GuideSheet Rodent Irradiation

X-ray irradiation has many applications in USC research, particularly when studying stem cells and immunotherapy agents in small animals (e.g., rodents). Its use in small animals, however, must be (a) scientifically justified and (b) articulated in terms of planned irradiation dose per species/strains when preparing applications for the Institutional Animal Care and Use Committee (IACUC) and the Radiation Safety Committee (RSC).

X-Ray Irradiator

The X-ray irradiator exposes sample material (e.g., cell lines) or organisms (e.g., rodents) to radiation but does not cause or induce radioactivity (i.e., the material does not become radioactive itself). USC's irradiator is self-shielded so that the radiation emitted from the system at any point five centimeters outside the external surface, or any door or port, does not exceed 0.5 milliroentgen in one hour. An interlock is built into the door such that if the door is opened while the irradiator is on, it stops generating X-rays to prevent exposure of users.

X-Ray Irradiator Training

All X-ray irradiator users must complete basic irradiator safety training which is comprised of a safety video and in-person instruction (see https://ehs.usc.edu/training/catalog/#IRR).

Experimental Guidelines for Rodent Irradiation

- ***Set up***. The dose set in each program refers to the dose delivered to the top surface of the shelf (50 cm from the x-ray source).
    - The dose rate given to the animal varies according to the radiation field size, the distance from the source, and the distance away from the radiation beam's central axis.
    - For **partial body exposure**, consult with Radiation Safety to determine a set up that correctly exposes the animal part and protects the rest of the animal.

    - For **whole body irradiation** in a pie cage or container, arrange the exposure to have the 50 cm distance pass through the midline of the average animal. This may require adjusting the shelf height. **Note:** Failure to adjust the shelf height will result in the top of the animal receiving several percent more dose than the dose indicated by the program setting.
    - Take into consideration the source and microbial status of the animals.
- ***Irradiation***. The use of a split dose is recommended to reduce animal mortality. If a high, single dose must be used, it must be scientifically justified in both IACUC and RSC applications.

For Principal Investigators (PI) that are new to rodent irradiation, it is important to consult with an experienced investigator. Contact Radiation Safety for assistance at radsafety@usc.edu or (323) 442-2200.

For novel animal strains with no published dose maximums available, a pilot dose-verification study using a few animals is highly recommended; it may be required in certain cases. The PI must then incorporate the pilot study in both IACUC and RSC applications with details of the number of animals and strain to be irradiated, test doses, and final readout parameters. Upon completion of the pilot study, the PI must submit a report to the IACUC and RSC for review. Following IACUC and RSC approval, the originally proposed experiments may then proceed.

Post Irradiation Care

Table 1 lists the health risks to rodents that undergo total body irradiation. Tables 2 and 3 list published doses and dose responses for mice and rats, respectively.

Irradiated animals are at risk of bacterial translocation and infections post-irradiation. For studies lasting longer than seven days, antibiotics and purified or acidic water should be administered post-irradiation and donor cells transplanted to allow animals to regain immune system competence. Antibiotic administration must be described and approved in the IACUC protocol.

Monitor irradiated animals closely for signs of radiation illness and/or transplant failure during the reconstitution period. Nursing and supportive care, in consultation with a Department of Animal Resources (DAR) veterinarian, must be provided to animals showing signs of illness. Humane endpoints must be described in the IACUC protocol and Euthanasia should be considered for animals not fully recuperated within 21 to 28 days after the procedure.

References

Radiation Biology: A Handbook for Teachers and Students. 2010. International Atomic Energy Agency

Irradiation of Rodents. 2019. Boston University IACUC

Monitoring Mice Post Irradiation. 2016. The University of Melbourne IACUC

The Guide for the Care and Use of the Laboratory Animals 8th edition, NRC 2011

California Code of Regulations Title 17 Subchapter 4

Table 1. Health risks associated with total body irradiation of rodents

Risk	Description of Risk	Dose Range (cGy)	Survival period
Neurovascular Syndrome	Cardiovascular and neurological dysfunction	> 2000[1]	Hours to days
Hematopoietic Syndrome	Myeloablation (destruction of bone marrow) leads to immune system suppression	300 to 1000[1]	12 to 30 days
Gastrointestinal Syndrome	Severe damage to the mucosal lining of the GI tract leads to infection, loss of electrolytes, and fluid imbalance	800 to 1200[1]	1 to 7 days
Graft Versus Host Disease	Inadequate immune system suppression leads to donor cells attacking the recipient's body's cells	Inadequate irradiation dose	-
Secondary Neoplasia	Formation of abnormal tissue growth in long term studies	Probability increases as dose increases	-

[1] Doses leading to these syndromes vary greatly between species and strains. The listed doses are most relevant to C57BL/6 mice, which are the most radiation resistant.

Table 2. Published mouse irradiation doses

Strain	Protocol	Dose	Response to Dose	Reference
C57BL/6	TBI* and BMT**	2 x 550 cGy 3 hour interval	Survival	The Mouse in Biomedical Research. Vol 3. Fox J.G et al. 2nd Ed. 2007. Academic Press. ACLAM Series. 453.
C57BL/6	Hematopoietic ablation	2 x 550 cGy 3 hour interval	-	UCSF IACUC Standard Procedure. Production of Hematopoietic Chimeras
C57BL/6 C3H/HeN	TBI* and BMT**	2 x 600-650 cGy 4 hour interval	Survival	Y-Z Cui, et al. 2002. Optimal protocol for total body irradiation for allogenic bone marrow transplantation in mice. Bone Marrow Transplant. 30: 843-849
C57BL/6	TBI* and BMT**	950 cGy	All mice died within 30 days.	Frasca, D. et al. 2000. Hematopoietic reconstitution after lethal irradiation and bone marrow transplantation: effects of different hematopoietic cytokines on the recovery of thymus, spleen and blood cells. Bone Marrow Transplant. 25: 427-433.
MRL/lpr	TBI* and BMT**	2 x 500 cGy 4 hour interval	Survival	Ikehara, S. 1989. Long-term observations of autoimmune-prone mice treated for autoimmune disease by allogenic bone marrow transplantation. Proc. Natl. Acad. Sci. USA. 86: 3306-3310.
BALB/c	LD/50	880 cGy	-	Duran-Struuck, R. 2009. Principles of Bone Marrow Transplantation (BMT**): Providing Optimal Veterinary and Husbandry Care to Irradiated Mice in BMT** Studies. Journal of the American Association for Laboratory Science. 48(1): 11-22.
NSG	TBI* and human B-cell lymphoma engraftment	150 cGy	Median survival of 27 days	Chadalavada, D. 2014. Irradiated Compared with Nonirradiated NSG Mice for the Development of a Human B-Cell Lymphoma Model. Comparative Med. 64 (3): 179-185.
NOD/SCID/ IL-2γc-/-	TBI* and BMT**	270 cGy	-	Andrade, J. 2011. Effects of Sub-lethal Irradiation on Patterns of Engraftment after Murine Bone Marrow Transplantation. Biol Blood Marrow Transplant. 17 (5): 608-619.

* TBI - Total Body Irradiation ** BMT - Bone Marrow Transplantation

Table 3. Published rat irradiation doses

Strain	Protocol	Dose	Response to Dose	Reference
Wistar rats M 100 g	TBI*	1440 cGy	Rats begin to die on day 5 post-exposure	Kassayova, E. et al. 1999. Two-Phase Response of Rat Pineal melatonin to Lethal Whole-Body Irradiation with Gamma Rays. Physiol. Res. 48: 227-230.
Wistar rats M 100 g	TBI*	960 cGy	Rats begin to die on day 10 post-exposure	Kassayova, E. et al. 1999. Two-Phase Response of Rat Pineal melatonin to Lethal Whole-Body Irradiation with Gamma Rays. Physiol. Res. 48: 227-230.

* TBI - Total Body Irradiation