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ALTERNATIVES TO CAESIUM IRRADIATORS FOR BIOLOGICAL SCIENCES AND BLOOD TRANSFUSION SERVICES

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Alternatives to Caesium irradiators for biological sciences research and blood transfusion services

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ABSTRACT

Global concerns on the potential for malevolent use of high activity sealed sources of ionising radiation have prompted moves to identify options for their replacement with alternative technologies. Sealed sources of caesium-137 are in common use in biological sciences research and in the production of blood products in blood transfusion services. This report considers the current uses of caesium-137 sources in biological sciences and blood transfusion services in the United Kingdom and potential alternatives. The characteristics of caesium-137 radiation fields are considered as well as those of X-ray alternatives. It is identified that suitable alternatives, including X-irradiators and non-radioactive methods, are available for all current applications but in some cases individual users and researchers may be required to test and develop the alternatives before they can be routinely adopted. A decision tree is provided to aid current users of caesium-137 sources to identify suitable alternative technologies

DECISION TREE TO GUIDE SELECTION OF ALTERNATIVES TO CAESIUM-137 IRRADIATION FOR SPECIFIC PURPOSES

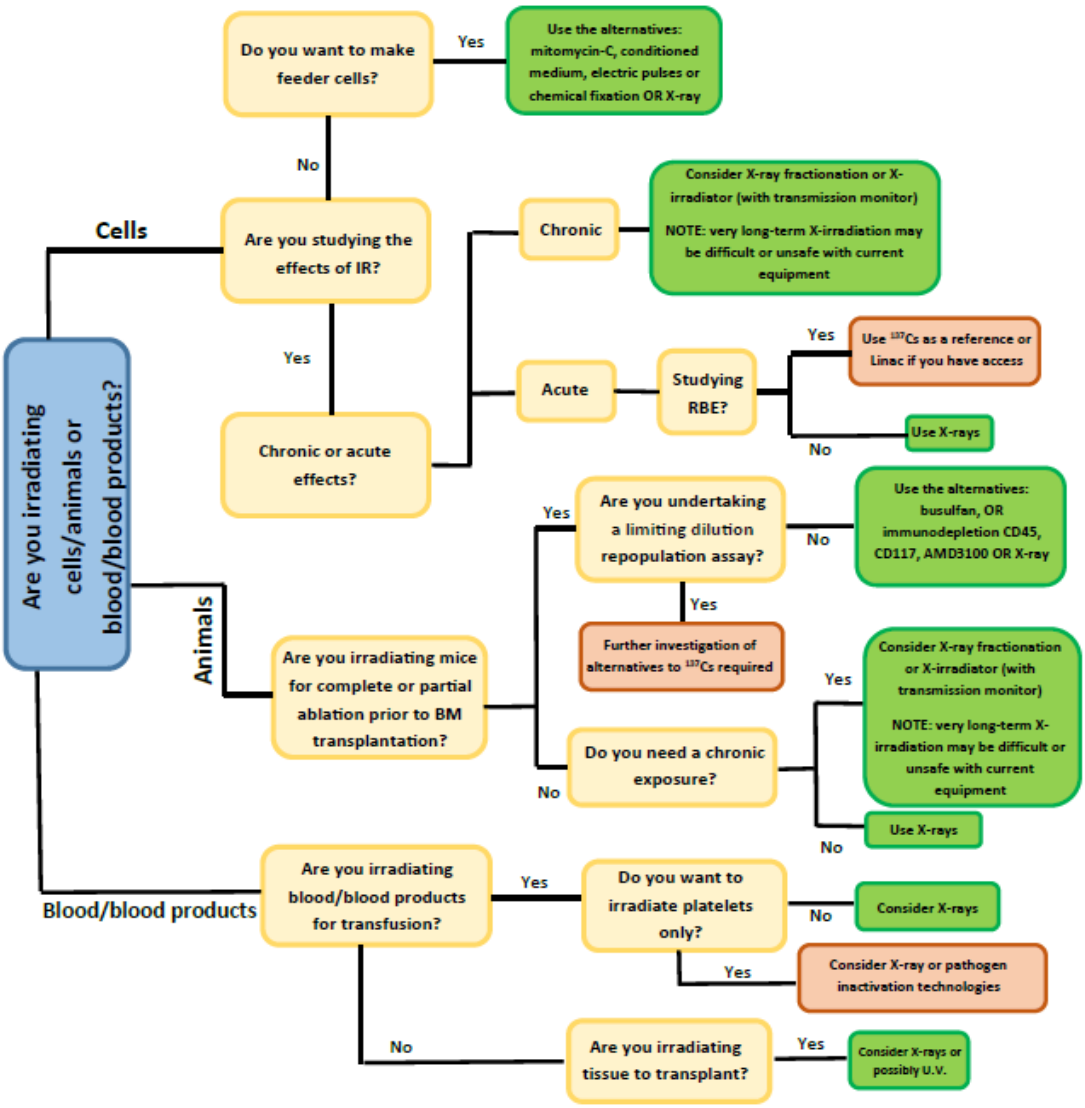

Figure 1. Decision tree to decide on the most appropriate alternative to ^{137}Cs irradiation. Users should consider the subject they wish to irradiate, the reason for irradiating and the duration of the exposure required as prompted within the blue and yellow boxes. Where there are clearly acceptable alternatives to ^{137}Cs irradiation for the given application they are summarised in the green boxes to the right. Where ^{137}Cs may be the only method available a red box would have appeared, but no such applications were identified in this report. The amber box for a few applications indicates that there is an alternative, but further work may be necessary or access may be limited. Full details and the rationale can be found in this report. IR: ionising radiation, RBE: relative biological effectiveness, BM: bone marrow, CD45/117: cluster of differentiation 45/117, AMD3100: an immunostimulant and U.V.: ultraviolet radiation.

CONTENTS

1 INTRODUCTION

This report considers the uses of caesium irradiators in biological sciences and blood transfusion services in the UK. The focus is to provide information on the available alternative technologies to the use of caesium-137 irradiators, and to identify any gaps where alternatives are not currently available. Additionally, a decision tree is provided to assist current users in determining the best available alternatives.

The use of irradiators and radioactive sources is common in the field of biological sciences and in blood transfusion services. Many advances have been made in our understanding of the biological effects of radiation, and the characterisation of radiation responses, through studies using radiation exposure. This continues to be of major importance in the context of improving radiotherapy and in contributing to the development of radiation protection standards that adequately protect individuals from the health effects of radiation exposure. Irradiation is also used in the biological sciences to produce 'feeder cells' used in the *in vitro* culture of several cell types, and to ablate, or deplete, the haematopoietic system of animals in preparation for bone marrow transplantation. In addition to uses in research applications, irradiation has led to improvements in healthcare services, including the exposure to radiation of blood used in transfusion in order to reduce the incidence of transplant-related immunological responses such as transfusion-associated graft-versus-host disease (TA-GvHD) [Gibson et al., 2015b]. TA-GvHD is characterised by profound bone marrow hypoplasia and mortality in over 90% of cases [Treleaven et al., 2011]. Thankfully, this is a relatively rare occurrence, which is effectively addressed by irradiating the blood components to inactivate any residual lymphocytes that may cause an immunological response.

These advantageous uses have contributed to the increases in the number of radiation and radioactive sources in operation across the UK and internationally, and while not exclusively so, many irradiators utilise caesium-137 (^{137}Cs) as their source of ionising radiation. Whilst the benefit of irradiators is clear, the risk of theft and malicious use must also be taken seriously. These concerns lead to the consideration of alternatives to, and replacement of, ^{137}Cs irradiators to reduce the risks of source loss and/or malicious use. An estimated 150 countries possess and use such radiation sources [Bieniawski et al., 2017], there is a global initiative to try and reduce the numbers of sources in use (see bleow).

^{137}Cs dispersal was a consequence of both the Chernobyl and Fukushima accidents, with elevated levels of the isotope still being detected in the environments surrounding both sites and much further afield. Several individuals were accidently exposed to radiation in 1987, when a ^{137}Cs source was removed from a radiotherapy unit [Sakamoto-Hojo, 2018] and become ruptured at a junkyard. Whilst this incident was not malicious, four people died from tissue reactions [IAEA], thus highlighting the potential hazard and risk of ^{137}Cs ownership.

^{137}Cs is a radioactive isotope of the element caesium, a fission product of uranium. ^{137}Cs is used commonly as a source of gamma-radiation, as is cobalt-60 (^{60}Co), both of which provide high energy gamma radiation and have relatively long half-lives. The reduction and replacement of ^{137}Cs sources has been identified by the Nuclear Threat Initiative as an urgent issue that will require a global effort to limit risk [Bieniawski et al., 2017].

The International Atomic Energy Agency (IAEA) place ^{137}Cs blood irradiators at the highest category 1 or 2 (1 – 5 scale, 1 being most dangerous based on activity and needing control, 5

being least), requiring heightened safety and security where they are located [IAEA, 2003]. The expense of housing and using ^{137}Cs sources, given the increased security associated with them, has led to increasing interest and desire to move towards alternative technologies with lower running costs and reduced security concerns, with X-ray irradiators often providing a potential substitute [Gibson et al., 2015b].

X-irradiators have been reported to be substantially cheaper to purchase than gamma-source irradiators [Janatpour et al., 2005]; a comparison of quotes by one research group revealed X-irradiators being around one sixth of the cost [Gibson et al., 2015b], although the cost-benefit of X- over gamma-irradiator is largely dependent upon source age and throughput. A report by Bakken *et al.* (2013) performed a cost-benefit comparison between ^{137}Cs and X-irradiators within the field of blood irradiation. Cost comparisons were performed at three levels, low, medium or high use, depending on the number of blood units irradiated per year. The social costs were also incorporated. The study reported that in most cases, there is a net benefit to switching to X-irradiators, when considering costs incurred from securing and disposing of caesium sources as well as running costs and maintenance [Bakken, 2013]. However, space requirements and contingency plans need to be considered, as well as installation and maintenance costs and training of staff; costs are likely to vary between models and manufacturers, the frequency of servicing, etc. and so it is not possible to provide a completely accurate set of figures for the comparison of whole-life costs for the use of X-irradiators as compared to 137Cs irradiators. A significant cost is associated with the disposal of ^{137}Cs sources, up to several hundreds of thousands of pounds, and this is an important consideration when considering a purchase or disposal [Dodd and Vetter, 2009, Murphy and Kamen, 2019, Kamen et al., 2019].

Shielded ^{137}Cs irradiators have been in use for many years and are routinely used during pre-clinical radiation research during early *in vitro* and *in vivo* studies, and to trial novel methodologies. With improvements in techniques and technology, both X- and gamma-irradiators are reported to be equally as effective in many radiobiological experiments performed in animals and in cell culture [Brodin et al., 2016]. Currently, both gamma- and X-irradiators appear to be in routine use for irradiating blood and blood products in both the UK [Bashir et al., 2011] and the US [Janatpour et al., 2005, Kamen et al., 2019].

The migration from ^{137}Cs and ^{60}Co gamma irradiators to X-irradiators over a 9-year period has recently been successfully completed in one of the largest US medical centres based in New York. This was achieved with minimal compromise to patient care or research studies [Kamen et al., 2019]. The replacement was facilitated by performing a series of comparison studies, not only in terms of the physics and dosimetry of gamma- as opposed to X-irradiation, but also in the various biological research studies performed at the centre, such as bone marrow ablations in mice, cell cycle inhibition and tumour treatment in mice. The differences between X- and gamma-irradiations were mostly of little consequence in the comparisons, which favours the use of X-irradiators due to economic benefits and reduced security measures required [Kamen et al., 2019].

A recent report by the Nuclear Threat Initiative considered a number of countries that have also began to make significant steps in phasing out ^{137}Cs and move towards alternative technologies as a replacement where possible, including Japan, France and Norway [Bieniawski et al., 2017]. Most notably, France has replaced all ^{137}Cs irradiators with X-ray units for use in blood transfusion services and some in research settings, and likewise Norway have so far replaced all blood irradiators with X-ray units. Japan has been phasing out ^{137}Cs

sources during the last two decades, and is estimated to have replaced over 80% of ^{137}Cs irradiators with X-ray equivalents [Bieniawski et al., 2017].

In the US, the Office of Radiological Security's *Cesium Irradiator Replacement Project (CIRP)* has been in place for around four years. CIRP is a voluntary initiative offering financial incentives to licensees who choose to replace ^{137}Cs irradiators with alternative, non-radioisotopic technologies. While there are no mandatory national recommendations or replacement schemes, several individual medical and research facilities have already adopted alternative technologies, including the University of California and a New York medical centre [Kamen et al., 2019, Murphy and Kamen, 2019, MacKenzie et al., 2020]. There is a recent comprehensive report on the available alternatives to caesium irradiators in all sectors, including biological sciences and blood transfusion services produced by the US Department of Homeland Security[*], exploring non-radioisotopic alternatives during irradiations.

2 CURRENT USES OF CAESIUM IRRADIATORS IN BLOOD TRANSFUSION SERVICES AND BIOLOGICAL SCIENCES RESEARCH

Caesium irradiation is in widespread use across the UK biological sciences research sector and in the blood transfusion services. Here we summarise the current uses of such irradiators within these areas.

2.1 Human blood transfusion and regenerative medicine

Blood and blood products used for transfusion are typically irradiated using gamma-irradiators to reduce the risk of TA-GvHD in humans. Fortunately, TA-GvHD is not common, but must be avoided as it is a potentially fatal complication of transfusion and transplantation and is often under-recognised despite well documented clinical symptoms and their timings [Pritchard and Shaz, 2016]. Individual patients most at-risk of the disease are often required to receive irradiated blood and/or blood products [Pritchard and Shaz, 2016]. Most blood gamma-irradiators use ^{137}Cs. Around 10% of all donated blood is irradiated prior to transfusion [Sullivan et al., 2007]. Clinically insignificant differences were reported in terms of red cell membrane permeability between X- and gamma-radiation use [Janatpour et al., 2005], with both being equally effective at reducing TA-GvHD.

A recent 2016 survey conducted in the U.S. (but including some international organisations) reported that the most frequent requirement for irradiated blood products is during transfusion from blood relatives in conjunction with histocompatibility testing of the human leukocyte antigen (HLA) gene complex matching [Pritchard and Shaz, 2016]. Neonatal exchange transfusions, intrauterine transfusions, and pre-term or low birth-weight babies also have a high requirement for irradiated blood, although patient groups deemed to be less at risk of TA-GvHD may still require irradiated blood products; there are differences in the requirements for the use of irradiated blood between nations and organizations [Pritchard and Shaz, 2016]. The minimum dose delivered to blood during ^{137}Cs irradiations is internationally recommended to be between 15 – 25 Gy (25 Gy minimum recommended in the U.K., ISO/ASTM FDIS 51939:2016(E); https://www.transfusionguidelines.org/red-book/chapter-7-specifications-for-

[*] https://www.cisa.gov/publication/non-radioisotopic-alternative-technologies-white-paper

blood-components/7-31-irradiated-components) to sufficiently inhibit an immune response in the recipient and prevent TA-GvHD [Mohammadyari et al., 2014]. An error in dose calculation could be fatal, therefore gamma sources such as ^{137}Cs and ^{60}Co require accurate and regular dosimetry, given the natural radioactive decay which they undergo.

The British Society for Haematology, during an extensive literature review, concluded that X- and gamma-irradiation can be considered equivalent in their ability to reduce TA-GvHD incidence [Treleaven et al., 2011]. The use of irradiated blood or blood products is recommended by the British Committee for Standards in Haematology/British Society for Haematology in the following situations: (i) to avoid TA-GvHD, (ii) for at risk patients all red cell, platelet and granulocyte concentrates except cryopreserved red cells after deglycolization, (iii) all donations from first and second-degree relatives even when the patient is immunocompetent, (iv) all HLA-selected components even where the patient is immunocompetent, (v) if the patient is at particular risk of hyperkalaemia, e.g. intrauterine or neonatal exchange transfusion, (vi) all granulocyte components 24 hours before issue and use with minimum delay, (vii) all blood for intrauterine transfusion and neonatal exchange transfusion, (viii) platelets transfused in utero for allo-immune thrombocytopenia, (ix) all cellular blood components for T-lymphocyte immunodeficiency syndrome patients, (x) transfusions for all recipients of allogeneic haematopoietic stem cell transplantation, (xi) transfusions for patients undergoing bone marrow or peripheral blood stem cell harvesting, (xii) transfusions for all patients undergoing autologous bone marrow transplant or peripheral blood stem cell transplantation, (xiii) red cells and platelet transfusion for all adults and children with Hodgkin lymphoma, (xiv) transfusions for all patients treated with purine analogue drugs and (xv) transfusions for all aplastic anaemia patients receiving immunosuppressive therapy with horse anti-thymocyte globulin [Treleaven et al., 2011].

The field of regenerative medicine is a growing one, likely to rely on the continued use of human embryonic stem cells and human induced pluripotent stem cells, the maintenance of both depends on the use of ‘feeder cells’ that provide essential cytokines and other factors supporting the growth of specific stem- and progenitor-cell populations. Therefore, the production of feeder cells will be discussed in this report both in the light of this application and applications in biological research.

2.2 Animal exposures

Within biological science research, rodents have become the predominant model animal for the study of transplantation. Bone marrow transplantation of hematopoietic stem cells and adoptive cell transfer are extensively studied [Gibson et al., 2015a, Duran-Struuck and Dysko, 2009]. Rodents, particularly mice, share a multitude of physiological similarities with humans and disease pathologies can be closely related in the two species, hence what we can learn and achieve in mice can often be translated to human use with few difficulties. Whilst there are several methods to pre-condition or immunosuppress the recipient animal to ensure survival following engraftment and transplantation, X- or gamma-irradiation of the recipient’s bone marrow is by far the most commonly practised approach [Duran-Struuck and Dysko, 2009].

Total body gamma-irradiation of mice with the aim of myeloablation is a longstanding and commonly used method for preconditioning animals and the haematopoietic system to allow the engraftment of donor haematopoietic cells in a recipient mouse [Duran-Struuck and

Dysko, 2009]. Myeloablative doses are in the range of 7 – 13 Gy of gamma radiation [Duran-Struuck and Dysko, 2009]. The high doses of radiation received by the mouse and subsequent compromised immune response result in strict veterinary and husbandry care requirements to ensure the welfare of the animal. Unsuccessful engraftment will typically result in the death of the animal due to the lack of an immune response. Therefore, the efficiency of myeloablation (most importantly the correct dose calculation) is key to a successful transplantation.

This approach is widely used during bone marrow transplantation and in gene therapy research. However, some cytotoxic effects have been reported as a result [Gao et al., 2019]. Hematopoietic stem cell transplantation (HSCT) requires the recipient's bone marrow to undergo myeloablative conditioning to create niches in the bone marrow, typically performed by irradiation or chemotherapy. However, some unwanted side effects are experienced with regards to mortality and bone marrow damage resulting in treatment-induced complications [Ibrahim et al., 2019]. Some methods employ a partial body radiation exposure, often utilising gamma irradiation from ^{137}Cs to part-ablate the haematopoietic system and allow the generation of chimeric mice. Commonly, an excess number of donor bone marrow cells is transplanted, but some studies require a strictly limited number of cells to be used in so called limiting dilution assays [Ivanovs et al., 2011, Azzoni et al., 2018, Zhang et al., 2020, Zhang et al., 2019]

Myeloablation by irradiation (by both X- and gamma-radiation) in mice has shown some disadvantageous effects including death from secondary radiation-associated complications such as toxicity, GvHD, tissue damage and subsequent inflammatory responses [Kallman, 1962]. Therefore, alternative approaches to irradiation are being investigated, such as non-genotoxic preconditioning techniques [Gao et al., 2019, Li et al., 2019] which are discussed within this report. Ionising radiation causes DNA double strand breaks [Asaithamby and Chen, 2009, Barnard et al., 2013, Rothkamm and Lobrich, 2003], of which not all will be repaired following high radiation doses. This may lead to cell death by apoptosis or tissue necrosis. As in humans, the response to myeloablation in mice appears to be age-dependant [Ordemann et al., 2002], with younger animals showing greater survival. The dose of irradiation needed to induce immunosuppression, as well as the strain of mouse, must also be factored into experimental design [Duran-Struuck and Dysko, 2009]. C57BL/6 mice tend to be the strain of choice as a recipient for donor cells as they show greater radio-resistance [Gibson et al., 2015a, Roderick, 1963, Worgul et al., 2005, Bakthavachalu et al., 2010, Mukherjee et al., 2014] and can tolerate the higher radiation doses than other strains, reportedly up to 11 Gy, needed during myeloablation [Duran-Struuck et al., 2008]. It is possible to use alternative strains with lower dose regimes, and dose fractionation or protraction can be considered.

Irradiation of animals is also used in radiobiological research to assess the consequences of exposure to health and in terms of disease development. Common areas of interest relate to the relative effectiveness of differing radiation types (e.g. Gamma- as opposed to neutron-irradiation or X-irradiation), the pathological consequences of differing doses and/or dose-rate(s) of exposure. Ionising radiation is a known carcinogen; therefore, many studies consider cancer as an endpoint. However, other late developing health effects are of increasing concern, including circulatory disease, cognitive impairment, and cataract formation. In pre-clinical studies of radiotherapy, animal irradiation is used to assess potential radiotherapeutic exposures, the occurrence of damage to normal tissues (e.g. skin damage,

fibrosis, impacts on urinary tract and bowel function) that can limit radiotherapy exposures. Irradiation of animal models is used in the development and assessment of potential medical countermeasures and treatments for radiation injury.

Irradiation of animals is also used in more fundamental studies of DNA damage response. In recent years, there have become available an ever-increasing range of genetically modified animals (largely mice), and the role of specific genes in disease development can be investigated [Wells, 2010]. The use of such models does not necessarily impact upon the requirements in terms of radiation dose, dose-rate or type where irradiation is used. Radiations of many types, including X-, gamma- and alpha-radiation sources have been frequently used to provide a DNA damaging stimulus [Rothkamm et al., 2003].

2.3 Exposure of *in vitro* cultured cells

Growth-arrested feeder cells have been in common use for the *in vitro* culture of many cells types for years, and is essential to many *in vitro* biological studies, in particular, where the cell population(s) of interest are stem cells. Feeder cells do not divide, causing them to produce excess extracellular secretions that can support the proliferation and growth of other cell types [Llames et al., 2015]. Feeder cells support the growth of cells in culture by providing this complex mixture of extracellular matrix components and various growth factors to promote the division and growth of the cells they support [Llames et al., 2015, Li et al., 2017]. The term 'co-culture' is often used to describe the interaction between feeder and stem cells, however, this term is not strictly correct as only one cell type is capable of growth and proliferation. Typically, the feeder cells facilitate the growth of stem cells and are mitotically inactivated by radiation exposure, commonly using either X-rays or ^{137}Cs irradiation [Li et al., 2017] or less frequently, the non-radiation alternative mitomycin-C [Lemoine et al., 1988]. The optimal dose needed during gamma-irradiation is reported to be 80 Gy to effectively inactivate fibroblasts for use as feeder cells [Lemoine et al., 1988]. Mouse embryonic fibroblasts represent the most commonly used feeder cell type, used in the co-culture of mouse embryonic stem cells. The demand for feeder cell production is high, with inactivation by gamma radiation proving the most economic method for large-scale generation of cells [Li et al., 2017], however, some alternative techniques have been explored.

Researchers investigating radiation damage responses, health effects of radiation or biological dosimetry will likely have a desire to consider the relative biological effectiveness (RBE), of differing radiation types during their studies. RBE is the efficacy of a given radiation type in a given assay by comparison with that of a standard radiation type, which is often gamma irradiation from a ^{137}Cs source [Hall, 1973]. Many researchers will have existing reference values for ^{137}Cs gamma vs X-radiation for a range of doses and energy spectra. However, existing reference data may not be adequate to support RBE studies utilising novel techniques or endpoints previously undiscovered. The RBE for a given X-ray unit will be filtration-dependant; therefore, it is possible to alter the RBE by using different materials or different thickness of filtration during setup of the X-ray source.

Radiation can be used as an external stressor/DNA damaging agent in more fundamental studies of DNA damage responses in cell culture studies as in animal studies (see above).

2.4 Sterilisation

Radiation, including that from X- and gamma- sources, can inactivate microorganisms efficiently and has therefore been used for the sterilisation of equipment and reagents. Historically, this has been considered a safe and cost-effective method for use in medical and research facilities, with the increasing use of single-use plastics [Yusof, 2018].

The practise of tissue sterilisation in countries of high malaria incidence using radiation is also common. Similar to the irradiation of blood, tissue donors also require screening and the malaria parasite is particularly sensitive to radiation, making this method of sterilisation highly effective [Myint, 2018]. Tissues are heat sensitive, therefore autoclaving or heat sterilisation is not possible prior to grafting [Yusof, 2018]. Typically, gamma-radiation or electron beams are required, with very high doses between 25 – 40 kGy reported as acceptable (tissue preservation method dependant) [Yusof, 2018, Nguyen et al., 2007]. In these instances, radiation-induced tissue damage can be reduced by modifying the preservation method prior to irradiation for tissue banking [Dziedzic-Goclawska et al., 2005].

2.5 Additional considerations

Some instances within both *in vivo* and *in vitro* radiation experimentation may require doses to be delivered in a protracted/chronic fashion, whereby the dose would be delivered over many hours, days or even weeks. Gamma-irradiation is often favoured in these instances due to a perceived stability in energy, accuracy of dosimetry and the lack of heat generation and electricity usage required by X-irradiators. The reliance of X-irradiators upon electricity make them susceptible to power outages or failures, which would interrupt exposure and significantly affect an experiment, such concerns do not apply to ^{137}Cs irradiators.

2.6 Uses in botanical sciences and agriculture

The above subsections outline the areas whereby gamma, and increasingly X-rays, are used to irradiate biological tissue, blood and organisms. Other less substantial uses have been reported across the world, with some very recent examples given here, albeit not in current use in the UK. In Brazil, researchers have used gamma irradiation (^{60}Co) at doses of 50 – 200 Gy to investigate the radiosensitivity of caster bean seeds to genetic alteration [Lopes et al., 2017]. Citrus seeds, budwoods (wood consisting of strong young shoots bearing buds suitable for use in propagation by budding) and nodal segments (cuttings taken just below a leaf node for propagation) are gamma-irradiated in an attempt to improve genotypes for fruit production (25 – 156 Gy) [Perez-Jimenez et al., 2020]. Stored grains are reportedly irradiated with an optimum dose of 650 Gy gamma-radiation to disinfect them of insects and their associated eggs and larvae, this method is deemed more environmentally friendly than using chemical pesticides such as methyl bromide [Hammad et al., 2020]. Irradiation leaves no residue in/on the product either.

Insects are routinely exposed in research applications as is the case for mammals, as discussed above; radiation is also used for disinfecting grain from insects [Hammad et al., 2020]. Gamma-irradiation doses of up to 350 Gy applied to the crop-damaging moths Ostrinia nubilalis Hubner and Helicoverpa armigera Hübner was recently demonstrated as a method to sterilise the insect to reduce reproduction [Osouli et al., 2020, Hallman and Hellmich, 2009,

Kim et al., 2015] and reduce crop spoilage. Similarly, irradiation of the invasive light brown apple moth to doses of 250 Gy gamma radiation successfully halts their reproduction [Follett and Snook, 2012] protecting apples and pears from destruction. Since the 1950s, a number of studies report the irradiation of potential malaria-carrying mosquitos which successfully induced dominant lethality in these insects [Dame et al., 2009]. Earthworms represent an increasingly relevant model for studying the effects of irradiation, for example radiation-induced mutations [Wilding et al., 2006], ecological risk assessment of gamma-exposures [Hertel-Aas et al., 2011, Hertel-Aas et al., 2007] and radiation-induced bystander responses [Rusin et al., 2019] to name a few. However, theses doses are unlikely to exceed 25 Gy and can therefore likely be performed using X-irradiation (dependant on dose-rate requirement when modelling environmental contamination/radiation).

At the time of writing, we are unaware of any UK examples of irradiation for crop sterilisation requiring these high doses of gamma-radiation, but such studies are included for completeness. There are also non-irradiation alternatives using novel genetic methodologies to achieve insect sterilisation, as demonstrated in the Coding moth in Canada [Thistlewood and Judd, 2019].

3 GENERAL CHARACTERISTICS OF IRRADIATION FIELDS

3.1 Photon fields

Photons are fundamental particles that are generally characterized by their frequency, wavelength or energy: they are described according to their frequency/wavelength and according to how they are generated. The photons of interest for this report have enough energy to ionize atoms and are typically termed gamma-rays or X-rays. However, the biological effectiveness of a photon is determined by its energy, not whether it is technically a gamma-ray or X-ray.

Gamma rays are emitted when a nucleus changes energy state, with the energy of the gamma-ray being determined by the difference between the initial and final energy levels. The excited state of the nucleus can be populated by a variety of means, but most typically following emission by the parent of a β-particle or α-particle. The well-defined energy of gamma-rays (γ-rays) is very attractive for experiments and calibrations, but many radionuclides emit gamma rays of a few or even many energies, and if the daughter is not stable, the field can become even more complex. Gamma-ray energies can range from low kilo-electron volts (keV) to several mega-electron volts (MeV), perhaps spanning a large range from a single radionuclide.

X-ray is a term used to cover two distinct types of photon. The X-rays used in most applications are fundamentally the bremsstrahlung photons first described by Röntgen [Röntgen, 1895]. These X-rays are generated by the stopping of electrons in a metal after they have been accelerated across a vacuum, with the photons emitted covering a broad range of energies: the maximum energy in electron volts is equivalent to the potential between the electrodes, but the energy with the highest probability is about ⅓ of the maximum energy. Consequently, X-ray fields of this type have broad energy distributions, though preferential attenuation of the lower energy components using filters is routinely used to narrow the energy distribution somewhat.

Photons emitted during the relaxation process of ionized atoms are termed “characteristic” X-rays. These are more like gamma-rays in nature, having energies precisely determined by the difference between the energy levels in the atomic rather than nuclear structure. They are relatively low in energy, with the maximum energy correlating with the atomic number, *Z*: for low *Z* elements, they are as low as 10 eV, but for higher *Z* elements they can reach 80-90 keV. These are a feature of bremsstrahlung X-ray spectra, because the target material emits characteristic X-rays, typically those of tungsten or copper, though filters are often used to remove them from the field that is used for whatever purpose.

The photons used in radiotherapy are also generated by bremsstrahlung, but the electron energies in a linear accelerator (linac) are typically higher, so the maximum energy can be tens of MeV. They are the same as the X-rays from an X-ray tube, with the maximum and peak energies following the same pattern, though they are not generally referred to as X-rays. Because the electron is accelerated in several or many steps, higher energy electrons can be produced without the risk of unwanted electrical discharge that would be a risk for a conventional X-ray tube that attempted to achieve the same energy in a single step.

Accelerators can also be used to produce beams of other charged particles, which can also be used to produce higher energy photon fields via interactions with the target nucleus [ISO, 2019]. Examples of this are the reactions $^{19}F(p, \alpha\gamma)^{16}O$ and $^{12}C(p, p'\gamma)^{12}C$, both of which produce much higher energy photons in the 2-7 MeV energy range. The dose rates are, however, too low to be useful in the types of exposure being considered in this report.

There are other means of photon generation, such as annihilation of particle-antiparticle pairs. The most common of these are the 511 keV pair of photons produced by positron annihilation. These are neither gamma-rays nor X-rays, and are not useful in the contexts considered in this report. Similarly, electron synchrotron accelerators are quite high-profile sources of photons for experiments, but these are enormous, very expensive facilities, and the photons produced are often relatively low in energy.

Where there is a need to deliver significant dose rates, higher energy photons are very advantageous for obvious reasons. This is evident from the ratio of the air kerma* to the fluence† ratio [ICRU, 1998, Hubbell and Seltzer, 1995] (Figure 2) where the dose deposited by higher energy photons is evident. There is also a significant increase at low energies, but this is not useful for blood irradiation or most biological experiments because the radiation is very rapidly attenuated in tissue.

Photons of different energies produce electrons with different energies, and those electrons will have different ranges when interacting with different materials before they get absorbed. In terms of absorbed dose to tissue, the mechanism by which photons interact with the body is generally of little importance. However, for small scale structures, either small tissues or substructures within a tissue, the range of the electrons generated becomes important. In terms of tissue structures, the complex structure of bones or the lens of the eye are specific examples, whereas inside cells the energy deposition within the nucleus is critical to the RBE of the radiation. For tissue, these effects begin to become quite significant below about 30 keV [Hubbell and Seltzer, 1995], so a switch to a source of photons of low energy might make it difficult to compare results to data obtained with ^{137}Cs or ^{60}Co sources. However, photons

* Kerma in J kg^{-1} or gray (Gy) is the kinetic energy released per unit mass of material. It is closely related to the dose, but is preferred in some calibration applications

† The fluence is simply a measure of the number of particles per unit area.

above 100 keV are broadly similar in terms of the secondary electrons generated in terms of RBE, so they ought to be comparable to the radionuclide sources. It would, in any case, be hard to generate the required dose rates using low energy photons.

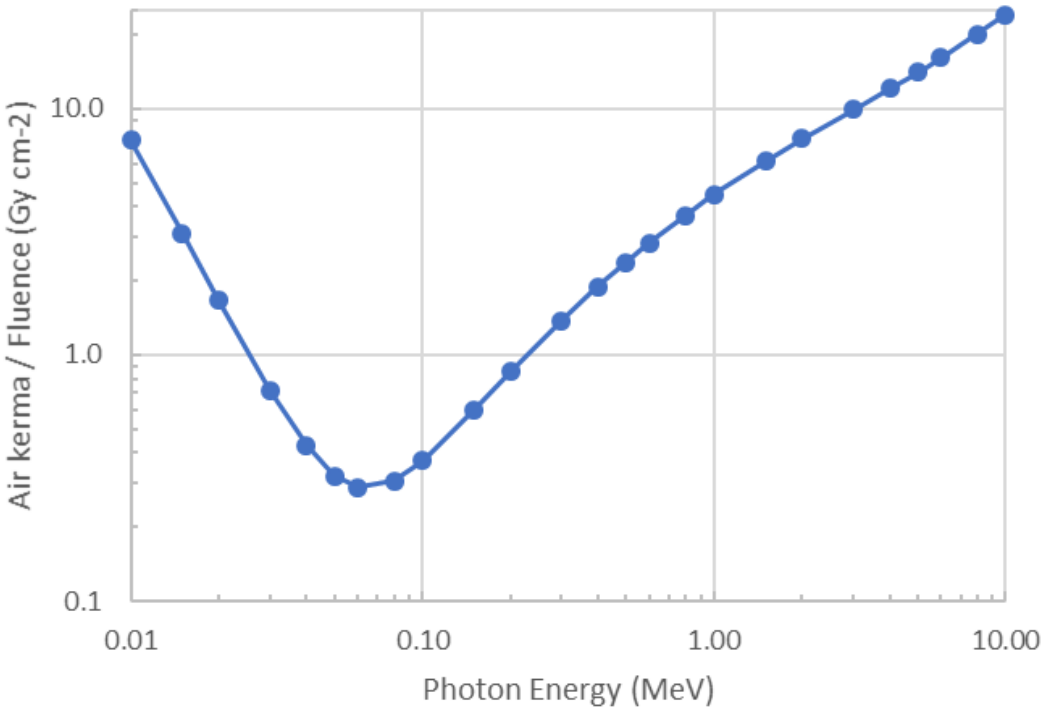

Figure 2. Ratio of air kerma to fluence for photons

There are differences in the local deposition of energy caused by the changes in the materials for small scale structures within the body. A good example of this is the bone marrow, which contains small structures and differing tissue components in the different structures. The tissues involved have different densities and elemental compositions, with significant amounts of iron and calcium, both of which are relatively high *Z* for tissue. Therefore, there are more photoelectrons generated, so that the local energy deposition has strong local variations. The impact of this has only a small effect on the effective dose to people [ICRP, 2010], but at lower energies, the effect within the bones is significant (Figure 3): below 100 keV the local energy deposition begins to have a significant impact, with the local absorbed dose to these radiation sensitive tissues increasing by a factor of two or more below about 50 keV.

The increased energy deposition by lower energy photons is relative to that of ^{137}Cs or ^{60}Co. Experiments with small animals would show similar effects, so it would hence be inadvisable to use fields with significant components of the dose deriving from photons with energies less than 100 keV. The example chosen here is the worst-case scenario because of the small scale radiosensitive structures and the high *Z* elements present. However, structures in small animals will be finer, so the effects could be either more or less pronounced.

These effects are hard to translate to the impact on blood irradiation, but it is possible that the presence of iron in the blood could have an impact if the field has a significant low energy component. ISO/ASTM 51939:2016 recommends using an X-ray field with energy “from 40

keV to 300 keV" which is necessary if it is to be assumed that a given dose from the X-rays is to be no more effective than an equal dose from one of the radionuclide sources.

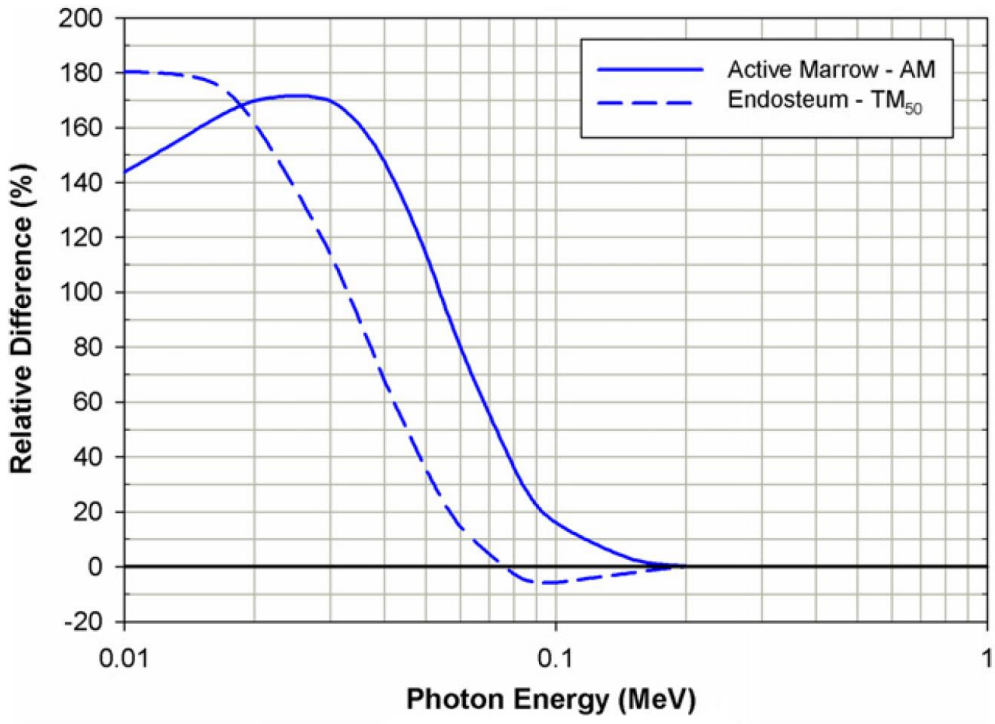

Figure 3. Ratio of the absorbed dose in the active marrow and endosteum for full transport of secondary electrons and using the kerma approximation, which assumes the electrons deposit their energy where the interaction takes place. The absorbed dose is seen to be much higher below 100 keV. The figure is taken from ICRP Publication 116 [ICRP, 2010].

Photons can be characterized in terms of their depth-dose distribution, which is essentially a measure of the absorbed dose within a material as the various physical processes change the field. These changes are caused by the attenuation and scatter of the photons, but also by changes in the secondary electron field within the material. Inevitably, as the photons are attenuated and scattered to lower energies, the dose versus depth falls, but near the surface, there can be increases, because the secondary electrons may have quite long ranges and deposit dose deeper in the material than the location of the primary interaction. For low energy photons, the attenuation is the dominant effect, with the differences for different photon energies becoming greater and greater as the depth increases.

For higher energy photons, when looking at shallow depth effects, it is routine in dosimetry to use "secondary charged particle build-up", which essentially means the use of a thin plastic sheet to generate enough secondary electrons to ensure equilibrium is obtained within the material that is being irradiated. This avoids a build-up of dose with depth in the material. However, this becomes quite a significant effect for ^{60}Co and higher energy irradiations, but it should not impact on the potential switch between ^{137}Cs and X-rays.

The significance of different depth-dose distributions depends on what is being irradiated. For small biological samples, the effect may not be very significant, whereas for a thicker sample there may be significant differences between the doses deposited at a given depth by photons of different energies. These effects do need to be considered when switching irradiation fields, especially if the photon energy is significantly reduced.

In practice, the field is characterized using half value layers (HVLs). These are simply the thickness of a specific material required to halve the dose rate in a reference instrument. For practical reasons, the material is generally a metal, typically aluminium, copper or lead. A higher HVL means more material is needed to reduce the beam intensity, which indicates a higher mean photon energy.

3.2 Radionuclide gamma-ray sources

The range of gamma-ray sources that is available to buy is much wider than the range of sources typically used in blood irradiators or for biological experiments. Most would be inappropriate or impractical because they have short half-lives or complex emissions which are either less well known or are not of appropriate energy. In practice, only three radionuclides are recommended by the International Organization for Standardization (ISO) as providing “reference fields” [ISO, 2019]: ^{137}Cs, ^{60}Co and ^{241}Am, though the last of these is not considered by ISO to be well enough known in terms of its emissions to provide the highest quality calibration fields. The 60 keV gamma-rays emitted by ^{241}Am are also relatively ineffective at delivering high dose rates, unless the source is of very high activity, which would make it very bulky. The source would then have problems with self-attenuation because americium absorbs 60 keV photons very effectively.

Other radionuclides are used for other applications, such as industrial radiography, with ^{192}Ir, ^{75}Se and ^{169}Yb being typical, but these have relatively short half-lives, which make them very impractical as sources to be held for extended periods, and the relatively low energy of their emissions.

In most applications, the shorter half-life of ^{60}Co when compared to ^{137}Cs requires the sources to be replaced more frequently, which is not a desirable feature. However, the higher energy γ-rays from ^{60}Co and the relatively high activity that can be achieved from an equivalent amount of radionuclide, make it attractive for the delivery of higher dose rates.

The standard for the dosimetry of blood irradiators [ISO, 2017] recommends that the practice should use ^{137}Cs, ^{60}Co, or “low energy X-radiation (bremsstrahlung) produced by an X-ray tube”. In this context, low energy means a maximum energy of 160 keV, which would not be considered a low energy for radiation protection. The filtration used is not specified, though it recommends that the lowest energy should be restricted to 30 keV. This is quite prescriptive, but it seems to reflect practice. The use of higher energy X-rays would offer significant benefits in terms of dose delivery, but perhaps shielding is the main reason for limiting the tube potential.

3.3 X-ray fields

In principle, an X-ray tube with any potential could be produced, but the single stage acceleration process makes electrical insulation difficult. The current reference irradiation fields [ISO, 2019] use a maximum potential of 400 kV, whilst online searches do not reveal X-ray tubes with a potential greater than 600 kV*. However, the peak of the emission spectrum being about 1/3 of the accelerating potential means that for 400 kV, the peak will be just above

* https://www.comet-xray.com/getmedia/cb6a19c8-3f60-43c3-898f-51c382031bc6/pdf_xrs_modules_and_generators_v1_1.aspx

100 keV and for 600 kV the peak will be around 200 keV. Both energies are significantly lower than the 662 keV of ^{137}Cs, though the RBE of the photons may be expected to be comparable.

If X-ray fields are to be used, it is necessary for them to be well defined so that good practice and consistency can be ensured between laboratories, which indicates that they should ideally be taken from a standard. The International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC) specify recommended fields for instrument and dosemeter calibration in BS ISO 4037-1:2019 [ISO, 2019] and medical imaging in BS EN 61267:2006 [IEC, 2006] respectively. The medical fields are relatively low in energy, with the highest tube potentials being only 150 kV, whereas the calibration fields go up to 400 kV. Given that ISO/ASTM DIS 51939:2016 [ASTM, 2016] recommends fields with energies from 40 keV to 300 keV with no recommendation on how those fields are specified or selected, the discussion in this document is focussed on the calibration fields from BS ISO 4037-1:2019, which are more likely have similar RBE to ^{137}Cs.

For the photon reference fields generated by calibration laboratories, the fields are filtered. All X-ray fields have a level of inherent filtration because they are sealed tubes, but that filtration is minimized by using low *Z* metals such as aluminium or beryllium. External filters are then used to “narrow” the X-ray field by preferentially attenuating the lower energy component. This produces quasi mono-energetic fields to compare with gamma rays using a combination of aluminium, copper, tin and lead filters [ISO, 2019]. The higher *Z* materials are used for higher energies, because the materials are especially effective below the K-edge*, but even for lead this is only 88 keV, so for higher energy X-rays the field cannot be narrowed appreciably using even a lead filter.

The available calibration fields are termed High (H), Wide (W), Narrow (N) and Low (L), with the amount of filtration increasing in this sequence. The fields are more monoenergetic in the same sequence as a result, with the H field being broadest in energy and the L field being the narrowest. The fields are not monoenergetic, even for the N series with quite a lot of filtration (Figure 4), but the RBE is not likely to vary much within the energy range spanned by even these broad peaks. The logarithmic x-axis used in Figure 4 should be noted, because the energy width of the peak increases with tube potential.

The bigger problem is that the dose rate falls significantly as the filtration is increased, so the highest fluences and doses are generated for the H series, and the lowest for the L series (Figure 5). The dose rate is approximately proportional to the tube current, but limits on the tube current vary with the applied tube potential, which imposes real limits on the dose rate that any X-ray set can produce. Biological experiments and blood irradiation are hence unlikely to be viable with the L or N fields because of their relatively low dose rates, so the W or even H series are more likely to be appropriate replacements for the radionuclide fields. However, these substitute fields are far from monoenergetic.

ISO 4037-1:2019 only defines the highest energy X-ray fields for the H and N series, as H400 and N400, where the 400 refers to the 400 kV applied to the tube. To produce these fields a laboratory needs a bipolar X-ray tube which uses two insulators to avoid electrical breakdown. The mean energy of the N400 field is 328 keV, about half the energy of the ^{137}Cs gamma-ray. For the H400 field the mean energy is only 190 keV, but the dose rate is 150 times higher for the same current. ISO does not recommend a W400 field, but it can be inferred that this would

* The photoelectric absorption cross section falls rapidly above the “K-edge”, the energy of the K-shell X-rays, which generally increases with *Z*.

have a lower mean energy but perhaps twice the dose rate of the H400 field. This presents the essential difficulty for X-rays: attenuating the lower energy component to produce a field that is more like a gamma ray reduces the dose rate, thereby removing one of the key advantages of using X-rays for experiments that need a high dose rate.

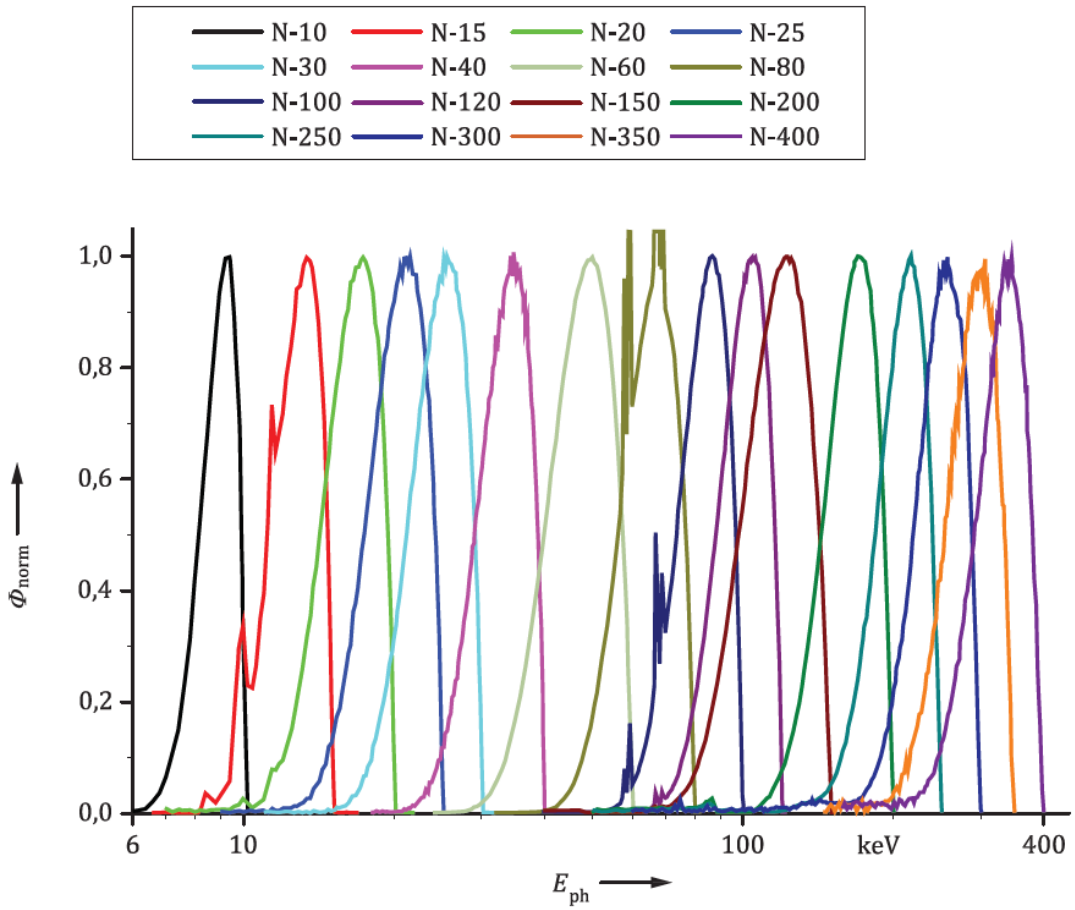

Figure 4. Narrow series X-ray fields, normalized to a peak fluence of 1, for the full range of kV values used in BS ISO 4037-1:2019 [ISO, 2019]

The practice of switching to X-rays from ^{137}Cs or ^{60}Co is established practice in calibration laboratories, because radiation protection instrumentation is designed to operate accurately and alarm at dose rates that relate to accidents and emergencies. Few calibration laboratories have radionuclide sources that can deliver the dose rates required at an appropriate distance from the source. It is hence necessary to switch to the H or W series from ISO 4037-1:2019, optimally at the highest available kV and current, so that high dose rates can be achieved. In such instrumentation, it is found that the calibrations using W300 or H300 are consistent with those performed using ^{137}Cs or ^{60}Co, though this is generally related to the energy deposition in silicon, a scintillator material or a gas, not biological material. However, because the highest energy available is generally used, the irradiations are at much higher energies than may be expected to show significant differences between the detector material and tissue.

The available X-ray fields have some significant differences from the radionuclide source fields that are currently used in biological experiments and blood irradiators:

- The photon energies are inevitably lower with maximum energies around 600 keV being the highest that are available.
- Most calibration or research laboratories do not have X-ray tubes that operate much above 300 kV, so the photon energies are not likely to be above 300 keV, with mean energies considerably lower.
- Filters that make the energy distribution more acceptable produce big reductions in the dose rate.

The essential question is then: does the difference in the energy distribution matter for the experiments and irradiations that are being conducted? For the highest tube potentials, even with relatively little filtration, most of the dose will be deposited by relatively long range secondary electrons, so the RBE is likely to be quite comparable to that for the radionuclide sources.

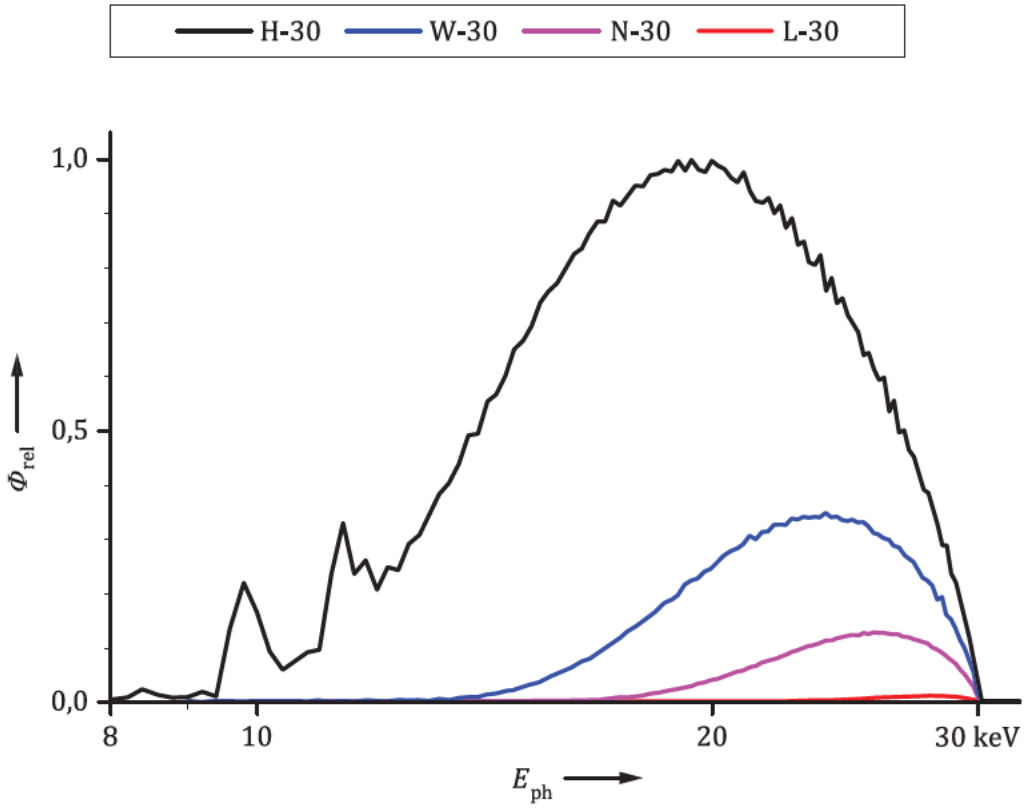

Figure 5. H, W, N and L series X-ray fields, unnormalized from BS ISO 4037-1:2019 [ISO, 2019]

3.3.1 Alternative X-ray irradiators

Reviews that have considered X-ray alternatives to radionuclide sources [MacKenzie et al., 2020, Murphy and Kamen, 2019] have looked at a wide range of experiments to determine the optimum X-ray alternatives to radionuclide sources. The factors considered include the energy distribution, dose rate and uniformity of the field, as well as the RBE determined by the experiment. The presence of low energy X-rays, which have a very shallow depth-dose curve, is clearly disadvantageous for small animal exposures because of the risk of skin burns. This

means filtration is required, but the high RBE of low energy photons [Nikjoo and Lindborg, 2010] also means that such fields would inevitably offer poor replacements for ^{137}Cs or ^{60}Co. However, the experiments surveyed [MacKenzie et al., 2020] that used ~ 300 kV fields, had HVLs from 1 mm to 4 mm of copper. This is a very significant range that indicates a lack of consistency in the filtration applied.

One review [Murphy and Kamen, 2019] looked specifically at three devices, the "RS2000 Series Biological Irradiator"*, "X-RAD 160 - Precision X-Ray"† and the "X-RAD 320 - Precision X-Ray"‡ . All of these devices are designed to irradiate biological samples, but the fields they generate do not conform to either the fields used in instrument and dosemeter calibration [ISO, 2019] or medical exposure [IEC, 2006] standards. Comparing the available fields for tube potentials around 160 kVp is difficult because of the incomplete information available (Table 1). The energy distributions are not readily available, and comparison between ideal calibration fields in low scatter facilities with an enclosed chamber is inevitably unreliable. However, the HVL for the field is perhaps the best comparator available.

Table 1. Specifications of the ISO 4037 instrument and dosemeter calibration fields, the IEC 61276 medical calibration fields and a typical X-ray calibration unit.

Field	Inherent filtration	Additional filtration	HVL
L170[a]	4 mm Al	1.5 mm Pb + 3 mm Sn + 1 mm Cu	3.4 mm Cu
N150[a]	4 mm Al	2.5 mm Sn	2.3 mm Cu
W150[a]	4 mm Al	1 mm Sn	1.78 mm Cu
H150[a]	4 mm Al	0.15 mm Sn	0.81 mm Cu
RQR10[b] (150 kV)	Not specified	2 mm Al	6.57 mm Al
RQA10[b] (150 kV)	Not specified	45 mm Al	13.3 mm Al
RQT10[b] (150 kV)	Not specified	0.3 mm Cu	10.1 mm Al
X-RAD 160[c]	0.8 mm Be	2 mm Al	Not specified
^{137}Cs[d]	n/a	n/a	34.5 mm Al or 10.7 mm Cu
^{60}Co[d]	n/a	n/a	46.5 mm Al or 14.8 mm Cu

a ISO 4037-1:2019
b IEC 31267:2006
c https://www.accela.eu/precision-x-ray/x-rad-160
d [Thoraeus, 1965]

The HVL can be specified in terms of any material, but in the case of the medical fields, which are all relatively lower energy, this is done in terms of aluminium, whereas the instrument/dosemeter fields are quoted mainly in terms of copper. Here the HVL for ^{137}Cs is

* https://www.radsource.com/wp-content/uploads/2016/06/RS2000_EN.pdf

† https://www.accela.eu/precision-x-ray/x-rad-160

‡ https://www.accela.eu/precision-x-ray/x-rad-320

seen to be much higher any of the X-ray fields, which indicates that a higher kVp would be preferable.

Comparison between the well characterized fields with tube potentials around 320 kV, shows that the HVLs are closer to that of ^{137}Cs (Table 2) than the ~160 kVp fields were. There are no medical fields in this table because such high energies are not used in imaging. One of the commercial systems quotes the HVL for their fields, which for lower dose rates is quite close to the H300 field. The W300 and N300 fields have higher HVLs, but lower dose rates because of the greater filtration.

Table 2. Specifications of the ISO 4037 instrument and dosemeter calibration fields, the IEC 61276 medical calibration fields and a typical X-ray calibration unit.

Field	Inherent filtration	Additional filtration	HVL
N300[a]	4 mm Al	5 mm Pb + 3 mm Sn	5.96 mm Cu
W300[a]	4 mm Al	6.5 mm Sn	5.03 mm Cu
H300[a]	4 mm Al	2.2 mm Cu	3.22 mm Cu
X-RAD 320[b] (3 Gy/min)	Not specified	Not specified	≈ 1 mm Cu
X-RAD 320[b] (1 Gy/min)	Not specified	Not specified	≈ 3 mm Cu
RS2000[c] (350 kV)	4 mm Be	Not specified	Not specified
^{137}Cs[d]	n/a	n/a	10.7 mm Cu
^{60}Co[d]	n/a	n/a	14.8 mm Cu

a ISO 4037-1:2019
b https://www.accela.eu/precision-x-ray/x-rad-320
c https://www.radsource.com/wp-content/uploads/2016/06/RS2000_EN.pdf
d [Thoraeus, 1965]

The X-ray fields that have been used in biological experiments are diverse, but the studies that used those fields were exposing very different samples and looking for different endpoints. The RBE values determined do not correlate very well with the HVL of the field used, which probably reflects the diversity of the experiments performed. However, it seems evident that the field used for reference RBE determinations needs to be close to ^{137}Cs in effect, so that the historical data can be used in comparison, which mitigates in favour of using fields for which the HVL approaches 10.7 mm of copper.

It can be difficult to decipher exactly what was done in a study if there are details missing from the description of the irradiation conditions. An example of this is the work of Gibson et al [Gibson et al., 2015a], which showed increased morbidity in mice exposed to 130 kVp X-rays when compared to mice exposed to an equivalent dose of ^{137}Cs gamma rays. This is an important study when considering a switch to X-rays instead of ^{137}Cs. It is, however, hard to work out what the cause of the different morbidity was for several reasons:

- The ^{137}Cs doses were delivered from top and bottom, whereas the X-ray doses were delivered from the top only.

- The ^{137}Cs dose rate was determined using Fricke dosemeters, which are presumed to be calibrated against ^{137}Cs, which will reduce potential bias, but there are many potential sources of uncertainty including traceability to national standards.
- The X-ray doses were measured using a "real-time dosimeter (model no. 2026c, Radcal, Monrovia, CA)", but the 2026c is not a dosemeter, it is a "radiation monitor controller" which uses an ionization chamber to measure the radiation dose. The ionization chamber is the crucial component here, but the model is not specified. Most likely it is accurate to within 5% down to some 10s of keV, but without the model being specified it is not possible to quantify the potential bias in the doses delivered.
- The "dose" is presumably absorbed dose to Fricke gel in the case of the ^{137}Cs and air kerma for the X-rays.
- The reference specifies that a "Rad Source 2000 Xray Biologic Irradiator" was used to generate the 130 kVp field but does not state what filtration was used, nor does it specify the half value layer for the field.
- The brochure* for this irradiator states that it has a 4 mm thick beryllium window, which will not remove much of the low energy X-ray component. If this inherent filtration is the only filtration used, then the HVL of the field will be a few mm of aluminium rather than the 10.7 mm of copper for a ^{137}Cs field. Consequently, a lot more dose will be delivered by low energy photons, which might explain the higher morbidity of the mice.

Whilst the morbidity data are unequivocal, it is not possible to dismiss X-rays as a substitute for ^{137}Cs based on this study. The difference is most likely caused by the X-rays used being a poor alternative to ^{137}Cs, since the X-ray field needs a higher kV and more filtration to better replicate ^{137}Cs.

Commercial animal irradiators that use higher kV are available, but there are pragmatic reasons for not using them. The price of the irradiators increases as the kV increases, as does the overall size of the irradiator. For example the Rad Source RS2000 irradiators used in the Gibson et al study [Gibson et al., 2015a] increase in mass by a factor of 4.5 in going from the 160 kV to the 350 kV model. Adaptation of existing facilities to accommodate a high kV irradiator may not be straightforward.

Table 3. Variation in size and mass for different kV specifications of the Rad Source RS2000 (Custom Built X-ray Irradiators with 450 kV or 600 kV are available)

kVp	160 kV	225 kV	350 kV
External dimensions	106.7 x 182.9 x 83.8cm	115.2 x 182.9 x 83.8cm	155.2 x 182.9 x 89.8cm
Mass	567kg	645kg	2560kg
Internal dimensions	43.2 x 68 x 38.1cm	43.2 x 68 x 38.1cm	52.5x 72 x 52.5cm

3.4 Linear accelerator fields

The photon fields generated by linear accelerators (linacs) are the same in their method of production as the photons from an X-ray tube, the difference being that they can be generated with much higher energies. Medical linacs operate at energies up to 25 MeV or even 30 MeV,

* http://www.radsource.com/small-animal-irradiation/?gclid=CjwKCAjw5vz2BRAtEiwAbcVILy8nhqBr6EDHcn80NWpWZ2q3CB_95EM8Ivw4kALA81YsltiQ3Rz9NBoCtdYQAvD_BwE

though the clinical benefit of these highest energies is no longer evident, so such energies are rarely used now. The benefit of very high energies for the irradiation of small animals or blood samples is not clear and it would generate very significant shielding issues, stray neutron fields and cost an excessive amount of money. Consequently, for this review, only lower energy linac fields are considered.

Work using a 3.5 MV linac [Bordy et al., 2019] has produced a field with a broad energy distribution with a mean energy of 640 keV using an accelerating potential of 3 MV. The field contains fluence from about 50 keV to 3 MeV, but the full width half-maximum (FWHM) ranges from about 80 keV to 2 MeV. Such fields are highly tuneable, but it is not possible to achieve much reduction in the FWHM using filters, because the energy of the photons is so high compared to the K-edge of possible filter materials.

The linac facility that has been built at ATRON METROLOGY, Cherborg en Cotentin, France [Dusciac et al., 2016, Bordy et al., 2019] has been designed to offer a close approximation to a ^{137}Cs source using bremsstrahlung photons, but it has been designed for instrument and dosemeter calibration. It is based on a 3.5 MV Singletron accelerator*, and is housed in a relatively large facility: the room housing the accelerator is approximately 11.5 m long by 6.5 m wide, with concrete walls more than 1.5 m thick. The beam is extracted into a separate room 6.7 m long by 3 m wide, which is also shielded by thick concrete. The beam generated is horizontal, whereas biological experiments are likely to require a vertical beam, which would require a design more like a medical linac with bending magnets to change the direction of the electron beam.

The thick shielding is required because, although the mean photon energy is close to that of ^{137}Cs, the photon energy distribution extends up to 3 MeV. To install a linac like this to replace a ^{137}Cs source would hence be likely to require a new building or extensive modifications to an existing facility. It is difficult to estimate the cost, but it would inevitably be much more expensive than an X-ray tube system, if a laboratory had the space available to install a linac.

Whilst linacs are quite attractive as a replacement for ^{137}Cs, because they can be used to generate a field with the same mean energy, the added cost of building the facility and shielding it are likely to leave X-ray tubes as a more attractive and practical option. However, if a facility exists already, using it for biological experiments is a realistic proposal, though it is hard to envisage it being practical to set up a blood irradiator using this technology.

3.5 Summary

The discussion above provides an overview of the characteristics of the radiation fields generated by gamma sources and X-ray sources. There are differences between the fields, but with appropriate attention to the filtration of X-ray sources they can very often be seen as suitable alternatives to ^{137}Cs. When considering switching from ^{137}Cs to X-rays, investigators should consider carefully the size and geometry of the sample to be irradiated as this will inform the choice of a suitable alternative. In many cases it would be prudent for investigators to undertake comparative studies before adoption of the X-ray alternative. Linac fields can be

* http://www.highvolteng.com/media/Brochures/Singletron_broch.pdf

more similar to ^{137}Cs fields but routine use in research labs or within blood transfusion services is unlikely due to high cost and requirements for shielding.

4 ALTERNATIVES TO CAESIUM IRRADIATION FOR SPECIFIC PURPOSES

The US Department of Homeland Security has recently published a comprehensive analysis of the alternatives to the use of caesium irradiators in all sectors*. This provides a valuable resource to all that are considering moving away from the use of caesium irradiators. The report concludes that there exist alternatives to caesium irradiation in the blood transfusion and biological research sectors. In this chapter, we aim to describe potential alternatives in these sectors and consider some specific applications and the available alternatives

Outside of the UK, experience with the replacement of caesium irradiators with alternatives is available in France, Norway and Japan. As also noted in the Introduction, the US is currently engaged in activities to seek alternatives as exemplified by the Department of Homeland Security report. More specifically there is well documented experience in certain US institutes. The University of California owned 42 ^{137}Cs irradiators for a wide range of purposes such as treating blood, exposing cells and small animals. A recently published report from the university identified 88% of their irradiators could be removed and replaced with X-ray irradiators [MacKenzie et al., 2020]. The report identified that 320 or 220 kVp X-irradiators make suitable replacements during animal exposures, and low 160 kVp X-irradiators being comparable to ^{137}Cs for *in vitro* cell exposures [Murphy and Kamen, 2019]. Several considerations must be taken into account when replacing a ^{137}Cs irradiator with a suitable X-irradiator; as discussed, RBE values can have a wider range in evaluations using X-rays depending on the energy used in comparison. However, achieving a comparable RBE value is possible with a ^{137}Cs reference [MacKenzie et al., 2020] and knowing the desired depth dose will also help identify the correct X-irradiator (and setup) required [Murphy and Kamen, 2019].

4.1 Human blood transfusion

Gamma-irradiation appears to be the most commonly used form of irradiation, although X-irradiation and pathogen inactivation technologies are becoming increasingly used alternatives to prevent TA-GvHD during transfusion in humans. The prevalence of gamma- over X-irradiations is most likely due to a lack of incentive to use an alternative due to economic or practical reasons rather than a biological advantage of one over the other.

In many cases, X-irradiators are an appropriate alternative to ^{137}Cs irradiators [Dodd and Vetter, 2009]. Whilst the energy of X-irradiators is different to that of ^{137}Cs, the former is more than capable of delivering the 25 Gy dose required with uniformity and stability [Dodd and Vetter, 2009] and with no clinically significant adverse effects [Janatpour et al., 2005]. A comparison of X- and ^{137}Cs irradiators was recently performed in a UK NHS Blood and Transplant unit, where the effects of both were analysed on the quality of red cell concentrates (RCC), to measure the effectiveness of each approach [Bashir et al., 2011]. Blood units for

* https://www.cisa.gov/publication/non-radioisotopic-alternative-technologies-white-paper

intrauterine or neonatal exchange transfusions were analysed and in terms of RCC quality, acceptable levels of haemolysis and potassium leakage were achieved compared to current gamma-irradiation results. X-irradiation of blood and blood products is now in routine use across England [Bashir et al., 2011]. A comparable study in the US reached similar conclusions, with red blood cell permeability being the measure of effectiveness of irradiation. Non-clinically important differences were observed, indicating that X-irradiation is an appropriate alternative to a gamma irradiation [Janatpour et al., 2005].

In 2019, the Component Development Laboratory, NHS Blood and Transplant (NHSBT), Cambridge, UK, performed controlled paired studies to compare the effect of either gamma- or X-irradiation on the storage quality of red cell blood components (publication in preparation). The investigators used adult-size red cell concentrates and paediatric-size red cells in saline adenine glucose mannitol, as well as intrauterine transfusion and neonatal exchange transfusion red cells in citrate phosphate dextrose plasma. All components were irradiated at 25-50Gy at the latest point in storage, while paediatric top-up red cells were also irradiated early in storage. Components were tested for quality parameters over storage until the end of shelf life including haemolysis, supernatant potassium, lactate, adenosine triphosphate and 2,3-diphosphoglycerate. Each parameter was analysed for equivalence between paired components that were either gamma- or X-irradiated. Despite differences identified that suggested slight worsening of red cell quality following X-irradiation compared with gamma-irradiation, these were not considered clinically significant; therefore, X-irradiated red cell components studied here were of acceptable storage quality. [Dr Athinoula Meli, NHS Blood and Transplant, personal communication]

As an alternative to irradiation, UV systems have been trialled in the US for blood pathogen reduction or inactivation [Schubert et al., 2018, Seghatchian and Tolksdorf, 2012] during platelet transfusion. FDA approval for the use of one system with red blood cells and whole blood is currently pending in the US.

A recent non-irradiation development in transfusion services is pathogen inactivation for platelet concentrates. Multiple techniques are available to perform the inactivation including amotosalen and UVA, riboflavin and UVA/B and using UVC light, each method uses a photosensitiser and/or UV light [Feys et al., 2019]. The principle here aims to decrease the chances of infectious disease transmission from the host to the donor. In addition, the Pathogen Inactivation of Platelets 2014 report of the advisory committee on the Safety of Blood Tissues and Organs working group, concluded that the pathogen inactivation technologies reviewed can replace irradiation of platelets for the prevention of transfusion-associated graft-versus-host disease. (SaBTO, 2014, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/324354/SaBTO_platelets_report.pdf).

Currently such techniques are not recommended for blood for transfusion as not all components can be successfully treated, but these techniques may become feasible in the not too distant future with successful further development. In fact, gamma-radiation has been reported as an ineffective alternative to non-irradiation pathogen inactivation [Bello-López et al., 2016].

4.2 Animal irradiations

Animals are irradiated for a variety of purposes, studying the effects of radiation through to ablation techniques for transplantation studies. Mice are the most commonly used species in such studies, although a range of species are used experimentally, from zebrafish [Praveen Kumar et al., 2017] through to primates such as baboons [Port et al., 2018]. The standardisation of animal irradiation when using X-irradiators has led to the design of an X-ray dosimetry protocol by the European Late Effects Project Group (EULEP) to improve the comparison of results achieved by different institutes [Zoetelief et al., 2001]. Such guidance makes the use of X-irradiators over gamma-irradiators both desirable and accessible and reduces the need for extensive dosimetry that would be continually needed when using gamma-radiation.

A number of specifically designed small animal X-irradiators are commercially available. X-irradiators generally deliver a uniform and homogenous dose for animal exposures and are a favourable alternative in several experimental approaches. However, restrained vs. free moving mice will be an important factor to consider when performing the dosimetry. Permitted mouse restraint procedures in the UK are largely dependent upon individual Home Office licence requirements for the use of animals for experimental purposes. This is unlikely to restrict the use of an X-ray alternative to ^{137}Cs.

A recent study comparing both ^{137}Cs and X-ray irradiators during mouse bone marrow ablation demonstrated that either source was fairly comparable to peripheral blood reconstitution following bone marrow ablation. However, the researchers highlight some distinct physiological differences in response between the two that would need consideration when choosing the optimal source to be employed [Gibson et al., 2015b]. Significant variation was observed between B, T, myeloid cell reconstitution between the two sources as were differential effects on morbidity, which would need careful consideration when replacing the ^{137}Cs irradiation source during such studies (see also Section 3.3.1).

In the investigation into treatment for haemophilia A, mice have traditionally been total body irradiated (TBI) using ^{137}Cs as a preconditioning of hematopoietic stem cells, with some undesired cytotoxic effects associated. Recently, a non-genotoxic alternative preconditioning technique has been demonstrated to be an effective and favourable replacement to irradiation [Gao et al., 2019]. The technique utilised within the study involved CD45.2- and CD117-targeting cell-specific antibody-drug conjugate (ADC) preconditioning, which effectively allows engrafting of haematopoietic stem cells, resulting in successful platelet lineage reconstitution in haemophilia A mice. Similarly, combatting the genotoxic effect of irradiation and chemotherapy appears to be a driver for alternative techniques during transplantation. Another non-genotoxic conditioning treatment trialled in mice involves the immunosuppression and selective depletion of recipient hematopoietic stem cells again using a CD117-ADC [Li et al., 2019]. The results were promising, with only a small elevation in liver enzymes reported during the first week post-conditioning, presenting an alternative to irradiation for immunosuppression.

Bone marrow transplantation is a very promising treatment approach with the possibility of improving health in a number of diseases and also greater success in solid organ transplantation [Xu et al., 2019]. Bone marrow transplantation research is heavily reliant on *in vivo* experimentation chiefly using mice [Duran-Struuck and Dysko, 2009], prior to human practice. Such studies have been demonstrating a number of irradiation and ablation

alternative approaches. One example is the recent use of busulfan conditioning [Peake et al., 2015]. Avoiding myeloablation, this conditioning method uses the chemotherapeutic agent busulfan which has been reported to be well tolerated by mice when administered by intraperitoneal injection. This approach does not require donor cell support for successful engraftment or survival [Peake et al., 2015] and eliminates the need for specialised irradiation facilities used for animal exposures. This provides a potentially invaluable tool for studying the hematopoietic system, TA-GvHD and a range of immunobiology issues.

Bone marrow transplantation can be avoided by stimulating the release of haematopoietic stem cells (HSC) into the peripheral blood (referred to as mobilised peripheral blood HSC), a method that has the benefits of rapid engraftment reducing infection incidence [Xu et al., 2019, Dey et al., 2007]. This approach has been successfully performed in mice using AMD3100 as a rapid mobilising agent releasing HSC into the blood of B6 mice. AMD3100 was co-administered with Flt3 ligand (FL) and granulocyte colony-stimulating factor (GCSF), referred to as FL/GCSF/AMD3100. The blood from the B6 was then extracted and transplanted into recipient BALB/c mice conditioned by both ablative and non-ablative approaches. No engrafted mice were observed to suffer from TA-GvHD, therefore the use of non-myeloablative conditioning during this mobilised HSC approach represents a promising alternative to irradiation if the principle is also successfully adapted to human patients [Xu et al., 2019].

A recently investigated alternative technique in mice involved the pharmacological inhibition of plasminogen activator inhibitor-1 (PAI-1) activity and was demonstrated as an effective myeloablative conditioning alternative.

Hematopoietic stem cell transplantation (HSCT) is an important therapy for patients with hemoglobinopathies, congenital immunodeficiency and several similar conditions. However, conditioning with irradiation can cause implications due to genotoxicity. To avoid this, and reduce TA-GvHD, these cells can be conditioned by targeting CD45 in a non-genotoxic way [Palchaudhuri et al., 2016]. Immunocompetent mice have successfully been conditioned using this method resulting in successful engraftment of donor cells, and it has been found more effective than irradiation conditioning with fewer undesired effects.

In most experimental studies of bone marrow/HSCT transplantation an excess of donor bone marrow/HSCs is transplanted, in some situations however very limited numbers of cells are transplanted in order to identify rare cells that have particularly high repopulating capacity, these assays are generally known as limiting dilution assays (Ivanovs et al, 2008; Azzoni et al, 2018; Zhang et al, 2019, 2020). From the available literature, it is apparent that there is a preference for the use of ^{137}Cs (and less commonly ^{60}Co) for ablation of host bone marrow prior to transplantation. While in principle other methods, including X-irradiation should be suitable for use on such procedures, work to establish these as acceptable alternatives will be required.

The use of proton therapy is increasing in medical facilities, and this in turn has triggered a slight increase in RBE related research to further understand and improve the effectiveness of this treatment. It is envisaged that some access to gamma-irradiators would be needed to continue these investigations to allow appropriate comparisons between radiation sources. Therefore, there may be a continued need in research to retain some ^{137}Cs (or ^{60}Co) irradiation sources in order to carry out further research and improve knowledge about the health effects of radiation [Gibson et al., 2015b].

Most of the studies and situations discussed here require relatively acute exposures, but there may be occasions when chronic or protracted exposures to radiation are required. X-irradiators will likely be suitable for this purpose, with some planning. For example, X-irradiators may be prone to overheating when used at maximum voltage and current for prolonged periods. However, such practical issues will be location and environment specific; the availability of air-conditioning, the desired dose etc. High-energy X-ray tubes, which are commercially available, are also a possibility for higher dose or dose-rate exposures and may be considered if such exposures are desired. If very high dose-rates are needed for a purpose, higher than feasibly possible with an X-irradiator, it is likely the researcher or medical facility would consider electron fields generated by a linear accelerator (linac) rather than pursue high-energy X-rays.

4.3 Cell culture

The type and structure of cell and tissue culture *in vitro* will influence the type and delivery of ionising radiation needed to irradiate them. Traditional 2D cell culture means that the cells are typically grown in a single layer within a flask or dish, making the dosimetry during irradiation straightforward. Increasingly, 3D cell culture, including spheroids, allow cells to be sustained or grown over more complex matrixes of material and demonstrate several benefits over 2D culture in simulating important cellular characteristics relevant to *in vivo* environments [Imamura et al., 2015, Liu and Chen, 2018]. This allows more cells to be supported within a given area as well as being more physiologically relevant when trying to mimic *in vivo* cell and tissue environments during *in vitro* drug testing and investigation of radiation responses etc. As a result, 3D cell culture would require depth dose to be considered during exposure to ionising radiation to ensure all cells receive a similar and accurate dose. There are no obvious limitations regarding the use of X- over gamma-radiation and vice versa. However, the field of feeder cell production and subsequent growth arrest seems to be moving towards non-radiation techniques, that will also be covered within this section.

Exposure of feeder cells to gamma-radiation successfully inhibits proliferation, but also has been reported to have some negative effects such as inducing undesired cell apoptosis affecting the secretion of the soluble factors produced by the cells needed to ‘feed’ stem cell expansion [Villa-Diaz et al., 2013]. One of the most commonly used alternatives to gamma-irradiation in feeder cell inactivation is not X-radiation, but the antitumoral antibiotic mitomycin-C (MMC), which has clearly been demonstrated to be as effective as gamma-irradiation [Ponchio et al., 2000] and is increasingly applied during feeder cell production. MMC can treat less cells than treatment with gamma-irradiation in relative terms, due to the requirement of an incubation period during exposure which can last from three hours to overnight [Ponchio et al., 2000], but MMC treatment appears to successfully inhibit mouse embryotic fibroblast (MEF) growth just as effectively. MMC is readily available and at a considerably lower cost (compared to irradiations, particularly caesium and cobalt gamma sources) and less time consuming [Llames et al., 2015, Ponchio et al., 2000, Chugh et al., 2015]. This alternative to irradiation, coupled with three-dimensional suspension method (3DSM) culture, could provide a highly economic and large-scale feeder cell production method to replace existing gamma irradiation-driven techniques [Li et al., 2017]. MMC has been demonstrated to cause no adverse effects when the target cells are hematopoietic stem cells [Ponchio et al., 2000]. An early comparison between MMC and gamma-irradiation treated feeder cells suggested a

slightly better efficiency of radiation exposed cells in sustaining target cell expansion; however, this advantage may be cell type specific. MMC treatment represents a viable and cost-effective alternative to gamma-irradiation, however the benefit of the alternative is likely to be cell (feeder or target) dependant. Therefore, it is recommended that scientists perform similar comparisons using their cells of interest when considering MMC as an alternative method to irradiation. In general, both gamma-irradiation and MMC treatments are considered equivalent, achieving the same desired outcome only by different mechanisms [Llames et al., 2015].

In the absence of a gamma-radiation source, feeder cells can also be produced via X-irradiation. Recently a comparison between X- and gamma-radiation on K562 cells alongside IL-2 and IL-15 supplementation was performed for use in natural killer cell expansion (used in anti-tumour immunotherapy studies). The study suggested no significant difference between the two radiation types [Kim et al., 2018].

Other novel treatments are less commonly used but are also reported to be effective under certain conditions where gamma-irradiation or MMC treatment are not possible. Ultrashort electric pulses have been demonstrated to penetrate cells and induce intracellular responses without affecting cell viability [Llames et al., 2015, Schoenbach et al., 2004]. This method has been recently demonstrated to prepare good quality feeder cells for culture [Browning et al., 2010]. Chemically fixed feeder cells have also been reported to effectively support the growth of stem cells incorporating very mild treatment with glutaraldehyde or formaldehyde which induces growth arrest and protein immobilisation, inhibiting proliferation whilst maintaining cell viability [Ito et al., 2006]. This method has the added benefit of allowing the feeder cells to be easily stored and used at a later date, which would be a benefit during *in vitro* research where there is a reduced throughput of production needed [Ito et al., 2006, Llames et al., 2015].

In certain situations, the use of feeder cells can be avoided altogether by using conditioned medium to achieve the same induction of proliferation of target cells [Llames et al., 2015]. Haematopoietic stem cells have recently been transplanted from one mouse to another without the need for preconditioning at all, due to the development of an albumin-free culture system that successfully supported long term expansion of function stem cells and engrafted into the donor animal with reduced toxic effects [Wilkinson et al., 2019]. This approach has important implications for future haematopoietic stem cell transplantation therapies in humans, removing the need for preconditioning with radiation completely.

It is noteworthy that the role of feeder cells in co-culture techniques is envisaged to be replaced by synthetic feeder cell substitutes, however this alternative replacement is unlikely to be available in the short term [Llames et al., 2015].

As noted in the previous section on animal irradiation, there are particular considerations when chronic irradiation is required, with ^{137}Cs being the current standard. With modification, it seems likely that X-irradiation can provide a suitable, stable and safe alternative.

5 DISCUSSION AND SUMMARY

Techniques involving ^{137}Cs irradiators are in use in the UK biological sciences and blood transfusion services sectors. Given global concerns on the potential malevolent use of ^{137}Cs,

there are sound reasons for considering alternatives technologies to replace ^{137}Cs irradiators. This report identifies several current uses, including:

- Irradiation of blood and blood products to avoid transfusion-associated graft-versus- host disease
- Irradiation of experimental animals to facilitate bone marrow transplantation for research purposes
- Irradiation of experimental animals as models for studies of radiation-associated disease in humans and in investigations of the impacts of radiation on the environment
- Irradiation of plant materials for a limited range of agricultural and botanical sciences applications
- Irradiation of cells cultured *in vitro* to provide 'feeder cells' to support the growth of particular cell types
- Irradiation of cells cultured *in vitro* for radiobiological investigations

Particular advantages to the use of ^{137}Cs include the stability of the radiation field and the well characterised nature of the gamma radiation produced. ^{137}Cs has a long half-life meaning that the sources can be used over many years without the need for replacement. By comparison X-ray fields are more complex, and can be less stable due to the operating characteristics of X-ray tubes. Nonetheless X-irradiation can frequently provide a suitable alternative to ^{137}Cs irradiation for most applications.

The well-defined nature of radionuclide fields has advantages in terms of consistency. Suppliers of commercially available biological X-irradiators will need to provide detailed information on the field characteristics or allow the user to tailor those field characteristics. The use of higher tube potentials provides better comparison with historical data that use ^{137}Cs or ^{60}Co, especially if high-*Z* filters such as lead are used, but there is a corresponding loss of dose rate, which could be problematic for blood irradiation and for some experiments. Consistency will be lost if laboratories all choose different X-ray fields for their experiments.

Linac generated photon fields have been shown to better replicate ^{137}Cs than is possible with X-ray tubes, but there will be greater difficulties replacing existing irradiators with this technology. The cost of the accelerator will be much higher. The space and shielding requirements are likely to be impossible to accommodate in most laboratories. The scientific benefits of the field relative to a field generated with a high potential X-ray tube are likely to be marginal and not justified by the additional cost.

For some applications, fully non-radiation alternatives are identified, notably for uses in generating feeder cells and the ablation of bone marrow in experimental animals prior to bone marrow transplantation for research purposes. Only few situations were identified where it is not currently clear if X-irradiation or other alternatives could be suitable – (i) limiting dilution repopulation of ablated mouse bone marrow with haematopoietic stem cells, and (ii) studies of the relative effectiveness of ^{137}Cs with other radiation types in studies of newer radiotherapy techniques and for determining the health impacts of ^{137}Cs. When an X-ray or other alternatives are being considered it is advisable for the researchers to conduct preliminary experiments to ensure the comparability of the methods and ensure results are compatible. A decision tree is provided to help users and researchers identify suitable alternatives to their current uses of ^{137}Cs irradiation. The maintenance of a limited number of ^{137}Cs sources for

studies of the relative health impact of this radiation source, which is an important constituent of some accidental releases, is considered appropriate.

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7 REFERENCES

ASAITHAMBY, A. & CHEN, D. J. 2009. Cellular responses to DNA double-strand breaks after low-dose gamma-irradiation. *Nucleic Acids Res,* 37**,** 3912-3923.

ASTM, I. 2016. Standard Practice for Blood Irradiation Dosimetry. West Conshohocken, PA: ASTM International.

AZZONI, E., FRONTERA, V., MCGRATH, K. E., HARMAN, J., CARRELHA, J., NERLOV, C., PALIS, J., JACOBSEN, S. E. W. & DE BRUIJN, M. F. 2018. Kit ligand has a critical role in mouse yolk sac and aorta-gonad-mesonephros hematopoiesis. *EMBO Rep,* 19.

BAKKEN, E., CARY, K., DERRICK, A., HILDEBRAND, H., SCHROECKENTHALER, K., TAALBI, M. 2013. Cost-benefit analysis of switching from cesium-chloride to x-ray blood irradiators.

BAKTHAVACHALU, B., KALANKE, S., GALANDE, S., RAMANAMURTHY, B., PARAB, P., KOHALE, K. N. & SESHADRI, V. 2010. Dense cataract and microphthalmia (dcm) in BALB/c mice is caused by mutations in the GJA8 locus. *J Genet,* 89**,** 147-154.

BARNARD, S., BOUFFLER, S. & ROTHKAMM, K. 2013. The shape of the radiation dose response for DNA double-strand break induction and repair. *Genome Integr,* 4**,** 1.

BASHIR, S., NAIK, F., CARDIGAN, R. & THOMAS, S. 2011. Effect of X-irradiation on the quality of red cell concentrates. *Vox Sang,* 101**,** 200-207.

BELLO-LÓPEZ, J. M., HERNÁNDEZ-RODRÍGUEZ, F. & ROJO-MEDINA, J. 2016. Bactericidal effect of γ-radiation with (137)Cesium in platelet concentrates. *Transfus Apher Sci,* 55**,** 347-352.

BIENIAWSKI, A., ILIOPULOS, I. & NALBANDIANM, M. 2017. Case Study: An Analysis of Several National Approaches to Alternative Technologies for Radioactive Sources. *58th Instritute of Nuclear Materials Management (INMM) Annual Meeting.* Indian Wells, California.

BORDY, J.-M., BLIDEANU, V., CHAPON, A., DUPONT, G., DUSCIAC, D., GOURIOU, J., LAINE, F. & LE ROY, M. 2019. Primary reference in terms of air kerma for ionizing radiation fields produced by an electrostatic electron accelerator. *International Congress of Metrology***,** 15003.

BRODIN, N. P., CHEN, Y., YAPARPALVI, R., GUHA, C. & TOME, W. A. 2016. Dosimetry Formalism and Implementation of a Homogenous Irradiation Protocol to Improve the Accuracy of Small Animal Whole-Body Irradiation Using a 137Cs Irradiator. *Health Phys,* 110**,** S26-38.

BROWNING, L. M., HUANG, T. & XU, X. H. 2010. Electric pulses to prepare feeder cells for sustaining and culturing of undifferentiated embryonic stem cells. *Biotechnol J,* 5**,** 588-595.

CHUGH, R. M., CHATURVEDI, M. & YERNENI, L. K. 2015. An evaluation of the choice of feeder cell growth arrest for the production of cultured epidermis. *Burns,* 41**,** 1788-1795.

DAME, D. A., CURTIS, C. F., BENEDICT, M. Q., ROBINSON, A. S. & KNOLS, B. G. 2009. Historical applications of induced sterilisation in field populations of mosquitoes. *Malar J,* 8 Suppl 2**,** S2.

DEY, B. R., SHAFFER, J., YEE, A. J., MCAFEE, S., CARON, M., POWER, K., TING, D. T., COLBY, C., PREFFER, F., BALLEN, K., ATTAR, E., SAIDMAN, S., TARBELL, N., SACHS, D., SYKES, M. & SPITZER, T. R. 2007. Comparison of outcomes after transplantation of peripheral blood stem cells versus bone marrow following an identical nonmyeloablative conditioning regimen. *Bone Marrow Transplant,* 40**,** 19-27.

DODD, B. & VETTER, R. J. 2009. Replacement of 137Cs irradiators with x-ray irradiators. *Health Phys,* 96**,** S27-30.

DURAN-STRUUCK, R. & DYSKO, R. C. 2009. Principles of bone marrow transplantation (BMT): providing optimal veterinary and husbandry care to irradiated mice in BMT studies. *J Am Assoc Lab Anim Sci,* 48**,** 11-22.

DURAN-STRUUCK, R., HARTIGAN, A., CLOUTHIER, S. G., DYSON, M. C., LOWLER, K., GATZA, E., TAWARA, I., TOUBAI, T., WEISIGER, E., HUGUNIN, K., REDDY, P. & WILKINSON, J. E. 2008. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptprca mice to commensal bacteria after whole body irradiation in translational bone marrow transplant studies. *J Transl Med,* 6**,** 10.

DUSCIAC, D., BORDY, J.-M., DAURES, J. & BLIDEANU, V. 2016. High energy photon reference for radiation protection: technical design of the LINAC beam and ionization chambers; and calculation of monoenergetic conversion coefficients. *EPJ Web Conf.,* 124**,** 00004.

DZIEDZIC-GOCLAWSKA, A., KAMINSKI, A., UHRYNOWSKA-TYSZKIEWICZ, I. & STACHOWICZ, W. 2005. Irradiation as a safety procedure in tissue banking. *Cell Tissue Bank,* 6**,** 201-219.

FEYS, H. B., VAN AELST, B. & COMPERNOLLE, V. 2019. Biomolecular Consequences of Platelet Pathogen Inactivation Methods. *Transfus Med Rev,* 33**,** 29-34.

FOLLETT, P. A. & SNOOK, K. 2012. Irradiation for Quarantine Control of the Invasive Light Brown Apple Moth (Lepidoptera: Tortricidae) and a Generic Dose for Tortricid Eggs and Larvae. *Journal of Economic Entomology,* 105**,** 1971-1978.

GAO, C., SCHROEDER, J. A., XUE, F., JING, W., CAI, Y., SCHECK, A., SUBRAMANIAM, S., RAO, S., WEILER, H., CZECHOWICZ, A. & SHI, Q. 2019. Nongenotoxic antibody-drug conjugate conditioning enables safe and effective platelet gene therapy of hemophilia A mice. *Blood Adv,* 3**,** 2700-2711.

GIBSON, B. W., BOLES, N. C., SOUROULLAS, G. P., HERRON, A. J., FRALEY, J. K., SCHWIEBERT, R. S., SHARP, J. J. & GOODELL, M. A. 2015a. Comparison of Cesium-137 and X-ray irradiators by using bone marrow transplant reconstitution in C57BL/6J mice. *Comparative medicine,* 65**,** 165-172.

GIBSON, B. W., BOLES, N. C., SOUROULLAS, G. P., HERRON, A. J., FRALEY, J. K., SCHWIEBERT, R. S., SHARP, J. J. & GOODELL, M. A. 2015b. Comparison of Cesium-137 and X-ray Irradiators by Using Bone Marrow Transplant Reconstitution in C57BL/6J Mice. *Comp Med,* 65**,** 165-172.

HALL, E. J. 1973. *Radiobiology for the radiologist*, Hagerstown, Md. : Medical Dept., Harper & Row, [1973] ©1973.

HALLMAN, G. J. & HELLMICH, R. L. 2009. Ionizing radiation as a phytosanitary treatment against European corn borer (Lepidoptera: Crambidae) in ambient, low oxygen, and cold conditions. *J Econ Entomol,* 102**,** 64-68.

HAMMAD, A., GABARTY, A. & ZINHOUM, R. A. 2020. Assessment irradiation effects on different development stages of Callosobruchus maculatus and on chemical, physical and microbiological quality of cowpea seeds. *Bull Entomol Res***,** 1-9.

HERTEL-AAS, T., BRUNBORG, G., JAWORSKA, A., SALBU, B. & OUGHTON, D. H. 2011. Effects of different gamma exposure regimes on reproduction in the earthworm Eisenia fetida (Oligochaeta). *Sci Total Environ,* 412-413**,** 138-147.

HERTEL-AAS, T., OUGHTON, D. H., JAWORSKA, A., BJERKE, H., SALBU, B. & BRUNBORG, G. 2007. Effects of chronic gamma irradiation on reproduction in the earthworm Eisenia fetida (Oligochaeta). *Radiat Res,* 168**,** 515-526.

HUBBELL, J. H. & SELTZER, S. M. 1995. Tables of X-ray mass attenuation coefficients and mass energy-absorption coefficients 1 keV to 20 MeV for elements Z= 1 to 92 and 48 additional substances of dosimetric interest. National Inst. of Standards and Technology-PL, Gaithersburg, MD (United ….

IAEA 1988. The Radiological Accident in Goiana. Vienna: IAEA.

IAEA 2003. Categorization of Radionuclide Sources. Vienna: IAEA.

IBRAHIM, A. A., YAHATA, T., MUGURUMA, Y., MIYATA, T. & ANDO, K. 2019. Blockade of plasminogen activator inhibitor-1 empties bone marrow niche sufficient for donor hematopoietic stem cell engraftment without myeloablative conditioning. *Biochem Biophys Res Commun,* 516**,** 500-505.

ICRP 2010. Conversion Coefficients for Radiological Protection Quantities for External Radiation Exposures. Publication 116. . *Ann. ICRP* 40.

ICRU 1998. Conversion Coefficients for use in Radiological Protection Against External Radiations. ICRU Report 57. Bethesda, Maryland: ICRU.

IEC 2006. Medical diagnostic X-ray equipment — Radiation conditions for use in the determination of characteristics. BS EN 61267:2006. *Geneva: IEC.*

IMAMURA, Y., MUKOHARA, T., SHIMONO, Y., FUNAKOSHI, Y., CHAYAHARA, N., TOYODA, M., KIYOTA, N., TAKAO, S., KONO, S., NAKATSURA, T. & MINAMI, H. 2015. Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. *Oncol Rep,* 33**,** 1837-1843.

ISO 2017. Practice for blood irradiation dosimetry. Geneva: International Organization for Standardization. ISO/ASTM 51939:2017

ISO 2019. Radiological protection — X and gamma reference radiation for calibrating dosemeters and doserate meters and for determining their response as a function of photon energy — Part 1: Radiation characteristics and production methods. Geneva: International Organization for Standardization.

ITO, Y., HASAUDA, H., KITAJIMA, T. & KIYONO, T. 2006. Ex vivo expansion of human cord blood hematopoietic progenitor cells using glutaraldehyde-fixed human bone marrow stromal cells. *J Biosci Bioeng,* 102**,** 467-469.

IVANOVS, A., RYBTSOV, S., WELCH, L., ANDERSON, R. A., TURNER, M. L. & MEDVINSKY, A. 2011. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. *J Exp Med,* 208**,** 2417-2427.

JANATPOUR, K., DENNING, L., NELSON, K., BETLACH, B., MACKENZIE, M. & HOLLAND, P. 2005. Comparison of X-ray vs. gamma irradiation of CPDA-1 red cells. *Vox Sang,* 89**,** 215-219.

KALLMAN, R. F. 1962. The effect of dose rate on mode of acute radiation death of C57BL and BALB/c mice. *Radiat Res,* 16**,** 796-810.

KAMEN, J., HSU, W. Y., BOSWELL, B. & HILL, C. 2019. Successful Migration from Radioactive Irradiators to X-ray Irradiators in One of the Largest Medical Centers in the US. *Health Phys,* 117**,** 558-570.

KIM, G. H., DANG, H. N., PHAN, M. T., KWEON, S. H., CHUN, S. & CHO, D. 2018. X-ray as Irradiation Alternative for K562 Feeder Cell Inactivation in Human Natural Killer Cell Expansion. *Anticancer Res,* 38**,** 5767-5772.

KIM, J., CHUNG, S. O., JANG, M., JANG, S. A. & PARK, C. G. 2015. Developmental inhibition and DNA damage of Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) by gamma radiation. *Int J Radiat Biol,* 91**,** 827-832.

LEMOINE, F. M., HUMPHRIES, R. K., ABRAHAM, S. D., KRYSTAL, G. & EAVES, C. J. 1988. Partial characterization of a novel stromal cell-derived pre-B-cell growth factor active on normal and immortalized pre-B cells. *Exp Hematol,* 16**,** 718-726.

LI, P., WANG, S., ZHAN, L., HE, X., CHI, G., LV, S., XU, Z., XIA, Y., TENG, S., LI, L. & LI, Y. 2017. Efficient feeder cells preparation system for large-scale preparation and application of induced pluripotent stem cells. *Sci Rep,* 7**,** 12266.

LI, Z., CZECHOWICZ, A., SCHECK, A., ROSSI, D. J. & MURPHY, P. M. 2019. Hematopoietic chimerism and donor-specific skin allograft tolerance after non-genotoxic CD117 antibody-drug-conjugate conditioning in MHC-mismatched allotransplantation. *Nat Commun,* 10**,** 616.

LIU, Y. & CHEN, Y. G. 2018. 2D- and 3D-Based Intestinal Stem Cell Cultures for Personalized Medicine. *Cells,* 7.

LLAMES, S., GARCIA-PEREZ, E., MEANA, A., LARCHER, F. & DEL RIO, M. 2015. Feeder Layer Cell Actions and Applications. *Tissue Eng Part B Rev,* 21**,** 345-353.

LOPES, A. M., BOBROWSKI, V. L., SILVA, S. D. & DEUNER, S. J. A. D. A. B. D. C. 2017. Morphophysiological and biochemical alterations in Ricinus communis L. seeds submitted to cobalt60 gamma radiation. 89**,** 1925-1933.

MACKENZIE, C., IWAMOTO, K. S. & SMITH, K. 2020. University of California Replacement of Cesium Irradiators with Alternative Technologies. *Health Phys,* 118**,** 209-214.

MOHAMMADYARI, P., ZEHTABIAN, M., SINA, S., TAVASOLI, A. R. & FAGHIHI, R. 2014. Dosimetry of gamma chamber blood irradiator using PAGAT gel dosimeter and Monte Carlo simulations. *J Appl Clin Med Phys,* 15**,** 3952.

MUKHERJEE, S., SAINIS, K. B. & DEOBAGKAR, D. D. 2014. F1 hybrids of BALB/c and C57BL/6 mouse strains respond differently to low-dose ionizing radiation exposure. *J Genet,* 93**,** 667-682.

MURPHY, M. K. & KAMEN, J. 2019. Deciding between an X-Ray and 137Cs Irradiator - It's not just about Energy Spectra. *Radiat Res,* 192**,** 493-506.

MYINT, P. 2018. Application of gamma irradiation knowledge in tissue sterilisation: inactivation of malaria parasite. *Cell Tissue Bank,* 19**,** 197-200.

NGUYEN, H., MORGAN, D. A. & FORWOOD, M. R. 2007. Sterilization of allograft bone: is 25 kGy the gold standard for gamma irradiation? *Cell Tissue Bank,* 8**,** 81-91.

NIKJOO, H. & LINDBORG, L. 2010. RBE of low energy electrons and photons. *Physics in medicine and biology,* 55**,** R65.

ORDEMANN, R., HUTCHINSON, R., FRIEDMAN, J., BURAKOFF, S. J., REDDY, P., DUFFNER, U., BRAUN, T. M., LIU, C., TESHIMA, T. & FERRARA, J. L. 2002. Enhanced allostimulatory activity of host antigen-presenting cells in old mice intensifies acute graft-versus-host disease. *J Clin Invest,* 109**,** 1249-1256.

OSOULI, S., ATAPOUR, M. & AHMADI, M. 2020. Effect of gamma radiation on the biology and inherited sterility in European corn borer Ostrinia nubilalis Hubner (Lep: Crambidae). *Appl Radiat Isot,* 160**,** 109096.

PALCHAUDHURI, R., SAEZ, B., HOGGATT, J., SCHAJNOVITZ, A., SYKES, D. B., TATE, T. A., CZECHOWICZ, A., KFOURY, Y., RUCHIKA, F., ROSSI, D. J., VERDINE, G. L., MANSOUR, M. K. & SCADDEN, D. T. 2016. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol,* 34**,** 738-745.

PEAKE, K., MANNING, J., LEWIS, C. A., BARR, C., ROSSI, F. & KRIEGER, C. 2015. Busulfan as a myelosuppressive agent for generating stable high-level bone marrow chimerism in mice. *J Vis Exp***,** e52553.

PEREZ-JIMENEZ, M., TALLON, C. I. & PEREZ-TORNERO, O. 2020. Inducing mutations in Citrus spp.: Sensitivity of different sources of plant material to gamma radiation. *Appl Radiat Isot,* 157**,** 109030.

PONCHIO, L., DUMA, L., OLIVIERO, B., GIBELLI, N., PEDRAZZOLI, P. & ROBUSTELLI DELLA CUNA, G. 2000. Mitomycin C as an alternative to irradiation to inhibit the feeder layer growth in long-term culture assays. *Cytotherapy,* 2**,** 281-286.

PORT, M., MAJEWSKI, M., HERODIN, F., VALENTE, M., DROUET, M., FORCHERON, F., TICHY, A., SIRAK, I., ZAVRELOVA, A., MALKOVA, A., BECKER, B. V., VEIT, D. A., WALDECK, S., BADIE, C., O'BRIEN, G., CHRISTIANSEN, H., WICHMANN, J., EDER, M., BEUTEL, G., VACHELOVA, J., DOUCHA-SENF, S. & ABEND, M. 2018. Validating Baboon Ex Vivo and In Vivo Radiation-Related Gene Expression with Corresponding Human Data. *Radiat Res,* 189**,** 389-398.

PRAVEEN KUMAR, M. K., SHYAMA, S. K., KASHIF, S., DUBEY, S. K., AVELYNO, D., SONAYE, B. H., KADAM SAMIT, B. & CHAUBEY, R. C. 2017. Effects of gamma radiation on the early developmental stages of Zebrafish (Danio rerio). *Ecotoxicol Environ Saf,* 142**,** 95-101.

PRITCHARD, A. E. & SHAZ, B. H. 2016. Survey of Irradiation Practice for the Prevention of Transfusion-Associated Graft-versus-Host Disease. *Arch Pathol Lab Med,* 140**,** 1092-1097.

RODERICK, T. H. 1963. THE RESPONSE OF TWENTY-SEVEN INBRED STRAINS OF MICE TO DAILY DOSES OF WHOLE-BODY X-IRRADIATION. *Radiat Res,* 20**,** 631-639.

RÖNTGEN, W. C. 1895. Ueber eine neue Art von Strahlung., Aus den Sitzungsberichten der Wuerzburger Physik. *Medic. Gesellschaft, Wuerzburg*.

ROTHKAMM, K., KRUGER, I., THOMPSON, L. H. & LOBRICH, M. 2003. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol Cell Biol,* 23**,** 5706-5715.

ROTHKAMM, K. & LOBRICH, M. 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A,* 100**,** 5057-5062.

RUSIN, A., LAPIED, E., LE, M., SEYMOUR, C., OUGHTON, D., HAANES, H. & MOTHERSILL, C. 2019. Effect of gamma radiation on the production of bystander signals from three earthworm species irradiated in vivo. *Environ Res,* 168**,** 211-221.

SAKAMOTO-HOJO, E. T. 2018. Lessons from the accident with (137)Cesium in Goiania, Brazil: Contributions to biological dosimetry in case of human exposure to ionizing radiation. *Mutat Res Genet Toxicol Environ Mutagen,* 836**,** 72-77.

SCHOENBACH, K. H., JOSHI, R., KOLB, J., BUESCHER, S. & BEEBE, S. 2004. Subcellular effects of nanosecond electrical pulses. *Conf Proc IEEE Eng Med Biol Soc,* 7**,** 5447-5450.

SCHUBERT, P., JOHNSON, L., MARKS, D. C. & DEVINE, D. V. 2018. Ultraviolet-Based Pathogen Inactivation Systems: Untangling the Molecular Targets Activated in Platelets. *Front Med (Lausanne),* 5**,** 129.

SEGHATCHIAN, J. & TOLKSDORF, F. 2012. Characteristics of the THERAFLEX UV-Platelets pathogen inactivation system - an update. *Transfus Apher Sci,* 46**,** 221-229.

SULLIVAN, M. T., COTTEN, R., READ, E. J. & WALLACE, E. L. 2007. Blood collection and transfusion in the United States in 2001. *Transfusion,* 47**,** 385-394.

THISTLEWOOD, H. & JUDD, G. J. J. I. 2019. Twenty-five Years of Research Experience with the Sterile Insect Technique and Area-Wide Management of Codling Moth, Cydia pomonella (L.), in Canada. 10**,** 292.

THORAEUS, R. 1965. Attenuation of Gamma Radiation from 60Co, 137Cs, 192Ir, and 226Ra in Various Materials Used in Radiotherapy. *Acta radiologica: therapy, physics, biology,* 3**,** 81-86.

TRELEAVEN, J., GENNERY, A., MARSH, J., NORFOLK, D., PAGE, L., PARKER, A., SARAN, F., THURSTON, J. & WEBB, D. 2011. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. *British Journal of Haematology,* 152**,** 35-51.

VILLA-DIAZ, L. G., ROSS, A. M., LAHANN, J. & KREBSBACH, P. H. 2013. Concise review: The evolution of human pluripotent stem cell culture: from feeder cells to synthetic coatings. *Stem Cells,* 31**,** 1-7.

WELLS, D. J. 2010. Genetically modified animals and pharmacological research. *Handb Exp Pharmacol***,** 213-226.

WILDING, C. S., TRIKIC, M. Z., HINGSTON, J. L., COPPLESTONE, D. & JANET TAWN, E. 2006. Mitochondrial DNA mutation frequencies in experimentally irradiated compost worms, Eisenia fetida. *Mutat Res,* 603**,** 56-63.

WILKINSON, A. C., ISHIDA, R., KIKUCHI, M., SUDO, K., MORITA, M., CRISOSTOMO, R. V., YAMAMOTO, R., LOH, K. M., NAKAMURA, Y., WATANABE, M., NAKAUCHI, H. & YAMAZAKI, S. 2019. Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature,* 571**,** 117-121.

WORGUL, B. V., SMILENOV, L., BRENNER, D. J., VAZQUEZ, M. & HALL, E. J. 2005. Mice heterozygous for the ATM gene are more sensitive to both X-ray and heavy ion exposure than are wildtypes. *Adv Space Res,* 35**,** 254-259.

XU, H., ZHU, Z., HUANG, Y. & ILDSTAD, S. T. 2019. FL/GCSF/AMD3100-mobilized Hematopoietic Stem Cells Induce Mixed Chimerism With Nonmyeloablative Conditioning and Transplantation Tolerance. *Transplantation,* 103**,** 1360-1371.

YUSOF, N. 2018. Advances of radiation sterilisation in tissue banking. *Cell Tissue Bank,* 19**,** 175-186.

ZHANG, X., KARATEPE, K., CHIEWCHENGCHOL, D., ZHU, H., GUO, R., LIU, P., YU, H., REN, Q., LUO, X., CHENG, T., MA, F., XU, Y., HAN, M. & LUO, H. R. 2020. Bacteria-Induced Acute Inflammation Does Not Reduce the Long-Term Reconstitution Capacity of Bone Marrow Hematopoietic Stem Cells. *Front Immunol,* 11**,** 626.

ZHANG, Y., SHEN, B., GUAN, X., QIN, M., REN, Z., MA, Y., DAI, W., DING, X. & JIANG, Y. 2019. Safety and efficacy of ex vivo expanded CD34(+) stem cells in murine and primate models. *Stem Cell Res Ther,* 10**,** 173.

ZOETELIEF, J., BROERSE, J. J., DAVIES, R. W., OCTAVE-PRIGNOT, M., REZVANI, M., VERGARA, J. C. & TONI, M. P. 2001. Protocol for X-ray dosimetry in radiobiology. *Int J Radiat Biol,* 77**,** 817-835.