Terms of Reference for Radioecology WG

Title and acronym:

Transgenerational Effects and Species Radiosensitivity

Topical area

The issue of biological effects of low doses of ionising radiation along with the 'hot question' from the public/media on the potential hereditary effects for both humans and wildlife is of major concern. This has been reinforced after the Fukushima accident, especially with respect to the quantification (and reduction if needed) of the magnitude of risk to individual (human) and population (human and biota) health when exposed at low doses/dose rates for multiple generations. This concerns both chronic exposure over several generations, and intermittent exposure (e.g. consequences of a short exposure in one generation to the others). The challenge is clearly to improve our understanding of the cascade of responses, from primary interactions of ionising radiation with biomolecules to adverse outcome for physiological functions, including reproduction, and ecosystem function.

Addressing the issue of biological consequences induced by low doses, we need to improve our knowledge on **long term and transgenerational effects**. This includes the study of genetic changes (mutations) but also the role of epigenetic mechanisms, both determining the adaptation ability of organisms. In particular, the role of epigenetics in genomic instability and inheritance in organisms/cells exposed to radiation/radionuclides and also in adaptation of organisms under conditions of a pressure selection must be better understood. In perfect complementarity, mutation rates and types are to be assessed and quantified in parallel. This will enable distinguishing between epigenetic and genetic induced changes.

The second topic of interest in this working group deals with differences of **radiation sensitivity** across species and phyla are poorly understood, but have important implications for understanding the overall effects of radiation and for radiation protection: sensitive species may require special attention in monitoring and radiation protection; and differences in sensitivity between species also lie behind overall effects at higher levels (community, ecosystem), since interactions between species will be altered. Understanding the mechanisms of inter-species radiation sensitivity may also help us understand mechanisms behind intra-species variation.

Leadership

Christelle Adam-Guillermin (IRSN, France) and Nele Horemans (SCK CEN)

Partners with a brief description of their assigned role

Interested Organisation	Assigned role
IRSN/Laboratory for radionuclide ecotoxicology, Christelle Adam-Guillermin (France)	WG coordination Use of (epi)genomic, transcriptomic and/or proteomic data to identify fingerprints/key processes, genes involved in transgenerational effects and radiosensitivity such as adverse hereditary or adaptive effects, induced by ionizing radiation (gamma, alpha irradiation, tritium) • role of genetic and epigenetic mechanisms in transgenerational effects, for 1 to 4 generations exposed under laboratory controlled conditions to ionizing radiation (in zebrafish, nematodes, daphnids) • role of genetic and epigenetic mechanisms in adaptation mechanisms, for 20 generations exposed under laboratory controlled conditions to ionizing radiation (nematodes) • study of organisms isolated from radionuclide contaminated sites (Chernobyl, Fukushima) to study resistance/adaptation mechanisms (nematodes) ; same goal but studying structural parameters of soil ecosystems (nematode species composition) • characterization of biomarkers of ionizing radiation effects from laboratory and field (frogs, birds) • role of protein carbonylation in transgenerational effects, adaptation and radiosensitivity : comparison of acute vs chronic data (nematodes, zebrafish) • comparison with other stressors (chemicals Evogenerate SETAC group, natural stressors) ; natural variability • role of metabolism in transgenerational effects, adaptation and radiosensitivity : use of Dynamic Energy Budget (DEBtox) models • comparison of environmental protection criteria obtained from one generation vs several generations
Helmholtz-Zentrum Dresden – Rossendorf, Karim Fahmy (Germany)	• In combination with genetic analyses and genetic engineering, the acquired data allow identifying genes that are critically involved in radionuclide-resistance and are prime candidates to identify transgenerational effects and radiosensitivity. • Real time monitoring of low dose radiation responses using metabolic heat measurements in bacterial cultures and multicellular organisms. • In combination with the isolation of model organisms from radionuclide-enriched sites, the research activities at the IRE provide quantitative reference data on radio- and chemitoxicity at low doses that can be directly related to molecular mechanisms that are under genetic control. • Adaptive effects will be revealed for microbial isolates from mining waste piles. The establishment of low dose-responsive model organisms and the molecular understanding of particle energy-dependence of biological effects will lay an important ground for defining knowledge-based radiation risk assessments and protection standards in radioecology.

Ukrainian Institute of Agricultural Radiology (UIAR), Valery Morozova (Ukraine)	The goal of our research work is to get the "dose-effect" dependence to develop approaches of the environment protection from radiation. Results will be obtained on the basis of the scientific monitoring of the radiobiological effects of the chronic ionizing radiation on the reference plant species (Arabidopsis and Scots pine) in the areas radioactively contaminated by Chornobyl NPP under the different levels of external and internal exposure. These results will allow the improvement of understanding of the mechanism of the organism adaptation to ionizing radiation. Besides it, future results will contribute to improve the number and quality of reliable protection criteria for ecosystems and their sub-organisational levels with respect to exposure to radioactive substances. These criteria are needed to support emerging policy in the field of radioprotection of the environment per se as this is now explicitly mentioned in both the International Basic Safety Standards (BSS) from IAEA, and the updated EURATOM BSS. • study of the functional state of the cell antioxidant defense system of the leaves and roots of Arabidopsis (Arabidopsis thaliana) and needles of Scots pine (Pinus sylvestris L.) and tissues of the sprouted in the laboratory seedlings of these plants. • estimation of morphological changes in plant samples. • cross comparison with the results of similar researches under the laboratory conditions.
NERC Centre for Ecology and Hydrology Molecular Ecotoxicology and Ecotoxicology Group, Dave Spurgeon (UK)	Study of the epigenetic and transgenerational effects of chemicals. From the projects we are running, we will have a growing set of working analytical and bioinformatic tools available for looking at transgenerational and epigenetic effects. • using next generation sequencing to map changes in the epigenome to expression change. • run transgeneration experiments for a range of chemical exposures (PhD). • study of epigenetic changes in earthworms in the field (Chernobyl) (European project Comet) and of transgenerational gamma effects (PhD). The two PhDs are collaborative with Prof. Peter Kille in Cardiff who has the expertise in the design and analysis of next generation sequencing for various type of assessment (RNAseq, RAD-seq, bisulphide etc.)
NERC Environmental Contaminants Group Centre for Ecology & Hydrology CEH Lancaster, Nick Beresford (UK), Jan Baas	Involved in TREE Program (NERC) with different research axes • data obtained from more than 40 wildlife trap cameras in Chernobyl could be used to model abundance of medium-large mammals in three study areas (TREE work in collaboration with Salford University); • study of ionizing radiation effect on feather hormone (PhD starting in October in collaboration with Christelle Adam IRSN) • study of soil functional parameters, like turnover processes, by using bait laminae in CEZ this year • use of dosimeters/GPS set on mammals in CEZ from next spring for better dose estimates. Use of these data for better characterization of dose-response curves (*both WGs*). • DEBtox modeling incorporating the metabolic rate

University of Portsmouth, School of Earth and Environmental Sciences, School of Biological Sciences, Adélaïde Lerebours (UK)	The research aims at studying the effects of low doses of ionizing radiations on natural populations of fish from the CEZ. This work could contribute to a better understanding of the molecular and physiological changes in fish induced by radiation exposure and the identification of relevant biomarkers for the effects of radiation. Specifically, the work is focusing on long-term consequences of radiation exposure on the reproduction of fish. • The consequences of parental gonad exposure to low doses of radiation will be investigated by examining the early life stages of their offspring. This will highlight the significance of transgenerational effects. • In parallel, our laboratory experiments aim to determine the effects of low doses of ^{32}P exposure on gonads and early life stages of the 3-spined stickleback. The work include biometric, histological, genotoxicity and transcriptomic analyses.
Russian Institute of Radiology and Agroecology, Laboratory of Plant Radiobiology and Ecotoxicology, Stanislas Geras'kin (Russia)	• Cytogenetic effects, isozymes polymorphism, enzymes activity, morphological and reproductive characteristics in chronically irradiated Scots pine populations inhabiting sites contrasting in level of radioactive contamination within Bryansk Region of Russia and Poleski Radioecological Reserve of Belarus will be estimated. • effects in natural populations and levels of technogenic impact as well as meteorological conditions will be performed • Analysis of relationships between manifestation of biological; time-dynamics and ecological-genetic variability in irradiated populations will be studied • the role of antioxidant enzymes in adaptation of plants to chronic influence will be estimated
SCK•CEN, Biosphere Impact Studies (BIS), Nele Horemans (Belgium)	Study of possible long-term or transgenerational effects of ionizing radiation on plants. • Epigenetic mechanisms leading to long term and transgenerational changes induced by low-dose radiation is being studied in two plant species (Arabidopsis and Lemna). • Additionally we are interested in mechanistically analyse if long-term exposure of plants to above background levels of radionuclides like U will induce an altered tolerance or sensitivity of the plants to the radionuclide it is being exposed to or to an alternative stressor. • This includes also priming experiments where plants are exposed to e.g. a low concentration of U or gamma radiation and the effect of a subsequent exposure to the same or other stressor is studied within the same generation or over several generations. • *On the topic of Radiosensitivity - SCK•CEN could in future contribute to study the functional mechanisms leading to radiosensitivity in plants. This will be accomplished by comparing the molecular profile and the regulation of the molecular mechanisms in at least two molecular model plant species (Arabidopsis thaliana and Oryza sativa) differing largely in radiation sensitivity and at different life stages of the plant. In addition these data can contribute to a larger comparison of interspecies sensitivity.*

Gent University, GhEnToxLab, Karel De Schamphelaere (Belgium)	• We could mainly contribute with bioinformatics of whole genome bisulfite sequencing data; with daphnia or other organisms. • Possible contribution to transgenerational effects part with Daphnia omics in general, and gene expression (whole genome transcriptomics) and genome wide DNA methylation (methylome) specifically. • *We are also interested in variation of responses between different daphnia species, between daphnia clones (within species) and between different populations (e.g. living in historically impacted environments).* • We are also interested in combined stress (e.g. U both as a toxic metal and at the same time resulting in radiation exposure, or radiation in combination with other chemical stressors).
Stirling University, David Copplestone (UK)	Study of long-term & transgenerational effects of ionizing radiation on insects (bees, drosophila) freshwater microcrustacean (daphnids), birds in the laboratory and in the field (Tchernobyl)
CEA, Laboratory for cellular bioenergetics, Laurence Blanchard, Arjan Degroot (France)	Study of long-term effects of ionizing radiation on bacteria : investigation of toxicity and resistance mechanisms
Norwegian University of Life science, (Deborah Oughton (Norway)	Study of long-term & transgenerational effects of ionizing radiation on worms (nematodes) and fish (zebrafish, in the laboratory (Co-60 irradiator) and in the field
GIG, Bogusław Michalik (Poland)	Study of transfers and dose characterization

Starting date and estimated duration of the WG to accomplish its plan

Starting date: April 2016

Duration of the WG: 5 years

Intended activities (task, approach, steps to accomplish, expected outcomes)

The intended activities contribute to the understanding of transgenerational and long-term effects, including resistance/adaptation effects, in three main tasks :

The work described here aims is focusing on the use of (epi)genomic, transcriptomic and/or proteomic data to identify fingerprints/key processes, genes involved in transgenerational effects such as adverse hereditary or adaptive effects, induced by ionizing radiation (gamma and alpha irradiation, tritium, ^{32}P, U) in the laboratory or in the field.

Approach:

Task 1 B**iomarkers of transgenerational/adaptation effects** : laboratory (and possibly field) studies will be undertaken to identify genes/proteins/epigenetic marks that are critically involved (i) in transgenerational effects or (ii) in radionuclide-resistance, and are prime candidates to identify transgenerational effects. Experiments will be performed in the laboratory on three-spined stickle bass (UoP), zebrafish, nematodes, daphnids (IRSN), Arabidopsis and Lemna (SCK•CEN), Scott pines (RIRA), bacteria (HZD and CEA).

The role of anti-oxidizing compounds (RIRA) and protein carbonylation (IRSN, CEA), will be specifically investigated. A system biology approach will be used to integrate the biological responses.

The acquired data will be integrated in population dynamics models, allowing a comparison of protection thresholds for the individuals or the population levels.

Task 2 Radioadaptation : priming experiments will be performed by exposing organisms to a low dose ; the effect of a subsequent exposure to the same or another stressor will provide knowledge in physiological adaptation within the same generation or over several generations (SCK•CEN, IRSN). Adaptation will also be studied by exposing several generations to dose rates acting as a selection pressure in order to better understand the (epi)genetic main actors of selection/adaptation/resistance mechanisms. Organisms isolated from field situations (e.g. mining area, Fukushima and Chernobyl Exclusion zone) will also be used to study resistance/adaptation mechanisms (nematodes (IRSN), bacteria (HZD), Scott pines (RIRA, UIAR), Arabidopsis (UIAR, SCK•CEN).

Transgeneration/adaptation mechanisms will also be studied in populations and ecosystems by characterizing effects of ionizing radiation on natural populations exposed in situ. This will include the study of structural changes of soil nematode (IRSN), earthworms (CEH, NMBU) and medium-size, large mammals populations (CEH). It will also include the study of functional changes of soil ecosystem by the use of bait lamina (CEH) and of plant communities (UIAR and RIRA).

Task 3 Role of metabolism in transgenerational/adaptation effects : the metabolic activity of cells and organisms can provide a highly reproducible biological signature of radiation effects (studied in bacteria at HZD). This approach could be applied to other organisms and be directly used in a DEBtox model to infer on the possible role of metabolic activity in transgenerational effects.

Steps to accomplish

Tasks 1 and 2 integrate within the framework of COMET WP4 Initial Research Action, dedicated to understanding the role of epigenetic mechanisms in transgenerational effects. As such, deliverables from this COMET WP4 will serve as basic information to better define future work to be accomplished. This will drive to write a position paper in 2016 to give guidelines on how to perform transgenerational studies taking into account genetic and epigenetic effects. In addition to these guidelines, a rationale for performing such studies will be given, integrating the major factors or key questions to take into account (e.g. specificity of biological functions or biomarkers of effects, dose-effect relationship if any, core of conserved mechanisms among biological models or specific genes/proteins explaining radioadaptation). From this paper will possibly come out a common project to be submitted to a European call for proposal, in collaboration with the other platforms (e.g. CONCERT second call in 2017). A specific pilot study could also be designed to answer an explicit biological hypothesis.

Several guidelines could be written : guidelines for setting up laboratory experiments with specific objectives and this for all the model species ; guidelines for conducting field experiments and given specific aims ; guidelines for specific analysis (gamma H2 AX, methylation grade) in order that people wanting to do the same work, applying the same techniques do not have to go through the same pitfalls.

In addition, a literature review and knowledge exchange will be performed among partners to better identify the common basic knowledge on transgenerational/radioadaptation effects, acquired even in acute exposure conditions or in the toxicology field. This approach will enable to identify candidate genes or processes for the context of radioecology in the chronic low dose context and will allow a better characterization of research needs.

Another action could be focused on the study of dose-response relationships. In the FREDERICA database, build under the European project ERICA (6^{th} FWP Euratom) and gathering all referenced effect studies, there are several datasets on mutational effects and other molecular level endpoints. These data could be further analysed in order to try to better define the range of dose rates by which such molecular responses are observed, for each considered organism. This will probably lead to define a benchmark based on this type of mutational response, that would be useful to interpret several in situ date. The shape of the curves could be studied, trying to identify the relevance of linear-models for these low dose rates range. Furthermore, these analyses could help in defining the term “low dose” for non-human species. This outcome would be a great step towards the integration of molecular responses in the regulatory framework, and would help to better define radiological protection criteria for non-human species.

Expected outcomes

The work accomplished in these three tasks will result in a better identification of radio-induced effects for the context of chronic low doses of exposure, highly relevant for natural sites and their non-human inhabiting species, for contexts such as routine radioactive discharges, high background natural radioactivity area and post-accidental conditions. Along with this knowledge, a better characterization of natural variability will be possible in wild organisms challenging highly variable nutrition, predation, multipollution.

Some specificities of the dose-responses in these conditions will be investigated through modelling approaches (non-linear dose-response curves, hormetic responses).

The intensity of biological responses induced by ionizing radiations will be compared with other pollutants such as metals by favouring collaborations with ecotoxicologists (e.g. through the evogenerate SETAC working group).

From a regulatory perspective, the understanding of low dose effect relationship will help to derive environment protection criteria.

In addition to these scientific outcomes, this working group will contribute to knowledge dissemination, in terms of workshops, student exchanges, shared experiments and platforms.

Work plan

Planned research activities and time scale: tasks, responsibilities, participants, use of observatory sites, use of large scale facilities, milestones, deliverables, resources committed by partners (estimated man.months, indoor funds), requested funds and targeted calls (EC Call, other calls)

Partner	Task	Means	Resources
IRSN, SCK•CEN, NERC CEH, NMBU, Stockholm Univ. …	Coordination Task 1 : paper position from COMET WP4 Task 2 : guideline on field studies Task 3 : DEBtox modelling integrating metabolic rate	T1 : COMET WP4 T2 : Ecorad ? (Biodiversa) CONCERT 2nd call T3 : DEBtox	T1 : 24 MM (post-doct), 72 MM PhD (2015-2018 ; 2016-2019) T2 : in function of project selection 24 MM (post-doc)

Major elements of the communication plan (workshops, publications, guidance documents…)

Partner	Activity
IRSN	COMET meetings, workshop, publications in peer reviewed journals, guidelines, share PhD students, sterring committee of PhD ? Position paper

Links with other activities identified at the national and the international levels

Partner	Activity
IRSN	COMET WP4 (epigenetics on zebrafish, nematodes, and in situ on frogs & birds). Contract with Electricité de France on low dose effects (tritium and gamma external irradiation). National research programs on epigenetics (NEEDS). IRSN research programs on low dose effects. 2 PhD and 1 post-doc on epigenetics changes induced by ionizing radiation.
HZ-IRE	Research Programme of the Helmholtz-Gemeinschaft: Nuclear Waste Management, Safety and Radiation Research
UIAR	COMET WP4, national project NUBiP of Ukraine No. 110/72f

NERC CEH MEEG	Working on two projects on the epigenetic and transgenerational effects of chemicals. One has been using next generation sequencing to map changes in the epigenome to expression change. The second is running transgeneration experiments for a range of chemical exposures. Also new PhD project on epigenetic changes in earthworms in the field due to start. Work in Comet on transgeneration gamma effects on earthworms. The two PhDs are collaborative with Prof. Peter Kille in Cardiff who has the expertise in teh design and analysis of next gereation sequencing for various type of assessment (RNAseq, RAD-seq, bisulphide etc.)
NERC CEH CG	TREE Project (see in partners description section)
UoP	Transfert Exposure and Effects project (NERC funded).
SCK•CEN	COMET WP4
Ugent	AquaStress project: we have one pH d student working on multigenerational effects of metals and heat stress (simultaneous and sequential exposure)
RIRA	Russian Scientific Foundation (grant 14-14-00666) Analysis of adaptation mechanisms in plant populations to technogenic impact
Stirling Univ	
CEA	
NMBU	
GIG	

Expected problems, gaps/lack of knowledge, etc. that might prevent the accomplishment of the research