BIO Web of Conferences **14**, 08001 (2019) https://doi.org/10.1051/bioconf/20191408001
HEIR 2018

Comments on actinide radiotoxicology research and the 3Rs remit – Replace, Reduce and Refine

Nina M. Griffiths[1,*], *Anne* Van der Meeren[1], *Jaime F.* Angulo[1]

[1]Laboratoire de RadioToxicologie, CEA, Université Paris-Saclay, 91297 Arpajon, France

1 Introduction

Radiotoxicology addresses the diverse biological effects and handling of radionuclides in humans, animals and in the environment. To characterise the health effects of incorporated radionuclides (HEIR) many experiments have been conducted in different animal models. Alpha particle-emitting actinides, such as plutonium, have been (and still are) the focus of many experiments with diverse aims and in multiple species. These range from (1) applied (quantification of tissue uptake/retention, decorporation therapy efficacy), (2) fundamental (gene expression, cancer induction), (3) biokinetic models for dose estimation and (4) environmental (food chain accumulation). The ICRP have used animal data, in part, for development of different biokinetic models to improve dose calculations in humans. Current societal demands now question the use of *in vivo* approaches for risk assessment, an integral part of actinide radiotoxicology research. Future research is needed concerning biokinetics and decorporation strategies of unknown, complex radioelements from different environments of new nuclear fuel and decommissioning. It is now timely to incorporate the replacement, refinement and reduction of animals (the 3Rs) for this research.

2 The 3Rs

The concept of the 3Rs, developed by Russell and Burch, has become embedded in the guidelines and legislation for animal experimentation in many countries. This concept encompasses *Replacement* – use of alternative methods (cell culture, mathematical modelling …), *Reduction* – use of an adequate number of animals to generate significant informative and reproducible data – requires good experimental design and help from statisticians, *Refinement* – use of improved animal welfare and husbandry, reduced invasiveness and stress as well as better assessment of pain. This presentation has for objective to privilege the remit of the 3Rs in radiotoxicology. The aim however is not to advocate prohibition of animal experiments, no model has the perfect answer but to present examples of specific questions pertaining to actinide radiotoxicology.

3 Actinide biokinetics, biological effects and decorporation

Biokinetics Actinide biokinetics and transfer from exterior to interior across the pulmonary barrier are governed by the physicochemical nature of the compound. Extensive

* Corresponding author: nina.griffiths@cea.fr

in vivo experiments have been carried out using pulmonary actinide administration to evaluate actinides ranging from insoluble ("S") to soluble ("F") forms. What answers could be given by replacement *in vitro* approaches using different actinide physicochemical forms? Simple approaches using dissolution techniques provide invaluable information on solubility and transferability. However, more complex *in vitro* models are required to mimic better cellular and acellular environments that may interact with actinides. For example, part of the soluble fraction may be bound to lung parenchyma "lung bound fraction" and further *in vitro* experiments can be used to determine respective affinities for biological ligands. Alveolar macrophages play a major role in lung actinide biokinetics to take up insoluble particles and again *in vitro* experiments can provide information on the differential uptake of various actinide physicochemical forms. In animal experiments the initial amount of actinide is known, the form is known and excretion is measured as are actinide tissue levels – all of have provided invaluable information for model construction. Historic data from humans (USTUR) or animal experiments are continually reassessed due to improved mathematical analyses and as such, actinide radiotoxicological research has been following at least two elements (replace, reduce) of the 3Rs remit for many years.

Biological effects Inhalation of actinides is a potential health risk for people handling actinides for either civil or military purposes. The different biological effects, particularly lung tumour induction, of these alpha emitters have been extensively studied in many different animal species since initial production of plutonium. The biological effects of retained actinides, either in pulmonary epithelial cells or in macrophages may be evaluated *in vitro* and compared to historic experimental or human data (e.g. actinide-induced lung tumours). Advances in cell culture, particularly 3D-printed cell culture platforms as well as stem cells can perhaps answer pertinent questions on lung dosimetry, lung binding and lung effects of actinides. These approaches could facilitate real time analyses without using large numbers of animals as it is always important to consider that lung tumours are an end-point and the questions of initiating events and which cells are irradiated by the alpha particle-emitting actinides remain unresolved. This is the concept of Adverse Outcome Pathways.

Decorporation Many animal experiments have also been carried out to evaluate the efficacy of potential decorporation agents. DTPA is the recognised treatment for decorporation of certain actinides (Pu, Am, Cm). Other approaches are feasible and have indeed been used – chemical affinity, molecular design and *in vitro* studies. However, potential new compounds may be very good chelators in a test tube in non-physiological media, but may be sequestered rather than eliminated, may produce toxic metabolites or may form an unstable actinide-chelator complexes *in vivo*. In this case, animal experiments can provide important pharmacokinetic and pharmacodynamic data. Moreover, the question of whether it is more advantageous to administer DTPA to the lungs directly compared to systemic dosing is important. Again judicious planning of animal experiments may allow the elaboration of a better biokinetic model for actinide behaviour associated with DTPA.

4 Conclusions

Within the 3Rs remit future research on actinide radiotoxicology should incorporate (1) Replace – development and use of appropriate, validated *in vitro* models where possible, (2) Reduce – use of *in vitro*, *ex vivo* and *in silico* models for "predictive screening" of potential decorporation or decontamination agents before *in vivo* testing and (3) Refine – use of improved experimental procedures, animal welfare with a prior harm-benefit analysis. Future research on actinides *in vivo* taking into consideration the 3Rs remit will undoubtedly be necessary to address the question of complex actinide forms likely to be encountered in new nuclear fuels and nuclear facility decommissioning.