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Review

The importance of standardization and challenges of dosimetry in conventional preclinical radiation biology research

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Abstract

To fully exploit the prospects presented by the increasing focus on biological approaches for enhancing radiotherapy outcomes, improvements in repeatability and translatability of radiobiological and preclinical studies are required. This requires the development and adoption of appropriate dosimetric standards and reproducible approaches to increase confidence in the studies, enabling inter-laboratory validation and facilitating clinical translation. An Institute of Physics and Engineering in Medicine (IPEM) working party reviewed the current status and challenges associated with dosimetry of medium-energy X-rays and make recommendations with the aim to optimize the potential clinical significance of radiobiological preclinical investigations. The paper discusses the currently available resources with technical recommendations for performing dosimetry in medium-energy X-rays, along with the consequences of lack of standardization and implications of dose inhomogeneity. It is clear that there is still a gap in understanding the needs for standardization of dosimetric aspects of preclinical and radiobiological studies. It is recommended that these radiobiology studies should be conducted in partnership with medical/radiation physicists. This collaboration ensures the correct utilization of suitable dosimetry systems, thus guaranteeing accuracy and consistency of dose delivery. Appropriate calibration and traceability to national/international standards laboratory, along with regular quality assurance of radiation devices, are paramount to reproducibility. Additionally, it is critical that experimental details and associated dosimetry are sufficiently reported to ensure accurate replication that enables reanalysis including evaluation of dose distributions. Increasing awareness among the researchers and the funding bodies was identified as a crucial step to improve translatability and appropriate resources are budgeted to increase the value for money of research proposals. The proposed recommendations will serve as a vital resource for researchers, encouraging uniformity in experimental design and improving the translatability of preclinical research to clinical settings.

Keywords: radiobiology; X-ray irradiators; dosimetry; beam characterization; QA.

Introduction

With the increasing focus on biological approaches to improve radiotherapy, using techniques such as molecular therapeutics and immunotherapeutic approaches, there is an urgent need to improve reproducibility and translatability of preclinical data to fully exploit these opportunities.[1] Fundamentally, there is a critical need to develop appropriate dosimetric standards and reproducible approaches, which will lead to more robust experimental designs, better results during inter-laboratory validation, and ultimately increase confidence in these studies, facilitating their clinical translation. A recent review[2] highlighted how the endemic failure to report basic experimental details of the physics and dosimetric irradiation techniques applied to biological samples can have far-reaching implications.

Specialized image-guided small animal irradiators have recently become commercially available (eg, SARRP from Xstrahl Ltd[3] and SmART+ from Precision X-ray[4]). They have the capability to deliver relatively complex image-guided plans, which closely resemble clinical radiotherapy

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treatment plans. A recent comprehensive review of published papers showed the potential of image-guided irradiators in supporting translation of preclinical and radiobiological research into clinical trials.[5] Moreover, in another recently published topical review, a panel of experts showcases a roadmap for photon-based precision and image-guided preclinical radiotherapy, discussing the recent technological evolution in dosimetry, imaging, irradiation, and monitoring as well as potential future developments.[6] However, these types of radiation devices are expensive, sophisticated, and currently being used only by a select number of established research groups. As a result, conventional cabinets (whether kV X-ray or gamma-ray emitting radioisotopes) are still employed worldwide for both *in vitro* and *in vivo* radiobiological studies, especially in the initial stages of these studies. Moreover, these cabinets are often used with little or no physics and dosimetry support. This is particularly critical when controlling the dose delivered using shielding approaches, complex geometries, and/or irradiation of a variety of biological samples. This poses significant challenges to the accuracy and reproducibility of the radiation dose applied, and subsequent interpretation of data.

Due to concerns of the potential misuse of high-activity sealed sources (HASS), there is growing governmental interest in the use of medium-energy X-ray cabinet irradiators as a replacement for existing Cs-137 irradiators[7,8] for a variety of biomedical uses. According to the definition by the UK IPEMB code of practice, the medium-energy range refers to X-rays of half-value layers between 0.5-4 mm Cu or equivalently above 8 mm Al, covering approximately those generated at tube voltages in the range 160-300 kV. The AAPM code of practice has a slightly different definition considering medium-energy X-rays those generated by a tube potential in the range 100-300 kV, HVL: 0.1-4 mm Cu. In addition to radiobiology studies, cabinet X-ray irradiators are also routinely used for techniques such as total body irradiations (TBIs) for immune ablation, which is commonly used for bone marrow transplantation (BMT) studies.[9] Here, accurate dosimetry and appropriate dose distributions are important to ensure sufficient depletion of the bone marrow cell population while minimizing normal tissue toxicity. The complexity and lower accuracy of dose calculation algorithms are exacerbated by the heterogeneous nature of the bone marrow inside the bone. In those conditions, measurements are difficult to perform and prone to larger uncertainties. Image-guided total marrow preclinical irradiation techniques, aiming to reduce dose to vital organs while enabling bone marrow dose escalation, have been reported in the literature.[10] However, the resulting irradiation plans are complex, difficult to implement and to dosimetrically verify.

More recent studies and human evidence-based compilations, which consider the different effects of systematic and random dose uncertainties on tumour control probability (TCP) and normal tissue complication probability (NTCP), place the overall required accuracy at 3% for the dose delivered to the patient at the dose specification point, and between 3% and 5% for absorbed dose distributions.[11] Considering that animal models are to mimic the human response, a similar dose-response sensitivity is to be expected; therefore, the 5% accuracy suggested for humans should also apply to animals.[12,13] Moreover, there is certain level of agreement between the preclinical and radiobiological researchers that a similar level of accuracy should apply to preclinical research aiming to translate results into clinical radiotherapy. In order to reach that goal, several aspects of the determination of the reference absorbed dose, the quality assurance (QA) of the devices, and the end-to-end verification of the radiation pathway need to be standardized to similar levels as to those achieved in clinical radiotherapy.

In this paper, we will briefly summarize the key challenges for the delivery of reproducible and traceable dosimetry in translational radiation biology research using conventional X-ray cabinets, in the context of United Kingdom best practice, and make a number of recommendations. We also highlight current available guidance, particularly for researchers who do not have access to local medical physics support.

Sources of uncertainties

Radiation biology research is usually challenged by systematic and statistical uncertainties related to the biological endpoints, alongside the errors associated with the physical aspects of the determination of the value of dose delivered.[14] In that context, radiation-induced molecular responses may be already complex at the cellular level. This is further magnified at the tissue level due to the presence of multiple cell types and their spatial distribution.[15] Ultimately, accurate assessment of response to dose will depend on the correct determination of the delivered 3D (dimensional) dose distributions, which is based on the understanding of complex physical interactions of the radiation with the matter, and the dosimetry protocols and measurement methods used.

Examples of biological and physical sources of uncertainties contributing to the complexity of the evaluation of a preclinical study are shown in Figure 1A and B.

On one side of the subject, biological dose response can show a large variation for different tissues, species, strains, and ultimately cell types. On the other side, selection criteria, that is, age, weight, sex, food intake, sleeping patterns, tumour grade and size, tumour microenvironment, and implantation, as well as the use (if any) of concomitant forms of therapy, can be more uniformly represented in rodent-based than in human-based studies. That could lead to expectations of a more uniform biological response for rodents, and the possibility that some radiobiology studies may not require precision of absorbed dose across the study group of better than 10%.[16] However, there are not yet enough studies that would have disentangled the complexity of the relationship between the different factors affecting the biological response and their association with the accuracy of the physical dose delivered. Moreover, several biological endpoints are particularly sensitive to changes in the radiation dose and require a higher delivery accuracy. Examples include induction of myelopathy following spinal cord irradiation, evaluation of gastrointestinal injuries, successful bone marrow transplant while minimizing normal tissue toxicity, lung responses and lethality, and cell survival curves for the determination of relative biological effectiveness (RBE).[16] TCP and NTCP are closely linked to cell survival because both probabilities are based on radiobiological models that describe how cells respond to radiation. Cell survival curves, derived from *in vitro* radiobiological experiments, provide critical parameters that connect these probabilities to radiation dose and biological response. Along variations in the biology of the tissue, TCP/NTCP, the models are highly sensitive to uncertainties in radiation dose delivery. Understanding and managing these

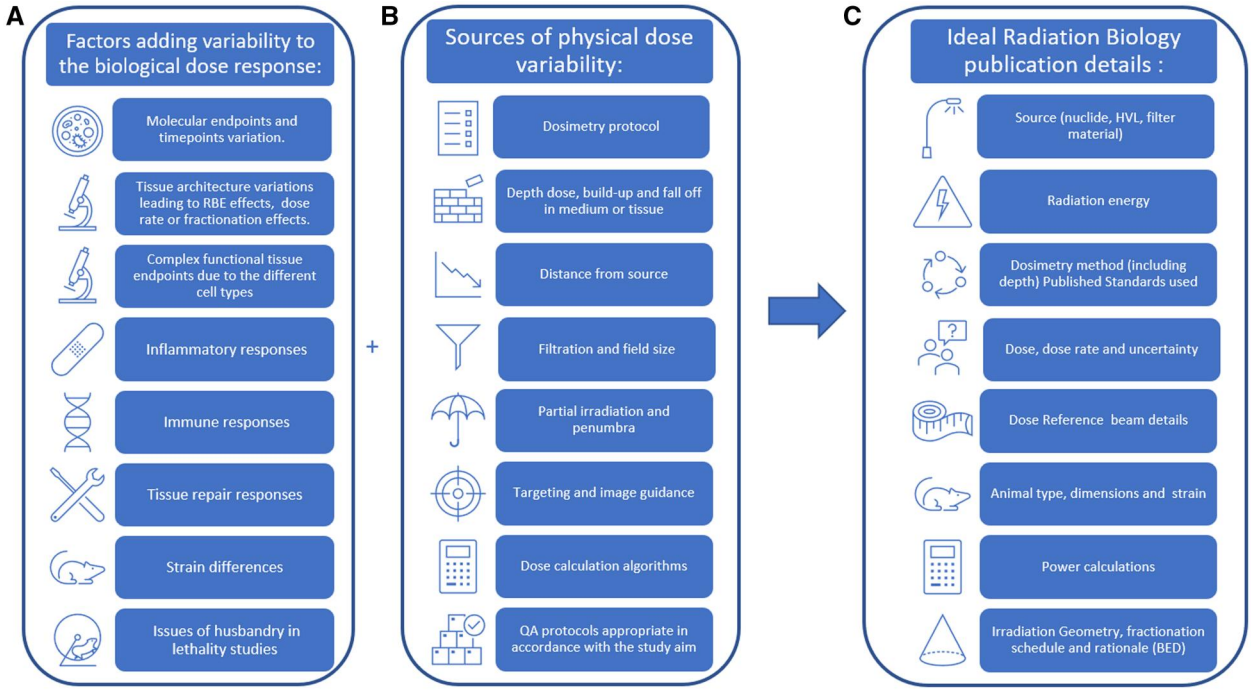


Figure 1. Sources of (A) biological and (B) physical variability in radiobiology, and (C) the recommendations for details to be included as part of preclinical publications with further details given in Table 1.

uncertainties is critical in radiobiology studies and their translational output. Ideally, preclinical radiation research should be delivered with an accuracy that is closer to what is required for the delivery of clinical radiotherapy plans (within 5% of prescribed dose), to resolve both dose-related and non-dose-related variabilities.

The introduction of a minimum set of reporting guidelines, containing the minimum required irradiation and associated protocol details to be included in published research, is therefore strongly recommended. An example of what should be reported to facilitate the replication of studies across institutions is shown in Figure 1C. Interestingly, it has been noted that in part due to strict article length requirements, highly cited journals and articles are more likely to be missing critical details.[2] A number of recent publications have presented what they believe to be essential or strongly recommended details, to be reported in either the main text, as supplementary data or as a reference to published standard operating procedures (SOPs), and indicated that this should be a requirement for the publication when preclinical irradiations are reported.[16-19] These have been reviewed, and an overview of the recommendations is presented in Table 1.

Challenges associated with dosimetry in preclinical settings

The dosimetry, if any, provided by the suppliers of X-ray cabinets is generally presented in the form of air-kerma rate (Air-kerma is equal to dose to air under conditions of charged particle equilibrium.), measured at a given point in air or at various points on the surface of a shelf, rather than in terms of absorbed dose (reported as either dose-to-water or dose-to-medium, eg, tissue) to the sample of interest. Accurate measurements of absorbed dose to a sample in conventional irradiators are not straightforward, as there are a number of elements that need to be considered to be able to establish the dose to a point, or the 3D dose distribution through the sample. These will depend on factors such as the X-ray fluence profiles, energy spectra, distance from the source, field size, and importantly the atomic composition, density, and geometry of the sample and any surrounding structures (Figure 1B). The presence of dosimeters may also perturb the dose distributions, depending on their size/geometry and composition.

Consequently, the dose delivered to samples in each experimental setup requires careful evaluation with the use of appropriate dosimetry systems and suitable protocols. Ideally, dosimetry measurements should be performed as close to the experimental setup as possible, with the dosimeter at a position in the sample with minimal perturbation of the radiation field. This will minimize the need for the application of correction factors. For *in vitro* experiments, this may include irradiating radiochromic films (such as Gafchromic EBT3 or EBT4), at the same position as the cells, by replacing media with water equivalent material of the same thickness within the flask/dish. With film dosimetry, there is an advantage of obtaining a 2D dose distribution (EBT3 films are not going to be further available; they are being replaced by EBT4 films from the same company, Ashland Advanced Materials LLC, Niagara Falls, NY). With rodents, dose verification should include using an appropriate dosimeter in a tissue-equivalent mouse phantom. The use of a simple rodent-sized geometric phantom (eg, an appropriately sized cuboid or cylinder), with detectors with traceable calibration, not only gives a more realistic determination of dose than in-air measurements but also allows for an initial basic intercomparison of the dose delivered by different preclinical research centres. These phantoms are easier and cheaper to replicate and position within the irradiators. With instructions for irradiations under similar conditions and prescription to an identifiable point within the phantom, such comparisons could provide

Table 1. Recommendations for relevant information for inclusion when reporting results of radiation biology *in vitro* and *in vivo* studies (based on previously published recommendations[16-19]).

Recommendation	Comments
Name of irradiator	• If commercial: model, company, country • If bespoke: reference to details of the main components and characteristics
Source specification	• If radionuclide: type (eg, Cs-137, Co-60), activity, irradiation geometry, delivery method • If X-ray: kVp, mA, filter (material and thickness), and half value layer (HVL)
Absolute dosimetry and beam calibration	• Standards/protocols used • Details of detectors used • Details of setup • Dose calibration and traceability to national standard • Details of any dose monitoring methods during beam delivery (if performed) • Planning/optimization and dose calculation method for multiple beams
Dose specification	• Radiation absorbed dose delivered to biological sample • Dose-to-water-in-water, dose-to-water-in-medium, or dose-to-medium-in-medium/tissue[a] (as opposed to air-kerma[b]) • Dose rate (including any variations) • Fractionation schedule (eg, number of exposures and intervals) • Dose prescription at a specific location (eg, midline dose) or within a specific volume within the sample (eg, the heart) • Calculated dose to sample or measured dose in a phantom • 2-3D dose distributions if available and how measured/calculated
Sample specification	• Animal/cell type • Geometry/orientation of sample and array of samples if multiple samples are to be irradiated simultaneously • Geometry and composition of sample holder or animal restraint (eg, pie cages or bespoke jigs) • Any animal/sample rotations
Irradiation geometry	• Field size (including details of any collimation/shielding) • Source geometry (eg, number, individual activities and distribution in the case of radionuclide sources) • Sample distance from source • Number/size/geometry/orientation of beams
Irradiator quality assurance programme	• Details of different aspects of the irradiators' quality assurance programmes, including tests performed and agreed frequencies
Dose uncertainties	• Dose associated with sample's imaging (if applicable) • Details of additional dose offset associated with deployment and retraction of radiation sources or ramp up of kV/mA for X-ray exposure • Details of any factors contributing to dose uncertainty (eg, backscatter, lateral scatter, differences between calibration and irradiation geometry, tissue heterogeneity, etc.)

[a]It is important that the users of the cabinets are able to identify the dose that has been reported: dose-to-medium-in-medium is most commonly obtained from Monte Carlo simulations, dose-to-water-in-medium can also be reported from calculations from MC simulations or from measurements with a dosimeter calibrated in terms of dose to water, and finally, dose-to-water-in-water can be derived from formalism and simple dose calculations without considering the differences in the material composition alongside the path of the radiation beam.
[b]Air-kerma is equal to dose to air under conditions of charged particle equilibrium.

valuable information to the research community.[20,21] Ideally, an anatomically correct phantom should be used for that purpose, resulting in a more realistic dosimetry assessment. However, although several publications refer to the development of anatomically correct phantoms,[22,23] the efforts of an international dosimetric intercomparison that would make available the same phantom to all laboratories have not yet materialized. In general, there are few commercially available zoomorphic phantoms for preclinical radiation research that would accurately match the geometry of the mouse being irradiated, and furthermore, the radiobiology and preclinical research community lacks awareness about their existence. Moreover, further research on validation of the phantoms' material composition and their effect in the accuracy of the dose calculations is needed.[24] The use of well-characterized and calibrated dosimeters is also crucial in order to convert the dosimeter response into absorbed dose to the sample. If detailed dosimetry measurements are not possible or available, then accurate dosimetry measurements should be reported for a well-defined configuration. Even from single measurements, as long as the dosimetry protocol and irradiation setup are described in sufficient detail, it should be possible to determine 3D dose distributions using Monte Carlo calculations.[25]

A final crucial factor contributing to the challenge posed by the dosimetry aspects of preclinical and radiation biology research is the lack of adequate medical/radiation physics expertise which can support the planning and data interpretation phase of the studies. Radiation biologists generally lack the necessary training to implement and perform QA procedures for radiation devices, including measurements of absorbed dose in reference or other experimental setups.

These are important to guarantee the traceability of the dose delivered, which stresses the need for appropriate physics support as a prerequisite for the adequate realization of radiobiological studies.

Consequences of the lack of standardization of preclinical dosimetry

The lack of standardization of the dosimetry aspects described above has been shown to result in large dose differences reported between laboratories that performed the same type of investigations. For example, a relatively recent dose verification survey of 5 X-ray facilities using a mouse phantom showed that only 1 of the institutions delivered the target dose within 5%, while dose differences delivered by the remaining 4 irradiators varied from 12% to 42%.[26] Among other reasons, this large level of spread has been attributed to the lack of consultation between radiation biologists and radiation physicists at the time of designing, documenting, and validating the irradiation protocols.[2]

The importance of an appropriate dosimetry characterization and the consequences of an inappropriate dosimetry chain at the time of reporting the dose delivered by preclinical irradiators are illustrated by the examples below.

Implications of changes to the irradiation conditions and their effect on the homogeneity of the dose delivered

Uniformity of the radiation field in radiobiology experiments is essential for obtaining reliable and meaningful results, as significant variations in the absorbed dose across the sample can lead to gross average effects and misleading outcomes. For cabinet irradiators, the dose rate will typically fall with increasing radial distance, with the heel effect resulting in some asymmetry, unless corrected for by using a uniformity filter. The effect is more pronounced closer to the X-ray tube and for lower beam energies (<150 keV) and can result in significant non-homogeneous dose distribution across irradiation samples. An example of the effect of the lack of homogeneity through the radiation field is shown in Figure 2A, with different doses per well, in a multi-well dish, depending on its orientation within the irradiation beam.

Other examples on the effect of changes in the irradiation conditions, for example, variation of the distance from the source to the sample (SSD) and the field size, on the surface dose and dose profiles are shown in Figure 2B(ii) and (iii). Homogeneity of dose with depth is also highly influenced by the beam quality. Given that there is a wide variety of beam qualities available for preclinical irradiators, the characterization of individual devices is important. The most commonly available biological irradiators have X-ray tubes which can deliver beams ranging from 40 to 350 kVp. Beam qualities can be modulated by the use of filtration, which reduces the low-energy component of the X-ray spectrum, with the consequent increase in the average energy. In that regard, unfiltered (or low filtration) X-ray beams should be avoided for both *in vitro* and *in vivo* experiments.[28] With the use of unfiltered beams for *in vitro* investigations, the dose to cells will also vary, depending on the amount of medium above them. This is because a steepest absorption of the low-energy range component of the unfiltered X-ray spectrum (which dominates the total spectrum) will happen within few millimetres from the sample surface (eg, see Figure S1). Similarly, for *in vivo* experiments, these low-energy X-rays would result in high skin dose and potentially lead to normal tissue toxicity.

While Cs-137 γ-ray irradiation of rodents results in a relatively uniform dose distribution through the body, the use of lower energy X-ray cabinets would result in a significant dose variation with depth. The degree of variation can be reduced by using high voltage X-ray tubes in combination with an adequate added filtration (Figure 3A-C). Uniformity can also be improved by using parallel-opposed beams, with the rodent irradiated from both sides of its body.[30]

The variation in beam quality also results in differential absorption in tissues due to differences in their average atomic number and density. As a result, for instance, the dose to bone can be significantly higher than that to surrounding tissues with lower atomic number and density. While the dose to the cortical bone is usually of limited interest (apart from when reducing the dose in tissues beyond the bone), the larger number of photo-electrons produced by the interaction of lower-energy X-rays in the bone will travel to the adjoining bone marrow. In small animals (such as mice) with sub-millimetre bone marrow, this can result in higher (up to 31%) average bone marrow dose. This is not only important with respect to the efficacy of TBI experiments but also when higher incidence of hemopoietic symptoms could jeopardize the results expected from the end-point of the irradiations. The effects of tissue heterogeneity and choice of beam quality on the dose distribution are illustrated in Figure 3D and E.[28]

Experimental setup for irradiation of samples for immunofluorescence investigations provides another example of the challenges of performing accurate dosimetry in preclinical studies and the possible oversights. These experiments are often performed by irradiating cells attached to glass coverslips rather than plastic dishes in order to facilitate the subsequent imaging procedures. Due to the higher atomic number and density of glass compared to plastic, the cells grown on glass will receive a higher dose for a given X-ray exposure caused by an increased backscatter contribution from the glass material.[31,32] In this type of cases, Monte Carlo methods can be useful to determine the dose across interfaces and resulting dose to the cells.

Partial irradiation, targeting

Conventional X-ray cabinets are often used to irradiate specific volumes within the samples. Examples of the above include partial irradiations of flasks/well-plates and of specific anatomical regions of the small animals. Targeted irradiations can be achieved either by using bespoke collimators attached to the X-ray source or by placing lead sheets just above the areas that need to be spared (Figure 2B and C).

Any misalignment between the shielding device, the radiation source, and the sample will result in a wider penumbra rather than in a sharp dose threshold. The size of the geometric penumbra depends on the position of the collimator relative to the X-ray source and the sample, as shown in Figure 2B(iii). It also depends on the focal spot selected for the irradiations and the size of the shielding aperture/collimator.[33] Moreover, any offset of the unshielded area from the centre of the beam/shelf will result in an offset of the transmitted beam from the unshielded region, with the offset distance increasing with the increasing depth (Figure 2C). If these effects are not accounted for, it could invalidate the radiobiological investigation.

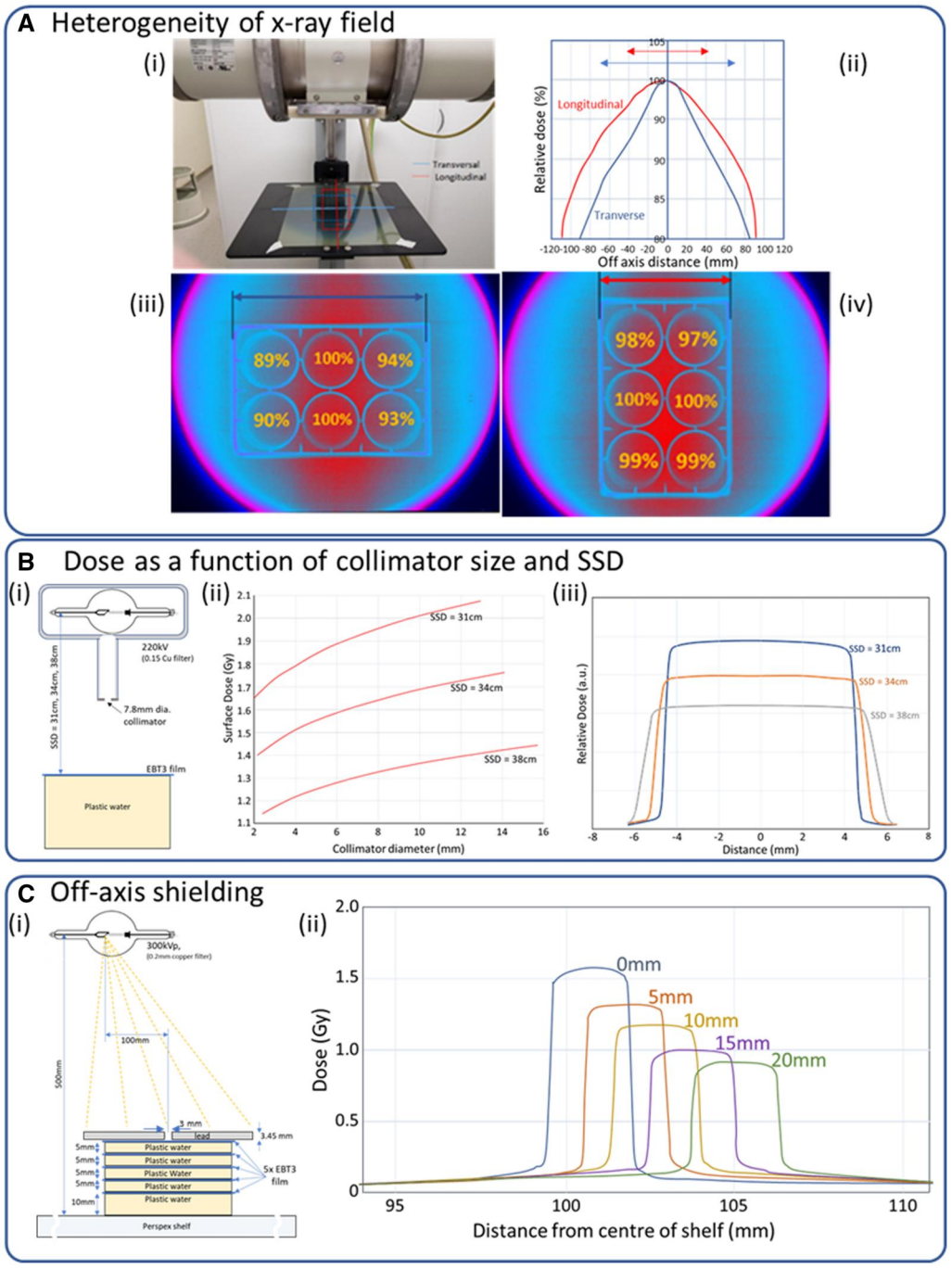


Figure 2. Illustration of X-ray dose heterogeneity. (A) Implications for the irradiation of cell plates in a conventional X-ray cabinet. If the plate is irradiated with its larger dimension in the direction of the transversal profile (blue line), dose across the cells can vary more than 11% depending on configuration (data from measurements performed in an AGO HS X-ray irradiation system [250 kV, 0.25 mm Cu + 1mm Al], available on request[27]). (B) Dose at the surface of a sample decreasing with increasing source to surface distance (SSD) and decreasing field size. It is essential that the collimated beam is accurately aligned with the area to be irradiated (illustrative data from measurements performed using an Xstrahl SARRP irradiator [220 kV, 0.15 mm Cu], available on request). (C) Shielding can also be achieved using lead sheet in close proximity to the area to be shielded; however, if the unshielded area is offset from the centre of the X-ray beam/shelf, then the transmitted beam will be offset from the unshielded region, with off-set increasing with depth (X-ray 300 kV, 0.15 mm Cu. Data modified from Hill et al. 2024,[25] under Creative Commons Attribution 4.0 licence).

Shielded irradiations using small apertures and/or collimators are even more challenging. If no image-guided targeting is available, accurate modelling and/or measurement of the penumbra along with accurate targeting are critical to ensure the delivery of reproducible doses using small-collimated fields. The use of radiochromic films has proven useful in these conditions.[33] The dose delivered at the centre of a small, collimated field can be considerably lower than the dose delivered by an open field. That is caused by reduced contributions from phantom scatter and partial occlusion of the radiation source with penumbra overlap.[34,35] Although the IAEA TRS 483 refers to challenges posed by the dosimetry of small fields in megavoltage X-ray beams, the document presents a thorough explanation of all the aspects that need

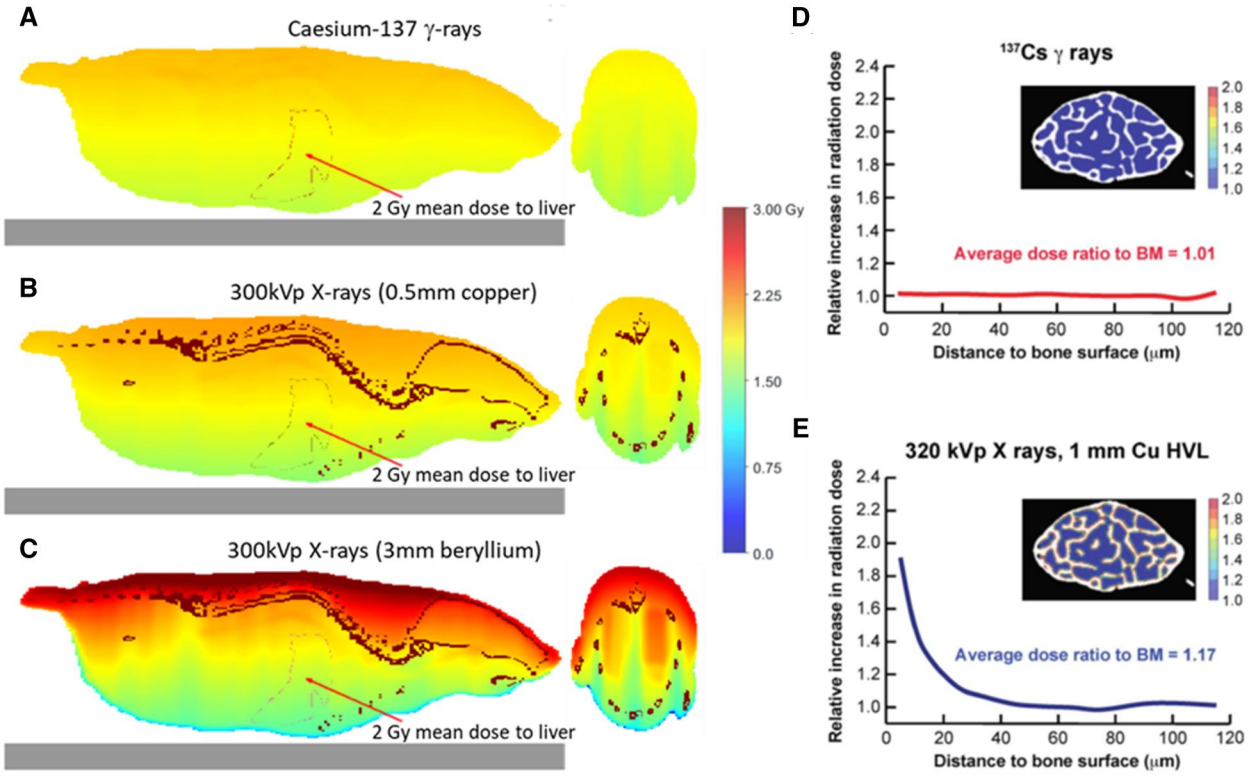


Figure 3. Illustration of the heterogeneity of dose distributions in a mathematical mouse model (MOBY)[29] at different energies and filtrations. Calculated sagittal and axial dose distributions to deliver a mean dose of 2 Gy to the liver when irradiated from above for (A) Cs-137 γ-rays, (B) 300 kVp X-rays with an inherent 3 mm Be, and additional 0.5 mm Cu filter and (C) 300 kVp X-rays with only the inherent 3 mm Be filtration (modified from Hill et al., 2024,[25] under Creative Commons Attribution 4.0 licence). Variation in dose distribution within trabecular bone and the variation in dose with distance from the nearest bone surface for (D) Cs-137 γ-rays and (E) 320 kVp X-rays (1 mm Cu HVL) (reproduced from Poirier et al., 2020,[28] © 2025 Radiation Research Society). Abbreviations: BM = bone marrow; HVL = half value layer.

to be considered, some of which would also apply to measurements in medium-energy-rays, that is, source occlusion, detectors' volume averaging effect, and material composition. Comprehensive and consensus-based guidelines for measurements in small-, medium-energy X-rays fields are yet to be produced. Measurements performed in a SARRP irradiator to illustrate the effect show that the absorbed dose ratio at the centre of the field of the small 1 cm × 1 cm brass collimator versus the open, not collimated field (~13 cm equivalent square), is around 0.6 (data from measurements performed in a SARRP device with alanine, film, and a plastic scintillator are available on request). Moreover, if the irradiation beam is larger than the shielded area, transmission from the unshielded regions combined with scatter within the animal (and/or potential scatter from the shelves or cabinet components) could result in a background dose to the shielded part of the sample. For well-designed shields, the contribution to the dose due to transmission can be low (eg, ~1% or less of the prescribed dose, for an X-ray system with 225 kV and 0.5 mm Cu added filtration, half value layer [HVL] = 1.3 mm Cu[36]). However, the leakage through inappropriately designed and wrongly placed shielding devices could lead to a miss-interpretation of the biological effect. Therefore, a critical evaluation of geometrical and dosimetric aspects of shielding design should always be considered.

Overall, and due to the intricate nature of shielded irradiation setups, achieving experimental reproducibility can be difficult. Variability in setup, equipment, and other factors can impact the ability to reproduce results over different experiments. Ensuring a uniform irradiation without image-guidance across the exposed portion of the samples, while minimizing the scattering contributions, is a challenging task. Although performing an accurate dosimetry assessment in these conditions is also challenging, this should always be performed. In preclinical *in vivo* irradiations, the field edge and dose inhomogeneity effects are further compounded by complexities related to the mouse anatomy and tumour biology variability. Achieving a uniform and conformal dose distribution within complex anatomical structures requires accurate targeting and should ideally be performed using image-guided small animal irradiators.[6,37,38] As well as enabling the target anatomy to be imaged, such systems typically also provide advanced treatment planning features allowing targeted X-ray beams to be delivered from multiple directions or arcs and therefore more conformal dose distributions.

Implications of scattering conditions

In addition to the dose from primary X-ray interactions, a significant contribution to the absorbed dose at a given point arises as a consequence of the scattered radiation resulting from interactions occurring at the sides, in front and behind the point of interest. This includes scatter, not just from the irradiated sample, but also from their surrounding materials, such as sample holders (eg, mouse jigs and flask walls and media). Other sources of scatter are shelves on which the

samples are placed and the cabinet walls. The contribution to the dose, resulting from the scattered radiation, depends on the field size and the X-ray energy spectrum.[39] Therefore, for an accurate evaluation of its impact, dosimetry measurement should be performed in the same geometrical setup as the one used during the experimental irradiations.

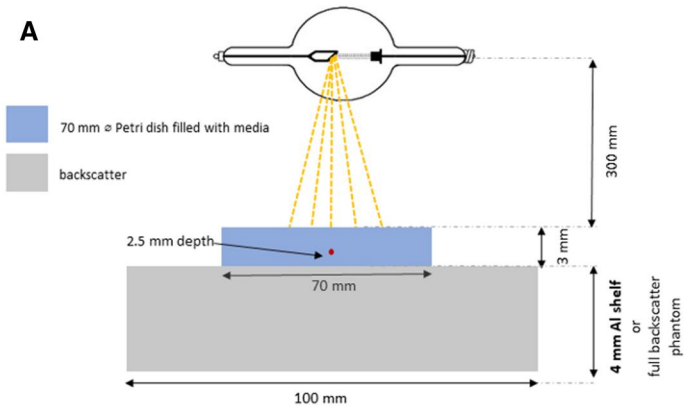
While conventional dosimetry protocols require the presence of considerable water equivalent material behind the sample to generate consistent and quantifiable backscatter radiation, this condition is very rarely fulfilled during preclinical irradiations. The Monte Carlo calculations in Figure 4D show that the dose delivered to the point of interest is significantly underestimated (up to 20%, for specific example shown). This is because full backscatter conditions are not met when a thin (4 mm) aluminium (Al) shelf is used. It is not only the difference in thickness but also the material composition of the supporting shelf which plays a key role at the time of evaluating the absorbed dose to the sample. Using metal shelves rather than plastic ones increases the contribution of photoelectric interactions, reducing the multiple-scatter component which, and depending on the beam quality, may reduce the amount of backscatter radiation. The use of metal shelves adds an extra degree of complexity to dose estimation. The calculations also show that in these irradiation conditions, the actual backscatter contribution will also vary, depending on the tube peak voltage and the additional filtration.

Variation in biological effectiveness with photon energy

While X-ray and γ-ray energy spectra can play a significant role with respect to the resulting 3D dose distribution, they can also impact on the resulting biological effectiveness per unit dose.[40,41] In general, with the decrease in the photon energy, the RBE for a variety of biological endpoints increases (eg, for survival, DSB induction, chromosomal aberration, mutations, neoplastic transformation). This is caused by the increased linear energy transfer (LET) of the secondary electrons. The value of RBE is dose dependent and increases with decreasing dose to a maximum, RBE_M, as the dose approaches zero. Moreover, spectral changes may also occur with depth, which may induce RBE changes as the beam penetrates the irradiated samples. A comprehensive discussion and review of available data on the RBE of low-energy photons for a variety of endpoints is available in references.[40,41] Differences have also been observed as a result of changes in the filtration of the X-ray tube. For example, for the induction of dicentric aberrations, the RBE_M for a 220 kV X-ray beam weakly filtered (4.05 mm Al + 0.5 mm Cu) is 3.7 ± 1.5 compared to ^{60}Co γ-rays, while for a heavily filtered 1 (2.0 mm Al + 3.35 mm Cu), the RBE_M is 2.1 ± 0.9. However, these RBE values do significantly decrease with increasing dose.[41] Therefore, it is essential that information on the quality of the radiation beam (eg, HVL) and/or parameters that impact the energy spectrum is reported. Overall, there is limited research on the magnitude of the variation of the RBE with changes in the energy spectrum of x-ray beams and dose delivered by conventional cabinets or image-guided small animal radiotherapy platforms.

Current available resources

While there are a number of codes of practice (CoPs) and guidelines designed for dosimetry measurements in low- and



B

TABLE: Underestimation of dose at 2.5 mm depth in Petri Dish placed on 4 mm thick Al shelf from the full scatter conditions

	nominal beam energy		
beam filtration	**160 kV**	**225 kV**	**350 kV**
filtered	19.9%	19.9%	12.4%
unfiltered	16.5%	18.8%	18.6%

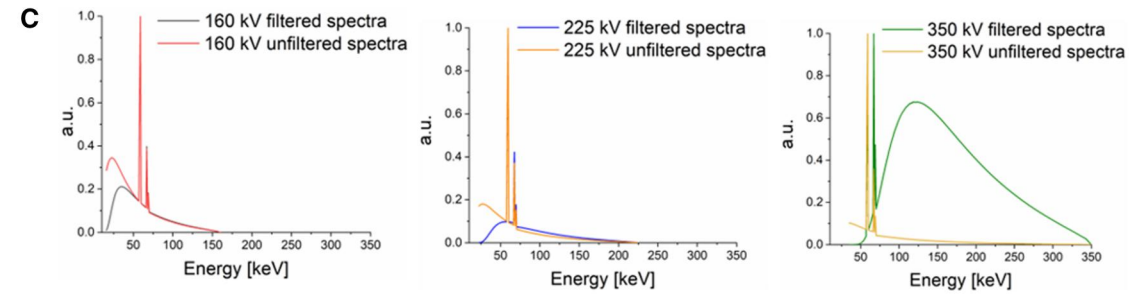


Figure 4. Demonstration of the effect of lack of full backscatter conditions on the determination of dose delivered (at 2.5 mm depth) to a Petri dish filled with a tissue culture media. (A) Petri dish placed on top of a 4-mm-thick aluminium shelf or on top of a 10 cm × 10 cm × 10 cm water phantom (full backscatter conditions), (B) Monte Carlo calculations of dose underestimation specifically performed to illustrate the effect of the scattering conditions for this paper (data available on request). Note: the beam diameter was set to 15 cm at the sample surface and (C) the filtered and unfiltered spectral shapes for X-rays at 160 kV (2 mm Al filter), 225 kV (0.3 mm Cu filter), and 350 kV (1.5 mm Al, 0.25 mm Cu, and 0.75 mm Sn filters).

medium-energy X-rays for radiotherapy and radiobiology,[42-44] such documents often relate to the determination of air-kerma or absorbed dose, at the surface or at a given depth in a large-idealized water phantom, conditions that are not realizable inside conventional radiobiological X-ray cabinets. Dosimetry protocols that would cover specific aspects of commissioning and reference dosimetry in the confined spaces of the X-ray biological irradiators are not currently available. However, some guidance can be found in EULEP-EURODOS protocols for X-ray dosimetry in radiobiology.[30] Several published studies[45-52] have addressed the limitations of CoPs designed for dosimetry in clinical low and medium-energy X-rays devices (eg, IPEMB,[42] IAEA TRS 398,[43] AAPM TG-61[44]) at the time of performing dosimetry in X-ray cabinets. Even though some had suggested adjustments and practical approaches to comply with international recommendations and ensure traceability and reproducibility of measurements, these are not straightforward to implement. Currently, the AAPM Task Group 319[53] is aiming to address these issues by suggesting new standards on how to perform and report experimental dosimetry in kilovoltage cabinet irradiators. However, these new guidelines are not yet published and will take time to implement, particularly as not all laboratories will have the resources to implement the suggested changes.

There is a collective agreement that users of conventional X-ray cabinets employed for *in vitro* and *in vivo* exposures should have access to appropriate dosimetry systems (see Table S1). Among these, a reference system, with a calibration traceable to a Secondary Standard Dosimetry Laboratory (SSDL) [https://ssdl.iaea.org/Home/Members] or to a Primary Standard Laboratory, as is the case of the UK National Physical Laboratory (NPL), should be available. Depending on their type, dosimetry systems would be used for absorbed dose determination (see footnote (a) in Table 1 about the quantities in which dosimeters are calibrated) and/or for measurements of beam parameters (eg, dose depth curves, beam profiles). Such measurements should be structured in a comprehensive QA programme that would regularly check the correct operation of the X-ray unit. The determination of the beam quality requires special attention, and it is recommended that it is assessed in terms of HVL, by adopting the setup suggested in the CoPs (ie, AAPM TG-6144 or Appendix C in the IPEMB42). However, accuracy of the measurements may be limited by the lack of space to fulfil the required measurement geometry and because not all irradiators have cable feed-throughs.

Considering the usually small dimension in the direction of the beam incidence of some radiobiological samples, such as the cell plates, assessment of dose-to-water at the surface of the sample is generally recommended. That can be achieved by placing a calibrated ionization chamber, in air, at the distance where the samples would be placed and following the in-air formalism as recommended by the aforementioned CoPs. That includes using the appropriate correction factors to account for the effect of (i) the lack of backscatter from the supporting shelves (which depends on its material and thickness), (ii) the radiation field size, and (iii) the attenuation in the materials of the sample. If preclinical samples are placed in irradiation conditions that simulate sufficient backscatter, useful data for the estimation of the dose absorbed at different depths within the sample can be found in the *British Journal of Radiology* Supplement 25.[54] For more complex irradiation geometries as may occur during preclinical *in vivo* research, a validation of the dosimetry chain, up to the dose at a point of interest inside the sample, will strengthen the value of the experimental results. For those cases, including exposure of partially shielded samples, the validation will require the use of appropriate detectors placed in the position (s) of interest inside phantoms that mimic the irradiation conditions. Detectors for these dosimetric validations should be selected based upon their response in similar irradiation conditions and in terms of their energy dependence, linearity with dose and dose rate response, among other characteristics. That type of information can be gathered from a literature review,[51] or from measurements performed in beams with similar characteristics to that of the irradiation cabinets.[55,56] Examples of detectors and their properties are presented in Table S1.

Ultimately, the selection of a dosimetry protocol depends on the specific application, the type of radiation beam, and the samples to be irradiated. While CoPs and general guidelines can provide a reference framework, for bespoke irradiations or complex irradiation setups, users should consult with experts from medical physics departments at universities, hospitals, or metrology institutes.

Independent validation of reference dosimetry and end-to-end types of verification across different research centres and/or radiation devices using a reference dosimetry system also provide a means to ensure that the units are correctly calibrated as well as confidence in the experimental results. They also help to identify where improvements can be made. This is standard practice in clinical settings, and it would be beneficial for preclinical centres too. Small animal phantoms[22,23,57,58] have been developed for such purpose, and more recently, phantoms resembling basic plasticware have been created using 3D printing or bolus materials. Such types of phantoms can be designed to accommodate certain types of passive or active detectors as described in Table S1 (eg, alanine and thermoluminescent dosimeters (TLDs)). Multi-institutional end-to-end tests are a reliable method to investigate sources of error and to obtain a better understanding of the accuracy of the dose delivered by the different participating centres.[59,60]

Finally, Monte Carlo simulations provide a useful tool to link a limited number of point dosimetry measurements to detailed 3D dose distributions through the irradiated sample.[25,46] This is particularly useful in more complex sample geometries, such as rodents. The application of modelling techniques can improve the reproducibility of reporting dosimetry findings in preclinical radiobiological research. This is a methodology that is complex to implement and currently not widely available to the radiobiological research community; however, this is currently being addressed.[25]

Summary of recommendations suggested by this working party

Accurate dosimetry is crucial in radiation biology research to ensure that the intended doses are delivered to the samples. This enables meaningful and reproducible results, including the inter-laboratory validation of irradiation techniques, as required to fully exploit the resulting biological and preclinical data in their successful translation into clinical trials. The following recommendations are proposed to facilitate meaningful radiation biology research. These recommendations are focused on the use of X-ray cabinets; however, with certain variations, mostly determined by differences in the

delivery workflow, they could also be applicable to image-guided small animal irradiators (with conventional and variable motorized collimators) as well as to γ-ray irradiators:

- *Staff qualification and training. Interdisciplinary approach:* It is important that leading researchers hire qualified staff, not only capable of utilizing the system but also to maintain adequate QA programmes for the irradiators. It is essential to formalize collaborations between medical/radiation physicists and radiobiologists to maintain robust dosimetry and QA programmes locally and nationally. It is important that the staff have the adequate training to be able to tailor the dosimetry approach to the specific requirements and types of experiments of each institution by adapting the existing CoPs and fully reporting the adopted procedures. Professional integration is crucial for further developing preclinical radiation research and its standardization.
- *Use of reference dosimetry systems:* It is essential that any dosimetry system used for reference measurements is appropriately calibrated, with the calibration traceable to a national/international standards laboratory (eg, NPL within the United Kingdom).
- *Dosimetry protocols:* To ensure accuracy and consistency of radiation protocols, appropriate dosimetry systems are needed to measure and/or verify the dose delivered to the samples. Verification measurements should be performed as close to the experimental setup as possible. If possible, detectors that required minimal corrections should be used. For cell irradiations, this may include radiochromic films (such as Gafchromic EBT4 film) in the positions of the cells. For verification of mice irradiations, this would include using an appropriate dosimeter (eg, alanine, TLD, film) in a tissue-equivalent mouse phantom. If detailed dosimetry measurements are not possible or available or 3D distributions required, then Monte Carlo calculations[25,46] should be considered.
- *Accurate reporting:* It is essential that the experimental details and associated dosimetry are accurately reported and in sufficient detail to ensure that the irradiation experiments can be replicated locally or by other laboratories. This will also allow subsequent reanalysis of the dose distributions and associated data. Detailed recommendations can be found in Desrosiers *et al.*[16] and the ESTRO-ACROP recommendations.[17] Appropriate reporting should be a prerequisite for acceptance of a manuscript for publication as it has been implemented by *Radiation Research* and the *International Journal of Radiation Biology*.[18,19] An overview of what is required is presented in Table 1.
- *Quality assurance:* Support should be provided during the procurement process of preclinical irradiators. Additionally, independent support-such as from medical physics or bioengineering specialists- should be available during the installation to ensure unbiased oversight. This will facilitate the device acceptance testing and that base line parameter records should be maintained for future performance comparisons. A comprehensive QA program must be established to ensure that the dose delivered corresponds to the dose planned. Reference absorbed dose measurements (in conditions recommended by the manufacturer or established CoPs) should ideally be performed once a year. The output stability of the irradiator should be frequently checked using dose rate measurements in a known, repeatable configuration. Regular checks for the beam quality, dose profiles, field size, and homogeneity should also be performed.
- *Regular audits and documentation:* We recommend that a national dosimetry service is available to institutions performing *in vitro* and *in vivo* radiation research. In addition to providing absolute traceable dosimetry measurements, such a service could implement a system of regular and independent dosimetry audits, including end-to-end type of tests. It could also provide support and guidance on the QA, and the purchase process for irradiation facilities and measurement equipment as well as on the design of irradiation setups and associated dosimetry. For centres without local medical/radiation physics support, it could supply technical expertise and equipment to perform reference measurements and dosimetry for specific experimental arrangements. Periodic revisions of the dosimetry data will help to highlight and address any issues and discrepancies.
- *Funding:* It is important that funding bodies are aware of the requirements and that provision for appropriate dosimetry support is a prerequisite for funding. While applying for grants, principal investigators should provide rationale for appropriate funds to cover the time and resources needed to conduct appropriate dosimetry assessment. Financial support for a multidisciplinary approach is paramount to the success of translatable preclinical radiation research.

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Supplementary material

Supplementary material is available at *BJR* online.

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Conflicts of interest

None declared.

References

1. Coleman CN, Higgins GS, Brown JM, et al. Improving the predictive value of preclinical studies in support of radiotherapy clinical trials. *Clin Cancer Res*. 2016;22:3138-3147. https://doi.org/10.1158/1078-0432.CCR-16-0069
2. Draeger E, Sawant A, Johnstone C, et al. A dose of reality: how 20 years of incomplete physics and dosimetry reporting in radiobiology studies may have contributed to the reproducibility crisis. *Int J Radiat Oncol Biol Phys*. 2020;106:243-252. https://doi.org/10.1016/j.ijrobp.2019.06.2545
3. Wong J, Armour E, Kazanzides P, et al. High-resolution, small animal radiation research platform with x-ray tomographic guidance capabilities. *Int J Radiat Oncol Biol Phys*. 2008;71:1591-1599. https://doi.org/10.1016/j.ijrobp.2008.04.025
4. Clarkson R, Lindsay PE, Ansell S, et al. Characterization of image quality and image-guidance performance of a preclinical microirradiator. *Med Phys*. 2011;38:845-856. https://doi.org/10.1118/1.3533947
5. Brown KH, Ghita M, Dubois LJ, et al. A scoping review of small animal image-guided radiotherapy research: advances, impact and future opportunities in translational radiobiology. *Clin Transl Radiat Oncol*. 2022;34:112-119. https://doi.org/10.1016/j.ctro.2022.04.004
6. Verhaegen F, Butterworth KT, Chalmers AJ, et al. Roadmap for precision preclinical x-ray radiation studies. *Phys Med Biol*. 2023;68:06RM01. https://doi.org/10.1088/1361-6560/acaf45
7. Barnard S, Ainsbury E, Daniels T, Eakins J, Tanner R, Bouffler S. Alternatives to caesium irradiators for biological sciences and blood transfusion services. 2020. Accessed November 28, 2024. https://www.ukhsa-protectionservices.org.uk/cms/assets/gfx/content/resource_4633cs96c974552b.pdf
8. Murphy MK, Kamen J. Deciding between an X-ray and 137Cs irradiator—it's not just about energy spectra. *Radiat Res*. 2019;192:493-506. https://doi.org/10.1667/RR15395.1
9. Park E, Evans MA, Doviak H, et al. Bone marrow transplantation procedures in mice to study clonal hematopoiesis. *J Vis Exp*. 2021;171:e61875. https://doi.org/10.3791/61875
10. Zuro D, Madabushi SS, Brooks J, et al. First multimodal, three-dimensional, image-guided total marrow irradiation model for preclinical bone marrow transplantation studies. *Int J Radiat Oncol Biol Phys*. 2021;111:671-683. https://doi.org/10.1016/j.ijrobp.2021.06.001
11. Thwaites D. Accuracy required and achievable in radiotherapy dosimetry: have modern technology and techniques changed our views? *J Phys Conf Ser*. 2013;444:012006. https://doi.org/10.1088/1742-6596/444/1/012006
12. Monson LA, Jing XL, Donneys A, Farberg AS, Buchman SR. Dose-response effect of human equivalent radiation in the mandible. *J Craniofac Surg*. 2013;24:1593-1598. https://doi.org/10.1097/SCS.0b013e31826cfeea
13. Ghandhi SA, Smilenov L, Shuryak I, Pujol-Canadell M, Amundson SA. Discordant gene responses to radiation in humans and mice and the role of hematopoietically humanized mice in the search for radiation biomarkers. *Sci Rep*. 2019;9:19434. https://doi.org/10.1038/s41598-019-55982-2
14. Williams JP, Brown SL, Georges GE, et al. Animal models for medical countermeasures to radiation exposure. *Radiat Res*. 2010;173:557-578. https://doi.org/10.1667/RR1880.1
15. McBride WH, Chiang CS, Olson JL, et al. A sense of danger from radiation. *Radiat Res*. 2004;162:1-19. https://doi.org/10.1667/rr3196
16. Desrosiers M, DeWerd L, Deye J, et al. The importance of dosimetry standardization in radiobiology. *J Res Natl Inst Stand Technol*. 2013;118:403-418. https://doi.org/10.6028/jres.118.021
17. Verhaegen F, Dubois L, Gianolini S, et al. ESTRO ACROP: technology for precision small animal radiotherapy research: optimal use and challenges. *Radiother Oncol*. 2018;126:471-478. https://doi.org/10.1016/j.radonc.2017.11.016
18. Trompier F, DeWerd LA, Poirier Y, et al. Minimum reporting standards should be expected for preclinical radiobiology irradiators and dosimetry in the published literature. *Int J Radiat Biol*. 2024;100:1-6. https://doi.org/10.1080/09553002.2023.2250848
19. Poirier Y, DeWerd LA, Trompier F, et al. Minimum reporting standards should be expected for preclinical radiobiology irradiators and dosimetry in the published literature. *Radiat Res*. 2023;200:217-222. https://doi.org/10.1667/RADE-23-00119.1
20. Almeida A, Togno M, Ballesteros-Zebadua P, et al. Dosimetric and biologic intercomparison between electron and proton FLASH beams. *Radiother Oncol*. 2024;190:109953. https://doi.org/10.1016/j.radonc.2023.109953
21. Polizzi M, Valerie K, Kim S. Commissioning and assessment of radiation field and dose inhomogeneity for a dual X-ray tube cabinet irradiator: to ensure accurate dosimetry in radiation biology experiments. *Adv Radiat Oncol*. 2024;9:101486. https://doi.org/10.1016/j.adro.2024.101486
22. Soultanidis G, Subiel A, Renard I, et al. Development of an anatomically correct mouse phantom for dosimetry measurement in small animal radiotherapy research. *Phys Med Biol*. 2019;64:12NT02. https://doi.org/10.1088/1361-6560/ab215b
23. Price G, Biglin ER, Collins S, et al. An open source heterogeneous 3D printed mouse phantom utilising a novel bone representative thermoplastic. *Phys Med Biol*. 2020;65:10NT02. https://doi.org/10.1088/1361-6560/ab8078
24. Schyns LE, Eekers DB, van der Heyden B, Almeida IP, Vaniqui A, Verhaegen F. Murine vs human tissue compositions: implications of using human tissue compositions for photon energy absorption in mice. *Br J Radiol*. 2019;92:20180454. https://doi.org/10.1259/bjr.20180454
25. Hill MA, Staut N, Thompson JM, Verhaegen F. Dosimetric validation of smart-RAD monte carlo modelling for x-ray cabinet radiobiology irradiators. *Phys Med Biol*. 2024;69:095014. https://doi.org/10.1088/1361-6560/ad3720
26. Pedersen KH, Kunugi KA, Hammer CG, Culberson WS, DeWerd LA. Radiation biology irradiator dose verification survey. *Radiat Res*. 2016;185:163-168. http://www.jstor.org/stable/44028548
27. Silvestre Patallo I, Carter R. *AGO HS X-Ray Irradiation System. Relative Film Dosimetry Assessment*. University College London, Cancer Institute, Internal Report; 2019.
28. Poirier Y, Belley MD, Dewhirst MW, Yoshizumic TT, Down JD. Transitioning from gamma rays to X rays for comparable biomedical research irradiations: energy matters. *Radiat Res*. 2020;193:506-511. https://doi.org/10.1667/RADE-20-00039.1
29. Segars WP, Tsui BMW, Frey EC, Johnson GA, Berr SS. Development of a 4-D digital mouse phantom for molecular imaging research. *Mol Imaging Biol*. 2004;6:149-159. https://doi.org/10.1016/j.mibio.2004.03.002
30. Zoetelief J, Broerse JJ, Davies RW, et al. Protocol for X-ray dosimetry in radiobiology. *Int J Radiat Biol*. 2001;77:817-835. https://doi.org/10.1080/09553000110050605
31. Hood SL, Norris G. Dosimetry of human cell cultures irradiated at the interface in plastic and in glass dishes. *Radiat Res*. 1961;14:705-712. https://doi.org/10.2307/3571011

32. Furre T, Bergstrand ES, Pedersen E, et al. Measurement of dose rate at the interface of cell culture medium and glass dishes by means of ESR dosimetry using thin films of alanine. *Radiat Res*. 1999;152:76-82. https://doi.org/10.2307/3580052
33. Ghita M, McMahon SJ, Thompson HF, et al. Small field dosimetry for the small animal radiotherapy research platform (SARRP). *Radiat Oncol*. 2017;12:204. https://doi.org/10.1186/s13014-017-0936-3
34. Feddersen TV, Rowshanfarzad P, Abel TN, Ebert MA. Commissioning and performance characteristics of a pre-clinical image-guided radiotherapy system. *Australas Phys Eng Sci Med*. 2019;42:541-551. https://doi.org/10.1007/s13246-019-00755-4
35. Palmans H, Andreo P, Huq MS, Seuntjens J, Christaki KE, Meghzifene A. Dosimetry of small static fields used in external photon beam radiotherapy: summary of TRS-483, the IAEA–AAPM international code of practice for reference and relative dose determination. *Med Phys*. 2018;45:e1123-e1145. https://doi.org/10.1002/mp.13208
36. Selvaraj J, Rhall G, Ibrahim M, et al. Custom-designed Small Animal focal iRradiation Jig (SARJ): design, manufacture and dosimetric evaluation. 2020;2:20190045. https://doi.org/10.1259/bjro.20190045
37. Verhaegen F, Granton P, Tryggestad E. Small animal radiotherapy research platforms. *Phys Med Biol*. 2011;56:R55-R83. https://doi.org/10.1088/0031-9155/56/12/R01
38. Tillner F, Thute P, Bütof R, Krause M, Enghardt W. Pre-clinical research in small animals using radiotherapy technology—a bidirectional translational approach. *Z Med Phys*. 2014;24:335-351. https://doi.org/10.1016/j.zemedi.2014.07.004
39. Chen Q, Molloy J, Izumi T, Sterpin E. Impact of backscatter material thickness on the depth dose of orthovoltage irradiators for radiobiology research. *Phys Med Biol*. 2019;64:055001. https://doi.org/10.1088/1361-6560/ab0120
40. Nikjoo H, Lindborg L. RBE of low energy electrons and photons. *Phys Med Biol*. 2010;55:R65-R109. https://doi.org/10.1088/0031-9155/55/10/R01
41. Hill MA. The variation in biological effectiveness of X-rays and gamma rays with energy. *Radiat Prot Dosimetry*. 2004;112:471-481. https://doi.org/10.1093/rpd/nch091
42. Klevenhagen SC, Aukett RJ, Harrison RM, Moretti C, Nahum AE, Rosser KE. The IPEMB code of practice for the determination of absorbed dose for x-rays below 300 kV generating potential (0.035 mm Al-4 mm Cu HVL; 10-300 kV generating potential). *Phys Med Biol*. 1996;41:2605-2625. https://doi.org/10.1088/0031-9155/41/12/002
43. Andreo P, Burns DT, Hohlfeld K, et al. Absorbed dose determination in external beam radiotherapy: an international code of practice for dosimetry based on standards of absorbed dose to water (V.12). *IAEA Technical Report Series* No *398*. Published online 2006. Accessed November 28, 2024.
44. Ma CM, Coffey CW, DeWerd LA, et al.; American Association of Physicists in Medicine. AAPM protocol for 40-300 kV x-ray beam dosimetry in radiotherapy and radiobiology. *Med Phys*. 2001;28:868-893. https://doi.org/10.1118/1.1374247
45. Wegner M, Frenzel T, Krause D, Gargioni E. Development and characterization of modular mouse phantoms for end-to-end testing and training in radiobiology experiments. *Phys Med Biol*. 2023;68:085009. https://doi.org/10.1088/1361-6560/acc566
46. Mahuvava C, Esplen NM, Poirier Y, Kry SF, Bazalova-Carter M. Dose calculations for preclinical radiobiology experiments conducted with single-field cabinet irradiators. *Med Phys*. 2022;49:1911-1923. https://doi.org/10.1002/mp.15487
47. McKerracher C, Thwaites DI. Calibration of an x-ray cabinet unit for radiobiology use. *Phys Med Biol*. 2006;51:3315-3329. https://doi.org/10.1088/0031-9155/51/14/002
48. Woo MK, Nordal RA. Commissioning and evaluation of a new commercial small rodent x-ray irradiator. *Biomed Imaging Interv J*. 2006;2:e10. https://doi.org/10.2349/biij.2.1.e10
49. Esplen N, Therriault-Proulx F, Beaulieu L, Bazalova-Carter M. Preclinical dose verification using a 3D printed mouse phantom for radiobiology experiments. *Med Phys*. 2019;46:5294-5303. https://doi.org/10.1002/mp.13790
50. Subiel A, Patallo IS, Palmans H, et al. The influence of lack of reference conditions on dosimetry in pre-clinical radiotherapy with medium energy x-ray beams. *Phys Med Biol*. 2020;65:085016. https://doi.org/10.1088/1361-6560/ab7b30
51. Hill R, Healy B, Holloway L, Kuncic Z, Thwaites D, Baldock C. Advances in kilovoltage x-ray beam dosimetry. *Phys Med Biol*. 2014;59:R183-R231. https://doi.org/10.1088/0031-9155/59/6/R183
52. Lawless MJ, Dimaso L, Palmer B, Micka J, Culberson WS, DeWerd LA. Monte Carlo and (60) co-based kilovoltage x-ray dosimetry methods. *Med Phys*. 2018;45:5564-5576. https://doi.org/10.1002/mp.13213
53. Task Group No. 319 - guidelines for accurate dosimetry in radiation biology experiments (TG319). 2018. Accessed January 2, 2024. https://www.aapm.org/org/structure/?committee_code=TG319
54. BJR. *Supplement 25: Central Axis Depth Dose Data for Use in Radiotherapy*. London: BJR; 1996.
55. Silvestre Patallo I, Subiel A, Carter R, Flynn S, Schettino G, Nisbet A. Characterization of inorganic scintillator detectors for dosimetry in image-guided small animal radiotherapy platforms. *Cancers (Basel)*. 2023;15:987. https://doi.org/10.3390/cancers15030987
56. Le Deroff C, Frelin-Labalme AM, Ledoux X. Characterization of a scintillating fibre detector for small animal imaging and irradiation dosimetry. *Br J Radiol*. 2017;90:20160454. https://doi.org/10.1259/bjr.20160454
57. Biglin ER, Price GJ, Chadwick AL, Aitkenhead AH, Williams KJ, Kirkby KJ. Preclinical dosimetry: exploring the use of small animal phantoms. *Radiat Oncol*. 2019;14:134-110. https://doi.org/10.1186/s13014-019-1343-8
58. Esplen N, Alyaqoub E, Bazalova-Carter M. Technical note: manufacturing of a realistic mouse phantom for dosimetry of radiobiology experiments. *Med Phys*. 2019;46:1030-1036. https://doi.org/10.1002/mp.13310
59. Biglin ER, Aitkenhead AH, Price GJ, et al. A preclinical radiotherapy dosimetry audit using a realistic 3D printed murine phantom. *Sci Rep*. 2022;12:6826-6813. https://doi.org/10.1038/s41598-022-10895-5
60. Silvestre Patallo I, Subiel A, Westhorpe A, et al. Development and implementation of an end-to-end test for absolute dose verification of small animal preclinical irradiation research platforms. *Int J Radiat Oncol Biol Phys*. 2020;107:587-596. https://doi.org/10.1016/j.ijrobp.2020.03.001

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References:
1. Gacci M et al. Bladder Instillation Therapy with Hyaluronic Acid and Chondroitin Sulphate Improves Symptoms of Postradiation Cystitis: Prospective Pilot Study. Clin Genitourin Cancer 2016; Oct;14(5):444-449. 2. Giannessi C et al. Nocturia Related to Post Radiation Bladder Pain can be Improved by Hyaluronic Acid Chondroitin Sulfate (iAluRil). Euro Urol Suppl 2014; 13: e592. 3. UK IQVIA data (accessed August 2024)

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