# Wageningen University and EcotoQ Research Symposium
# INRS Québec June 6th, 2025

## Schedule / Horaire

| | |
|---|---|
| 9-9h05 | Opening Remarks / Mot d'ouverture |
| 9h05-10h50 | Oral Presentations / Présentations orales |
| 10h50-11h | *Break / Pause* |
| 11h-12h | Poster Presentations / Présentations par affiche |
| 12h-13h | *Lunch* |
| 13h-14h10 | Poster Presentations / Présentations par affiche |
| 14h10-15h40 | Oral Presentations / Présentations orales |
| 15h40-15h45 | Closing Remarks / Mot de la fin |

INRS, Centre ETE
490 rue de la Couronne,
Québec, QC

# Wageningen University and EcotoQ Research Symposium

## Morning presentations

| Time | Presentations |
|---|---|
| 9h05 | **A systems-based approach to evaluating the risk of pesticides to black-tailed godwits (Limosa limosa), Barbara Pacheco Harrison Righetto (WUR)** |
| 9h20 | **Trophic transfer of microplastics: from the periphyton to a primary consumer, Linsey Yvette Mouatchô (INRS)** |
| 9h35 | **Pesticide exposure to birds, Aafke saarloos (WUR)** |
| 9h50 | **Voies d'exposition et transfert maternel des substances per- et polyfluoroalkylées (PFAS) chez le fou de Bassan, Laurianne Richard (INRS)** |
| 10h05 | **Investigating of legacy and unknown PFAS in an otter food web in The Netherlands, Ling Chen (WUR)** |
| 10h20 | **Investigating the toxicity effects of tire crumb particles and its leached chemicals on Daphnia magna, Ana Carrazco Quevedo (McGill)** |
| 10h35 | **All organisms have their limit: Using toxicodynamic modelling to identify main drivers of species differences in sensitivity to pesticides, Nick van Sabben (WUR)** |

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## Afternoon presentations

| Time | Presentations |
|---|---|
| 14h10 | **A PBK model-based QIVIVE approach for organophosphate pesticide risk assessment, Thijs Moernhout (WUR)** |
| 14h25 | **Using stream algal biofilms to monitor pesticide contamination, Laura Malbezin (INRS)** |
| 14h40 | **Marine oil toxicity: Can the humble cockle survive?, Edith Etor (WUR)** |
| 14h55 | **Accumulation of radium 226 and its effect on the growth of the unicellular green alga Chlamydomonas reinhardtii, Flavie Desreac (INRS)** |
| 15h10 | **Urban stressors on avian immune sefenses: Impact of pollution on viral challenge via polyI:C in great tit and blue tit nestlings, Shivani Ronanki (WUR)** |
| 15h25 | **Toxicity of radium-226 on early life stages of Lymnaea stagnalis, Léna Guimard (INRS)** |

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| # | Poster Presentations |
|---|---|
| 1 | Ecdysone receptor agonism adverse outcome pathway validation for insect-specific in vitro assay development, Rebeka Darmati (WUR) |
| 2 | Generation of intestinal tissue-resident macrophages and dendritic cells for an immunocompetent intestine-on-chip system, Donovan O'Brien (WUR) |
| 3 | ToxoP : occurrence and toxicity of oxodegradable plastic mulch, Isabelle Lavoie (INRS) |
| 4 | Toxicity of true-to-life microplastics to human induced pluripotent stem cell-derived intestinal epithelium correlates to their protein corona composition, Hugo Brouwer (WUR) |
| 5 | Studying the role of chylomicrons in intestinal absorption of lipophilic chemicals in vitro, Germaine Aaldreink (WUR) |
| 6 | Assessment of platinum and palladium toxicity to two freshwater organisms, Julien Michaud-Valcourt (INRS) |
| 7 | Risk assessment innovation for low-risk pesticides with Caenorhabditis elegans as ecotoxicological model, Xupeng Yu (WUR) |
| 8 | Development of immunocompetent liver toxicity models to unravel the role of the hepatic immune system in hepatotoxicity, Gijs van Slobbe (WUR) |
| 9 | Toxicité de plusieurs métaux (Pd, Pt, 226Ra) pour la lentille d'eau Lemna minor, Océane Hourtané (INRS) |
| 10 | The integration of in vitro cell-based and computational approaches to improve the toxicological hazard assessment of nephrotoxicants, Kiri Romano Olmedo (WUR) |

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| # | Poster Presentations |
|---|---|
| 11 | A multi-omics approach to study the mechanism of neurotoxicity induced by advanced glycation end products (AGEs), Haomiao Wang (WUR) |
| 12 | Effet de le présence de métaux sur l'ozonation catalytique de l'antibiotique Norfloxacin et son impact écotoxicologique, Djidja Roumaissa (UQAM) |
| 13 | Comparative hazard assessment of titanium and silica-based nanomaterials for antimicrobial coatings using intestinal cell models, Tanne Meuwissen (WUR) |
| 14 | Evaluating the functionality of xenobiotic biotransformation enzymes and transporters in In vitro intestinal models, Jingxuan Wang (WUR) |
| 15 | Effect of pH on metal bioaccumulation in freshwater biofilm exposed in the laboratory, Frederique Warren (INRS) |
| 16 | Alterations in bile acid secretion profile after Rifampicin- treatment in HepaRG- liver- on- chip, Katharina Nitsche (WUR) |
| 17 | Advancing in vitro models of inflammatory bowel disease: Investigating the impact of foodborne contaminants in novel culture systems, Tom Walraven (WUR) |
| 18 | Fleurs sous surveillance : mesurer les résidus de pesticides pour protéger les pollinisateurs, Michael Tremblay (Université Laval) |
| 19 | Oral toxicity of weathered microplastics studied using an in inflamed triple culture human intestinal cell model, Rizal  Malkarim (WUR) |
| 20 | Studying the relationship between the structure and apparent permeability of flavonolignans in silymarin in in vitro intestinal models, Xuan Zhang (WUR) |

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## Presentation Abstracts

A systems-based approach to evaluating the risk of pesticides to black-tailed godwits (Limosa limosa), **Barbara Pacheco Harrison Righetto** (WUR)

Though loss of habitat is pointed out as a key driver of bird population declines worldwide, exposure to contaminants has been shown to lead to immune and reproductive alterations that can have severe long-term populational effects. However, comprehending the risk of contaminants to wildlife species is often hindered by their exposure to multiple chemicals simultaneously and, for migratory animals, by the spatial/temporal variability of exposure. In that sense, the main goal of this study is to determine which pesticides a migratory farmland species the black tailed godwit (Limosa limosa) is exposed to during its annual migration cycle and to investigate if such compounds, individually or jointly, present a risk to two key biological processes: endocrine response and maintenance of oxidative balance. To determine exposure we will quantify, using LC-MS/MS, individual pesticides in both soil and prey samples, collected along the godwits' migration route, and blood samples, collected from adult godwits. To determine effects of exposure, individual compounds will be assessed for their capacity of 1) binding to nuclear receptors involved in reproductive processes and 2) generating ROS or oxidative damage. The toxicity of mixtures will be estimated by concentration addition and independent action approaches. To further characterize effects of exposure to pesticides, biomarkers of endocrine disruption and oxidative stress will also be analyzed in the blood of godwits. The data obtained will be used to assess the risk of chemicals to migratory black tailed godwits, incorporating the exposure to mixtures and the spatial/temporal variability of exposure. In the presentation, initial observations of exposures will be provided based on soil samples from different locations along the East Atlantic Flyway.

Trophic transfer of microplastics: from the periphyton to a primary consumer, **Linsey Yvette Mouatchô** (INRS)

In light of the growing demand for and the continuously increasing production of plastic materials, concentrations of plastic debris in the environment will also increase. The risk of microplastics (MP) exposure of aquatic invertebrates is not well documented, especially in freshwater environments. It is essential to evaluate the risk of plastic contamination in aquatic food webs, starting at the base of the trophic chain. In this study, we used algal biofilms (periphyton) and the freshwater gastropod Lymnaea stagnalis as model organisms for a MP transfer experiment. The objective was to assess the short-term (48-h) uptake and elimination of MP trapped in periphyton by the primary consumer L. stagnalis. Additionally, the study aimed to determine whether MP particle size and/or shape influence the uptake and transfer of MP. With regard to shape, particular attention was paid to microfibers (MF) as their effects on biota are relatively less studied compared to those of other forms of MP such as microbeads (MB). Preliminary results revealed a greater uptake of MF compared to MB by the gastropod. In terms of elimination, MB was more easily egested than MF.

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### Pesticide exposure to birds, **Aafke saarloos** (WUR)

Two spontaneous mass mortality incidents in two different swallow bird species were studied. The first incident was observed in a sand martin (Riparia riparia) colony near Utrecht, the Netherlands. More than 30 individuals showed direct mortality and 15 individuals died soon after being caught by bird ringers. The birds showed remarkable neurological symptoms on their behavior: proptosis (all looked dull, some had bulging eyes), hypotonia (they span their head) and ataxia (all performed uncoordinated flights using only one wing while hipping in circles, and some of the swallows tumbled down). Interestingly, a similar case was observed in barn swallows (Hirundo rustica) in Noord-Holland, the Netherlands. To determine the potential cause of death, a comprehensive virological, pathological and pesticide exposure assessment was performed. Liver, feather, stomach and brain samples were analyzed and screened for their presence of 650 pesticides. The pesticide analyses revealed the ubiquitous presence of the pyrethroid insecticides permethrin and tetramethrin in all feather and some brain samples. Only traces were detected for some other pesticides, but most pesticides were not detected. At the moment, we are evaluating if these pesticides contributed to their spontaneous death. Concerning is that current environmental risk assessment of chemicals does not always adequately consider the impact of direct pesticide exposure to wildlife, and knowledge on potential effects of this exposure route is lacking. This may limit proper conservation of bird populations and the protection of environmental health. In particular because the current study suggests that dermal exposure is the exposure route of relevance.

### Voies d'exposition et transfert maternel des substances per- et polyfluoroalkylées (PFAS) chez le fou de Bassan, **Laurianne Richard** (INRS)

Les substances per- et polyfluoroalkylées (PFAS) représentent une classe de polluants d'origine anthropique qui sont très persistants dans l'environnement, ce qui entraîne une accumulation et des effets néfastes chez les animaux et les humains. L'objectif principal de cette étude était d'identifier la relation entre la concentration de PFAS et les variables biologiques chez la femelle fou de Bassan (i.e. la condition corporelle, les aires d'hivernage, la position trophique et l'aire d'alimentation) ainsi que de déterminer l'impact sur le transfert maternel. Pour se faire, 35 femelles de l'île Bonaventure (Golfe du Saint-Laurent, Québec, Canada) ont été échantillonnées. Le sang et les œufs ont été analysés pour 17 PFAS, dont 13 acides carboxyliques perfluorés (PFCAs) et 4 acides perfluorosulfoniques (PFSAs). Le PFOS, acide perfluorooctanesulfonique, est le composé le plus présent dans le sang et les œufs des femelles fous de Bassan, avec une représentation ~50% de la somme totale des PFAS. Un transfert préférentiel de la femelle à l'œuf pour les PFSAs ainsi que les PFCAs à longue chaîne (plus de 10 atomes de carbone) a été observé. La condition corporelle des femelles est positivement corrélée avec le ratio de transfert maternel. Les premières données de géolocalisation montrent un effet des aires d'hivernage sur la charge en contaminants des femelles, pouvant influencer le transfert maternel. Les plans futurs comprennent l'incorporation de d'autres données de géolocalisations (migration) et d'isotopes stables (position trophique et zone d'alimentation) en relation avec les PFAS. De plus, des biomarqueurs moléculaires des gènes du métabolisme des xénobiotiques, de la réponse immunitaire, du stress oxydatif et de la voie des hormones thyroïdiennes seront développés pour évaluer la réponse toxique des femelles à l'exposition aux PFAS.

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### Investigating of legacy and unknown PFAS in an otter food web in The Netherlands, **Ling Chen** (WUR)

The widespread use of Per- and polyfluoroalkyl substances (PFAS) has led to their ubiquity in the environment. Most of these chemicals currently do not have accurate analysis and detection methods. The updated guidelines heighten the necessity of developing ultrasensitive detection methods. Therefore, it is urgent to develop new analytical methods. CIC (combustion ion chromatography) is a good way to capture the total organic fluorine, which could provide a more comprehensive measurement of PFAS contamination. HRMS (high-resolution mass spectrometry) can be used to identify unknown PFAS. LC-MS/MS method could give more detailed and quantitative information on individual PFAS, currently we aim to develop and validate a novel analytical method for the simultaneous identification and quantification of 56 anionic and neutral PFAS. By changing the mobile phase and adjusting the pH to 8.0, we may achieve higher sensitivity. The combination of these methods can estimate the fraction of unidentified organic fluorine and create a complete picture of PFAS. Since the vast majority (over 95%) of PFAS are released into the water, we focus in the current proposal on fish and their predator (Lutra lutra) in the aquatic environment. This research focuses not only on the development of instrument-based analytical methods but also on the investigation of the PFAS accumulation patterns in otter food. Derived from this, we will not only assess potential risks for otters, but may also be able to provide insights into risks for humans on a fish diet.

### Investigating the toxicity effects of tire crumb particles and its leached chemicals on Daphnia magna, **Ana Carrazco Quevedo** (McGill)

Crumb rubber is widely used in urban landscaping, playgrounds, sports fields, and as an additive in asphalt for road construction. Over time, environmental factors (such as weathering) and normal usage can lead to the release and accumulation of particles in the environment, especially during rainfall, when runoff carries these particles into storm drains and waterways. Additionally, this process may facilitate the leaching of harmful contaminants, including heavy metals, volatile organic compounds, and polycyclic aromatic hydrocarbons, which pose significant threats to aquatic ecosystems. We investigated the effects of crumb rubber particles on the well-known aquatic model organism, Daphnia magna. Through a series of filtration steps and dialysis, we separated crumb rubber debris into three fractions: Fraction 1 (chemicals + particles), Fraction 2 (chemicals), and Fraction 3 (particles). The acute toxicological effects of each fraction were tested on D. magna neonates (<24 h old) over 24 and 48 h of exposure. Neonate survival was recorded, along with molecular responses such as total reactive oxygen species (ROS) production and lactate dehydrogenase (LDH) activity. Additionally, the internalization of the particles and disruptions in swimming behavior were analyzed. The production of ROS exhibited the highest activity in F1, followed by F2, and then F3 at 24 h. A similar pattern was observed in cell membrane damage (LDH assay), with F1 showing the greatest effect, followed by F2 and F3 at this time point. However, both LDH and ROS activities slightly decreased after 48 h compared to 24 h. Exposure to the different fractions also disrupted the swimming behavior of the D. magna neonates. Overall, these results suggest that the neonates were able to mitigate some of the damage caused by the fractions, activating protective mechanisms to cope with the adverse effects after 48 h. These preliminary steps in evaluating the impact of crumb rubber components on aquatic organisms offer valuable insights into the potential risks posed by these contaminants to aquatic ecosystems.

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All organisms have their limit: Using toxicodynamic modelling to identify main drivers of species differences in sensitivity to pesticides, **Nick van Sabben** (WUR)

In industrialized nations, food production depends on plant protection products (PPPs), which protect crops but can harm non-target species. Toxicity testing of PPPs is currently limited to a few species, and no universal method exists to extrapolate findings across diverse species. Safety factors are used to take species sensitivity differences into account, but they serve as precautionary rather than predictive tools. Some organism traits, such as size, partially explain differences in sensitivity between species. However, sub-organismal processes are less thoroughly studied. These processes include those important for what the chemical does to the organism (toxicodynamics). We investigate the toxicodynamic processes utilizing the Adverse Outcome Pathway (AOP) framework. We assess whether the molecular initiating events and key events consistently correlate with adverse outcomes across species. This data is integrated into a Toxicokinetic-Toxicodynamic (TKTD) model to estimate the rates at which key events progress over time. Observed interspecies differences in these rates will help identify underlying biochemical or genetic traits responsible for sensitivity variability. To populate the models with detailed and high-quality data, we now perform initial toxicity experiments to create a species sensitivity distribution for a carbamate pesticide, focusing on six aquatic arthropod species. We employ a General Unified Threshold model of Survival (GUTS) to quantify hazard rate, damage level, threshold damage, uptake rate constants and elimination rate constant. In the presentation I will illustrate the differences in these model parameters, the results from assays examining toxicodynamic differences and what these tell us about processes important for interspecies differences in sensitivity.

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### A PBK model-based QIVIVE approach for organophosphate pesticide risk assessment , **Thijs Moerenhout** (WUR)

As part of the Virtual Human Platform for Safety project (VHP4Safety), in vitro and in silico approaches for chemical risk assessment are developed to predict acute and chronic neurotoxicity of organophosphate (OP) pesticides in humans. OP pesticides act by blocking acetylcholinesterase (AChE) enzymes, causing overstimulation in cholinergic neuronal connections in both pests and non-target species. With the aim of investigating OP induced neurotoxicity in humans according to 3R principles, PBK models were developed to facilitate quantitative in vitro to in vivo extrapolation (QIVIVE). Metabolism was characterized in in vitro studies to describe these processes accordingly in the PBK model. Furthermore, validation of model accuracy was performed by developing a model for rats and comparing to in vivo data from literature. For 5/8 OP pesticides tested, the model predicted internal concentrations mostly within a 2-fold difference, while the other three seemed to be part of a subclass of OP pesticides, which is metabolized via a different metabolic process. Lastly, the potency of multiple OP pesticides towards AChE inhibition was determined and translated to the in vivo situation using QIVIVE to predict doseresponse curves, which in turn are used for benchmark dose modelling and further risk assessment.

### Using stream algal biofilms to monitor pesticide contamination, **Laura Malbezin** (INRS)

Traditionally, pesticide monitoring is based on punctual water measurements, which may not always represent bioavailable concentrations affecting the biota. In addition, concentrations of pesticides in the water are highly variable over time. While their application in contaminant biomonitoring remains limited, river biofilms (consortia of microorganisms at the basis of aquatic and terrestrial food webs) provide meaningful insights on the health of aquatic ecosystems and bring interesting complementary information to existing monitoring tools.

The objectives of this project were: (1) to determine whether biofilms from agricultural streams (Quebec, Canada) have accumulated pesticides from surface water, and (2) to explore the relationship between pesticide concentrations in surface water and accumulated concentrations in biofilms, with the aim of using biofilms as indicators of pesticide exposure. Concurrently, we aimed to (3) analyze microbial communities from stream biofilms in areas of "moderate" and "intense" agricultural pressure and to determine the extent to which this pressure can modulate their structure.

Biofilm samples were collected at 26 sites over a five-year period in streams included in the Quebec government monitoring program. (1) Analyses of pesticide concentrations in biofilms using HPLC-MS/MS revealed the accumulation of several pesticides, particularly atrazine, S-metolachlor, and chlorantraniliprole. (2) A comparison of the concentrations of these compounds in the water and in the biofilm revealed a linear relationship. Finally, the metabarcoding results of rRNA genes 16S (prokaryotic), the 18S (eukaryotic algae), ITS (fungi) and CO1 (metazoan) showed a difference in community structure between areas with "moderate" and "intense" agricultural pressure, reflecting a combination of spatial and local environmental influence of agricultural activities.

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### Marine oil toxicity: Can the humble cockle survive?, **Edith Etor** (WUR)

Several marine species live in the intertidal zone of the marine ecosystem. These species are vulnerable to tidal stressors and potentially crude oil spills from oil transport. After a spill, crude oil, a complex component combination, will weather due to environmental conditions including temperature, water currents, and tidal streams, which may change the composition of the fresh oil. Light and heavy poly cyclic aromatic hydrocarbons (PAHs) are key to the complex composition of oil. At high tide, the intertidal zone is flooded bringing plankton and debris with relatively cold, nutrient-rich saltwater, while at low tide organisms may be exposed to extreme temperature and other stressors and may not be able to feed in this period. Water-soluble lightweight PAH components also known as water accommodated fractions (WAFs) are accessible to intertidal sentinel species via filter feeding, which may act as a stressor on the organisms as well. Tidal movement may also affect filter feeders as they stay submerged under the flood and have more time to siphon the WAFs. Unfortunately, there is not much information about the toxicity of WAFs of crude oil to marine invertebrates in synergy with the tide. Therefore, this study identifies and characterizes the mixtures in the WAFs of fresh and weathered crude oil exposed to a model invertebrate, Cerestoderma edule. In the presentation, impacts on sublethal and biomarker effects of WAFs exposed to C. edule will be illustrated, and related to tidal and stagnant conditions.

### Accumulation of radium 226 and its effect on the growth of the unicellular green alga Chlamydomonas reinhardtii, **Flavie Desreac** (INRS)

Canada is one of the world's leading producers of uranium, a radioisotope that can decay to radium 226 (226-Ra). Uranium has been found to occur naturally in many tailings from other mining activities, which can lead to the release of 226-Ra into natural surface waters. However, knowledge of the toxicity of 226-Ra to aquatic organisms remains limited. Furthermore, in order to assess the potential risks of radium transfer to aquatic food webs, it is essential to study its bioaccumulation in phytoplankton, which is the basis of these webs. Radium toxicity and bioaccumulation were therefore studied in the unicellular green alga Chlamydomonas reinhardtii. To determine the effects of 226-Ra, microalgae were exposed to different doses of Ra-226 ranging from 0 to 150Bq/L for 72 hours, in MHSM-1 medium at pH 7.0. Exposures to 150 Bq/L were also conducted for subcellular distribution and proteomics analyses, to determine the localization of radium in the cell and to potentially identify which proteins bind radium. The results obtained demonstrate a growth inhibition, with the effective concentration threshold of 76.57 ± 9.40 (EC20), and an accumulation of radium in organelles and the heat stable peptides and proteins fraction of the cytosol. Subsequent to this study, subcellular distribution will be carried out on a second algal species, Chlorella fusca, which, according to bioaccumulation and toxicity results, appears to be more resistant to 226-Ra.

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### Urban stressors on avian immune sefenses: Impact of pollution on viral challenge via polyI:C in great tit and blue tit nestlings, **Shivani Ronanki** (WUR)

Urbanization alters landscapes, increases emissions, and disrupts habitats. This affects biodiversity, especially birds adapted to city life, where chemical exposure is an indirect outcome. Urban areas have elevated air pollution via ozone, metals, nitrogen oxides, and PMs, exposure to which induces oxidative stress, disrupting cellular balance. The innate and acquired immune systems, regulated by antioxidant systems, are susceptible to oxidative stress. While studies often report baseline immune impacts in urban birds, few employ standard approaches like immune challenges. Given rising avian influenza rates, understanding how pollutants affect birds' ability to establish anti-viral responses is crucial. To address this, a field experiment was conducted on great tits (Parus major) and blue tits (Cyanistes caeruleus) nestlings in polluted and clean sites in the Netherlands. Passive NO samplers were and on Day 13 post hatch, four chicks within each box were selected, with two receiving a Poly I:C injection, and two saline as control. Two days later, a blood sample was extracted, and immune parameters, including haptoglobin(acute phaseprotein), immune cell counts, and Poly I:C specific gene expression (TLR3, RIG-1, IL-6, & IRF-7) were quantified. Preliminary results suggest atmospheric NO levels were four times higher in polluted sites than in clean sites and positively correlated with haptoglobin. However, when challenged with Poly I:C, nestlings in clean sites exhibited a stronger immune response, with increased haptoglobin and immune cells compared to nestlings in urban sites. Birds in polluted sites show a proinflammatory state at baseline but struggle to mount an effective immune response during infections.

### Toxicity of radium-226 on early life stages of Lymnaea stagnalis, **Léna Guimard** (INRS)

In Canada, mining can lead to the contamination of surface waters by natural radioelements. Among these, radium-226 (226Ra) is of particular concern because of its long half-life (1600 years), its potential for bioaccumulation in organisms and its radiotoxicity. To better understand the effects of this radioelement on aquatic organisms, we examine the toxicity and bioaccumulation of 226Ra in Lymnaea stagnalis, a pulmonate snail commonly found in Canadian freshwater. It is expected that this calcifying organism will bioaccumulate 226Ra and that this accumulation will cause toxicity. To test these two hypotheses, we carried out two chronic toxicity tests with early life stages of the great pond snail: a 28-day test with juveniles (0 - 28 days post-hatching) and a 52-day test on two early life stages (< 24 h embryos - 40 d post-hatching juveniles). Our results suggest that 226Ra increases mortality and the activity of an antioxidant enzyme (glutathione peroxidase) in juvenile snails exposed to ≥ 50 Bq/L of 226Ra from the embryonic stage. Exposure to 226Ra had no significant effect on the growth of embryos and juvenile snails, but the heart rate of embryos increased from 0.1 Bq/L of 226Ra. This study indicates that $^{226}$Ra can induce a physiological stress in L. stagnalis, but not at activity concentrations found in the most contaminated natural waters in Canada (<1 Bq/L).

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