RadoNorm
Managing risks from radon and NORM

Deliverable D4.9

Concise summary report on cumulative risks on non-human biota exposed to uranium-series radionuclides and chemicals

Work Package 4

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Executive Summary

The primary objective of subtask 4.9.2 in RadoNorm was to advance the modelling of combined stressor effects on natural biota by using more relevant and environmentally realistic exposure conditions. This was done through two subtasks: i) incorporating explicitly acclimation processes and adaptive phenotypic plasticity into population models, ii) consider multigenerational and multi-exposure scenarios for evaluating transgenerational effects, delayed responses, and possible carry-over mechanisms, which are increasingly recognized as key components of ecological risk.

This report assesses ecological risks posed by uranium-series radionuclides and associated toxicity on non-human biota, with particular focus on freshwater organisms. It aims at providing an update on the ecotoxicological evidence and best practices in risk analysis. It also describes how advanced modelling and Adverse Outcome Pathway (AOP) can help to better describe the effects of combined exposures.

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Glossary

AO	Adverse Outcome
AOP	Adverse Outcome Pathway
KE	Key Event
KER	Key Event Relationship
MIE	Molecular Initiating Event
NORM	Naturally Occurring Radioactive Material
WoE	Weight of evidence

1. Mechanisms of uranium toxicity

Uranium is a naturally occurring actinide element in the Earth's crust, but its environmental mobilization can significantly increase due to anthropogenic activities such as uranium mining, nuclear fuel cycle operations, and the widespread application of phosphate fertilizers containing uranium-bearing apatite. These activities enhance uranium's transport into surface waters, sediments, and groundwater systems. Once released into aquatic environments, uranium rarely exists in isolation. It is typically associated with a collection of co-occurring trace metals and metalloids, such as manganese (Mn), iron (Fe), aluminium (Al), barium (Ba), and cadmium (Cd), which can interact to produced combined effects on the natural biota.

Under environmentally relevant conditions, uranium's toxicity is primarily governed by chemical toxicity, with radiological effects generally negligible, except in contexts involving enriched uranium or significant accumulation of radioactive decay products in the ^{235}U or 238U decay chains (for instance radium-226, polonium-210) [1, 2].

At the cellular and molecular levels, uranium predominantly exerts toxicity through redox imbalance and oxidative stress pathways, in a way similar to other heavy metals like cadmium [3, 4]. Indeed, the hexavalent form of uranium [U(VI)], typically present as the soluble uranyl ion (UO_2^{2+}), is a potent oxidant. Upon cellular uptake, often via phosphate or calcium transporters, uranyl ions stimulate the intracellular generation of reactive oxygen species (ROS). This ROS overproduction overwhelms antioxidant defence systems and can leads to oxidative damage to critical biomolecules including lipids (through peroxidation of cellular and mitochondrial membranes), proteins (via oxidative denaturation and carbonylation), nucleic acids (through strand breaks and base alterations) and mitochondria, leading to altered membrane potential, energy imbalance, and ultimately apoptosis [5–8]. On top of these, uranium can form stable complex to phosphate-rich biomolecules such as ATP, nucleotides, and DNA phosphodiester backbones, interfering with DNA replication, and transcription fidelity [9]. Together, these interactions result in genotoxic, cytotoxic, and immunotoxic outcomes, which have been demonstrated in both vertebrate and invertebrate models. At higher levels of biological organization, these events can lead to sublethal physiological impairments such as reduced growth, reproductive dysfunction, altered behaviour, and ultimately, reduced fitness and population viability in affected species [10–13].

Importantly, the toxicodynamics of uranium are highly modulated by environmental conditions and co-contaminant interactions. The presence of other metals can alter uranium uptake and toxicity through competitive, synergistic, or antagonistic interactions at membrane transport sites, detoxification pathways, or within shared molecular targets [14]. For instance, calcium (Ca^{2+}) and magnesium (Mg^{2+}) can compete with uranyl for binding sites, potentially mitigating toxicity, while metals like cadmium or manganese may amplify oxidative stress responses or disrupt repair mechanisms. Moreover, environmental parameters (pH, redox potential, ionic strength, and the presence of organic ligands) significantly influence uranium speciation, bioavailability, and reactivity which can alter observed toxicity by up to three orders of magnitude. In oxidizing and acidic aquatic environments, uranium exists primarily as the free uranyl ion (UO_2^{2+}) (a highly soluble, mobile, and bioavailable species considered the most ecotoxicologically relevant form) [14]. In contrast, under alkaline, carbonate-rich, or neutral conditions, uranium forms less bioavailable hydroxylated and carbonate complexes, such as $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$. The formation of such complexes can significantly reduce uranium uptake by aquatic organisms. Furthermore, complexation with natural organic matter (NOM), including humic and fulvic acids, leads to the formation of colloidal or organo-metallic uranium species, which are typically less bioavailable due to steric hindrance and reduced membrane permeability [15, 16]. Field-based geochemical modelling suggests that in riverine systems, over 90% of total dissolved uranium

may exist in such non-bioavailable forms, thereby substantially reducing its toxic potential [17, 18] (STAR (Contract Number:Fission-2010-3.5.1-269672) DELIVERABLE 4.2, 28/05/2015).

Despite uranium's well-documented environmental persistence and mobility, chronic ecotoxicity data under environmentally relevant conditions remain limited compared to other heavy metals. Existing datasets cover approximately 50 freshwater species, including primary producers (algae, diatoms), primary consumers (crustaceans, molluscs), and secondary consumers (fish and amphibians), as well as several benthic invertebrates [19, 20]. These studies provide the empirical basis for the derivation of Predicted No-Effect Concentrations (PNECs), which exhibit substantial variability, ranging from a few micrograms per liter to several milligrams per liter, depending on species sensitivity, life stage, and water chemistry.

2. Transgenerational and multigenerational effects of uranium on natural biota

Multigenerational effects and carry-over mechanisms are increasingly recognized as critical determinants of ecological risk. Evidence shows that chronic uranium exposure induces cumulative impacts that can extend across multiple generations (including transgenerational inheritance of damage). These processes have profound implications for both ecological integrity and risk assessment, highlighting the need to move beyond single-generation toxicity testing toward frameworks that capture long-term hereditary effects [21]. Studies in fish demonstrate that uranium can be directly transferred from females to offspring via the yolk, inducing heritable perturbations that affect growth, reproduction, and metabolism. Importantly, these effects extend beyond the F1 generation, indicating that uranium toxicity is not solely a consequence of maternal transfer in eggs, but can involve genuine heritable effects. Consistent impacts of both depleted uranium (DU) and natural uranium (NU) on multigenerational endpoints are detailed below.

In *Daphnia magna*, chronic natural uranium (NU) exposure across successive generations (F0-F2/F3) leads to progressive declines in growth and fecundity, with decreasing no-effect concentrations largely driven by maternal transfer and cumulative physiological damage, as characterized through toxicokinetic–toxicodynamic (TKTD) and Bioenergetic modelling (DEBtox) approaches [13, 21, 22] Histological alterations, notably damage to the digestive epithelium, have also been reported and are proposed to underlie reduced assimilation efficiency under chronic exposure to NU [13]. Complementary evidence shows that a short-term (48 h) exposure to a 2% dilution of uranium mine effluent or to equivalent concentrations of waterborne uranium (NU) induces genotoxicity and growth defects in parental individuals and early broods. However, following transfer to clean water, DNA integrity was restored by the third brood, with no significant impacts persisting in subsequent offspring life-history traits, suggesting that at least part of these effects may be transient under this experimental design [23].

In a 16-generation experimental evolution study, *Caenorhabditis elegans* populations were chronically maintained either under control conditions (MGC) or exposed to 1.1 mM natural uranium (MGU) [24]. At specific generations (P0, F2, F3, F10, F12, F16), both populations were challenged with a concentration range from 0.1 to 1.2 mM uranium. Comparison of the two lineages revealed both acclimation and evolutionary divergence. Chronic uranium exposure caused a significant brood size reduction of ~60% at concentrations ≥0.9-1.1 mM, with effects becoming more pronounced after approximately 10 generations of exposure (F10-F16). Bioenergetic modelling (DEBtox) confirmed that uranium acts primarily by impairing energy assimilation already at sub-millimolar concentrations (≥0.5-0.9 mM), thereby shifting energy allocation strategies. Both exposed and control populations evolved toward larger maximal body size, slower growth, reduced reproductive output, and decreased elimination rates. Maternal effects were visible up to about the third generation (F3), after which they diminished. Beyond

this point, the uranium-exposed lineage diverged: by generations F10-F16 at 1.1 mM, individuals were significantly larger (+7% maximal length) but also showed increased sensitivity to uranium, as reflected by decreasing no-effect concentrations (NEC). These findings demonstrate that chronic uranium stress at millimolar levels induces not only short-term phenotypic plasticity but also heritable adaptive trade-offs, leading to populations that are larger but more vulnerable to uranium toxicity.

In vertebrate models, particularly zebrafish (*Danio rerio*), transcriptomic analyses of F1 offspring derived from depleted uranium (DU)-exposed parents revealed deregulation of oxidative stress–related genes, including sod1, sod2, and cat, as well as evidence of mitochondrial damage in skeletal muscle and altered expression of genes involved in early embryonic development, brain function, and gonad formation [25, 26]. At the epigenetic level, F1 embryos exhibited a significant, approximately two-fold increase in global DNA methylation as early as the prim5 stage (24 hpf), which persisted through 96 hpf, even though uranium had already been cleared from the embryos at the later stage, indicating a lasting epigenetic imprint from parental exposure [27]. These findings underscore both physiological and epigenetic inheritance of uranium-induced damage, with heritable transcriptomic shifts persisting across generations.

In mammalian models, rodent studies further support the heritable effects of NU exposure. Chronic ingestion in rats induces global DNA hypomethylation in F2 sperm and persistent sperm morphological defects, indicating disruption of reproductive fitness through epigenetic mechanisms [28]. Multigenerational exposure also alters sperm metabolomic profiles and morphometric parameters, demonstrating uranium's capacity to impact heritable traits beyond conventional toxicological endpoints [29]. These findings underscore the value of integrating multiple omics layers, including epigenomics, transcriptomics, and metabolomics, to improve the consistency, relevance, and mechanistic resolution of toxicology studies [30, 31].

Uranium exposure can also affect survival indirectly by inducing behavioural alterations that compromise feeding, predator avoidance, and reproductive behaviours. In this context, endocrine disruption is a key mechanism, with pronounced perturbations of the hypothalamic–pituitary–thyroid (HPT) axis observed in fish and altered motility [32]. However, these observations are currently limited to F0 embryos, and data on behavioural and endocrine effects in F2 and F3 progeny remain lacking. Finally, mitochondrial dysfunction is documented in zebrafish larvae, linking uranium (NU and DU) exposure to impaired energy metabolism and reduced organismal fitness [25, 33]. Given that mitochondria are maternally inherited, dysfunctions in these organelles and their transmission through successive generation could represent a major mechanism underlying the transgenerational effects of uranium.

3. Environmental relevant exposures

The Environmental Quality Standards (EQSs) designed to protect aquatic biodiversity across Europe remain highly heterogeneous, ranging from 0.015 µg/L in Denmark to 24 µg/L in the Czech Republic [34]. This variability stems from differences in incorporation of bioavailability and speciation data (as well as different methodological choices). Risk assessment increasingly relies on bioavailability-adjusted approaches that incorporate water chemistry parameters directly into predictive models. Among these, the Biotic Ligand Model (BLM) accounts for competitive interactions at biological uptake sites (e.g., with Ca^{2+}, Mg^{2+}, H^{+}), and integrates pH, hardness, and dissolved organic carbon (DOC) to refine uranium toxicity predictions under site-specific conditions (STAR (Contract Number: Fission-2010-3.5.1-269672) DELIVERABLE 4.2, 28/05/2015).

The traditional hazard quotient approach, which compares measured environmental concentrations to PNECs or EQSs, continues to be used for regulatory screening. However, this method is inherently conservative and often inadequate under complex, low-dose, and multi-contaminant exposure

scenarios. In particular, it fails to address the interactive effects of uranium with other metals, which can alter both bioaccumulation dynamics and toxicological endpoints.

In this context, assessing the combined toxicity of uranium (U) and other metals is essential to validate toxicity thresholds derived from laboratory studies. A study by Margerit et al. (2015) [35] investigated the joint effects of U and cadmium (Cd) on the growth and reproduction of *Caenorhabditis elegans*, a model organism in ecotoxicology. This study employed an integrated approach that accounted for metal concentrations in both the exposure medium (agar) and the bacterial food source (the primary uptake route for *C. elegans*). It demonstrated a significant antagonistic interaction between U and Cd, where the presence of one metal mitigated the toxicity of the other. This antagonism was evident for both endpoints: maximal body length increase and total brood size. To interpret these joint effects, the authors used Concentration Addition (CA) and Response Addition (RA) models alongside deviation models, specifically Dose-Ratio (DR) and Dose-Level (DL) dependent interactions. The results demonstrated that complex deviation models (DR and DL) provided a better fit to the experimental data than simple synergism/antagonism models, indicating that the combined toxicity of U and Cd deviates from classical additivity in a concentration- and dose-dependent manner. Moreover, the study identified two nested antagonistic interactions. The first occurred in the exposure medium, where U was shown to significantly reduce the transfer of Cd from the medium to the bacteria, thus decreasing Cd bioavailability. The second interaction likely occurred within the organism, either during toxicokinetics (e.g., competitive uptake mechanisms) or toxicodynamics (e.g., interactions at molecular targets or within stress response pathways).

In another study, the acute toxicity of U (under the form of hexavalent uranium (U(VI)) was tested alone and in binary mixtures with cadmium (Cd) and copper (Cu), on the freshwater ostracod *Cypridopsis vidua* [36]. The 96-hour LC_{50} value for U was 14.244 mg/L, indicating moderate toxicity under the GHS (Globally Harmonized System of Classification and Labelling of Chemicals) criteria and significantly lower toxicity than Cd (0.158 mg/L) and Cu (0.898 mg/L). When combined with other metals, U-Cd and U-Cu mixtures exhibited intermediate toxicity—greater than that of U alone but lower than the more toxic partner—demonstrating that U contributes to toxicity in mixtures, but its effect is modulated by the presence of more toxic metals. Modelling of mixture effects showed that U-Cd interactions were best described by the Concentration Addition (CA) model, suggesting that U and Cd may share similar modes of action or toxicological targets. In contrast, the U-Cu combination showed concentration-dependent deviations from additivity, reflecting more complex or variable interactions.

Finally, *in situ* field studies provide indispensable data for validating laboratory-derived thresholds. For instance, Le Guernic et al. (2016) [37] conducted a 28-day caging experiment with the three-spined stickleback (*Gasterosteus aculeatus*) in ponds contaminated by uranium mining effluents. Fish from impacted sites exhibited significant bioaccumulation of uranium and co-occurring metals such as Mn and Al, and displayed alterations in antioxidant enzyme activity (e.g., catalase, superoxide dismutase), immune response parameters (e.g., lysozyme activity, phagocytosis), neurotoxicity biomarkers (e.g., acetylcholinesterase inhibition), as well as histopathological lesions and DNA strand breaks. At the community level, compositional changes in periphytic diatom assemblages downstream of uranium discharges have been observed, including shifts toward metal-tolerant taxa, reduced diversity, and impaired productivity [38]. Although diatoms are sensitive bioindicators of metal stress, attribution of effects specifically to uranium remains challenging due to confounding factors such as natural variability and the co-occurrence of other stressors. Table 1 summarizes the different studies describing the effects of uranium in combination with other metals or chemicals.

Table 1 - Summary of ecotoxicological effects of uranium in combination with other metals or chemicals

Study System / Type	Metals / Factors Examined	Organism / System	Combined Effects / Key Findings	Reference (full article title)
Duckweed lab test (synthetic soft water)	U + Cu	*Lemna aequinoctialis*	Antagonistic: combined EC_{50} ≈ 1.35 toxic units	Charles A. L., Markich S. J., Ralph P. J. (2006). *Toxicity of uranium and copper individually, and in combination, to a tropical freshwater macrophyte (Lemna aequinoctialis).* Chemosphere, 62(8), 1224–1233.
Ostracod acute lab assays	U + Cd, U + Cu, Cd + Cu	*Cypridopsis vidua*	U–Cd fits Concentration Addition (CA); Cd–Cu fits Independent Action (IA); U–Cu shows mixed interactions depending on concentration range.	Chen L. et al. (2022). *Sensitivity of Ostracods to U, Cd and Cu: The Case of Cypridopsis vidua.* Toxics, 10(7), 349.
Soil nematode lab assays	U + Cd	*Caenorhabditis elegans*	Strong nested antagonism: one interaction in exposure medium, another at toxicokinetic/dynamic phase. Growth and brood-size impairments were less-than-additive.	Margerit A., Lecomte-Pradines C., Svendsen C., Frelon S., Gomez E., Gilbin R. (2015). *Nested interactions in the combined toxicity of uranium and cadmium to the nematode Caenorhabditis elegans.* Ecotoxicology and Environmental Safety, 118, 139–148.
Sediment bioassays	U, As, Ni	*Hyalella azteca* (juveniles & adults)	Uranium most toxic; tissue residue correlated linearly with survival/growth thresholds; juveniles much more sensitive.	Goulet R. R. & Thompson P. (2018). *Bioaccumulation and toxicity of uranium, arsenic and nickel to juvenile and adult Hyalella azteca in spiked sediment bioassays.* Environmental Toxicology and Chemistry, 37(9), 2340–2349.
In situ biomonitoring (fish near uranium mine)	U (natural field contamination)	*Gasterosteus aculeatus* (three-spined stickleback)	Seasonal exposure (~20 µg U L^{-1}) led to measurable uranium accumulation in otoliths; observed concentrations aligned with lab-derived toxic thresholds.	Le Guernic A. et al. (2016). *In situ effects of metal contamination from former uranium mining sites on the health of the three-spined stickleback (Gasterosteus aculeatus, L.).* Ecotoxicology, 25(6), 1234–1259.
Aged agricultural soil ecotoxicity tests	Uranium (aged soil) only	Grass (*Elymus lanceolatus*), *Eisenia andrei* (earthworm), *Folsomia candida* (springtail)	No adverse effects for plants or earthworms up to ~1,000 mg U/kg; springtail EC_{20} was ~85–130 mg U/kg, supporting PNEC estimates ~100–250 mg U/kg.	Sheppard S. C. & Stephenson G. L. (2012). *Ecotoxicity of aged uranium in soil using plant, earthworm and microarthropod toxicity tests.* Bulletin of Environmental Contamination and Toxicology, 88, 43–47.
Earthworm bioaccumulation comparison	Natural vs depleted uranium	*Eisenia fetida*	No mortality or weight change up to ~600 mg U/kg; low-level genotoxic and lysosomal damage seen—natural uranium more toxic than depleted.	Giovanetti A. et al. (2010). *Bioaccumulation and biological effects in the earthworm Eisenia fetida exposed to natural and depleted uranium.* Journal of Environmental Radioactivity, 101, 509–516.

Polymetallic mine-soil microbial adaptation	U rich mine soil	Soil microbial communities in antiquated spoil soil	Long-term co-contaminants shaped microbial enzyme activity; organic matter reduced bioavailability; U contributed to community shifts.	Joner E. J. et al. (2007). *Bioavailability and microbial adaptation to elevated levels of uranium in an acid, organic topsoil forming on an old mine spoil.* Environmental Toxicology and Chemistry.
Mine-area field soil multi-species bioassay	U + Cd + As + others	Ostracods (*Cypridopsis*, *Heterocypris*), *Vibrio fischeri*, *Vicia faba*	High-contaminant soils caused elevated mortality and toxicity responses across species	Zhang Z. et al. (2023). Study on *Ecotoxic effects of uranium and heavy metal elements in soils of a uranium mining area in northern Guangdong.* Toxics, 11(2), 97.
Natural uranium–rich soils & bacterial communities	High U content as natural variable	Indigenous soil bacteria	Elevated uranium levels drove changes in community composition, enriching taxa like *Geobacter*—evidence of long-term microbial adaptation.	Mondani L. et al. (2011). *Influence of uranium on bacterial communities: a comparison of natural uranium-rich soils with controls.* PLoS One, 6(10): e25771.
Diatom biomonitoring – field monitoring	U (plus $BaCl_2$, $Al_2(SO_4)_3$)	Periphytic diatoms on artificial substrates in stream	Community structure significantly altered downstream of treated uranium effluent discharge; diatom assemblages shifted toward acidophilic, uranium-sensitive taxa, despite no change in periphyton biomass or photosynthetic activity.	Herlory O., Bonzom J.-M., Gilbin R., Frelon S., Fayolle S., Delmas F., Coste M. (2013). Use of diatom assemblages as biomonitor of the impact of treated uranium mining effluent discharge on a stream: case study of the Ritord watershed (Center-West France). Ecotoxicology, 22(8), 1186–1199.

4. Advanced modelling of combined exposures and ecological risks in the RadoNorm task 4.9

The importance of considering situations of multiple exposure throughout life, from (pre)conception to death to study the (human and animal) health effects of environmental exposures, is increasingly recognized, as illustrated by the rapidly growing use of the “exposome” concept which aims at considering all environmental factors impacting life from its conception until death. In spite of this, the joint effects of exposure to NORM and other environmental exposures (e.g., chemical, physical agent) remain poorly documented. To better characterize joint effects (e.g., additivity, synergy, antagonisms), understanding and characterizing the underlying mechanisms of the response to NORM in combination with other stressors is of primary importance. To this end, the empirical evaluation/quantification of adverse outcomes resulting from joint exposures through experimental, epidemiological (for human health) and ecological observations (for environmental risks assessment) is needed. The Adverse Outcome Pathway (AOP) framework offers a promising approach to assess these multi-level assessments by allowing the qualitative and quantitative description of events from the molecular initiation of the toxic effects to the adverse effects observed at the organism and populations levels [39–41]. It describes the fundamental biological events from a Molecular Initiating Event (MIE) and propagate through essential Key Events (KE) across the different biological scales to results into an Adverse Outcome (AO). The AOP describes thus, in a simple way the scientific knowledge of the toxic effects at the different biological levels of organization from molecular mechanisms to the occurrence of the disease (Figure 1).

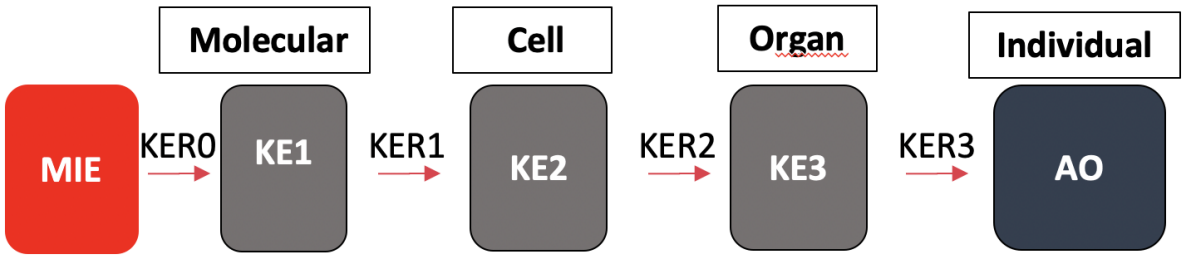

Figure 1 - Description of the AOP framework.

Population model with inclusion of acclimation processes

A population-level model based on a system of ordinary differential equations (ODEs) was implemented to simulate key biological processes, including mortality, morbidity, reproduction, and acclimation [42]. This mechanistic framework allows for the exploration of both additive and non-additive effects (i.e., synergistic or antagonistic interactions) resulting from combined chemical and radiological exposures. The model is governed by key parameters such as the radiation dose rate (D), chemical concentration (C), and an interaction term (γ), which together define the combined effects equation:

$$\emptyset = \alpha D + \beta C + \gamma DC,$$

where α and β are scaling coefficients for individual stressors. Additional biologically relevant parameters include fecundity (r), natural death rate (d), and the kinetics of repair and acclimation mechanisms (κ, η, μR, μF), which collectively capture the population's resilience and its potential tipping points under environmental stress.

This modelling framework offers significant flexibility, enabling the simulation of diverse exposure scenarios, from chronic low-dose exposure to accident-related peak doses, and provides a valuable platform for evaluating both short- and long-term population dynamics. Importantly, the inclusion of acclimation processes, though currently based on theoretical assumptions, opens promising avenues for studying adaptive responses and their implications for long-term population sustainability. Ultimately, this approach provides a blueprint for mechanistic population modelling in the context of complex, multi-stressor environments. The parameters identified through this model could be used as index quantities for site classification in integrated chemical, radiological, and ecological risk assessments. By bridging the gap between mechanistic understanding and regulatory application, this modelling framework contributes meaningfully to both scientific research and the development of evidence-based environmental protection strategies.

Development of AOP for combined exposure to natural biota

By delineating a sequence of causally linked key events (KEs) that follow a molecular initiating event (MIE), AOPs facilitate the identification of mechanistic intersections where different stressors elicit overlapping biological responses [43, 44]. This is particularly useful when evaluating additivity and stressor interaction such as synergism or antagonism, where non-additive effects are frequently reported for environmental-relevant stressors [4, 45–47].

To enhance predictive accuracy, AOPs can be quantitatively parameterized using dose– or dose-rate–response data in conjunction with statistical tools. Quantitative AOPs (qAOPs) employ approaches such as benchmark dose (BMD) modelling to derive points of departure (PODs), structural equation modelling (SEM), and Bayesian networks to assess and quantify causal relationships between stressors and biological events [48–52]. When individual AOPs are integrated into quantitative AOP networks (qAOPNs), they can simulate cumulative effects of stressor mixtures across levels of biological organization [43, 44].

In ecotoxicology, this framework supports extrapolation across species and ecological contexts, particularly when combined with toxicokinetic-toxicodynamic (TKTD) or dynamic energy budget (DEB) models that connect sub-individual responses to organismal fitness and population dynamics [53]. Nevertheless, several challenges persist: the limited taxonomic representation of current AOPs, insufficient validation under environmentally relevant exposure conditions, and poor integration of non-targeted and transgenerational effects [54–57].

5. Knowledge gap and research priorities

The consistent ecotoxicological findings across multiple studies indicate that uranium can exert toxic effects even at low concentrations. These effects are modulated by various environmental factors, including water chemistry and the presence of co-contaminants. Field studies and long-term exposure investigations further enhance the ecological relevance of these findings by validating laboratory results and revealing chronic effects.

While the current body of evidence is generally scientifically sound, significant gaps remain. These gaps are particularly evident in studies concerning multi-generational effects and the toxicity of complex contaminant mixtures, where low number of studies are available. Research addressing the combined effects of uranium with other metals is limited, and there is virtually no data on interactions between uranium and organic compounds or other contaminant classes. The limited available data suggest complex interactions, including antagonistic, additive, and synergistic effects. This complexity highlights the weak pertinence of traditional single-contaminant risk assessment approaches (developed from laboratory experiment on single species and single stressors) under realistic environmental conditions (which include different species in interactions and mixture of stressors). Current regulatory frameworks may be overly conservative or insufficiently robust in such contexts.

To address these limitations and enhance the ecological relevance of uranium risk assessments, there is a growing shift toward mechanistic, process-based modelling approaches. These approaches explicitly link environmental exposures to biological outcomes. For instance, the Biotic Ligand Model (BLM) incorporates site-specific water chemistry parameters—such as pH, hardness, and dissolved organic carbon—to predict uranium bioavailability more accurately, thereby refining toxicity estimates. Similarly, Dynamic Energy Budget (DEB) models simulate energy allocation within organisms under toxic stress, enabling extrapolation from molecular and cellular effects to critical biological endpoints like growth, reproduction, and survival. Additionally, the Adverse Outcome Pathway (AOP) framework provides a structured approach for connecting molecular initiating events, such as reactive oxygen species generation or DNA damage, to adverse effects at organismal and population levels. This facilitates cross-species extrapolation and regulatory application.

Despite their theoretical advantages, the application of these mechanistic models to uranium ecotoxicology remains in its early stages. Critical obstacles include a lack of uranium-specific parameters within BLM and DEB frameworks, a scarcity of chronic low-dose exposure data across diverse ecologically relevant species, and limited incorporation of mixture toxicity and metal interaction

effects. Moreover, field validation of these models is notably lacking, impeding their refinement and confidence in real-world scenarios.

Significant knowledge gaps continue to constrain the robustness of uranium risk assessments. Chronic exposure studies at environmentally relevant concentrations, particularly those involving metal mixtures, are rare. The application of mechanistic biomarkers, such as indicators of genotoxicity, oxidative stress, and immunotoxicity, remains limited in ecological contexts despite their importance for early effect detection. Furthermore, transgenerational and reproductive impacts of uranium, especially in combination with other contaminants, are poorly understood and scarcely studied in non-model aquatic species. Exposure pathways associated with sediments and benthic organisms, which are critical components of aquatic ecosystems and likely exposed to uranium contamination, have been insufficiently investigated.

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