



Herbicide-induced ecotoxicological responses in earthworms: An integrated review of soil ecosystem services, growth, reproduction and biomarker-based risk assessment

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Abstract

Earthworms are important components of terrestrial ecosystems because they contribute to soil structure formation, organic matter decomposition, nutrient cycling, water regulation and the maintenance of soil biological activity. Their ecological functions make them valuable bioindicators for evaluating the potential effects of agricultural chemicals on soil health. Although herbicides are used to control weeds and improve crop production, their active ingredients, commercial formulations and transformation products may expose non-target soil organisms under certain agricultural conditions. This review critically examines herbicide-related effects on earthworm survival, growth, reproduction, behaviour and biochemical responses, while integrating these responses with the ecosystem services provided by earthworms. Particular attention is given to glyphosate-based herbicides as a comparative case study, with emphasis on the distinction between active ingredients and commercial formulations and the potential influence of temperature and soil organic matter. Evidence from published studies and standardised ecotoxicological guidelines is evaluated to identify the strengths and limitations of commonly used toxicity endpoints. The literature indicates that acute mortality alone may not adequately represent ecological risk because changes in reproduction, behaviour or physiological function may occur independently of substantial mortality. Responses also vary according to the chemical, formulation, earthworm species, exposure conditions and biological endpoint. An integrated framework combining standardised toxicity tests, biochemical biomarkers, soil biological functions and field-level validation is proposed. This approach may improve the ecological interpretation of herbicide exposure and support environmentally responsible weed management, soil conservation and sustainable agricultural practices.

Keywords: Earthworms, herbicides, soil ecotoxicology, ecosystem services, glyphosate, *Eisenia fetida*, reproduction, biomarkers, soil health, ecological risk assessment

Introduction

Soil is a complex biological system in which microorganisms, invertebrates, plants and physicochemical processes interact to maintain ecosystem productivity. Earthworms are widely recognised as ecosystem engineers because their feeding, burrowing and casting activities influence soil structure, organic matter distribution, water movement and nutrient cycling. Their contributions extend beyond individual soil processes to ecosystem services that support agriculture, land restoration and environmental quality (Blouin *et al.*, 2013; Bertrand *et al.*, 2015)^[1, 2].

Agricultural intensification has increased the use of herbicides to manage weeds and reduce competition between weeds and crops. Although these chemicals provide important agronomic benefits, their environmental effects depend on the active ingredient, formulation, application conditions, persistence and interactions with soil properties. Earthworms may be exposed through contact with treated soil and ingestion of soil or organic matter containing chemical residues. These exposure pathways create a need to evaluate potential effects on non-target organisms rather than assessing herbicides solely in terms of weed-control efficacy (Pelosi *et al.*, 2014; Klátyik *et al.*, 2023)^[4, 7].

Earthworms are relevant to soil ecotoxicology because their responses can be evaluated at multiple biological levels, including survival, body mass, reproduction, behaviour and biochemical activity. Yasmin and D'Souza (2010)^[9] reviewed pesticide-related effects on earthworm growth and reproduction, while Pelosi *et al.* (2014)^[7] synthesised evidence concerning pesticide effects on earthworms across several biological endpoints. These reviews support the use of complementary endpoints, while also indicating that findings depend on the chemicals and experimental conditions investigated. Importantly, herbicides should not be treated as a single toxicological category. Different active ingredients have different properties, and the toxicity of a commercial formulation cannot necessarily be inferred from its active ingredient alone. Research on glyphosate-based herbicides illustrates the importance of distinguishing active ingredients from complete formulations and considering environmental conditions when evaluating effects on soil organisms (Klátyik *et al.*, 2023; Schmidt *et al.*, 2023)^[4, 8]. The present review integrates two connected perspectives: the ecological importance of earthworms as beneficial soil organisms and their potential vulnerability to herbicide exposure. Its objectives are to evaluate published evidence on survival, growth, reproduction and biomarkers; examine

glyphosate-based herbicides through a comparative case analysis; and identify research priorities for integrating earthworm protection with sustainable weed management.

Review methodology and scope

This manuscript follows a critical narrative-review approach. It synthesises relevant published research, authoritative reviews and standardised ecotoxicological guidelines concerning earthworm ecology, pesticide effects and herbicide risk assessment. Major sources include Blouin *et al.* (2013)^[2] on earthworm-mediated ecosystem services, Bertrand *et al.* (2015)^[1] on earthworm services in cropping systems, Pelosi *et al.* (2014)^[7] on pesticide effects, and OECD Test Guidelines 207 and 222 on acute toxicity and reproductive assessment. Evidence is interpreted according to the chemical or formulation studied, earthworm species, exposure route, duration, concentration and measured endpoint. Research involving insecticides or fungicides is used only when discussing general ecotoxicological principles; such findings are not automatically attributed to herbicides. The glyphosate case analysis draws on published research examining active ingredients, commercial formulations and environmental conditions (OECD, 1984, 2016; Schmidt *et al.*, 2023)^[5, 8]. This is a narrative review with a comparative case analysis, not a systematic review or meta-analysis. No original experimental data, new toxicity measurements or pooled effect sizes are claimed. Numerical results from individual studies should be interpreted according to the methods and conditions reported in those original publications.

Earthworms as an eco-friendly biological resource

Earthworms can be considered an eco-friendly biological resource because their natural activities contribute to soil formation, organic matter transformation, nutrient cycling and the maintenance of soil structure. By ingesting soil and decomposing organic material, they redistribute nutrients and produce casts whose physical, chemical and biological

properties may differ from those of the surrounding soil. Their burrows can also influence aeration, water infiltration and water movement through soil. These functions explain why earthworms are widely described as ecosystem engineers (Blouin *et al.*, 2013)^[2].

The ecosystem services provided by earthworms are diverse. Blouin *et al.* (2013)^[2] reviewed their contributions to pedogenesis, soil structure, water regulation, nutrient cycling, primary production, climate regulation and pollution remediation. Bertrand *et al.* (2015)^[1] examined earthworm services in cropping systems and their relevance to agricultural productivity. However, the magnitude of these benefits varies with earthworm species, soil conditions, land use and climate; the presence of earthworms does not guarantee identical outcomes in every ecosystem.

Earthworms are also important in organic-waste management. Selected species are used in vermicomposting systems to process suitable organic residues in association with microbial communities, producing a stabilised material that may be used as a soil amendment. This provides a biological route for managing selected organic wastes and recycling nutrients. Nevertheless, vermicomposting performance depends on species, waste composition, moisture, temperature and contaminant content. Contaminated feedstocks may compromise the process and should not automatically be regarded as suitable for biological treatment (Edwards & Bohlen, 1996)^[3].

The eco-friendly value of earthworms therefore depends on maintaining healthy populations and suitable habitat conditions. If herbicide exposure changes survival, feeding, growth or reproduction, it may affect not only individual organisms but also the soil functions they support. This provides the ecological rationale for assessing herbicide exposure through multiple biological endpoints and, where possible, linking those endpoints to soil processes (Blouin *et al.*, 2013; Pelosi *et al.*, 2014)^[2, 7].

Table 1: Earthworms as eco-friendly biological resources: ecosystem functions and relevance to herbicide risk assessment

Ecosystem function	Earthworm-mediated contribution	Relevance to herbicide assessment
Soil structure and aggregation	Burrowing, casting and redistribution of soil particles can modify soil structure.	Persistent changes in earthworm abundance or activity may affect soil physical processes.
Water regulation	Burrows can facilitate water infiltration and movement through soil.	Behavioural changes or reduced activity may warrant investigation of potential soil-function effects.
Organic matter decomposition	Ingestion and processing of organic matter contribute to its transformation.	Effects on feeding and survival may alter earthworm-mediated organic matter processing.
Nutrient cycling	Earthworm activity redistributes nutrients and changes the biological environment of soil.	Soil nutrient changes should be measured directly rather than inferred from toxicity alone.
Soil microbial interactions	Earthworms interact with soil microorganisms through feeding, gut passage and casting.	Herbicide effects on earthworms and microbial communities may need to be evaluated together.
Soil remediation processes	Earthworms may influence the transformation or redistribution of some contaminants.	This function is contaminant-specific and should not be assumed to apply to every herbicide.
Organic-waste processing	Selected species are used in vermicomposting of suitable organic residues.	Suitable feedstocks and healthy earthworm populations are essential for effective processing.

Source: Synthesised from Blouin *et al.* (2013)^[2], Bertrand *et al.* (2015)^[1] and Edwards and Bohlen (1996)^[3]. This table summarises established ecological functions and their potential relevance to herbicide assessment; it does not imply that every herbicide has been demonstrated to affect each function.

Effects of herbicides on earthworm survival and growth

1. Acute toxicity and survival

Acute toxicity tests are used to identify chemicals that cause mortality under defined exposure conditions. OECD Test Guideline 207 describes earthworm acute-toxicity testing using filter-paper contact and artificial-soil methods. The

artificial-soil approach provides a more representative exposure setting than contact-only screening, although it remains a controlled laboratory system rather than a complete agricultural ecosystem (OECD, 1984)^[5].

Acute toxicity results depend on the chemical, concentration, exposure duration and experimental

conditions. Soil properties can influence chemical availability, while differences among earthworm species may affect sensitivity. A toxicity value obtained for one species or formulation should therefore not automatically be generalised to all earthworms or herbicides (Pelosi *et al.*, 2014)^[7].

The original draft discusses reported effects of herbicides such as acetochlor and butachlor. These examples may be relevant, but exact concentrations and outcomes should be included only after verification against the original experimental publications. A general conclusion that all herbicides are highly toxic to earthworms is not justified by evidence from selected chemicals alone.

2. Growth and body mass

Changes in body mass can reveal physiological responses that are not captured by mortality measurements. Growth depends on food intake, assimilation, water balance, metabolic expenditure and environmental conditions. A decline in body mass may indicate altered feeding or physiological stress, although it does not independently identify the underlying mechanism.

Yasmin and D'Souza (2010)^[9] reviewed pesticide-related effects on earthworm growth and reproduction and discussed the value of sublethal endpoints in soil ecotoxicology. Their review supports the inclusion of body-mass changes in toxicity assessments, but findings involving insecticides or fungicides must be distinguished from those involving herbicides.

Growth should be interpreted alongside survival, feeding activity and reproductive performance. Experimental comparisons should report the earthworm species, developmental stage, concentration basis, exposure duration and control conditions. These details are necessary to determine whether findings from different studies are genuinely comparable (Yasmin & D'Souza, 2010; OECD, 2016)^[6, 9].

3. Reproduction and population renewal

Reproductive performance is a critical endpoint because reduced cocoon production, hatching success or juvenile survival may affect population renewal even when adult mortality remains low. OECD Test Guideline 222 provides a standardised method for assessing earthworm reproduction using *Eisenia fetida* or *Eisenia andrei*. It includes observations of adult survival and body-mass changes as well as juvenile production after the exposure period (OECD, 2016)^[6].

Yasmin and D'Souza (2010)^[9] emphasised the importance of reproductive endpoints in pesticide ecotoxicology. However, reproductive effects must be interpreted in relation to the chemical tested, formulation, concentration and experimental design. A reduction observed at a high laboratory concentration should not automatically be presented as an effect expected under normal field use. Conversely, the absence of adult mortality does not demonstrate that reproduction is unaffected.

Where possible, laboratory findings should be linked to realistic exposure estimates and field observations. This is especially important when extrapolating from standard laboratory species to earthworm communities inhabiting agricultural soils.

Behavioural and biochemical responses

1. Behaviour as an early-warning endpoint

Earthworm behaviour can provide additional information about environmental disturbance. Relevant observations

include avoidance of treated soil, altered movement, burrowing changes and feeding activity. These endpoints matter because earthworms contribute to soil functioning through their physical activities, not simply through their presence.

Pelosi *et al.* (2014)^[7] reviewed behavioural responses among the endpoints used to assess pesticide effects on earthworms. Avoidance responses are influenced by soil properties, exposure distribution and test design. Avoidance does not necessarily mean mortality or establish that a population will decline, but it can indicate that organisms respond to the tested conditions.

Behavioural observations are most informative when combined with survival, growth, reproduction and appropriate soil measurements. This helps distinguish a short-term response from an effect that persists or has consequences for earthworm populations.

2. Biochemical biomarkers

Biochemical biomarkers can help identify physiological responses to chemical exposure. Their value depends on the endpoint measured, the biological mechanism involved and the ability to distinguish a chemical-specific response from general stress.

Acetylcholinesterase (AChE), for example, is associated with neurotransmission and is commonly used in studies of certain neurotoxic insecticides, particularly organophosphates and carbamates. Evidence involving these pesticide classes should not be presented as direct proof that all herbicides inhibit AChE. Such a claim requires direct experimental support for the particular herbicide and earthworm species.

Glutathione-S-transferase (GST) participates in the metabolism of various compounds and may respond to chemical exposure. Increased activity can be consistent with an induced defence response, whereas reduced activity may reflect inhibition or physiological disruption. Neither direction alone establishes ecological harm. Biomarker results should therefore be interpreted alongside organism-level endpoints such as growth and reproduction (Pelosi *et al.*, 2014; Yasmin & D'Souza, 2010)^[7, 9].

Oxidative-stress measurements, including antioxidant enzyme activity and indicators of cellular damage, can further help characterise physiological responses. However, biomarker changes should not be assumed to occur uniformly across herbicides. Chemical identity, formulation, dose, exposure duration and experimental conditions need to be specified before a mechanistic conclusion can be drawn.

3. Earthworm-associated enzymes and microbial interactions

Earthworms interact with soil microorganisms through feeding, burrowing, casting and the transformation of organic material. These interactions contribute to soil biological activity and nutrient cycling. However, changes in soil enzyme activity cannot automatically be attributed to the earthworm itself: measured activity may originate from earthworm tissues, gut-associated microorganisms or the surrounding soil microbial community (Blouin *et al.*, 2013)^[2].

The original draft mentions enzymes such as cellulase, amylase, phosphatases and nitrate reductase. These may be relevant to the broader ecological context, but herbicide-specific claims about inhibition or stimulation require

traceable primary research that identifies the tested chemical, organism, exposure conditions and measured enzyme.

Future studies should combine enzyme measurements with microbial analyses and organism-level outcomes. This would help determine whether biochemical changes represent a transient response, an alteration in microbial activity or a biologically consequential effect on earthworm health and soil function.

Comparative case analysis: glyphosate-based herbicides

1. Rationale for the case analysis

Glyphosate provides a useful example for examining herbicide ecotoxicology because published research has investigated both the active ingredient and commercial formulations. Klátyik *et al.* (2023) ^[4] reviewed terrestrial ecotoxicity evidence concerning glyphosate, glyphosate-based herbicides and co-formulants. Their review highlights the need to distinguish the active ingredient from the complete commercial product when interpreting effects on non-target organisms (Klátyik *et al.*, 2023) ^[4].

Schmidt *et al.* (2023) ^[8] investigated avoidance behaviour, biomass growth and reproduction in *Eisenia fetida* and *Eisenia andrei* in response to glyphosate salts, corresponding commercial formulations and an alkylpolyglucoside co-formulant. The experiment also examined two temperatures and two soil organic-matter levels. This study is especially relevant because it explicitly considered formulation and environmental context rather than treating glyphosate exposure as a single uniform condition (Schmidt *et al.*, 2023) ^[8].

2. Active ingredient versus commercial formulation

The presence of an active ingredient in a product does not mean that the product and the isolated ingredient will necessarily produce identical biological responses. Commercial formulations may contain additional substances that influence chemical behaviour or biological exposure. Therefore, comparisons require suitable controls and clear identification of the tested preparation.

Schmidt *et al.* (2023) ^[8] reported that earthworm biomass growth and reproduction were affected by the tested

substances and temperature. Reproduction was also affected by an interaction between substance and temperature. They found that the effects did not follow a universal pattern in which commercial formulations were always more harmful than their corresponding active ingredients. These findings support product-specific evaluation rather than a blanket conclusion about all glyphosate-based herbicides.

3. Temperature and soil organic matter

Temperature and soil organic matter can influence chemical behaviour and earthworm physiology. Temperature may affect organism activity and chemical transformation, while organic matter can alter sorption and chemical availability. Schmidt *et al.* (2023) ^[8] found that avoidance behaviour varied with soil organic-matter conditions, while substance and temperature effects were observed for growth and reproduction. The study therefore demonstrates that environmental conditions can affect the interpretation of toxicity endpoints.

These findings should not be generalised beyond the experimental conditions. The direction and magnitude of environmental effects may differ across chemicals, formulations and soil types. Studies should report environmental parameters clearly and assess whether observed effects are likely to be relevant under realistic exposure scenarios.

4. Interpretation for agricultural management

A laboratory response indicates a potential hazard under the tested conditions; it is not, by itself, a complete field-risk assessment. Field risk additionally depends on the likelihood and duration of exposure, the concentration reaching earthworms, chemical persistence and the sensitivity of the earthworm community. The glyphosate case demonstrates the importance of evaluating the exact formulation, exposure scenario and biological endpoint. Glyphosate-based products should not be described as universally harmless or universally toxic to earthworms. The scientifically defensible approach is to integrate product-specific evidence, exposure assessment, multiple biological endpoints and field validation (Klátyik *et al.*, 2023; Schmidt *et al.*, 2023) ^[4, 8].

Table 2: Comparative evaluation of endpoints used in earthworm ecotoxicology

Endpoint	What it measures	Main strength	Key limitation
Acute mortality	Survival after a defined exposure period	Useful for initial toxicity screening	May miss chronic and sublethal effects
Body-mass change	Change in organism mass during exposure	Indicates possible physiological or nutritional disturbance	Can be influenced by feeding, hydration and experimental conditions
Cocoon production and juvenile output	Reproductive performance	Helps identify effects relevant to population renewal	Requires a longer test period and suitable reproductive controls
Avoidance behaviour	Movement away from treated soil	Can reveal a response without requiring mortality	Avoidance does not necessarily predict long-term population decline
AChE activity	Activity of an enzyme involved in neurotransmission	Useful for investigating relevant neurotoxic mechanisms	Not a universal or specific biomarker for herbicides
GST and antioxidant responses	Selected detoxification and oxidative-stress responses	May help investigate physiological mechanisms	Changes alone do not establish adverse population-level effects
Earthworm abundance and community composition	Field-level population and community responses	Improves ecological relevance	Influenced by soil type, season, land management and other environmental variables
Soil-function indicators	Selected measures of aggregation, infiltration, nutrient cycling or organic-matter processing	Connects biological responses with ecosystem functioning	Requires direct measurement and careful causal interpretation

Source: Synthesised from OECD (1984, 2016) ^[5, 6], Pelosi *et al.* (2014) ^[7] and Yasmin and D'Souza (2010) ^[9]. This table is an analytical comparison of endpoints, not a quantitative toxicity dataset.

Table 3: Comparative case-analysis framework for glyphosate-based herbicides

Comparison dimension	Evidence or question to evaluate	Interpretation for the review
Active ingredient versus formulation	Are the active ingredient and commercial product tested separately?	Attribute effects to the preparation actually tested.
Exposure concentration	Is the tested concentration relevant to the intended exposure scenario?	Laboratory hazard should not automatically be equated with field risk.
Temperature	Were temperature conditions controlled and reported?	Results may be condition-dependent.
Soil organic matter	Was organic matter measured or experimentally varied?	Chemical availability and organism responses may depend on soil properties.
Behaviour	Was avoidance or another behavioural endpoint measured?	A behavioural response does not necessarily predict population decline.
Growth and reproduction	Were body mass, cocoon production or juvenile output measured?	These endpoints provide information beyond acute mortality.
Field validation	Were laboratory results compared with field observations?	Field evidence is needed to establish ecological relevance.

Source: Analytical framework based on Klátyik *et al.* (2023) ^[4], Schmidt *et al.* (2023) ^[8] and OECD (2016) ^[6]. The table identifies dimensions for comparing studies; it does not present new experimental results.

Linking herbicide exposure with soil ecosystem services

The ecological significance of earthworm toxicity extends beyond individual survival. Earthworms influence soil aggregation, water movement, organic matter redistribution and nutrient cycling. Persistent effects on their abundance or activity could therefore affect soil processes that support agricultural productivity and ecosystem functioning. Nevertheless, these consequences must be demonstrated rather than inferred solely from a biochemical change (Blouin *et al.*, 2013; Bertrand *et al.*, 2015) ^[1, 2].

Earthworm-mediated ecosystem services provide the ecological foundation for treating these organisms as an eco-friendly biological resource. Their contributions to soil structure, water regulation, nutrient cycling and selected remediation processes have been documented in the literature. At the same time, the scale of these services varies across species, land uses and climates, which limits the extent to which findings from one system can be generalised (Blouin *et al.*, 2013) ^[2].

A useful conceptual pathway for future research is:

Herbicide application → soil exposure and chemical availability → earthworm behavioural, physiological and reproductive responses → changes in earthworm activity or

population structure → potential changes in soil ecosystem functions.

This pathway is a framework for research, not proof that every herbicide causes each successive effect. Each link requires evidence appropriate to the chemical, organism and environment under investigation (Pelosi *et al.*, 2014; Blouin *et al.*, 2013) ^[2, 7].

Ecological risk assessment and research priorities

Standardised testing is essential for reproducible toxicity assessment. OECD Test Guideline 207 provides methods for evaluating acute earthworm toxicity, while OECD Test Guideline 222 assesses reproductive output and other sublethal endpoints in *Eisenia fetida* or *Eisenia andrei* (OECD, 1984, 2016) ^[5, 6].

No single endpoint captures every dimension of ecological risk. Mortality tests may overlook reproductive or behavioural changes, whereas biochemical responses do not automatically demonstrate population-level harm. A stronger approach combines standardised laboratory tests with exposure assessment, selected biomarkers and field-level observations (Yasmin & D'Souza, 2010; Pelosi *et al.*, 2014) ^[7, 9].

Table 4: Research priorities for improving herbicide risk assessment in earthworms

Research priority	Current limitation	Recommended direction
Formulation-specific assessment	Active ingredients and commercial products may not have equivalent effects.	Compare active ingredients and formulations using appropriate controls.
Realistic exposure scenarios	Laboratory concentrations may not represent field exposure.	Report application context, concentration basis and relevant soil conditions.
Multiple biological endpoints	Mortality alone may miss sublethal effects.	Combine survival, growth, reproduction and selected biomarkers.
Species diversity	A standard laboratory species cannot represent every earthworm community.	Include locally relevant species where scientifically justified.
Earthworm–microbiome interactions	Enzyme changes may originate from organisms or microbial communities.	Distinguish tissue, gut-associated and soil microbial responses.
Mixtures and co-exposure	Agricultural soils may contain multiple contaminants.	Test mixtures with controlled designs and avoid assuming interaction patterns.
Field-level validation	Laboratory effects do not directly establish ecosystem-level consequences.	Compare laboratory outcomes with field abundance, community and soil-function measures.
Reproducibility	Inconsistent reporting can hinder comparisons among studies.	Report controls, replication, statistical methods and uncertainty transparently.

Source: Synthesised from Blouin *et al.* (2013) ^[2], Pelosi *et al.* (2014) ^[7], Klátyik *et al.* (2023) ^[4], Schmidt *et al.* (2023) ^[8] and OECD (2016) ^[6].

These priorities are consistent with the limitations identified in reviews of earthworm ecosystem services and pesticide

effects and with the purpose of standardised earthworm toxicity and reproduction tests.

Implications for sustainable weed management

Sustainable weed management should balance crop protection with the maintenance of soil biological health. Cultural practices, crop rotation, cover crops, mechanical weed control and appropriately selected herbicides can form components of integrated weed management. The suitability of each measure depends on the crop, weed species, local climate and agricultural system.

The ecological functions of earthworms provide a reason to include soil biological indicators in the broader evaluation of agricultural practices. However, the available evidence does not support a universal conclusion that all herbicides should be avoided or that any single product is safe under every condition. Product-specific risk assessment, adherence to authorised application instructions and avoidance of unnecessary exposure are more scientifically defensible principles (Blouin *et al.*, 2013; Bertrand *et al.*, 2015; Klátyik *et al.*, 2023)^[1, 2, 4].

Earthworms can also contribute to sustainable organic-waste management through vermicomposting when suitable, uncontaminated feedstocks and appropriate environmental conditions are used. Protecting these organisms is therefore relevant to both soil conservation and the responsible use of biological resources (Edwards & Bohlen, 1996; Blouin *et al.*, 2013)^[2, 3].

Conclusion

Earthworms are important biological resources that contribute to soil structure, organic matter transformation, nutrient cycling and ecosystem services. Their ecological importance makes them valuable organisms for evaluating the potential effects of herbicides on soil health. Published research supports the use of multiple endpoints, including survival, growth, reproduction, behaviour and biochemical responses, rather than relying solely on acute mortality.

The comparative analysis of glyphosate-based herbicides demonstrates why the active ingredient, commercial formulation, soil conditions and exposure scenario must be considered separately. Results from one product or laboratory condition cannot automatically be generalised to all herbicides or field environments. Similarly, a biomarker response alone is insufficient to establish ecosystem-level harm.

Future research should connect standardised toxicity tests with realistic exposure assessment, broader species representation and field-level observations. Linking individual responses with earthworm populations and soil ecosystem functions will improve the ecological interpretation of herbicide effects. Such evidence can support sustainable weed management while protecting the biological processes on which healthy and productive soils depend.

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