Stacked Pie Cage In Vivo Mouse Dosimetry Involving Multiple Sources and Energies

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ABSTRACT

The study measures the dose for phantom mice within three stackable pie cages, using all of the most common irradiation devices used in radiobiology today. It facilitates determination of net scatter factors for irradiation caused by the cages and mice being in the beam, at energies ranging from 160 keV to 15 MV. In vivo radiation measurements using TLDs on phantom mice were used to approximate the dose at each pie cage stack level. Irradiation devices include a particle accelerator at 6, 10 and 15 MV, cobalt unit at 1.253 MeV, cesium unit at 662 keV, and a standard x-ray unit at 160 keV. Energy dependence was observed with respect to the dose given. Dose change irregularities were consequentially a result of attenuation and scatter throughout the pie cage. This dose dependence on energy was found to increase with increasing photon energy. Dose results from stacked cage irradiation were found to be within SF=0.701-2.508 (-30% to +251%) of the calibrated dose output. Radiation measurements suggest considerable dosimetric differences exist to mice at each level when pie cages are stacked. Results varied remarkably when also including a change in the incident photon energy beam. Researchers may make use of plots offered in this research to estimate corrections to the calculated irradiation time needed to arrive at the prescribed radiation dose to mice at any level. Data provided directly correlate to the dose given to real mice at any energy using this same geometry.

I. INTRODUCTION

Mice are the most common lab animal to be used in experiments in the last century. Mice reproduce within weeks and can therefore be bred quickly, for a replenishing supply and at low cost. With their size also being very small, they can be easily contained and handled. Perhaps the greatest benefit of all is the biological upside in using mice. Their physiological construction, genetic content and behavioral characteristics are closely similar to the human model, even for complications like cancer, which can be replicated in mice. To date, research remains heavy with mice in the fields of neurology, psychology, hematology, biochemistry and radiobiology. For cancer research specifically, xenografting is a common technique used by radiobiologists to test the characteristics of disease growth, inhibiting drugs, and tumor responses from radiation by the injection of tumors into immunodeficient mice. Having an immunodeficient research subject, such as the nude mouse is greatly beneficial since it removes the single most important degree of freedom; tumor rejection.[1] Radiobiologists have valued this animal model for sustained grafted tumor growth, permitting fractionation studies like for humans in radiation therapy.[2] Still, radiobiology researchers find it is a difficult task to accurately assess the dose given to mice.[19] The difficulty is compounded when the groups of mice are irradiated in stackable pie cages. Here, we have tasked ourselves to dissolve much of this issue.

We first selected a phantom mouse to be used in the place of living mice. The phantom mouse used was determined ideally suited to permit radiation dose measurements *in vivo* using miniature detectors. We then considered the most common sources of radiation by a review of literature in radiobiology. To date, there is an upward trend in radiobiological experiments with mice having been performed with kilovoltage x-ray machines.[5,12] However, some researchers have shown a preference in using higher photon energies, such as those from radioactive ^{137}Cs (Cesium) and ^{60}Co (Cobalt) irradiators.[1,18] Linear accelerators have not been investigated as much as either machine, although more and more researchers are now also trending towards using this technology. Clinical trials are more commonly requiring results to be based on the delivery of radiation at the same nominal energy and beam intensity as would be required to treat a human with cancer.[8,10-11,17]

Given that the vast majority of published research for mice irradiation involves the use of x-ray machines, cesium irradiators, cobalt irradiators or particle accelerators, we chose to introduce this experimental research using each of these systems identically. We further extended the research to consider the need to irradiating groups of mice at the same time in pie cages. Since each of these machines accommodates cages that are stackable, we provide dosimetry at each level for phantom mice, while accounting for dose deposition losses from scatter and attenuation through the pie cage. From this investigation, it is expected that future researchers will then be able to accurately gauge the amount of radiation dose given to the mouse, depending on the source used, and the distance to the mouse at each level in a triple stacked pie cage. Specifically, the practical application is that for any given machine used to provide external beam radiation, whether x-ray unit, cesium irradiator, cobalt irradiator or particle accelerator, mice can be irradiated with radiation detector confirmed dose accuracy. Additionally, the stackable pie cage concept exemplifies the benefit of treating more than one cage at a time, as done traditionally. To date, no third party experience has benefitted from stackable pie cage irradiation mechanisms, as this product evaluated was only recently made available. However, the approach we presented is expected to not only speed up the time frame for mice irradiation, but enable greater accuracy in dose estimation for mice regardless of pie cage location or photon energy used.

II. MATERIALS AND METHODS

Ia. Stacked Pie Cages and Mouse Phantom

Study commencement first included preparation of the phantom environment. A Best® Theratronics, Ltd. (Fullerton, VA) Theratronics Model RadDisk Micro4 Cell Holder was the pie cage to be used for the study. It consisted of medical grade polycarbonate housing with a nylon screen and lid. The cage had a cylindrical cross-section shape at 12.1 cm diameter and 4.8 cm height. Internally the cage was divided into three separate yet identical wedge-shaped compartments. A rubber block was determined to be ideally suited to represent the mouse within each compartment. The rubber mouse phantom had dimensions of 6.0 cm x 2.5 cm x 2.0 cm. Given the interest to irradiation multiple cages at once, it was predetermined that all radiation delivery machines could accommodate the height of three stacked pie cages.

In order to establish the effect on the dose at each level as a result of the cage and phantom mice present, an air phantom was constructed without the presence of either. A Huestis Medical Model SFB102 block was acquired, having composition made up of only The Dow Chemical Company® (Midland, MI) Styrofoam®. With the extruded foam being only 98% air, no measured attenuation would be observed by any radiation delivery unit. The foam block dimensions on purchase were originally 25.4 cm x 25.4 cm x 5.1 cm, but were cut down to an area of 7 x 7 cm^2 in various slices up to a combined total thickness of only 10.1 cm. With the detector positions known to be at 1.2 cm, 5.6 cm and 10.1 cm in the stacked pie cages for each level, care was taken to enable a single detector to be placed within the foam block at the same height above the table.

Ib. Radiation Detector

The absorbed dose to the phantom mouse was made possible using Best® Dosimetry Services Model DXT-107H thermoluminescent dosimeters (TLDs). The TLD is a nominally 70 μm grain known as Model TLD-100H® (LiF:Mg,Cu,P) natural lithium fluoride doped with magnesium, copper and phosphorus powder supported by a polyimide film and surrounded by a metal ringlet with numeric identification (Luo 2002). Prior to use, each TLD was read on a Harshaw® (dba Thermo Fisher Scientific®, Inc.; Waltham, MA) Model 8800PC Automatic Card Reader. The temperature of the powder was increased from 22°C by 15 °C/s to 255 °C in low ultraviolet lighting. At this maximum temperature, glow curves were achieved. The resolution of the TLD with respect to calibrations against standard dose calibrations from the National Institute for Standards and Technology (NIST) is 1 cGy[†]. Histories of each detector were maintained over the time of use. These suggest measurement precision at ± 5%, albeit with incident photon radiation energy dependence. Two TLDs were numerically logged into a manifest and then placed on two rubber blocks in the pie cage. This process was repeated for all three stacked levels, such that there were a total of two detectors at each level and 6 detectors in

[†] The gray (Gy) is the International System of Units standard unit for absorbed dose, defined as the absorption of 1 Joule of ionizing radiation by 1 kilogram of matter (i.e. human tissue or water). It is equivalent to 100 cGy or 100 rads.

total for each irradiation. A depiction of the set-up with detectors is illustrated in Figure 1, where the mouse phantom is shown with a size proportioned to that of living mouse. For irradiations in Cesium and the kilovoltage x-ray unit, an alanine dosimeter was placed in the third chamber of each level, helping to provide additional insight on dose delivered. Each alanine dosimeter was calibrated at their respective energy, with calibration traceable to NIST or its equivalent.

Figure 1. Localization of the TLD wafers on the mouse phantom.

Ic. Particle Accelerator

A Varian Medical Systems, Inc.® (Palo Alto, CA) Model TrueBeam® linear accelerator (LINAC) was acquired to provide the highest megavoltage treatment energy levels. The unit was used daily for the treatment of cancer patients at the clinic operating it.[6,15-16] The unit featured machine created bremsstrahlung radiation beams having energies 6 MV, 10 MV and 15 MV. The dose-rate at 100 cm permits delivery of radiation at up to 600 cGy/min under calibration conditions. For delivery, a field size of 40 x 40 cm^2 was set. This insured that the radiation beam size encompassed the entire phantom, while also assuring maximal flatness and symmetry. To begin, the treatment couch was raised to 100 cm. The cages were stacked and centered in the beam using the light field of the accelerator. The LINAC was programmed to deliver 1,000 cGy over a time of 1.7 min for a 6 MV beam. After completion, the detectors were replaced and irradiated identically for a 10 MV beam. The process was then duplicated for the remaining 15 MV beam.

Id. Radioactive ^{60}Co Irradiator

The system obtained for low energy megavoltage delivery was the Best® Theratronics, Ltd. (Ontario, CANADA) Model Equinox™ 100 Cobalt-60 unit. Cobalt (^{60}Co) is a sealed source radioactive material. It was formerly known as the principal means for cancer patient treatment leading up to the 1980's in the United States, and remains the primary device for veterinary clinics and third world countries.[3,14] This isotope emits two common gamma energies on decay; 1.173 MeV and 1.333 MeV. The energies are so close that for radiobiological concerns, the average energy of 1.253 MeV is used when describing it. It decays with a half-life of 5.27 years. As such, users must keep track of production dates to insure decay has been accounted for. Since the ^{60}Co irradiator is a machine designed initially as a teletherapy unit, it has the same basic design features as the LINAC, with a treatment couch and a gantry. The distance from the source to the couch for this device is also 100 cm. The maximum field size is 30x30 cm^2, due to the presence of a 60 leaf collimator. Similarly, this large field aperture insured the entire pie cage stack would be fully irradiated in the incident beam and with maximum uniformity. The maximum activity of ^{60}Co allowed in the unit was 15,000 Ci, but the unit used had activity of 1,492 Ci at the time of irradiation. The dose-rate achieved in this experiment was 29.4 cGy/min at 100 cm from the source.

Ie. Radioactive ^{137}Cs Irradiator

Irradiation in the high kilovoltage range was provided by the Best® Theratronics, Ltd. GammaCell® 3000 Elite Model-A Cesium irradiator. Cesium (^{137}Cs) is also a sealed source radioactive material and is the most common means that hospitals use to irradiate blood specimens.[4,19] During decay to the daughter nucleus, it emits its main gamma ray peak at 662 keV. This is markedly less than LINAC energies and roughly half of the energy of the cobalt unit. Similarly, there is a half-life to recognize. However, at 30.2 years the activity does not change as drastically as does cobalt. The cesium irradiator works differently in that the stacked cages are automatically pivoted inside in order to provide radiation. The source is retracted from its shielded environment to provide radiation to the specimen not from the top, but rather from the side. During irradiation, the entire stacked cage assembly endured radiation. The ^{137}Cs irradiator has an activity of the irradiator 940 Ci. This source activity produces a dose rate of 450 cGy/min at the center of the canister. The source is a pencil source, achieving 1.3:1 dose uniformity.

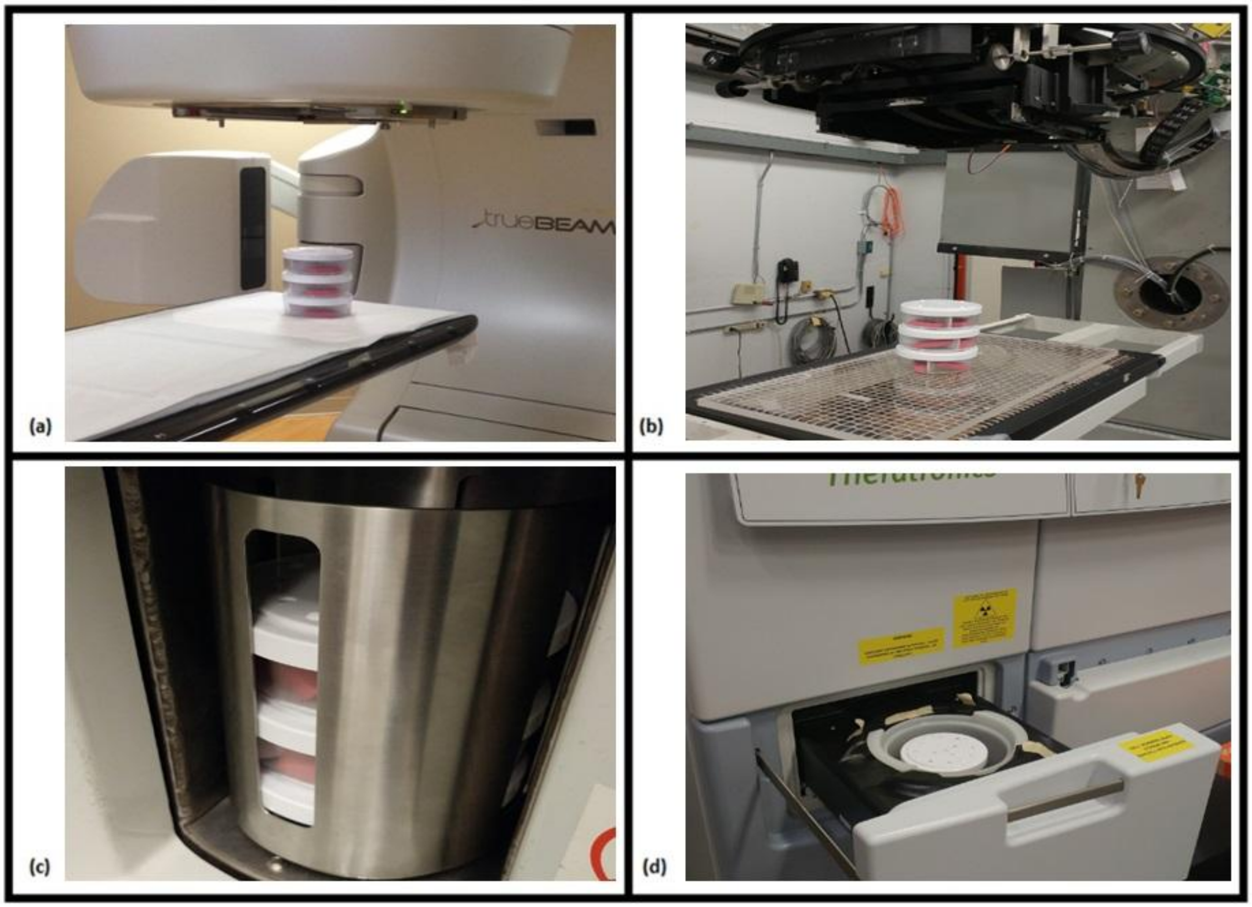

Figure 2. Set-up geometry for all testing with pie cages; (a) Varian Medical Systems, Inc.® TrueBeam® particle accelerator, (b) Best® Theratronics, Ltd.™ Equinox 100 Cobalt-60 unit, (c) Best® Theratronics, Ltd. GammaCell® 1000 Elite Cesium irradiator, and (d) Best® Theratronics, Ltd. Raycell® MK2 KV irradiator

If. X-ray Unit

Finally, low energy kilovoltage radiation was obtained using a Best® Theratronics, Ltd. Model Raycell® MK2 KV irradiator; 3.5 L Version. The machine operates in similar to x-ray machines found in Radiology today. However, it has been substantially miniaturized, enabling it to be housed in university-type research environments and blood banks.[9] Its popularity as a replacement for Cesium-based irradiators is growing, due to increased regulatory burden placed on radioactive materials since 9/11. The only energy available for selection is at 160 kV. Based on internal Monte Carlo simulation, the average energy of photons emitted by this unit is approximately 70 keV. It is a machine that generates characteristic x-rays using two opposing x-ray tubes. The height of the shelf is 152.5 cm from source to source. The center of the shelf is therefore at 76.3 cm from either source. With opposing sources, the dose delivery is given from the top and from the bottom simultaneously. A central dose-rate of 890 cGy/min is the device's specification. The calculated dose-rate on either side (top or bottom) of the pie cage is then higher as the position would be closer to one of the two sources.

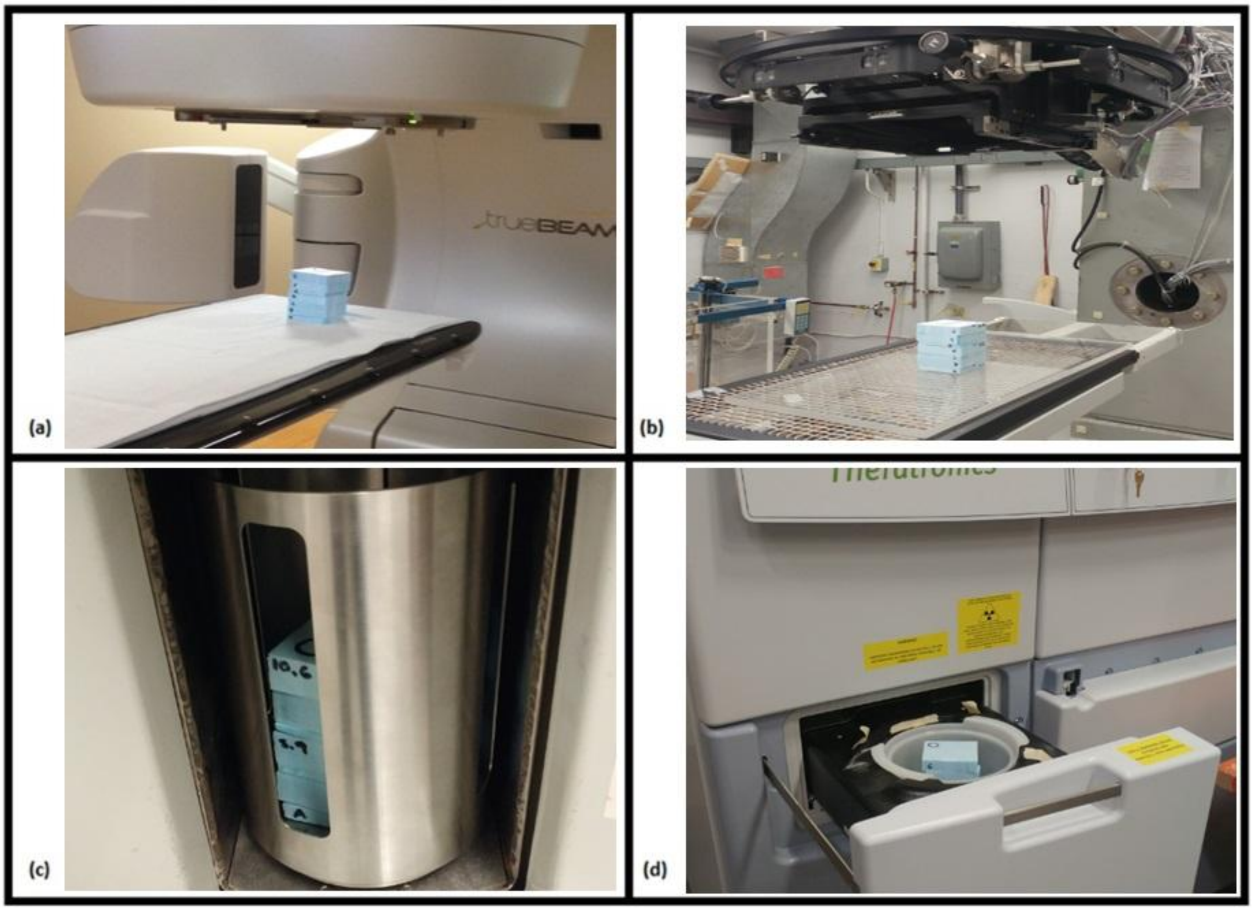

Figure 3. Set-up geometry for all testing without pie cages; (a) Varian Medical Systems, Inc.® TrueBeam® particle accelerator, (b) Best® Theratronics, Ltd.™ Equinox 100 Cobalt-60 unit, (c) Best® Theratronics, Ltd. GammaCell® 1000 Elite Cesium irradiator, and (d) Best® Theratronics, Ltd. Raycell® MK2 KV irradiator

III. RESULTS AND DISCUSSION

The results from all dosimeter processing were used to calculate the ratio of dose with the cage present to the dose with the cage absent. This ratio, defined by us as a net scatter factor (SF), represents the change in combined scatter from the table and phantom. For irradiations with the cage present, scatter radiation exist as a results of interactions with the table as well as with the present cage, mouse phantom and detector. For irradiations without a cage present, there is no associative scatter contribution from it, only the detector mounted in Styrofoam®. Table 1 provides the data and resulting scatter factor for all energies at each level of the pie cage. Figure 4 was generated from these data.

Table 1. All measured data from TLD analysis (dose: cGy)

	Lower Pie Cage			Middle Pie Cage			Upper Pie Cage		
Energy	Cage	Foam	SF	Cage	Foam	SF	Cage	Foam	SF
160 keV	675	962	0.701	743	924	0.804	-	-	-
662 keV	1,025	1,156	0.887	1,110	1,150	0.965	1,194	1,335	0.894
1.253 MeV	960	826	1.162	1,093	1,009	1.083	1,290	645	1.998
6 MV	906	433	2.093	1,203	689	1.745	1,205	481	2.508
10 MV	1,033	493	2.095	1,110	589	1.885	1,184	495	2.392
15 MV	979	526	1.861	1,135	547	2.074	1,059	546	1.941

We considered the dose estimates to have some error associated with measurement, although not with geometry. It was known at the time of testing that our chosen TLD-100H® powder alanine dosimeters have an estimated uncertainty of 10%. This error magnitude is reflected within the plot of Figure 4 in the form of 10% error bars. The error bars indicate a more critical consideration at megavoltage energy ranges for the dose to mice in each cage level.

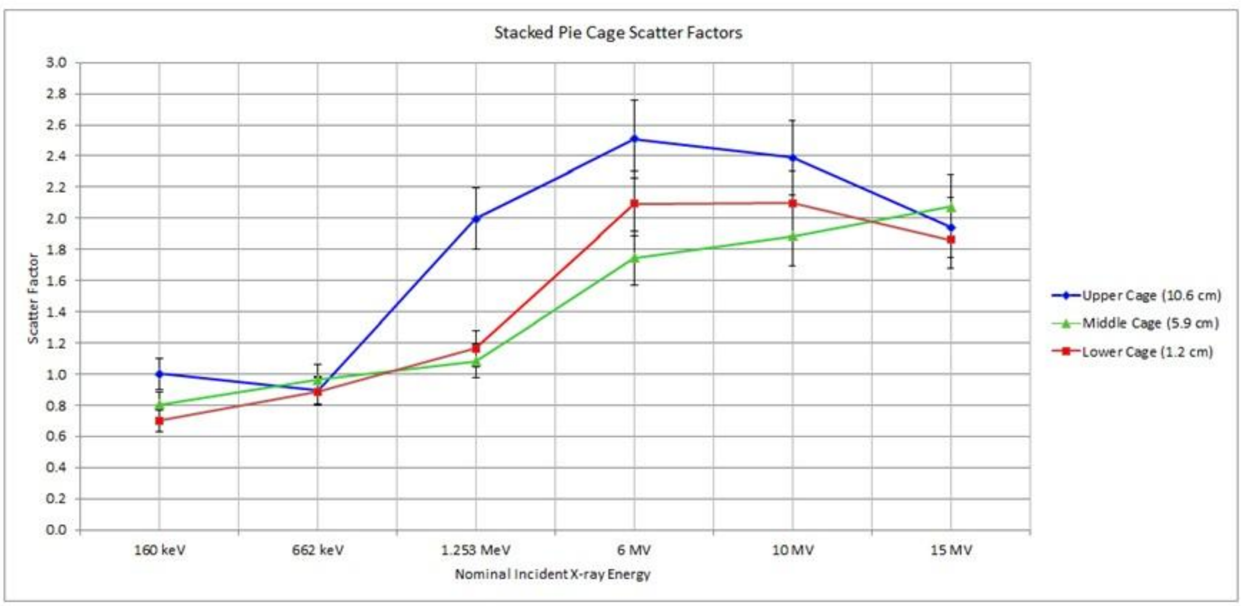

Figure 4. The average result projects the measurement for pie cage dosimetric net scatter at each level and energy. Dose to inserted mice can then be interpolated from these factors.

Results show that at the lower x-ray energy, the dose output in the lower and middle cage are less than the known calibrated dose output of the beam. For the x-ray irradiator the correction factor for dose at those cage levels range from SF=0.804-0.701 (-20 to -30%). For the cesium irradiator, at all cage levels the dose is also less than the known calibrated dose output, although within 10% nominally. At the 1.253 MeV average energy of the cobalt teletherapy unit, the lower and middle cage doses are closely related to the known calibrated dose output. However, for the upper cage the results indicate a dose increase that is considerably higher. With a higher dose than expected, the scatter contribution must be much more pronounced (SF=1.998; +200%). An alternative explanation is the lack of build-up for the top position TLD in the foam phantom, which is valid for all photon energies. At higher megavoltage energies from the medical accelerator, the dose is markedly higher than the known calibrated dose output at each cage level. The change in dose relative to the known calibrated output is found to be between SF=1.745-2.508 (+175 to +251%) for all accelerator energies and cage levels.

Limitations within the study were experienced in the geometry for irradiation. Although the limitations did not cause any undesired error in the study, it is appropriate to discuss those findings. First, the x-ray irradiator has two opposing sources, rather than one incident beam. Second, the position of the sources within the shielded environment limits the height of the phantom to only two cage levels, not three. The cesium irradiator requires that the cages be placed in a stainless steel holder (Figures 2c). Therefore, the scatter factor for cesium also includes the consequential interaction of this steel holder. One can conceive that with relative measurements, the scatter factor for the steel holder cancels out. However, we note to the reader that the interaction between the steel holder and cages, as compared to the steel holder and Styrofoam®, are too complex to allow such simplified consideration. Finally, we consider the fact that the cesium irradiator is a pivoting device. Once the doors of the cesium irradiator are closed and the unit is programmed to deliver radiation, the phantom is rotated around a turret to the shielded irradiation doors. This kind of geometry also places the phantom and detectors closer to the source as previously seen for the dual opposing source x-ray irradiator. Since the experiment involved an identical setup for both cage dose delivery and Styrofoam® dose delivery, there was no further experimental difference in testing delivered.

IV. CONCLUSIONS

Radiation measurements suggest considerable dosimetric differences exist to mice at each level when pie cages are stacked. These results vary remarkably when also including a change in the incident photon energy beam, predominantly as a result of interaction processes and cross-sections. While interactions at lower photon energies are dominated by the Photoelectric Effect, interactions at megavoltage levels are dominated by the Compton Effect. The cross-sections for these interactions are different. At higher energies, secondary photons from scatter provide for many more interaction combinations. It is therefore the interaction process and the associated increased probability of interaction at higher energies that cause the dose increase seen in Figure 4.

It was found in this study that it is more critical to account for dose output increases for megavoltage beams, since scatter was shown to increase dose output by up to 240%. Researchers may make use of the table and plot provided to estimate corrections to their irradiation time, which directly correlate to the dose given to real mice at any energy using this same geometry. It is noteworthy that users be weary of using these data tables for a different geometry or with a different number of cages, since scatter processes and dose may be substantially different.

V. ACKNOWLEDGEMENTS

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VI. REFERENCES

1. **Agarwal JK, Ayappan P, Santhamma AV.** 1975. Carcinogenic effect of radiation on the mouse embryo; physical aspects of radiation dosimetry. Strahlentherapie **150**(6):618-623.

2. **Allam A, Perez LA, Huang P, Taghian A, Azinovic I, Freeman J, Duffy M, Efird J, Suit HD.** 1995. The effect of the overall treatment time of fractionated irradiation on the tumor control probability of a human soft tissue sarcoma xenograft in nude mice. Int J Rad Oncol Biol Phys **32**(1):105-111.

3. **Cadman PF, Paliwal BR, Orton CG.** 2010. Co-60 tomotherapy is the treatment modality of choice for developing countries in transition toward IMRT. Med Phys **37**:6113-6115.

4. **Gibson BW, Boles NC, Souroullas GP, et al.** 2015. Comparison of Cesium-137 and X-ray Irradiators by Using Bone Marrow Transplant Reconstitution in C57BL/6J Mice. Compar Med **65**(3):165-172.

5. **Gorodetsky R, Mou X, Fisher DR, Taylor JM, Withers HR.** 1990.0Radiation effect in mouse skin: dose fractionation and wound healing. Int J Rad Oncol Biol Phys **18**(5):1077-1081.

6. **Gossman MS, Das IJ, Lopez JP, Sharma SC, CM Howard, PP Claudio.** 2011. A novel phantom model for mouse tumor dose assessment under MV beams. Health Phys J **101**(6):746-753.

7. **Greco A, Benedetto AD, Howard CM, Kelly S, Nande R, Dementieva Y, Miranda M, Brunetti A, Salvatore M, Claudio L, Sarkar D, Dent P, Curiel DT, Fisher PB, Claudio PP.** 2010. Eradication of therapy-resistant human prostate tumors using an ultrasound guided site-specific cancer terminator virus delivery approach. Molec Ther **18**:295-306.

8. **Jaffe DR, Williamson JF, Tim G.** 1987. Ionizing radiation enhances malignant progression of mouse skin tumors. Carcinog **8**(11):1753-1755.

9. **Janatpour K, Denning L, Nelson K, Betlach B, et al.** 2005. Comparison of X-ray vs. gamma irradiation of CPDA-1 red cells. Vox Sang **89**(4):215-219.

10. **Kumar S, Kolozsvary A, Kohl R, Lu M, Brown S, Kim JH.** 2008.0Radiation-induced skin injury in the animal model of scleroderma: implications for post-radiotherapy fibrosis. Rad Oncol **3**:40.

11. **Kuroda M, Inamura K, Tahara S, Kurabayashi Y, Akagi T, Asaumi J, Togami I, Takemoto M, Honda O, Morioka Y, Kawasaki S, Hiraki Y.** 1999. A new experimental system for irradiating tumors in mice using a linear accelerator under specific pathogen-free conditions. Acta Med Okayama **53**(3):111-118.

12. **Lo Y, Taylor JMG, McBride WH, Withers HR.** 1993. The effect of fractionated doses of radiation on mouse spinal cord. Int J Rad Oncol Biol Phys **27**(2):309-317.

13. **Luo LZ, Velbeck KJ, Rotunda JE.** 2002. Evaluating two extremity dosimeters based on LiF:Mg,Ti or LiF:Mg,Cu,P. Rad Prot Dosim **101**(1-4):211-216.

14. **Minard P, Dean DA, Jacobson RH, Vannier WE, Murrell KD.** 1978. Immunization of Mice with Cobalt-60

Irradiated Schistosoma. Am J Trop Med Hyg **27**:76-86.

15. **Miranti A, D'Ambrosio A, Cattari G, Garibaldi E, Bresciani S, Gabriele P, Stasi M.** 2016. NOD-SCID mice irradiation with medical accelerators: Dosimetric and radiobiological results. Phys Med **32**(11):1453-1460.

16. **Nande R, Greco A, Gossman MS, Lopez JP, Claudio L, Salvatore M, Brunetti A, Denvir J, Howard CM, Claudio PP.** 2013. Microbubble assisted p53, pRB, and p130 gene transfer in combination with radiation therapy in prostate cancer. Curr Gene Ther **13**(3):163-74.

17. **Stüben G, Budach W, Schick KH, Stuschke M, Stapper N, Muller S, Feldmann.** 1994. A time-saving system for irradiations of experimental tumors. Strahlenther Onkol **170**(1):36-41.

18. **Urano M, Nishimura Y, Kuroda M, Reynolds R.** 1998. Are hypoxic cells critical for the outcome of fractionated radiotherapy in a slow-growing mouse tumor? Radiother Oncol **48**(2):221-228.

19. **Yoshizumi T, Brady SL, Robbins ME, Bourland JD.** 2011. Specific issues in small animal dosimetry and irradiator calibration. Int J Rad Biol **87**(10):1001-1010.