CHAPTER 1

CHALLENGES IN RADIOECOTOXICOLOGY

B. SALBU AND L. SKIPPERUD

Isotope Laboratory, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway

Abstract: *Radioecotoxicology* refers to responses, usually negative and detrimental responses, in living organisms exposed to radionuclides in ecosystems contaminated with artificially produced radionuclides or enriched with naturally occurring radionuclides. The key focus is put on the link between radionuclide exposures and the subsequent biological effects in flora and fauna. Radioecotoxicology is therefore an essential ingredient in impact and risk assessments associated with radioactive contaminated ecosystems. The radionuclide exposure depends on the source, release scenarios, transport, deposition and ecosystem characteristics as well as processes influencing the radionuclide speciation over time, in particular bioavailability, biological uptake, accumulation and internal distributions. Radionuclides released from a source may be present in different physico-chemical forms (e.g. low molecular mass species, colloids, pseudocolloids, particles) influencing biological uptake, accumulation, doses and biological effects in flora and fauna. Following releases from severe nuclear events, a major fraction of refractory radionuclides such as plutonium will be present as particles, representing point sources if inhaled or ingested. When organisms, and especially sensitive history life-stages, are exposed to radionuclides, free radicals are induced and subsequently, effects in several umbrella biological endpoints (e.g. reproduction and immune system failures, genetic instability and mutation, increased morbidity and mortality) may occur. However, radionuclides released into the environment rarely occur alone, but may co-occur in a mixture of other contaminants (e.g. metals, pesticides, organics, endocrine disruptors), which potentially could lead to synergisms or antagonisms. Thus, the relationships between radionuclide exposure and especially long-term effects are difficult to document and quantify, reflecting that the challenges within radioecotoxicology are multiple.

Keywords: ecotoxicology; multiple stressors; radiation

C. Mothersill et al. (eds.), Multiple Stressors: A Challenge for the Future, 3–12.

Introduction

When radionuclides are released from a source, the receiving ecosystems might be affected by *radioactive contamination*. To identify the degree of contamination, sampling and analysis are needed, and for alpha and beta emitters *radiochemistry* is needed that is the separation of radionuclides of interest from the bulk of interfering radionuclides prior to analysis. To assess the environmental impact and risks associated with the radionuclide contamination, information based on *radioecology* is needed; that is knowledge on the behaviour of radionuclides, in particular radionuclide species (Salbu, 2000; Salbu et al., 2004), in affected ecosystems. As ecosystem characteristics are essential for the behaviour of trace amounts of substances (e.g. pH in soil water, redox conditions, interacting components such as humic substances and clays) knowledge and principles from *ecology* should be implemented in radioecology. Serious consequences from radioactive contamination refer most often to negative or detrimental biological effects in exposed organisms such as man or organisms living in the affected environment. Since some organisms and some history life-stages are more susceptible to radiation exposures than others, knowledge from *radiobiologi* is essential. To evaluate biological early responses, toxicity or detrimental effects from radionuclide exposures, knowledge from *human toxicology* or *ecotoxicology* is needed. The radiation characteristics of radionuclides, their environmental mobility, bioaccumulation and doses are important determinants of the exposure and thereby the magnitude of the consequences following radionuclide releases. Thus, the phrase *radioecotoxicology* refers to the responses, usually negative or detrimental responses, in living organisms exposed to radionuclides, that is, key focus is put on the link between radionuclide exposures and the subsequent biological effects in flora and fauna.

The exposure (i.e. the radionuclide composition, their amounts and the radionuclide speciation) depends on the source, and in many cases several sources may contribute to the contamination (Fig. 1). Furthermore, the release scenarios (temperature, pressure, presence of air) may influence on the speciation of radionuclides deposited in an ecosystem. Following a severe nuclear accident a major fraction of refractory radionuclides such as plutonium is present as radioactive particles (Salbu et al., 1994, 2000; Salbu 2001; Salbu and Lind, 2005). Following an explosion under high temperature and pressure conditions, radioactive particles can be rather inert towards weathering, while during a fire the released oxidised particles are more readily dissolved Salbu et al., 2004. Thus, for radionuclides mobilised from oxidised particles, the soil to vegetation and animal transfer is rapid, while delayed for inert particles. External exposures reflect contaminated (gamma, high energy beta emitters) habitats, while the internal exposure depends on the presence

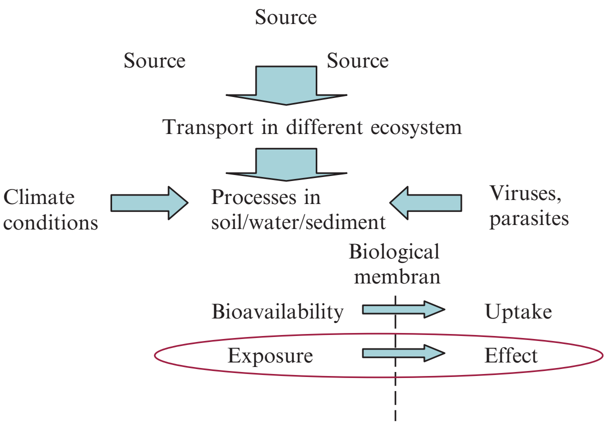

Fig. 1. Many variables influences on the exposure – biological effects relationship.

of bioavailable radionuclide species, biological uptake, accumulation and delivered doses. The radionuclide speciation depends, however, on the source term and interactions with other components during transport in air (e.g. association with soot particles), during transport in the aquatic environment (e.g. association with humic substances), and during deposition on ground (e.g. interactions with clay). These interactions may change the speciation of radionuclides released, and transformation processes influencing mobility, biological uptake, accumulation and doses take place over time. If mobile species are present, ecosystem transfer is relatively fast, whereas the ecosystem transfer is delayed if particles are present (Salbu, 2000; Salbu et al., 2000).

Relationships between exposure and especially long-term effects (responses in biological endpoints) are often difficult to document and quantify, although biological responses from molecular to ecosystem level have been identified for different organisms in contaminated areas. When organisms and especially sensitive history life-stages are exposed to radionuclides, free radicals and ROS are induced and subsequently, effects in several umbrella biological endpoints (e.g. reproduction and immune system failures, genetic instability and mutation, increased morbidity and mortality) may occur. However, radionuclides released into the environment rarely occur alone, but may co-occur in a mixture of other contaminants (e.g. metals, pesticides, organics, endocrine disruptors), which potentially could lead to effects in the same umbrella endpoints. Thus, multiple stressor exposures may induce synergetic or antagonistic effects in exposed organisms, and other factors such as climatic conditions and pathogens can also add to the stress (Salbu et al., 2005). Thus, a series of factors, including the interactions from other stressors, represents challenges within radioecotoxicology.

Sources and Release Scenarios

A significant number of nuclear sources has contributed, is still contributing, or has the potential to contribute to radioactive contamination of different ecosystem. As the Arctic ecosystems are believed to be most vulnerable, the present focus is put on this region (Fig. 2). The major sources contributing to radioactive contamination of long-lived radionuclides in Arctic ecosystems in the past are the nuclear weapon

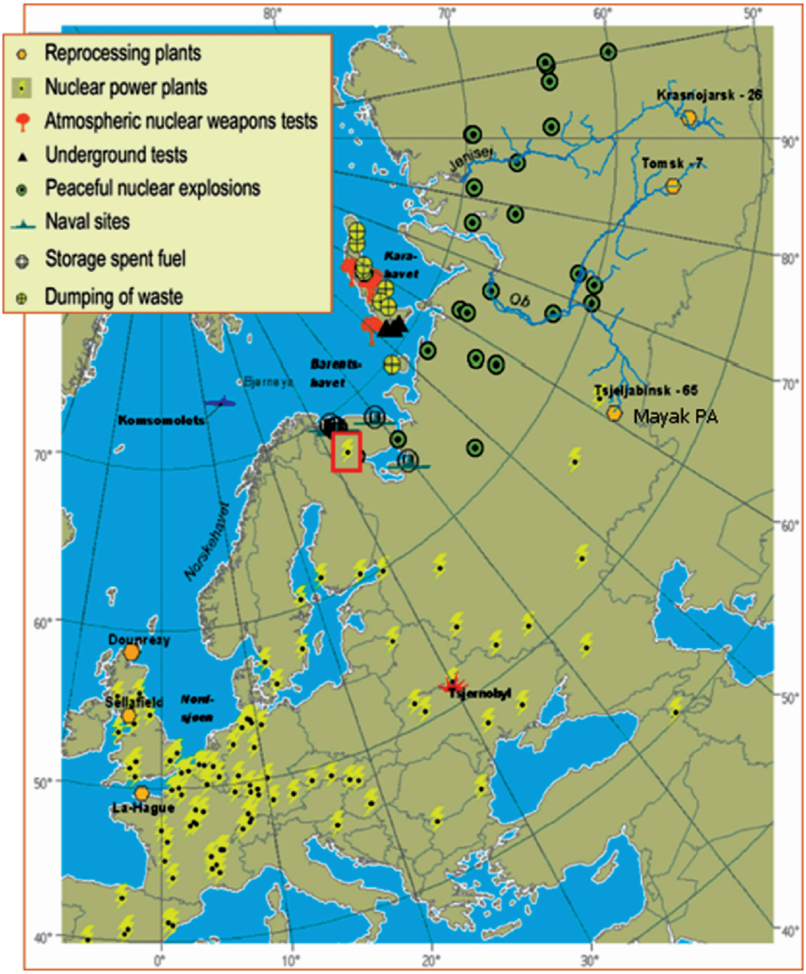

Fig. 2. Nuclear sources, which have contributed, are contributing, or have the potential to contribute to radioactive contamination of Arctic ecosystems.

tests (i.e. atmospheric tests resulting in global fallout, underground and underwater weapon tests at Novaya Zemlya resulting in local contamination), dumped liquid and solid radioactive waste in the Barents and Kara Seas and the Chernobyl accident (AMAP, 2002). Marine transport of artificially produced radionuclides from European reprocessing plants (i.e. Sellafield and Dounreay, UK, and La Hague, France, since 1950ies) and of Radium-, Lead- and Polonium-isotopes from the North Sea oil industry is ongoing (Salbu et al., 2003) together with river transport from Ob and Yenisey having large drainage areas affected from global fallout and from several nuclear installations (Mayak PA, Tomsk-7, Krasnoyarsk (Skipperud et al., 2004; Lind et al., 2006)). In addition, accidents (e.g. the US B52 bomber accident at Thule, Greenland, the COSMOS satellite accident in Canada, the Komsomolets submarine accident at Bear Island) have contributed to local contamination.

A series of sources may also potentially contribute to radionuclide contamination of the Arctic in the future; such as nuclear weapons, old land-based reactors such as the Kola NPP and Bilibino NPP, reactor fuelled submarines in operations or waiting for decommissioning, as well as spent fuel stored under non-satisfactory conditions at the Kola peninsula (AMAP, 2002).

The source and the release scenarios, including characteristics such as temperature and pressure, are important determinants of the radionuclide speciation, and thereby their mobility, biological uptake and effects, that is, consequences for affected ecosystems. Source terms are usually estimated from the inventory, for example the amount of radionuclides released; their isotopic composition; physical-chemical form of release (i.e. gas, solution, aerosols); time development of the release; release point and plume height; and the energy content of the release. Following a serious event involving nuclear fuel, however, a major fraction of released refractory radionuclides such as actinides will most probably be associated with particles (Salbu and Lind, 2005). The particle matrix, the refractory radionuclide composition and isotopic ratios will reflect the specific source (e.g. burn-up), while the release scenarios (e.g. temperature, pressures, redox conditions) will influence particle characteristics of biological significance. The composition, particle size distribution and specific activity are essential for acute respiration and skin doses, while factors influencing weathering rates such as particle size distribution, crystallographic structures, porosity, and oxidation states are essential for long-term ecosystem transfer 5]. For areas affected by particle contamination, impact and risk assessments will suffer from large uncertainties unless the impact of particles is included. Therefore, radionuclide speciation as well as processes influencing speciation, uptake, accumulation and biological effects are essential for estimating exposures to living organisms.

Ecosystem Transfer

Radionuclides released from a source may be present in different physico-chemical forms (e.g. low molecular mass species (LMM), colloids, pseudo-colloids, particles) influencing biological uptake, accumulation, doses and biological effects in flora and fauna. LMM species and colloids are believed to be mobile, while particles are easily trapped (Salbu, 2000). If mobile species are present, ecosystem transfer is relatively fast, whereas the ecosystem transfer is delayed if particles are present. Soil–water or sediment–water interactions are usually described by distribution coefficients, K_d, assumed to be constants at equilibrium. The speciation of radionuclides deposited in the environment will, however, change with time due to interactions with components in soils or sediments (Hinton et al., 2007; Skipperud et al., 2000a, b). Due to interactions with humic substances, the mobility of LMM-species is reduced. Due to weathering of particles, associated radionuclides are mobilised and the ecosystem transfer increases with time (Salbu, 2000; Skipperud et al., 2000a,b). Thus, the distribution of radionuclides between solid and solution is a time depended process and the thermodynamic constant should be replaced by a time-function.

The speciation of radionuclides is of importance for biological uptake, accumulation and biomagnification. LMM-species can cross biological membranes, directly or indirectly after interactions with ligands or carrier molecules. LMM organic ligands such as citrate may stimulate the uptake, while high molecular mass (HMM) organics (e.g. Prussian Blue) reduce uptake and are used as countermeasures for Cs-isotopes (Salbu, 2000; Salbu et al., 2004). Information on bioavailable forms is, however, still scarce. For soil-to-plant transfer, transfer coefficients; TC (m^2/kg), and for soil-plant-animal transfer aggregated transfer coefficients; T_{agg} (m^2/kg), are utilised for modelling purpose, and depend on several factors (e.g. soil types, microbial activities, plant- and animal species, dietary habits, trophic levels) and in particular on radionuclide speciation. Uptake in fish and invertebrates depends on ionic species interacting with external organs (gills, skin) or by digestive uptake. In filtering organisms, however, particles and colloids are retained and radionuclides may accumulate due to changes in bioavailability in the gut (digestion) or through phagocytosis. Bioconcentration factors (BCF) vary according to the radionuclide species in the exposure, degree of biomagnification and can be distinguished for different compartments within organisms. The link between Radionuclide speciation - K_d - T_{agg} - BCF, being time functions, represents a challenge within radioecology, with important implications for *radioecotoxicology* (Hinton et al., 2007; Salbu, 2007).

Effects from Radiation Exposure

Biological uptake, bioaccumulation and the radiation characteristics of radionuclides are important determinants of the magnitude of the environmental consequences following release. The receiving environments themselves also influence the scale of the consequences, since some organisms are more susceptible to incorporating radionuclides into exposure chains than others. Relationships between accumulation, dose and short and long-term effects (biological endpoints) are difficult to document and quantify, although biological responses from molecular to ecosystem level have been identified for different organisms in contaminated areas. When biological systems are exposed to radiation, free radicals are formed due to excitation and ionisation of water molecules in cells and Haber–Weiss and Fenton reactions (Fig. 3). Free radicals produced (·H, ·OH) are extremely reactive, will recombine and produce various reactive compounds in cells (e.g. HO_2, H_2O_2, H_2, O_2). Free radicals and the formation of reactive oxygen species (ROS) may affect membrane integrity and damage proteins and nucleic acids (DNA, RNA). Radiation induced free radicals can be identified as ROS and as enzymatic activity of antioxidant repair enzymes for example, superoxide dismutase (SOD), catalase or glutathione cycle enzymes (glutathione reductase and peroxidase). To identify early effects, expressions of metal-responsive genes, for example, cellular antioxidants, antioxidant enzymes, heme oxygenase, metallothionein, and information of cellular integrity and protein kinetics are equally important.

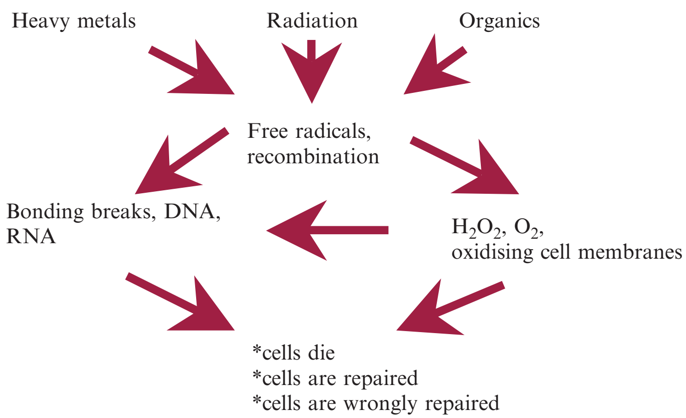

Fig. 3. Radiation induces free radicals in organisms, affecting sensitive biological endpoints: reproduction and immune system failures, genetic instability and mutation, increased morbidity and mortality. Other stressors such as metals and organics can also induce free radicals in organisms.

Dose-effect-risk relationships are based on a variety of biological endpoints ranging from molecular to ecosystem level. However, the evaluation of internal and external doses to biota using dosimetry models (e.g. equilibrium absorbed dose constant models, point source distribution models such as Loevinger's expression and Monte Carlo simulations) represents a challenge (Ulsh et al., 2003; Broion et al., 2006), in particular for uneven internal distribution of radionuclides. Recent data on Relative Biological Effectiveness (RBE) indicates that radiation with high linear energy transfers (LET) causes a greater degree of biological damage than low LET radiation for a given absorbed dose. Recent data implies also that RBEs for flora and fauna probably are different to those of humans, and calls for more research. Also at the mechanistic level there are gaps in knowledge with respect to low dose non-targeted effects of radiation such as genomic instability and bystander effects (Mothersill et al., 2006, 2007). These have been shown to occur in fish and mammals, but their impact on risk is uncertain, that is whether they are adaptive responses or they can magnify the damage.

The role of radionuclide speciation and internal distributions, exposure time associated with episodic accumulation and uneven distribution of doses (micro-dosimetry) inducing effects in sensitive biological endpoints for sensitive history life-stages for organisms is, however, still not understood, and improvement of speciation - low dose - early effect models is needed (Hinton et al., 2004). Problems also arise when benchmark concentrations are used to regulate doses and effects in the environment. The benchmark concentrations represent upper limits which, if exceeded, may result in harm to the environment. However, most benchmark values are derived from extrapolating toxicity data; from acute to chronic effects, from laboratory to field conditions, from effect concentration to no-effect concentration and from isolated test-species to complex systems. Thus, proper uncertainty estimates are essential.

However, radionuclides released into the environment rarely occur alone, but may co-occur in mixtures with other contaminants (e.g. metals, pesticides, organics, endocrine disruptors), which potentially could lead to effects in biological endpoints sensitive to radiation (Mothersill et al., 2006). One single stressor may induce multiple biological effects, if multiple interactions occur or if interactions with different biological targets take place. In mixtures with several different stressors, multiple types of interactions and interactions with multiple target sites may occur. Thus, the interactions may be concentration additive (1+1=2), synergetic (1+1=3 or 4) or antagonistic (1+1=0).

It is internationally recognised that there are severe gaps in basic knowledge with respect to biological responses from multiple stressor exposures. Identification of biological responses from low dose chronic exposure calls for early warning biomarkers, utilising modern molecular and genetic tools. Furthermore, information on dose-response relationships (on/off mechanisms), sensitivity (detection limits, thresholds), and synergetic and antagonistic effects, as well as the role of protecting agents such as antioxidants is highly needed. Development of analytical strategies, methods and biomarkers that can be utilised to increase the knowledge on biological impact from multiple stressors represents also a challenge for the future.

Conclusions

Radioecotoxicology is an essential discipline in environmental impact and risk assessments associated with radioactive contaminated ecosystems, linking radionuclide exposures to the subsequent biological effects in flora and fauna. However, a series of factors influencing the exposure must be taken into account, when doses are assessed or predicted. Similarly, a series of factors influencing the biological responses must be considered, contributing significant to the overall uncertainties in assessments. Adding the multiple stressor exposure and multiple response concept, the uncertainties increase with order of magnitude. Thus, meeting the challenges within *Radioecotoxicology* is essential to reduce the overall uncertainties in environmental impact and risk assessments for contaminated areas.

References

AMAP 2002. Arctic pollution Issues: A state of the Arctic environment report. Oslo, Norway.

AMAP 2002. AMAP Assessment 2002: Radioactivity in the Arctic. Oslo, Norway.

Brown JE, Hosseini A, Borretzen P, Thorring H. 2006. Development of a methodology for assessing the environmental impact of radioactivity in Northern Marine environments. *Mar. Pollut. Bull*. 52:1127–1137.

Hinton TG, Bedford JS, Congdon JC, Whicker FW. 2004. Effects of radiation on the environment: a need to question old paradigms and enhance collaboration among radiation biologists and radiation ecologists. *Radiat Res* 162:332–338.

Hinton T, Garten CT, Kaplan DI, Whicker FW. 2007. Major biogeochemical processes of radionuclide dispersal in terrestrial environments. *Rad. Ass*. in press.

Lind OC, Oughton DH, Salbu B, Skipperud L, Sickel M, Brown JE, Fifield LK, Tims SG. 2006. Transport of low 240Pu/239Pu atom ratio plutonium in the Ob and Yenisey Rivers to the Kara Sea. *Earth Planet. Sci. Lett*. in press.

Mothersill C, Bucking C, Smith RW, Agnihotri N, Oneill A, Kilemade M, Seymour CB. 2006. Communication of radiation-induced stress or bystander signals between fish in vivo. *Environ Sci Technol* 40:6859–6864.

Mothersill C, Salbu B, Heier LS, Teien HC, Denbeigh J, Oughton DH, Rosseland BO, Seymour CB. 2007. Multiple stressor effects of radiation and metals in salmon (Salmo salar). *J. Environ. Radioact*. April 9th epub.

Salbu B. 2000. Speciation of radionuclides in the environment. In Meyers RA, ed, *Encyclopedia of Analytical Chemistry*, John Wiley & Sons Ltd, Chishester, pp 12993–13016.

Salbu B. 2001. Actinides associated with particles. In Kudo A, ed, *Plutonium in the Environment*, First ed, Elsevier, Tokyo, pp 121–138.

Salbu B. 2007. Speciation of radionuclides – Analytical challenges within environmental impact and risk assessments. *J. Environ. Radioact*. April 30th epub.

Salbu B, Lind OC. 2005. Radioactive particles released from various nuclear sources. *Radioprotection* 40:27–32.

Salbu B, Krekling T, Oughton DH, Ostby G, Kashparov VA, Brand TL, Day JP. 1994. Hot particles in accidental releases from chernobyl and windscale nuclear installations. *Analyst* 119:125–130.

Salbu B, Janssens K, Krekling T, Simionovici A, Drakopoulos M, Raven C, Snigireva I, Snigirev A, Lind OC, Oughton DH, Adams F, Kashparov VA. 2000. X-ray absorption tomography and – XANES for characterisation of fuel particles. *ESRF Highlights* 24–25.

Salbu B, Skipperud L, Germain P, Guegueniat P, Strand P, Lind OC, Christensen G. 2003. Radionuclide speciation in effluent from La Hague reprocessing plant in France. *Health Phys*. 85:311–322.

Salbu B, Lind OC, Skipperud L. 2004. Radionuclide speciation and its relevance in environmental impact assessments. *J. Environ. Radioact*. 74:233–242.

Salbu B, Rosseland BO, Oughton DH. 2005. Multiple stressors – a challenge for the future. *J. Environ. Monit*. 7:1–2.

Skipperud L, Oughton D, Salbu B. 2000. The impact of Pu speciation on distribution coefficients in Mayak soil. *Sci. Total Environ*. 257:81–93.

Skipperud L, Oughton DH, Salbu B. 2000. The impact of plutonium speciation on the distribution coefficients in a sediment-sea water system, and radiological assessment of doses to humans. *Health Phys*. 79:147–153.

Skipperud L, Oughton DH, Fifield LK, Lind OC, Tims S, Brown J, Sickel M. 2004. Plutonium isotope ratios in the Yenisey and Ob estuaries. *Appl. Radiat. Isot*. 60:589–593.

Ulsh B, Hinton TG, Congdon JD, Dugan LC, Whicker FW, Bedford JS. 2003. Environmental biodosimetry: a biologically relevant tool for ecological risk assessment and biomonitoring. *J Environ Radioact* 66:121–139.