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1 **Commentary: Minimum Reporting Standards Should be Expected for Preclinical Radiobiology**

2 **Irradiators and Dosimetry in the Published Literature**

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24 **Introduction**

25 Preclinical researchers have long acknowledged the critical need for accurate dosimetry in the
26 conduct of radiation-exposure studies involving living systems. Although radiation dosimetry is required to
27 be National Institute for Standards and Technology (NIST)-traceable for human irradiation protocols, there
28 were previously only limited requirements stipulating accurate radiation exposures for preclinical radiation
29 studies funded by the National Institutes of Health (NIH). In one of the earliest identified publications
30 (1956) that addressed radiation dosimetry in murine studies, Hurst *et al.* (1) discussed their calculations
31 surrounding estimating neutron responses, and “distribution of of absorbed energy versus LET”. In 2009,
32 Zoetelief *et al.* (2) outlined European efforts to harmonize X-ray dosimetry studies in radiation biology,
33 which resulted in the establishment of a protocol to improve dosimetry oversight and allow for better
34 comparison of results across different institutions. It is, however, unclear if these early efforts substantially
35 changed the approach of biologists to ensuring accurate and reproducible dosimetry in their radiation
36 research.

37 The lack of consideration of dosimetry in *in vitro* and *in vivo* work was further explored in 2011
38 during the “Radiation Dose is More than a Number” workshop convened by NIST, and co-sponsored by the
39 National Institute of Allergy and Infectious Diseases (NIAID), and the National Cancer Institute (NCI). The
40 report that followed (3) made nine recommendations that ranged from coordinating with radiation physicists
41 during the experimental design phase and including more setup and dosimetry details in publications, to
42 establishing working groups to develop protocols, and implementing formal dosimetry intercomparison
43 programs. In an attempt to address the latter suggestion, and ensure rigor and reproducibility in their funded
44 research portfolio consistent with NIH grants policy¹, the NIAID Radiation and Nuclear Countermeasures
45 Program (RNCP) released a request for proposals (NIAID-NIH-RFP-NIHAI201800020²) in 2019, seeking
46 to make a single contract award for “RNCP-Wide Dosimetry Guidance & Monitoring of Sources and
47 Irradiation Protocols”. This funding opportunity requested that offerors develop a consistent means of
48 dosimetry comparison and reproducibility, as well as provide the administrative foundation necessary to

¹ <https://grants.nih.gov/policy/reproducibility/index.htm>

² <https://sam.gov/opp/360d070bdf5d437f83e88c13442fa23f/view>

49 facilitate and coordinate dosimetry activities in partnership with the NIAID. Respondents were asked to
50 provide services, facilities, expertise, and capabilities to develop a centralized dosimetry harmonization
51 effort that fits the resources and circumstances of projects across the funded RNCP portfolio. The contract
52 award was made in 2020 to the University of Wisconsin (Principal Investigator Larry DeWerd), who
53 established a harmonization protocol to encompass all the irradiators in use across the RNCP-funded
54 portfolio of grants, contracts, and inter-agency agreements.

55 In 2019, the RNCP also became concerned with the specifics of establishing and reporting neutron
56 dosimetry and convened a “Neutron Radiobiology and Dosimetry Workshop” with presentations from 17
57 subject matter experts, planned in collaboration with the Department of Defense (DoD), Defense Threat
58 Reduction Agency (DTRA), and the National Aeronautics and Space Administration (NASA). The meeting
59 report that was published in 2021 (4) explored historical neutron exposure research and highlighted the need
60 for harmonized reporting of exposure and experimental parameters. The authors concluded that efforts
61 should be made to update computer coding for neutron dosimetry estimates, government policies needed to
62 be re-visited in terms of expected radiation energy spectrums, and funding agencies must make key
63 investments in consideration of neutron dosimetry, to improve science and ensure radiation emergency
64 preparedness.

65 All of these early attempts to ensure the use of accurate dosimetry in research laboratories
66 culminated in the current effort to take the next step and make strong recommendations that sufficient
67 information be included in manuscripts or supplemental data files, such that reviewers and editors can be
68 assured that the irradiations were carried out correctly, and so readers of the published work have the
69 necessary information to replicate published experiments.

70

71 **Background and Rationale for Dosimetry Reporting**

72 Good science must be well described to be understood, compared, and replicated. For radiation
73 biology, this requires the combination of both biology and radiation physics – two very different expertises,
74 typically provided by different individuals with specialized training. While biological systems can be

75 difficult to control, particularly for *in vivo* models, radiation follows well-understood physical processes,
76 which can be controlled and measured to a very high level of accuracy, precision, and reproducibility.
77 Therefore, it is both feasible, and important, to require radiobiological studies to describe their irradiation
78 protocol.

79 Unfortunately, experimental irradiation protocols are currently not well described in the majority of
80 published radiobiology studies. This was already true in 2011, during the original NIST workshop (3), and
81 has since been confirmed by multiple meta-studies showing no apparent improvements in reporting since
82 the original symposium (5-9). In a recent analysis of 1758 published pre-clinical studies employing ionizing
83 radiation, some parameters, such as radiation dose, are nearly always provided (>94%). Many important
84 details, such as the equipment producing the radiation, the radiation type, beam energy (or quality), and dose
85 rate, are usually, but not always, reported (>50-80%). Details pertaining to experimental design, such as the
86 number and geometry of the radiation fields, the use of shaping collimators, or rotating tables to ensure
87 uniform delivery to multiple animals, are seldom shared (>25%). Finally, details relating to experimental
88 validation of the delivered radiation dose through some form of dosimeter (ideally traceable to national
89 standards), are nearly always absent (<15%). Reference to published standards or protocols was as low as
90 1.3% of published studies in Draeger *et al.* (9).

91 As originally illustrated in the report following the NIST workshop, “investigators cannot report
92 what they have not considered.”(3). When experiments are not described, a reviewer cannot assess what an
93 author might or might not have considered, or the soundness of the experimental design at all. In some
94 instances, metastudies reveal that certain publications contained glaring contradictions in their irradiation
95 protocols. These include descriptions of irradiators that would be incapable of producing the experiment
96 described in the methods, non-existent radionuclides, or typographical errors (e.g., kV to MV), which
97 translate into a thousand-fold difference in energy. These findings suggest that many studies may be
98 erroneously described, even in cases where there is no clear contradiction in the few details available. For
99 instance, irradiating multiple mice placed at varying distances from the center of the field will often result in

100 a different dose (and therefore a different biological effect) delivered to each, but this will not be apparent in
101 a manuscript that simply describes the source used and dose prescribed.

102 In a vacuum, a lack of rigor in describing experimental protocols might not imply anything, but
103 insufficient communication among authors, or a propensity to copy and combine old protocols without
104 sufficient rigor can be problematic. When combined with repeated reports outlining difficulties in
105 harmonizing radiation dosimetry across separate laboratories, (2, 7, 8) a more concerning picture emerges.
106 As stated in the original NIST report, “Few students or researchers using ionizing radiation in biological
107 research have training in basic radiation physics. This leads to the difficult situation that when ‘one does not
108 know what one does not know’ dosimetry design and documentation go unaddressed”. And if it is not
109 described, a journal or grant reviewer cannot assess whether the experiment is sound.

110 One particular concept not initially addressed in the first NIST workshop concerns the definition of
111 radiation dose. Indeed – not all dose is created equal. An X-ray tube can be calibrated in terms of exposure,
112 air kerma, or adsorbed dose to water (D_w), depending on which correction factors are used. Reference
113 dosimetry can assume, or not, the presence of backscatter. Or, an investigator can refer to the radiation dose
114 being delivered to animals under experimental conditions. Each of these descriptions is scientifically sound,
115 and yet can lead to differences of up to 50% in reported doses. As described in the next section, the use of
116 Roentgen (exposure) vs dose for ^{137}Cs exposures will introduce an error of ~11%. Without further
117 description, it is impossible to know which quantity an investigator is referring to. To address this, the
118 American Association of Physicists in Medicine (AAPM)’s Task Group 319 is developing new standards on
119 how to perform and report experimental dosimetry in kilovoltage cabinet irradiators. However, these new
120 guidelines have yet to be published, and their implementation will not be instantaneous. In the meantime,
121 investigators should be careful in how they describe their irradiation protocol in scientific communications.

122
123 **U.S. Efforts in Dosimetry Harmonization**

124 Inconsistent dosimetry was first outlined by the 2011 NIST panel³, and follow-up publications in
125 2016 by Seed, *et al.*, (8) and Pedersen, *et al.* (7) suggested that only one in five laboratories deliver dose
126 within 5% of a specified target level, with variations up to 42% observed. From 1971 to 1999, the EULEP-
127 EURADOS initiative performed 8 similar intercomparisons and consistently found errors of up to ~20%,
128 and found the majority of institutions could not satisfy a $\leq 10\%$ homogeneity requirement (10). Radiation
129 biology research often employs orthovoltage X-ray systems and ¹³⁷Cs sources where dosimetry is based on
130 manufacturer recommendations. For X-rays, results may not be traceable to NIST standard beams because
131 of the differing maximum beam energy and the filtration used for a certain energy. In addition to traceability
132 issues, low-energy X-rays introduce variability in dosimetry and radiobiological response in multiple ways.
133 First, low energy bremsstrahlung radiation is more readily absorbed in tissues than are higher energy
134 photons from isotope irradiators. Second, low-energy X-rays are more readily absorbed by cortical bone,
135 inducing differential biological effects in bone marrow and different biological responses (11-13).

136 From 2020 to 2023, The NIAID/RNCP program for harmonization of radiation dose evaluated >30
137 irradiators (X-ray and cesium), and found that upon initial survey, 50% of machines were outside of $\pm 5\%$ of
138 the targeted 4 Gy dose, the benchmark for acceptable irradiation, as is shown in Figure 1. Traceable NIST
139 beams for X-ray beams were used for cabinet X-ray systems by matching half value layers (HVL) for
140 radiobiological X-ray beams, although kVp and second HVL are much different. Phantoms for total-body,
141 and partial-body irradiation were established, and dose was measured using LiF:Mg,Ti thermoluminescent
142 dosimeters (TLDs) calibrated using the NIST-traceable HVL matched X-ray beams.

143 Variations from the 4 Gy target dose ranged from +/-12% to 25%. After consultations and retesting,
144 results for nearly all institutions were brought within the required 5% of target dose, and doses were
145 resolved for the remaining institutions after consideration of physics issues. Also, precision mapping of the
146 radiation field using multiple TLDs was important and permitted the resolution of dose discrepancies that
147 aligned with variations in dose. To summarize, there are three problems that are frequently encountered at
148 institutions that must be addressed. First, the correct physics dose quantity and other characterizations

³ <https://www.nist.gov/programs-projects/medical-archive-workshop-radiobiology-dosimetry-standardization>.

149 should be employed, especially for X-ray machines. An example of this that was found with some
150 radionuclide sources involving a dose discrepancy of ~11 %. The institutions in question were using the
151 manufacturer-supplied radiation exposure quantity in Roentgens, rather than the absorbed dose to water; and
152 the correction needed to achieve absorbed dose to water was 11% - matching the observed error perfectly. A
153 second problem is not understanding the physics or the measurements given; consultation with a medical
154 physicist would be of great benefit, but not all sites conducting radiation biology research have access to the
155 support of a certified medical physicist. Of note, not all certified medical physicists are familiar with
156 irradiation conditions and calibration protocols of preclinical systems, which can be vastly different than
157 clinical systems. Finally, an inadequate understanding of dose distribution within the radiation field,
158 especially in cabinet-style orthovoltage X-ray irradiators, is a significant problem. It is important to
159 understand the isodose plot of the field, and in many cases, rotation of the subject animals is necessary to
160 average out the dose. The goal of this NIAID-funded program is the harmonization of dose among
161 institutions regardless of the source used to establish the radiation.

162

163 **French Efforts in Dosimetry Harmonization**

164 In France, the Institute of Radiological Protection and Nuclear Safety (IRSN) has a long tradition of
165 collaboration between dosimetrists and radiobiology researchers. Technical support has always been
166 provided to define the best irradiation conditions and reference dose rates, whether for gamma irradiators,
167 conventional X-ray facilities, or for irradiation at reactors (e.g., the SILENE facility in Valduc, France) with
168 a concern for the traceability of these data. A dose rate in the most appropriate dosimetric quantity and a
169 homogeneity level are defined for each irradiation configuration. For example, for cell irradiations, a
170 configuration is defined for a given container, filling volume, and number of containers. It is not only a
171 question of defining a reference rate in D_w or in air kerma according to the reference protocols in use (such
172 as the AAPM's TG-61 or the IAEA TRS-398), but also taking into account factors that can affect the dose
173 rate, and defining it at the level of the biological material of interest (14). For small animals, whether for
174 total-body or localized irradiations, specific phantoms are produced using molds. For programs involving

175 study of bone tissue, electron paramagnetic resonance (EPR) spectroscopy, which enables the dose actually
176 delivered to the bone to be estimated, has been introduced (15). These skills have been regularly made
177 available to other national or European research institutes, private companies, and European projects such as
178 RENEb (16). In Figure 2, the accuracy of the reference dosimetry of beams used to establish calibration
179 curves in biological dosimetry was evaluated with alanine dosimeters calibrated in Dw. Deviations from
180 reference dose (10 Gy) range from a few % up to 50%, with about 50% of results within +/- 5%. Dose
181 quantities used, when known by participants, were either air kerma or Dw. The impact of using different
182 dose quantities and/or beam quality was noticeable in dicentric assays, but had not been accounted for when
183 analysing results of biological dosimetry for this inter-laboratory comparison. With the arrival of more
184 complex new small animal X-ray conformal irradiation platforms to the IRSN (SARRP, Xstrahl), and the
185 increased use of a linear accelerator (LINAC), the need to have a dedicated physicist on a full-time basis
186 was felt to be important, due to the greater complexity of use and dosimetry aspects. This recommendation
187 has also resulted in a dedicated research program at IRSN on the latter aspect, notably to develop new
188 dosimetry approaches (e.g., for small fields), new protocols for dose rate measurement at biological sites,
189 verification of manufacturer data, and quality control. A similar need among other users of SARRP type of
190 X-ray platforms has resulted in hiring additional physicists to ensure the optimal performance of these
191 machines. This small community quickly organized itself to share experiences, protocols, and methods, and
192 endeavored to set up a program to verify manufacturer data and provide intercomparison and technical
193 assistance for machines without local physicist expertise, notably in the framework of a new French national
194 network of irradiation platforms for radiobiology studies (Réseau des Plateformes National d'Irradiation;
195 ResPlanDir)⁴.

196

197 **Proposed New Instructions for Authors for Dosimetry Reporting**

198 Given the concerns outlined above regarding accuracy and reproducibility of radiation dosimetry in
199 radiobiological research, and in the interest in ensuring rigor and reproducibility of irradiation experiments,

⁴ <https://www.france-hadron.fr/en/plateforms/features-modalities.html>

200 the authors of this commentary, in agreement with another recent commentary on this subject by Marcu et
201 al. (17), propose that journals engaged in publishing radiobiology research introduce new ‘Instructions for
202 Authors’ addressing minimum standards for reporting radiation dosimetry information. Implementing the
203 recommendations below would also address issues sometimes encountered in successful study replication.

204 Therefore, the following minimum irradiation parameters are suggested:

- 205 • Name of the irradiator (company, country, model)
- 206 • Type of source (radionuclide (e.g. cesium, cobalt), X-ray)
- 207 • Energy (kVp, mA; for X-ray systems)
- 208 • Filtration (for kV X-ray systems, half value layer if known)
- 209 • Radiation dose and dose rate, source to surface distance (SSD), field size,
- 210 • Sample being irradiated, and
- 211 • Irradiation geometry (nature of animal restraint (e.g., pie or other jig), the material of the sample
212 holder, shielding (if appropriate), orientation to the source, any animal(s)/sample rotation)
- 213 • Dose quantity (e.g., how radiation dose was measured - D_w , air kerma, midline tissue, etc.)
- 214 • Daily pre-exposure device calibration method
- 215 • Planning and dose calculation method (required for multiple field conformal dose delivery,
216 particularly orthovoltage small animal conformal dose delivery systems, optional otherwise)
- 217 • Reporting of any in-run dose measurements (if performed)

218

Figure 1

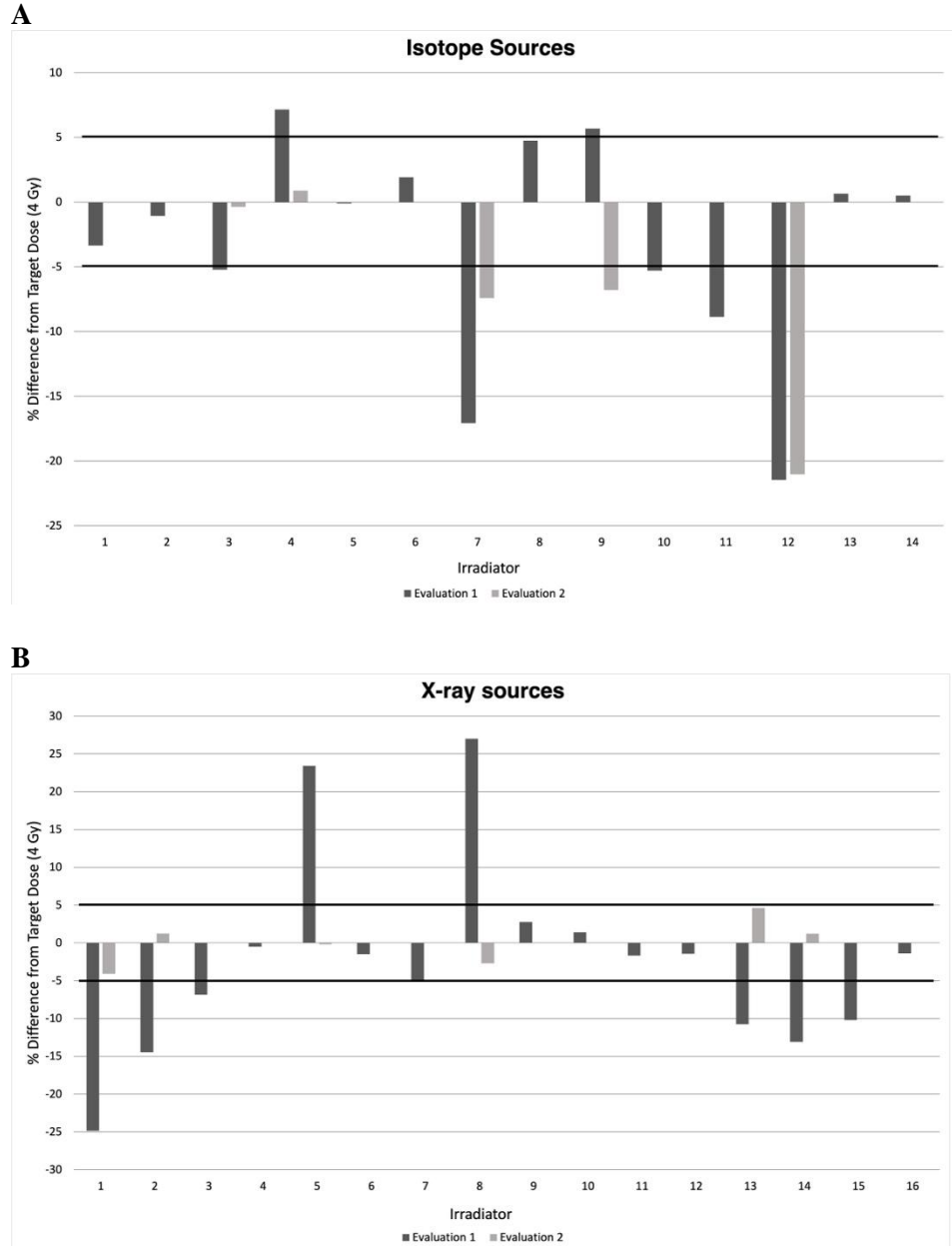


Figure 1. A) Performance of U.S. institutions with isotope irradiators within NIAID-supported sites; B) Performance of institutions with orthovoltage X-ray irradiators within NIAID-supported sites. The bold horizontal lines in each panel represent the +/-5% cutoffs for acceptable variance from the 4 Gy target dose. Gray bars indicate paired final retesting results for irradiators with initial test doses that were \geq +/- 5% of the target dose.

Figure 2

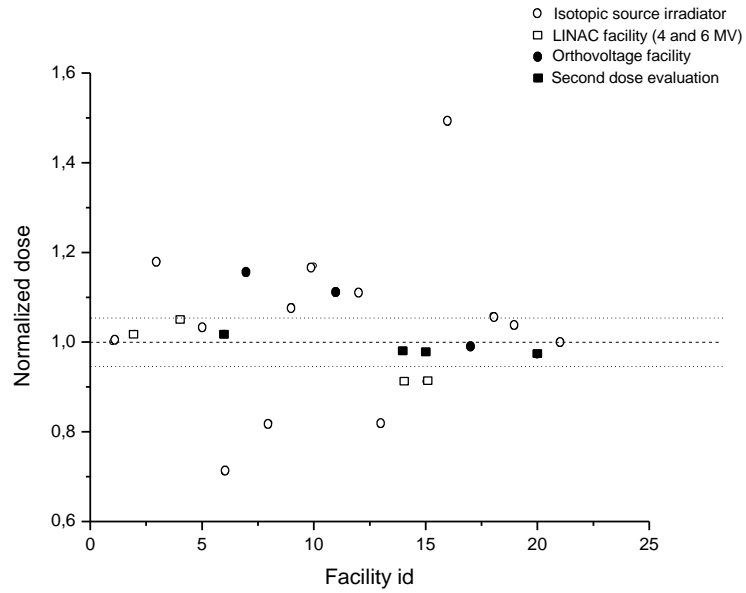


Figure 2: Doses measured at 21 European facilities with alanine with a calibration in terms of dose in water and normalized to the dose delivered at the French primary laboratory (modified and used with permission from) (16).

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