Radioecology

Editorial

Radioecology (radiation ecology) is the scientific field encompassing the relationships between ionizing radiation or radioactive materials and the environment or subunits thereof. Its study constitutes an important component of radiological protection of both humans and environment through its relevance in understanding and describing environmental exposure pathways and quantifying radionuclide transport along them. Such pathways can be described as the route radioactive substances take from their source to their end point and how humans or biota can be exposed to the substance. There is acceptance that one can not assume that if humans are protected from radioactive contaminants, then so is the environment. This acceptance has led to consideration of environmental exposure pathways that are not strictly directed towards the human endpoint but which are of relevance to wildlife species. This expansion in the scope of the pathways and species that must be considered has presented new challenges for radioecology. With greater reliance on nuclear power to meet the increased energy demand, there is need for a system to protect the environment from ionising radiation. The focus has been on collecting relevant information and developing approaches to enable regulatory assessments to demonstrate whether the environment is adequately protected from exposure to ionizing radiation resulting due to authorized releases. There is an international debate on a possible evolution of the current system of radiological protection in order to make the system more coherent, concise and cost-effective.

This bulletin introduces the basics of radioecology and discusses the role of radioecology with respect to environmental exposure pathways for radioactive contaminants. It presents the fundamental concepts of radioecology, the tools used by radioecologists with respect to the study of exposure pathways and future directions of research in the field. I am thankful to Dr. S.K. Jha for his excellent support as the Guest Editor.

CONTENTS

Understanding the Transfer of Radionuclides in the Environment

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Introduction

After many years of reluctance to consider nuclear energy as an alternative to conventional or renewable energy production systems the situation in the last couple of years has changed also due to climate change and the corresponding need for reduction of carbon emissions. Nowadays countries specifically from the developing world are exploring the potential to embark on nuclear power although it is a high investment and not fully accepted by the public; in addition "experienced users" are considering the extension of lifespan of existing Nuclear Power Plants. With this often cited "nuclear renaissance" the need to deeper and more comprehensively understand the behaviour of radionuclides in the environment is becoming increasingly important as nuclear energy production results in releases of radionuclides into the environment and thus in exposures and radiation doses to humans and biota. Also the management of radioactive waste is a general problem which will need further considerations and requires sustainable solutions.

Protection of the environment has become one of the key issues in the processes for approving any industrial activities in many countries. The level of societal concern for the environment internationally is indicated in documents reflecting international consensus, notably the report of the Brundtland Commission (Bruntland Commission, 1987), the Rio Declarations on Sustainable Development (United Nations, 1982), and a Joint Convention of the IAEA (1997) stating that present generations should not compromise the ability of future generations to fulfil their needs for living in a healthy environment.

Many data in the radioecological science and research have been produced either in field or laboratory experiments, or are derived from models and applications of concentration factors of stable elements and analogues in corresponding media, most of them in the European, Nordic and Northern American countries. However it is well understood that the site specifics of radionuclide environmental behaviour is one of the critical factors influencing the resulting dose as does consumption attitudes and behaviours which all can vary spatially tremendously. Therefore still only few experimental or measured data are available of have been published for tropic or special environments such as the Antarctic, the Rainforest or arid areas, and data for a limited number of radionuclides only are published in the open literature or are available.

Due to new techniques and developments in Nuclear Energy production and the increasing use of nuclear science worldwide the fate and behaviour of a variety of radionuclides, their environmental behaviour and resulting impact during operations, releases and final disposition needs to be studied in more detail and should cover other non-European and Northern American conditions, cultures and practices than presently. Specifically radioecological sensitive or vulnerable areas or populations groups resulting in higher than expected radiation doses due to environmental parameters or special behaviour such as consumption and living habits need to be identified, and accounted for in order to ensure adequate environmental sustainability and radiation protection.

In the following recent and ongoing activities initiated by the IAEA with the support of scientists in its Member States are presented addressing above items and providing recommendations and solutions to some of the problems identified.

IAEA Documents Related to Protection of the Environment

Since its foundation the IAEA promotes the peaceful and safe use of various nuclear technologies within a safety and security regime including protection of the general public and the environment against ionizing radiation. In particular, in March 1960, the Board of Governors of the IAEA approved main directions for the IAEA involvement in nuclear safety and stated that "the Agency's basic safety standards will be based, to the extent possible, on the recommendations of the International Commission on Radiological Protection (ICRP). Since then, the IAEA is promoting the establishment and application of world standards, and provides accordingly supporting documents, for the protection of humans and the environment against ionizing radiation in close cooperation with the ICRP and in considerations of its relevant publications (ICRP Publication 2006, 2007, 2008).

Currently, the system of the IAEA environment related documents includes two main categories of publications, namely, documents of the IAEA Safety Standards Series and other IAEA environment related series.

The publications by means of which the IAEA establishes safety standards and radiation protection measures are publications of the IAEA Safety Standards Series. These publications cover all nuclear and radiation safety aspects, and are structured into the Safety Fundamentals, Safety Requirements and Safety Guides. Safety Fundamentals state basic objectives, concepts and principles for safety and radiation protection. The current Safety Fundamentals provides basic safety principles building the bases for the IAEA safety standards and its safety related programme (IAEA, 2006). Principle 7 on protection of present and future generations declare that "People and the environment, present and future, must be protected against radiation risks". Safety Requirements

provide the requirements that must be satisfied to ensure safety and radiation protection while Safety Guides recommend actions, conditions or procedures for meeting defined safety requirements. However, it should be recognised that all these requirements, recommendations and guides are not legally binding for any Member State but can be used as a basis for updating and revision of national regulations by adopting international recommendations for own activities and demands. At the same time the standards are binding for the IAEA in relation to its own operations and are binding for States when assisted by the IAEA e.g. via technical cooperation (TC) projects.

The IAEA Basic Safety Standards (BSS) series is the best example of such international regulating documents. The first BSS was issued by the IAEA in 1962 (IAEA, 1962) and successively revised in 1967, 1982 and 1996. The most recent BSS (IAEA, 1996), which is currently undergoing revision, was mainly based on the ICRP publication 60 (ICRP). The new BSS will be based on ten main principles which were first presented in the Safety Fundamentals published in 2006 (IAEA, 2006) and adopts statements and recommendations presented in the ICRP publication 103 (ICRP, 2007). Although, the present system of radiation protection measures generally accounts for appropriate protection of ecosystems against harmful effects of radiation exposure, the international trends in this field clearly show an increasing awareness of the vulnerability of the human environment. They also indicate the need to demonstrate (rather than to assume) that the environment is protected against effects of any industrial pollutants, including radionuclides, for a wider range of environmental situations, irrespective of any human connection with them. The new BSS statements mainly follow this line, stating also that radiation impacts on a particular environment constitute only one type and in most cases may not be the dominating impact of a particular facility or activity but is subject to a variety of confounding factors. Further, the assessment of any impact on the environment should be viewed in an integrated and holistic manner with all other key features of the system to be able to establish the conditions applicable to a particular source. Overall the new BSS is designed to identify protection of the environment as an issue to be assessed, while leaving flexibility to incorporating relevant results into the appropriate decision making processes.

Thus, the BSS establishes requirements as given in the IAEA Safety Standards Series No. WS-G-2.3 "Regulatory Control of Radioactive Discharges to the Environment" (IAEA, 2000) or Safety Standards Series No. WS-R-3 "Remediation of Areas Contaminated by Past Activities and Accidents" (IAEA, 2003) mainly describing how to apply the Safety Fundamentals and the BSS in the control of discharges of radionuclides to the environment from normal operation and sources within practices.

Further the IAEA also disseminates and fosters the exchange of information on peaceful nuclear activities and serves as an intermediary among its Member States for this purpose. Reports on nuclear safety and protection are issued in other report series, in particular the IAEA Safety Report Series, as informational publications. Safety Reports may describe good practices and give practical examples and detailed methods that can be used to meet safety requirements. They do not establish requirements or make recommendations.

In particular, the IAEA Safety Report Series 19 (Generic Models for Use in Assessing the Impact of Discharges of Radioactive Substances to the Environment) provides a set of models, methods and parameters for calculating doses arising from radioactive discharges into the environment (IAEA, 2001). Recently published SRS 64 (IAEA, 2010) provides information on the design and operation of sources, environmental monitoring programmes and systems to control radionuclide releases and public exposure due to direct radiation from both nuclear and non-nuclear facilities and in emergency situations, whilst SRS 34 describes good practices in management of radioactive waste in the oil and gas industry (IAEA, Safety Reports Series No.34, 2003).

Other IAEA informational publications are the Technical Report Series, the Radiological Assessment Report Series, Technical documents and the INSAG Series. The IAEA also issues reports on radiological accidents and updates information in the TECDOC Series, the Provisional Safety Standards Series, the Training Course Series, the IAEA Services Series and the Computer Manual Series, and Practical Radiation Safety Manuals and Practical Radiation Technical Manuals.

Overall, these documents provide data for use in radiological assessments of routine discharges of radionuclides to the environment. In particular, two publications of the TRS level, namely, Sediment Kds and Concentration Factors for Radionuclides in the Marine Environment (Technical Reports Series No. 247), published in 1985 (IAEA, 1985), and the Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments (Technical Reports Series No. 364), published in 1994 (IAEA, 1994), provided a full set of available transfer parameter values for the marine, freshwater and terrestrial environments. For many years, these publications have served as key references for radioecologists, modellers and authorities for risk assessments. Another key TRS documents was TRS 433 published in the early 1990th which summarised all available up-to-date information on biological effects of radiation and established for the first time the radiation doses which could be considered as safe for non-human species at the population level (IAEA, 1992).

Publications of the Radiological Assessment Reports Series present results of environmental assessment studies performed in particular sites, such as the Chernobyl accident affected region (IAEA, 2006a, 2006b), sites of nuclear weapon testing as the atolls of Mururoa, Fangataufa and Bikini, Semipalatinsk test site in Kazakhstan, former French

nuclear test sites in Algeria (IAEA, 1998 a,b,c; IAEA, 2005) or other radiation legacy sites (IAEA, 1998d; IAEA, 2000).

The TECDOC series provides technical details describing key transfer processes, concepts and models that were found to be important for radiation safety including examples of best practices. The key environment related examples of this series are the IAEA- TECDOC 1616 uantification of Radionuclide Transfers in Terrestrial and Freshwater Environments for Radiological Assessments" (IAEA, 2009), IAEA-TECDOC-918 Health and Environmental Aspects of Nuclear Fuel Cycle Facilities, IAEA-TECDOC-918 (IAEA, 1996) and a set of TECDOCs describing various aspects of the environmental remediation (IAEA, 1998e,f; IAEA 1999 a,b and IAEA, 2002).

The Revision of the Technical Report Series: Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments

In 1994, the IAEA published the Technical Reports Series No. 364 (TRS 364) "Handbook of parameter values for the prediction of radionuclide transfer in temperate environments". Although of its wide use and valuable information provided the TRS 364 had three major limitations: First, the parameters given were mainly limited to temperate environments. Second, it gave parameters for equilibrium conditions, and, therefore, they often cannot be applied for environmental assessments in accidental situations or to situations with variable release of radionuclides into the environment, and third, the there was no information on transfers of some radionuclides that are of importance for waste management.

Since the publication of the TRS 247 (in 1985), covering marine environments and TRS 364 (in 1994), covering terrestrial and freshwater environments many publications, specifically as a consequence of studies following the Chernobyl accident, have been published which merit to be taken into consideration and to be included for environmental assessments. In 2003, within the framework of the Environmental Modelling for Radiation Safety (EMRAS) project several initiates were started to address existing gaps.

The overall objective of the TRS-364 revision therefore was to provide both, updated and revised transfer parameter values and completing missing data, as well as to provide information on key transfer processes, concepts and models which have been found to be important for radiation safety. The TRS 472 has been published in 2009 as andbook of Parameter Values for the Prediction of Radionuclide Transfer in Terrestrial and Freshwater Environments and is accompanied by the IAEA TECDOC 1616 "Quantification of Radionuclide Transfer in Terrestrial and Freshwater Environments for Radiological Assessments" intended for presentation and justification of radioecological information used to derive reference values, radioecological concepts and models facilitating simultaneously the use of reference values in specific situations. The TECDOC provides additional information to tables recommending transfer parameter values definitions, concise descriptions of processes and parameters, and referring to the details in the according TECDOC chapters.

As mentioned above, it is essential that parameters used for radiological assessment are kept up-to-date and this, in itself, is a strong argument for regular revisions. TRS 364 was based only on information collected until 1992, and most of relevant literature derived from research conducted after the Chernobyl accident was published later. Besides, the scope of TRS 364 had three major limitations: Firstly, the parameters given are limited to temperate environments. Secondly, parameters apply mainly for equilibrium conditions, and, therefore often cannot be applied for accidental situations or for situations with variable release of radionuclides into the environment. Finally, TRS 364 does not provide information on transfers of some radionuclides that are of importance in waste management practice.

The new document comprises all available information on arctic, temperate, tropical and subtropical environments and all radionuclides as appropriate. At the same time, the data remain to be relevant mainly in equilibrium conditions, although some data relevant to time dependency are included in this document such as weathering and translocation during foliar uptake, long-term dynamic of root uptake, and behaviour of some semi-natural ecosystems.

In the frame of the TRS 364 revision, new soil and plant classifications have been developed providing straightforward links with the International (FAO) and other national classification systems. Such unified classifications used across the document provide a harmonised presentation of the transfer parameters and facilitate their use for application for site-specific situations.

Parameter values presented in the updated document clearly documented to help the modeller to choose with full knowledge the different values presented in the tables. The data in this new TRS are given with geometric mean, geometric standard deviation, number of observation, observed minimum and maximum values where possible. Beside corresponding information on the context, the limitations of the use of the data are indicated in every chapter. In cases where values are not available, the analogue approach is suggested to compensate for missing data and to give guidance, on how to derive, with caution, parameter values.

The individual chapters cover the classical radioecological processes such as deposition, foliar and root uptake, radionuclide mobility in soil, transfers to animals, radionuclide transfers in forest and freshwater ecosystems including contamination routes, physical processes and radionuclide accumulation in food products and biota species. However the documentation now provides more information and contains dynamic models which allow a more accurate description of the processes and derive more reliable prediction results for environmental assessments and radiation protection purposes.

Environmental Dimension of the INPRO Project

The International Project on Innovative Nuclear Reactors and Fuel Cycles (INPRO) was launched in the year 2000, based on resolutions of the IAEA General Conference. INPRO is intended to help to ensure that nuclear energy is available in the 21st century in a sustainable manner, bringing together interested Member States, both technology holders and technology users, to jointly consider actions to achieve desired innovations (IAEA, 2005).

The INPRO project is playing a unique role for the sustainable use of nuclear energy, and its mission is to provide a forum where experts and policy makers from industrialized and developing countries can discuss technical, economical, environmental and all other aspects of the deployment of Innovative Nuclear Energy Systems (INS) in the 21st century (IAEA, 2005). Protection of the environment has become a major consideration in the processes for approving industrial activities in many countries and is a central theme within the concept of sustainable development. There is a growing understanding that nuclear power supports sustainable development by providing much needed energy with relatively low burden on the environment.

The term "environment" is defined within the laws and regulations of various jurisdictions. It generally includes the following components: human beings; non-human biota; abiotic components, including soil, water and air, natural resources and landscape; and interactions among these components. Figure 1 illustrates the sources, stressors, environmental pathways and end points involved in any assessment of environmental effects and as used within the INPRO methodology.

Environmental effects considered within the INPRO assessment approach include: physical, chemical or biological changes in the environment; health effects on people, plants and animals; effects on quality of life of people, plants and animals; effects on the economy; use/depletion of resources; and cumulative effects resulting from the influence of the system in conjunction with other influences on the environment.
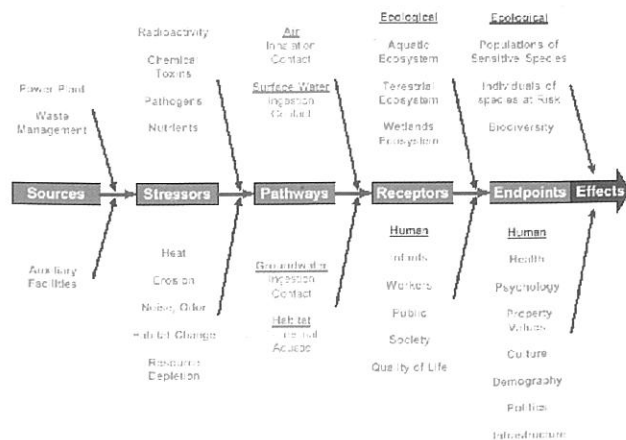

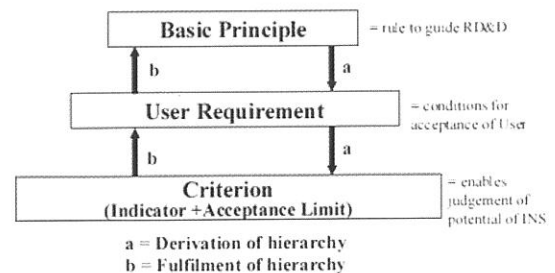Fig. 1   Factors in environmental assessments

Fig. 2   INPRO hierarchy of demands on innovative designs of nuclear energy systems

The INPRO methodology comprises Basic Principles (BP), User Requirements (UR), and Criteria (CR) and the INPRO method of assessment in a hierarchical structure, as demonstrated in Fig. 2. The INPRO Indicators (IN) and Acceptance Limits (AL) are also defined for each INPRO element.

The purpose of the INPRO assessment is either to confirm an adequate environmental performance of the INS, i.e. fulfilling all INPRO environmental criteria, or the identification non-compliance with INPRO criteria requiring corrective actions to become adequate.

Two major basic principles are defined for environment within the INPRO methodology to achieve the above purpose:

Basic Principle 1 (BP 1) requires acceptability of expected adverse environmental effects and reads as: "The expected (best estimate) adverse environmental effects of the innovative nuclear energy system shall be well within the performance envelope of current nuclear energy systems delivering similar energy products". Figure 3 illustrates this statement.

Each stressor in either the INS, or in a current nuclear energy system (CNS) chosen for comparison, is represented by a vector whose length is proportional to the level of the stressor. The radius of the circle passing along the vector represents the standard for that stressor. In this way each stressor can be represented relative to its standard, and all standards will lie on the circumference (red circle in Fig. 3). The number of stressors illustrated is arbitrary and the relative magnitude of vectors representing different stressors is not meaningful. Stressors arising from the CNS are shown as blue arrows, and their magnitude is denoted as L_{CNS-i}. The green arrows represent the stressors arising from an INS and their magnitude is denoted by L_{INS-i}. Each environmental stressor from an INS must be located inside the red circle (i.e. must meet its standard). A current system may or may not be entirely inside the circle, depending on whether the current standards are different from those that were applied when the CNS was implemented, as illustrated by L_{CNS-4}. When all the stressors are considered, the performance envelope of an INS (dotted line, green) should be well within the performance envelope of the CNS (dashed line, blue). This does not mean that the magnitude of all stressors of the INS
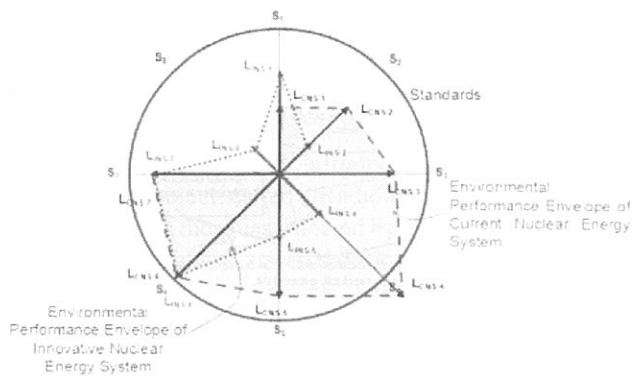
Fig. 3   Environmental performance envelopes

must be smaller than that of the stressor in the current energy system; rather, that, on balance, any stressor that is greater in the INS should be more than compensated by other stressors being lower.

The second user requirement is focused on application of ALARP (as low as reasonably practical) concept, demonstrating that all practical means have been used to protect the environment, i.e. confirming that any further reduction of risks (stressors) would be impracticable or grossly disproportional to the improvement that could be gained.

Within these assessments the assessor should prove that the environmental stressors from each part of the INS over the complete life cycle are controllable to levels meeting or superior to current standards and to demonstrate that the likely adverse environmental effects attributable to the INS should be as low as reasonably practicable, social and economic factors taken into account.

The second environmental basic principle (BP2) declare that the INS shall be capable of contributing to the energy needs in the 21st century while making efficient use of non-renewable resources (Fitness for purpose). This BP results in a need to consider a set of used requirements including: consistency with resource availability without running out of fissile/fertile material or other non-renewable materials and demonstration that the energy output of the INS should exceed the energy required to implement and operate the INS within an acceptably short period.

The INPRO basic principles and user requirements call upon the accomplishment of the environmental sustainability level thus resulting in an increasing public acceptance of an INS; the assessor should strive to achieve a better environmental performance of the INS compared to existing nuclear energy systems, or at least not worse for all of its components (exceptions for a limited number of stressors must be justified on the basis of compensation either in the component or in other components, to make the INS 'better' as a whole).

TECDOC 1575 provides practical applications within the INPRO Manual-Environment (Volume 7). Besides recommendations on how to use of the INPRO methodology,

this document also provides general guidelines on data, calculation methods and computer tools to be used to evaluating stressors, availability of resources and impacts on humans and biota.

Environmental Remediation

After contamination of ecosystems, independent upon its contamination scenario, remediation actions will be implemented to reduce exposures to man and biota and to mitigate negative effects. However these measures also contribute to pertain or regain trust by the public and the affected population in the authorities' actions and reactions. However, experience has shown that physical and chemical large scale remediation actions –although they might be effective- will depend on many additional factors which have to be taken into account when trying to recover or remediate, and even rehabilitate a radioactively contaminated area (Voigt & Fesenko 2009). A variety of examples such as the EUT (East Ural Trail(and the Mayak releases in the Russian Federation, be it releases due to nuclear bomb testing in Semipalantisk, Kazakhstan or Novosemlija in Russia, or Maralinga and the Bikini atolls have clearly demonstrated that further considerations specifically the inclusion of the affected population and local stakeholders is a must to be fully successful (Fesenko et al., 2009).

Further, not only procedures and physical and chemical treatments which will help to reduce the radioactivity concentrations and as a consequence internal or external radiation exposures will have to be implemented, also the contamination scenario, local conditions, the nature of the ecosystem affected und human interaction with the affected ecosystem for recreation, food production and any other income, has to be taken into account. The social-ethical consequences of radioactive contamination are often of much higher impact on health conditions than the radiation exposure itself. Thus the contributions of both to a deteriorating physical and mental health status of an affected population are difficult to differentiate and epidemiological studies often lack statistical significance due to the low cohort numbers, limited follow up times or lack of comparable reference groups.

In addition side effects resulting from remediation activities might become sometimes more costly or impacting on natural resources more seriously as originally planned or even anticipated. These might stem from the remedial action itself or from the changed ecological conditions and often result on a tremendous cost implication in the years to follow. Thus these costs have to be part of the cost-efficiency calculations when starting with any remediation strategy and action.

Because of these multiple factors affecting the efficiency and suitability of each countermeasure, generalised recommendation which do not account for diversity often result in inadequate decisions when applied at a local scale. These considerations have led to a need for a development of practical environmental decision support
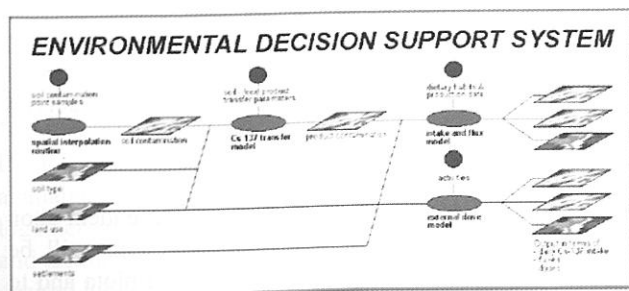
ENVIRONMENTAL DECISION SUPPORT SYSTEM

Fig. 4 *Schematic presentation of the EDSS to model radionuclide transfer through the food chain*

Fig. 5 *Decision making tree on the application of countermeasures in contaminated forests (Fesenko et al. 2006)*

systems (EDSS) which take into account the temporal and spatial variation in the above factors and are capable for providing advice on countermeasure strategies at different levels of the decision making process.

Due to modern technology, IT and developed tools such as Geographical Information Systems and Multi-Criteria Decision Support systems which allow to integrate spatial with temporal and additional criteria into one decision making system. The general structure of a environmental decision support system is outlined in Fig. 4 while Fig. 5 illustrates a framework of decision making tree for the application of countermeasures considering contaminated forests as an example. Today it is possible to make sound decisions taking even factors into account which are difficult to quantify but which allow a qualitative assessment, and thus to reach more holistic and sound recommendations. These tools have been developed but did not yet find its full implementation into regular emergency preparedness systems or into remedial strategies from the past.

Following the Chernobyl accident different EDSS have been produced (Fesenko et al., 1996; Van der Perk et al., 1998; Gillet et al., 2001) which have enhanced the ability to optimize remediation strategies in contaminated areas.

In September 2009, the IAEA established the Network of Environmental Management and Remediation (ENVIRONET) with the aim to facilitate sharing and exchange of knowledge and experience amongst organizations with advanced environmental management and remediation programmes in place. This provided a forum to discuss good practices, identifying and treating improper past operations and assuring the longer term knowledge management in support of public and environmental protection and site monitoring (to be published in 2010/11). The ENVIRONET has been established to disseminate international experience in the application of best and proven practices for remediation of radiologically contaminated sites including issues related to stakeholder involvement (communication, participation in the decision making process, etc). The network covers both
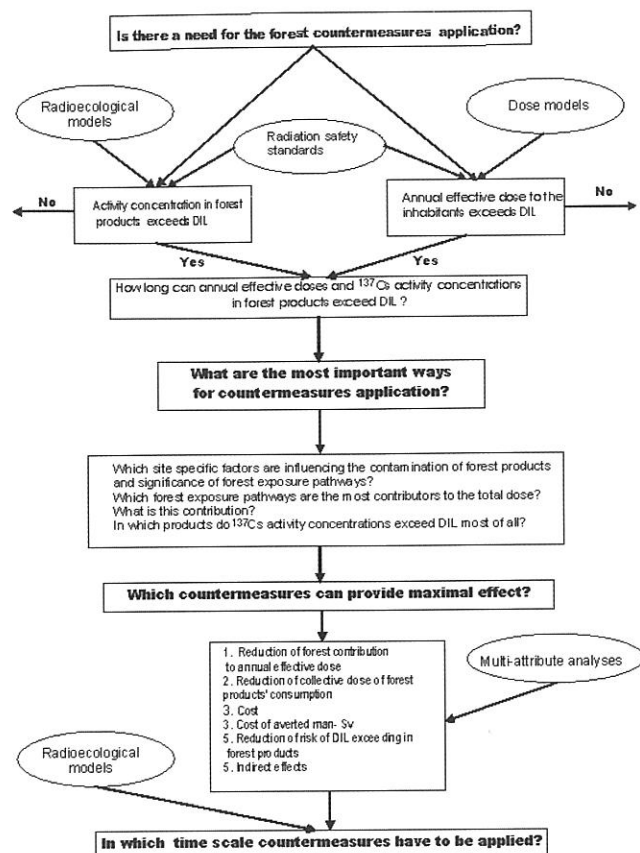
the radiation legacy sites and on-going the life-cycle facilities.

Additionally, a set of Technical Cooperation projects were recently started in 2008 with overall objectives to support national and regional supports in remediation of sites contaminated mainly by NORMS. Regional TC project entitled "Supporting Preparation for Remediation of Uranium Production Legacy Sites in Central Asia" and national projects: "Establishment of Radioecological Monitoring and of Rehabilitation Programmes for the Contaminated areas of the Absheron Peninsula" (Azerbaijan), "Monitoring and Assessing Naturally Occurring Radioactive Materials from the Oil Industry in Kuwait", Managing Naturally Occurring Radioactive Materials in the Oil and Gas Industry in Libya can be considered as good examples of such a cooperation (see more www.TC-iaea.org).

More recently the International Commission on Radiological Protection (ICRP, 2007) revised its approach to characterize situations in which exposure to humans may occur and introduced a concept of a Representative Person for radiation protection purposes[1]. These changes led to a need for corresponding revisions of current approaches to

[1]The Representative Person is defined as a person, "who will ... be a hypothetical construct, receives a dose that is representative of the more highly exposed individuals in the population". (ICRP, 2007)

remediation planning and optimizing remediation strategies. First attempt to apply this new concept for decision making was made within the TC regional Chernobyl project (Jacob et al., 2010).

In 2008, the IAEA initiated a regional technical cooperation project called "Radiological support for the rehabilitation of the areas affected by the Chernobyl nuclear power plant accident" and to date continues this activity. In the framework of these projects, a new methodology as well as a software tool called "ReSCA – Remediation Strategies after the Chernobyl Accident" for optimising rehabilitation strategies for the affected areas were developed and validated (Jacob et al., 2009, Ulanovsky et al., submitted). The tool is based on two decades of experiences in implementation of countermeasures against radioactive contaminations in the aftermath of the Chernobyl accident (IAEA, 2006). The dose to the Representative Person is a main radiological criterion in optimizing process of remediation within the ReSCA tool. The ReSCA tool considers remedial options in three aspects: radiological, economic and social ones. Overall, such approach provides an opportunity of making flexible decisions within the limitation on funds allocated for remedial purposes.

The expression below is used for the prioritization of the remedial actions:

$$\beta \cdot \frac{\min(CD_r)}{CD_r} + (1-\beta) \cdot DA_r$$

where CD_r is the cost of 1 man-Sv being averted as a result of application of the remediation option r; DA_r is the degree of acceptability of the corresponding action. Parameter β allows the user to give preferences either to economic or to social aspects of the remediation planning. Thus, for the value of $\beta=1$ the remedial actions are ranked according to the costs per averted dose, while for the minimum $\beta=0.01$ the ranking is mainly based on acceptability of remediation actions. The remediation strategy is being sequentially built as a list of separate remediation actions until the total cost exceeds available funds allocated for remediation purposes or there are no more settlements with annual dose exceeding control dose limit or there are no more possible remedial actions to undertake or the remaining possible actions are too costly (typically, more than 100 thousand Euro per man-Sv averted). Thus, for the given input and model parameters, several strategies can be generated varying the amount of available funds and/or user priorities.

In order to illustrate the tool applications we used data compile for areas affected by the Chernobyl accident. The assessments were made based on information obtained from rural settlements, where annual effective doses exceeded 1 mSv in 2004. For each of these settlements, calculations of the effective doses to the Representative Person defined by the sum of the averages of the upper deciles of the effective dose distributions from external and internal exposures were made at the initial stage. All such settlements, where according to the ReSCA calculations 2004 annual doses in 2004 exceeded 1 mSv, were defined as 'affected settlements' and were therefore eligible for consideration as a subject of remedial actions implementation.

Among the 545 settlements, listed in the national dose catalogues, there were only 290 settlements, in which in 2004 the effective dose for the Representative Person exceeded 1 mSv. In total, these affected settlements had 78 172 inhabitants and most of them (57 960) lived in the Russian settlements. The number of settlements with annual doses exceeding 1 mSv is expected to decrease due to natural processes and radioactive decay until 2020 to 121 settlement with 35044 inhabitants. Thus, without remedial actions the number of inhabitants in settlements with annual doses exceeding 1 mSv would decrease slowly. Collective dose assessed for 2004 for the affected settlements is about 65 man-Sv, three quarters of this occurring in Russia (Jacob et al., 2009). The distributions of the doses of external and internal exposures in the affected settlements differ in the three countries: in Belarus, external exposure dominates; in Russia, both pathways are equally important; in Ukraine, the dose is mostly due to internal exposure. In about half of the Belarusian and Russian affected settlements, the annual dose from consumption of mushrooms and forest products is comparable to the annual dose from milk. In Ukraine, however, milk is a major source of internal exposure in most of the affected settlements.

As in the case of the test settlements, in order to consider possible alternatives the assessments were made for two different remediation strategies for the year 2010: the social strategy which gives a higher importance to public acceptability of the suggested remedial actions, and the radiological strategy which is based on minimisation of costs per averted dose. Social remediation strategy was derived for β equal to 0.1, i.e., the degree of acceptability of the remedial actions was considered to be important for developing the strategy. Radiological remediation strategy was derived for β equal to 1.0, i.e., only costs per averted dose are taken into account in the process of optimisation.

The effect of the remediation is dependent on both site-specific factors, which are directly included in the analysis, and availability of funds for remediation purposes. Thus, the relationships, reflecting dependence of averted doses on the remediation costs, turn out to be different among affected countries (Fig. 6). In Belarus the trends, reflecting increase of the averted dose with the invested funds, become similar for two strategies considered if funds available for remediation purposes exceeds 1 M and in Ukraine if they are higher 0.1 M (Fig. 4). In Russia, the significant difference between two strategies persists up to several M of available funds, because of the larger number of the affected settlements (Fig. 4).

In contrast to Belarus and Ukraine, the cost-effectiveness of two strategies is similar in Russian affected settlements, if the available resources are below 0.5 M, while the social strategy begins to be less cost-effective
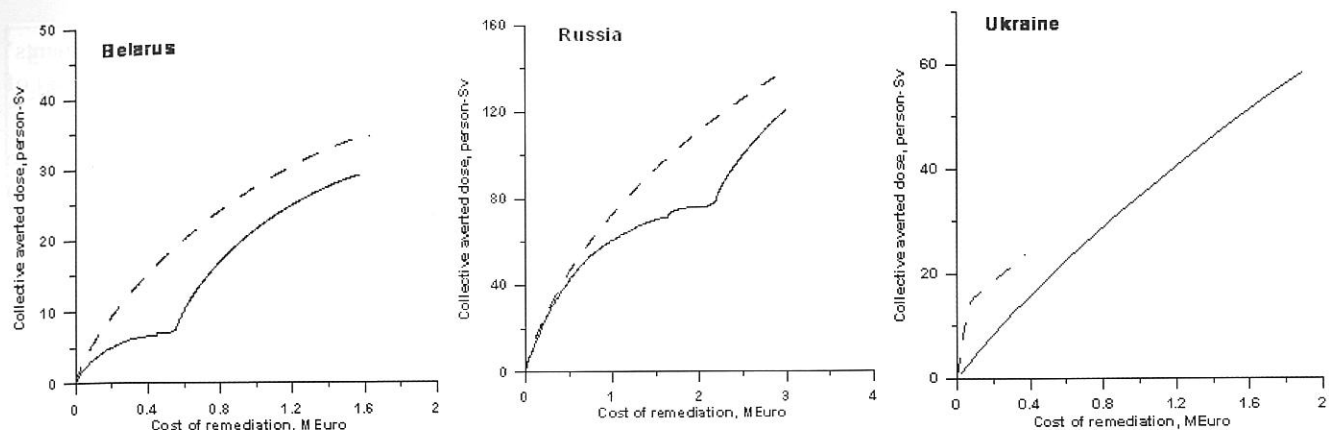
Fig. 6   Total averted collective dose (person-Sv) as function of funds invested in remediation in three countries. (Solid line corresponds to the social strategy and dashed line to the radiological strategy)

compared to the radiological strategy at higher expenditures (see Fig. 4).

The radiological strategy pays more attention to chemical effectiveness: here first to applications of ferrocyn to cows, this action has to be applied continuously, and then, in Belarus and Russia, the strategy recommends removal of highly contaminated soil from the populated areas. This second action, although being quite effective in reduction of external doses to the population, raises substantial problems (and costs) with disposal of the contaminated soil.

Overall, the social strategy is considerably less cost-effective and requires larger resources for remediation. However, compared to international values for the cost-effectiveness of actions for reducing occupational exposures, both remediation strategies are still quite cost-effective, varying from 14 k /person-Sv (Ukraine, the radiological strategy) to 47 k /person-Sv (Belarus, the social strategy). It should be also mentioned that in terms of the averted collective dose the effectiveness of these strategies is similar and quite high, averting 120-130 person-Sv depending on the remedial actions implemented.

Conclusion

Despite that efforts have been undertaken and improvements have been achieved to describe and understand the behaviour of radionuclides in different ecosystems many items remain unsolved and deserve further studies. This specifically applies to regions where in the past no nuclear activities have been conducted but which are considered now to be explored for potential nuclear energy production, mining, waste disposal or any other activity in the nuclear fuel cycle. Such ecosystems have been hardly investigated and the human use of and interaction with such ecosystems need to be covered to understand such environments and assess their radioecological sensitivity. With this new challenges are for radioecologists worldwide: 1) to share information so that already existing information is not been lost 2) to provide training and education to inexperienced new and young radioecologists and 3) to create a worldwide new network of radioecologists and radioecological laboratories and modellers to cover all different climatic zones, ecosystems and environments to be prepared for potential accidents or routine releases of radionuclides of a variety of nature, waste disposal requirements and decommissioning of nuclear facilities in the future. In a present situation of retirement and decreasing number of active radioecologists these will be a challenge that instead of risk to loose expertise to create a hub of radioecological knowledge for future generations of scientist and environmentalist, but also decision makers.

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Fasset Project: Recommended Protocols for Assessing Genotoxic Effect of Radiation on Non-Human Biota

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The main objective of the FASSET (Framework for ASSessment of Environmental ImpacT (FASSET) Project is to assess the impact of radiation on environment. Several European countries e.g. Finland (Radiation and Nuclear Safety Authority), France (Institute for Radioprotection and Nuclear Safety), Germany (German Federal Office for Radiation Protection, German National Centre for Environment and Health), Norway (Norwegian Radiation Protection Authority), Spain (Spanish Research Centre in Energy, Environment and Technology), Sweden (Swedish Radiation Protection Authority, Swedish Nuclear Fuel and Waste Management Company, Kemakta Konsult AB, Stockholm University) and UK (Environment Agency, Centre for Ecology and Hydrology, Westlake Scientific Consulting Ltd., Centre for Environment, Fisheries and Aquaculture Sciences, University of Reading) have participated in this project. Several reference organisms both from terrestrial and aquatic ecosystems have been used in this project. Some of the terrestrial ecosystem consists of soil micro-organisms, soil invertebrates (worms), plants and fungi, burrowing mammals. Herbaceous layer includes bryophytes, grasses, herbs, crops and shrubs. Above ground invertebrates are herbivorous mammals, carnivorous mammals, reptiles, vertebrate eggs, amphibians, birds, canopy trees, invertebrates. An aquatic ecosystem includes sediment e.g. benthic bacteria, benthic invertebrates (worms), mollusks, crustaceans, vascular plants, amphibians, fish, fish eggs, wading birds, sea mammals. Water column organisms includes: Phytoplankton, zooplankton, macroalgae, fish and sea mammals.

The overall FASSET project is classified in to four main work packages (WP).

WP 1: Dosimetry. This work package deal with radiation dosimetry models for a set of reference organisms relevant to different exposure situations

WP 2: Exposure. The objective of this work package is to assess transfer, uptake and turnover of radionuclide in European ecosystem and identify main components of the ecosystems where external and internal level may be high.

WP 3: Effect Analysis. This package aims to critically examine reported data on biological effects on individual, population and ecosystem levels as a point of departure for characterizing the environmental consequences of e.g. a source releasing radioactive substances into the environment.

WP 4: Framework. To review existing frameworks for environmental assessment used in different environmental management or protection programs and to integrate project findings into assessment frame work.

The work package 3 on effect analysis, mainly deals with the effect of environmental radiation on non-human biota. Major interest in the effect analysis falls in to four main categories, e.g. studies dealing with morbidity, mortality, reduced reproductive success and mutation analysis.

I.   Morbidity: These studies should include experiments pertaining to growth rate, effects on the immune system and the behavioral consequences of damage to the central nervous system from radiation exposure in the developing embryo.

II. Mortality: These studies should include stochastic effect of somatic mutation and its possible consequence of cancer induction, as well as deterministic effects in particular tissues or organs that would change the age-dependent death rate.

III. Effect on reproductive success: These studies should include experiments pertaining to fertility and fecundity.

IV. Mutation: These studies should focus on both gene and chromosomal mutations in somatic and germinal cells.

Methods for Quantification of Geno-toxicity

Following geno-toxicological techniques have been recommended to be carried out in organisms belonging to non-human biota.

1.   Micronucleus test

2.   Alkaline comet assay

3.   Metaphase chromosomal aberrations

4.   Fluorescence in situ hybridization (FISH)

5.   Mini- and micro-satellites

We describe here two most widely used methods for mutation detection in detail e.g. micronucleus test and alkaline comet assay.

I. Bone Marrow Micronucleus Test in Mammals

The mammalian in vivo micronucleus test is used for the detection of genetic damage induced by any physical or chemical agent on the chromosomes or the mitotic apparatus. This test can be used to detect genetic damage in any dividing tissue e.g. bone marrow cells, peripheral blood lymphocytes, human buccal epithelial cells, germinal cells, etc (Chaubey et al. 1978a; Romagna and Staniforth, 1989; Mavourin et al. 1990). Bone marrow micronucleus assay is one of the most useful cytogenetic methods to detect the genetic damage in mice and several other species under in vivo conditions

(Jenssen and Ramel, 1976; Chaubey et al.1978b). This assay was developed independently by Schmid and Heddle in early 1970's, and since then it is being increasingly used to assess the clastogenic effect of ionizing radiation (Heddle et al. 1983) and chemical mutagens in mammals (Schmid, 1975; Heddle, 1973; Collaborative Group, 1995, Hayashi et al. 1983, 1994). A large number of physical and chemical agents have been assessed for their potential clastogenicity using this assay in a variety of organisms (Chaubey et al. 1993, Bhilwade et al. 2004, 2010). This is one of the accepted methods by Regulatory Authorities to assess the genetic damage induced by any drug or chemical in vivo in mammals. The test serves as an useful indicator of cytogenetic damage and till to date it has proved to be a convenient and rapid method for the detection of radiation and chemical induced chromosomal damage in vivo in mice with about the same level of sensitivity as that of bone marrow metaphase analysis (Schmid, 1975, McGregor et al. 1983, 1987; Hayashi et al. 1990).

Principle of micronucleus Test

The test is based on the principle that chromatid, chromosomal fragments or even the whole chromosome, which may be, produced by the clastogenic agents or spindle poisons lag behind during anaphase due to the lack of centromere and are not included into the nucleus of the daughter cells. These small fragments of chromatid or even whole chromosome subsequently give rise to micronuclei, which are present in the cytoplasm of the daughter cells. Mouse bone marrow micronucleus test is a well accepted and adapted method for mutagenicity evaluation of radiation and chemical mutagens. (Chaubey et al. 1978; Heddle et al. 1983).

Chemicals

1. Fetal Calf Serum
2. Giemsa Stain
3. May-Gruenwald Stain
4. DPX Mount
5. Centrifuge Tubes
6. Polished Cover Glasses (Haemocytometer Polished glass)

Preparation of Stock Solution

Giemsa Solution

Giemsa powder	: 800 mg
Glycerol	: 50 ml
Methanol	: 50 ml

1. Dissolve Giemsa powder in glycerol at 60°C with regular shaking.
2. Cool the above solution to room temperature and then add methanol.

3. The solution should be mixed thoroughly for 5 to 10 min. and allowed to stand overnight.
4. Next day filter the solution and keep it in the dark bottle and store at 4°C.

May-Gruenwald Giemsa (MG)

| May-Gruenwald Powder | : 250 mg |
| Methanol | : 100 ml |

1. Dissolve May-Gruenwald powder in methanol.
2. Mixed well for 5 min.
3. Filter with Whatman filter paper 541 and use it for staining.
   (Note May-Gruenwald stain is prepared freshly just before use).

Procedure for Bone Marrow Smears

1. Twenty-four hours after the scheduled treatment with chemicals or radiation exposure sacrifice the animals by cervical dislocation, remove both the femur bones and clean them by cotton gauge from the adhering muscle and tissues.
2. Aspirate the bone marrow cells slowly into a centrifuge tube containing fetal calf serum (FCS) using syringe with 25 g needle and centrifuge at 1000 rpm for 5 min.
3. Remove the supernatant with the help of a Pasteur pipette.
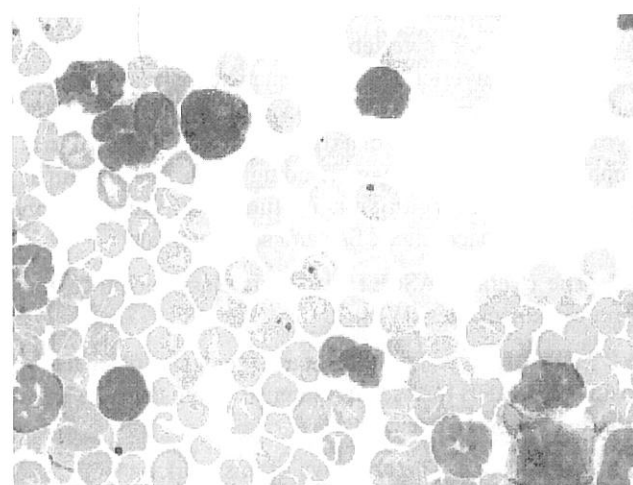
Fig. 1 *Photomicrographs showing bone marrow micronucleated erythrocytes in mouse exposed to 2 Gy of gamma radiation.*

4. Mix the sediments thoroughly in the capillary part of the Pasteur pipette and make smear onto a clean slide.
5. The air-dried smears should be stained in May-Grunwald Giemsa and mounted in DPX or euparal (Schmid, 1975; Chaubey et al, 1993).

General Experimental Design

Sl. No.	Treatment groups	Number of animals/ group	End point	Number of cells to be counted
1	Control	6	Miconucleated polychromatic cells (mn-PCEs) and Miconucleated Normonucleated Normochromatic erythrocytes (mn-NCEs)	2 Slides per animal. 1000 PCEs and the corresponding number of NCEs per slide
2	Solvent control	6	mn-PCEs and mn-NCEs	Same as above
3	Positive control	6	mn-PCEs and mn-NCEs	Same as above
4	Test compound T1	6	mn-PCEs and mn-NCEs	Same as above
5	Test compound T2	6	mn-PCEs and mn-NCEs	Same as above
6	Test compound T3	6	mn-PCEs and mn-NCEs	Same as above
7	Test compound T4	6	mn-PCEs and mn-NCEs	Same as above

Recording of Micronucleus Test Data

Sl. No.	Stage Number (X- and Y-coordinate)	Polychromatic erythrocytes (PCEs)	Micronucleated Polychromatic erythrocytes (mn-PCEs)	Normochromatic erythrocytes (NCEs)	Micronucleated Normochromatic erythrocytes (mn-NCEs)	PCEs/NCEs ratio

Staining

1. Stain air-dried bone marrow smears in undiluted May-Gruenwald solution for 5 min. and then in diluted May-Gruenwald (1 : 1 May-Gruenwald with distilled water) for 3 min.

2. Rinse the slides in distilled water.

3. Stain the smear with diluted Giemsa solution (one part Giemsa, six parts distilled water) for 10 min.,

4. Rinse it again in distilled water.

5. Blot gently and dry with in two layers of filter paper.

6. Cleared it in xylene for 5 min and embed in DPX.

Scoring

All the slides should be randomly coded before scoring. The slides should be initially observed under 40 X magnification to select area with optimum cell density and good staining. These areas are generally located towards the tip of the smears. The cells from the well stained areas with clear morphology should be selected and scored at 100X magnification in oil under bright field microscope. The immature or polychromatic cells are stained bluish (PCEs) while the mature or normochromatic cells (NCEs) are stained golden yellow. From each slide, score 1000 polychromatic erythrocytes (PCEs) and corresponding number of normochromatic erythrocytes (NCEs) with or without micronuclei (mn) and record them separately. From each animal at least 2000 PCEs and the corresponding NCEs should be recorded.

Criteria for Detecting Artifacts

The criteria for identifying micronuclei from artifact should be based on their morphology, staining pattern, size, and location within the cell and the reflectance characteristic after focusing the field. The presence of potential artifact should be judged by the recommendations of MacGragor et al. (1987).

Test Report

The test report should include the following information:

1. Test animals.

2. Species/strain used.

3. Number, age and sex of animals.

4. Source, housing conditions, diet, etc.

5. Individual weight of the animals at the start of the test, including body weight range.

6. Mean and standard deviation for each group.

Data Presentation

The data can be presented in a tabular form as given in a model Table 1.

TABLE 1. Micronucleated polychromatic erythrocytes (mn-PCEs) in the bone marrow of different genotypes of mouse exposed to various doses of gamma radiation.

Sl. No.	Dose (Gy)	% mn-PCDEs	PCEs/NCEs ratio
Swiss	0	3.35±0.32 (27/8065)	1.00±0.01
	0.125	10.63±0.97 (86/8094)	1.00±0.01
	0.25	13.58±1.20 (110/8098)	0.93±0.03
	0.50	22.87±0.43 (187/8178)	0.90±0.02
	1.00	34.81±2.52 (284/8159)	0.91±0.01
C57BR/cd	0	3.47±0.21 (28/8068)	0.96±0.03
	0.125	11.90±1.26 (96/8067)	0.92±0.02
	0.25	17.16±2.60 (138/8042)	0.87±0.03
	0.50	24.60±1.56 (199/8090)	0.89±0.02
	1.00	36.31±1.18 (293/8069)	0.80±0.03
C57BL/6	0	2.86±0.59 (23/8055)	1.02±0.02
	0.125	9.70±0.61 (78/8038)	0.93±0.03
	0.25	16.41±0.98 (133/8103)	0.88±0.03
	0.50	27.82±3.10 (226/8125)	0.90±0.02
	1.00	37.19±2.73 (302/8120)	0.81±0.03
C3H	0	3.22±0.53 (26/8080)	1.02±0.02
	0.125	11.86±0.94 (96/8097)	0.98±0.01
	0.25	12.02±0.53 (97/8072)	0.93±0.02
	0.50	22.85±1.27 (187/8185)	0.94±0.03
	1.00	39.42±1.22 (318/8067)	0.87±0.05
CBA	0	2.47±0.20 (20/8084)	0.98±0.04
	0.125	6.36±0.24 (51/8015)	0.91±0.02
	0.25	16.54±1.90 (133/8041)	0.93±0.01
	0.50	21.45±1.80 (173/8067)	0.85±0.02
	1.00	35.48±1.95 (285/8032)	0.86±0.04
DBA	0	2.35±0.32 (19/8096)	0.92±0.04
	0.125	6.93±0.19 (56/8082)	0.94±0.02
	0.25	12.56±0.66 (102/8118)	0.82±0.04
	0.50	20.65±1.45 (167/8086)	0.86±0.03
	1.00	36.65±2.19 (297/8104)	0.87±0.04
AKR	0	3.20±0.66 (26/8130)	0.97±0.03
	0.125	9.14±0.44 (74/8093)	0.95±0.02
	0.25	14.69±1.10 (119/8102)	0.94±0.02
	0.50	21.80±0.70 (176/8075)	0.89±0.03
	1.00	34.47±2.39 (278/8066)	0.87±0.05

Bhilwade H.N., R.C.Chaubey and P.S.Chauhan (2004) Mutation Res. , 560, 19-26

Test Conditions

1. Positive and negative (vehicle/solvent) control data.

2. Data from range-finding study, if conducted.

3. Rationale for radiation dose level selection.

4. Details of the radiation exposure.

5. Tissue used for study.

6. Detailed description of treatment and sampling schedules.

7. Methods of slide preparation.

8. Methods for measurement of toxicity.

9. Criteria for scoring micronucleated immature erythrocytes.

10. Number of cells analyzed per animal.

11. Criteria for considering studies as positive, negative or equivocal.

Results

1. Signs of toxicity

2. Proportion of immature erythrocytes among total erythrocytes

3. Number of micronucleated immature erythrocytes, given separately for each animal

4. Mean ± standard deviation of micronucleated immature erythrocytes per group

5. Dose-response relationship, where possible

6. Statistical analyses and method applied

7. Concurrent and historical negative control data

8. Concurrent positive control data

II. Alkaline Comet Assay

Introduction

Single-cell gel electrophoresis (SCGE) or comet assay was first introduced as the microelectrophoretic technique for the direct visualization of DNA damage in individual cells (Ostling and Johanson 1984). In this technique cells are layered on to the slides in agarose gel matrix and then the cells are lysed in the presence of detergents and high salts. The liberated DNA fragments are electrophoresed under neutral/alkaline conditions. The electric current pulls the negatively charged DNA from the nucleus in the direction of the anode and that result in characteristic images that look like a comet with a head and a tail. The cells are stained with a fluorescent dye, and the extent of movement of the DNA fragments is proportional to the extent of DNA damage in the cells. Initially this technique was employed at pH less than 10 but was then modified by Singh et al. (1988) who introduced electrophoresis under alkaline conditions (pH>13), which enabled detection of not only frank strand breaks but also alkali labile sites, DNA cross-linking and incomplete excision repair sites. Under alkaline conditions, (pH 13 or above) DNA base pairing is disrupted and the strands tend to separate. Unwinding occurs from the ends of the molecule and also from the internal DNA single strand breaks (SSBs). If lysis is carried out in neutral pH condition DNA base pairing is not disrupted and only DNA double strand breaks (DSBs) are detected.

Currently the technique of Singh et al (1990, 1994) is the most widely used SCGE method for detection of DNA damage. Exposure to radiation or any chemical mutagen results in the formation of DNA strand breaks. During electrophoresis broken DNA fragments move towards the anode. After staining with any suitable DNA specific fluorescence dye e.g. Ethidium bromide, propidium iodide the cells appears as a comet with brightly fluorescing head and with reducing fluorescent intensity in the tail region. The distance migrated by DNA fragments from the nucleus (also called as the tail length) is proportional to the extent of damage. Larger the DNA damage higher the tail length. Undamaged cells appear as intact nuclei (comet heads) without tails, a bright fluorescence core is seen with a less intense edge of fluorescence facing the anode. Singh and colleagues (1994) reported that the tail length reflects the amount of DNA breakage in a cell.

Principle of Comet Assay

The cells are embedded in a thin layer of agarose on a fully frosted microscope slide. The cells are lysed with any detergent or high salt solution to remove all cellular proteins and the liberated DNA is electrophoresed under alkaline or neutral condition. Depending on the size and total negative charge, the DNA fragments produced by any physical or chemical mutagen migrate to different distances towards anode. After electrophoresis the cells are stained with any DNA specific dye and observed under a fluorescence microscope. The cells appear as a comet with brightly fluorescing nucleus and diminishing fluorescence intensity in tail. Distance migrated by DNA fragments from the nucleus is taken as the measure of genetic damage (tail length). Using digital imaging software, other characteristics of comets e.g. percent DNA in the tail (%DNA-T), tail moment (TM: product of fraction of DNA in the tail and tail length), or percent DNA in head (%DNA-H) can also be measured, which are considered to be more consistent and reliable parameters of DNA damage.

Parameters of Comets to be Measured

Tail Length (TL), Tail moment (TM) and % DNA in tail region are the most common parameters which are being used by most of the soft wares, which have been developed for the detection of DNA damage by comet assay. The major advantage of using TM and % DNA-T expresses both the migration of various DNA fragments forming the tail and their relative amount of DNA as one number (Sasaki et al.1997 Tice et al. 2000, Chaubey et al. 2001a, 2001b, 2001c, Fairbairn et al. 1995).

Materials and Chemicals Used for SCGE

Chemicals to be used for comet assay should be of high purity. All the chemicals used in this assay can be obtained from Sigma Chemicals Inc, St Louis, MO, USA.

1. Agarose (Low Melting)

2. Dimethyl Sulphoxide

3. Ethylene Diamine Tetra Acetic acid disodium salt (EDTA)

4. Ethidium bromide

5. Propidium iodide

Sensitivity of various Assays for detection of DNA Single Strand Breaks

Assays	Single strand breaks in Daltons of DNA	Lower limit of detection of DNA X-ray dose (in Rad)
Alkaline sucrose sedimentation (Lett. et al.)	1 break /2-5 x10^8	500
Nucleoid sedimentation (lipetz et al., 1982)	1 break/2x 10^9	33
Alkaline elution (Kohn et al., 1986)	1 break / 2-3 x 10^9	30
Alkaline gel electrophoresis (Freeman et al., 1986)	1 break / 3 x 10^9	30
Alkaline unwinding (Rydberg, 1980)	1 break /6-9 x10^9	10
Alkaline microgel electrophoresis (Singh et al., 1994)	1 break / 2 x10^{10}	3.2

6. SYBR Green-II
7. Triton-X-100
8. Trizma base (Tris [hydroxy methyl amino methane])
9. 5,6-carboxyfluorescein diacetate (Molecular Probes)
10. Micropipettes, 10-100 μl and 200 - 1000 μl (Nichipet, Nichiryo, Japan)
11. Microfuge tubes (1.0, 1.5 2.0 ml) and Microtips (Axygen Scientific, California, USA)

Following materials and chemicals listed below can be obtained from local suppliers.

1. Acetone
2. Cover glass (24x60 mm)
3. Glycerin
4. Heparin
5. Sodium hydroxide
6. Sodium chloride
7. Sterile needles
8. Sterile syringes
9. Steel tray

Equipments

1. Electrophoresis power supply (Pharmacia Biotech, Sweden).
2. Horizontal gel electrophoresis unit
3. Microwave oven
4. Fully frosted microscope slides
5. Fluorescent microscope with filters for DNA specific dyes (Carl Zeiss, Germany).
6. Horizontal gel electrophoresis unit.
7. Semi-automatic imaging software for comet assay "SCGE-Pro" developed in our Division in collaboration with Electronics Division of Bhabha Atomic Research Centre, Mumbai-400 085 or any other commercially available software for comet assay.

Reagents for Comet Assay

Agarose Gel Preparation

For each experiment prepare fresh 0.8% low melting agarose in 0.9% saline were prepared just before experiment.

Lysing Solution for Alkaline Comet Assay (SSB)

Prepare lysing solution for alkaline comet assay was prepared by dissolving

NaCl	: 2.5 M
Disodium EDTA	: 100mM
Triton-X100	: 1%
DMSO	: 10%

(Always fresh lysing solution was prepared before the experiment and kept at 4ºC)

Electrophoresis Buffer for Alkaline Comet Assay (SSB)

Prepare electrophoresis buffer for alkaline comet assay was prepared by dissolving NaOH: 300 mM, Na_2 EDTA: 1 mM and pH was adjusted to 13. Lysis solution and electrophoresis buffer has to be prepared just before use. Stock solutions of 500mM Na2 EDTA 10M NaOH and 5M NaCl can be prepared and kept.

SYBR Green-II

SYBR Green- II stain can be used for the detection of single stranded DNA. It is supplied as 10,000X stock solution in DMSO. Diluted working solution is protected from light. Working solution (1:5,000 dilutions) was prepared by adding 2ìl SYBR Green-II in 10 ml of T.E. buffer

1X TE Buffer

Tris-HCl: 10 mM, EDTA: 1 mM, pH of the buffer was adjusted to 8.0

Methods

Experimental Protocols for Comet Assay

Comet assay can be used to detect genotoxicity of γ-radiation or chemicals in any mammalian species or any tissue of non human biota. While designing the experiment the following groups, viz. control, solvent control, positive control (a known mutagen) and 3 to 4 doses of radiation or the test compound should be used. Viability of the cells can be checked by using trypan blue dye exclusion test. All the samples should be checked for viability after making single cell suspension. For DNA repair study, the animals should be exposed to radiation or treated with test compound and blood sample can be collected from the tail vein at different time intervals e.g. 15, 30, 45, 60, 90 and 120 min post treatment and processed for comet assay.

Trypan Blue Exclusion Test

Trypan blue is a cationic chromophore that intercalates into DNA. This is one of the most commonly used techniques to check the viability of cells. In a viable cell, the plasma membrane is intact and prevents entry of trypan blue into the cell. At the onset of cell death, the membrane permeability is lost and the dye readily enters into the nucleus, and the dead cells appear blue, while the viable cells appear transparent. In this test 20-ìl of cell suspension is mixed with 180 ìl of 0.2% of trypan blue in phosphate buffered saline (PBS) and cells are counted with the haemocytometer at 40X magnification. The cell density is adjusted to 5×10^6 cells/ml of medium.

Slide Preparation

There are two basic procedures to make gel on the slides: the original three layers or the sandwich method and single layer method. In sandwich method the cells are suspended in low melting agarose and are layered between the two layers of normal agarose. In single layer method the cells are directly suspended in agarose and placed on the slide and allowed to solidify.

Detection of DNA Single Strand Breaks by Alkaline Comet Assay

For human and mouse studies, about 50 - 100 ìl of heparinised whole blood was mixed with 1.0 to 1.5 ml of 0.8% agarose at 38°C and poured on fully frosted slides uniformly. After solidification, the slides were kept in lysing buffer, (2.5 M NaCl, 100 mM Na₂-EDTA with freshly added 1% Triton X-100 and 10% DMSO), for 1h at 4°C. After lysis, the slides were washed with alkaline buffer (300mM NaOH, 1mM Na₂EDTA, pH 13.0) and placed on a horizontal electrophoresis tank, which was filled with freshly prepared alkaline buffer for 20 min at room temperature to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was carried out using a compact power supply. The electrophoresis conditions were set up depending upon the experiment. After electrophoresis, slides were washed gently to remove the alkali and detergents by placing them horizontally in 0.4 M Tris-HCl

buffer, pH 7.5. After neutralization, slides are stored on wet paper in dark boxes at 4° C before staining and observation. Slides were stained with SYBR Green II. Approximately 50-60 ìl of the working solution of SYBR Green II was spread on the slide and observed under fluorescence microscope at 40x magnification using filter 09 filter set (BP 450-490/FT510/BP515-565).

Other Fluorescent Dyes which can be used for Staining

A number of DNA specific dyes e.g. Ethidium bromide (EB), propidium iodide (PI), YOYO-1 etc are commercially available, which can also be used for staining DNA in comet assay.

Slide Scoring

In each experiment two slides should be prepared per dose point. At least 50-75 cells per slide and a total of 150 cells per group should be scored from the coded slides to get reproducible data.

Digital Imaging System for Comet assay

A number of imaging software is commercially available for comet assay, like Fenestra Comet, developed by Confocal Technologies Ltd. U.K, Comet IV, Comet Score, CASP etc., can also be used. CASP which is also an effective software can be down loaded at (Site: www.casp.of.pl/) and used for measuring comet parameters. In our laboratory we have developed dedicated imaging software, SCGE-Pro for automated image analysis and data processing for comet assay. The digital imaging system consists of following components:

Fluorescence Microscope

A Carl Zeiss Axioplan microscope with epi-fluorescence facility (HBO 50 high pressure mercury lamp) and suitable filter 09 (450-490, FT510, LP520)

Video Camera

A high performance color video camera (KY-F55BE 3CCD, JVC, Japan) has been used in this system. This camera is provided with I/3 inch 440,000 pixels CCD with on chip lens and it delivers high quality pictures with an Signal / Noise ratio of 58 dB and sensitivity as high as 2000 flux at F 5.6. It also has outputs for composite video, RGB and composite sync (JVC Victor Company of Japan Ltd).

Video Frame Grabber

The system has Integral Flashpoint Intrigue frame grabber. Flash Bus MV uses the PCI bus for real-time transfer of video to system memory. The Integral Flashpoint Intrigue frame grabber accepts color composite video output of the camera. It digitizes each of RGB planes at a total resolution of 24 bits per frame. It has spatial resolution of 768 x 576 per frame. (Integral Technologies Inc., 9855 Cross Blvd., suit 126, Indianapolis, IN-46256-3336, USA)
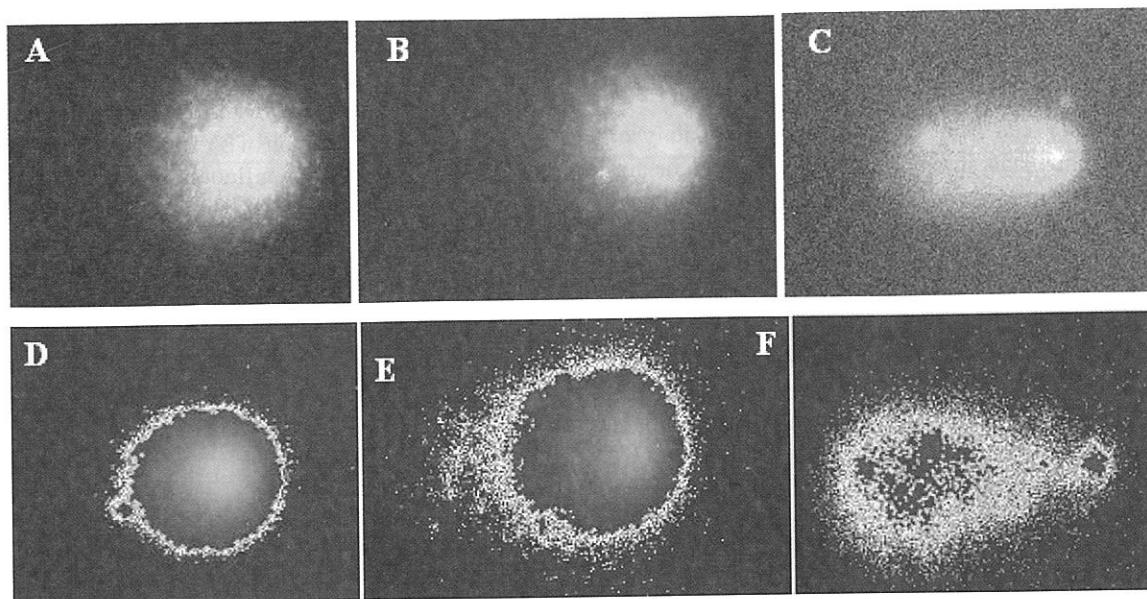
Fig. 1   Images A, B, C are photomicrographs of comets obtained after alkaline comet assay and stained with SYBR green II as seen under the fluorescent microscope at 40X magnification. Comets shown here were obtained from cells of Swiss albino male mice. A- Undamaged (Control) cell, B- Moderately damaged (Treated) cell, C- Highly damaged (Treated) cell. Images D, E, F are photomicrographs of comets obtained after the images were processed by the software SCGE-PRO, D-Control undamaged cell, E- Moderately damaged (Treated) cell and F- Severely damaged (Treated) cell with almost all the DNA in the tail of the comet.

Computer

Pentium-IV computer with super VGA color monitor, CD-ROM drive, 80 GB Hard disk, a CD writer for image storage, and a HP Desk Jet printer. The images of the individual comets are captured and stored in separate files. The software SCGE-Pro allows quantitative measurements of total fluorescence of the comet, fluorescence of the tail, length of migrated DNA fragments and finally calculates the tail moment, an internationally most accepted parameter for comparing the DNA damage. Fig.1. shows the digital imaging system and the software SCGE-Pro for comet assay.

Steps involved in the measurement of DNA damage using SCGE-Pro system

The images of the individual comets are captured using a 3-CCDcamera and stored in a separate files. The acquired images are pre-processed to remove acquired artifacts, if any. The total SYBR Green II fluorescence intensity is taken as total DNA content in the comet. The software allows quantitative measurements of total fluorescence of the comet, fluorescence of the tail, length of migrated DNA fragments and it finally calculates the tail moment (product of fraction of DNA in the tail and tail length), an internationally most accepted parameter for comparing the DNA damage. This software allows clear discrimination between the head (nucleus) and tail of the comet. The head of the comet was seen very clearly with the original color of dye used while tail regions showed original dye color superimposed with the high contrast pseudo color to give a more precise lower threshold (LT) setting. Figure 1 shows different extent of DNA damage from control and treated animals. Figure 2 shows various steps involved in the

measurement of comet characteristics by the software SCGE-Pro.

Statistical Analysis

Calculate the mean, standard deviation, standard error. The statistical significance for mean and standard deviation should be checked by using One-way ANOVA and Student's T-test. Values will considered significant at $P < 0.05$.

Following precautions should be taken while performing comet assay experiments

1. Fully frosted microscope slides should be washed with distilled water, dried and sterilized in a microwave to prevent any contamination. Just before use the slides should be dipped in ethanol and cleaned with muslin cloth.

2. All the steps involved in comet assay are photosensitive; hence steps from single cell suspension preparation to staining and observation should be carried out in dark or in yellow light to prevent additional DNA damage.

3. Preparation of single cell suspension from tissues should be handled delicately to prevent cellular damage. The viability of cells should be always measured before treating the cells with any genotoxic agent. Apoptotic or dead cells produce very different type of comet image. In this type of comet most of the DNA will appear in the tail region or there will be very little DNA in the head region which appear like a balloon. These cells should be recorded separately and should not be included in the main data.
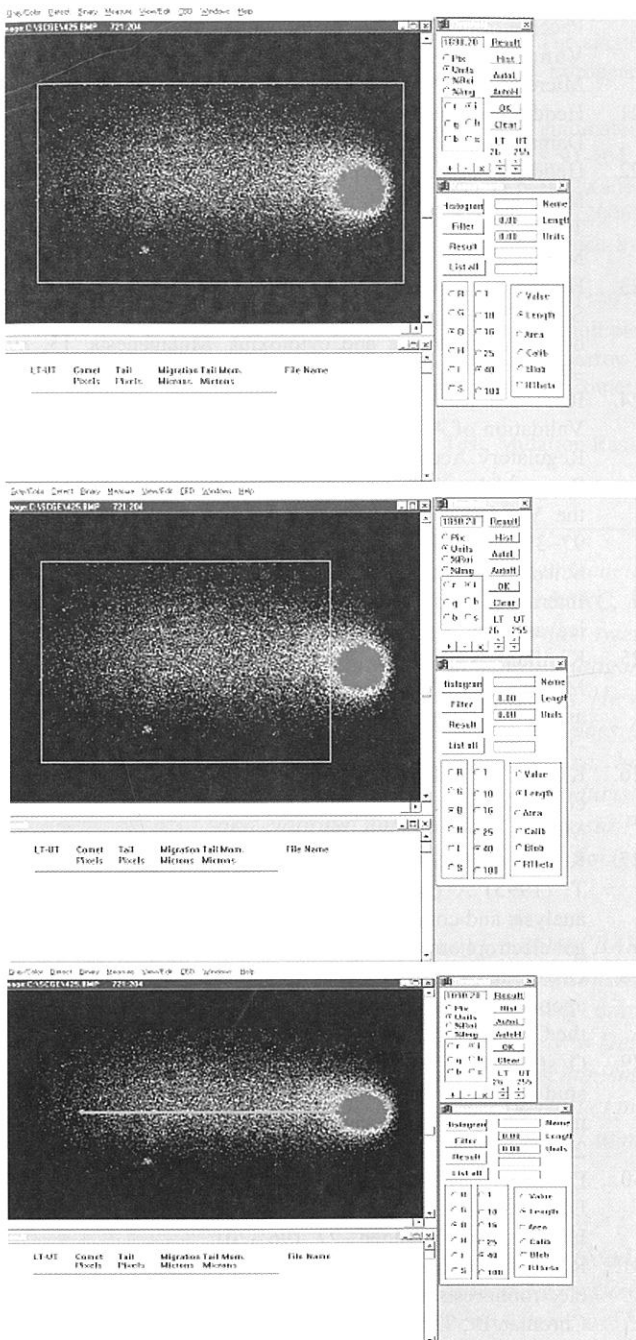
Fig. 2 Measurement of comet parameters using the software SCGE-PRO

4. Electrophoresis conditions: Depending upon the nature of experiment, electrophoresis time, voltage and current has to be adjusted. The slides inside the electrophoresis tank should be kept side by side with the agarose end facing towards the anode. Always prepare fresh electrophoresis buffer and the level of the buffer should be approximately 0.25 cm above the slides.

5. All the experiments should be conducted under identical conditions to prevent intra- or inter-run differences in DNA migration. Utmost care should be taken with regard to reagents and preparation of solutions, preparation of agarose gel, conditions of lysis, alkali unwinding and electrophoresis.

Advantages of Comet Assay

1. The SCGE Assay is of particular importance because it allows the detection of intercellular differences in DNA damage and repair in virtually any eukaryotic cell population that can be obtained as a single cell suspension (Anderson et al. 1998, Basu et al 2005, Bocker et al. 1999, Chaubey et al. 2005, 2006a, 2006b Gedic1992, ICCVAM, 1997, Bhilwade et.al. 2010).

2. Another important advantage of this assay is the requirement of extremely small sample size (as low as 25 μL of blood cells or 10^3 to 10^5 cells).

3. Apart from image analysis, which greatly facilitates and enhances the possibilities of comet measurements, the cost of performing the assay is relatively low.

4. This assay can be fully automated and results can be obtained in a single day.

5. This technique can also be used in several other areas of bio-medical research e.g. in genetic toxicology, radiation biology, clinical and molecular epidemiology and as a predictive assay in cancer radiotherapy (Kizilian et al 199, Klaude et al. 1996, Kobayashi et al. 1995, McKelvey-Martin et al. 1993, Olive et al. 1993, Pfuhler and Wolf 1996, Rojas et al. 1999, Malladi et al 2007, Sandhya et al. 2006).

Shortcomings of Comet Assay

1. In spite of being such a valuable technique, it has a few shortcomings – The biological importance of DNA alterations that are measured in the Comet Assay is not clear as the majority of the lesions may be repaired long before being fixed as mutations.

2. There is a lack of agreement on a single appropriate comet parameter capable of adequately describing the observed damage.

3. There are also wide variations in the methodologies followed during alkali treatment and electrophoresis.

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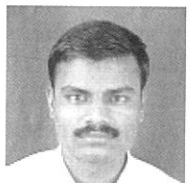
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New Challenges in Marine Radioecology

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Summary

This paper aims to firstly present an overview of the evolving socio-economic and environmental context within which the new requirements for marine radioecological science are becoming better clarified. Some examples are given of the laboratory-based experimental research programme that is being conducted at the IAEA Marine Environment Laboratories, in contribution to these identified needs for radioecology and where further opportunities for national and international research are seen to exist. Some conclusions about the increasing significance and societal role of marine radioecology are proposed to perhaps motivate a new generation of radioecologists to undertake the science that is needed in support of both societal advancement and environmental protection.

Introduction

The science of radioecology includes investigation of the environmental behaviour of radionuclides after their release from nuclear facilities. It is particularly focussed on environmental transfer and bioaccumulation mechanisms by which radionuclides may be taken up by biological systems, with their attendant potential to deliver radiological dose to humans or biota. These data provide the basis for the subsequent quantitative assessment of radiological doses received by both the community and the environment and as such are fundamental to the demonstration of their adequate protection from potential adverse radiological impacts associated with environmental releases on radionuclides.

Historically the societal needs for radioecology have been geographically linked to nuclear power industries (as well as other nuclear facilities) that have been focussed in Western Europe, Japan, Russia and North America, and typically in temperate and cold-temperate geographical regions (Fig. 1), with their particular biological communities. There are recent expansions of nuclear power programs into different geographic regions that may also be expected to increase in the future. The new regions of growth have their attendant contrasts in human demography and diet, biodiversity and climatic regimes relative to these centres of historical radioecological research, that bring new challenges particularly for coastal marine radioecology. The increasing international interest in radiation protection regimes that can be demonstrated to holistically protect humans, environment and biodiversity is another challenge and opportunity for advances in radioecology. Moreover, the high CO_2 world brings with it increasing temperature regimes and ocean acidification. The potential impacts of these overarching physical and chemical changes on marine radioecological processes cannot be ignored and now also need to be evaluated experimentally to assess their potential radioecological significance.

The crucial role that nuclear energy can play in the mitigation of carbon emissions by 50% at 2050 has been recently articulated in the OECD roadmap (2008) jointly launched by the International Energy Agency and the OECD Nuclear Energy Agency in 2010, with almost one quarter of global electricity being nuclear-generated. Radioecology has an essential role to play in supporting its societal acceptance for the broader environmental benefits that nuclear power can bestow for future generations.

The Increasing Socio-economic Importance of Aquatic Radioecology

The recent and near-future expansions of civilian nuclear power programmes bring a new significance to radioecology and particularly marine radioecology for the following reasons.

Geographical Shifts in Nuclear Power

Their shift into different geographic regions, particularly East and South Asia, has radioecological importance because of their occurrence in different biogeographical regions where the boundaries of major faunal changes are primarily determined by temperature in shallow sea communities which are characterised by greater diversity in fishes and corals for example (Cox and Moore, 2005). Their occurrence particularly in the tropical marine biogeographical realm will expose groups of biota that are quite distinct and more diverse compared to those that have typically been the focus of previous radioecological investigation, typically in the cold and cold-temperate biogeographical realms (Cox and Moore, 2005; Lomolino et al, 2006). Consequently there exists the potential for different transfer pathways for radionuclides in different coastal marine regions that will need to be investigated to demonstrate that they do not lead to unduly enhanced exposures to radionuclides in seafood-consuming communities or the marine biota themselves. This geographical gap in essential radioecological information has been identified during the production of a recent IAEA handbook (in prep) of parameter values for the prediction of radionuclide transfer to wildlife. This handbook will provide equilibrium concentration ratio values for wildlife groups in terrestrial, freshwater, marine and estuarine environments.

Increasing Populations and Redistributions into Coastal Areas

In Figure 2a is shown a prediction of the global population densities in 2015 that confirms both the higher densities within the regions where most increases in nuclear power plant build is and will take place (Fig 1), and with
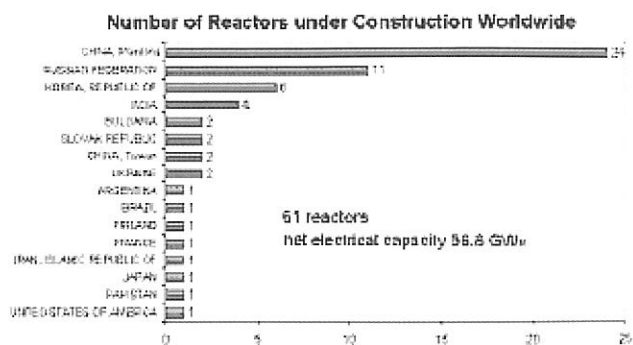
Fig. 1a Numbers of nuclear power reactors under construction in different countries
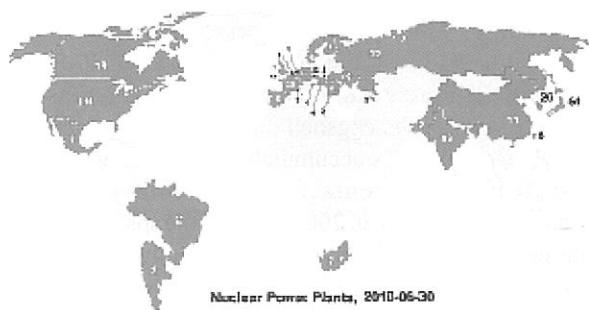

Fig. 1b Current geographical distribution of nuclear power plants

particularly high and increasing densities in coastal areas (GESAMP, 2001), as shown geographically in Figure 2b.

Enhanced regional importance of seafoods in Human Diet and the 'blue Revolution'

The role of seafood in global food security and supply is already very well established (Smith et al., 2010) and its importance in this roles is expected to continue to grow as the international aquaculture industry positions itself for a 'blue revolution'. The Blue Revolution is the aquatic analogue of the agricultural 'green revolution' that began in the 1960's, and it is viewed as necessary in order to fill much of the projected shortfall in food production from agriculture that will be needed to feed the increases in world population over the coming decades (Lubchenko, 2003; Sachs, 2007). The consumption of seafoods as a proportion of total diet is already elevated in the regions of nuclear growth. Figure 3a shows both the total fish catch for the marine regions of the world (2002) and also fish production (2001) for the highest 12 countries. The regions of the Pacific North West and Pacific Western Central are in the two highest categories of fish catch and highest levels of fish production, ie. in the general region of expanding nuclear power production, thus confirming a geographical coalescence of the socio-economic importance of seafoods with nuclear activities.

Relevant Experimental Studies in Marine radioecology at IAEA-MEL

Our experimental studies have had an emphasis on marine biota that are valued as seafoods such as bony fishes,
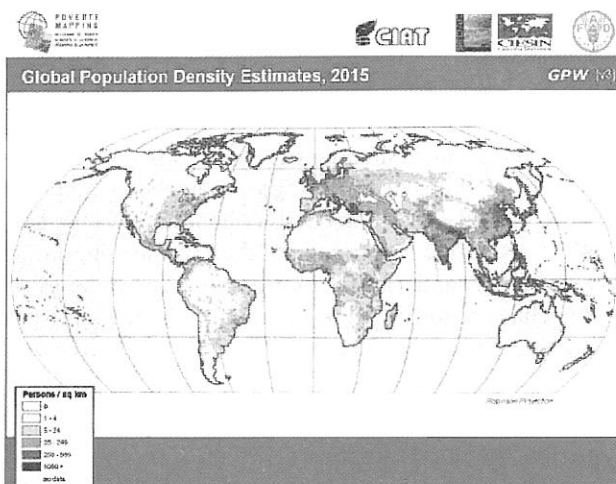

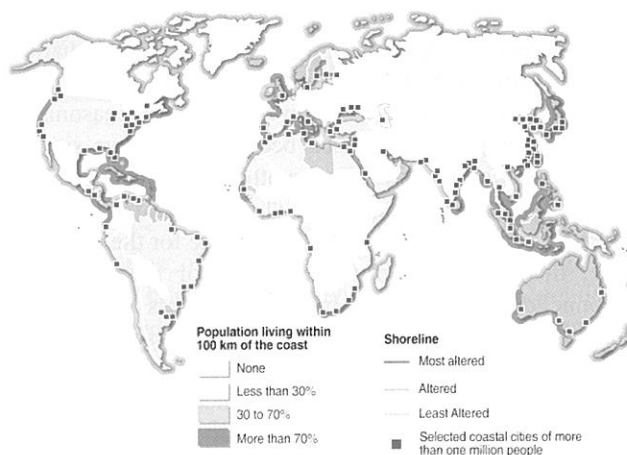Fig. 2a Global population density estimates for 2015.

Fig. 2b Coastal population, urban conurbations and altered land cover in coastal zones (100 km of coastline).The graphic shows the proportion of the population that lives within 100 km of the coast, for each of the world's nations and also where there are coastal zones with high degree of human alteration. In addition, the locations of selected larger coastal cities are presented. From Burke et al, World Resources Institute (WRI), Washington DC, 2001; Paul Harrison and Fred Pearce, AAAS Atlas of Population and Environment 2001, AAAS, University of California Press, Berkeley

because of their socio-economic significance, and they have also included a representative ICRP Radiological Reference Organism, the turbot (family Pleuronectidae). There has also been an emphasis in our investigations on taxa that have been less investigated to make our radioecological studies more representative of marine biodiversity. Other questions have concerned the effect of life-history stage on radionuclide transfer factors and also maternal transfer to young, as this pathway has been so little studied compared to the classical aquatic pathways, ie. transfer from water, food and sediment. Furthermore, the over-arching effects of ocean acidification and increasing water temperature on some of these

radioecological transfer pathways have begun to be investigated.

Phylogeny and Ontogeny in Radioecology

Our experimental comparisons among two major phylogenetic groups of marine fishes, namely the bony fish (Teleosts) and the sharks and rays (Chondrichthyans), have shown distinctive contrasts in their bioaccumulatory characteristics and tissue distributions for a range of radionuclides absorbed from seawater. These taxon-based differences have clear implications for radiological dose assessments (Jeffree et al. 2006; Jeffree et al. 2010).

These studies have provided evidence that supports the hypothesis that radionuclide bioaccumulation characteristics may be regarded as relatively unique to the biology of a species, and that the differences between taxa may be greater the longer the period of their evolutionary divergence. With regard to the extent to which a pleuronectid teleost is representative of fish biodiversity in its radiation exposure from radionuclides accumulated from seawater, our results for three teleosts investigated are reasonably compatible with this approach because they have similar bioaccumulation patterns. On the other hand our results for chondrichthyans do not accord with this interpretation and, depending on the radionuclide responsible for the radiation dose, they may be more exposed for many of the radionuclides examined in this study but less exposed for radio-caesium. Phylogenetic divergence may similarly lead to differentiation in radiological exposure regimes. Hence the choice of additional marine reference organisms to be more representative of marine biodiversity and more 'biologically or phylogenetically equitable' may be supported by radioecological investigations of those less studied taxa that are most separate in evolutionary time from the currently selected species. Such a process may also give a more biologically sound basis, being grounded in evolutionary and related phylogenetic theory, to support the selection and evaluation of reference organisms.

The identification of bioaccumulation patterns that are taxon-specific indicates the possibility for better prediction of the susceptibility of marine fishes to contamination from both stable and radioactive elemental pollutants in seawater, according to their taxonomic status. Our results and interpretation would also point to the need to consider the taxonomic compositions of marine biota and seafoods from the biogeographical regions within which there is expanding nuclear power programmes (Fig. 1).

Ontogeny refers to an organism's developmental history during its life from the fertilisation of the egg through to the mature adult. Our ontogenetic radioecological studies have been undertaken on the eggs and subsequent life stages of the cartilaginous dogfish (Scyliorhinus canicula) (Jeffree et al. 2006; Jeffree et al. in prep) and the cuttlefish Sepia officinalis (Mollusc: Cephalopoda) (Lacoue-Labarthe et al., 2009) to show the following;

(i) the unexpectedly high concentrating ability of the dogfish egg and in particular its protective collagenous egg-case for a range of radionuclides, with water: egg case CF's extending to more than 10^4, relative to water levels The accumulatory capacity of the egg-case effectively surrounds the developing embryo within an enhanced radiation field than continues to increase for some radionuclides over more than 100 days of embryogenesis prior to the hatching of the pup,

(ii) In general, Cephalopods play a key role in many marine trophic networks and constitute alternative resources for fisheries, as fin fisheries continue to be depleted by over-fishing. Cephalopods quickly die after mating leading to population dynamics that are highly dependent on the hatching success of the eggs. Along the European coast, the eggs of the cuttlefish, Sepia officinalis, are characterized by an increasing permeability of the eggshell during their development leading to a selective accumulation of essential and non essentials elements in the embryo (e.g. Lacoue-Labarthe et al. 2008a). From the spawning date up to 1 month of development radioactive silver was taken up efficiently by the eggs, reaching load/concentration ratio (LCR) over 10^3. From this time onwards, ^{110m}Ag activity continued to increase in eggs, passing through the eggshell from day 30 onwards and was then accumulated in the embryo, which contained more than 40% of the whole egg metal burden at the end of the exposure period. During depuration conditions, Ag continued to accumulate in the embryo indicating translocation processes from the eggshell and also a high affinity of the metal for the embryonic tissues.
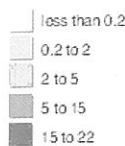
Both of these studies are instructive and cautionary in that they show enhanced vulnerability of the embryonic life stages to either direct contamination with radionuclides or enhanced radiation exposure regimes, due to their unexpectedly high accumulatory capacities relative to more mature phases of the life-cycle of these taxa.

How many other radioecological surprises await discovery, particularly in the less explored biogeographical regions?

Maternal Transfer of Radionuclides

Both the species that were employed in these ontogenetic studies were also investigated to determine if, and to what degree, radionuclides could be transferred from the mother's radio-labelled food to their eggs and embryos (Lacoue-Labathe; 2008b; Jeffree et al, in prep). In the dogfish the derived maternal: egg transfer factors for four radionuclides were measured over a 60 day exposure and were ranked as ^{134}Cs>^{65}Zn>^{60}Co>^{241}Am; they ranged over an order of magnitude from 2×10^{-4} for ^{134}Cs to 2×10^{-5} for ^{241}Am. During a maternal post-ingestion phase extending over c. 170 days we measured the percentage changes in the maternal transfer of these radionuclides to eggs, that were
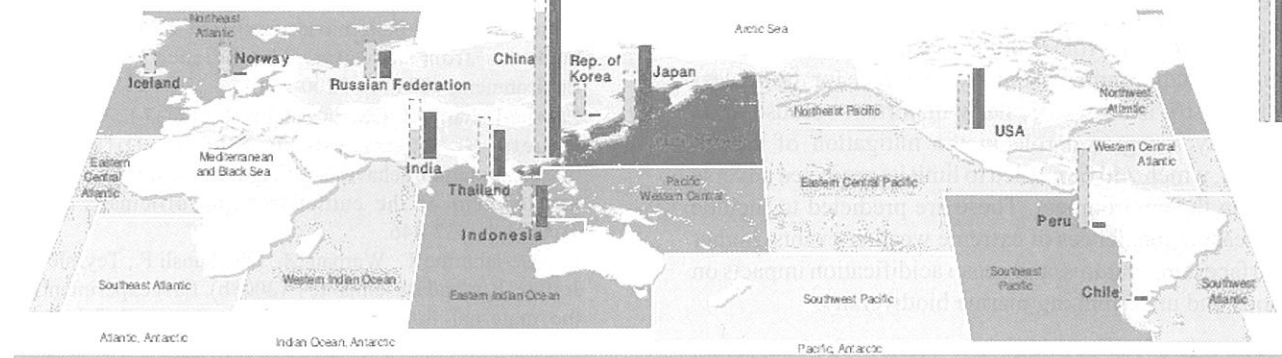
Fig. 3 Fish catch and production. Source: FAO (2004)

revealed to be radionuclide-specific. ^{65}Zn levels actually increased by a factor of two prior to its decline to very low percentages after 50 days; ^{60}Co showed a monotonic decline over the first 50 days to consistently low percentages that continued up to 170 days; and both ^{134}Cs and ^{241}Am also show a general their major declines within the first 50 days but with higher and more variable retentions subsequently, particularly for ^{241}Am.

The aim of the study on cuttlefish was to provide a first insight on the incorporation of eight metals in the eggs of the cuttlefish Sepia officinalis via maternal transfer, using radiotracer techniques (^{110m}Ag, ^{241}Am, ^{109}Cd, ^{60}Co, ^{134}Cs, ^{54}Mn, ^{75}Se and ^{65}Zn). The cuttlefish was fed daily with radio-labelled crabs for two weeks; after which they spawned every three days. Among the eight tracers, only ^{110m}Ag, ^{75}Se and ^{65}Zn were significantly transferred to the eggs. The radiotracer distribution among the egg compartments showed that ^{75}Se and ^{65}Zn were accumulated predominantly in the yolk whereas ^{110m}Ag was found in similar proportion in the yolk and the eggshell. During the embryonic development, ^{75}Se and ^{65}Zn contained in the yolk were progressively transferred to the embryo, probably to supply its metabolic needs in these essential elements. Although it has no known biological functions, Ag contained in both yolk and eggshell was also transferred to the embryo. Overall, our results confirmed that transfers of Ag, Se, and Zn do actually occur from a female cuttlefish to its eggs, for at least the last two weeks before spawning.

Such studies of maternal transfer are needed to complete the full picture of radioecological exposure via all four transfer pathways in aquatic biota.

Radioecology and Environmental Change

A series of experiments on cuttlefish eggs also investigated how their embryogenesis and bioaccumulation of radionuclides may be modified by temperature and pH, two critical factors that affect the metabolism of marine organisms in the coastal shallow waters and that are predicted to be changed in a high CO_2 world (Lacoue-Labarthe et al. 2009; Orr et al. 2005). In this study, we investigated the effects of pH and temperature through a crossed (3x2; pH 8.1 (pCO2, 400 ppm), 7.85 (900 ppm) and 7.6 (1400 ppm) at 16 and 19°C, respectively) laboratory experiment. Seawater pH showed a strong effect on the egg weight and non-significant impact on the weight of hatchlings at the end of development implying an egg swelling process and embryo growth disturbances. The lower the seawater pH, the more ^{110m}Ag was accumulated in the tissues of hatchlings. The ^{109}Cd concentration factor (CF) decreased with decreasing pH and ^{65}Zn CF reached maximal values at pH 7.85, independently of temperature. Our results suggest that pH and temperature affected both the permeability properties of the eggshell as well as embryonic metabolism. To the best of our knowledge, this is one of the first studies on the consequences of ocean acidification and ocean warming on metal uptake in marine organisms, and our results indicate the need to further evaluate the likely radioecological and eco-toxicological impact of the global change on the early-life stages of the cuttlefish and other marine organisms.

The Societal Role and Contributions of Radioecological Science

Radioecology provides an important and key role in the process of nuclear power production with regard to the assurance that it provides to both the local community and

the opinion-forming general public that their national nuclear power plants cause no adverse radiological exposure to either humans or biodiversity and also does not alienate commercially valuable seafoods from consumption and trade due to perceived risks of contamination by radionuclides. This requirement is even more pronounced in countries with littoral communities that have greater reliance on coastal aquaculture production and wild fisheries for both national consumption and regional and international trade (Fig. 3a).

Radioecology's role in the provision of reliable science in support of environmental quality assurance for the nuclear power industry is also particularly important because of its increasingly recognised role in the mitigation of carbon emissions, which will be needed to limit its projected adverse effects on the environment. These are predicted to include the increasing incidences of extreme weather events, higher sea-surface temperatures, and ocean acidification impacts on seafoods and underpinning marine biodiversity.

The role of radioecology is to perform the transparent and quality- assured science in support of the production of low-carbon nuclear energy that is required for economic development but with a mitigation of the attendant destructive environmental effects predicted to be associated with carbon emissions.

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Strategy and Methodology for Radioecology Studies

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Introduction

During a survey of certain nuclear sites or, considering marine radioecological studies in the field, in general, researcher(s) is/are faced with problems to accurately measure and determine concentration factors (CFs) and biological half-lives or half-times in biota. At times, this fact results in very low concentrations of natural radioactivity in the water and in the organisms so that concentration may be beneath the detection limits of the equipment used for measurements. Moreover, it is very rarely possible to follow the kinetics of radioisotope behaviour (accumulation and loss) in marine organisms in the field, especially in those species which are commercially important and/or of economic value. Such difficulties and problems may be surmounted only by undertaking well defined laboratory for radiotracer studies to determine these parameters.

Scope

Laboratory studies with living organisms should, therefore, serve for obtaining such information under defined laboratory conditions that is difficult or impossible to achieve in the field. The results which are most important should enable us to extrapolate the information obtained in the laboratory to field conditions. The knowledge of the behaviour and physiology of radionuclides in fish is of general importance because fish represent the higher trophic levels in the marine ecosystem, and thus constitute one of the most direct transfer routes of radioactivity back to human beings.

Equipment

Aquaria

Fishes are normally the most difficult marine organisms to maintain in a healthy state in the laboratory (aquaria), since they have precise requirements for temperature, salinity, light, food, diet, and adequate volumes of water. For the purpose of experimentation, it is advisable to start with a stock population of fish in an open-circulation system in order to furnish an adequate supply of fresh water to maintain the fish in a healthy state. Moreover, fish should remain for a sufficiently long time (weeks) in the stock aquaria for acclimatisation to the artificial environment and to ensure good health.

The experiments with radioisotopes should be carried out in a system with closed circulation of water in order to avoid loss of radioactivity. Thus, two distinct aquaria sets are necessary, the stock population of fish, acclimated to the artificial, unnatural conditions in the laboratory, and the smaller experimental group of fish generated from the stock population to be used in radioecological studies.

After a certain time of acclimatisation only healthy organisms will be chosen for the experiments in order to extrapolate the data obtained to the natural environment. Radiotracer concentration, pH, S%0, T 0C and oxygen should be kept constant and monitored. A change may alter metabolism of organism and physiochemical forms.

Radioisotopes

It is preferable to use gamma-emitting radionuclides with suitable and appropriate physical half-lives (days, weeks, months) according to the duration of the experiments. The commercial market offers a variety of radionuclides (beta-, and gamma-emitters) with half-lives which easily match the requirements of the laboratory experiments. The gamma-emitters also have the advantage to enable a non-destructive measurement of the experimental organisms, since the living organisms can be measured for accumulated radioactivity and analysed again in the same experiment. This possibility will decrease experimental variability between organisms and the quantity of radioactivity to be used. Furthermore, the same experimental animals can be utilised for the total length of the experiments which reduces the numbers of animals necessary and, hence, the size of the aquaria and the quantity of water which is contaminated.

Counting Facilities

Unfortunately, most of the commercial counting facilities are unsuitable for radiobiological or radioecological purposes because they are constructed for general purpose use, therefore, normally have relatively small crystals and, hence, also very small volumes for counting vials. These facilities may be used for small mussels or crustaceans but are unsuitable for fish. Thus, often it will be necessary to construct counting facilities with bigger crystals and a counting chamber which may respond to the requirements of the measurements.

Multi-Compartmental Experiments

In laboratory experiments on uptake and accumulation of radioactivity, usually only two compartments are considered, (e.g. water and fish, or food and fish), in order to enable following the kinetics of uptake in the biota. In more complicated systems, involving several compartments like water, sediments, prey organism, predator, and second stage predator, often the results and/or observations are difficult to explain. Therefore, it may be advisable to make a step by step approach to such a complicated system and/or food web by considering transfers of radioactivity in food chains using separate experiments using different trophic levels.

Uptake Pathways

Uptake pathways are quite variable according to species and their habitats. A fish in nature normally encounters different uptake routes for radionuclides present in the environment. Radioactivity will enter the organism via contaminated food or will be accumulated directly from the surrounding water body through drinking, by absorption across the gills, and to a minor extent by absorption through total surface of the fish. Fish living on bottom sediments often will feed on bottom dwelling organisms and, hence, will eat sediment particles together with the food.

Uptake from Water

Various factors may influence the uptake of radionuclides from water by fish. These are the initial concentration of the radioisotope in the water, the physico-chemical state of the selected radioisotope because it may be soluble, colloid, or in a particulate form, all of which may influence the effective uptake. The stability of the tracer in the water is also important since it may form a compound or absorb to surfaces, container walls or other parts in the system. The loss of the initial radioactivity in solution has to be corrected in order to maintain a relatively constant concentration in the experimental system. A varying concentration of radioactivity will not result in a reliable value of the concentration factor (CF).

The radioisotopes used may or may not be regulated by the organism. If a radioisotope of an element is used which is metabolically regulated by the fish, the behaviour of the radioisotope in the organism changes with respect to an isotope which is not regulated and the resulting concentrations factors may vary considerably. This holds for chemical analogues for example Ca (Sr) and K (Cs) which are regulated and treated by the organism as physiologically essential elements.

Concentration Factor (Measure of Uptake from Water)

In the literature different denominations for the term "concentration factor" exist; these are "concentration coefficient", "concentration ratio", "bioaccumulation factor", etc.. All these terms mean basically the same thing and they refer to the ratio between the activity in the organism (fresh weight, assuming a relative density of 1), divided by the activity found in the same quantity (or volume in this case, g/ml) of water. Thus, the definition of the concentration factor is a ratio of activities in equal units of the organism and water.

Furthermore, CFs refers to equilibrium conditions between the organism and the surrounding water and can by definition be calculated only at steady-state. This means that equilibrium exists where intake of radioactivity by the organism equals the excretion rate, so that the concentration of radioactivity in the body remains constant.

$$CF = \frac{\text{activity (cpm, Ci, Bq) / g organism (FW)}}{\text{activity (cpm, Ci, Bq) / ml water}}$$

where, CF is the concentration factor, cpm is counts per minute and FW is fresh weight

The scope of the calculation of concentrations factors in the environment is to relate all concentrations of radionuclides in environmental samples to a common value, and this value is the corresponding activity in the water. The disadvantage of the use of concentration factors is that the CF is just a ratio and not a numeric or an absolute value. Concentrations factors originally refer to uptake from water and not from other sources like food in which case the term "transfer factor" should be normally used.

Potential Parameters influencing the Calculation of Concentration Factors

The calculation of a concentration factor is strongly influenced by environmental as well as by inherent factors of the organisms. The relative concentration of radioactivity in water may vary by a factor of 100 whether filtered or unfiltered water is considered (e.g. total water: particulate matter, phyto-, zooplankton). The use of the type of filters is crucial, of course, because the definition of the "soluble phase" of filtered water depends on the mesh size (e.g. 0.45 or 0.25 um). Therefore, the question arises where the limit between particulate and soluble can be set because some "soluble" components will not pass through a filter of 0.22 μm. With respect to organisms the parameters which influence the CF are season, temperature and salinity of the water, as well as the general physiology, sex and size of the experimental organisms.

Considering uptake from food other parameters may influence the CF. This depends mainly on whether natural food or artificial food is used in the experiments and how the radioactivity was accumulated in the food. Whether the radioactivity was introduced artificially or if it was accumulated by the organism physiologically (i.e. assimilated) the CFs obtained may change considerably. In the latter case the type of compound in the food may be readily bioavailable for the organism of the next trophic level. This is also true for artificial food; however, the situation may be somewhat less clear because all characteristics and constituents of the food have to be known (stable element content, physico-chemical state of the radioisotope, the different compounds of the radionuclide in the artificial food) in order to assess the bioavailability of the radioactivity in that specific food. Some examples in the literature serve to illustrate the influence of size of organisms on the CF (Fig. 1).

Accumulation from Water

Accumulation from water can be expressed by a simple model, i.e. accumulation is intake minus excretion; therefore, at equilibrium intake equals excretion. This can be derived from the following formula where the change in concentration with time is equal to intake minus the concentration at any time multiplied by the coefficient k,
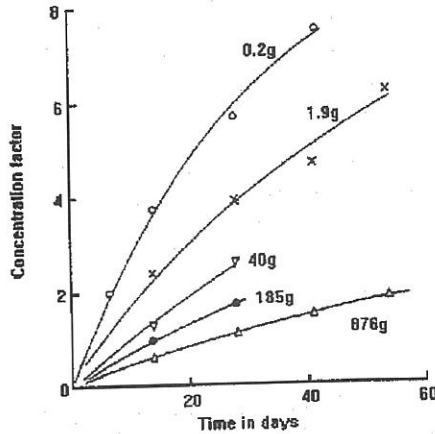
Fig. 1 Concentration of ^{134}Cs by plaice of different weights [1]

which is the absorption or fixation coefficient and also the excretion coefficient.

At equilibrium between the organism and the environment (steady state) intake balances excretion that means the maximum value for the CF will reach maximum value of the accumulation curve.

$$\frac{dC_t}{dt} = I - kC_t; \quad I = C_{SS}$$

$$\left(\text{steady site; asymtotic value: } C_{SS} = \frac{I}{k} \right)$$

$$\frac{dC_t}{dt} = k C_{SS} - k C_t = C_t = C_{SS}(1 - e^{-kt});$$

$$k = \frac{\ln 2}{T_{1/2(b)}} = \frac{0.693}{T_{1/2(b)}}$$

where, C_t is the concentration of a radioisotope at time t, I is the intake/unit weight/unit time, C_{SS} is the concentration at equilibrium (steady state), k is a constant, i.e. the coefficient of adsorption or fixation (excretion) and $T_{1/2(b)}$ is the biological half-life

Accumulation from Water and Food

On some occasions one has to consider the combined uptake of radionuclides from water and from food. Coming back to the initial formula, the simple model describes uptake or accumulation as intake minus excretion. The formula remains practically the same as before except that two factors, which correspond to the intake from food plus the intake from water, must be introduced. Furthermore, one has to consider various parameters and factors mentioned previously which potentially influence the uptake. All those factors enter into the formula, i.e. concentration of the radioisotope in the water and food, body size (weight) of the organism, and the temperature (uptake velocity is dependent on temperature and weight of the organism at a certain time).

$$\frac{dC_t}{dt} = I - kC_t; \quad \text{(Accumulation = Intake - Excretion)}$$

$$\frac{dW_t C_t}{dt} = (I_{ft} + I_{wt})W_t - k_T W_t C_t$$

where, C_t is the concentration of a radionuclide (Bq/g) at time t, W_t is weight of organism (g) at time t, I_{ft} is intake from food, I_{wt} is intake from water and k_t is the fixation rate (elimination) in function of temperature and weight (W_t)

The former formula does not consider growth since it is sufficiently difficult to control all the afore-mentioned parameters of the experiment. Nevertheless, under normal experimental conditions one should maintain growth. However, sometimes it may be impossible to control growth; thus, during the time of the experiment exponential growth of the organisms, especially when using small organisms, may occur. That situation will complicate the formula because organisms, depending on the initial weight at time zero, will exponentially change weight according to their growth constant, i.e. it will increase.

$$W_t \ W_0 e^{\lambda_g - t}; \ \lambda_g = \text{growth constant / day}$$

$$C_t = \frac{I_{wt} + I_{ft}}{k_T + \lambda_g}\left[1 - e^{-(k_T + \lambda_g)t}\right]$$

If intake from water and food are considered separately, then the following formulas can be used. Once again it holds that uptake or accumulation is intake minus excretion. In both cases the same parameters as before have to be considered, i.e. the weight of the organism, the temperature at time t, and the increase in weight per day.

$$C_t = \frac{I_{wt}}{k_T + \lambda_g}\left[1 - e^{-(k_T + \lambda_g)t}\right]$$

$$I_{wt} = \frac{C_t (k_T + \lambda_g) + I_{ft}}{\left[1 - e^{-(k_T + \lambda_g)t}\right]};$$

at equilibrium (steady state): $C_t = C_{SS}\left[1 - e^{-(k + \lambda)t}\right]$

$$I_{wt} = C_{SS}(k_T + \lambda_g)$$

Elimination

Once a certain value of CF is reached or a certain quantity of the radioisotope has accumulated in the organism, which may be close to the steady state condition, it may be of interest to examine the loss, or elimination rate of the radioisotope by the organism. For this reason an elimination or loss experiment has to be performed in uncontaminated aquaria. Generally, the contaminated organism which had accumulated a certain amount of radioactivity is placed into a non-contaminated environment. This can be done in two ways, either in a flow-through sea water system or in a aquarium with a re-circulation system for uncontaminated water. Normally, aquaria with a sufficiently large volume are satisfactory in order not to change the water frequently.

The quantity of radioactivity in the organism at the time of start of the elimination experiment (Co) is measured at 100%, regardless of the absolute quantity in the organism.

The elimination or loss followed over time can be described by the following formula.

$$C_t = C_0 \, e^{-k_{eff} \cdot t}; \; k_{eff} = \frac{0.693}{T_{1/2eff}}$$

(constant of effective estimation)

where, C_t is the concentration at time t and C_0 is the concentration at time t_0 (body burden) which can reach C_{SS} (100%).

This correlation shows that the concentration at time t is dependent on the concentration of the radionuclide in the organism at time zero. That means 100% in this case, i.e. $C_0 e^{-k_{eff} t}$. This constant of effective (eff) elimination consists of two rates or two factors of elimination: a real physiological elimination of the quantity of radioactivity in the organism, and the elimination by physical decay. This may be of varying importance according to the radioisotope used. The coefficient of effective elimination can be derived from the formula for effective half-life ($k_{eff} = 0.693/T_{1/2eff}$)

The calculation of the effective half-life or half-time of a radioisotope in an organism is a relation between physical half-life or decay and the biological half-life which still has to be determined. The equation can be solved for the biological half-life and elimination rate or turnover time in the organism.

$$T_{1/2eff} = \frac{T_{1/2(p)} \times T_{1/2(b)}}{T_{1/2(p)} + T_{1/2(b)}}; \; T_{1/2(b)} = \frac{T_{1/2(p)} \times T_{1/2eff}}{T_{1/2(p)} + T_{1/2eff}}$$

p = physical and b = biological

Graphical determination of parameters

The parameters of for the elimination equation can be determined graphically and this is often used to determine the elimination coefficient and the percentage of radioactivity in the different compartments. The loss curve (points) drawn on semi-logarithmic paper will be represented by one or more straight lines. The number of lines to be fitted to the experimental points should correspond to the number of compartments found in the organisms by dissection.

If the graphic presentation of the experimental results for radionuclide loss indicates a single straight line, then the organism can be considered as only one compartment. In this case the quantity of radionuclide transferred (accumulated) per unit of time from water to the organism is proportional to the concentration of the radionuclide in the water (Cw) and a constant (kw.org)' the coefficient of adsorption, while the quantity eliminated from the organism (lost from the organism to water) per unit of time is proportional to the radionuclide concentration in the organism itself and the constant <korg.w), the coefficient of elimination or excretion. Under equilibrium conditions, the two rates, kw argo and korg.w' are equal, i.e. the velocity of transfer of radioactivity from the water to the organism (accumulation, intake), depending directly on the concentration of the radioisotope in the water, balances the velocity of loss from

the organism to the water. This indicates steady state where intake equals loss or release.

At equilibrium (steady state):

$$k_{w\,org} \cdot C_w = k_{org\,w} \cdot C_{org}; \; \frac{C_{org}}{C_w} = CF_\infty = C_{SS} \text{ (steady state)}$$

$$\underset{\text{(intake)}}{k_{w\,org}} = \underset{\text{(loss)}}{k_{org\,w}} \cdot C_{SS}$$

where, $k_{w\,org}$ is the rate constant water → organism and $k_{org\,w}$ is the rate constant organism → water

A practical demonstration of the procedure of the graphical method is given using the experimental data for a crab (Pachygrapsus mannoratus) obtained in a laboratory study (Fig. 2).
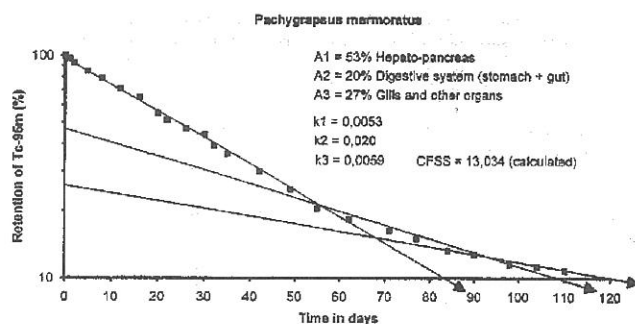
Fig. 2   Graphical determination of the percentages of radioactivity present in different component of crab

The straight lines arbitrarily drawn correspond to three different velocities and compartments in the organism. During the observation phase of 120 days, no more than three compartments could be identified. A longer observation time would perhaps have revealed additional compartments with very slow elimination velocities like bone.

Starting with the slowest compartment one can directly read the percentage of radioactivity which was present at time zero for this part of the curve, in this case 27%. For the faster compartments the differences between each other and 100% are 20% for the intermediate and 53 % for the fastest compartment. By dissecting the organisms afterwards one may find the corresponding compartments which show similar contents (percentages) of the total radioactive body burden. In this particular case the three percentages could be attributed to hepato-pancreas (53%), digestive system (stomach and gut, 20%), and gills and other organs (27%), the latter which had the slowest exchange or turnover rate.

The remaining elimination coefficient k necessary to describe completely the elimination process can be calculated by the formula k = 0.693/biological half-life(days). The biological half-life (time necessary to reduce the initial activity 50%) of a certain radioisotope e.g. Tc in the different organs can be read directly from the graph by subtracting the slow loss components from the faster ones since the value of 100% is the sum of all components.
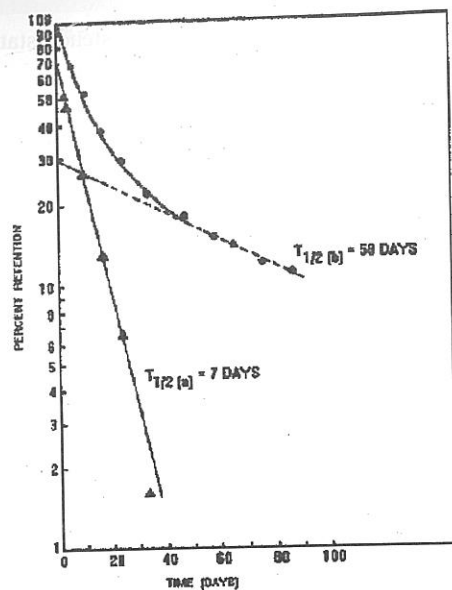
Fig. 3   Retention of Tc-95m by Haliotis rufescens following direct uptake from sea water

The practical execution of the graphical determination of biological half-lives and the percentages present in the different compartments is demonstrated in the Fig. 3. In this two component loss curve the low velocity component, which represents 30% of the total body burden, has to be subtracted from 100%. The resulting fast velocity component which represents 70% has to be back-extrapolated to the initial original part of the loss curve. Then starting from 70%, the biological half-life can be found by reading the value on the abscissa vertically below the point of intersection between 35 % and the constructed line (7 days). The same holds for the low velocity component.

The question arises whether the field or the laboratory data have to be considered as artefacts. The difficulty to measure and determine CFs in the natural environment is caused by the high uncertainty of correct measurements of radioactivity concentrations in the natural water body. Often very low concentrations of a certain radionuclide are found which are difficult to detect (large water volumes to be handled) or vary considerable between locations and within short time periods, so that it is difficult to decide on a realistic value

Conclusion

Although the experimental approach to measurements and determinations of radioecological parameters in marine biota (concentration factors, assimilation rates, biological half- lives etc.) seemed to be quite simplistic, experience on the general behaviour of radionuclides in marine species gained over several decades has demonstrated that much useful information can be obtained from laboratory simulations providing proper controls on the experiments and their conditions were set and maintained. However, data obtained under laboratory conditions with species deprived of their natural environment can only be but indicative since

realistic values can only be found and/or measured in the field. For several reasons direct measurements of concentration factors and still more seldom biological half-lives or residence-times are often very time consuming and difficult to obtain in the field because of fluctuating concentrations of the radionuclide or high infra-species variability. Nevertheless, data from laboratory experiments have often given "values" that could have been otherwise obtained much more difficultly or not at all with field measurements. In conclusion, one should consider laboratory experiments, even if performed as close as possible to natural conditions, as a means, technical tool and/or simulation of what may happen under natural conditions. Therefore, data and results obtained in the laboratory should always be taken with care and, if possible, be validated against measurements in the field.

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Understanding and Evaluation of Exposure Effects to Non-Human Species from Radionuclides: Recent Advances and Perspectives in Nuclear Ecotoxicology Research in France and Europe

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Abstract

Funding for radioecology in Europe escalated after the Chernobyl accident, and remained elevated for some 15 years. The enhanced funding permitted Europeans to explore new directions in radioecology, particularly in the area of effects to non-human biota. Herein we highlight several recent advances and attempts to merge environmental risk analysis methods for radioactive contamination with those for other pollutants using species sensitivity distributions; to extrapolate damages observed in individual organisms to potential effects in their populations; and the use of advanced models to estimate and explain changes in an individual's energy allocation as a result of exposure to low doses of radionuclides. We conclude by presenting the radioecology Alliance which is an international network under construction in Europe. The Alliance goal is to integrate resources in order for radioecology to efficiently fill in the gaps of knowledge and improve radiological risk assessments tools to support both human and environmental radioprotection.

Background

Basic Lines

Radioecology is a branch of environmental sciences devoted to a specific category of stressors i.e. natural and artificial radioactive substances. This science includes key issues (i) common with other groups of pollutants, particularly metals (e.g., transport, fate, speciation, bioavailability, biological effects at various organisational levels) and (ii) specific to radionuclides (e.g., external irradiation pathway, radiation dosimetry, decay products) [1].

On an operational point of view, radioecology gathers all the environmental-related knowledge necessary to provide the key elements to perform the assessment of the impact or risk of radioactive substances on man and the environment. Ideally, integrated Environmental Impact (Risk) Assessment (EIA/ERA) may be done in parallel for both non-human species (demonstrating the protection of the ecosystem per se) and for humans (and for both chemicals and radioactive substances). Ecological (Human) Risk is an estimation of the probability (or incidence) and magnitude (or severity) of the adverse effects likely to occur in an ecosystem or its sub-organisational levels (in human individuals or groups), together with identification of uncertainties. The Environmental Impact (Risk) Assessment is generally implemented through a tiered-approach, from screening tier using simple models and conservative assumptions to higher tier using site specific models and data associated with Sensitivity/Uncertainty analysis for a proper interpretation of the impact or risk. The basic components of any EIA/ERA are presented on Figure 1. They comprise exposure and effects analyses integrated through risk characterisation. Today, even if existing knowledge on transport, transfer, dosimetry and biological effects has been extensively used to gain the capability of implementing an ERA-type approach, it still remains major research challenges in radioecology. This paper only deals with one of
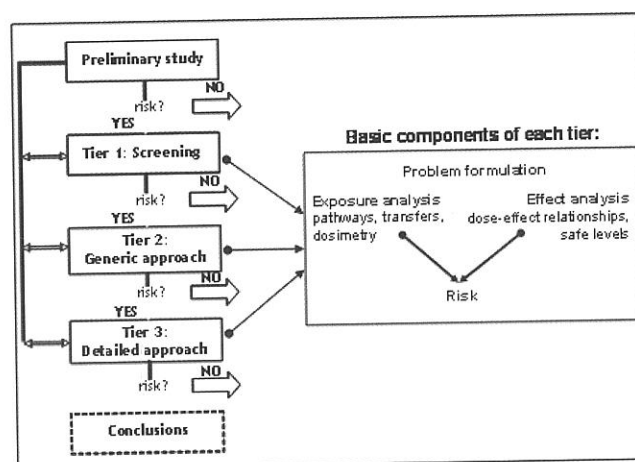

Fig. 1 *General scheme of a tiered approach (left side) and its basic components (right side) used for Environmental Impact or Risk assessment whatever the object of protection (human or ecosystems) and the category of pollutants (stable or radioactive substances).*

the discipline of radioecology, i.e. nuclear ecotoxicology which operational outcomes are useful for regulatory needs in the field of environmental radioprotection.

Focus on the Radioprotection of the Environment

The need for a system to protect the environment from ionising radiation has, over the past decade, been recognised internationally. The ICRP has addressed environmental protection as an element of the revision of its recommendations [2] and released underlying concepts [3] and environmental protection is now referred to in the International Atomic Energy Agency's Fundamental Safety Principles [4]. Moreover, both the International and Euratom Basic Safety Standards (BSS) are currently under revision, considering the inclusion of radioprotection of the environment. Today, within Europe only the UK, Sweden and Finland appear to currently regulate specifically to

protect the environment rather than relying on previous ICRP statements. The recommendations of the ICRP and changes in both the IAEA and the Euratom BSS are likely to lead to a change in this situation. As more member states regulate specifically for the environment in forthcoming years, regulators and industry will require the support of radioecological expertise.

Within this framework, this paper reports on two examples to illustrate the major advances in the following topics : 1) advances in making consistent environmental risk analysis methods for radioactive contaminants with those for other pollutants; 2) advances to extrapolate damage observed in individual organisms to potential effects in their populations which are more relevant wih regard to ecological protection. The conclusion lists the priority lines of research that have been identified to enhance our capability of predicting environmental effects of radionuclides under the realistic conditions in which organisms are actually exposed, i.e. mainly chronic low dose exposure and muti-contamination.

Some Examples of Recent Advances in Nuclear Ecotoxicology

The major overarching challenge for nuclear ecotoxicology is to develop efforts to align environmental risks assessments from radiological exposures to the methods used in ecotoxicology for other types of contaminants [5]. The science of ecotoxicology, however, is also troubled with procedural difficulties, large uncertainties, and the current need to extrapolate results generated on individual organisms to predicted responses at the population and community levels [6]. To counter the uncertainty, extrapolation factors (also named Assessment Factors AFs) are used to incorporate precautionary safety in the risk estimates. AFs increase the conservatism in risk estimates by safety factors up to 1000, and indicate the level of uncertainty in predicting environmental effects. Reducing extrapolation and the associated conservatism in environmental risk assessments has been identified as a major research need in environmental sciences [7]. New knowledge and methods are needed in both ecotoxicology and radioecology that will permit the development of science-based rules experimentally validated for confidently extrapolating from simple to more complex biological/ecological systems [8].

How to Estimate the Exposure to Non Human Biota from Radionuclides ?

Several generic assessment approaches have been developed for exposure dose (rate) assessment of non human biota. These approaches are generally based on the definition of Reference organisms [9], i.e. "a series of imaginary entities that provide a basis for the estimation of radiation dose rate to a range of organisms which are typical, or representative, of a contaminated environment. These estimates, in turn, would provide a basis for assessing the likelihood and degree of radiation effects. It is important that
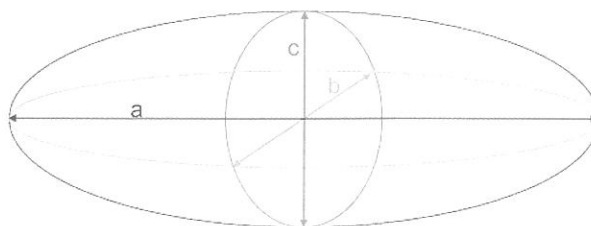

Fig. 2   EDEN equivalent ellipsoid and its three axes

they are not a direct representation of any identifiable animal or plant species" [10]. In this aim, the ERICA approach [5] was developped, including radionuclide transfer models for the calculation of external and internal radionuclide concentrations, and tables of dose conversion coefficients for the calculation of absorbed dose (rate) to a number of representative geometries for different reference organisms. Non-human biota dose calculations are conducted on the basis of a simplified (e.g. ellipsoid) geometry, representative of the dimensions of the organism. Dose conversion coefficients (DCCs) for both internal and external exposure to radionuclides are specific to these organisms and geometries.

As an example, the estimation of DCCs are evaluated at the IRSN applying the EDEN software. EDEN is a tool based on modules that allow any user to define his own case study [11]. It is obtained through the combination of the elementary data required to fully describe the exposure scene: shapes, compositions and source term (or energies). In EDEN, each organism shape is assimilated with an ellipsoid, characterised by its thee axes, following the Fig. 2. The shape of the ellipsoid depends then on the values of the axes a,b and c respectively.

The composition of the different media (air, soil, water, biota) can be found in elswere [13] and EDEN uses a grid of basic energies for each radiation type, to evaluate the associated monoenergetic Dose Conversion Coefficient (DCC). Three environmental scenes may be considered in EDEN: one single medium, two or three media (Fig. 3). The considered organisms have then to be located in their environment following these possibilities and their lifestyle, an occupancy factor being associated with the situation involving several media.

External radiation exposure depends on various factors including contamination levels in the environment, the geometric relationship between the radiation source and the organism, habitat, organism size, shielding properties of the medium and the physical properties of the radionuclides present. External DCCs are the factors that allow the absorbed dose rates to an organism to be estimated from the average concentration of a radionuclide in an environmental compartment (soil, sediment, water) of a reference ecosystem (expressed in, for example, μGy per hour per Bq/kg). External dose rates are calculated using the DCCs, taking into account the proportion of time that an organism

A: one external medium   B: two external media   C: three external media
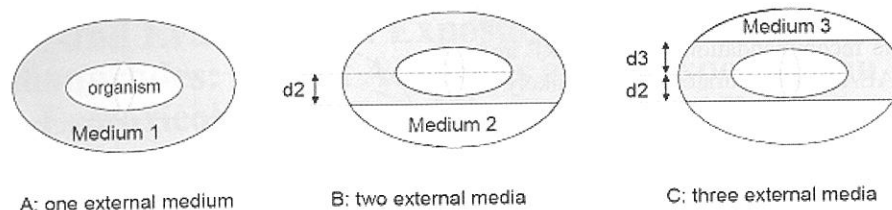
Fig. 3   Exposure geometries described in EDEN

spends in different compartments of the reference ecosystem (occupancy factors).

In the case of internally incorporated radionuclides, the internal concentration of radionuclides in the organism is required. Internal exposure arises following the incorporation of radionuclides by the organism via pathways such as ingestion, root uptake... The magnitude of internal doses is determined by the activity concentration in an organism, the size of the organism, and the type and energy of emitted radiation. Internal activity concentrations are generally calculated through the application of concentration ratios, which relate the concentration in an organism to that in the surrounding environment. Concentration ratios assume a uniform distribution of the radionuclide within the organism (i.e. with no accumulation of radionuclides within individual tissues). Internal DCCs are then applied that relate the average concentration of a radionuclide in a reference organism to the dose rate.

Finally, once external and internal DCCs calculated for each radionuclide and organism geometry of interest, the key parameters utilised in the calculation of absorbed dose (rates) for non human biota are thus (1) the occupancy factors; (2) the concentration ratios; and (3) the concentration in the media (Sediment, water, air, soil).

How to Integrate Environmental Risk Assessment Methods for Radionuclides and Chemicals?

In Europe, the technical guidance document for chemicals [12] and recently the ERICA integrated approach for radionuclides [14] recommend to implement, in a first step, a Screening Tier (or Screening Level Ecological Risk Assessment, SLERA). SLERA is used to evaluate whether the emissions can put the receptor ecosystems at risk or not. Beyond this ERA-type approach, one major challenge still remaining is to gain the capability for assessing radiological impact in a comparative unbiased way to what is done for other stressors such as chemical substances. Recently we proposed to adapt a Life Cycle Assessment derived methodology to solve this issue [15]. Actually, concerning releases from nuclear facilities under authorization, any SLERA is a challenging task because of (1) the large number of substances, (2) the various quantities that may be emitted to the ecosystems and (3) the various types of ecosystems to be considered. This task must be performed for two categories of pollutants, radionuclides and chemicals, each exhibiting specificities in terms of concentration in the exposure medium (- or dose) -effect relationships.
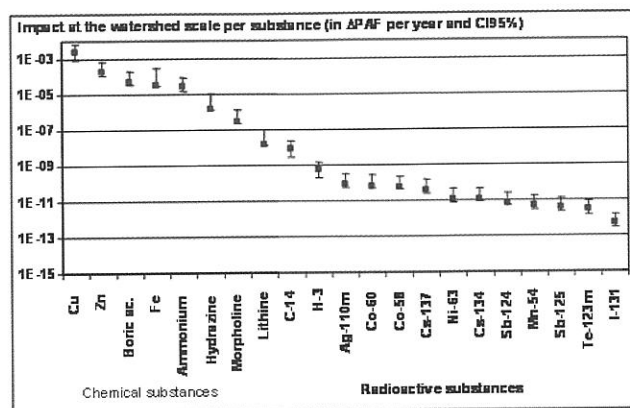

Fig. 4   Ranking of the substances realeased in the year 2005 from electronuclear power plants alongside the Rhône river according to their calculated impact at the watershed scale expressed in change of potentially affected fraction (PAF) of species [15].

Briefly, the method consists in calculating the ecotoxicological impacts in ecosystems. It comprises a fate-analysis step described by a fate factor (calculation of the change in exposure from a given release) and an effect-analysis step described by an effect factor (calculation of the change in effect per unit change of exposure) [15, 16]. Ecotoxicological exposure-response is mostly based on the Species Sensitivity Distributions (SSD) and the potentially affected fraction (PAF) of species as indicator of ecosystem damages. The PAF value expresses the toxic pressure put on ecosystems due to the presence of a given pollutant and can be easily calculated homogeneously whatever the category of stressors. As an example, the method allowed us to rank the routinely released substances from nuclear power plants alongside the Rhône river, on the basis of the associated ecotoxicological hazard for the watercourse and therefore to identify high-risk chemicals and/or radioactive substances for ecosystems (Fig. 4). Such comparative method represents a first step towards an integrated impact assessment whatever the category of stressors considered. The next step is to take account for potential interactions between the different types of stressors.

How to Extrapolate Damages Observed in Individual Organisms to Potential Effects at Population Level?

EU regulations for protecting the environment target protection of populations and ecosystems; yet, most laboratory research on toxic effects is carried out at individual level (e.g., responses in growth, reproductive
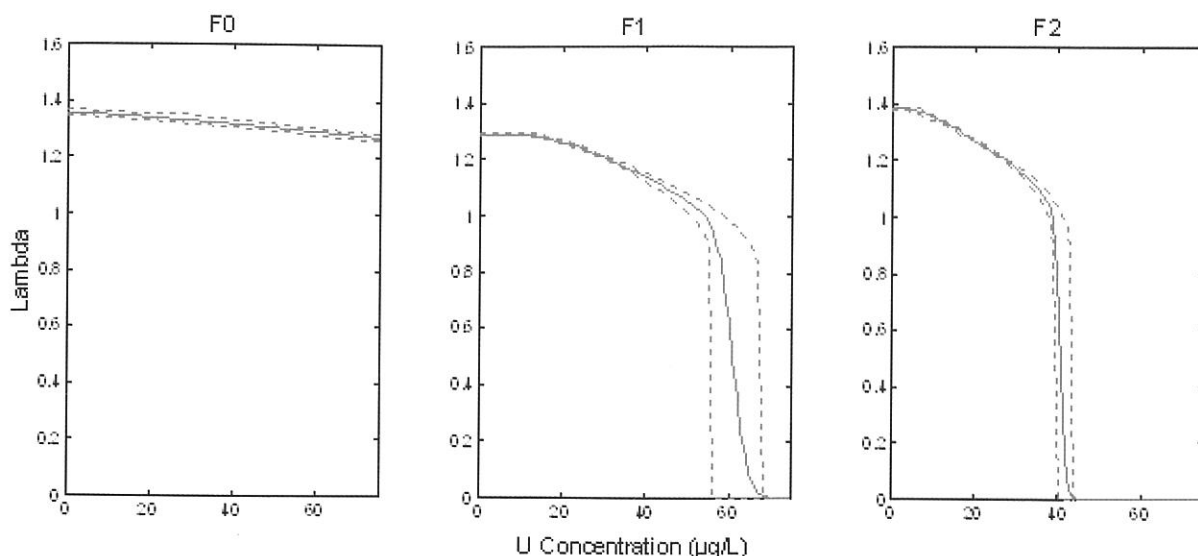
Fig. 5 Changes in population growth rate (λ) of D. magna at equilibium as a function of uranium water concentrations (μg/L) in 3 successive exposed generations [18]. Threshold at which significant effects are detected at organismal level in F2 is 2.0 μg/L. Above this value, population grows at reduced rate and extincts at 39 μg/L (when λ<1).

capacity, survival). One of the most important challenges is to extrapolate measured effects from the individual level to the population level (e.g., responses in abundance, age or size structure, population growth rate, carrying capacity, genetic diversity). From a management perspective, those population-level endpoints are ecologically more relevant than health or survival of individual organisms, excepting for endangered species. Focusing only on individuals can lead to inaccurate estimates of risk to populations, due to the complexity and nonlinearity in the relationship between effects on individual survival, reproduction or growth and population dynamics [17].

One approach recently adopted at IRSN and currently applied to a limited number of stressors combines the use of Dynamic Energy Budget (DEB) and Leslie matrices [18]. Based on simple rules for metabolism [19], the DEB theory describes how individual organisms acquire energy from food and allocate it to survival, growth, maturity and reproduction and how underlying physiological functions are perturbated under toxicant exposure. Traditionnally used to simulate population dynamics, Leslie matrices allow to integrate effects on individual survival and reproduction in terms of population growth rate. Here, we combined both approaches to predict consequences of increasing dose rates for population dynamics based on survival, growth and reproduction responses measured at individual level. As an example, we report briefly on effects of multigenerational exposure to waterborne uranium in a freshwater invertebrate (Daphnia magna). Under controlled laboratory conditions, toxic effects on daphnid life history and physiology increased over a 3-generation period [18]. Uranium primary mechanism of toxicity was identified as an inhibition of food assimilation, with strong consequences for somatic growth and reproduction. Combining DEBtox-Leslie matrix approaches, we integrated effects and predicted changes in population growth rate (over the range of uranium concentration. In the second offspring generation, exposure to uranium caused toxic effects above a concentration of 2 μg.L^{-1}, the resulting decline in leading to population extinction above 39 μg.L^{-1} (Fig. 5). Such combination of the DEBtox and matrix population models has already been successfully applied to metals and organics [20,21]. This approach appeared as a powerful tool to identify and compare underlying mode of toxic actions among stressors and to assess ecotoxic consequences at the individual and the population levels. This work will be followed up under the STAR EC-funded network of excellence with a comparative investigation of the population consequences of chronic external gamma exposure vs. alpha irradiation.

Perspectives Under the European Alliance in Radioecology

During the last decades, research in the field of radioecology has led to a widely recognized expertise in Europe. As pointed out in the FUTURAE project [1], there are today clear signs that key elements of this expertise are endangered. One major reason of this decline is that research effort, that was intensive during the years following the Chernobyl accident, is now regularly decreasing. Most of the National and EU funded radioecology programmes of the last decade have focused on modeling efforts and data summaries whether very few attention was paid to the acquisition of new knowledge, especially through experiments. Although this situation has no visible consequences on the short term, it can be anticipated that, on middle and long term, the lack of competences and expertise in radioecology could have important consequences for example in the case of a nuclear accident or for new builds. Moreover, given the worldwide so called "nuclear rennaissance" and the increased concern of the public for the impact of human activities on health and the environment, it

is essential to ensure the long-term maintenance and enhancement of expertise, infrastructures and resources relating to radioecology.

In mid 2009, eight european organisations forming the European Alliance in Radioecology acted together to operate a "revival" programme. They signed a memorandum of understanding for establishing a long-term strategic research agenda in order to (i) enhance the efficiency of research, (ii) share infrastructures, (iii) maintain and enhance radioecological expertise through education, training, mobility, knowledge management and dissemination. The first concrete action was to establish a proposal for a network of excellence in radioecology submitted in spring 2010 to the EC. The Alliance was successful and STAR, a Strategy for Allied Radioecology, will be launched in early 2011.

Several lines of research will be pursued over the next 10-15 years to address our current inability to accurately predict the environmental impacts of radioactive contaminants. Concerning more specifically nuclear ecotoxicology, we propose to focus on environmentally relevant low dose and low dose rates, including:

- multiple generational effects;
- comparative sensitivity among organisms with different life history traits; inter- and intra-species;
- comparative biological efficiency of different types of exposure pathways and radioactive emissions;
- primary modes of action from which effects are generated;
- propagation of effects to various levels of biological and ecological organisation;
- relationships of exposure concentrations (chemical speciation, bioavailability, dose) and effects
- mixed contaminants and multiple stressors.

The research conducted in this area by our Alliance will be based on hypothesis-driven laboratory and field work, with concomitant modeling and meta-analysis of data.

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Dr. Thomas Hinton has developed a broad, diverse knowledge in radioecology with 60+ peered-reviewed manuscripts that span topics on the transport and fate of radioactive contaminants in aquatic and terrestrial ecosystems; effects of low-level, chronic irradiation on biota; transgenerational effects from contaminant exposures; remediation of radioactively contaminated ecosystems; contaminant transport models; as well as human and ecological risk analyses. Dr. Hinton was hired by IRSN in 2009 and brings an international perspective to the European team that constitutes the Strategic Network for Integrating Radioecology (STAR).

Dr. Jacqueline Garnier-Laplace, has been working for IRSN for 20 years. She is currently Head of Department on studies of radionuclides behaviour in ecosystems (50 permanent staff involved in radioecology and ecotoxicology R&D and in environmental modelling and risk assessments). She was involved in the EC funded projects suite (FASSET, ERICA, PROTECT, FUTURAE) and has published ca. 60 peer-reviewed papers. She will be involved in STAR European Network of Excellence as a leader of the work package devoted to Ecologically Relevant Low Dose Effects.

Dr. Rodolphe Gilbin, head of Radioecology and Ecotoxicology Laboratory at IRSN (24 permanent staff involved in radioecology and ecotoxicology R&D), is a PhD ecotoxicologist of 10 years experience. His main areas of research are the bioavailability of trace elements in freshwaters and multipollutants in ecological risk assessment. He has published ca. 25 peer-reviewed papers. He is a member of the IAEA EMRAS II Biota Working Group and IUR Task Forces Waste and Mixtures and will be involved in the STAR European Network of Excellence.

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Dr. Karine Beaugelin-Seiller is an expert in environmental risk assessment, especially regarding wildlife exposure to ionising radiation, with about 20 years experience in radioecology. She is involved in the conception and development of methods and tools in this area at the international level (ICRP committee 5 dosimetry task group, EMRAS I and II Biota Modelling Group, FASSET, ERICA and PROTECT EC projects) and will be involved in the STAR European Network of Excellence.

Radio-ecological Aspects of Environmental Surveillance Around Nuclear Facilities

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Abstract

Among the major challenges of radiological surveillance, quantitative evaluations of the biological hazards, if any, resulting from prevailing radioactivity in the environment is of prime importance. The impact of ionizing radiation on human through direct / indirect pathways are relatively well understood and documented. However interaction between radiation and a wide range of biological species (plants and animals) is not very well understood because of their large variation with regard to their life cycles, their life spans and their exposure pathways in the environment. Thus, it is imperative to undertake the studies to know how radionuclides in their different chemical forms interact with nature; how different mechanisms affect the radionuclide's migration and uptake in food chain of ecosystems. Therefore, investigations in radioecology might include aspects of field sampling, laboratory experiments and the development of predictive simulation models. This paper discusses the various aspects of radio-ecological aspects related to radiological environmental surveillance around nuclear facilities.

Introduction

Radioecology may be defined as the study of biological species and their interaction with external environment in relation to ionizing radiation. The prevailing radioactivity in the environment has two components; one relates the effect of ionizing radiation on individual species, their population, communities and ecosystem while the other refers to its movement or transfer of radionuclides in the biosphere and their accumulation within the specific matrices of the environment, such as soil, sediment, water and biota.

The entry of radioactivity in the environment is mainly through naturally occurring radionuclides from primordial origin, fallout radioactivity due to weapon testing or mishaps of nuclear devices and radioactive effluent released from nuclear facilitates. In order to assess the fate of environmental radioactivity, it is essential to monitor the levels of radionuclides in various matrices of environment. This needs to follow a comprehensive environmental surveillance around individual operating nuclear facilities.

Indian nuclear programme of peaceful uses of atomic energy involves operations of various facilities to complete the stages of nuclear fuel cycle process. As a matter of the environmental policy of the Department, comprehensive radiological environmental surveillance is being persuaded at all the nuclear installations in the country to assess the impact of operations of the facility on the environment. The environmental monitoring covers a radial distance of 30 km around each of the facility.

However, detail studies on the migration and accumulation of radionuclides in biological species through various components of environment is of utmost importance to assess the effects of radiation on biological species at their cellular levels. Such studies form the part of radio-ecological aspects of environmental surveillance around operating nuclear facilities.

Environmental Surveillance Programme

The radiological surveillance is carried out by well established Environmental Survey Laboratories (ESL) located in the vicinity of each nuclear facility in India. The ESLs have been established and operated by Health, Safety and Environment Group, BARC.

In fact the environmental monitoring commences at least two years prior to the commissioning of the nuclear facility to collect the baseline data on natural and fallout radioactivity in the environs of proposed facilities and the later phase of monitoring continuous throughout the operating period of the facility to assess the impact of operation of the facility on the environment. The monitoring includes estimation of radioactivity in different environmental matrices, including dietary items to evaluate radiation doses received by the members of public, residing in the vicinity of nuclear facilities. The well defined and executed environmental radiological surveillance programme around each nuclear facility shows that radioactive contamination is insignificant and demonstrated that the doses to the members of public are far below the regulatory limits.

Scope of Radioecological Studies

The ICRP believes that the standard of environmental control needed to protect man to the degree currently thought desirable will ensure that non-human species are not put at risk. ICRP publication - 103 further emphasized to assess the relationships between exposure and dose, and between dose and effect, and the consequence of such effects, for non-human species, on a scientific basis. The biological effects of radiation on plant and animals have long been of interest to radioecologists. The basic understanding of the effects of radiation at a cellular level is extremely important to know the mechanisms by which radiation damage is caused. Further, because of the potential for radioactive contamination in the food web which can adversely impact human and ecological health, as well as in the interest to understand the relative contributions to local and global sources to current and future trends of radionuclides in environmental components, the studies are essential. Scientific efforts are therefore needed to study radioecology in the surroundings of nuclear facilities. To address the issue,

numerous ecological risk assessments have been carried out at several sites abroad to study the potential effects of ionizing radiation on a variety of plants and animals.

Taking a note of the above, it is imperative to study the radiological risk to non-human biota around Indian nuclear facilities. A few projects on such studies have been granted to universities to study these aspects in the vicinity of NPP sites under DAE - BRNS. These projects are focused to assess the effects on non-human biota through Screening Index methodology, which has been developed by Framework for Assessment of Environmental Impact (FASSET, 2003, 2004).

Assesment of Radiological Risk to Non-human Biota

A number of approaches for evaluating radiological risks to non-human biota have been published and are widely used by IAEA, US NCRP, US DOE and ICRP. All approaches involve simplifications of the actual environment. In general the following steps are needed to be undertaken to evaluate the ecological risk owing to the releases of radioactive effluent from the facility.

Identification of Nuclear Facility

Any facility of nuclear fuel cycle right from mining & milling of nuclear fuel to radioactive waste management, areas of enhanced naturally occurring radionuclides which are associated with the releases of radionuclides in the environment may be considered for such studies.

Identification of Radionuclides in the Effluents Released from the Nuclear Facility

Once the study area is identified, the next step is to identify the radionuclides discharged into the environment. For example, the fission & activation products in case of nuclear power plants, isotopes of uranium & thorium and their daughter products in case of front end nuclear fuel cycle sites and trans-uranic radio-nuclides for fuel reprocessing sites. The identified radionuclides are then orderly bench marked as per their potential to deliver external / internal exposure to the ecological receptors. This needs information on their physical (such as their half life), chemical (such as their chemical form) and biological (such as metabolic) characteristics.

Biodiversity of Non-human Species

In simple terms the biodiversity provides the details on variety and abundance of flora and fauna in the study area. The biodiversity of non-human species in the study environment needs to be assessed; it would be appreciated if such studies could be conducted during pre operational phase of the nuclear facility so that a clear cut comparison of their variety and abundance of non-human species may be established during operational phase of the faculty.

Identification and Characterization of Non-human Biota

This is the next step which involves the development of a conceptual model of the study area and, an understanding of the sources & route of exposure, and the selection of representative non-human biota for risk assessment purpose. It is important to understand that organisms considered as indicator species in a particular ecological risk assessment need to be representative of the specific location and thus the indicator species will vary from location to location.

Criteria for Selection of Reference Non-human Biota

The term "reference non-human biota" has been defined as: "a series of entities that provides a basis for the estimation of the radiation dose rate to a range of organisms / plants that are typical, or representative, of a contaminated environment." These estimates, in turn, provide a basis for assessing the likelihood and degree of radiation effects. The selection criteria used for different biota dose assessment models in the selection of reference organisms was reviewed by Biota Working Group formed by IAEA. The group reviewed various approaches suggested by ICRP, RESRAD-BIOTA, ECOMOD, AECL, IRSN, ERICA-FASSET and USDOE.

The identification of possible transfer pathways for the radionuclide of interest in the food web of the ecosystem is the first step. The next step is to pinpoint the different target organism groups (functional groups) and select relevant species from these groups according to following criteria:

1. The most abundant species in the ecosystem

2. Resistance to ionizing radiation

3. The group of species of importance in food webs leading to human consumption

4. The representative species of importance for human consumption

5. The species established as keystone i.e. bio-indicator /sensitive for the radionuclides of interest (Transfer Factor). The details on evaluation of transfer factors are given in a book (Environmental Transfer Factors, 2008)

6. The species with different size and fat contents

7. Amenability for sampling and monitoring

For example, the probable selected reference organisms to marine ecosystem such as oysters and sponges for Zn-65 and Co-60 respectively and benthic organisms for naturally occurring radionuclides of uranium & thorium and trans-uranics.

Determination of Radionuclide Concentration in the Environmental Matrices and Non-human Biota

Generally equilibrium models, where the radionuclides are assumed to reach equilibrium within each of the environmental compartments is relevant to the dose assessment. This needs the knowledge on various environmental concentration factors for the radionuclides of interest. These factors would provide the information on migration / accumulation of a particular radionuclide in the abiotic/biotic compartment of the environment. This may help to identify the critical radionuclide and the indicator

species which preferentially concentrate the radionuclide of interest.

In general the following ecological receptors may be considered for evaluation of ecological risk assessment:

Aquatic Environment

Aquatic plants, Benthic invertebrate, phytoplankton, zooplankton, fish, aquatic birds etc.

Terrestrial Environment

Terrestrial plants, small terrestrial animals, riparian animals, terrestrial birds, etc.

Dosimetry Model

A dosimetry model is then used to convert exposure from external / internal radiation to absorbed dose in non-human biota. Factors which are important for external dose include geometrical relations between the source of radiation and the receptor biota, its size and characteristics of the radionuclides.

Factors important to estimate dose from internal radionuclides include, the fraction of emitted energy that is absorbed in the non-human biota, its distribution within the biota and radiation weighting factors to account for the relative biological effectiveness of different kinds of radiation. Such considerations are described in the literature [US NCRP (1984), Woodhead (1979), IAEA (1976), US DOE (2002) and FASSET 2003].

Radiation Dose to Non-human Species

Once the radionuclide is selected, its concentration in the medium is known, and the reference biota & its geometrical relationship with the contaminants is identified, the projected dose rate / radiation dose to the non-human biota may be obtained. Similar to the calculations of external and internal dose to the members of public, these doses with respect to non-human biota may also be evaluated by multiplying the concentration of radionuclide in the medium (in which the biota is being exposed) with external and internal dose conversion factors respectively.

Evaluation of Radio-ecological Risk to Non-human Biota

A common approach to assessing the effects on non-human species is the use of Screening Index (SI). The SI is a simple ratio of the estimated dose rate to an individual non-human biota to the reference radiation dose rate. The reference radiation dose rates for terrestrial plants and aquatic organisms are given in literature (IAEA,1994), NCRP(1991) and UNSCEAR (1996).

$$SI = \frac{\text{Estimated dose rate, mGy / d}}{\text{Reference dose rate, mGy / d}}$$

When the radiation dose to the non-human biota is evaluated over a known period, the SI values can be computed by dividing with reference dose rates of 10 mGy/d as given in UNSCEAR, 1996.

The reference radiation dose rate refers to the levels below which potential health effects to the population of non-human biota are not expected. This comparison assumes that numerator and denominator of SI are based on a common assessment of dose relevant to the end point of interest i.e. mortality, reproductive capacity etc. In practice, the estimated dose rates are for a uniform exposure over the whole organism. When SI <1, it is expected that an effect to a biota is unlikely. When SI >1, an effect may be possible and further more detailed evaluations are carried out to investigate whether an actual effect might be possible. Such follow up evaluation may be iterative in nature with increasing use of site specific information and realistic assumption at each stage of iteration. It is evident that there are many complex factors to consider in the extrapolation from effects of dose on an individual non-human biota to a population of non-human biota.

In case if SI exceeds unity, which is highly improbable, an investigation sort of analysis is required to be carried out to ascertain the delivery of actual dose to the receptor and if so the levels of radionuclides in different parts like organs and sensitive cells of the receptor is required for risk assessment. This involves sensitive techniques and knowledge to obtain the correct risk assessment.

Techniques and Experiments for Risk Assessment

The basic requirements for evaluating risk to plants and animals are as follows. One must know the migration and accumulation of radionuclides in various component of environment. These aspects can be studied by simulating experiments in the field or laboratory conditions.

1. Irradiation chambers are used for studying the effect of external radiation on plant and animals/organisms.

2. The simulated artificial terrestrial and aquatic environs are required to be created to study the migration / accumulation of radionuclides to assess the effect of internal radiation on them.

3. Various geno-toxicological techniques such as Metaphase chromosome analysis, Micronucleus test, Single cell gel electrophoresis, Comet assay, Mini & micro satellites etc are required to be exercised to assess the risk at the cellular levels of the biological species.

The most essential and important aspect of such studies is to perform the experiments and use of techniques is to follow the standard protocol. The protocols for carrying out radiological surveillance and assessment of radiation dose to the members of public around nuclear facilities and for assessment of risk to plant and animals/organisms using radio-ecological aspects has been established. This protocol is prepared to provide a common methodology in terms of sampling, processing and measuring of radiological parameters in various environmental matrices while keeping the site specific requirements also in place. The experimental methodologies to be adopted for evaluating the effect of internal as well as external exposure of selected non-human

test species are also given. The protocol also includes the details of various genotoxicological techniques required for the risk assessment.

Radiation Effect Analysis

The effect analysis, mainly deals with the effect of environmental radiation on non-human biota. Major interest in the effect analysis falls in to four main categories, e.g. studies dealing with morbidity, mortality, reduced reproductive success and mutation analysis.

Morbidity

These studies should include experiments pertaining to growth rate, effects on the immune system and the behavioral consequences of damage to the central nervous system from radiation exposure in the developing embryo.

Mortality

These studies should include stochastic effect of somatic mutation and its possible consequence of cancer induction, as well as deterministic effects in particular tissues or organs that would change the age- dependent death rate.

Effect on reproductive success

These studies should include experiments pertaining to fertility and fecundity.

Mutation

These studies should focus on both gene and chromosomal mutations in somatic and germinal cells.

In order to peruse the R & D activities on radio-ecological studies in terrestrial and aquatic environs of inland nuclear facilities a Radioecology Centre has been established at GJUST, Hisar and another such Centre has been planned at Central Institute of Fishries education, Mumbai to study marine radio-ecological aspects in the environs of coastal nuclear facilities.

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