Abstract Book

Low Dose Radiation Risks: Present Research & Future Perspectives

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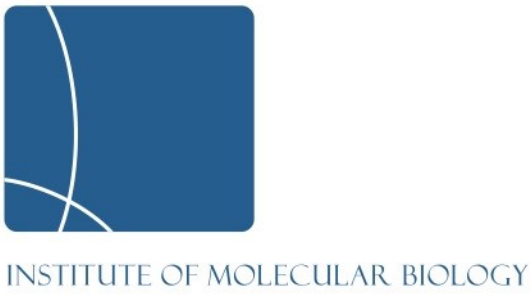

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Session 1 *– Low-dose radiobiology and environmental toxicology background*

Low Dose Radiobiology – an overview

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We have known for over 30 years that there are mechanisms occurring in the low radiation dose range, that are different to those seen following high dose exposure. These include bystander effects and genomic instability which are triggered by very low acute doses and appear to be stress *responses* rather than direct mechanical effects of radiation interactions with biological material. Because what we measure after low dose exposure is a response, it does not necessarily obey a linear dose effect relationship such as that assumed by the LNT model. This means that extrapolation from high to low dose does not "work" in the sense of permitting accurate estimation of harm from low doses exposure. The evidence for this has been published and extensively reviewed by multiple authors over the last 30 years. Critically, responses to low dose exposures appear to operate at the population level, involving signalling and communication which modulate and presumably optimise, the response. This area is where environmental radiation protection and human radiation protection goals diverge and where social and ethical issues come into to the debates about radiation protection. Human radiation protection seeks to "protect" all individuals with cancer being the main outcome we are seeking to prevent. However the environmental protection goal is aimed at protecting populations and ecosystems, meaning that low dose population-based responses and transgenerational impacts such as those associated with genomic instability or bystander effects are critical to understand and include in protection frameworks. Chronic irradiation also has different mechanisms and effects. Radiation protection frameworks currently deal with chronic doses by assuming a "weighting factor" or "scaling factor" to account for the sparing effect of protracted doses versus the same dose received in one go. Experimental approaches to attempt to determine the appropriate scaling factor usually use relatively high fractionated doses (which are not the same as a chronic low dose) and show sparing factors of about 2. The only field data which can inform us come from Chornobyl data obtained in the first year, accidents or deliberate releases in the former Soviet Union, or data from the experimental site at Colorado, or test sites in the US. Human data are very scarce and often anecdotal, coming mainly from high background areas such as Ramsar and Kerala but since these data come from adapted populations, where there is poverty and where medical care may not be ideal, it is difficult to draw conclusions about the impacts of low chronic versus acute doses in humans. The ecological data are complicated by multiple stressor issues, adaptive effects the lack of ecosystem level markers of effect. This last issue means that at best, measurements of effects are based on measurements of impacts on reproduction of individuals in what we decide are key stone species within populations. This hardly captures the complexity of ecosystem interactions but at present, the tools are just not there to do better.

Radiation impact to ecosystems at low doses, issues and challenges ahead

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What are the effects from low dose of radiation still is a debated question which has traditionally been focused on DNA, thought to be the major relevant target within cells and their assembly within individual organisms. In the purpose of risk assessments, the effects from high doses which are today fairly understood are simply extrapolated to low doses according to an assumption that is referred to as the Linear No Threshold Theory (LNT). A number of experimental results however do challenge this theory. When one moves to understanding the potential effects from radiation on ecosystems, the relevant units of the environment, risk assessments are currently also being driven through an extrapolation, from known effects described on individual organisms to potential effects on populations and ecosystems. Here again, a series of experiments and observations do question this extrapolation strategy. It is argued that incorporating ecosystem understanding, such as structure and functioning, is a prerequisite to adequately address ecological risk assessments of low doses impact on ecosystems. When doing so, new issues and challenges are revealed and will be discussed. Low doses drive automatically to a multi-pollution context where radiation forms but one of many potential stressors. Ecomarkers would then need to be considered in the context of ecosystems behaving as integrators of multiple stress. The resilience status of ecosystems might be the largest driver of their behavior upon stress, puzzling what could be the effects from low doses. Especially at low doses where effects are expected to be small by definition, impact would not be essentially driven by dose itself, but radiation would act as a triggering mechanism leading to potential major unbalances. This is why responses at this level of organization are no longer linear, a small stress being capable of producing a large effect. Furthermore, ecosystem response to radiation may not be driven by dose proper, but rather by differences in radiosensitivity between species, ultimately causing unbalances.

Multiple stressor exposures

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Living organisms are exposed to a myriad of physical, chemical and biological factors that can change their normal functioning and well-being, by introducing stress reactions. Anthropogenic activities such as nuclear activities are also increasing the variety and intensity of stressors that potentially are acting in concert. Thus, environmental contaminants such as radionuclides, trace metals as well as organics rarely occur alone, and organisms in mixed contaminated areas are exposed to a cocktail of stressors. i.e. multiple stressors.

Following multiple stressor interactions, production of free radicals and recombination may result in reactive oxygen substances (ROS), damaging membrane integrity, degrading key biomolecules and thereby inducing genetic effects such as DNA/RNA damage. Thus, a series of downstream responses may occur which potentially could lead to umbrella biological endpoints such as reproduction and immune system failure, genetic instability and mutation, as well as morbidity and mortality.

When stressors are acting in concert, combined effects can be additive (equal to the sum of individual stressors, (1+1=2), antagonistic (less than additive, (1+1=0)), or synergistic (more than additive, 1+1= 3or 4). Mixed exposure could therefore significantly influence ecological impact and risk assessments. However, all effects may not be negative as DNA repair can be stimulated, and stress tolerance and adaptation can be developed. Therefore, information on uptake, accumulation, dose-response relationships (on/off mechanisms), sensitivity (detection limits/thresholds), and effect responses at molecular and individual levels and furthermore to population and ecosystem levels are essential.

Multiple stressor interactions are described according to the applied null model. Without interactions between stressors, combined effects can be predicted based on single-stressor effects. The assumptions of concentration addition (CA) are utilized for stressors having similar modes of action, while effect addition (EA) is utilized for stressors with different modes of action. These models have been applied to predict additive effects of multiple stressors, while deviation from null models would indicate synergism or antagonism.

The paper will discuss problems associated with assessing, predicting, and managing combined effects induced from multiple natural and anthropogenic stressors, still being a challenge, not only in science but also for regulatory purposes.

Low dose and low-dose-rate studies of neutrons and gamma rays on cancer in cadence in large-scale animal studies

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US Department of Energy conducted a series of large-scale animal studies during the cold war era using internal emitters and external beam exposures of dogs (24,000), rats (30,000), mice (50.000) and other species to understand short- and long-term effects of radiation. The databases and preserved tissues reside in the Woloschak lab at Northwestern University as part of the Northwestern University Radiobiology Archive. The tissues and datasets can be used to explore new questions about radiation effects using technologies that were not available at the time these experiments were originally performed. Recently work includes new statistical analyses of animal datasets to define low-dose-rate effects using both externally exposed and internally exposed animals. In addition, analysis of dogs exposed to internal emitters via different routes (injection, inhalation) using X-ray fluorescence microscopy has permitted the identification of radionuclides and daughter products in different tissues and tissue sublocations in the animals. Details of this work will be shown.

Environmental Toxicology and Transgenerational Effects in Non-Human Species

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Introduction: Species that inhabit radio-contaminated regions can be exposed to ionizing radiation over the course of multiple generations. To date, the systems of radiological protection do not quantify or consider the possibility for effects that may manifest subsequent generations following the initial exposure. A Task Group (TG121) of the International Commission on Radiological Protection (ICRP) was launched to study the effects of ionizing radiation in offspring and next generations.

Objectives: The goal of this paper will be to review the literature on both multi-generational (in which the exposure continues across multiple generations) and trans-generational (in which later generations are not exposed during a recovery period) effects in non-human biota.

Methods: Multiple online databases (Google Scholar, PubMed, Scopus) were searched on the topics of multi- and trans-generational effects of ionizing radiation in non-human species. Both laboratory-controlled experiments and field studies (mainly inhabitants of the Chernobyl Exclusion Zone or Fukushima-Daiichi prefecture) were considered.

Results: Studies were grouped into categories based on the model organism used, which includes species of bacteria, nematodes and annelids (largely *Caenorhabditis elegans*), crustaceans (largely *Daphnia magna*), insects, amphibians, birds, fish, mammals and plants. Effects on altered reproductive parameters were reported multiple different study models. In some studies, decreased offspring survival was reported, however these studies typically involved chronic, persistent exposure of numerous generations to a radiation field or following very large acute doses in trans-generational studies. There were also studies that reported changes in genetic and epigenetic endpoints, with transmission of epigenetic changes into subsequent generations previously described as a possible mechanism for multi- and trans-generational irradiation effects.

Conclusion: The reported effects in altered reproduction represent an area of potential concern, due to the importance of population and ecosystem structure within ecological radiation protection. This contrasts with human radiation protection, which considers effects at an individual level. Future work of ICRP Task Group 121 will continue to review this literature, with a final ICRP Publication that will be published for the radiation protection community.

Beyond the AOP: causal inference engines in coupled human and natural systems (CHANS) for radioecology

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The Adverse Outcome Pathway (AOP) was originally developed as a regulatory tool to model cause-effect relationships between molecular initiating events (MIEs) and measurable adverse outcomes (AOs) through a series of key events (KEs) linked by key event relationships (KERs). AOPs are structured symbolic graphs constructed based on experimental and observational studies, with plausibility assessed using modified Bradford-Hill criteria, such as temporality, consistency, and dose-response relationships. Expert domain knowledge is essential in constructing and evaluating these pathways.

Recently, quantitative AOPs have emerged, where causative edges are mathematically defined, but there is no agreed-upon strategy for their development. Challenges include uncertainties in causative edges, confounding factors, data inconsistencies, and integrating different data types. Despite these challenges, AOPs have significant advantages due to their modular structure, allowing the creation of interlocking and branching AOPs. This has made them particularly useful in radioecology, where they model the causative chains linking radiation exposure to ecological impacts.

AOPs can help infer long-term population-level effects from molecular and cellular responses by systematically identifying key biological events. However, their structure depends on available knowledge, limiting the ability to include unknown confounders. Additionally, AOPs lack a formal mathematical framework to establish causality.

The concept of causation has been debated philosophically and mathematically. The goal is to create a framework where causality can be definitively established, aligning with David Hume's classical definition that when one object or event is consistently followed by another it may be deemed a cause. Achieving reliable assertions of causality are critical. It is only when we can do this that we can gain predictive power from our studies and only then that we can justifiably use that predictive power to drive policy.

Judea Pearl's foundational work on causal inference introduced causal Bayesian networks as a more rigorous approach to causality. Unlike traditional statistical models that rely on correlation, causal inference methods construct graphical representations of causal relationships, where domain experts define causative edges. A key innovation is the do-operator, which simulates interventions by manipulating variables in the network. Through do-calculus, these methods can analyze confounding factors, distinguish between mediators and confounders, and explore counterfactual scenarios. Unlike AOPs, causal inference engines can mathematically prove causality and generate numerical predictions from validated models. They also allow the integration of existing biological knowledge, such as metabolic pathways and protein interaction networks, into causal frameworks.

Causal inference methods are well-suited for radioecology due to their flexibility in handling complex systems, such as ecospheres. Traditional causation approaches assume that factors influencing, for example, radiation dose rates as an initiating factor do not independently affect the outcome, and that species interactions are unaffected by radiation exposure to other populations. In real-world ecological contexts, particularly in coupled human and natural systems (CHANS), these assumptions do not hold. This makes it particularly difficult to address confounders when trying to link radiation exposure to adverse ecological effects.

Applying causal inference to radioecology provides a rigorous framework for establishing causal links, particularly in situations where historical data is lacking, such as near newly established small modular reactors (SMRs). This method can guide targeted data collection, improve AOP development, and enhance the reliability of causal inference in environmental assessments.

Enhancing radiation risk assessment with 3D culture models

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Understanding the biological effects of ionizing radiation is essential for accurate risk assessment in medical, occupational, and environmental contexts. Compared to traditional 2D cultures, three-dimensional (3D) cell culture models provide a more physiologically relevant platform by better replicating cell-cell interactions, extracellular matrix composition, and dynamic responses. While 3D models have demonstrated superior performance across various biomedical fields, their application in radiobiology is still in its early stages. So, advancing 3D in vitro systems is crucial for improving the predictive accuracy of radiation-induced biological effects. This study aimed to conduct a comparative analysis of ultrashort pulsed electron beam (UPEB) and X-ray irradiation in human 3D cell culture models. Cell viability and reactive oxygen species (ROS) levels were assessed in human cancerous and healthy 2D and 3D in vitro cell models following irradiation at varying doses. Viability was evaluated using Calcein-AM staining and quantified via the Cytation C10 confocal imaging system. ROS generation was analyzed using CDFDA staining and detected through two-photon microscopy. It was shown that UPEB irradiation leads to a steeper dose-response curve, with fewer surviving cells at equivalent doses compared to X-rays. In 3D cultures, X-rays exhibit a differential effect, with proliferative outer layers being more radiosensitive and hypoxic deeper layers showing greater radioresistance. UPEB irradiation induced a higher extent of cell death in 3D cultures compared to X-rays, whereas X-ray-induced cell death exhibits greater dependence on oxygen availability. Cells grown in 2D cultures were more sensitive to both types of irradiation than those in 3D cultures. This difference was most pronounced at lower doses of irradiation, while at higher doses, it became less significant. These findings underscore the importance of 3D cell culture models in radiation risk assessment. The differences between 2D and 3D models being most pronounced at low doses, emphasizing the critical role of the modeling system in low-dose irradiation studies.

Session 2 - *Low-dose effect mechanisms*

Toxic, but not cytotoxic nor genotoxic - Low-Dose ionizing radiation can trigger senescence and loss of multi-potency in adult stem cells.

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The most prominent and well studied cellular effects of ionizing radiation are those that are irreversible (like changes in DNA, heritable mutations) or develop relatively fast after exposure (like apoptosis or clonogenic cell death). Changes in the epigenetic program of cells are less well understoud, not least because they depend on the cell type, metabolic state and also because they are very dynamic and can even be reversed.

Low doses or chronic LDR experiments not only on cells, but also on entire organisms frequently show contradictory results, depending on the tissue type or the biological endpoint. Usually, effects like the above mentioned cytotoxic or genotoxic changes are almost impossible to dissect from experimental noise.

We have argued before (1) that adult stem cells due to their extremely long persistens in the organism have the capacity to accumulate LDR radiation exposure, without being affected by cell death or mutations. We show here results of studies on mesenchymal stem cells (MSC) and adipose derived stem cells (ADSC) treated by low-dose X-rays (0.2 Gy - 2 Gy). We found these types of adult stem cells show signs of premature ageing and senescence (2). Whole transcriptom profiling using RNA-sequencing indicates that genetic pathways of WNT (stem cell potency) and Bmi1 (self-renewal, immortality) are affected (3). Only higher X-ray doses (>2Gy) are able to induce autophagy in those cells, a cellular defense to remove proteins and lipids which are damaged by ROS.

Since MSCs are the main source for the regeneration of connective tissue and bone marrow stroma, and also play important roles during wound healing, smooth muscle maintenance around blood vessels or even formation of the myelin sheath arouind peripheral nerves, such long-term detrimental effects might impair long-term organ or tissue regeneration in higher organisms.

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An Issue for Late Health Effects? In: Genetics, Evolution and Radiation,V.L. Korogodina et al. (eds.),St.Petersburg 2016, DOI 10.1007/978-3-319-48838-7_32

(2) Yentrapalli R et al, The PI3K/Akt/mTOR pathway is implicated in the premature senescence of primary human endothelial cells exposed to chronic radiation. PLoS One. 2013 Aug 1;8(8):e70024

(3) Schuster M et al, In vitro cellular and proteome assays identify Wnt pathway and CDKN2A-regulated senescence affected in mesenchymal stem cells from mice after a chronic LD gamma irradiation in utero. Radiat Environ Biophys. 2021 Aug;60(3):397-410.

How low dose ionizing radiation engages immune networks

Dörthe Schaue

Radiation signals “danger” that the immune system responds to in many ways. The most ancient and primitive way is through innate, primarily myeloid cells that are the first line of defense protecting hosts against microbial, physical, and chemical invasion. The pathways that are engaged are evolutionarily conserved but remained relatively obscure until the 1990’s when mutations in pattern recognition receptors (PRR) revealed their critical role in sensing pathogen-associated molecular patterns (PAMPS) and more recently also damage-associated molecular patterns (DAMPs). All DAMPs and PAMPs have in common the ability to generate free radicals in tissues and all converge to stimulate potent pro-inflammatory, self-amplifying cytokine signaling networks. The result is a sustained pro-oxidant response through induction of NF-kB and other transcription factors associated with inflammation combined with oxidative stress from mitochondrial damage, NADPH-oxidase activation, ER stress, and other endogenous sources of reactive oxygen intermediates. Anti-oxidant, anti-inflammatory counter-responses can be generated in an attempt to limit tissue damage and promote tissue healing. Ionizing radiation (IR) can drive these same canonical pathways to culminate in normal tissue radiation effects, with perhaps greater emphasis on recognizing DNA damage than some other stimuli. The chronicity of radiation effects in atomic bomb survivors certainly indicates the long-term persistence of such inflammatory networks. Danger signaling additionally provides the impetus for myeloid cells to mature into specialized antigen presenting cells that become competent at presenting antigenic fragments to B cell and T cell receptors on lymphocytes, essentially engaging the adaptive immune arm in a concerted response. IR can therefore in certain circumstances not only activate innate but also adaptive immunity. The complex interplay between opposing pro- and anti-inflammatory forces and potential involvement of adaptive immunity will ultimately dictate the immune effector mechanisms underlying short and long-term radiation effects, including cell death, regeneration, fibrosis, scarring and risk of carcinogenesis. It is reasonable to assume that different thresholds exist for different radiation-induced effects. As dose increases, different, perhaps more “complete”, pathways kick-in to contribute and there may be a semblance of dose linearity or even non-linearity. Clearly, understanding how the immune rheostat might be set by low dose radiation, the importance of radiation dose, dose rate, and quality, as well as the impact of collateral signals in the context of danger or non-danger signaling is relevant to many life shortening and carcinogenetic events.

Modelling the Effects of Low-Dose Radiation: Studying Its Implications in Clinical Radiotherapy and Radiation Protection

Gibin Powathil

Traditionally, mathematical models of radiation effects focus on estimating cell death due to direct irradiation, often overlooking other direct and indirect effects. However, advances in molecular biology have broadened this perspective, revealing that signals from irradiated cells can also affect non-irradiated cells or organisms in various ways, especially when they are exposed to low-dose radiation. This expanded understanding has significant implications across multiple fields, warranting further experimental and mathematical exploration. Here, we will discuss a hybrid multiscale mathematical model that examines both the direct and indirect effects of radiation, with applications in clinical radiotherapy, experimental research, and radiation protection.

Approaches to developing holistic models in radioecology

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The issue of determining likely outcomes after low dose exposure to radiation is complex and controversial. Currently the Linear-non-Threshold (LNT) model is used to justify the linear extrapolation of (adverse) outcomes from high doses, where effects are clearly seen, to low doses where effects are very difficult to detect and even more difficult to ascribe to the measured radiation exposure. Among the factors hindering the development of a more precise system are the lack of reliable predictors of system health. While biomarkers indicating health of individual cells or organisms exist, they fail at low doses due to the complexity of cause effect relationships and the multiple factors contributing "stress" to the system as a whole, (whether "whole" is a whole organism, a population or an ecosystem). Approaches to capture this complexity include Adverse Outcome Pathway (AOP) analysis which looks at multiple levels of organisation from gene to ecosystem. In this review we examine the role of non-targeted effects (NTE) such as genomic instability (GI) and bystander effects (BE). These mechanisms involve transmission of information between different levels of organisation. In the case of BE signals from exposed to unexposed cells or organisms coordinate response at higher levels of organisation permitting population responses to radiation to be identified and, potentially, mitigated. GI is more complex as it involves not only signalling but also trans-generational transmission of genetic or epigenetic changes and may lead to long-term adaptive evolution. GI may also be involved in memory or legacy effects, which contribute a further component to the dose effect measured in legacy sites. Our recent analysis of the contributions of memory and legacy effects to the total effect using data sets from Chernobyl and Fukushima (voles, birds and butterflies) suggest this type of analysis may help reduce uncertainties over laboratory to field extrapolations. A focus on novel but widespread NTE mechanistic pathways may open the way to successful prophylaxis and development of new biomarkers for better risk assessment after low dose exposures.

Session 3 – *Multiple Stressors and markers*

Time to x LNT and base radiation protection regulations on science

Marek K. Janiak

Misinterpretation and omission of data compounded by the (undeserved) Nobel prize afforded to Joseph Hermann Muller led in the late 1950s to formulation of the unscientific LNT hypothesis which became and still is the basis for calculations of cancer risk associated with exposures to ionizing radiation.

Since the renowned statement by Lauriston Taylor, the founding chairman of the NCRP, who in 1980 pointed out that we had had enough data to claim that exposure at a dose rate of 2 mGy per day (i.e., about 730 mGy per year) was harmless, many new data have been amassed to confirm the safety of irradiations at low doses and dose rates. Regardless, the standing regulations keep obliging us to „protect" people from exposures at extra 1 mGy/year (general public) and extra 20 mGy/year (staff exposed occupationally).

No wonder therefore that such stringent regulations lead to very high costs of production and employment of ionizing radiation in many areas of human activity (including saving lives) and also to many absurdities and even tragic consequences in everyday life.

Hence, it is high time to base the rules of radiation protection on solid scientific data which have repeatedly demonstarted that exposures to low-level ionizing radiation is not only harmless, but in many instances protect against the growth of cancer, improve our general condition and prolong life. We, therefore, have to dispose of the LNT hypothesis and replace it with the threshold or hormetic models of the dose-reponse relationship.

Radiation in the City: Evaluating Environmental Radioactivity and Public Health Risks

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Introduction: Urban environment, especially soil, undergoes substantial alterations. Consequently, the most effective approach for assessing human exposure to naturally occurring radionuclides and the most abundant artificial ^{137}Cs in urban areas is soil geochemical survey.

The objective of this study was to investigate the spatial pattern of naturally occurring ^{226}Ra, ^{232}Th, ^{40}K, ^{222}Rn and artificial ^{137}Cs in urban environment of major Armenian cities: Yerevan, Gyumri, and Vanadzor.

Methods: Soil survey (scale: 1:100000) was implemented in major cities of Armenia. Activity concentration of radionuclides in soil was determined using HPGe-base gamma spectrometry system. Survey of ^{222}Rn in soil gas was carried out in Yerevan, taking into consideration the geological structure of the area.

Results and Conclusion: Among all the cities studied, Yerevan exhibits the highest activities of both natural radionuclides and ^{137}Cs. However, the distribution of natural radionuclides in Yerevan is strongly influenced by the city's geological characteristics, making it distinct among the investigated locations.

In Yerevan, ^{40}K and ^{226}Ra concentrations were highest in the northwest, while ^{232}Th was more elevated in the northeast, suggesting that quarries may serve as a secondary factor in the redistribution of radionuclides. The southern part of Yerevan, which hosts potential anthropogenic sources of radionuclides (Yerevan Thermal Power Plant and molybdenum industrial enterprises) exhibited the lowest activity levels for all natural radionuclides. Soil gas radon activity ranges from 483.4 to 38375 Bq/m^3 in summer period. The highest radon activity was recorded in soils with high sand content (up to 81%) derived from sedimentary rocks (alluvial, proluvial sediments and sandstones). Activity of radon in soil gas correlated negatively with ^{226}Ra and ^{232}Th in soil. Th/Ra ratios varied from 0.8 to 1.51.

In Gyumri, the highest activity levels for natural radionuclides were recorded in the western region, where black tuff reserves and quarries are present. Notably, Gyumri also had the highest ^{40}K concentrations among the studied cities.

Vanadzor exhibited the lowest activity levels of natural radionuclides among all the research cities.

The annual effective dose equivalent and excess lifetime cancer risk largely reflect the spatial distribution of natural radionuclides, highlighting their significant contribution to overall dose and risk formation.

Genotoxic effects of low LET irradiation by electron pulses in human cells

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Genotoxic effects caused by accelerated electrons as a source of low LET radiation present a promising approach in radiation oncology. For their induction, we applied the AREAL accelerator (Armenia), which produces electron pulses generated from a photocathode by a UV laser and accelerated using a high-gradient RF resonator, thus leading to precisely formed beam parameters. It provides high stability and reproducibility of electron beam and radiation characteristics, shorter pulse duration (sub-ps vs. ps), and a higher ultra-high pulse dose rate (up to 10^7 Gy/sec). To evaluate the genetic instability in human cells induced by radiation, we used the following DNA damage markers:

- DNA damage by the DNA-comet assay: Estimates DNA damage and repair after irradiation with various durations and doses of accelerated electrons that lead to isolated DNA lesions, including single-strand and double-strand breaks of DNA. The application of electron irradiation allows for the description of primary radiobiological effects. Therefore, the detected radiation-induced DNA damage is primarily caused by initial lesions that form at the moment of irradiation.
- DNA damage by FOCI: Dynamic microstructures are formed during the recognition and repair of double-strand breaks. They contain hundreds to thousands of copies of various proteins involved in these processes. The quantitative analysis of the foci of repair proteins and their localization/colocalization in the post-radiation period allows determining not only the number of DNA breaks and their spatial distribution in the cell nucleus, but also the efficiency and mechanisms of their repair.
- Micronuclei induction: Compared to X-rays, electron irradiation exhibits a slight increase in micronuclei formation alongside a strong induction of apoptosis. This effect can be attributed to the elimination of cells with highly damaged DNA through apoptosis rather than cytogenetic disturbance, observed only after electron irradiation, suggesting its greater potential for radiation therapy.
- DNA copy number variations: Persist for several days post-exposure and are useful for characterizing the genetic effects of accelerated electrons.
- Other biomarkers of genomic instability: e.g., telomeres shortening.

The results obtained will help to select the strategy for further research to understand the possibilities of applying ultrashort pulsed electron beam radiation in cancer therapy.

Long-Term Pathway Activation in Cardiac Ventricular Tissues Following Low-Dose Gamma and simGCRsim Irradiation

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Space irradiation (SIR) represents a significant health risk for deep-space exploration, yet its long-term effects on cardiovascular function remain poorly understood. While previous studies have highlighted transcriptional changes in left ventricular (LV) and right ventricular (RV) tissues post-irradiation, a systems-level understanding of pathway activity deregulation is lacking. To address this gap we applied the Pathway Signal Flow (PSF) algorithm to analyze long-term pathway activity alterations in LV and RV tissues of C57Bl/6J mice exposed to Gamma irradiation (100 cGy 137Cs-γ) or simulated Galactic Cosmic Ray (simGCRsim, 50 cGy 500 MeV/n) irradiation (IR). RNA sequencing data were analyzed to assess pathway activity changes, sex-specific effects, and ventricular differences 440 days post-irradiation. We observed marked sex- and ventricle-specific differences in pathway deregulation. LV tissues in females exhibited broad signaling pathway alterations after simGCRsim exposure, particularly in immune response, cytoskeletal remodeling, and survival-related pathways (e.g., NF-κB, VEGF, and MAPK). In contrast, male RV tissues demonstrated higher pathway deregulation than LV, particularly in PPAR, NF-κB, and HIF-1 pathways, implicating metabolic disruption and survival adaptations. SimGCRsim induced greater long-term pathway perturbations than Gamma-IR, suggesting persistent stress adaptation mechanisms post-exposure. Our findings suggest that sex-dependent and ventricle-specific signaling alterations contribute to long-term cardiovascular risks following space irradiation. Notably, VEGF and NF-κB signaling emerge as key regulators of cardiac adaptation. Future studies in larger cohorts, incorporating early-stage molecular responses and broader pathway analyses, are needed to refine cardiovascular risk assessments for space travel.

Deciphering molecular mechanisms of plant radiosensitivity

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Plants provide the fundament of ecosystem productivity and health. History has shown that the environmental consequences of ionizing radiation-related incidents affect plant communities most profoundly. A long-withstanding conundrum of radioecology has been the large span in radiosensitivity among different plant species.

In this study we present a systematic investigation of phenotypic and molecular responses in five different plant species including the unicellular green algae *Chlamydomonas reinhardtii*, the aquatic macrophyte *Lemna minor*, the herbaceous species *Arabidopsis thaliana*, and the conifer tree species Norway Spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*).

All species were subjected to a chronic low to medium dose rates of ^{60}Co gamma radiation and we analyzed molecular, cellular and phenotypic responses for each species. We present data on cellular ROS-formation, DNA-damage, photosynthesis effects and gene-expression responses. Compiled data-analysis revealed relatively similar dose-rate dependent DNA-damage, but a huge difference in initiation of repair mechanisms. Radioresistant species modulated a comprehensive response at low exposure dose-rates thus preventing cell loss, while radiosensitive species modulated defenses at higher exposure levels where cell death and mortality were evident. This suggests that resistant plant species are more poised to rapidly respond to ionizing radiation induced stress, while radiosensitive species do not vigilantly protect DNA and cell integrity.

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Session 4 *– Modelling approaches and novel tools*

On the possibility of using physical methods in the tasks of bioindication of small doses of radiation

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The task of bioindication of small doses of radiation has a number of difficulties due to the specificity of the individual nature of the manifestation of the biological consequences of the damaging effect of ionizing energy at the supracellular, organelle and higher levels of biological organization. In other words, the effect of ionizing energy in any small amount, while showing the same effect at the molecular and atomic level, biologically shows different qualitative and quantitative shifts for different organisms in the same region. From this point of view, monitoring the contingent of this region is actually quite a labor-intensive and expensive job when using classical methods of biodosimetry (immunological, genetic, hematomorphological, etc.). This, in turn, complicates the effectiveness of solving the corresponding radioecological problems.

In this regard, it seems advisable to combine the use of physical methods for indicating radiation damage and assessing the severity of radiation exposure in the area of low doses of radiation. In early studies, we found that in the early post-radiation period (after 2 and 3 days), shifts in the transverse dimensions of red blood cells are observed towards microcytosis, after which there is a restoration to normal. For these studies, we developed a laser diffractometric method. It is known that the most sensitive method for recording the dose-effect relationship in the area of low doses of ionizing energy is EPR spectroscopy using spin probes. We are talking about assessing shifts in rheological parameters of blood from the norm based on an assessment of the change in the permeability of the erythrocyte membrane depending on the radiation dose.

The aim of this study is the simultaneous use of the two mentioned physical methods for studying blood from one organism, to establish a correlation of data obtained by the two methods and to assess the possibility of using them to solve prognostic problems.

Delayed effects of chronic radiation exposure on aquatic life across biological organization levels in the chernobyl exclusion zone

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The results obtained over the past 15 years in the most radionuclide-contaminated water bodies of the Chernobyl Exclusion Zone (CEZ) are presented. Representatives of higher aquatic plants, fish and invertebrates were studied at various levels of biosystem organization. The current gradient of radiation dose rate for different species was in range of 0.04-220 μGy/h. Control levels did not exceed 0.05-0.08 μGy/h. A direct relationship has been registered between the absorbed dose rate and the frequency of chromosomal aberrations in cells of root meristems of aquatic plants. It has been established that against the background of a certain stabilization of chromosomal mutagenesis, which is 2-3 times higher than the spontaneous level, an increase in the number of cells with multiple aberrations is observed. The hematopoietic system of fish reacts to chronic radiation exposure by activating compensatory-adaptive processes, which are manifested in changes in the number of leukocytes, leukocyte indices, as well as the redistribution of individual types of cells in the leukogram. At the same time, a significant increase in pathological changes in the structure of the nuclei and cytoplasm of erythrocytes was noted. In juveniles of various fish species of the CEZ an increased level of axial skeleton disorders was detected, localized mainly in the caudal and abdominal parts. The main anomalies were recorded in the form of additional branches of neural and haemal processes, fusion and deformations of vertebrae, curvature of spine (kyphosis, lordosis and scoliosis), as well as rib deformations. Analysis of pollen grains of aquatic plants revealed a significant proportion of sterile grains, as well as changes in their morphometric indicators in the most contaminated reservoirs. Species richness and diversity, as well as indicators of abundance and biomass of benthic invertebrates of the reservoirs of the CEZ are quite low. In general, the assessment of the abundance indicators of all studied water bodies may indicate a depressed state of aquatic invertebrate communities. However, no significant relationship was found between these indicators and the external dose. It is also assumed that the chronic radiation exposure to higher aquatic plants did not cause significant changes in the species and coenotic composition of macrophytes in the studied water bodies. The studies were supported by the National Research Foundation of Ukraine (projects 2020.02/0264, 2023.03/0156).

Organically bound tritium (OBT) chronic exposures over multiple generations of rodents

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A multigenerational mammal study was conducted to further the understanding of organically bound tritium (OBT) doses and associated biological effects. The parent generation of mice was exposed, starting at seven weeks of age, to three different forms of OBT (approximately 15,000 Bq/L) and the exposures were continuous until pups from the third breeding cycle reached thirty weeks of age. One group was exposed to three essential tritiated amino acids (AAs) – leucine, arginine, and lysine - through drinking water, another to the same tritiated AAs through feed, and the last group was exposed to tritiated arachidonic acid, a fatty acid (FA), through feed. Stable FA was added to the tritiated AAs feed and stable AAs were added to the tritiated FA feed. A control group, with stable AAs and FA added to the feed, was also included in the study.

When each generation of mice was six and thirty weeks of age, Tissue Free Water Tritium (TFWT) and OBT were measured in the body of exposed animals, along with a series of biological markers. Measures of body weight at the time of dissection correlated with treatment group, generation, age and sex. Hematology (white blood cells, lymphocytes, neutrophils, red blood cells and hematocrit parameters) correlated with treatment group and generation. The white blood cell and the lymphocyte counts also correlated with sex. The neutrophil counts correlated also with age and sex. The red blood cell count correlated also with age. Blood chemistry parameters (alkaline phosphatase and blood urea nitrogen) correlated with treatment group, generation and age. Alkaline phosphatase also correlated with sex. Gamma-H2AX foci, in gonads, correlated with treatment group, generation, age and sex. Fatty acid composition in liver and muscle was also correlated with treatment group, with liver showing a greater number of types of fatty acid affected. Each fatty acid studied correlated differently with generation, age and sex.

Low-dose radiation effects on stem cells in wild animals in Fukushima

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Since the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, wildlife within the alert zone have been exposed to low-dose-rate (LDR) radiation. We have been investigating the effects of chronic and LDR exposure associated with the FDNPP accident on wildlife, particularly on stem cells / progenitor cells. In 2014, we captured Large Japanese field mouse at control and contaminated areas and observed no significant difference in the frequency of DNA damage, measured by chromosome analysis, in bone marrow cells and spleen cells. However, there was a decrease in the number of hematopoietic progenitor cells (HPC) colonies in two contaminated areas (ambient dose-rate: 178–240μGy/day and 377-564μGy/day) compared to control area determined by CFU assay. In addition, we captured freshwater planarians (Dugesia japonica) in two contaminated areas in 2018-2020 (ambient dose-rate: 43μGy/day and 101μGy/day), and observed accumulation of radionuclides in their bodies, reduced regenerative activities in contaminated areas. These results suggest that LDR radiation affected the number and regenerative activities of stem/progenitor cells in wild animals.

Current work of the ICRP related to environmental radiological protection

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The International Commission on Radiological Protection (ICRP) is dedicated to developing recommendations in support of protecting people, animals, and the environment from deleterious effects of ionizing radiation. The scope of work for several task groups within the ICRP includes various aspects of environmental radiological protection (ERP) with emphasis on contemporary issues ranging from conservation to sustainable development. For example, modern statistical methods and models are being explored in one task group (TG99) for application in improved robustness and transparency in the development of derived consideration reference levels (DCRLs) (TG105). Another task group is evaluating ecosystem services as a potential tool (TG125) complementary to the existing conservation-based approach in ERP in support of broadening perspective with respect to environmental impacts associated with radiation and radioactivity. This presentation reviews these and other efforts related to ERP within the ICRP in the context of the broader, on-going reflection on the System of Radiological Protection in preparation for the development of the next set of general recommendations.

Imaging Molecule to Man - Unveiling the implications in Low Dose Radiation Research

Dr. Rao Papineni

Understanding the biological impact of low-dose ionizing radiation (LDR) is crucial for environmental protection and public health. Recent advances in molecular imaging technologies are poised to revolutionize our understanding of LDR-induced biological responses. Building on over two decades of experience in developing molecular tools and imaging techniques, our team has created innovative technologies that are now employed in nearly 1000 laboratories worldwide. These innovations have played a pivotal role in elucidating radiation-induced effects at the molecular, cellular, and tissue levels.

Our early work in functional imaging paved the way for real-time visualization of radiation-induced bystander effects — a key finding that has scope for utilization in LDR research paradigms to evaluate effects on multiple physiological systems. For instance, our use of luminol-based optical probes such as L-012 has enabled precise imaging of radiation-induced reactive oxygen species (ROS) and oxidative stress in vivo. More advanced and clinically translatable probes, such as 64Cu-diacetyl-bis(N4-methylthiosemicarbazone) (64Cu-ATSM), have emerged as powerful tools for quantifying ROS, hypoxia, and metabolic responses following low-dose radiation exposure.

These probes, when integrated with established molecular imaging platforms, provide powerful insights into oxidative stress, DNA damage, and adaptive cellular responses in LDR environments. Additionally, magnetic resonance imaging (MRI) has proven invaluable in assessing radiation-induced changes in tissue perfusion, metabolism, and functional alterations.

Emerging techniques such as positron emission tomography (PET) using radiolabeled biomarkers offer real-time insights into cellular stress responses. Although the use of PET imaging in LDR research may raise concerns due to radiation dose considerations, efforts will be made to justify and present its potential in LDR research, highlighting its ability to provide unique and valuable information. Meanwhile, elemental imaging via X-ray fluorescence microscopy (XFM) has shown exceptional promise for mapping elemental distributions in biological tissues, shedding light on radiation-induced molecular changes at the atomic level.

Furthermore, our image-based microdosimetry platform, leveraging cutting-edge CMOS technology, will play a pivotal role in elucidating LDR effects in cells, organoids, and other models. By combining these modalities with established ROS imaging techniques, we present a powerful toolkit for investigating the mechanistic underpinnings of LDR effects. This integrated imaging approach not only enhances our ability to assess cellular stress responses but also reduces uncertainties in risk assessment models and improves environmental protection strategies.

By refining imaging tools and adopting innovative AI-powered computational approaches, we can accelerate progress in understanding the subtle yet significant effects of low-dose ionizing radiation exposure — insights that are crucial for developing evidence-based policies and improved protective measures for public health and environmental safety.

This presentation aims to stimulate expert discussion on leveraging molecular imaging to advance LDR research, ultimately informing the development of more effective radiation protection strategies.

Session 5 *– Learning from other disciplines about multiple stressor interactions*

Learning from Emergency and ARS planning

Michael Abend

Learning from Ecotoxicology in Shaping Future Directions in Radioecology

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Developments in radioecology in recent decades have rejected the assumption that radiological protection of the environment is automatically ensured by the enforcement of benchmarks developed for humans, and called for a more ecocentric approach. Such an approach would ensure greater efficacy of radiological protection while also embedding radioecological research in the context of current environmental crises. Ecotoxicology, itself a branch of environmental toxicology, has been incorporating more ecological realism in its approach and tools, somewhat getting ahead of radioecology in its advancement. Examples of advancements in ecotoxicology that exceed knowledge in radioecology include the greater taxonomic breadth of species studied, the adoption of advanced monitoring and modeling tools, and more holistic and sophisticated approaches to multi-species and multi-stressor questions. This contribution will review similarities and differences between ecotoxicology and radioecology, and propose several areas in which ecotoxicology may inspire progress in radioecology. Both radioecology and ecotoxicology require multidisciplinary expertise for connecting physico-chemical processes to their ecological consequences. In practical terms, both radioecology and ecotoxicology fundamentally require measures of exposure, with dose estimation a better risk estimator and better ground for causal inference than environmental estimates and nominal exposures. More broadly, the two fields largely share the same fundamental challenges: the identification and validation of biomarkers of exposure; the extrapolation from biomarkers of effect to their significance at higher levels of biological organization, like population and ecosystems; the understanding of factors that explain variation in exposure and sensitivity. Reassuringly, progress in one discipline may create advancements in the other, provided that gainful dialogues and exchanges are established.

Quantum signaling directs IR effects on T-helper (Th) lymphocyte-mediated immune responses: A framework for understanding and treating irreversible osteopenia and cardiovascular disease after deep space exercises/sojourns

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Plasma membrane (PM)- derived, extracellular vesicles (EVs) may express opposing biological signals that exhibit quantum properties (N. Dainiak, *Radiat Res* 2024;202:328-354). Superposition (the simultaneous existence of multiple states of an object in the same physical location) of these signals and entanglement of EV-associated molecules with external energy forces such as ionizing radiation (IR) and microbial EVs, may explain their relative expression at a given time point. Atomic force microscopy studies determined that protein arrangement correlates with the dipolar direction and conducting states of cytoskeletal proteins that drive EV release from the PM. EVs exhibit conformational flexibility and hydrophobic channels that are subject to quantum-chemical effects, mimicking transport phenomenon in biological membranes. EV formation, release and reentry involves calcium flux through voltage-dependent channels, calcium sensors and SNARE proteins. The rate of vesiculation is increased or decreased by IR exposure, depending on its quality and dose and on cell type. Quantum properties may be associated with immunity at multiple additional levels, including T-helper (Th) activation/differentiation, T cell receptor (TCR) degeneracy, and TCR-peptide MHC interactions that discriminate between self and non-self. Exfoliated HLA-DR molecules (the human version of MHC class II molecules) play a critical role in antigen presentation to Th cells. Here, we further explore the relationship between IR and the quantal response of EV-associated monocyte HLA-DR (mHLA-DR). Studies in irradiated NHPs with infection and humans with sepsis demonstrated low mHLA-DR levels. Yeast-derived rhu GM-CSF (sargramostim) reversed sepsis and prolonged survival in NHPs and normalized the mHLA-DR level in 19/19 septic patients. These effects are presented in the context of environmental noise from IR and endogenous microbes that induce opposing biological effects.

Quantum immunology is relevant to manned NATO operations in space. Astronauts returning from space develop irreversible cardiovascular disease and/or osteopenia. EV signaling mediates cardiomyocyte function/regeneration and bone regeneration. EV therapeutic and cytokine approaches may mitigate and/or prevent these disorders that develop after exposure to IR (and possibly other radiations) present in space.

Complex chromosome aberrations in human populations as biomarkers of LET: Challenges and future considerations.

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Ionising radiation deposits energy in the form of radiation tracks of varying structure. At low doses, individual low and high-linear energy transfer (LET) radiation tracks are known to influence the quality of DNA damage initially induced and in-turn, the type and complexity of chromosome aberration induced. For instance, complex chromosome aberrations are a broad classification of aberration type known to be characteristically induced after low doses of high-LET but not low doses of low-LET radiation. Technical advances such as 24-colour karyotyping (M-FISH), enable the visualisation of complex aberrations however this is at a financial (equipment, specialist probes) and time (analysis time/cell) cost. For low dose exposures of unknown and/or mixed LET exposure scenarios, the informative value of identifying complex aberrations may outweigh these costs. The potential usefulness, challenges and future considerations of detecting complex aberrations in human populations will be discussed. For this, two studies will be described; 1. Cancer patients undergoing ^{223}Ra and IMRT (mixed LET exposures) and 2. Veterans of historical nuclear test sites (unknown and mixed LET exposures).

Adverse Outcome Pathways in Radiation Research and Impact Assessment

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The Adverse Outcome Pathway (AOP) framework has become an essential tool in (eco)toxicology and hazard assessment, providing a structured approach to linking molecular initiating events (MIEs) to adverse outcomes (AOs) across different levels of biological organization. Originally developed for chemical hazard assessment through international initiatives, the knowledge framework is increasingly being applied to other stressors, including ionizing radiation. By capturing mechanistic understanding in a transparent and systematic way, AOPs facilitate the transition from traditional descriptive (eco)toxicology to predictive, pathway-based approaches that may support regulatory decision-making. Both qualitative and quantitative AOPs (qAOPs) are now being developed for radiation, integrating empirical data and computational modeling to improve dose-response assessments. These models help characterize the biological effects of radiation at different levels of biological organization, from DNA damage to organismal and population-level impacts after exposure to ionizing radiation. While early AOP applications have primarily focused on single stressors, it is increasingly used to also address combined toxicity to co-occurring stressors, such as chemical pollutants and UV radiation (i.e. multiple stressors). Expanding AOP networks to capture such multi-stressor interactions is crucial for improving ecological relevance and support regulatory applicability. By providing a structured framework for evaluating radiation effects, AOPs contribute to the refinement of (eco)toxicological testing, evaluate causality between effects, develop predictive tools for hazard assessment, and supports harmonization of international research and regulatory activities in the field of radiation impact assessment. Future efforts should focus on advancing AOPs towards larger more quantitative models that can assist risk assessment and enhance predictive accuracy and regulatory utility.

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Systems ecology and wicked problems.

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We live within a complex socio-ecological system composed of multiple nested feedback loops, each operating on variable spatial and temporal scales. The so-called hard sciences, especially physics and chemistry have revealed much about the rules that constrain the commonly understood four dimensions that comprise space and time. Over the past several decades, we have begun to appreciate a fifth dimension, not the band, but freedom, the exploration of how things could be constrained as they are by the four dimensions that determine how things are (as defined by Timothy Snyder in *On Freedom*, 2024). It is the interplay of values (and chance) that determines the trajectory of ecological systems, the underlying biology, and the prominence of social interactions that shape societies. Wicked problems were first defined in 1967 and formalized in 1973 by Rittel and Webber. Subsequent authors have pared the original ten attributes to five or six. These key elements of wicked problems are that the problems are not understood until after a solution has been formulated, there are no stopping rules, there are no right or wrong solutions, each problem is unique, and therefore each must have its own array of solutions. These conditions are highly disturbing to many scientists and engineers; some. however, have embraced active adaptive management as a reasonable approach with the recognition that stochastic interplay can and often does render a plausible solution to be ineffective. In essence, solutions to wicked problems must be negotiated among affected stakeholders and must reflect the prominent values of the community in its larger societal context. What works in one setting cannot be transported to another without recalibrating the uniqueness of the new set of conditions, including the societal values. The presentation will be illustrated with a select set of examples drawn from environmental management challenges.

When is Change Bad? Low Dose Radiation Research from an Ethical and Social Science Perspective

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Natural scientists study the effects of ionising radiation on living organisms by testing for statistically significant changes in various endpoints as a function of increased radiation dose. While some of those endpoints can be directly linked to harmful effects in populations or individuals (e.g., cancer incidence, morbidity or reproduction), others have a more tenacious link with undesired effects (e.g., molecular changes, gene expression or epigenetic effects). Changes in endpoints require extrapolation in order to make judgements on the potential for negative impacts at higher levels. Such decisions are not only a matter of natural science, but encompass a series of value judgements about both what type and what level of change is unacceptable. Could genetic changes lead to more radiation resilient wildlife populations? And is that a good or a bad thing for ecosystems? When does reduction in reproduction in one species lead to an unacceptable risk to other species? Who decides which species are most valuable in an ecosystem? Do disputes about tolerability of radiation exposures in humans come down to disagreements about the size of the risk or the acceptability?

This paper argues that assessment and management of low dose radiation risks would benefit from more transparency in those value judgements. The presentation will give examples of how social science and ethical evaluation can support low dose radiation research, including other methods of gathering data, such as through citizen science.

Risk communication: how should we effectively engage the public?

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As nuclear projects, particularly Small Modular Reactors (SMRs), begin to take shape in Canada, it is increasingly clear that meaningful community engagement is crucial. To empower communities to make informed decisions that reflect their values and priorities, innovative approaches to education, relationship-building, and consultation are essential. Indigenous peoples, in particular, require focused attention. A primary driver behind the development of SMRs in Canada is the shift from diesel to nuclear power in remote communities, many of which are Indigenous. In the Canadian context, "Indigenous" refers to First Nations, Inuit, and Métis peoples. To gain a deeper understanding of the unique challenges faced by these communities, we plan to conduct interviews with Indigenous leaders, students, and community members. The communities closest to McMaster University in Hamilton, Ontario, are the Mississauga's of the Credit and the Six Nations of the Grand River First Nations. These communities will also be consulted to investigate approaches for weaving Indigenous knowledge with Western science to better engage Indigenous youth into science, technology and engineering programs. Beyond simply disseminating reports, we aim to develop initiatives that enhance risk communication for all communities interested in nuclear development. This presentation will explore community informed engagement strategies, highlighting the complex relationship Indigenous peoples have with their land and the potential impact of nuclear projects in their territories.

Poster Session

Comparison of radiation effects on DNA damage and cell survival in 2D and 3D cell culture models

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Introduction: Three-dimensional (3D) cell culture models outperform traditional 2D cultures, better mimicking human physiology through distinct mechanical forces, cell interactions, and ECM barriers [1, 2]. Understanding their differential radiation response may improve the accuracy of radiation risk assessment.
Objectives: This study aimed to investigate the differential effects of X-ray irradiation on DNA damage and cell survival in 2D and 3D cell culture models.
Methods: 2D and 3D cell culture models were established using A-549 (human lung adenocarcinoma) and F-98 (rat glioma) cell lines. DNA damage was analyzed using the Comet assay, while cell viability was assessed via the colony-forming assay at 4 h, 24 h, and 14 days post-irradiation. Cell culture models were irradiated using a MultiRad 365 kV irradiator at a dose of 1 Gy.
Results: Comet assay results indicated an increase in DNA damage in A-549 cells, with no significant differences between 2D monolayers and 3D spheroids. In contrast, F-98 cells exhibited a higher frequency of DNA breaks in 2D cultures compared to 3D spheroids. Overall, the F-98 cell line demonstrated greater resistance to radiation at 1 Gy, showing lower levels of DNA damage than A-549 cells in both 2D and 3D models. A-549 cells retained 93% survival compared to control in 2D culture, while in 3D culture, survival fraction (SF) was reduced to 92% of the control. For F-98 cells, SF at 1 Gy was 86% of the control in 2D culture and 96% of the control in 3D culture, indicating greater radioresistance in the 3D model.
Conclusion: The radiation response differed between A-549 and F-98 cell lines in both 2D and 3D culture models. While A-549 cells exhibit similar radiation sensitivity in both culture conditions, F-98 cells in 3D culture demonstrate increased resistance to radiation-induced damage. The incorporation of 3D culture systems into radiation biology research is important to enhance the predictive accuracy of in vitro studies.

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Tradescantia-Micronucleus assay of undisturbed Aragats massif (Armenia) soils genotoxicity considering their radioactivity

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In the biomonitoring system, plants are considered the most sensitive and reliable indicators of biosphere pollution. The *Tradescantia*-Micronucleus (Trad-MCN) bioassay is one of the tests used in the International Program on Plant Bioassay (IPPS) under the auspices of the UNEP. Using chromosome damage as the indicator of the carcinogenic properties of environmental agents, the Trad-MCN bioassay is a quick and efficient tool for screening carcinogens and toxins in both liquid and soil forms.

Biotesting of the genotoxicity of soil samples with various activity concentrations of naturally occurring (^{226}Ra, ^{232}Th, ^{40}K) and artificial (^{137}Cs) radionuclides in soil from the territory of the Aragats Massif (Armenia) was carried out using the *Tradescantia* micronucleus (Trad-MCN) bioassay of the *Tradescantia* (clone 02) model test object in the soil-plant system. Undisturbed soil sampling was performed on the southern slopes of Aragats from different altitudes (from 1000 to 3200 m above sea level). The assays were conducted according to the standard protocol. The following two marker test criteria were considered: the frequency of tetrads with micronuclei and the frequency of micronuclei in tetrads.

The soils in the region exhibited a typical background level of naturally occurring materials (NORM), with the highest variability found in ^{40}K activity concentrations. Levels of genetic damage were significantly higher for plants of *Tradescantia* grown on the soils from Aragats than in control soil. As a result of the study of the frequency of genetic disorders in the sporogenic cells of *Tradescantia* (clone 02), the genotoxic effects of the components of the studied soil samples were shown, which is expressed in a significant increase in the frequency of the appearance of MNs in tetrads of microspores. A positive correlation was observed between the frequency of MCN in tetrads and tetrads with MCN, and specific activity radionuclides ^{40}K and ^{226}Ra ($p < 0.05$; $p < 0.01$). *Tradescantia* (clone 02) accumulated mainly ^{40}K.

In terms of the main marker indicators, the Trad-MCN assay shows high sensitivity of the test system based on *Tradescantia* for assessing the mutagenic potential of soils to natural and artificial radionuclides in the soil environment.

Applicability of Commercially Available Admixed Plastic Filaments for Simulating Bone Tissue in 3D-Printed Dosimetry Phantoms

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Precise estimation of dose exposure to humans is a key issue in the field of radiation protection. Experimental measurements using specialized dosimetry phantoms are one of the primary methods to achieve this. This approach has found widespread application in various fields, including radiation therapy and imaging, monitoring dose exposure to the human body in cosmic radiation environments, and assessing radiation exposure in nuclear facilities, among others. Dosimetry phantoms can vary in shape and material composition. The use of 3D-printed anthropomorphic phantoms has been extensively discussed in recent studies [1-3]. While soft tissues can be accurately mimicked using standard plastic filaments, simulating bone tissue presents a more complex challenge. This study investigates the applicability of several commercially available admixed plastic filaments for this purpose. Particularly, we considered PLA plastics filled with metal powders (copper, aluminium, bronze) and ABS plastics filled with various ceramics. Hounsfield units of samples printed from these filaments with different filling factors were evaluated as the eligibility criterion. CT scans of the printed samples were performed using a Philips Brilliance 64 clinical tomography system."

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Exposure to Radon in Tap Water: Risk Assessment and Spatial Trends in Yerevan, Armenia

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Introduction. Exposure to radon (Rn-222) constitutes the most significant component of human irradiation from natural sources. One of the sources of Rn-222 in tap water, which can affect the human body through ingestion, inhalation due to the degassing of tap water as temperature increases, and the dissolution of Rn-222 in blood.

Objective of this study was the assessment of spatiotemporal distribution of Rn-222 activity in tap water of Yerevan city from 2023 to 2025 and to evaluate risks associated with Rn-222 applying both deterministic and probabilistic approaches.

Methods. Tap water were collected monthly from all administrative districts of Yerevan and measured using the RAD8 radon meter. The obtained data were compared with drinking water consumption habits among different groups of the adult population, based on data from a Food Frequency Questionnaire (FFQ).

Results and Conclusion. The study results revealed significant variations in Rn-222 activity (ranging from 1.57 to 11.4 Bq/L) across districts within the city, however Maximum Permissible Concentration (60 Bq/kg) was not exceeded. Activity concentration of Rn-222 in water was found to be higher in the northern and western districts than in the eastern and southern ones. The highest average concentration was detected in Davtashen (7.07 Bq/L), while the lowest was recorded in Kanaker-Zeytun (1.57 Bq/L). These substantial variations are attributed to the different sources of drinking water. The deterministic approach to radon risk assessment (the total annual effective dose that is additive sum of dose from water ingestion, inhalation of Rn-222 released from tap water into indoor air during household activities, and dissolution of Rn-222 in the blood) indicated that the obtained values did not exceed the individual dose criterion (IDC) of 0.1 mSv/year. However, probabilistic Monte Carlo modeling of the “Worst Case Scenario” indicated that individuals consuming more than 3 L of water per day with maximal activity concentration detected in the period 2023-2025 (11.4 Bq/L) have an 86.26% probability of exceeding the IDC. For those consuming 2.1 L per day, the probability of exceeding the IDC is 7.02%.

This study highlights the necessity of Rn-222 monitoring in tap water and the revision of legislation to include an "action level" instead of Maximum Permissible Concentration.

Telomere Lengths of Human Chromosomes as Potential Biomarkers of Genomic Instability Induced by Electron Beam Radiation

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Telomeres are specialized nucleoprotein structures located at the ends of linear eukaryotic chromosomes which play an essential role in DNA replication and cell proliferation. The guanine triplet repeats in telomeric sequences (5'-GGG-3') have been shown to be a target for reactive oxygen species induced by ionizing radiation, resulting in telomeric lesions and shortening. Considering that DNA damage in telomeric DNA can lead to replication stress and genomic instability, our study aims to reveal telomere length (TL) alterations in normal and cancer cells induced by low-dose radiation with laser-generated ultrashort electron beams. TLs in metaphases and interphases of human leukocytes and K562 leukemic cells irradiated with 0.5, 1.5, and 3.0 Gy at 2 Hz were analyzed using quantitative fluorescence in situ hybridization (Q-FISH. A dose-dependent negative correlation was observed between TL and radiation doses in both normal and leukemic cells. K562 cells were more sensitive to increasing doses of ultrashort electron beam radiation compared to normal blood cells. Radiation-induced changes in the distribution of TLs in normal and cancer cells populations were revealed. The obtained results open new insights for the Q-FISH analysis of TL changes in human cells as a biological endpoint of genotoxic impact of ultrashort electron beam radiation.

Possible Mechanism of the Impact of Radiation on Living Organisms

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Scientific literature contains numerous reports on phenomena described as low-energy nuclear transmutations. Studies of this phenomenon confirm the occurrence of atomic transformations in water under certain conditions when subjected to electric fields [1,2] or steep mechanical impacts [3-5]. In [6], the emergence of X-ray radiation under such influences on water was reported. It was demonstrated that this radiation is bremsstrahlung emitted by electrons in aqueous solutions.

Based on these findings, there is growing interest in investigating the phenomenon of bremsstrahlung generation when water is exposed to radioactive radiation. This paper presents the results of experimental studies on the process of bremsstrahlung radiation formation when water comes into contact with metallic tungsten containing strontium impurities, which are subject to beta decay, and optical filters with uranium impurities, which undergo alpha decay. For comparison, the results of experimental measurements conducted on a pure tungsten sample are also provided. It is shown that when samples undergoing β-decay come into contact with water, X-ray radiation with an energy of 5.67 keV is generated. However, in the case of similar contact with materials undergoing α-decay, no bremsstrahlung formation is observed.

Considering that more than 75% of the human body consists of water, it is hypothesized that under the influence of β-radiation, nuclear transformations may occur in living cells, leading to changes in the composition of organic molecules. The channel for bremsstrahlung and quasi-neutron formation is proposed as follows:
$e^- + H^+ \rightarrow n^* + \upsilon + X$,
where *e* is an electron, H^+ is a hydrogen ion, n^* is a quasi-neutron, υ is the neutrino, and *X* is an X-ray photon with energy of 5.67 keV. It is evident that the formation of quasi-neutrons can induce nuclear transformations via the (n,γ) reaction channel, leading to structural modifications in organic molecules.

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