RadoNorm
Managing risks from radon and NORM

# Deliverable D4.8

# Effects of multiple stressor exposures (NORM, metals and particles) of selected organisms

Work Package 4

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**Disclaimer**



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## Executive Summary

The overall objective of task 4.8 has been to improve knowledge on transfer and effects, including underlying mechanisms, of combined exposures to radon or NORM and other stressors. This included studies on exposure situations relevant for humans, notably radon combined with tobacco smoke or nanoparticles via inhalation, and other organisms. A summary of work carried out on human relevant exposures has been reported in Deliverable 4.7. This deliverable presents an overview of studies carried out on nematodes (*C. elegans*) and daphnia. Since much of the work is still being prepared for publication, this document provides a brief summary of work carried out to date. Co-exposure studies have been carried out with *daphnia magna* (uranium, uranium nanoparticles and microplacstics) and *C. elegans* (uranium, radium and nanoplastics). In combination with synchrotron-based x-ray fluorescence mapping (Diamond Light Source, UK), results have demonstrated differences in uranium uptake, distribution and toxicity between uranium and uranium nanoparticles in daphnia. A significant reduction in uptake of palladium-tagged nanoplastics (100 nm) body burdens in daphnia was seen when co-exposed to uranium (30 µg/l), likely resulting from impairment of intestinal function. Toxicity tests have been carried out for nematodes exposed to Ra-224 (t1/2), and a series of dose-response relationships established for reproduction endpoints. Dosimetry has been performed in collaboration with MTA-EK, with reduction in numbers of offspring seen at total doses of about 1.6 Gy. Experiments looking at combined exposure of Ra-224 and nicotine are ongoing and will include both toxicity tests and biomarkers.

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# 1. Introduction

For organisms exposed to NORM combined with metals and particles, ecosystem transfer (uptake and accumulation) depends on exposure routes and interactions between stressors (toxicokinetics), while effects depend on competition between stressors to interact with key biological targets (toxicodynamics). In order to investigate which mechanisms a series of experiments looking at organism uptake, effect and interactions between NORM (uranium and radium) and other stressors (nanoplastics, nanoparticles and nicotine) have been carried out. The overarching aim has been to link exposure to uptake, internal distributions and responses to understand why combined exposures could depart from additivity along the source - adverse outcome pathway.

# 2. Uranium uptake and distribution in daphnia

As part of the foundation for studies on interactions of uranium with other stressors, a series of studies has been carried out that combine synchrotron based elemental analysis and acute toxicity tests [1,2]. *Daphnia magna* was exposed to uranium nanoparticle (UNPs, 3 – 5 nm) or to uranium reference ($U_{Ref}$) solutions, and the biodistribution and adverse effects were investigated. Adverse morphological changes to the midgut and the hepatic ceca organs were visualized with CT (2 µm voxel size) in daphnia exposed to both treatments. Elemental imaging by means of 2D µ-SRXRF (1 µm beam size) showed the morphological changes in these digestive tract organs, which are critical to digestion and nutrient absorption, to be co-localized with relatively high U concentrations in lumen contents. Furthermore, U distributions indicated U uptake in epithelial tissues.

## 2.1 Results

Speciation analysis revealed similar size distributions between exposures, and toxicity tests showed similar acute effects (UNP: 402 µg $L^{-1}$ [95% CI: 336 - 484], $U_{Ref}$: 268 µg $L^{-1}$ [95% CI: 229 - 315]). However, the U body burden was 3- to 5-fold greater in UNP exposed daphnia, while analysis of survival as a function of body burden revealed a ~ 5-fold higher specific toxicity from the $U_{Ref}$ exposure. High resolution (2 µm) XRF elemental maps of intact, whole daphnia derived from sublethal, acute exposures revealed similar biodistributions between treatments, with high U accumulation onto the gills (epipodites) as well as within the hepatic ceca and the intestinal lumen. Uranium uptake into the haemolymph circulatory system was visible in organs such as the heart and the maxillary gland, an organ of the daphnia excretory system. The substantial uptake in the maxillary gland and nephridium suggest that these organs play a role in U removal from the haemolymph and subsequent excretion. Maternal transfer was demonstrated by U signals observed in the embryos and remnants of the chorion. Histological analyses of microtome slices (1 µm thickness) showed stressed, irregular, and, in some cases, destroyed epithelial cells in hepatic ceca and midgut tissues that underpin the morphological changes observed in the CT renderings. Furthermore, nano-SRXRF identified U-bearing structures within the lumen, including partially digested algae (*Raphidocelis subcapitata*), that contribute to the sequestration of U within the digestive tract (Figure 1). The results in the present work support the notion that disruption of intestinal assimilation of nutrients is an important toxic mode of action for U in *D. magna* and that midgut epithelial cells as well as the hepatic ceca are major target organs of U toxicity. The reported whole organism biodistribution links the exposure to toxic effects in *D. magna* and provides novel insights that should improve the understanding of uptake and elimination pathways.

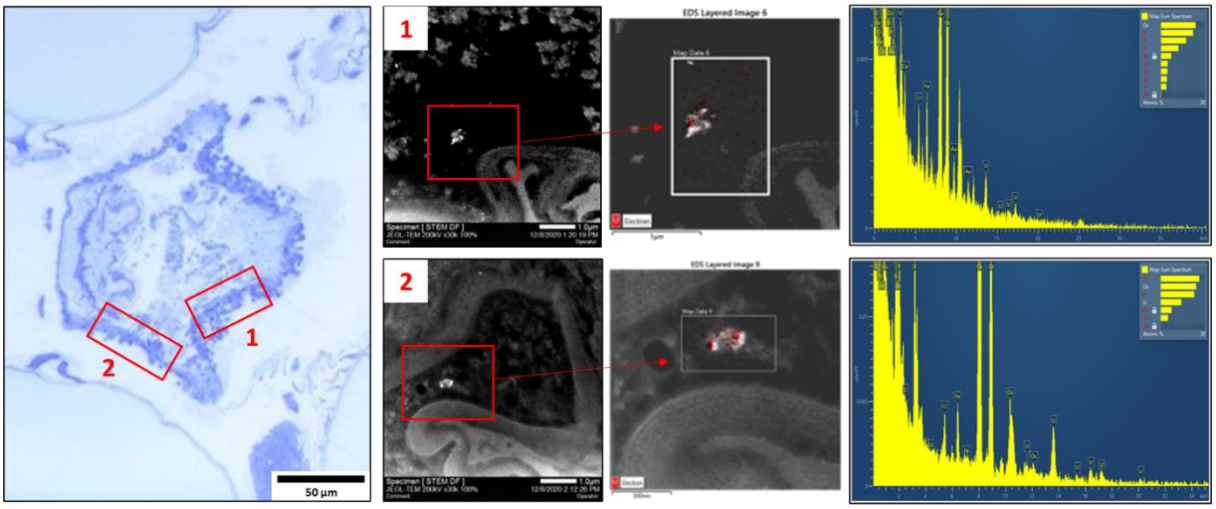


*Figure 1. Transmission electron microscopy examinations of midgut sections from UNP exposed D. magna. Two investigation sites are noted where small (< 250 nm) high density aggregates were found along the brush border of the epithelial cells. X-ray analysis shows the particles contain U indicating that UNP aggregates are present at least along the epithelial cells setup and the endpoints [2].*

# 3. Combined Uranium and Nanoplastic in the model organism *Daphnia magna*

A potential combined exposure in aqueous environments include potentially hazardous radionuclides and nanoscale plastics. Building on previous work studying the uptake, and effects of uranium and uranium nanoparticles, these experiments investigated the uptake, retention, and adverse effects of uranium (U) and 200 nm palladium (Pd) -doped polystyrene nanoplastics (NPs) in *Daphnia magna*. A series of separate and combined acute toxicity tests were carried out wherein daphnia were exposed to 1) aqueous uranyl nitrate: 0 – 400 µg U $L^{-1}$; 2) palladium (Pd) tagged polystyrene spheres (100 nm): 0 – 10 mg PLAST $L^{-1}$; and 3) Combinations: 50 – 200 µg U $L^{-1}$ and 0.1 – 10 mg PLAST $L^{-1}$. Elemental biodistribution analysis at the i18 beamline of the Diamond Light Source (UK).

## 3.1 Results

Exposure media characterization indicated little interaction between U and NPs and no significant changes in acute toxicity were observed. However, NP body burden was reduced following co-exposure with U. *Daphnia magna* exposed to U concentrations approaching the WHO drinking water threshold (30 µg U L-1) exhibit lower nanoplastic (~100 nm) body burdens likely resulting from impairment of intestinal function in line with the results found in [2]. Synchrotron-based X-ray fluorescence elemental mapping of intact, whole daphnids supported these data showing detailed NP retention in the digestive tract when exposed without U. When co-exposed with U, NPs were only found in the anterior of the hindgut or not at all. The reduction in NP body burden when co-exposed with U indicates that feeding behaviours of daphnids are perturbed leading to adverse effects in line with previous U toxicity studies. These results highlight potential challenges associated with impact and risk assessments for multiple stressor exposure situations involving naturally occurring radionuclides and NP contamination.

# 4. Chronic alpha exposure in $^{224}RaCl_2$ using the model organism *C. elegans*

Ra-224 ($^{224}Ra$) is a representative of alpha (α)-emitting Naturally Occurring Radionuclide Material (NORM) as the fifth member of $^{232}Th$ decay series. It occurs at very low concentrations yet is present in virtually all rocks, soil, waters, and even in plants and animals [4]. The large mass and high positive charge confer the strong ionizing capacity inherent to alpha particles through both Coulomb interactions and direct collisions with atomic electrons [5]. Among the naturally occurring isotopes of radium, $^{224}Ra$ has the shortest half-life of 3.6 days, making it a great candidate for modern targeted α-particle cancer therapies. Historically, $^{224}Ra$ has historically been used in medical treatments for conditions such as anemia, leukemia and ankylosing spondylitis.

Despite its application in medical treatments, concerns persist regarding the potential risks associated with these treatments and α-particle exposure in general. One of the decay products of $^{224}Ra$—$^{220}Rn$ together with $^{222}Rn$—were shown in previous epidemiological studies increase the risk of lung cancer [6,7]. Moreover, the health effects of α-particle radiation in humans include not only carcinogenesis but also genetic diseases, teratogenesis, and degenerative disease [8]. In organisms other than human, such as in plant model *Tradescantia,* reduced fecundity and increased somatic mutations were observed at 0.01−3.32 mR/hr [9]. Increased chromosome breakage was exhibited among *Drosophila melanogaster* that had been subjected to α-particles during larval stages [10]. In zebrafish embryos, increased apoptosis was identified following α-particle exposure ranging from 2.8 to 11.2 mGy [11]. Additionally, experiments at cellular levels have shown that α-exposure induces DNA double strand breaks (DSBs), cell cycle arrest and micronuclei formation [12]. Despite these findings, significant knowledge gaps remain regarding organism-level phenotypes, including effects on somatic growth, fertility, germ-cell apoptosis, the level of reactive oxygen species (ROS), and transcriptomic regulation.

To fill the knowledge gaps, we utilized the model organism *Caenorhabditis elegans* (*C. elegans*) to conduct α-exposure in $^{224}RaCl_2$ solutions (the highest activity level is 2300 Bq/mL) within close glass vials. As one of the most radio-resistant species, *C. elegans* enables comprehensive assessment of whole-organism phenotypes, transcriptomic responses and uptakes following a 65-hour α-exposure regime in $^{224}RaCl_2$ solutions.

## 4.1 Materials and methods

Several strains of *C. elegans* have been used in the current study including wild-type N2 Bristol, fluorescent apoptosis reporter ZH1963 [*ced-1p::2xFYVE::GFP (enIs59)*], and the SOD-1 fluorescent reporter GA508 [*wuIs54(pPD95.77 sod-1::GFP, rol-6(su1006))].*

All experiments were designed as dose-response exposures with three biological replicates per activity concentration (Figure 2. *$^{224}RaCl_2$ exposure setup and the endpoints.*

). For each replicate, approximately 200 synchronized L1 larvae were transferred into closed glass vials and exposed for 65 hours in $^{224}RaCl_2$ solutions (0, 144, 575, 2300 Bq/mL). Prior to each exposure, the activity levels and equilibrium status of the $^{224}RaCl_2$ solution (Oncoinvent, Oslo, Norway) were verified using a liquid scintillation counter (LSC, Hidex 600 SLe, Hidex Oy, Turku, Finland). For measurement, 100 µL of $^{224}RaCl_2$ solution was mixed with 0.5 mL HCl [1 M], 900 µL dd$H_2O$, 18.5 mL LSC cocktail AB.

Following the exposure, nematodes were washed with moderate hard water (MRHW, 0.96% $NaHCO_3$, 0.6% $CaSO_4 \cdot 2H_2O$, 0.6% $MgSO_4$, 0.04% KCl, HCl [1M]) transferred onto Ø6 NGM plates (freshly

seeded with OP50). Developmental progression was closely monitored until relevant phenotypes were measurable (90 hours post-L1). All samples were blinded prior endpoint assessments.

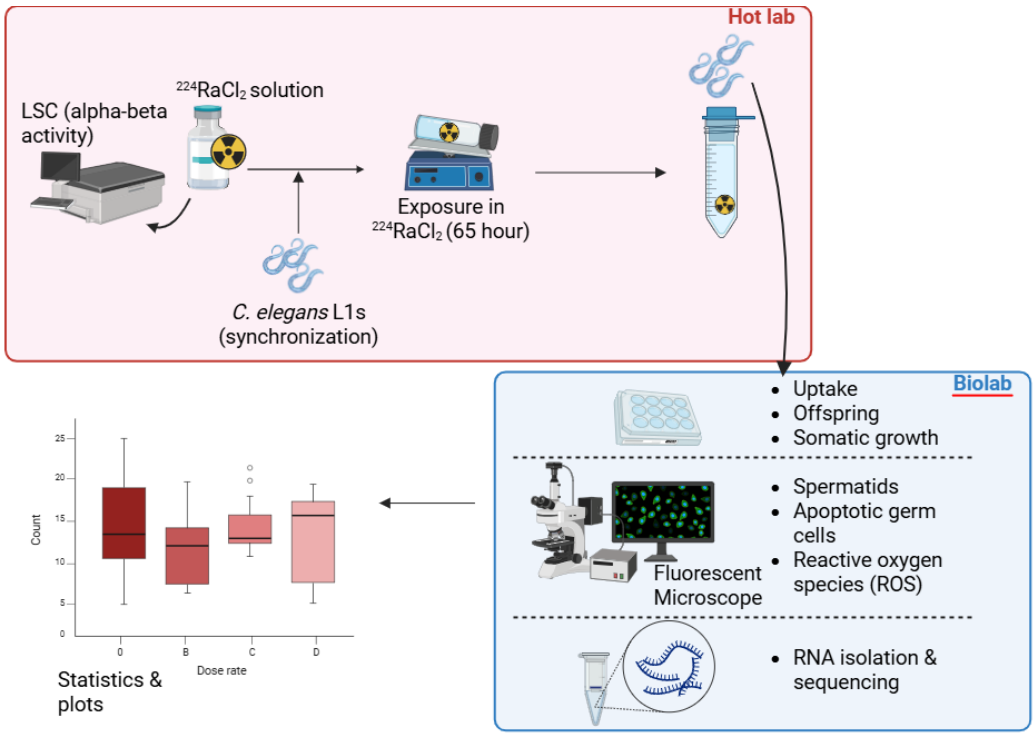


*Figure 2. $^{224}RaCl_2$ exposure setup and the endpoints.*

## 4.2 Results

The somatic growth of *C. elegans* larvae appeared normal regardless of the activity levels of $^{224}RaCl_2$ (up to 2300 Bq/mL) to which they were exposed. In contrast, reproductive phenotypes were markedly sensitive to α-exposure. Total brood size was reduced by 39.8% at the highest activity level (2300 Bq/mL). Dosimetry estimates in collaboration MTA-EK, equate this to total doses of about 1.6 Gy. Spermatid counts declined by 18.6%, 23% and 35.5% following exposures to 144, 575, and 2300 Bq/mL, respectively, compared with unexposed controls. Consistently, germ cells—the progenitors of both spermatids and oocytes—exhibited increased apoptosis, with 1.76- and 1.62-fold elevations at 575 and 2300 Bq/mL, respectively. Furthermore, the expression of SOD-1, a superoxide dismutase that converts superoxide radicals into hydrogen peroxide and oxygen, showed a dose-dependent increase of 2.15-, 2.6- and 2.7-fold at 144, 575, 2300 Bq/mL, respectively. Transcriptomic analysis revealed that the molecular mechanisms underlying the toxicity of $^{224}Ra$ chronic alpha exposure were characterized by upregulation of protein phosphorylation and dephosphorylation, coupled with downregulation of genes involved in chromatin remodeling, cell division, and regulation of DNA-templated transcription.

Building on the results of these studies, experiments are ongoing to investigate the interaction of radium with nicotine, thus further investigating the interactions observed in 4.7. The final results will be available in September 2025.

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