

## **SOPS For General Surgery Labs:**

**Title: Irradiation of Rodents**

**Date: 6-2012**

**Last Updated: Unknown**

Boston University is committed to observe federal guidelines and AAALAC International guidelines for humane care and use of animals.

This policy deals with ionizing irradiation of mice and rats exposed to gamma irradiation emanating from a <sup>137</sup>cesium source. Experimental reasons for exposing rodents to ionizing radiation are used in the fields of immunology and cancer biology. Irradiation to produce myeloablation preparatory to bone marrow transplantation (BMT) is frequently used. BMT procedures are addressed elsewhere in a separate IACUC policy.

### **DEFINITIONS**

A. Gamma irradiation is one type of ionizing irradiation. Source may be <sup>137</sup>Cesium, <sup>60</sup>Cobolt or high-energy X-rays. This policy refers to the <sup>137</sup>cesium irradiator at BUMC.

B. Gray (Gy) is the SI unit of absorbed radiation dose due to ionizing radiation.

C. Rad is a largely obsolete unit of absorbed radiation dose. It is now superseded in the SI by the "gray". 100 rads = 1 gray (Gy).

D. Fractionation of dose: The total irradiation dose is split into two or more equal parts separated by a time interval in order to minimize morbidity and mortality.

Example Post Procedure Monitoring Form

### **POLICIES FOR ANIMALS ON STUDIES EXPOSED TO IRRADIATION**

A. Animals exposed to irradiation must be monitored and findings documented daily for the first 14 days. However, after protocols have been established and PI has experience with the particular strain of mice and dose, three (3)X/week monitoring and

documentation is acceptable. If animals experience morbidity or mortality daily checks are required. Procedures for care of irradiated rodents must be observed.

B. Irradiation must be scientifically justified in the IACUC protocol.

C. The planned dose of irradiation, or dose range, must be specified.

D. Fractionated dose may be useful in certain studies if practical, since animal morbidity and mortality will be lower.

E. Unless literature references are available, a pilot study to determine best dose is recommended if the PI is starting a new study or using a new strain of mice.

F. If control animals (animals that are lethally irradiated but not reconstituted with bone marrow) will be used, the PI must justify the use of these animals, use as few animals as possible, use historical controls as much as possible, and comply with Humane Endpoints Policy.

G. The IACUC will place non-reconstituted lethally irradiated control rodents with death as an endpoint in USDA Category E. Scientific justification is required.

H. Prior to starting an irradiation experiment, the PI is required to submit a form to LASC Intent to irradiate rodents: Request for supplies needed for post-irradiation animal care

## **EFFECTS OF WHOLE BODY IRRADIATION**

Ionizing irradiation causes breaks in the DNA double-strand, thus it mostly affects mitotically active cells. This damage leads to cell death through either necrosis or apoptosis primarily affecting the cells of the hematopoietic and gastrointestinal tract (Duran-Struuck, R. and Dysko, R, 2009).

### **A. Weightloss**

Lethargy, inappetance, diarrhea resulting in anorexia, and body weight loss, which peaks at about 7 days post-irradiation. Depending on the dose and whether immune reconstitution had been provided, recovery will usually occur in 2-3 weeks. Mice may never regain the body weight they had before irradiation.

B. Anemia.

C. Infection: Severe bacteremia may occur as a result of translocation of bacteria from the GI tract (Duran-Struuck, R. et.al., 2008).

D. Intestinal bleeding.

E. Transplant failure: Successful survival of a bone marrow graft requires suppression of the host's immune system. If the irradiation dose has been too low, Graft Versus Host Disease (GVHD) will ensue. As in humans, older mice are more prone to develop GVHD.

F. Graying of hair coat: Black mice, such as C57BL, will frequently turn gray after irradiation.

G. Development of secondary neoplasias: The development of neoplasia after irradiation has been reported in humans and many large animal species. This may occur in mice on long-term studies as well.

H. Incisor damage: One non-neoplastic illness reported in mice is incisor damage and subsequent difficulty in eating. Giving softened food during the recovery phase is required.

## **DOSES OF IRRADIATION**

There are numerous literature references reporting difference in sensitivity in radio resistance or radio susceptibility in different strains of mice. If the PI is unfamiliar with the strain to be used or the radiation source, it is advisable to irradiate a small number of animals in a pilot study to determine to optimal dose for the project and strain under study. The age of the mouse is important as well; older mice being more resistant. For myeloablation in preparation for BMT, total whole body irradiation (TBI) is used. Doses of 700 to 1300 cGy are myeloablative (Duran-Struuck, R. and Dysko, R., 2009). Mice of the C57Bl strain are more resistant to irradiation damage, whereas BALB/c mice are more sensitive.

**Table 1****Published doses of irradiation for various rat and mouse stocks and strains****(2X indicates fractionated dose are given 3 hours apart)**

<b>MOUSE STRAIN</b>	<b>PROTOCOL</b>	<b>DOSE (GY)</b>	<b>RESPONSE TO DOSE</b>	<b>REFERENCE</b>
<b>C57BL</b>	<b>TBI and BMT</b>	<b>2 X 5.5</b>	<b>Survival</b>	<b>The Mouse in Biomedical Research. Vol 3. Fox J.G et. Al. Eds. 2nd Ed. 2007. Academic Press. ACLAM Series. P. 453.</b>
<b>C57BL</b>	<b>Hematopoietic ablation</b>	<b>3.5-6.0 2 X 5.5 G</b>		<b>UCSF IACUC Policy Source: 60Co irradiator</b>
<b>C57BL/6 BALB/c B6CF1</b>	<b>LD50/6 LD50/30</b>	<b>Source 60Co 0.8 Gy 1.4 Gy</b>	<b>The F1 hybrid is similar in radio-sensitivity to the C57BL/6 and different from the BALB/c</b>	<b>Hanson et. al. 1987. Comparison of intestine and bone marrow radio sensitivity of the BALB/c and the C57BL/6 mouse strains and their B6CF1 offspring. Radiat Res 110:340-352.</b>
<b>FVB/N C57BL/6J Rag1-/-iNOS FVB/N Tie2-GFP</b>	<b>TBI and BMT</b>	<b>Source 137Cs 10 – 22 Gy</b>	<b>Germ Free (GF) mice were much less sensitive to radiation enteritis than conventional mice</b>	<b>Crawford, PA and Gordon, JI Microbial regulation of intestinal radiosensitivity. PNAS 102(37); Sept. 13, 2005.</b>

<p>A/J C3H/HeMs C57BL/6J C.B.17/lcr-scid C3H-scid</p>			<p>Source 137Cs 10 – 60 Gy 10 – 50 Gy 10 – 50 Gy</p>	<p>Skin sensitivity Long-term damage Mild damage Less sensitive than A/J Severe skin damage Severe skin damage</p>	<p>Iwakawa, M. et. al. 2003. Different radiation susceptibility among five strains of mice detected by skin reaction. J Radiat Res (Tokyo) 44: 7-13.</p>
<p>C57BL/6NCr B6.Cg- Ptpcrca/NCr(Ly5.2)</p>		<p>TBI and BMT</p>		<p>More sensitive &amp; more prone to bacteremia Less sensitive</p>	<p>Duran-Struuck, R. et.al. 2008. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptpcrca mice to commensal bacteria after whole-body irradiation in translational bone marrow transplantation studies. J Transl Med: 6:10</p>
RAT STOCK	PROTOCOL	DOSE (GY)	RESPONSE TO DOSE	REFERENCE	
<p>Wistar rats M 100 g</p>	<p>TBI</p>	<p>14.4 Gy</p>	<p>Increased serum Corticosterone 60 minutes and 3 days after exposure. Rats began to die on Day 5 post-exposure.</p>	<p>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.</p>	

<b>Wistar rats M 100 g</b>	<b>TBI</b>	<b>9.6 Gy</b>	<b>Rats began to die on Day 10 post-exposure.</b>	<b>Same</b>
<b>Wistar rats M 100 g</b>	<b>TBI</b>	<b>4.8 Gy</b>		<b>Same</b>

## **PROCEDURES**

### **SELECTION AND PREPARATION OF MICE**

Animals to be lethally irradiated should be healthy and free from infectious agents, including *Pseudomonas aeruginosa* (Fox et al., 2007). Mice are tested for *P. aeruginosa* at the vendor and at the annual sentinel surveillance at Boston University LASC/LACF. The drinking water is tested for *P. aeruginosa* annually as well. Immunocompetent mice are not made sick even though they may carry commensal microbial agents. After exposure to TBI mice are immunocompromised and subject to infections not previously clinically important. If post-irradiation infections occur and interfere with recovery of the animals, this possibility should be taken into consideration.

### **IRRADIATING THE MICE - PREPARATION**

A. Purchasing the pie cage- Each investigator is required to purchase their own pie cage. The type recommended is a pie cage with filters which holds up to 20 mice and which can be autoclaved between uses. Pie cages must be sanitized between uses.

- These may be obtained from Braintree Scientific (<http://www.braintreesci.com/Products/Piecages.asp>. The dimensions of the irradiator slot is circular with diameter of 12 inches and depth 4 inches. There is plenty of space for either type pie cage used for mice or rats.)

B. Scheduling - PI must call the Radiation Protection Office (RPO) at 617-638-7052 to schedule time. If the dose is to be fractionated, this must be specified and time must be scheduled for 3 hours later as well.

C. The approved IACUC protocol number is given to the RPO staff.

D. RPO staff sets and runs the irradiator.

E. Dose - PI must provide RPO staff with a suitable dose required for their study.

F. Time of exposure - Animals are generally irradiated for a short period of time (< 15 minutes). The amount of time spent inside the irradiator varies depending upon the dose and the radioisotope decay charts.

G. Rodents are restrained in a rotating pie-shaped holder (to limit mobility and insure equal dose of irradiation). This may be done in the PI's laboratory in a biosafety cabinet (BSC), or in the rodent housing room in a BSC, and the rodents then transported to the irradiator inside the pie cage. Alternatively, rodents are transported to the irradiator in their home cage and transferred to the pie cage once inside the irradiator room. This may be necessary if more rodents are irradiated than can be contained inside one pie cage.

H. Rodents may or may not be anesthetized for irradiation.

I. Transporting the mice to the RPO - The IACUC Guidelines for Transportation of Rodents must be followed with the proviso that mice may be transported inside the pie cage.

J. Rodents can only stay in the pie cage for a maximum of one hour.

## **USE OF THE RPO IRRADIATION ROOM**

A. PI is responsible for bringing the following items:

1. Pie cage and rodents.

B. The following items are available in the irradiator room:

1. Chucks (diapers) to cover the work counter.
2. Paper towels
3. MB 10
4. Trash bags
5. PPE as needed. Minimal required PPE if mice are handled include:
  - disposable gown

- surgical mask
- gloves
- bonnet

## **PROCEDURES FOR HANDLING RODENTS IN THE IRRADIATOR ROOM**

A. If animals are transferred from home cage to pie cage in the irradiator room, PI or research staff are required to first don PPE.

B. After the irradiation is completed, PI or research staff are required to sanitize the counter and place all trash in a trash bag AND remove the trash bag from the irradiator room, take it back to the LASC, and dispose of it according to LASC policy.

C. If animals are transported in the pie cage it is not necessary to don PPE or sanitize the room.

## **IRRADIATING THE MICE**

A. After the pie cage is loaded, it is placed in the irradiator.

B. Mice must be returned to the animal housing room or laboratory as soon as the irradiation is completed.

## **CARE OF IRRADIATED MICE**

**A. After irradiator exposure mice must be placed in a cage and kept clean and quiet.** Sterilized caging, food and water is recommended. PI must order these from LASC prior to the start of irradiation.

**B. PI or designated research staff are responsible for caring for the irradiated animals.** LASC staff will provide special care in an emergency and in such a case the PI will be charged accordingly. For the first 14 days animals must be checked daily, or 3X/week, their condition and care documented on the rodent postprocedure monitoring sheet. Care, especially during the first week, must be dedicated to making animals as comfortable as possible, keeping them clean and quiet, and making sure that they stay



hydrated and have ready access to moistened food and Napa Nectar if indicated. Please refer to the Policies For Animals on Studeis Exposed to Irradiation listed above.

### **C. Use of antibiotics in the drinking water**

PI is responsible for placing rodents on SulfaTrim antibiotic water a few days before the planned irradiation in order for the animals to get used to the taste. Rodents are kept on the SulfaTrim water for at least 14 days and up to 28 days post-irradiation.

### **D. Making drinking water readily available**

Irradiated mice will suffer from irradiation sickness and will not feel well for the first 7-14 days. They may lose up to 25% of their body weight, which in the case of successful bone marrow transplant, will be mostly regained by Day 14-21 post-irradiation. It is important to provide easy access to water.

**E. Napa Nectar must be provided on the bottom of the cage during the first 14 days if morbidity or mortality is seen.** Napa Nectar is available in the animal room free of charge. Placing a new Napa Nectar in the cage daily can be done by PI or research staff or can be done by LASC for fee for service. The Napa Nectar becomes contaminated with fecal material and must be replaced daily.

### **F. Provision of softened food**

Giving softened food during the recovery phase is required. Powdered chow is available in the rodent housing rooms and should be mixed with water and served in a small Petri dish on the cage floor. Pellets moistened with water dry up easily and are not recommended. Placing a new moistened food dish in the cage daily must be done by PI or research staff or can be done by LASC for fee for service.

### **G. Housing**

It is important to realize that even after bone marrow transplantation, lethally irradiated mice are severely immunosuppressed for the first two weeks and providing a totally sterile housing environment is recommended (Fox et al. 2007, & Duran-Struuck, R and Dysko, R., 2009). Sterile caging, food and water is strongly recommended and is required if post-irradiation complications occur.

## **HUMANE ENDPOINTS FOR IRRADIATED RODENTS**

In the case of BMR, transplanted mice undergo a 5-10 day irradiation sickness period from which they generally recover within 14 days (Duran-Struuck, R and Dysko, R., 2009). Depending upon radiation dose, they may lose as much as 25% body weight by Day 7, but if successfully reconstituted will regain most of this weight during the following week. If mice have not received a bone marrow transplant they will remain sick and/or die; time of death depending upon the dose.

A. If animals by Day 14 are not well on their way to recovery, considerations should be given to euthanasia. Consultation with veterinary staff is highly recommended. Moribund animals must be euthanized.

B. Animals must be euthanized by Day 21 post-irradiation if not recovered.

## **REFERENCES**

1. The Mouse in Biomedical Research. Vol 3. Fox J.G et. Al. Eds. 2nd Ed. 2007. Academic Press. ACLAM Series. Pp. 453-454.
2. Duran-Struuck, R. and Dysko, R. Principles of Bone Marrow Transplantation (BMT): Providing Optimal Veterinary and Husbandry Care to Irradiated Mice in BMT Studies. JAALAS 48(1): 2009. 11-22. Highly recommended.
3. APHIS Policy 11. Painful procedures.
4. The Guide for the Care and Use of Laboratory Animals. NRC. ILAR. 1996. P.12.
5. Boston University IACUC Policy for Humane Endpoints
6. Duran-Struuck, R. et.al. 2008. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptprca mice to commensal bacteria after whole-body irradiation in translational bone marrow transplantation studies. J Transl Med: 6:10

**LASC SULFATRIM (SEPTRA) DOSES AND DILUTIONS**  
**REVISED APRIL 2008**

Sulfatrim Stock solution (oral suspension):

200 mg sulfamethoxazole and 40 mg trimethoprim/5 ml = 240 mg sulfatrim/5 ml

Mouse drinks 15 ml/100g/24 hours = 150 ml/kg/24 hours

Rat drinks 10 ml/kg/24 hours = 100 ml/kg/24 hours

**FOR MICE:**

3.5 ml stock → 250 ml water =  $\frac{3.5}{250} \times 240 \text{ mg} = 0.6720 \text{ mg/ml}$  drinking soln.

5 ml x 250 ml

**Mouse dose** = 0.6720 mg x 150 mg = 100.8 mg/kg ~ 100 mg/kg/24 hours PO

ml x kg x 24 hours

**FOR RATS:**

2.78 ml stock → 250 ml water =  $\frac{2.78}{250} \times 240 \text{ mg} = 0.5358 \text{ mg/ml}$  drinking soln.

5 ml x 250 ml

**Rat dose** = 0.5358 mg x 100 mg = 53.58 mg/kg ~ 54 mg/kg/24 hours PO

ml x kg x 24 hours

