



Swansea University
Prifysgol Abertawe



Cronfa - Swansea University Open Access Repository

This is an author produced version of a paper published in:
Environmental Research

Cronfa URL for this paper:
<http://cronfa.swan.ac.uk/Record/cronfa38187>

Paper:

Mothersill, C., Abend, M., Bréchnac, F., Iliakis, G., Impens, N., Kadhim, M., Møller, A., Oughton, D., Powathil, G., et al. (2018). When a duck is not a duck; a new interdisciplinary synthesis for environmental radiation protection.

Environmental Research, 162, 318-324.

<http://dx.doi.org/10.1016/j.envres.2018.01.022>

This item is brought to you by Swansea University. Any person downloading material is agreeing to abide by the terms of the repository licence. Copies of full text items may be used or reproduced in any format or medium, without prior permission for personal research or study, educational or non-commercial purposes only. The copyright for any work remains with the original author unless otherwise specified. The full-text must not be sold in any format or medium without the formal permission of the copyright holder.

Permission for multiple reproductions should be obtained from the original author.

Authors are personally responsible for adhering to copyright and publisher restrictions when uploading content to the repository.

<http://www.swansea.ac.uk/library/researchsupport/ris-support/>



Commentary

When a duck is not a duck; a new interdisciplinary synthesis for environmental radiation protection



Carmel Mothersill^{a,*}, Michael Abend^b, François Bréchnignac^c, George Iliakis^d, Nathalie Impens^e, Munira Kadhim^f, Anders Pape Møller^g, Deborah Oughton^h, Gibin Powathilⁱ, Eline Saenen^e, Colin Seymour^a, Jill Sutcliffe^j, Fen-Ru Tang^k, Paul N. Schofield^l

^a Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

^b Bundeswehr Institute of Radiobiology, Neuherbergstr. 11, 80937 Munich, Germany

^c Institute for Radioprotection and Nuclear Safety (IRSN) & International Union of Radioecology (IUR), Centre du Cadarache, Bldg 229, St Paul-lez-Durance, France

^d Institute of Medical Radiation Biology, University of Duisburg-Essen, Medical School, Hufeland Str. 55, 45122 Essen, Germany

^e Institute of Environment, Health and Safety, Biosphere Impact Studies, SCK-CEN, Boeretang 200, 2400 Mol, Belgium

^f Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK

^g Ecologie Systématique Evolution, Equipe Diversité, Ecologie et Evolution Microbiennes Université Paris-Sud, CNRS, and AgroParisTech, Université Paris-Saclay, F-91405 Orsay Cedex, France

^h Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Campus Ås, Universitetstunet 3, 1432 Ås, Norway

ⁱ Department of Mathematics, College of Science, Swansea University, Singleton Park, Swansea Wales SA2 8PP, UK

^j Low Level Radiation and Health Group, Ingrams Farm Fittleworth Road, Wisborough Green RH14 0JA, West Sussex, UK

^k National University of Singapore, Radiobiology Research Laboratory, Singapore Nuclear, Research and Safety Initiative, Singapore

^l Dept of Physiology Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

ARTICLE INFO

Keywords:

Radioecology

Environment

Reference animals and plants

Low dose

Radiation protection

ABSTRACT

This consensus paper presents the results of a workshop held in Essen, Germany in September 2017, called to examine critically the current approach to radiological environmental protection. The meeting brought together participants from the field of low dose radiobiology and those working in radioecology. Both groups have a common aim of identifying radiation exposures and protecting populations and individuals from harmful effects of ionising radiation exposure, but rarely work closely together. A key question in radiobiology is to understand mechanisms triggered by low doses or dose rates, leading to adverse outcomes of individuals while in radioecology a key objective is to recognise when harm is occurring at the level of the ecosystem. The discussion provided a total of six strategic recommendations which would help to address these questions.

1. Introduction

Radiobiology as a sub-division of Radioecology is often neglected. However moves over the last few years to protect the natural environment from harmful effects of ionising radiation, rather than assuming protecting humans also protects other species, has led to a need for radioecologists and radiobiologists to establish a closer and more meaningful dialog. Cross-fertilization in science resulting from bringing the fields together has often proven to be very effective in promoting

new theories and innovation (Jones, 2009; Van Noorden, 2015).

Radiobiology has essentially been developed in the context of understanding how radiation affects living tissues, and is aimed at helping to protect humans from deleterious effects of radiation such as cancer. It has therefore been focussed on biological materials derived from humans or from a few species considered as human surrogates.

Radioecology in turn, has been dominated in the decades since the accident at Chernobyl (Smith and Beresford, 2005) by a consideration of the environment as a simple mediator of transfer of radiation towards

E-mail addresses: mothers@mcmaster.ca (C. Mothersill), MichaelAbend@bundeswehr.org (M. Abend), francois.brechignac@irsn.fr (F. Bréchnignac), Georg.Iliakis@uk-essen.de (G. Iliakis), nimpens@skcen.be (N. Impens), mkadhim@brookes.ac.uk (M. Kadhim), anders.moller@u-psud.fr (A.P. Møller), deborah.oughton@mbu.no (D. Oughton), g.g.powathil@swansea.ac.uk (G. Powathil), eline.saenen@skcen.be (E. Saenen), seymou@mcmaster.ca (C. Seymour), jillsutcliffe1@gmail.com (J. Sutcliffe), tangfr@gmail.com (F.-R. Tang), pns12@cam.ac.uk (P.N. Schofield).

Abbreviations: IAEA, International Atomic Energy Authority; ICRP, International Commission on Radiological Protection; RAP, reference animals and plants; CAP, contextualised animals and plants; NTE, non-targeted effects; OECD NEA CRPPH, Organisation on Economic Cooperation and Development Nuclear Energy Agency Committee on Radiological Protection and Public Health; PCR, polymerase chain reaction; CpG, cytosine-guanine dinucleotides

* Corresponding author.

<https://doi.org/10.1016/j.envres.2018.01.022>

Received 28 December 2017; Received in revised form 18 January 2018; Accepted 19 January 2018

0013-9351/© 2018 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

man, therefore largely ignoring radiation effects on animals and plants and their related ecosystems, and the potential of these environmental perturbations to impact on humans indirectly (Caffrey et al., 2014; Salbu, 2009).

Responsibility for protection of the environment from anthropogenic ionising radiation has been a universally accepted principle since radiation safety was first put on an international and institutional footing. From both an anthropocentric point of view and from an ethical point of view, the imperative to protect the environment has been accepted and formalised in, for example recently, IAEA GSR Part 3 published in 2014 (European Commission and others, 2014). In this set of recommendations, protection of the environment is broadly defined as follows:

“include(s) the protection and conservation of: non-human species, both animal and plant, and their biodiversity; environmental goods and services, such as the production of food and feed; resources used in agriculture, forestry, fisheries and tourism; amenities used in spiritual, cultural and recreational activities; media, such as soil, water and air; and natural processes, such as carbon, nitrogen and water cycles.”

This definition provides a more complex articulation of environmental protection than just the sustainability and complexity of ecosystems, in that it also takes an anthropocentric view of the utility of the natural environment to humans. Thus it supports the main thrust of radiation protection legislation for which the primary purpose is protection of humans. The question addressed in the workshop was whether the current approach to environmental protection is sufficient to meet these aspirations, and whether in the light of more recent developments in our understanding of the action of low-dose ionizing radiation on the biosphere the approach currently adopted, that of using key indicator or “reference” species (RAPs) (ICRP, 2008) as surrogates for the effect of radiation on humans in the contaminated environment, is any longer adequate, especially in the case of chronic low dose exposure. The currently used RAPs are deer, rat, duck, frog, bee, earthworm, crab, trout, flat fish, pine tree, grass, seaweed. There is concern not only that the list is limited to 12 undefined organism types but about the fact that no number of organisms can represent the complexity and diversity of the ecosystem.

The recent move towards consideration of effects on wildlife presents new opportunities in radiobiology, since it has been known for many years that biodiversity presents a large spectrum of radiosensitivities within and between a wide range of species (Bowen, 1961) including humans (Bouffler, 2016). Unravelling the mechanistic reasons for these varied radiosensitivities has been quite poorly addressed so far. However, understanding these mechanisms should provide new insights that may prove to be very useful in better understanding human radiobiology, and may aid the development of a more holistic understanding of the impact of radiation on the ecosphere.

The workshop on which this paper reports represented an interdisciplinary attempt to address some of the key issues relevant to both fields, and to assess what changes in approach might be desirable to better realise the aspirations already articulated through international regulatory and advisory bodies; specifically the International Atomic Energy Authority (IAEA) and the International Commission on Radiological Protection (ICRP).

1.1. Key questions

In order to structure the discussion a key question was identified in each discipline and subdivided yielding topics for discussion. The key question in radioecology was identified as “how do we protect biodiverse ecosystems to the benefit of humans and other living organisms”? While in radiobiology the key question was “what are the risks to human and non-human animal health associated with low/chronic dose exposures”? From these overarching questions, the topics for discussion were as follows:

1. Development of big data approaches to gathering and analysing large scale complex ecosystem data: informatics, citizen scientists and ecological observatories
2. Segmentation of the ecosystem to provide niche and landscape data to facilitate integrated analysis
3. The impact of new knowledge concerning multiple stressors and synergistic interactions between radiation, other noxious agents and natural environmental challenges, such as climate change.
4. The impact of historic dose in the light of our new understanding of non- targeted effects (NTE)
5. The opportunity to exploit and validate new biomarkers coming from radiation biology, while recognising that population and ecosystem level biomarkers for fitness are required.
6. Impact of radiation induced epigenetic changes on individual organism and population fitness, adaptation and population genetic structure.

2. The discussions on these topics are summarised in the following sections

2.1. Development of big data approaches to gathering and analysing large-scale complex ecosystem data: informatics, citizen scientists and ecological observatories

A prominent challenge to gathering data from exposed ecosystems is the level at which data needs to be collected and consequently the scale and depth of that collection. While a known deficiency of human radiological protection studies is the inability to introduce existing knowledge about individual sensitivity into exposure limits and damage assessment, we know hardly anything about the radiation sensitivity of most species of animals and plants or its individual variation. Moreover the health issues of concern in human radiation protection, which are used to set endpoints for damage assessment such as cancer or cardiovascular disease are rarely concerns in non-human species. A third challenge is that human radiation protection objectives are set at individual organism level of one single species, leading to a fit-for-purpose methodology of risk assessment. Since environmental protection objectives are set at population and ecosystem levels, associated methods of risk assessment that would address these levels are required (Bradshaw et al., 2014; Brechignac and Doi, 2009; Brechignac et al., 2016). This means that human radiation protection approaches should not be repurposed to deal with environment protection.

The initial approach recommended by the ICRP includes the use of a small set of reference animals and plants (RAPs) which we discuss later. However these are difficult to justify given the range of biodiversity and the complexity of ecosystems. In fact, this approach is broadly speaking a simple extension from human radiation protection methods, involving the “reference man” concept (ICRP, 2008). As such, it does not take due account of the fact, stated above, that the object of protection is different, i.e. populations of interacting species and their ecosystems. It is therefore difficult to demonstrate that this meets the objectives asserted for protection of the environment (Bradshaw et al., 2014; Brechignac et al., 2016). While integration of human and other species in one same system of radiation protection may seem a laudable goal, it is currently more methodology-driven than conceptually-driven and does not capture the relative positions of human and other species within the ecosystem and how these interact with each other (Brechignac, 2017).

Ten years ago, an Expert Group created by the NEA CRPPH (Committee on Radiation Protection and Public Health) acknowledged that efforts were generally fragmented and involved a range of disciplines (radiation biology, radioecology, environmental toxicology, ecotoxicology and ecology). They also accepted that environmental data collected over the last half century by the nuclear industry for surveillance purposes had not been utilised in an efficient, coordinated, manner and recommended the development of an international network or “Observatory” (NEA/OECD 2007). This international network

was meant to allow researchers to coordinate and understand research in relevant fields based on past and on-going observations in the real environment and allow them to be linked with laboratory and theoretical developments. While observatories do currently exist at sites of known contamination, collection of much more data would be required to produce a picture of the impact of exposure on the ecosystem. In the area of human health there have recently been proposals for the establishment of a formal “exposome” i.e. a detailed, preferably life-time exposure assessment database (Wild, 2005). Unlike the genome the exposome is highly variable and dynamic, and its establishment would presumably require significant infrastructure, standards, and commitment to legal and financial sustainability. A smaller scale and more feasible approach could be as proposed in the Section 2.3 dealing with multiple stressors.

It was acknowledged by the group that the 12 reference organisms can make a useful contribution it was also strongly agreed that these organisms do not sufficiently represent key niches in the ecosystem, (e.g., decomposers, predators, keystone species). Observing the interactions between species in an ecosystem under stress is critical in understanding how that system is responding, and many secondary and tertiary changes brought about through, for example direct radiation effects causing reproductive damage, loss of fitness and changes in population size and composition can affect other species not immediately impacted by radiation exposure. The unit of response is therefore not the organism or even the species but the ecosystem, congruent with concept of “planetary health” which is based on the understanding that human health and human civilization depend on flourishing natural systems and the wise stewardship of those natural systems (Whitmee et al., 2015). This is a fundamentally different concept to that being currently implemented for human radiological safety. The subjects of data gathering will be case, environment and hypothesis dependent. For example the most useful organisms to study links between epigenetic effects and population level response need not be the same (or are unlikely to be the same) as those best suited to test ecosystem interactions between different species.

In all cases it would be necessary to pay more attention to the environment (or landscape/ ecosystem) those organisms inhabit. We believe that there needs to be increased focus on:

- Life history traits
- Seasonal variation in migration, breeding, nutrition and predation etc.
- Dose and dose rates to specific organs (e.g. reproduction) and timescales of important biological processes (e.g. spermatogenesis)
- Presence of other environmental stressors, both natural and anthropogenic.
- Other endpoint/markers (e.g. population and functional level)

With further consideration of:

- Homo/heterogeneous populations
- Presence of organism in both contaminated and uncontaminated environments
- Availability of DNA barcoding and individual identification
- Power of metagenomics for the microbiome and
- Factors likely to impact on radiosensitivity (e.g. ability to cope with oxidative stress)

Collection and processing of this data is likely to be significantly challenging. Collection may be helped with the encouragement of “citizen science” where tailored data capture tools can be rolled out to interested and enthusiastic non-experts to collect data. Such tools have already been prototyped and used in some environments such as “Scratchpads” (Smith et al., 2011) and automated recording tools for assessment of distribution of large mammals and birds have already been used at Chernobyl, including for example acoustic monitoring

(Fukasawa et al., 2017) and phototraps. New informatics approaches to capturing data from images, particularly from plants are being developed, (Lobet, 2017) and huge scale metagenomic analyses of complex ecosystems such as the oceans have already yielded enormous volumes of information (Sunagawa et al., 2015). Many of these problems and potential solutions are well understood and the current state of the art for biodiversity informatics discussed by Hardisty et al. (Hardisty et al., 2013), but the particular application of these methodologies at multiple levels in the projected radioecological observatories has yet to be explored.

2.2. Complexity challenges; necessity to focus on a niche or a landscape since an ecosystem is too complex

A key concern among the group was the complexity of ecosystems and the huge challenges of identifying chains of cause and effect, crossing intersecting levels of time, space and taxonomy. However the use of RAPs, while simple, does not really reveal anything about the actual situation facing an animal or plant in the field. Some species might be more sensitive to ionising radiation than others, and threatened with extinction. As part of a food chain, this may affect predators, irrespective their radiosensitivity. This is borne out by the instances where there is a lack of agreement between field and laboratory data (Beaugelin-Seiller et al., 2016; Garnier-Laplace et al., 2015).

The discussion breakthrough was to realise that instead of trying to understand what ionising radiation might do across a complex ecosystem, it might be useful to think in terms of the RAPs, or better “CAPs” (for “Contextualised Animals and Plants”), contextualised in a niche or “landscape”, instead of relying on selection of limited and isolated reference species. In radiobiology the idea of a microenvironment where the cell and its environment interact and communicate led to major advances in cell biology, in particular in suggesting new ways to understand and control cancer. RAPs, like cell types, can be understood only in the context of the niche or landscape they inhabit.

Analogies between adverse outcomes to humans and other species could be identified that directly affect the behaviour and functioning in the ecosystem. Such information may help to identify some key species in the ecosystem as a first approach to model the ecosystem. For example, low dose radiation might lead to eye lens opacity, an important issue in prey-predator interactions (Lehmann et al., 2016). In utero low doses of ionising radiation at critical developmental time points are known to induce persistent effects on the brain structure and functioning including behavioural consequences in mice (Verreet et al., 2016). Exposure to ionising radiation affects brain function in humans (Bazyka et al., 2014; Marazziti et al., 2016; Otake and Schull, 1998), and compromises reproduction e.g. (Hurem et al., 2017).

Modelling the effects/consequences of radiation on an ecosystem is a complex, spatial and temporal multiscale problem and has many similarities to modelling cancer growth by incorporating intra and intercellular changes and its interaction with its microenvironment (Powathil et al., 2015). These mathematical models can help in studying, qualitatively and/or quantitatively, various processes involved in the cancer progression and treatment, predicting potential new therapies or best possible treatment strategies; and form testable hypotheses (Powathil et al., 2013, 2016). The development of the model clearly depends on the questions that we are asking or details that we are interested in. Are we interested in general health of ecosystem or do we like to know what is happening within the complex ecosystem? A possible modelling technique might be multiscale modelling approach, that includes essential details of the ecosystem such as RAPs and their interactions with each other and microenvironment. Similar examples can be found in the context of cancer modelling (Powathil et al., 2015).

In a similar vein, we considered that it might be useful to think of “CAPs” selected as part of a simplified ecosystem that could both represent what is observed in the real environment, and be tested in

parallel in laboratory microenvironments. The interest here being to open a path to theoretical modelling that would inform about such aspects as how toxic stress affects ecosystem resilience, and how different radio-sensitivities interact with/may affect ecosystem balances.

The problem with the current RAP approach is that the organism is considered without reference to the context of its environment. While target shape and volume, and isotope transfer routes may be considered, little attention is given to behaviour, lifestyle, lifecycle or position in the ecosystem. We consider however that the whole ecosystem approach, on the other hand, is too complex to allow regulation based on dose limits to be applied.

During the meeting the idea of a compromise approach was discussed at length. This “Landscape approach” represents an attempt to hybridise the two so that selected organisms can be viewed in relation to their actual environment – i.e. the “contextualised duck” is examined as an entity within a duck habitat doing what ducks do, and subjected to chronic, acute and seasonal stressors, such as diseases, environmental toxins, predation, compromised nutrition, and climate. A similar approach could be taken for each reference organism. A key issue here is to be able to monitor and assess biotic and abiotic interactions which are critical to the survival of a viable ecosphere. A landscape approach would require a consideration of both aspects of the particular ecosystem under investigation. It would also encompass microenvironments, for example the earthworm gut and its importance as a source of microorganisms needed for a soil to sustain other organisms.

One major issue is whether the current RAPs represent keystone species – ie those which, for their number, have a disproportionate effect on their ecosystem. This will change for different environments suggesting that rather than there being a set of “Platonic” RAPs for all situations, selection of a CAP should be situation dependent. With this approach RAPs may still be adopted as environmental sensors but as contextualised CAPs, so that modelling parameters can be selected which relate to the real-world nature of the species and not just to abstractions used so far in modelling such as surface area or generic location in soil, air, water or sediment. The intrinsic “duckness” of the duck may thus be captured.

2.3. Contributions of multiple stressors in the environment

A key insight coming from the radioecologists in the group was that when looking for effects of radiation in non-humans it is critical to understand that ionising radiation is but one of many stressors impacting biota in the field. At this moment, a lot of data exist on the effect of a single stressor on different organisms. However when more than one stressor is used even in a laboratory situation, it can be difficult to discern in these organisms which responses are from radiation and which are from the other stressors. This makes the experiments much more complex than standard lab experiments where one can give one stressor at a time. Based on existing data, extrapolations are made to estimate effects in the field. However, from (Garnier-Laplace et al., 2013) it is clear that there is a mismatch between data obtained in the lab and in the field. As such, one of the major challenges in looking at low dose effects in real environments is that any convergence of effector processes or exposure outcomes makes it difficult to disentangle the contribution of radiation from other stressors such as pesticides or heavy metals. This is particularly true when endpoints are stochastic rather than deterministic.

This means that a given dose of incident radiation or isotopic exposure can generate a different bioresponse in different contexts, and what might be deemed a safe level in one environment might be significantly damaging in another. Knowledge of the context in which radiation effects are being examined is therefore critical, the “stressor milieu” but also the history of these stressors in the environment, where the time of exposure, the changing combinations, different background values and possible sequencing of mixed exposures, can all in principle contribute to their impact on the environment. For example,

(Vandenhove et al., 2010) found that the effects induced after exposing *Arabidopsis thaliana* plants to uranium and gamma exposure are different from the individually induced effects. In addition (Dallas et al., 2016) have shown that the genotoxic effect of tritium is dependent on the temperature, with more effect at higher temperature. This can be important in the light of the climate change, ultimately leading to higher environmental temperatures.

The confounding factors are particularly important during low or chronic dose exposures where they may dominate the outcome. The most important stressors in a certain ecosystem should be identified, and approached through techniques such as principal component analysis. Determining the share of ionising radiation in a complex mixture of stressors is of importance to optimise the radiation protection system, tailor-made to realistic exposure scenarios.

The group considered it essential to develop measures of stress effect or burden rather and to gather geographic and historical contextual data to better understand the impact of radiation within each environment. It is significant that an approach to radiological protection using such holistic descriptions of multiple environmental stressors and realistic scenarios has recently been incorporated onto the roadmap research plan for the MELODI, EURADOS, NERIS, ALLIANCE and EURAMED platforms coordinated by the European Commission (Impens et al., 2018).

2.4. Importance of historic dose?

This insight came from radiobiology and may have profound implications for radioecology. A concern in the radioecology field is that there appears to be a big discrepancy between field data and that predicted using the FREDERICA and other databases (Coppstone et al., 2008; Garnier-Laplace et al., 2015). The issue has resulted in heated, and sometimes acrimonious debate but an established phenomenon in radiobiology may hold the key. It has been known for over 30 years that persistent expression of genomic instability or lethal mutations can occur in progeny of irradiated cells which survive and reproduce apparently normally for many generations but carry a higher tolerance for mutation than the normal (unirradiated) population (Holmberg et al., 1993; Kadhim et al., 1992; Nagasawa and Little, 1992; Seymour et al., 1986); reviewed (Mothersill and Seymour, 2013). The mechanism has been shown to occur in vivo (Kadhim et al., 1994; Pampfer and Streffer, 1989; Watson et al., 1996) and is now thought to be driven by epigenetic or extragenetic factors including cell signalling (Al-Mayah et al., 2012; Hei et al., 2011) (reviewed in (Hei et al., 2011)), exosomes (Al-Mayah et al., 2012; Jella et al., 2014), and biophotons (Le et al., 2017). The typical dose response for these so-called non-targeted effects (NTE) is a considerable effect triggered by a very low dose which saturates and does not increase with increasing doses above 0.5 Gy (Prise et al., 2001; Seymour and Mothersill, 2000). The non-clonal and stochastic genomic instability effects persist for as many generations as have been measured (at least 400 population doublings in culture and for 20 years in bank voles trapped from the post-Chernobyl exclusion zone (Ryabokon and Goncharova, 2006)) (Marozik et al., 2007). During the discussion this was interpreted to mean there may be an additional component to consider when determining dose effect relationships in radioecology. In addition to the ambient dose and the cumulative effect of the decaying chronic dose, it may be necessary to determine the likely degree of long term genomic instability induced in the system (i.e. the impact of the initial or historic dose). Similar related concerns are reflected in considering long term epigenetic changes in the population – discussed below. The instability is not necessarily adverse. A previous exposure to radiation, can lead to more resistant/adapted organisms, the phenomenon of hormesis. For example, (van de Walle et al., 2016) have shown that *A. thaliana* plants exposed to gamma radiation for two consecutive generations can probably deal more efficiently with reactive oxygen species produced after exposure to gamma radiation.

One way to study the effects of historic and non-targeted effects of radiation on ecological field and species within is through mathematical modelling. Once the model is developed, it can be used to test various hypotheses using the available experimental and field data to understand the discrepancy between field and available databases. Furthermore, modelling can help in the dynamic prediction of radiation effects and to adopt or develop appropriate measures to address it, as discussed above. Recently, mathematical modelling techniques (Powathil et al., 2016) have been used to study the role of bystander effects at low dose radiations and the inferences made by the models are subsequently confirmed by experimental studies (Fernandez-Palomo et al., 2016).

2.5. Biomarkers from radiation biology can usefully be applied to individual organisms but population biomarkers of exposure and fitness are needed

This insight came from the realisation by the radiobiologists that in radioecology biomarkers of population fitness are needed. It is accepted that endpoints used to predict harm in individuals are not very useful in predicting harm to populations because of individual variation but on a higher level we are still some distance from defining how radiation-induced “harm” might be defined in an ecological context.

Identification of such markers is in a way paradoxical in that markers of population effects still have to be assayed in individuals, and measurements in individuals representative for a population at risk will continue to be the point from which to extrapolate, not only to population risk but also to the impact on other interconnected populations, at least until there is more consensus about the feasibility of developing useful ecosystem level markers. A major problem here is that the variables and uncertainties involved in this extrapolation are largely unknown; we suffer from having too many “unknown unknowns”. Molecular biology nowadays provides a wide variety of tools for screening (next generation sequencing) as well as validation purposes (quantitative PCR) on the DNA or RNA level.

Once again the analogy between the microenvironment of the tissue and the macroenvironment inhabited by biota is useful because it provides a way to bridge from individual level markers to those involving epigenetic processes operating at the population level. Epigenetic markers would include those controlling communication, methylation (controlling expression of characteristics) and those controlling signalling or behaviour which must have macromolecular analogues in the macroenvironment. The relevance of neuroscience biomarkers was suggested (Filiou and Turck, 2011).

2.6. Impact of radiation induced epigenetic changes on individual organism and population fitness, adaptation and population genetic structure

There is increasing evidence that the background mutation rate in plants and animals around Chernobyl and other contaminated sites is dramatically enhanced, in some cases estimated as giving rise to up to 44% of genetic variance in existing populations (Moller and Mousseau, 2015). Provocatively Kovalchuk et al. reported an increased mutation rate only ten months after planting wheat plants in contaminated land near Chernobyl (Kovalchuk et al., 2000). As this represents only one generation, the implication is that the rapid increase in genetic variance, entirely disproportionate to the amount of energy deposited by the known exposure, involves a non-classical mechanism of action which is not simply the deterministic chemical effect of ionising radiation on DNA.

More recently, evidence has accumulated that exposure to low dose ionising radiation can cause epigenetic changes, for example changes in CpG methylation which may be inherited across generations (Merrifield and Kovalchuk, 2013; Schofield, 1998; Schofield and Kondratowicz, 2017). The implication of this is that in addition to accumulated DNA mutations, either caused directly and deterministically by interaction with DNA or through induced genomic instability, the induction of

paramutations, or as often described, epimutations may have a significant effect on organismal phenotypes through alteration of patterns of gene expression. These changes, non-clonal, heritable and likely pleiotropic have received little attention as a potential aspect of radiation induced ecological damage. There is considerable evidence that these effects do occur in the wild and that both plants and animals are affected (Artemov et al., 2017; Herrera and Bazaga, 2010; Lighten et al., 2016; Xu et al., 2016). The impact of such heritable changes in patterns of gene expression is conceivably at two levels – that of the individual organism, where changes might be either deleterious or beneficial in the prevailing environment, but also at the level of the overall genetic structure of the population.

More subtle, yet potentially highly perturbing, effects on the ecosystem have not been considered. Naturally-occurring epigenetic changes have been examined for many years in natural ecosystems, with still controversial assessments of the impact of epigenetic variation on the genetic structure of populations and contributions to fitness variance under changing selective pressure (Charlesworth et al., 2017; Geoghegan and Spencer, 2013; van der Graaf et al., 2015). If epigenetic changes impact pleiotropically on genetically-determined quantitative traits it is conceivable that they could drive new alleles through the population faster than novel genetic variants alone, suggesting that we might see rapid selective sweeps of beneficial epigenetic and genetic alleles through populations, or genotypes and epigenotypes co-selected by their epistatic interactions. Modelling of “epigenetic drive” shows the potential for rapid spread of epigenetic and then beneficial genetic variants though the population under changing selective pressure (Geoghegan and Spencer, 2013; Kilvitis et al., 2014; Nishikawa and Kinjo, 2014). The potential for interaction between radiation enhanced genetic mutation rates reported to date and enhanced paramutation rates is highly significant, and made more complex by the possibility that with the population new DNA mutations may “piggyback” on rapidly induced epimutations if they affect related aspects of fitness in an environment where other effects of radiation are also affecting selective pressures.

There may also be complex founder effects if the same epigenetic changes are induced in multiple individuals at the same time. It is entirely conceivable that beneficial alleles, say affecting reproductive success, might occur in one species, but a rapid spread through the population towards fixation will likely impact on other species and the structure of the ecosystem. Without taking a holistic view, at least of a niche or part of the landscape, tracking either biological markers or established phenotypic markers in one species will not provide information on the secondary deleterious changes, which may occur in months to years depending on the life cycle of the organism.

3. Conclusions

In conclusion, the workshop pointed a way forward for an approach to environmental radiation protection which avoids dealing with the enormous complexities of ecosystems while recognising that a reference organism is defined not only by its physical characteristics but also by its environment and the niche it occupies in the landscape. The term CAP (contextualised animals and plants) encapsulates this new paradigm and makes RAP (reference animals and plants) more meaningful and more amenable to site specific modelling and analysis.

The workshop recognised the importance of an ecosystem-based approach to how long-term low dose effects are examined, including as appropriate the presence of multiple stressors. The primary approach to radiological protection – protecting humans through protecting the environment - has shifted subtly in recent years from the use of animals as surrogates for humans to understanding the concept that environmental damage itself leads to damage of the anthroposphere, broadening the concept of damage from its initial focus on human neoplasia and compromised reproduction (Ceballos et al., 2017; European Commission and others, 2014; ICRP, 2008). At a time when

anthropogenic damage to the ecosystems of the planet, with consequences for biodiversity and sustainability, this approach is much more aligned with our modern understanding of the interdependence of man and his environment, and the impact of anthropogenic ecological damage on humans themselves.

However it was recognised that the complexities of the ecosystem need to be simplified in order to allow identification and examination of potential ecological markers which could be used as indicators of ongoing processes in an ecosystem.

Analysing the role and impact of radiation in an ecosystem is a multiscale, complex problem, considering the amount of information and details that need to be incorporated within a wide range of spatial and temporal scales. A conclusion from the workshop was that in addition to potential predictive capabilities, multi-scale modelling approaches which use CAP can act as a tool to understand and dissect the role of various interactions within the overall behaviour of the ecosystem, which are usually nonlinear and less evident. The workshop recommendations also suggested that efforts be devoted to development of advanced conceptual methods to resolve the impact of multiple stressors. The low radiation dose issue, when dealing with most situations of environmental impact, involves a multiple stressor context and radiation impact cannot easily be disentangled from the impact of other stressors. This is because in real environments after low radiation dose exposure, there is usually no single dominant contaminant. This is likely to have important conceptual consequences because it means that the issue is about the cumulative effect of an addition of multiple small doses/concentrations of contaminants, which would be negligible if each one were considered in isolation.

Similarly, the workshop identified as important the consideration of mechanistic studies of low dose radiobiological phenomena such as delayed and non-targeted effects which could impact the dose response relationship, particularly if measurements of ambient dose are being made at time points distantly removed from the initial event.

This paper resulted from a multidisciplinary brainstorming approach to identify novel directions to cope with complexity. It is hoped it will stimulate further interdisciplinary efforts, by bringing together different but related communities in order to facilitate the emergence of a new conceptual methodology for truly demonstrating and achieving ecosystem protection.

Acknowledgements

The authors are grateful to the IUR for its continued and invaluable support, and acknowledge the assistance of the University of Duisburg-Essen in hosting the meeting.

Declarations of interest

None.

Funding sources

Funding was provided for this workshop by the International Union for Radioecology and the University of Duisburg-Essen.

References

- Al-Mayah, A.H., et al., 2012. Possible role of exosomes containing RNA in mediating nontargeted effect of ionizing radiation. *Radiat. Res.* 177, 539–545.
- Artemov, A.V., et al., 2017. Genome-Wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. *Mol. Biol. Evol.* 34, 2203–2213.
- Bazyka, D.A., et al., 2014. TERF1 and TERF2 downregulate telomere length in cognitive deficit at the late period after low-dose exposure. *Probl. radiatsionnoi meditsyny ta Radio.* 19, 170–185.
- Beaugelin-Seiller, K., et al., 2016. Should we ignore U-235 series contribution to dose? *J. Environ. Radioact.* 151 (Pt 1), 114–125.
- Bouffler, S.D., 2016. Evidence for variation in human radiosensitivity and its potential impact on radiological protection. *Ann. Icrp.* 45, 280–289.
- Bowen, H.J.M., 1961. Radiosensitivity of higher plants, and correlations with cell weight and DNA content. *Radiat. Bot.* 1, 223–228.
- Bradshaw, C., et al., 2014. Using an ecosystem approach to complement protection schemes based on organism-level endpoints. *J. Environ. Radioact.* 136, 98–104.
- Brechignac, F., et al., 2016. Addressing ecological effects of radiation on populations and ecosystems to improve protection of the environment against radiation: agreed statements from a Consensus Symposium. *J. Environ. Radioact.* 158–159, 21–29.
- Brechignac, F., 2017. Assessing ecological risk from radiation requires an ecosystem approach. In: Korogodina, V. (Ed.), *Genetics, Evolution and Radiation – Crossing Borders, the Interdisciplinary Legacy of Nilolay W. Timofeev-Ressovsky*. Springer International Publishing, Cham, Switzerland, pp. 207–224.
- Brechignac, F., Doi, M., 2009. Challenging the current strategy of radiological protection of the environment: arguments for an ecosystem approach. *J. Environ. Radioact.* 100, 1125–1134.
- Caffrey, E.A., et al., 2014. Radioecology: why bother? *J. Environ. Prot.* 05 (03), 12.
- Ceballos, G., et al., 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc. Natl. Acad. Sci. USA.* 114, E6089–E6096.
- Charlesworth, D., et al., 2017. The sources of adaptive variation (pii: rspb.2016.2864). *Roy. Soc. Proc. Biol. Sci.* 31 284 (1855). <http://dx.doi.org/10.1098/rspb.2016.2864>.
- Copplestone, D., et al., 2008. The development and purpose of the FREDERICA radiation effects database. *J. Environ. Radioact.* 99, 1456–1463.
- Dallas, L.J., et al., 2016. Exposure to tritiated water at an elevated temperature: genotoxic and transcriptomic effects in marine mussels (*M. galloprovincialis*). *J. Environ. Radioact.* 164, 325–336.
- European Commission and others, 2014. *Radiation Protection and Safety of Radiation; GSR Part 3. Sources: International Basic Safety Standards*. International Atomic Energy Agency, Vienna.
- Fernandez-Palomo, C., et al., 2016. Inter-relationship between low-dose hyper-radiosensitivity and radiation-induced bystander effects in the human T98G glioma and the epithelial HaCaT cell line. *Radiat. Res.* 185, 124–133.
- Filiou, M.D., Turck, C.W., 2011. General overview: biomarkers in neuroscience research. *Int. Rev. Neurobiol.* 101, 1–17.
- Fukasawa, K., et al., 2017. Acoustic monitoring data of avian species inside and outside the evacuation zone of the Fukushima Daiichi power plant accident. *Ecol. Res.* 32 (769–769).
- Garnier-Laplace, J., et al., 2013. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *J. Environ. Radioact.* 121, 12–21.
- Garnier-Laplace, J., et al., 2015. Radiological dose reconstruction for birds reconciles outcomes of Fukushima with knowledge of dose-effect relationships. *Sci. Rep.* 5, 16594.
- Geoghegan, J.L., Spencer, H.G., 2013. The evolutionary potential of paramutation: a population-epigenetic model. *Theor. Popul. Biol.* 88, 9–19.
- Hardisty, A., et al., 2013. A decadal view of biodiversity informatics: challenges and priorities. *BMC Ecol.* 13, 16.
- Hei, T.K., et al., 2011. Radiation induced non-targeted response: mechanism and potential clinical implications. *Curr. Mol. Pharmacol.* 4, 96–105.
- Herrera, C.M., Bazaga, P., 2010. Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.* 187, 867–876.
- Holmberg, K., et al., 1993. Clonal chromosome aberrations and genomic instability in X-irradiated human T-lymphocyte cultures. *Mutat. Res.* 286, 321–330.
- Hurem, S., et al., 2017. Parental gamma irradiation induces reprotoxic effects accompanied by genomic instability in zebrafish (*Danio rerio*) embryos. *Environ. Res.* 159, 564–578.
- ICRP, 2008. ICRP publication 108: environmental protection: the concept and use of reference animals and plants. *Ann. Icrp.* 38, 1.
- Impens, N.R.E.N., 2018. et. al. First Joint Roadmap Draft, EJP-CONCERT Deliverable D3. 4, Submitted. Contract H2020-662287. Eventual Weblink where the document will be available as soon as accepted by the EC <<http://www.concert-h2020.eu/>>.
- Jella, K.K., et al., 2014. Exosomes are involved in mediating radiation induced bystander signaling in human keratinocyte cells. *Radiat. Res.* 181, 138–145.
- Jones, C., 2009. interdisciplinary approach - advantages, disadvantages, and the future benefits of interdisciplinary studies. *ESSAI* 7.
- Kadhim, M.A., et al., 1992. Transmission of chromosomal instability after plutonium alpha-particle irradiation. *Nature* 355, 738–740.
- Kadhim, M.A., et al., 1994. Alpha-particle-induced chromosomal instability in human bone marrow cells. *Lancet* 344, 987–988.
- Kilvitis, H.J., et al., 2014. Ecological epigenetics. *Adv. Exp. Med. Biol.* 781, 191–210.
- Kovalchuk, O., et al., 2000. Wheat mutation rate after Chernobyl. *Nature* 407, 583–584.
- Le, M., et al., 2017. Exosomes are released by bystander cells exposed to radiation-induced biophoton signals: reconciling the mechanisms mediating the bystander effect. *PLoS One* 12, e0173685.
- Lehmann, P., et al., 2016. Fitness costs of increased cataract frequency and cumulative radiation dose in natural mammalian populations from Chernobyl. *Sci. Rep.* 6, 19974.
- Lighten, J., et al., 2016. Adaptive phenotypic response to climate enabled by epigenetics in a K-strategy species, the fish *Leucoraja ocellata* (Rajidae). *R. Soc. Open Sci.* 3, 160299.
- Lobet, G., 2017. Image analysis in plant sciences: publish then perish. *Trends Plant Sci.* 22, 559–566.
- Marazziti, D., et al., 2016. Ionizing radiation: brain effects and related neuropsychiatric manifestations. *Probl. Radiac Med Radiobiol.* 21, 64–90.
- Marozik, P., et al., 2007. Bystander effects induced by serum from survivors of the

- Chernobyl accident. *Exp. Hematol.* 35, 55–63.
- Merrifield, M., Kovalchuk, O., 2013. Epigenetics in radiation biology: a new research frontier. *Front Genet.* 4, 40.
- Moller, A.P., Mousseau, T.A., 2015. Strong effects of ionizing radiation from Chernobyl on mutation rates. *Sci. Rep.* 5, 8363.
- Mothersill, C., Seymour, C., 2013. Uncomfortable issues in radiation protection posed by low-dose radiobiology. *Radiat. Environ. Biophys.* 52, 293–298.
- Nagasawa, H., Little, J.B., 1992. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* 52, 6394–6396.
- Nishikawa, K., Kinjo, A.R., 2014. Cooperation between phenotypic plasticity and genetic mutations can account for the cumulative selection in evolution. *Biophysics* 10, 99–108.
- Otake, M., Schull, W.J., 1998. Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. *Int. J. Radiat. Biol.* 74, 159–171.
- Pampfer, S., Streffer, C., 1989. Increased chromosome aberration levels in cells from mouse fetuses after zygote X-irradiation. *Int. J. Radiat. Biol.* 55, 85–92.
- Powathil, G.G., et al., 2013. Towards predicting the response of a solid tumour to chemotherapy and radiotherapy treatments: clinical insights from a computational model. *PLoS Comput. Biol.* 9, e1003120.
- Powathil, G.G., et al., 2015. Systems oncology: towards patient-specific treatment regimes informed by multiscale mathematical modelling. *Semin Cancer Biol.* 30, 13–20.
- Powathil, G.G., et al., 2016. Bystander effects and their implications for clinical radiation therapy: insights from multiscale in silico experiments. *J. Theor. Biol.* 401, 1–14.
- Prise, K.M., et al., 2001. A review of studies of ionizing radiation-induced double-strand break clustering. *Radiat. Res.* 156, 572–576.
- Ryabokon, N.I., Goncharova, R.I., 2006. Transgenerational accumulation of radiation damage in small mammals chronically exposed to Chernobyl fallout. *Radiat. Environ. Biophys.* 45, 167–177.
- Salbu, B., 2009. Challenges in radioecology. *J. Environ. Radioact.* 100, 1086–1091.
- Schofield, P.N., 1998. Impact of genomic imprinting on genomic instability and radiation-induced mutation. *Int. J. Radiat. Biol.* 74, 705–710.
- Schofield, P.N., Kondratowicz, M., 2017. Evolving paradigms for the biological response to low dose ionizing radiation; the role of epigenetics. *Int. J. Radiat. Biol.* 1–13.
- Seymour, C.B., et al., 1986. High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 50, 167–179.
- Seymour, C.B., Mothersill, C., 2000. Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose-response curve. *Radiat. Res.* 153, 508–511.
- Smith, J., Beresford, N.A. (Eds.), 2005. *Chernobyl; catastrophe and consequences*. Springer, Berlin.
- Smith, V.S., et al., 2011. Scratchpads 2.0: a Virtual Research Environment supporting scholarly collaboration, communication and data publication in biodiversity science. *Zookeys* 53–70.
- Sunagawa, S., et al., 2015. Structure and function of the global ocean microbiome. *Science* 348.
- van de Walle, J., et al., 2016. Arabidopsis plants exposed to gamma radiation in two successive generations show a different oxidative stress response. *J. Environ. Radioact.* 165, 270–279.
- van der Graaf, A., et al., 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proc. Natl. Acad. Sci. Usa.* 112, 6676–6681.
- Van Noorden, R., 2015. Interdisciplinary research by the numbers. *Nature* 525, 306–307.
- Vandenhove, H., et al., 2010. Life-cycle chronic gamma exposure of *Arabidopsis thaliana* induces growth effects but no discernable effects on oxidative stress pathways. *Plant Physiol. Biochem.* 48, 778–786.
- Verreet, T., et al., 2016. Persistent impact of in utero irradiation on mouse brain structure and function characterized by MR imaging and behavioral analysis. *Front. Behav. Neurosci.* 10, 83.
- Watson, G.E., et al., 1996. Long-term in vivo transmission of alpha-particle-induced chromosomal instability in murine haemopoietic cells. *Int. J. Radiat. Biol.* 69, 175–182.
- Whitmee, S., et al., 2015. Safeguarding human health in the anthropocene epoch: report of the Rockerfeller Foundation-Lancet Commission on planetary health. *Lancet* 386, 1973–2028.
- Wild, C.P., 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomark. Prev.* 14, 1847–1850.
- Xu, J., et al., 2016. Quantitative trait variation is revealed in a novel hypomethylated population of woodland strawberry (*Fragaria vesca*). *BMC Plant Biol.* 16, 240.