity (e.g., number, expression, and diversity of detoxification genes such as CYP450s), as well as the presence, expression, and binding affinities of off-target receptors that can sequester chemicals away from sensitive targets. (B) TD factors influencing adverse outcome pathways (AOPs), from chemical interaction with target receptors to downstream processes leading to adverse outcomes. These include variation in receptor presence, expression, and binding affinity (including off-target receptors), differential susceptibility to varying types of damage, differences in damage repair gene repertoires and their responsiveness, and species-specific capacity to reallocate energy toward damage repair and mitigation.

### 1.2. Reduction of Animal Testing

In line with ethical and regulatory drivers to reduce and replace animal testing, accurate sensitivity predictions would decrease reliance on in vivo testing and support implementation of the 3Rs framework.[6]

### 1.3. Compliance with Local Regulation

Regulatory authorities often require toxicity data for locally relevant species that are closely related to existing test organisms. Reliable sensitivity prediction would help identify when additional testing is likely to alter hazard conclusions, avoiding unnecessary and costly retesting.

### 1.4. Improved Species Selection for Environmental Monitoring

Given limited resources for postauthorization monitoring, sensitivity prediction would allow monitoring efforts to focus on particularly sensitive species and on species prone to chemical accumulation and food-chain transfer risks.

## 2. INTRODUCING AND DEFINING "OMICS", SENSITIVITY: TKS AND TDS

A rapidly expanding range of omic approaches—including genomics, transcriptomics, proteomics, metabolomics, lipidomics, peptidomics and ionomics—is now available. Omics have long been applied in ecotoxicology, primarily in retrospective assessments of stressor impacts.[7] However, numerous studies highlight their potential to support prospective ecotoxicology and environmental risk assessment (ERA).[4,8−11] In particular, advances in high-throughput DNA sequencing[12] have driven rapid expansion of genomic and transcriptomic data for ERA-relevant species. These data are transforming toxicology for a limited set of model organisms, including some of environmental relevance (e.g., the Precision Toxicology Initiative[8]). Beyond these model species, and a small number of targeted efforts for key nonmodel taxa,[13,14] genomic resources still remain largely unexploited. This article focuses on the use of genomic and transcriptomic data from nonmodel species to support chemical risk assessment. While we acknowledge the potential contribution of other omic approaches, hereafter, "omics" refers specifically to genome and transcriptome data.

Chemical sensitivity is defined as variation among species in their responses to the same chemical exposure.[4] This broad definition encompasses more specific interpretations, such as differences in response at equivalent external (nominal) concentrations, or divergent responses at equivalent internal concentrations within organisms. Omics-based approaches could be important under any definition. However, the latter definition is particularly relevant to omics-based approaches, as sensitivity differences at comparable internal concentrations are more likely driven by suborganismal, potentially molecular,


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mechanisms, and thus more amenable to prediction using omic data. In contrast, sensitivity defined using the former definition may not only reflect molecular variation but also factors at higher levels of biological organization, such as organism behavior, or even superorganismal influences (e.g., different exposure matrices affecting bioavailability). Varied 'responses' to exposure can vary from lethality through to a wide array of sublethal effects, such as altered growth, development, reproduction, and/or behavior, where responses also depend on the extent exposures are varyingly acute or chronic. Chemical sensitivity (however defined) is shaped by multiple biological factors (Figure 1) and omics can provide insights into both toxicokinetic (TK) and toxicodynamic (TD) processes. For TK (Figure 1A), this means insight into processes influencing a chemical's internal concentration, namely a chemical's absorption, distribution, metabolism, and excretion (ADME). Specifically, omic variation in components mediating such ADME processes, e.g., elements of a species' "chemical defensome"[15,16] or "stressome"[17] may correlate with, or mechanistically explain, differential sensitivity. In practice, such 'defensomes' means assessing the composition and functional redundancy of xenometabolic gene families. This can be assessed through broad comparisons of genes involved in phase I, II, and III detoxification processes, including cytochrome P450 (CYP450) enzymes, transferases, and ATP-binding cassette transporters,[18] or by targeting the presence of particularly critical CYP450 genes.[19] Beyond gene inventories, transcriptomic data enable comparisons of baseline expression patterns within xenometabolic gene families[11] and assessments of xenometabolic responsiveness to chemical exposure.[20,21] Omics can also identify and compare expression of off-target receptors (Figure 1) that may divert chemicals away from classical target-mediated toxicity pathways, potentially reducing sensitivity when off-targets are associated with less consequential damage pathways.[22,23]

In terms of TDs, omics can reveal key differences in target receptors and chemical vulnerability between species (Figure 1B). For target receptors (defined below), sensitivity can be explained (and therefore predicted) by comparisons at multiple levels. Initially, by identifying the presence or absence of a target receptor in the genome and, in cases where receptors are present, comparing the presence/identity of specific amino acids critical to chemical-receptor binding.[24,25] Transcriptomic data allows an extension of this analysis to include expression pattern comparisons of target receptors possessing specific motifs.[22] High-throughput conversion of sequence data into accurate protein structures will also add a further dimension to chemical-receptor binding predictions.[26,27] It is worth noting that "receptor" may be a classical molecular receptor, such as the nicotinic acetylcholine receptor targeted by neonicotinoid insecticides,[28] or a broader biological target initiating a damage pathway, such as DNA in cases of ionizing radiation−induced damage.[29]

Outside target receptor analysis, the TD elements of the 'chemical defensome' could also be considered, specifically the differential capacities of species to cope with downstream damage following chemical exposure.[15,30,31] This includes presence and responsiveness of genes linked to damage mitigation and repair.[20] The potential for omic-based TD predictions is magnified when TD features are placed within an adverse outcome pathway (AOP) framework, a conceptual model that looks to remove the "black-box" connection between exposure and effect.[32] Specifically, AOPs describe a TD pathway as a series of mechanistically linked events from a molecular initiating event (MIE) to an adverse outcome (AO), where the linked pathway of events spans several levels of biological organization. In the context of ecotoxicology, the MIE may represent an interaction between a chemical and its target receptor and an AO by changes to reproduction, growth, feeding, or behavior, all of which could be reasonably linked to higher population level impacts. Combining omic data from untested but vulnerable species with those possessing fully developed AOPs can identify the extent critical AOP pathway components are conserved, thereby allowing predictions using multiple AOP components (so, beyond just the MIE) across different levels of biological organization.[33,34] This is being enhanced by efforts to address the "FAIRification" of AOP data, improving the (re)-use and reliability of AOP information.[35]

## 3. NONOMIC SENSITIVITY PREDICTIONS: LIMITATIONS AND INTEGRATION WITH OMICS

Given the importance of predicting species sensitivity, numerous predictive tools and approaches—mostly nonomic—have been developed, and their predictive capacities have been reviewed.[4,5] These approaches are not competing alternatives; rather, it is important to consider how omic-based predictions relate to and complement these nonomic methods, and how their integration can provide insights to guide future strategies for omic-based prediction.

Two widely used nonomic approaches for sensitivity prediction are interspecies correlation estimation (ICE) and phylogeny-based models. ICE predicts toxicity for an untested species based on known toxicity for a species with available data.[5,36,37] While effective for closely related species (up to the family level), predictive performance declines substantially with increasing phylogenetic distance.[36] Phylogeny-based models also provide some predictive power. For example, phylogenetic and physicochemical properties can be combined to help explain metal sensitivity.[38] As with any correlative approach, both ICE and phylogeny-based models have limitations,[4,5] notably providing little mechanistic grounding and relying on extensive toxicity data for matched species−chemical combinations, which are often unavailable. The growing availability of omic data therefore offers a gap-filling opportunity for poorly tested taxa that cannot be addressed by these data-intensive correlative models. Moreover, omics can be integrated with nonomic approaches to strengthen and explore mechanistic understanding; for example, comparing closely related species that show large discrepancies between observed sensitivity and ICE predictions may offer useful test cases for revealing molecular variation underlying differential sensitivity.

Phenotypic traits also influence the vulnerability of species to chemicals.[39] For example, differences in foraging behavior and anatomy can affect pesticide exposure in bees under field conditions.[40] Trait-based studies have linked vulnerability to behavioral, morphological, physiological, and ecological characteristics,[39] showing that traits such as life cycle duration and feeding mode are associated with vulnerability to chemicals with specific modes of action, albeit with varying mechanistic understanding.[41] Even when strong correlations with standard sensitivity metrics (i.e., $LC_{50}$, $EC_{50}$) are absent, weaker quantitative links between traits (or trait combinations) and specific TK processes remain informative.[39,42] Trait-based prediction is already used in regulatory contexts; for example,




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SPEcies At Risk pesticides (SPEARpesticides) incorporates traits such as generation time and migration ability to assess pesticide runoff risks to stream ecosystems.[43−45] As potential molecular predictors emerge—such as redundancy in xenometabolic genes[18] (see Section 5)—these could be readily integrated to strengthen trait-based predictions,[46] even when gene function is not fully resolved. Similar integrative strategies are well established in medical research, where correlations between metabolic gene expression and drug responses have been identified for humans.[47]

Omic data may ultimately dominate correlative sensitivity prediction, as trait-based approaches are often difficult to develop. Trait data is sometimes lacking and, even when available,[47] compiling them into reliable, searchable databases remains challenging due to limited coverage across species.[41,48] In contrast, the anticipated expansion of large-scale sequencing initiatives[49] suggests available and searchable omic data will soon be less limiting. Trait-based approaches also suffer from inconsistencies in trait nomenclature across databases.[5] Although genomics faces similar challenges,[50] systems for gene naming and ontologies are comparatively more unified than those for traits.[51,52] Ongoing efforts to standardize trait nomenclature,[53,54] alongside initiatives to harmonize molecular identifiers,[55] will be critical for advancing both trait- and omic-based sensitivity prediction.

In summary although all approaches are associated with various limitations, ICE, phylogenetic and trait-based approaches have genuine predictive power and offer a critical benchmark for judging the relative usefulness of omic-based prediction. To warrant substantial investment, omic-based prediction should either outperform these existing approaches or, at least, enhance predictive power when integrated with them. However, given the limitations of existing nonomic approaches and the sensitivity-relevant insights omic data can reveal, it is reasonable to suggest molecular data can be combined with existing predictive methods into a unified predictive framework.[5]

## 4. EXISTING OMIC TOOLS: A FOCUS ON TARGET RECEPTOR AND AOP COMPARISON

Using omics to predict species sensitivity is a relatively new but rapidly advancing field, with some notable pioneering advances. One of the first approaches used genome data from laboratory model organisms (e.g., rat, mouse, fruit fly, zebrafish) and two ecotoxicological species (*Daphnia pulex* and *Chlamydomonas reinhardtii*) to assess the presence of human drug targets across 16 species.[56] Target conservation was found to decline with increasing evolutionary distance from humans; however, more than 50% of drug targets were still identified in distantly related species such as *D. pulex*. This work has now been extended[25,57] and an expanding ECOdrug database was created that links orthologues and phylogeny for close to 1000 drug targets. Together, these approaches demonstrate that screening expanding genomic resources for target presence or absence provides a rapid, cost-effective source of predictive data.

In many cases, target receptors are present across species but exhibit sequence differences that alter chemical interactions. Implementing the SeqAPASS tool[58] (https://seqapass.epa.gov/seqapass) provides an omics-based framework for comparing target receptors across species.[24] SeqAPASS evaluates primary amino acid sequences, functional domains, and key ligand-binding residues to estimate whether orthologous receptors in untested species are likely to interact with a given chemical.

While both ECOdrug and SeqAPASS demonstrate the predictive value of comparing receptor orthologues, their limitations warrant consideration. To date, analyses have focused on relatively straightforward targets. For example, the acetylcholinesterase (AChE) receptor analyzed with SeqAPASS[24] is a highly conserved, single-subunit receptor with well-characterized pesticide-binding residues. Even in such 'simple' cases, sensitivity prediction extends beyond receptor presence and sequence homology. Despite high AChE conservation, sensitivities to organophosphate pesticides vary by at least 3 orders of magnitude,[42] indicating the importance of additional TK/TD factors such as species-specific xenometabolic capacity. AChE genes have also undergone lineage-specific duplications in invertebrates,[59] resulting in organophosphate-binding cholinesterases that are not associated with the sensitive synaptic function.[23,60,61] Moreover, non-AChE esterases can irreversibly bind organophosphates,[62] and species differ substantially in their complements of these enzymes.[63−65] Assessing only the classical target receptor sequence (e.g., via SeqAPASS) would not capture such off-target variation, although SeqAPASS analyses could be expanded and integrated with expression data to evaluate the influence of these additional receptors (a concept expanded on in Section 5 below).

Some recently developed approaches[33] already recognize that receptor orthologue comparisons can be strengthened by integrating additional toxicodynamic (TD) components within relevant AOPs.[32] If the chemical-receptor interaction represents a MIE in a well-developed AOP, i.e., one with clearly defined links between the MIE and AO via a pathway of verified Key Events (KEs), MIE-based prediction can be integrated with knowledge of other conserved AOP components. This integration provides stronger "weight-of-evidence" predictions by incorporating both MIEs and multiple KEs. The predictive value of cross-species AOP comparisons has been discussed,[66] including whether conserved subpathway modules (i.e., particular KEs) may be more informative than whole-pathway assessments for environmental risk assessment. More recently, the G2P-SCAN approach has been proposed to improve cross-species extrapolation of biological processes,[33,34] enabling integrated analyses of orthology and functional gene families to identify pathway-level conservation and susceptibility. Incorporating, reliably catalogued,[35] cross-species AOP information is thus extending sensitivity prediction beyond target receptor (MIE) comparisons alone.

## 5. OMICS AND ECOTOXICOLOGY: A RESEARCH PATH TOWARD BETTER PREDICTION

For some, the potential of omics in developing new in-silico ERA approaches is clear,[67] while others have questioned whether complex omic data across diverse species can be effectively incorporated into predictive frameworks.[5] Exploiting omic data will require identifying and comparatively ranking molecular differences underlying species sensitivity. Establishing structured research priorities would hasten this effort and ensure omics realizes its predictive potential. To this end, we have considered the resources, methodological approaches, and molecular understanding required to take omic-based predictions forward, identifying three key components for




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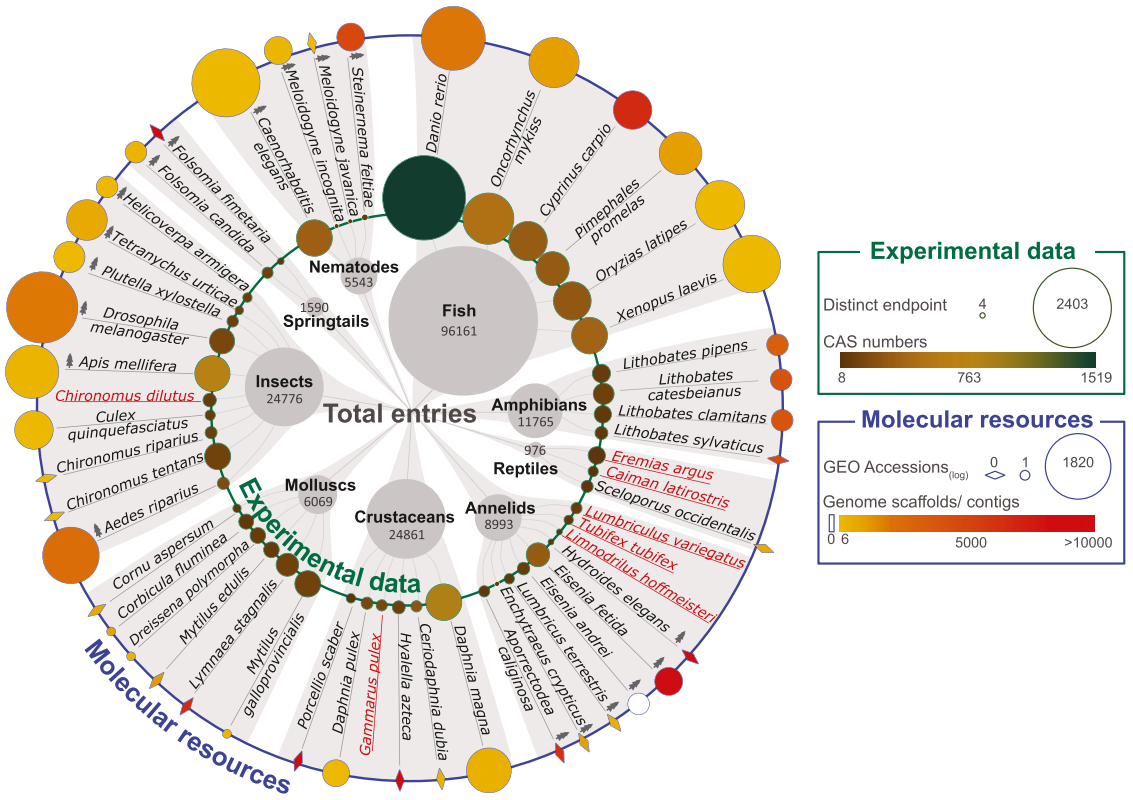


**Figure 2.** Chemical sensitivity and omic data across species.

developing, validating, and integrating omic-based approaches into broader predictive frameworks.

**Component 1: Linking sensitivity and omic data (detailed in Section 5.1).** Effective prediction requires connecting molecular differences (established using functional genetic tools and in vitro work) to observed chemical sensitivities. Progress depends on the availability of relevant ecotoxicological and omic data for the same diverse species.

**Component 2: Acquiring relevant functional knowledge (detailed in Section 5.2).** Accurate omic-based predictions depend on the validity of underlying molecular hypotheses and assumptions. Therefore, understanding the functional, biochemical, and molecular basis of sensitivity differences, and how to infer these differences from omic data, is essential.

**Component 3: Integrating omics into holistic predictive frameworks (detailed in Section 5.3).** Omic-based predictions should be appropriately incorporated into broader models that also consider phenotypic traits, phylogeny, known toxicology, ecological context, and landscape-level factors.

### 5.1. Component 1: Linking Sensitivity and Omic Data

The development of omic-based prediction requires the capacity to generate and validate hypotheses linking molecular variation to differential sensitivity. This effort requires the availability of comprehensive ecotoxicological data (e.g., LCx and ECx values for a range of chemicals and end points) and high-quality omic data for the same set of diverse species. But do such data exist, and where are the gaps? To address this, we assessed existing ecotoxicological data across taxa and aligned it with available omic resources. We used the US-EPA ECOTOXicology (ECOTOX) Knowledgebase (https://cfpub.epa.gov/ecotox), extracting data (December 2025) for species across nine high-level taxonomic groups (Figure 2). We focused on studies published from 2000 onward to capture currently used species, excluding mammals and birds, which are typically studied for human or veterinary toxicology. For each taxonomic group, we selected the five terrestrial and aquatic species with the highest number of publications (using a minimum cutoff of 10 publications), noting that some groups lacked representatives in both habitats (e.g., there are no terrestrial fish). Amphibians were treated as a single group, as species data often appeared in both aquatic and terrestrial repositories. This yielded 50 "ecotoxicological" species (24 aquatic, 21 terrestrial, 5 amphibian). Extracted data included total end points in the ECOTOX Knowledgebase (gray circles in the center of Figure 2), the number of distinct end point types, such as mortality, reproduction, behavior (see proportionally sized circles in the "Experimental data" in Figure 2), as well as the total number of chemicals (i.e., unique CAS numbers) tested (represented by circle color in the "Experimental data" in Figure 2). For "Molecular resources" (see "Molecular Resources" in Figure 2) the circle sizes are proportional (within the "expression profiling by high throughput sequencing" category), and the color represents genome quality (i.e., scaffold number). Colored diamonds represent cases where a genome exists but no GEO accessions. A white circle represents GEO accessions but no genome. Species names in red font signify no GEO accession or genome availability. Terrestrial species are identified with a black tree icon.

This survey shows that, although many species have some data, extensive ecotoxicological research is often focused on a single species within most taxonomic groups (e.g., see nematodes; Figure 2). Using these species for omic-based prediction requires high-quality, comparable omic resources. We therefore assessed omic availability for the 50 "ecotoxicological" species via the NCBI database (see "Molecular




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Resources" in Figure 2). Genomes with fewer scaffolds were considered higher quality, and a higher number of GEO accessions indicated greater transcriptomic data availability. The results reveal mixed genome quality and availability for the 50 "ecotoxicological species": some species lack genomes entirely (e.g., *Gammarus pulex*), while others have highly fragmented genomes (e.g., *Eisenia fetida*), complicating gene family characterization. Additionally, absent GEO accessions prevent assessment of basal expression for critical genes in many species (discussed more below).

**5.1.1. Identifying Useful Species and Filling Data Gaps.** Given the current availability of omic and ecotoxicological data, the following species represent an optimal taxonomic range for developing omic-based predictive approaches: *Danio rerio* (fish), *Xenopus leavis* (amphibian), *Caenorhabditis elegans* (nematode), *Daphnia magna* (crustacean), and *Apis melifera* (insect), with *Eisenia fetida* the best (though imperfect) annelid representative. Significant data gaps remain for key invertebrate taxa, particularly terrestrial species, necessitating targeted generation of ecotoxicological and/or omic data to establish representative molluscs (*e.g., increasing omics for Lymnaea and Mytilus*), annelids, and springtails. Priority actions include increasing gene expression data for molluscs, *Enchytraeus crypticus*, and the springtail *Folsomia candida*, and generating a high-quality genome for *E. fetida*. Notably, *Gammarus pulex*, a long-standing reference freshwater crustacean,[68] lacks omic resources in NCBI, while establishing *Porcellio scaber* as a terrestrial crustacean model would require both high-quality genomic and transcriptomic data.

Beyond omic gaps, in silico prediction would also benefit from targeted ecotoxicological data generation for underrepresented invertebrates. Despite their importance for nutrient cycling, soil health, and ecosystem services,[69−71] springtails and annelids remain relatively data-poor (Figure 2). These gaps could be efficiently addressed using high-throughput exposure systems for terrestrial invertebrates.[72,73]

**5.1.2. Good Genomes for "Ecotoxicological Species".** Sequencing initiatives such as the Darwin Tree of Life (DToL) will generate genomes for species directly used in ecotoxicological testing,[74] as well as other ERA-relevant taxa, including diverse pollinating insects.[75] The current scope of DToL shows that researchers in evolutionary biology, biotechnology, and medicine effectively advocate for their taxa of interest; both our analysis (Figure 2) and recent surveys indicate that ecotoxicologists must similarly coordinate efforts to prioritise ERA-relevant species.[76] Species can be nominated via the DToL "suggest a species" portal (https://www.darwintreeoflife.org/genomes/suggest-a-species-2/). This need is exemplified by *G. pulex*, which lacks an assembled genome[77] in NCBI (Figure 2) despite being a key ecotoxicological test species, although a sample has been submitted to the DToL Project.

Multiple genome assemblies often exist for a single species, with no clear criteria for identifying the most suitable version. Many public genomes remain highly fragmented (Figure 2), and while higher contiguity can be prioritised, this may reduce completeness. Assemblies may also be contaminated by DNA from parasites or symbionts, an issue likely for wild-caught ecotoxicological species that commonly host infections.[78,79] Fragmented, incomplete, or contaminated genomes can lead to incorrect inference of target receptors or xenometabolic components. However, established tools exist to assess assembly quality,[80−83] and newer methods such as BlobToolKit can identify contamination.[84] Such tools need to be utilized to assess genome completeness and contamination before being used for predictive omics. To this end, we suggest a "best practice" approach be developed for the deployment of such tools, at least while "ecotoxicological species" are represented by few and predominantly low-quality genomes. It is also critical to consider genome annotation. How best to annotate a genome is a developing field[85] and there is considerable variation across annotation approaches.[86] For these reasons, comparing the complexity of sensitivity-pertinent gene families across species requires genomes to be equivalently annotated.

**5.1.3. Comparable Transcriptomes for "Ecotoxicological Species".** Divergent expression of critical genes can drive species sensitivity,[87] making transcriptomic resources as important as genomes for omic-based prediction. Until large-scale initiatives deliver widespread high-quality genomes, Transcriptome Shotgun Assemblies (TSAs) often remain the only means of comparing key gene families.[88] However, as with genomic data, transcriptomic resources generated by high-throughput sequencing remain uneven across aquatic and terrestrial ecotoxicological species, with no accessions for approximately one-third of the 50 species assessed (Figure 2). Key gaps include terrestrial and aquatic annelids—important taxa for chemical assessment[89,90]—as well as terrestrial crustaceans and aquatic hexapods. Even among well-studied groups such as aquatic crustaceans, omic resources are largely limited to *D. magna* and *D. pulex*. While large-scale transcriptome initiatives exist for single-celled eukaryotes in specific environments,[91] there is currently no transcriptomic equivalent to the Darwin Tree of Life Project. In its absence, ecotoxicologists must generate transcriptomic resources themselves, prioritising ecotoxicological species lacking such data (Figure 2), followed by nonecotoxicological but ERA-relevant taxa that are of conservation concern, or that provide ecosystem services, such as pollinators, pest predators, and key detritivores. This raises questions about the nature of any such resources. Even basal transcriptomes (i.e., gene expression in the absence of chemical exposure) are highly context dependent, varying with life stage, tissue type, background stress, and nutritional status. Transcriptomic studies of chemical exposure add further layers of variation driven by experimental design choices. There are no simple solutions and no prospect of full standardization, as experiments are necessarily tailored to specific scientific questions. However, comparisons can be facilitated by collecting equivalent metadata and applying minimal but consistent data-formatting standards.[92] Even generating comparable transcriptomic data sets across ecotoxicological species raises fundamental questions about scope. A logical starting point is the basal transcriptome of unexposed organisms at life stages with the greatest likelihood of exposure. An open question is whether to prioritise whole body profiles or tissue specific transcriptome atlases, the latter offering greater resolution at sites of uptake and action. Ultimately, the most pragmatic approach will depend on the current availability of tissue specific data across ecotoxicological species.

As a detailed case study, we characterized omic data linked to NCBI bioprojects for the subclass Oligochaeta. This taxon was selected because—first, it is dominated by earthworms, an ecotoxicologically relevant group.[93] Second, the most studied species (i.e., *E. fetida*) represents close to the average in terms of the data extracted from the US-EPA Knowledgebase (i.e.,




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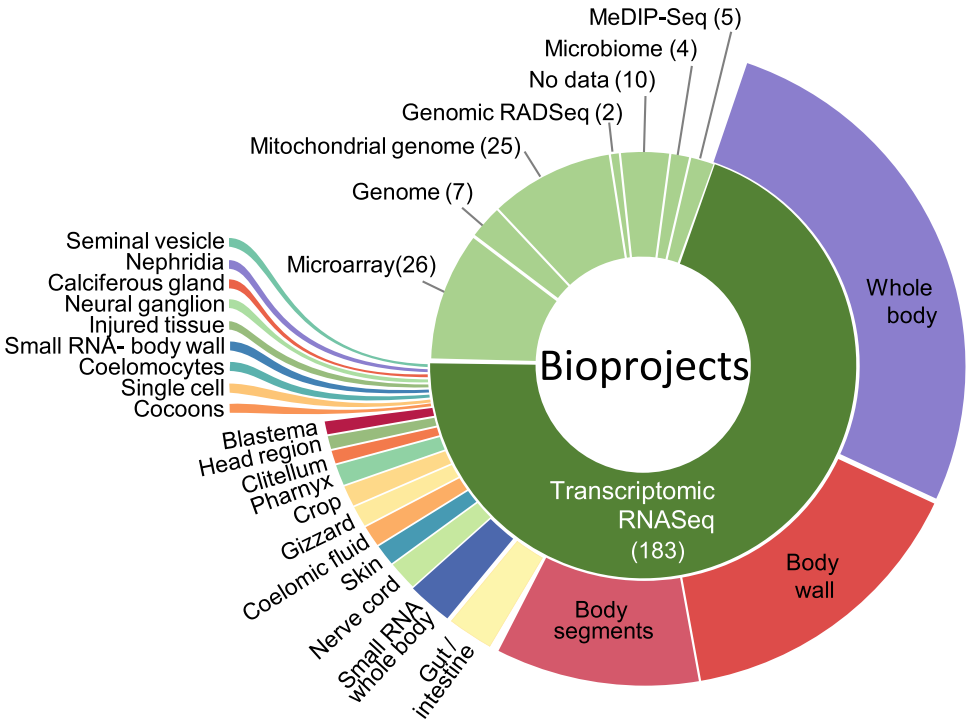


**Figure 3.** Categorisation of omic data sets associated with all NCBI bioproject accessions for the subclass Oligochaeta. Inner layer: types of omic data associated with NCBI bioproject accessions, number of bioprojects in brackets. Outer layer: RNA-Seq data sets associated with NCBI bioproject accessions categorized by tissue type.

~4800 total entries for *E. fetida* versus an average of ~3600 across the 50 species, see Figure 2). Third, oligochaetes represented in NCBI are generally large-bodied and amenable to dissection, making this group suitable for assessing how often tissue-specific expression data are generated when readily obtainable. Finally, it represents ecotoxicologically relevant taxa that lack a genetic model species but have been studied at the molecular, immunological, and ecological level.[94−96] The survey reveals a total of 221 NCBI bioproject numbers associated with 101 species (Figure 3). RNA-Seq entries constitute the majority of omic data (Figure 3, inner green circle). Promisingly, data is available across a diverse range of species. Subcategorization by tissue type (Figure 3, outer circle) shows most RNA-Seq data is generated from "whole body" (38%), then "body wall" (22%) and "body segments" (tail/body cross section) (15%). Despite the relative ease of generating tissue-specific libraries, such tissue/cell-specific data exists for only a few species, primarily the lumbricids *E. fetida* and *Lumbricus rubellus*. This suggests that current omic resources for key "ecotoxicological" taxa will (generally) be limited to predictions using the whole-body level transcriptomics, rather than tissues targeted by a given chemical, or tissues that dominate the detoxification processes. A longer-term goal should be to generate tissue-specific expression data for priority ecotoxicological species. For species not amenable to macro-dissection, this could involve techniques such as Laser Capture Microdissection[97] or spatial transcriptomics.[98]

Even where available, transcriptomic resources show greater variability than genomes, which must be accounted for to ensure meaningful comparisons. This includes aligning equivalent tissues and life stages, as well as less obvious sources of variation. For example, transcriptomic data are often generated from pooled RNA from genetically heterogeneous individuals, producing Transcriptome Shotgun Assemblies (TSAs) that contain multiple polymorphic sequences for the same gene.[22] Such artificial duplications arise because assembly software[99] cannot reliably distinguish polymorphic variants from closely related paralogs. These overassemblies compromise cross-species comparisons of gene number and expression and are particularly problematic in ecotoxicology, where studies commonly pool wild-caught organisms with high cryptic speciation and allelic diversity.[100]

Concerns about omic heterogeneity can be reduced through robust, user-friendly bioinformatic pipelines for efficient data preprocessing that are scalable across organisms and experimental designs. Such workflows can address nonuniform coverage (e.g., Rcorrector[101]), taxonomically classify reads to remove contamination (e.g., Kraken2[102]), and mitigate overassemblies by refining and filtering transcripts and selecting biologically relevant representatives (e.g., EvidentialGene[103]). The extent of TSA overassembly can be quantified using assessments of universal single-copy genes (BUSCO[82]), enabling comparison of artifactual duplication, sequencing depth and gene representation.[104] Beyond bioinformatics, RNA sources should be more carefully considered for future TSAs, prioritising single individuals or more clonal populations where possible, and applying DNA barcoding to ensure pooled samples do not contain cryptic species.[105]

### 5.2. Component 2: Acquiring Relevant Functional Knowledge

Understanding when and how molecular mechanisms drive species sensitivity is central to omic-based prediction. However, if such prediction requires species-specific molecular resolution, it risks perpetuating the "too many species" problem. Therefore, research should prioritise approaches that maximize predictive power by enabling sensitivity predictions across many species, particularly those providing


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key ecosystem services. Here, we outline the most cost-effective molecular and research priorities for translating expanding omic resources into improved predictive capacity.

**5.2.1. Beyond Classic Targets and AOPs: The Case for Off-Target Receptors.** Mechanistic understanding of the toxicokinetic (TK) and toxicodynamic (TD) processes underlying chemical sensitivity is essential for developing omic-based predictive tools. While current approaches focus on cross-species comparison of target receptors and downstream AOP components[24,33,34] (see Section 4), these frameworks must be extended beyond the MIE and classical TD pathways. In particular, they should incorporate the binding affinity and expression of off-target receptors involved in stoichiometric detoxification (i.e., chemical "sinks" or "sponges"). Sensitivity to the neonicotinoid imidacloprid in earthworms illustrates the importance of such mechanisms. Earthworm species show ~30-fold differences in imidacloprid sensitivity,[93] and similar uptake and elimination rates indicate a TD (rather than TK) explanation.[22] However, sequence and expression differences in nAChR target receptors could also not explain the sensitivity variation; instead, insensitive species exhibited high expression of acetylcholine binding proteins (AChBPs) predicted to have high affinity for imidacloprid. AChBPs are nAChR-like receptors[106] that bind acetylcholine and imidacloprid,[107−109] and appear to function away from the sensitive synaptic environment.[110] Unlike the target nAChRs, AChBPs can explain the differential sensitivity: insensitive species express high levels of high-affinity AChBPs, whereas sensitive species mostly express low-affinity AChBP variants. This suggests a mechanism in which high-affinity AChBPs sequester imidacloprid, limiting binding to synaptic nAChRs and reducing toxicity. Comparable off-target "sponge" mechanisms have been described elsewhere. Pyrethroid resistance in mosquitoes is linked to high expression of a pyrethroid-binding sensory appendage protein (SAP2) that protects neuronal sodium channels.[111] Similarly, non-neural esterases can bind organophosphate insecticides, limiting interaction with synaptic AChE and reducing sensitivity in some taxa.[23,60,61] Together, these examples highlight the need to move beyond classical target receptors and downstream AOPs to identify and incorporate noncanonical molecular drivers of sensitivity.

**5.2.2. Finding and Scoring "Core" Xenometabolic Genes.** Considerable effort is needed to characterize species' "chemical defensomes"[15,16] and link them to chemical sensitivity. This includes genes that mediate TK-related processes, such as CYP450 enzymes critical for chemical detoxification. Identifying the "key" genes driving this detoxification is challenging due to the size and complexity of xenometabolic repertoires.[112−114] Nevertheless, studies suggest that a relatively small gene subset can dominate compound metabolism. For example, of 57 human CYP450 genes, five metabolize most drugs, with CYP3A4 alone catalyzing over half of those reactions.[115,116] Identifying this "core set" of detoxification genes across taxa would greatly improve TK-based omic predictions. The challenge is determining which genes are most critical. When differential sensitivity is clearly linked to TK processes, such as variable chemical elimination, strong correlations may exist between sensitivity and the presence or expression of key TK genes, including CYP450s and ABC transporters. In addition to informative correlations, the growing literature on chemical resistance also highlights relevant xenometabolic genes, such as CYP6D1 in insects.[117] Although studied in pest species,[113] these findings reveal genes crucial for xenometabolic breakdown in ecotoxicologically relevant taxa. Xenometabolic repertoires, particularly CYP450s, vary widely even within taxonomic classes,[118,119] and dramatic gene-family expansions or elevated expression of resistance-associated CYP450s in less sensitive species can provide key insights. Moreover, expanding use of genetic tools for in vivo functional studies in understudied taxa[120] can also help uncover TK genes that drive sensitivity. While detailed functional molecular knowledge will clearly improve predictions, correlations can also be informative; for example, changes in metabolic genes are used to predict cancer cell sensitivity to drugs.[47]

Once the "core-set" of detoxification genes are known, we need to develop approaches to comparatively rapidly (but effectively) score these xenometabolic systems across species. Using available genomic data, it will be possible to compare gene composition and redundancy,[121] an approach that has provided key insights in bees.[18] Beyond genomic comparisons, we need to assess basal expression patterns of the core genes across species,[122] as this expression can be altered by chemical exposure over only a few generations.[123,124] It is also important to consider the responsiveness of detoxification pathways—i.e., how species induce expression changes in key xenometabolic genes upon exposure.[125−127] Therefore, we propose a scoring-system that considers TK-related genes at three levels: (1) xenometabolic potential (gene/gene family composition), (2) baseline xenometabolic capacity (i.e., basal expression at exposure), and (3) response competency (speed and extent of gene induction). Comparing this data across the "core-set" of detoxification genes could greatly improve the TK-dimension of omic-based prediction.

**5.2.3. Xenobiotic Receptors: Comparing Regulators of Detoxification Pathways.** In addition to comparing xenometabolic gene repertoires and their induction after chemical exposure, it may be possible to predict a chemical's likelihood of triggering these responses by examining species' xenobiotic receptors—i.e., the regulatory nuclear receptor transcription factors that bind chemicals and induce expression of metabolizing enzymes and transporters.[128,129] As an example, in vertebrates, differences in Aryl Hydrocarbon Receptor sequences predict dioxin sensitivity across bird species.[130] In invertebrates, there is also evidence that xenobiotic receptors can drive differential sensitivity. For example, crossbreeding *Tetranychus urticae* strains with contrasting pesticide sensitivities reveals allele-specific differences in CYP450 gene expression were largely driven by changes to trans-acting factors (possibly xenobiotic receptors), rather than variations in the promoters or enhancers of the CYP450 genes.[131]

Given their pivotal role, it may be valuable to compare xenobiotic receptor sequences, basal expression, transcriptomic responsiveness, and chemical-induced activation.[132−135] Emerging AI approaches for protein structural analysis[136,137] may support this process by enabling accurate species-specific predictions of chemical binding to xenometabolic receptors and, by extension, predict the magnitude of downstream xenometabolic activation. If reliable, predictions of xenometabolic response could be made from genomic sequences alone, expanding phylogenetic coverage of our predictions (given high-quality genome availability). They could be further refined by combining receptor binding/activation assays[134] with transcriptomics to link activation to specific detoxification components and pathways.[132]





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**5.2.4. Understanding Differential Damage Responses.** Genes associated with stress response and damage repair represent a sensitivity-relevant TD element of an organism's "defensomic" response.[15,16] Stress/repair pathways are under strong selection and diverge between taxa,[138] increasing the likelihood they explain differential sensitivity. The role such damage repair pathways play in sensitivity is revealed by bdelloid rotifers surviving extreme ionizing radiation, not by avoiding DNA damage, but via an expanded DNA repair gene repertoire.[139,140] This example suggests divergent DNA repair gene repertoires and induction capacities may predict sensitivity to DNA-damaging chemicals. More generally, species exposed to unpredictable environmental extremes may have evolved a more reactive defensome, rapidly modulating the expression of more stress response and damage repair genes to a greater extent.[30,141] We propose that differences in stress/damage-linked TD genes could be scored and correlated with chemical sensitivity, especially for compounds acting via general stress mechanisms, thus improving omic prediction by revealing the extent response capacities can inferred from genomic data alone.[140]

**5.2.5. Response Data without Extending the "Too Many Species Problem".** Few transcriptomic studies currently have the temporal resolution to untangle the hierarchy of gene activation needed to identify early responses, including xenometabolic and damage mitigation genes. Most data sets focus on a single, relatively late exposure time point and limited concentrations,[88] potentially missing early expression changes and conflating direct and indirect transcriptomic responses. We support building gene activation pathways using time-resolved transcriptomic studies, often used to investigate developmental expression,[142] and now increasingly being applied in ecotoxicology.[143] Such quantitative transcriptomics can generate gene expression points of departure following exposure,[144] a sensitive indicator for cross-species sensitivity comparisons. Advances in "3D" transcriptomic analysis[145] can further assess interactions between gene expression, exposure concentration, and time, enabling construction of xenometabolic response pathways across species and chemicals.

At the start of Section 5, we emphasized prioritising research that delivers the greatest predictive power, enabling sensitivity prediction for species providing key ecosystem services. This is particularly important for generating highly resolved (quantitative) chemical TK/TD response data, which is costly and time-consuming. Attempting to produce such data across many species−chemical combinations risks extending the "too many species/chemicals" problem into the molecular domain. Instead, alongside leveraging existing data sets,[146] we argue for comprehensive response profiling in representative species of key ecosystem services. These data can then be extrapolated, as many species share common detoxification mechanisms/genes (e.g., shared CYP450 gene use in insects[113]). Expanding genome availability then enables cross-species validation using rapid, cost-effective and high-throughput assays.[147,148] Integrating these approaches would substantially improve understanding of differential response capacities within realistic budget constraints.

**5.2.6. Epigenomes and Sensitivity.** Although the extent of its contribution is poorly understood, epigenetics represents an area of molecular biology that may be relevant to sensitivity prediction. Epigenetic mechanisms enable rapid gene expression changes following exposure[149] and contribute to environmental adaptation and early speciation,[150,151] suggesting a potential role in differential sensitivity. This is supported by evidence that DNA and histone modification systems vary widely among major taxonomic groups,[152] with species differing substantially in their complements of epigenetic regulators, such as DNA methyltransferases in ecdysozoans.[153] Patterns of epigenetic deployment and transgenerational maintenance likely reflect environmental variability experienced by species,[154] and species with expanded epigenetic regulator repertoires may therefore possess more dynamic genomes that better mitigate toxicity. Conversely, greater reliance on precise epigenetic maintenance could increase vulnerability to genotoxic chemicals, due to the need to accurately restore epigenetic marks after DNA repair. We propose that targeted experiments are needed to test whether differential epigenetic utilization is associated with sensitivity variation.

To this point, we have focused on sensitivity in directly exposed organisms, yet transgenerational responses to diverse stressors have been reported across ecotoxicologically relevant species.[7,155−157] Although such effects are not currently considered in risk assessment,[156,158] omic and other data may help identify species that are differentially vulnerable to chemical-induced transgenerational impacts. Modeling suggests that species in unpredictably fluctuating environments evolve complex epigenetic regulation enabling dynamic responses,[154] a capacity that may lead to better damage mitigation. The same environmental unpredictability may also favor reduced reliance on transgenerational epigenetic inheritance, as inherited marks are more likely to be maladaptive.[154] If correct, species from such habitats may also be less susceptible to transgenerational effects. In practice, predictive power could be enhanced by comparative surveys and functional analyses of genes critical for maintaining epigenetic modifications across generations. For example, DNA methyltransferase 1 (DNMT1) is central to methylation maintenance,[159−161] yet many ecdysozoan lineages lack this gene.[153] Therefore, the presence, absence, or expression of such genes in reproductive tissues could flag species likely to employ transgenerational epigenetic mechanisms. However, this approach is constrained by limited fundamental knowledge of epigenetics in many taxa. For instance, nematodes generally lack DNMT1 and the associated 5-methylcytosine DNA methylation,[153] yet still exhibit epigenetically mediated transgenerational sensitivity to chemicals.[157] Such transgenerational effects may instead be mediated by adenine methylation[162] or other mechanisms, such as histone acetylation and/or histone variant incorporation.[163,164] This reality highlights that multiple (and still poorly understood) transgenerational pathways likely operate, making omic-based predictions using epigenetic gene repertoires more complex.

Although knowledge remains limited, pioneering ecotoxicological epigenetic studies have been conducted, including in *C. elegans*[157] and *D. magna*.[165] Extending this work to a broader range of "ecotoxicological species" is needed, with priority given to organisms that can be cultured at scale and have short life cycles. In this context, the pot worm *E. crypticus* represents a suitable terrestrial annelid candidate,[166,167] despite its more limited use in ecotoxicology (Figure 2).

**5.2.7. Linking Omics, Sensitivity and Internal Concentration.** Assessing sensitivity using standard ecotoxicology data (such the US-EPA data used to generate Figure 2), allows us, in general, to determine differential sensitivity (either lethal




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or sublethal) to the same external (or nominal) chemical concentration. While such data reveals differential responses to across species to the same chemicals, it does not explain the underlying mechanisms. Phenotypic differences, such as feeding rates or cuticle thickness,[168] affect exposure levels, while variations in test methodology, including exposure matrix (e.g., water or soil) or absorption route (topical vs ingestion), influence chemical bioavailability. These multiple factors make it challenging to determine which molecular data (if any) should be prioritised for investigation.

Quantifying the relative contributions of uptake, biotransformation, internal distribution, and elimination kinetics can help identify when omic data are most informative. For example, it has been demonstrated that while imidacloprid bioaccumulation explains sensitivity differences among Gammaridae, it does not account for the insensitivity of *Lymnaea stagnalis*, implicating molecular differences in the nervous system.[169] Given the presence of off-target AChBPs in snails, which mediate imidacloprid toxicity in earthworms,[22] it is tempting to hypothesize a similar mechanism for *L. stagnalis*. Despite sensitivity data being available for many species (Figure 2), bioavailability and TK parameters remain understudied.[170] Targeted assessments of bioavailability across exposure media and species,[171] combined with radio-labeling, tissue residue analysis, and quantitative whole-body autoradiography,[22,169,172] would help identify cases where molecular difference—and thus omic data—are central to explaining sensitivity. To achieve this, we suggest the need for better integration of TK analyses and ecotoxicology,[169] with advances in sequencing,[22] and high-throughput chemical analytics.[173]

**5.2.8. Advancing Tools for Comparing Target Receptors.** Comparison of target receptors is arguably the most advanced component of current omic-based prediction[4] (see Section 4), with tools, mentioned above, such as SeqAPASS leading these efforts.[26] Although these approaches are useful, the further development of these orthologue-based approaches to improve assessment of complex multisubunit receptors would be beneficial. As would utilizing such receptor-comparison approaches to identify off-target receptors that may contribute to differential sensitivity.[22,60] SeqAPASS (or similar approach) could potentially be extended to identify such off-targets by comparing sequence similarity to known targets, then generating likelihood scores for species possessing chemical "sinks" or "sponges."

Emerging tools now extend beyond MIEs and target receptors by incorporating broader biological processes through cross-species AOP comparisons (e.g., G2P-SCAN).[33,34] These approaches appropriately rely on conservation of orthologous AOP components to support sensitivity prediction.[174] However, orthologue presence alone does not ensure functional conservation, as genes may retain altered or even opposing functions across divergent lineages.[33,175] For example, alternative splicing can generate functionally distinct isoforms from highly conserved genes.[176,177] Although fully accounting for functional divergence is not feasible, its extent could be better estimated by including data derived from applying high-quality genomic resources and long-read RNA-Seq data to ecotoxicological species.[96] Such data would rapidly enable improved assessment of gene duplication, functional divergence of isoforms, and tissue-specific expression differences, adding additional dimensions to the existing scoring of pathway component conservation.[33]

Emerging Artificial Intelligence (AI) approaches could substantially enhance predictive capacity in ecotoxicology, including the prediction of chemical−receptor interactions.[178] AlphaFold can predict protein structures with near-atomic accuracy from amino acid sequences alone, even in the absence of known homologous structures.[137,179] This capability is particularly relevant where receptor interactions of recently developed chemicals remain poorly characterized,[180] and has already motivated extensions of SeqAPASS to include structural comparisons of target receptors.[26] AI approaches may also transform the identification of off-target receptors. While some off-targets share sequence similarity with known targets,[22] others do not.[111] However, convergently evolved three-dimensional structures can be detected through structural and computational comparisons.[181,182] Although not originally designed for this purpose, AlphaFold models are increasingly being adapted to predict protein−ligand interactions,[136] and continued advances in these methods promise rapid in silico receptor−ligand binding predictions with high relevance for cross-species sensitivity assessment.

**5.2.9. Molecular Mechanisms Underlying Chemical Synergy.** To this point, we have considered species sensitivity to single compounds, yet real-world exposure most commonly involves chemical mixtures.[183] Understanding the effects of such complex exposures is a major ecotoxicological challenge.[184] Mixtures introduce the potential for chemical interactions, with synergism—where combined toxicity exceeds additive expectations—being of particular concern for risk assessment.[183,185] Modeling approaches to identify synergistic combinations are advancing,[72,186] and predicting species vulnerability to such interactions would be ecologically valuable.[183] As with intrinsic sensitivity, not all synergistic effects are best explained at the molecular level. For example, cypermethrin toxicity in *Apis mellifera* is limited by repellence, which is substantially reduced when coexposed with chlorothalonil.[187] However, some synergisms identified through TK/TD modeling and biochemical assays are amenable to omic-based prediction.[188] A key mechanism involves inhibition of xenometabolic enzymes responsible for detoxifying a second compound, thereby enhancing its toxicity.[63,189,190] Given limited knowledge of invertebrate xenometabolism, many such interactions likely remain undiscovered. Identifying a cross-species "core set" of CYP450 enzymes (see Section 5.2.2) would support in vitro testing to determine the extent specific CYP450s are inhibited by various chemicals, enabling synergistic risk flagging using omic data. In parallel, TK-based modeling and assays,[188] should be applied to explore mechanisms underlying less-well characterized synergistic interactions,[191,192] some of which involve chemicals with uncertain modes of action in nontarget species.

**5.2.10. The Role of Microbiomes in Species Sensitivity.** Organisms host diverse microbial communities (or microbiomes) in the gut, skin, and other organs,[193] which reflect the host's biology and ecology.[194] These microbiomes can be sensitive to chemical exposure, and their alteration has been linked to effects in nontarget species.[195,196] Such findings feed into a growing recognition of the ecotoxicological relevance of microbiomes.[197−200] Chemical−microbiome interactions may influence species sensitivity through multiple mechanisms. Even assuming two species have equal reliance on microbial symbionts, sensitivity differences may occur when microbiomes are not equally impacted by a chemical. Likewise,




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Table 1. Summary of Molecular Biology Linked to Chemical Sensitivity, with Alignment of This Biology with the Extent of Evidential Support, along with the Availability of Potential Tools for Risk Assessors[a]

| Sensitivity relevant biology | Evidence linking to differential sensitivity | Tools for risk assessment | Section(s) |
|---|---|---|---|
| Target receptors | Strong empirical support (e.g., ref 28) | Available (e.g., ref 24) | 4, 5.2.8 |
| Molecular components of AOP Key Events | Theoretical reasoning (e.g., ref 34) | Available (e.g., ref 33) | 4, 5.2.8 |
| Off-target receptors | Moderate empirical support (e.g., ref 22) | Available with modification of existing tools (e.g., ref 24). | 5.2.1 |
| Xenometabolic complexity and gene expression variability | Strong empirical support (e.g., ref 13). | Limited availability, feasible for some taxa (e.g., ref 13) | 5.2.2 |
| Microbiomes | Moderate empirical support (e.g., ref 203). | Not currently available | 5.2.10 |
| Xenobiotic receptors | Theoretical reasoning with supporting evidence (e.g., ref 131). | Available with modification of existing tools (e.g., ref 24) when key receptors are identified. | 5.2.3 |
| Damage responses and energy allocation capacity | Theoretical reasoning with limited evidence (e.g., ref 141). | Not available, more research required. | 5.2.4 |
| Epigenetic processes including transgenerational effects | Theoretical reasoning (e.g., ref 156−158). | Not available, major research required. | 5.2.6 |

[a]The in-text section/s dealing with this biology is also provided.

cases where microbiomes are similarly affected, species may differ in their microbial symbiont dependence. Additionally, distinct microbiomes may differentially enhance or reduce toxicity even when unaffected themselves.[197] For example, chlorpyrifos toxicity depends on microbial conversion to its active oxon form,[201] meaning natural variations in gut microbial metabolism could influence host sensitivity[202−204] or susceptibility to chemical synergism.[205]

Although understanding remains limited, available evidence suggests microbiomes may range from minor modifiers to major drivers of species sensitivity.[197] If so, omic approaches could support prediction. High-throughput sequencing can characterize microbiome composition (e.g., 16S rRNA profiling[206]), but taxonomic data alone may be insufficient, as function can vary widely among closely related taxa or be conserved across taxonomically distinct communities.[197] Future work should therefore focus on linking microbiome function to host sensitivity using metagenomics, metaproteomics, metabolomics, and transcriptomics.[197]

**5.2.11. Genetic Tools to Validate Hypotheses.** Functionally testing mechanistic hypotheses linking sensitivity to molecular biology—whether xenometabolic pathways, chemical binding to target/off-target receptors, or damage mitigation—would enhance the predictive power of omic data. However, relevant techniques, such as in vivo gene manipulation, are not yet widely optimized across the 50 "ecotoxicological species" (Figure 2). Some species are amenable to genetic tools, e.g., RNA interference in insects,[207] CRISPR−Cas9 in aquatic crustaceans,[208−210] and even homozygous knockout lines in marine annelids.[211] Despite this, greater effort is needed to develop such approaches for ecotoxicologically relevant species, even if this means initially applying them to species less commonly used for ecotoxicology. For instance, the enchytraeid *E. crypticus*, while not a standard terrestrial annelid test species (Figure 2), is suitable for functional studies due to its size, life cycle, amenability to culture, and omic resources.[166] Closely related surrogate species may also be used, as whole-mount in situ hybridization, CRISPR-Cas9, and gene knockdowns have been applied in marine annelids, leeches, and molluscs.[212−216] Where whole-organism studies are infeasible, combining tissue explants or cell lines[217,218] with transgenic methods[219,220] offers a promising alternative.

Alongside in vivo methods, in vitro approaches offer valuable routes to improve omic predictions. Subcellular inhibition assays measuring specific CYP450 activity,[221] can be correlated with sensitivity. Furthermore, novel in vivo enzymatic assays have been developed for both aquatic and terrestrial invertebrates to assess broad CYP450 responses (and inhibition) by chemicals.[222,223] Heterologous expression and functional in vitro experiments can also validate the roles of specific xenometabolic components in both model species (e.g., *D. melanogaster CYP6A2*[224]) and nonmodel species.[120] Though often applied to the study of pesticide resistance, we recommend the use of such methods are expanded to functionally link sensitivity to the presence and expression of xenometabolic components more broadly.

### 5.3. Integrating Omics into Holistic Predictive Frameworks

We have identified data gaps and outlined how tool development can support cross-species comparison of sensitivity-relevant omic data (Table 1).

However, truly predictive ecotoxicology requires integrating these comparisons into a holistic (or systems-based) modeling framework that combines omic data with phenotypic traits, phylogeny, toxicology, ecological knowledge, and nonomic AOP components. Developing such integrative in silico models remains a complex challenge.[4,5] The structure of predictive models has been somewhat discussed,[5] but this research remains at an early stage. Initially, it is necessary to identify optimal input data and determine which molecular and nonmolecular factors carry the most predictive weight, an endeavor requiring well-designed ecotoxicological, ecological, and molecular/genetic experiments.[131] Incorporating sensitivity prediction models into a species' broader chemical vulnerability will demand an interdisciplinary approach, integrating Molecular Biology, Ecology, Ecotoxicology, Bioinformatics, and Modeling expertise. Once created, models can be validated and refined by comparing predictions to observed sensitivity/vulnerability differences across increasingly complex exposure scenarios, from laboratory, to mesocosms and real-world environments. The ability of transcriptomics to detect subtle, sublethal, responses will also be part of validating sensitivity predictions by providing biomarkers of adverse outcome pathway initiation.[225] Considerable progress has been made in integrating complex data sets, for example through "digital twins", a concept applied in engineering, personalized medicine, and environmental modeling.[226,227] Combining AI with digital twin approaches offers a promising strategy to enhance predictions and environmental risk assessment.




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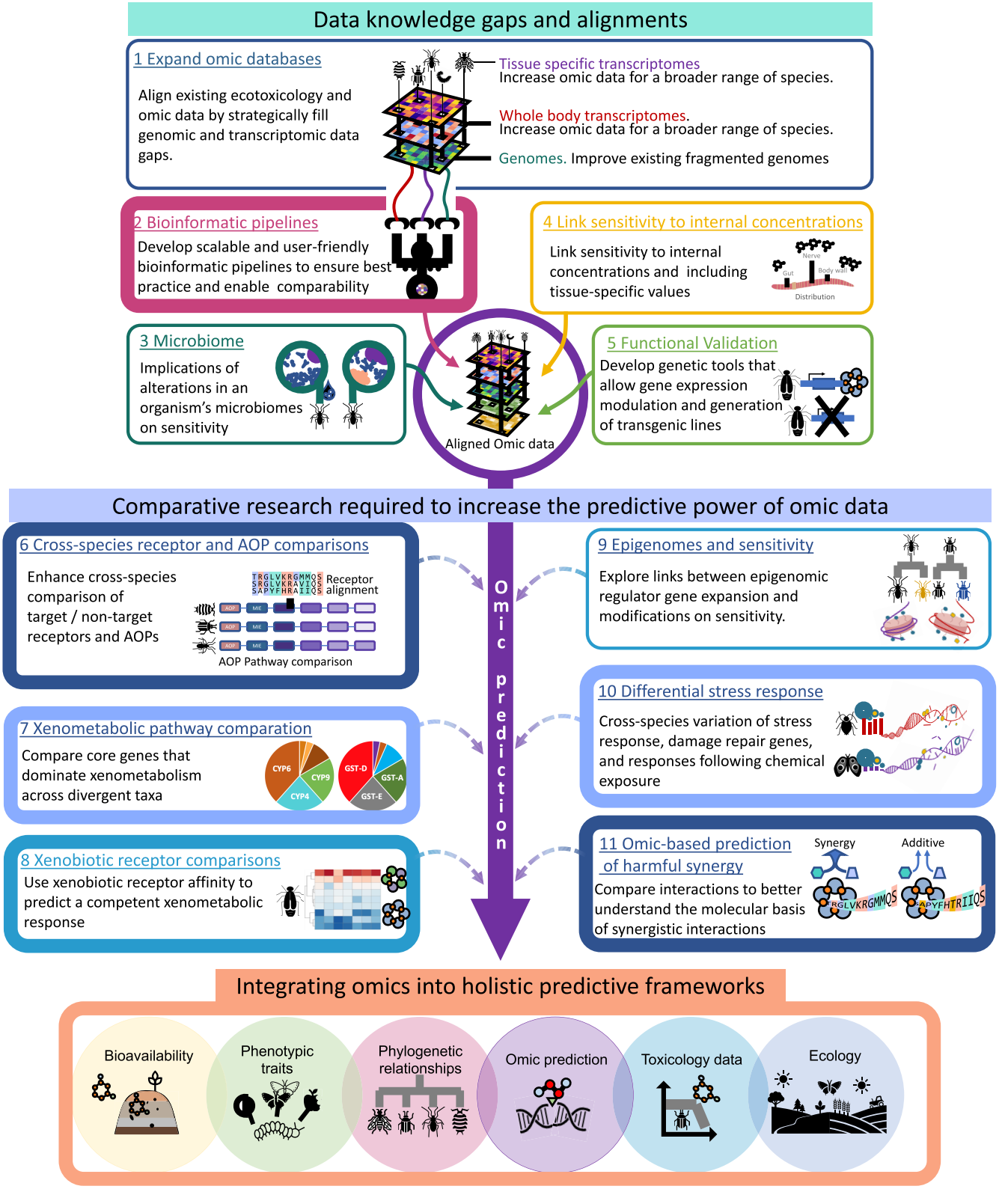


**Figure 4.** Summary of a suggested 12-point research path to facilitate exploitation of omic data for species sensitivity prediction. Top panel: Ecotoxicological and omic data gaps, bioinformatic pipeline optimization and background tools/research needed for omic-based prediction. Middle panel: comparative analyses required to generate sensitivity predictions when armed with sufficient omic data and functional knowledge. Bottom panel: integration of omic data into a holistic modeling framework that allows sensitivity to be predicted using a wide range of pertinent data.

Predicting a species' chemical sensitivity would benefit risk assessment, conservation, pollution monitoring, and could eventually reduce animal testing. We have shown how omic data can enhance predictive capabilities, identified data gaps for ecotoxicologically relevant species, and outlined research needed to improve sensitivity prediction (Figure 4). As an



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expanding resource, omic data provides insight into sensitivity-relevant biology. We hope this review guides ecotoxicologists and risk assessors in leveraging omic data to support environmental protection.

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### Notes

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