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# MEETING REPORT

# Animal Care in Radiation Medical Countermeasures Studies

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**Animal models are necessary to demonstrate the efficacy of medical countermeasures (MCM) to mitigate/treat acute radiation syndrome and the delayed effects of acute radiation exposure and develop biodosimetry signatures for use in triage and to guide medical management. The use of animal models in radiation research allows for the simulation of the biological effects of exposure in humans. Robust and well-controlled animal studies provide a platform to address basic mechanistic and safety questions that cannot be conducted in humans. The U.S. Department of Health and Human Services has tasked the National Institute of Allergy and Infectious Diseases (NIAID) with identifying and funding early- through advanced-stage MCM development for radiation-induced injuries; and advancement of biodosimetry platforms and exploration of biomarkers for triage, definitive dose, and predictive purposes. Some of these NIAID-funded projects may transition to the Biomedical Advanced Research and Development Authority (BARDA), a component of the Office of the Assistant Secretary for Preparedness and Response in the U.S. Department of Health and Human Services, which is tasked with the advanced development of MCMs to include pharmacokinetic, exposure, and safety assessments in humans. Guided by the U.S. Food and Drug Administration's (FDA) Animal Rule, both NIAID and BARDA work closely with researchers to advance product and device development, setting them on a course for eventual licensure/approval/clearance of their approaches by the FDA. In August 2020, NIAID partnered with BARDA to conduct a workshop to discuss currently accepted animal care protocols and examine aspects of animal models that can influence outcomes of studies to explore MCM efficacy for potential harmonization. This report provides an overview of the two-day workshop, which includes a series of special topic presentations followed by panel discussions with subject-matter experts from academia, industry partners, and select governmental agencies.** 

## INTRODUCTION

After the terrorist events of September 11, 2001, the U.S. Government focused additional medical response efforts on the nation's preparedness in the event of a radiological or nuclear incident. Multiple agencies were assigned the mission to support research to develop medical countermeasures (MCMs) to mitigate/treat injuries and to assess exposure (biodosimetry) following a mass casualty, or radiation public health emergency. One of these organizations was the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH), within the Department of Health and Human Services (HHS). Since 2004, with the inception of the Radiation and Nuclear Countermeasures Program (RNCP), the NIAID has supported MCM and biodosimetry advancement spanning all levels of radiation research: animal model development, basic research identifying and targeting molecular pathways involved in responding to radiation damage, as well as advanced product development and regulatory strategies leading to eventual licensure/approval/clearance of MCMs and devices by the U.S. Food and Drug Administration (FDA). The Biomedical Advanced Research and Development Authority (BARDA), also within the HHS, was initiated in 2007 and supports late-stage activities needed for product approval. BARDA is also responsible for the procurement of products to be placed in the U.S. Strategic National Stockpile (SNS).

Product development in this space is ultimately guided by the FDA's Animal Rule,[2] a pathway whereby a product can

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[2] FDA regulations commonly known as the *Animal* Rule: 21 CFR 314.600-650 for drugs; 21 CFR 601.90-95 for biologics.

be licensed (biologic) or approved (drug) based on animal efficacy studies since radiation testing in the human population would be unethical or unfeasible. For biodosimetry test development, the FDA published a Radiation Biodosimetry Guidance to advise sponsors on requirements for assay or test clearance (*1*). As such, the Animal Rule requires products to be tested in at least one well-characterized animal model relevant to the human condition. The biodosimetry guidance (which does not utilize the Animal Rule) requires the development of the signature or biomarkers in a large mammalian model that can be validated in clinical samples. Animal models must reflect different and complementary aspects of the clinical scenario with study endpoints that relate to the desired benefit in humans. For this reason, a single animal model won't completely replicate all these requirements. An inherent challenge in developing and using animal models is the limited human data available from actual radiation incidents (e.g., Chernobyl, Hiroshima, Nagasaki, etc.) to mirror in the laboratory animal. Hence, choosing an appropriate model and simulating radiation injury is difficult. While radiotherapy data from cancer patients can provide valuable insights, these data are often of limited value due to differences in the radiation delivery method and the fact that it is often localized to one part of the body. Radiotherapy is typically fractionated, whereas animal studies designed to simulate a mass casualty event normally employ a single acute, high-dose radiation exposure. To facilitate the evaluation of different MCMs, it will also be necessary to harmonize, to the extent possible, those variables that are amenable to standardization, such that experiments within a particular animal model performed at one institution may inform and be compared to experiments performed using the same or similar model at other sites.

To date, four MCMs to treat hematopoietic injuries resulting from radiation exposure have been approved under the U.S. FDA Animal Rule. These include granulocyte-colony-stimulating factor (G-CSF), also known as filgrastim (Neupogen®, March 2015); pegylated G-CSF, also known as pegfilgrastim (Neulasta®, November 2015); sargramostim (Leukine®, March 2018); and romiplostim (Nplate®, January 2021). While these approvals mark advancements in the field of radiation countermeasure research and position the U.S. to respond more adequately to a public health emergency involving radiation, treatment gaps remain. Other radiation-induced mass casualty-associated injuries with no approved MCMs include injury to the gastrointestinal (GI) tract, lungs, kidneys, cardiovascular system, and skin. In addition, as of the writing of this article, no biodosimetry test has been approved/cleared by the FDA for triage or determination of dose. Relevant animal models are currently under development for some of these areas, but more work is required to refine and optimize their use for systematic MCM efficacy testing. It is anticipated that the outcome of these efforts will result in the identification of promising candidates/tests to be advanced through the approval/licensure/clearance process.

On August 24–25, 2020, the NIAID RNCP, in partnership with BARDA, held a virtual workshop to discuss animal care protocols currently accepted in the field of radiation research, and to examine the multiple factors that can impact MCM efficacy studies. Specifically, the meeting goals were to identify and address gaps in knowledge, discuss solutions to facilitate the evaluation of MCM efficacy, and define variables amenable to standardization by sharing best practices for similar animal models from select institutions in the radiation field. A stated objective from the meeting includes the publication of this open-source document summarizing the meeting findings. In addition, information gathered from this meeting will assist the NIAID RNCP and other government/research partners to work together more effectively, facilitate ways to compare or replicate studies, and ensure accuracy and reproducibility in the field.

Subject matter experts along with meeting participants who took part in the panel exchanges on day 2 are listed in Table 1. Presentations and discussions from this two-day workshop have been captured in this meeting report and an outline of the topics addressed during the meeting is shown in Table 2. The audience included investigators from academia and private industry, as well as representatives from NIAID and BARDA, other federal government funding and regulatory agencies, and global research partners. The content of this meeting report contains only comments and information shared at this workshop and is *not* intended to be an official guidance document on animal care practices in radiation research.

## MEETING PROGRAM OVERVIEW

The workshop was structured as a two-day event, with Session I (day 1) consisting of animal model overviews followed by panel discussions on topic areas of interest with question prompts from the moderators. Session II (day 2) was comprised of talks about the individual components of animal care presented by radiation research experts, followed by a panel discussion. This document captures key points from both days of the workshop, including content from presentations and panel discussion dialogue. This meeting report is not meant to be a comprehensive review of all available literature on animal care.

## SESSION I: ANIMAL CARE FOR ANIMAL MODELS

Session I consisted of a series of focused talks on details of appropriate and humane care for animal models. Key animal care elements, natural history, and comparisons to the human condition were discussed. Specific topics addressed include the natural history of several models, and how they relate to the human condition, animal housing, infection control, concomitant medications, hydra-

**TABLE 1**
**Workshop Speakers and Areas of Expertise**

| Name | Affiliation | Area of EXPERTISE |
|---|---|---|
| Simon Authier, DVM, PhD | Charles River, Laval (CRL) | Safety pharmacology, irradiation, cell and gene therapy |
| Catherine Booth, PhD | Epistem, Ltd., Manchester, United Kingdom | Epithelial stem cell research, animal models, product development |
| Polly Chang, PhD | SRI International, Menlo Park, CA (SRI) | Radiation biophysics, product development |
| Sanchita Ghosh, PhD | Armed Forces Radiobiology Research Institute, Bethesda, MD (AFRRI) | Radiation countermeasures, molecular markers of radiation injury, delayed effects of ARS |
| Isabel Lauren Jackson, PhD | University of Maryland, School of Medicine, Baltimore, MD (UMSOM) | Normal tissue radiobiology; animal models of ARS/DEARE; MCM development under the AR |
| Matthew Lindeblad, BS | University of Illinois, Chicago (UIC) | Development of animal models of radiation injury, development and validation of biomarkers associated with ARS, and evaluation of MCMs |
| Thomas MacVittie, PhD | University of Maryland, School of Medicine Baltimore, MD (UMSOM) | Radiation biology; animal models, NHP and canine, medical countermeasure development, FDA AR |
| Meetha Medhora, PhD | Medical College of Wisconsin, Milwaukee, WI (MCW) | Rat models of radiation injury, DEARE, biomarkers, vascular effects of radiation |
| Maria Moroni, PhD | Biomedical Advanced Research Development Authority, Washington, DC (BARDA) | Animal models of radiation injury, MCM testing, pathophysiology of ARS, biodosimetry |
| Christie Orschell, PhD | Indiana University School of Medicine (IU) | Animal models of radiation injury, acute and delayed effects of radiation exposure, hematopoietic system, hematopoietic stem cell biology |
| Waylon Weber, PhD | Lovelace Biomedical Research Institute, Albuquerque, NM (LBRI) | Animal models, product development |
| Karen Wong, BSc | Charles River, Laval (CRL) | Irradiation, cell and gene therapy |

tion, diet, clinical condition, laboratory assessments, euthanasia criteria, and study design.

*Topic 1: Total-Body Irradiation in Rodents (Mice and Rats)*

*Natural History of the Models*. The total-body irradiation (TBI) mouse animal model, as described by Christie Orschell, allows for examination of the hematopoietic acute radiation syndrome (H-ARS), from a uniform total-body exposure administered in a bilateral/anterior-posterior or caudal-cranial manner. TBI elicits H-ARS that can include prolonged immunosuppression, impaired function of hematopoietic stem cells, and multi-organ, delayed effects of acute radiation exposure (DEARE) in long-term survivors of TBI doses (*2–10*). The Orschell laboratory has also developed young adult models of H-ARS with C57BL/6J mice exposed at three months of age (*9, 11–14*), Jackson Laboratories Diversity Outbred (JDO) mice exposed at eight weeks of age (*8*), C57BL/6J pediatric models where mice are exposed at three to eight weeks of age (*10*), and geriatric (12 and 24 months at exposure) models, all of which have been used for efficacy studies.

Several elements of animal care have been identified that can affect 30-day survival in these models. These components should be kept consistent for rigorous studies to yield high-quality data. Vendor and microbial barrier levels utilized have also been shown to impact survival, with lifespan differences noted in C57BL/6 mice bred and raised in different facilities (*15*). These differences were attributed to environmental factors that may impact mouse phenotype. For example, 24-month-old mice from Jackson Laboratory had >30% greater survival 30 days postirradiation, as compared to 24-month-old mice from the National Institute on Aging (NIA) ($P < 0.001$). It is noteworthy that mice from the Jackson Laboratory were received at 2.5–5 months of age and aged to 24 months at Indiana University (IU), whereas mice from NIA were received at 19–22 months of age, then aged to 24 months. Thus, the Jackson mice were exposed to the IU environment longer, influencing survival potentially due to differences in microbial barrier conditions. Vendor and microbial barrier conditions have also been found to influence the incidence of swollen muzzle syndrome, which can occur 2–5 days postirradiation (albeit rarely). Maximum microbial barrier housing conditions that employ sterilized individual ventilated caging and drinking water, and clean-room procedures where workers wear personal protection equipment, are correlated with a lower incidence of swollen muzzle syndrome (*13*). Swollen muzzle syndrome occurred more frequently in mice with the highest plasma levels of lipopolysaccharide (LPS), consistent with the presence of sepsis. Further, a single high radiation dose (25–30 Gy) to the snout alone does not cause swollen muzzles. Consistent with the GI source of swollen muzzle syndrome, antibiotics and/or bone marrow shielding decrease the incidence. Epistem has found that C57BL/6 mice are more susceptible to this syndrome than CBA mice, but this may reflect differences between vendors. Mice with swollen muzzles are often moribund or dead within 24 h of developing swelling of the muzzle, and these deaths can dramatically impact study results. Although differences in fecal microbiota diversity are found between rodents from different vendors, their contribution to the difference in radiosensi-

**TABLE 2**
**Meeting Sessions and Topic Areas**

| **Session I: Animal Care for Animal Models Speaker Series** |
|---|
| Presentation Topics: |
| □ TBI in rodents (mice and rats) |
| □ TBI in large animals (minipig, rabbit, NHP) |
| □ PBI in rodents (mice and rats) |
| □ PBI in large animals (NHP) |
| Panel Discussion Topics: IACUC and statistical considerations impacting study design (e.g., euthanasia criteria) |
| □ Inter-institutional data used to defend appropriate endpoints |
| □ Pros and cons of harmonizing animal models |
| □ Academic and corporate views and possible alignment |
| □ Major obstacles to harmonizing animal models and how they can be mitigated |
| □ Consideration of FDA-approved treatments, such as G-CSF, as standard of care in animal model development |
| **Session II: Baseline Animal Care in Radiation Research Panel Discussion Series** |
| Panel Discussion Topic 1: Animal Housing and Handling |
| □ Importance of group housing, social interactions, enrichment strategies |
| □ Designing experiments, standard procedures to minimize biological variables (e.g., stress, discomfort, pain, single vs. group housing) to enhance reproducibility and rigor |
| □ Consideration of how sex and age impact housing and handling (e.g., male vs. female pheromone exposure) |
| □ Minimizing stress during animal transport and how this affects study outcome (e.g., survival, CBC etc.) |
| Panel Discussion Topic 2: Infection Control |
| □ Use of antibiotics in irradiated animal models (e.g., prophylactically, therapeutically, trigger to treat, or not used) |
| □ Antibiotics commonly used (e.g., drug name, dose, and frequency administered) |
| □ How antibiotics can impact survival of irradiated animals |
| □ IACUC approval of antibiotic use in irradiated animals |
| □ Concerns of antibiotic resistance in irradiated animals |
| □ Staff procedures to minimize cross-contamination, such as use of special cages, room access, feed handling (e.g., non-irradiated vs. irradiated animals, etc.) |
| Panel Discussion Topic 3: Hydration and Diet |
| □ Considerations for adequate feeding of irradiated animals (e.g., nutritional gel packs, wetted chow, non-citrus fruits, soft food vs. hard food, to balance between loss of teeth and appetite) |
| □ Considerations for adequate hydration of irradiated animals (e.g., water gel packs vs. drinking water, acidified v. non-acidified water; self-watering systems or refillable water bottles |
| □ Best routes of administration for hydration and diet in irradiated animals (e.g., oral, intravenous, nasogastric, subcutaneous bolus) |
| □ Changes in survival based on dietary components in irradiated animals (e.g., isoflavones) |
| □ Consideration of feeding and fasting schedules and the impact on diurnal rhythm |
| Panel Discussion Topic 4: Euthanasia Criteria |
| □ Sharing information between institutional IACUCs to harmonize euthanasia criteria for specific models |
| □ Consideration of euthanasia criteria to ensure ethical treatment of animals without biasing endpoints (e.g., body weight loss, injection site injury) |
| □ Concerns about euthanasia criteria and confounding institutional lethality profiles |

tivity and lifespan is unclear (*16*). Given these data, consistency in the mouse vendor is crucial for studies.

Overall, the health status of animals should be assessed before the study start, and animals found with pre-existing conditions such as malocclusion, barbering, dermatitis, or malignancies should be excluded. Additionally, identification methods should be considered before the start of the study, allowing for recovery time. Some methods such as tail tattooing can lead to combined injury from the wounding and influence sensitivity to radiation exposure. In addition, sex, age, and weight have been shown to influence radiosensitivity. Radiosensitivity can also change with age, with 3-week-old mice showing the highest sensitivity. Sex-associated radiosensitivity has also been noted, with female mice being more radioresistant than males between 5 weeks and 3 months of age, but male mice being more radioresistant at $<$5 weeks or $>$3 months of age (*10*). For this reason, weight range should be controlled as much as possible, and outliers should be excluded before the study starts. Even when vendor and microbial barrier levels are controlled, other environmental variables may impact mice over time. Interestingly, observational data indicate that there has been a gradual increase over 14 years in body weights of 11-week-old C57BL/6 mice obtained from mouse vendor, Jackson Laboratory, with an increase in mean weight of $\sim$1.5 g for female mice and $\sim$2.7 g for male mice (data not published). Increases in mouse body weight seen over the years may be due to subtle changes in husbandry and unidentified environmental factors. These findings highlight the importance of periodically re-establishing institutional radiation dose-response curves for rodent irradiation studies.

*Irradiation Setup.* Factors to be considered include the radiation source, dose rate, best practice for dose validation, irradiation geometry and setup, and how long animals are left in the apparatus during irradiation are all factors to be considered. The Orschell Lab irradiates mice in a single Plexiglas chamber with rotation to ensure uniform exposure using a total-body dose of gamma radiation from a $^{137}$Cs radiation source (Gammacell 40 Exactor; Nordion International) at an exposure rate of 0.63–0.68 Gy/min. Once irradiated, mice are divided among treatment groups and each exposure is confirmed using in-run dosimetry (*11, 12*). Animal body temperature may increase with time spent in the pie jig or irradiator too long, affecting radiosensitivity. The time of day of irradiation is another factor, as mice have been shown to exhibit chrono-radiosensitivity. Mice are most radioresistant when exposed between 11 am to 1 pm, while mice irradiated in the early morning (9–11 am) or later in the afternoon were more radiosensitive (*11*).

*Husbandry and Handling.* Husbandry aspects such as bedding, and enrichment may also impact study results. Mice will segregate distinct sections of their cage into ''dirty'', ''clean'', and ''apartment'' areas, particularly when housed alone (*17*). Mice should receive food, clean bedding with enrichment (e.g., nesting materials), and water

regularly. Additionally, the use of acidified (pH 2.0–3.0) vs. non-acidified water has been shown to reduce the radiosensitivity of mice (*18*). Provision of wetted chow in Petri dishes for mice that experience postirradiation tooth loss can help with animal nutrition. Housing should also be standardized, with vented racks, microbial barrier cages, and animals housed in social groups. For example, the ''apartment concept'' has been a successful group-housing method for mice. This concept works because it supports the natural behavior of the mice. Geriatric mice, for example, huddle to make one apartment with less trampling, unlike younger mice that don't seem to mind the activity. Group housing is not always possible, as some strains are particularly aggressive and may need to be singly housed, and as animals get older, they may barber each other, requiring separation. Temperature, humidity, air changes, and the light/dark cycle of their environment are also important and should be controlled (*19*). Additionally, MCM dosing volume, administration route/site, and frequency, as well as any handling due to blood sampling, can influence stress levels and may impact mortality.

*Euthanasia Criteria.* Well-developed euthanasia criteria specific to each animal model are key to any well-constructed study. Animals are euthanized based on the clinical judgment of well-trained veterinary staff, based on criteria determined before the study start. Animal health should be monitored on a pre-established schedule with baseline status known before the start of the study. Ensuring all caretakers and staff are aware of the schedule and study needs is vital to the success of a study. At IU, mice are observed twice daily during the H-ARS timeframe and once daily at other times. Animals are given a score of 0–3 for each of the following criteria: 1. severity of hunched posture, 2. squinted/closed eyes, and 3. level of decreased activity (*11*). If the sum of the three scores is 8–9, or the mouse exhibits signs of central nervous system damage, it is humanely euthanized. For one study utilizing this system, 50% of decedents were euthanized while the other 50% were found dead. Other groups have found daily body weights to be helpful; however, this criterion is not always used since the added handling stress can impact survival. It is important to note that in 8–10-week-old C57BL/6 mice, deaths due to TBI H-ARS usually occur between 2–3 weeks, with the first death occurring around day 7–9, and most deaths or major morbidity occurring by day 24. The timeline can differ based on the mouse strain and/or animal model. After day 24, deaths are infrequent and only daily monitoring is sufficient; however, the monitoring schedule is dependent on Institutional Animal Care and Use Committee (IACUC) approval.

*Other Factors to Consider.* As with any animal model, there are often differences between the mouse model and the human condition. One of the main differences relevant to radiation MCM research is the species-specific effect of G-CSF on the recovery of blood count parameters during H-ARS. In humans, G-CSF promotes the recovery of neutrophils only, while in non-human primates (NHPs) and canines it also promotes the recovery of platelets (*20, 21*), and in mice, it promotes the recovery of all three lineages (neutrophils, platelets, and erythrocytes) (*22*). Lethal radiation doses are also different across species, with the $LD_{50/30}$ for C57BL/6J and JDO mice ranging from 8.5–9.0 Gy, while the $LD_{50/60}$ for humans is estimated to be 4 Gy without supportive care and 6–7 Gy with supportive care (e.g., antibiotics and fluids) (*23*). However, extensive administration of supportive care can be a confounder in mice, due to handling stress. As described previously, radiation sensitivity in the mouse H-ARS model appears to be age-dependent (*10*); however, age equivalence is also difficult to determine, as mice are estimated to mature at 150 times the rate of humans at 3-4 weeks of age, and 45 times faster than humans at 5 weeks to 3 months of age (*24, 25*). Yet another factor to consider is the period between the presentation of euthanasia criteria and death, a period that can be markedly short, thus making frequent observations important. Deaths that occur later in the expected range for H-ARS (2–3 weeks), tend to be preceded by a longer period of morbidity that could be identified with more frequent monitoring.

### *Topic 2: Total-Body Irradiation in Large Animals, Minipig (MP), Rabbit, Non-human Primate (NHP)*

*Natural History of the Animal Models.* Karen Wong presented background on several large animal TBI models developed at the Charles River Laboratories (CRL), Laval facility, including NHPs (specifically rhesus macaques), Göttingen minipigs (MPs), and New Zealand White rabbits. TBI animal models are typically used for H-ARS studies, resulting in bone marrow myelosuppression and possible myeloablation at high enough doses of radiation. This bone marrow impact can lead to lymphopenia, thrombocytopenia, neutropenia, and decreases in other blood cell parameters. These drops in blood cell counts manifest approximately 10–19 days postirradiation in these species; however, the timeframe of disease onset or critical period is highly dependent upon the animal model, radiation dose, receipt of supportive care, and other factors.

The natural history of the various animal models is akin to what would be expected in humans as clinical signs of radiation exposure seen; these include emesis (not seen in rodents), diarrhea, ulcerations of the oral mucosa, wound development, decreased activity, and reduced appetite that often leads to a decrease in body weight. During neutropenia, fluctuations in body temperature, particularly increases, have also been noted. Furthermore, changes in blood parameters measured via hematology, clinical chemistry, and blood coagulation as well as the development of systemic infections are noted as well. Macroscopic and microscopic changes to tissues and organs including hemorrhage, necrosis, and hypocellularity are also observed.

**TABLE 3**
**Antibiotic Selection and Schedule in Irradiated Animal Models**

| Mouse | Rat | Rabbit | Minipig | NHP |
|---|---|---|---|---|
| Acidified, autoclaved water | Enrofloxacin (~10 mg/kg/day) in drinking water from day 2 to 14 | Bactrim (sulfamethoxazole/trimethoprim, 30 mg/kg) once daily from day 3 to 30 | Amoxicillin (10 mg/kg, PO, BID from day 3 to 30), flavored tablet | Full supportive care model: Trigger to treat adapted from UMSOM |
| Ciprofloxacin in water - common prophylactic regimen in mice (UIC) | | | Gentamicin (2 mg/kg, PO, daily from Day 3 to 30) | Minimal supportive care model: Enrofloxacin, 10 mg/kg, PO or IM |
| Antibiotics in water no improvement in survival (UIC) | | | | |

*Irradiation Setup*. As with rodents, the factors to be considered for NHP irradiations include the radiation source, dose rate, best practice for dose validation, the radiation setup and geometry.

The setup for NHPs at the CRL facility allows for irradiation while animals are conscious or unconscious. In the conscious setup, NHPs are positioned in a crouch position with limbs restrained. In the unconscious setup, NHPs are sedated and placed in a supine position. For both conscious and unconscious irradiation, the exposure is split into two fractions, with half the radiation dose given anterior to posterior and the second half administered posterior to anterior. This is important to note because exposure can change based on the facility. For example, in exposures done at the Armed Forces Radiobiology Research Institute (AFFRI), both small and large animals can be irradiated from both sources simultaneously or sequentially, which can potentially have a different impact. During irradiation, animals are made as comfortable as possible, including the playing of soft music for all irradiation studies, to decrease stress for the animals and facility staff. There are also cameras to continually monitor animals during irradiation, and team members can stop the irradiation if needed. For the MP setup, animals are sedated and placed in a sling; the legs protrude through the bottom of the sling and are restrained. Similarly, rabbits are also sedated and placed in slings for irradiation, with the ability to irradiate two rabbits at a time. In both cases, the positioning is such that irradiation is lateral, with half of the dose received to the left and the other half to the right side.

*Husbandry and Handling*. General husbandry and animal handling are similar across different species. NHPs, MPs, and rabbits are all provided purified water via automatic sippers, and certified food, with commercial biscuits given to NHPs and commercial chow provided to MPs and rabbits. MPs and NHPs also receive edible enrichment, which includes fruits and vegetables. Irradiated large animals are singly housed to allow the team to monitor bloody feces, diarrhea, or emesis more precisely, facilitating individualized medical management. This practice also prevents contamination or infection between animals that may have skin lesions. NHPs are housed in ''squeeze back'' cages with perches, MPs are placed in cages with side doors, and rabbits in cages with underpads. Although housed separately with limited physical contact, the housing allows visual and scent cues to encourage socialization. In addition, other methods of enrichment include puzzles and movies for NHPs or rooting toys for MPs and rabbits.

Animals are handled while conscious before and after irradiation, so care must be taken, and all cage materials should be clean to avoid infection. It is also important to note that different methods of restraint have pros and cons. For example, slings can contribute to irritation to the limbs where restraints are fastened as well as irritation in the axillary, inguinal, or urogenital areas. For NHPs, the CRL facility employs chair restraints, which allows access to the femoral area for blood draws, as well as easier assessment of limb usage and function. In addition, chairs are available for MPs, where they sit on the seat like a saddle. Using this kind of restraint allows access to the cephalic and jugular veins for blood draws and seems to cause less stress to pigs than the sling. Rabbits are restrained with rabbit snuggle restraints that cover the eyes to decrease stress while allowing access to the ears for blood draws or treatments.

*Supportive Care: Prophylactic and Trigger to Treat*. Medical management is specific to each model, but the products and care provided to large animal models should mimic the human condition in the event of a nuclear disaster. This can be sub-divided into prophylactic (provided before symptoms appear), trigger-to-treat supportive care, or time-sensitive MCMs. Prophylactic supportive care includes antibiotics, analgesics, antiemetics, food, and fluid supplementation. Given that the immune system is suppressed, and pain and stress are highest during the critical period (i.e., the onset of disease), supportive care is provided during this time to alleviate symptoms and help with survival. Supportive care can include 1. antibiotics (Table 3); 2. analgesics (e.g., buprenorphine) by subcutaneous (SC) route of administration in NHPs and rabbits and using transdermal patches in MPs; and 3. antiemetics (e.g., ondansetron) pre-and postirradiation in NHPs and MPs.

The standard procedure of swabbing sites with alcohol and/or chlorhexidine before administration of prophylactic antibiotics or blood collections is of particular importance as animals may be more susceptible to infection after irradiation. If a blood sample is damaged or lost, a re-draw

is not permitted from the same animal to maintain consistency of blood volume collected across animals and to ensure that the overall blood budget is not affected. Certain blood collection sites like the femoral area for NHPs or jugular for pigs are avoided during certain critical periods because platelet counts can be low, increasing the risk of hemorrhage or hematomas. Trigger-to-treat supportive care is provided when symptoms manifest, per pre-set criteria, and can include additional antibiotics and analgesics, parenteral fluids, nutritional support, wound support, and blood products. For additional fluids, oral electrolyte replacement, like Pedialyte® (Abbott Laboratories) may be given. NHPs may also receive IV lactated Ringer's solution or oral carbohydrate and electrolyte replacement, Gastrolyte® (Sanofi), and MPs may receive juice. Chewing and digestion may be problematic following radiation. NHP nutritional support may include crushed biscuits with banana and/or fruit/vegetable buffet. Pigs may receive oral nutritional replacement Ensure® (Abbott Nutrition), fruit and vegetable buffets, hay, or grass, and rabbits may be provided Ensure. If NHPs or pigs develop open skin lesions, they may be treated with hydrotherapy, iodine, and topical antibiotics. NHPs may also have lesions flushed with cefazolin or treated with topical lidocaine, which minimizes animals picking at the wounds to prevent further irritation. NHP full supportive care models may include blood product administration at pre-determined triggers, where whole blood or packed red blood cells are given; however, this process is very labor-intensive and requires a set of donor NHPs for blood supply. In general, the NHP model of H-ARS developed through NIAID, includes blood transfusions as a part of standard medical management. All blood donor animals are in good health and are seronegative for simian immunodeficiency virus, simian T cell leukemia virus type 1, malaria, Herpes B virus, and tuberculosis (*26*).

When establishing a supportive care regimen, it is important to consider the types of supportive care that might be available to an affected human population in a mass casualty scenario. For example, some care can be self-administered orally (PO) or SC injection while others administered via intravenous (IV) route may require trained medical personnel potentially in a hospital setting. Overall, significant attempts are made to provide any injectable supportive care via a SC route of administration rather than intramuscular (IM) to avoid muscle tissue injury and possible hemorrhage. Hydration supplementation is provided buccally so animals can drink or eat it, rather than via oral gavage which may add stress and potential trauma. Another concern is the lack of supportive care that would be available in compromised hospitals or other infrastructure, and how feasible it would be to treat a large population with the same type of supportive care. For example, it may not be feasible to administer blood products or transfusions to a large population due to labor and resource limitations. These factors must be considered when conducting animal model studies. In some cases, animal facilities may be able to provide extensive supportive care including blood products, while others may have supportive care limited to analgesics, the minimum required by the Institutional Animal Care and Use Committee (IACUC). In all cases, planned supportive care programs must be scientifically justified and any limitations should be addressed before they are reviewed and approved by the IACUC.

With large animals, it is possible to monitor more endpoints throughout the in-life portion of the study, such as body weight, temperature, clinical chemistry, hematology, and coagulation. Additionally, for NHP studies, hemoculture for bacteriology may be assessed. These are usually scheduled to help assess euthanasia criteria, initiation of supportive care, and the general monitoring of the animal. One alternative to minimize animal handling for body temperature and/or blood pressure monitoring in NHPs is a surgically inserted telemetry device. This approach decreases stress from handling the animals and provides remote access to a continuous data output. However, this procedure requires a longer pre-treatment time for animals to recover from the insertion surgery before irradiation and can increase the risk of infection or other surgical complications. Additionally, the inserted telemetry device may not function. For NHPs, outside of the critical period, the femoral vein is the main source for blood collection as it is the easiest to access. The use of a telemetry device reduces this access port for blood draws during the study.

A balance between the frequency of animal monitoring and allowing the animals to rest is necessary. Recently, CRL instituted an infrared camera system, allowing stress-free remote monitoring during the lights-out night period to monitor for euthanasia criteria providing the implementation of humane endpoints. Staff only enter the room if an animal is suspected to meet euthanasia criteria. The current setup only allows for infrared monitoring of the upper cages, necessitating more cages and rooms for a study. In addition, due to the sensitive wiring setup, cage maintenance and exchange must be done carefully to avoid damage to the monitoring system.

*Euthanasia Criteria.* Main euthanasia criteria used across models at CRL include severe respiratory distress with increased and/or labored respiration, anorexia, or decreased appetence over multiple days with no interest in food treats, sustained and/or severe weight loss, recumbency or unresponsiveness, gross blood loss, or hemorrhage that cannot be controlled, seizure activity, or severe dehydration with hypo- or hyperthermia. An additional criterion that CRL has added is severe pain, which is considered inhumane if it cannot be significantly alleviated with analgesics. Finally, consistency in euthanasia criteria across studies in the same model is important for staff support considerations during the critical period, when a higher workload is anticipated. These considerations allow for a more predictable lethality curve and comparisons among studies of the same animal model.

*Other Factors to Consider*. Maintaining the same technical staff throughout a study allows animals to become accustomed to handling, thus decreasing animal stress. In addition, a consistent small team of personnel authorizing euthanasia criteria introduces less bias and variability. Technical staff should meet before, during, and after each study to communicate the details of each study. Research activities should be scheduled for the same time of the day, for comparison of results and to reduce stress on the animals. The frequency of endpoint sampling and assessments, both invasive and non-invasive, should be determined before the study start. Well-controlled pivotal studies should be conducted by blinded technical and veterinary staff, although this is not a requirement for proof of concept or animal safety studies.

The post-presentation discussion addressed several points of interest. For example, it was noted that no significant differences were seen between NHPs that received similar supportive care measures and were either conscious or unconscious (anesthetized) during irradiation, although CRL has not conducted a head-to-head analysis to verify this observation. NHP blood transfusions generally consist of irradiated whole blood (plasma and leukocytes) that is then analyzed with a hemanalyzer. Laboratory assessments of hematologic parameters are obtained by mid-morning to identify animals as candidates for blood transfusion later in the day. Donor animals are assessed based on recovery time from their previous donation. Blood is collected from these animals and taken to the radiation facility to be irradiated. Animals in need of a transfusion receive the blood the same day it is collected. Criteria used to determine the provision of whole blood treatment are different from criteria used for the provision of packed red blood cells, and treatment determination is based on platelet levels. If platelet levels are sufficient, animals are candidates for intervention with packed red blood cells. This approach is used particularly in larger studies, where medical management is being provided to as many animals as possible and whole blood is kept for animals who will require it. It has been noted that blood cell parameters undergo significant improvement following whole blood transfusion, which provides platelets and red blood cells, as compared to infusion of packed red blood cells which contain no platelets.

### *Topic 3: Partial-Body Irradiation in Rodent Models (Mice and Rats)*

*Natural History of the Models*. Catherine Booth presented information on the partial-body irradiation (PBI) mouse model, which uses male C57BL/6 mice (10–12 weeks of age, ~26 g weight) under uniform conditions of caging, handling, and feeding. In this model, Epistem Research Services has evaluated the natural history of GI-ARS, characterized by dramatic weight loss, diarrhea, and high radiation-dose dependent mortality that varies based on the use of concomitant antibiotics or partial body shielding. Mice lose weight dramatically and develop loose stools/diarrhea beginning 4 to 5 days postirradiation that may last 4–5 days. After this, mice are either moribund or recovering. Mice do not vomit, and nausea/stomach cramping cannot be assessed or quantified. Mortality manifests within a week of radiation exposure, though this timeframe may be extended with the use of antibiotics and is also delayed in PBI models compared to TBI. With TBI, all animals die within 2 weeks from either GI-ARS or H-ARS, similar to the human condition.

The PBI model provides minimal bone marrow sparing, which allows for the evaluation of mitigator efficacy in more than one syndrome – GI, hematopoietic, and/or lung; however, survival outcomes are significantly tied to IACUC criteria for animal care and euthanasia (*27*). Applying strict weight loss euthanasia criteria can result in earlier euthanasia of animals that may have otherwise recovered, particularly at higher dose levels (e.g., >12 Gy PBI). A 10-day difference was noted in the lethal dose curve of mice when employing weight only vs. multiple criteria at 12 Gy exposure (data not published) since weight alone does not consider other critical health factors that can impact whether an animal is euthanized. In some cases, animals may reach a weight loss cut-off but may have no other signs of distress and will recover. However, lowering the weight loss cut-off, in combination with other critical health factors, may result in more accurate decisions regarding euthanasia.

*Irradiation Setup*. In addition to the factors described previously (radiation source, dose rate, best practice for dose validation, radiation setup and geometry), Epistem minimizes variability in outcomes due to the circadian rhythm as well by irradiating animals at a prespecified time of day using a common X-ray source with uniform conditions of irradiation. Mice are placed in a subdivided plexiglass box, such that 10 mice may be irradiated at once. Epistem allows only a 2-h time window for irradiation per day to minimize variability, and in that timeframe, they can irradiate 60–80 mice. For large studies, groups are staggered over several days. Mice are anesthetized and restrained with the lower part of one hind limb (~2.5% of the bone marrow) shielded with a lead tube from the knee down.

*Husbandry and Handling*. Epistem purchases all mice from the same vendor to ensure the same microbial barrier level is maintained, which reduces variability between experiments. Ventilated cages are kept in a specific pathogen-free unit with a 12-hour light/dark cycle, temperature set to 21°C ±2°C, and mean relative humidity of 55% ±10%. When possible, mice are typically housed 5 per cage with nesting materials for enrichment; however, male mice are often housed individually. Handling, dosing, and weighing are conducted outside of cages in laminar flow workstations to prevent infection/contamination. Mice are provided acidified water and a consistent Harlen Teklad 2018 extruded rodent diet (same diet provided to mice at IU). Additionally, mice are acclimated for two weeks before any study start.

*Supportive Care: Prophylactic and Trigger to Treat.* Supportive care (e.g., analgesia and antibiotic) can have a significant impact on study outcomes, thus it is important to determine their effect on the efficacy of experimental mitigators (*19*). For example, antibiotic use can alter the incidence of diarrhea and even platelet counts and increase survival after irradiation (*11*), suggesting an impact beyond infection. Hydration is also important, as sick animals drink less, and animals with diarrhea become prone to dehydration. Fluid supplementation can increase survival but handling and dosing for supplementation in sick animals can reduce survival; therefore, a balance must be struck when determining the duration, volume, and frequency of fluid supplementation. At Epistem, PO and SC fluid administration during GI-ARS, at a dose of 5–10 mL/kg or 0.1–0.2 mL volume once or twice daily, showed some benefit at lower radiation doses, but the stress of handling at higher radiation doses outweighed the benefit. Providing wetted chow or gel packs proved a less stressful means of supplementing hydration in mice receiving GI-ARS doses of radiation.

Diet may also influence the study outcome; calorie restriction before irradiation has been shown to benefit intestinal stem cell regeneration and crypts (*28, 29*). Glucose regulators such as GLP1/2, ghrelin, octreotide, and metformin given before or after radiation exposure can also modulate radiosensitivity and crypt recovery, potentially due to their influence on underlying endocrine pathways. Additionally, vitamins D and E, antioxidants, and soy derivatives in the diet may also mitigate GI-ARS. For these reasons, acclimation to a new chow is very important and must be factored into a study design. Whenever a new type of chow (such as one containing a potential mitigator) is introduced, mice may be initially averse to the taste or texture, thus decreasing caloric intake and impacting survival. It is recommended that diet change not be introduced abruptly during the in-life portion of the study. It is also important to assess if the mitigator being tested has analogs or inhibitors included in the chow. Additionally, dosing with excipients, such as oils given orally, may affect satiety and caloric intake.

*Euthanasia Criteria.* At Epistem, welfare checks include body weight and stool consistency measurements at least once per day, increasing to 2–4 times per day during the critical period (every 6 h at peak deterioration). As noted by other speakers, observations include weight loss, as well as changes in posture, grooming, respiration, and interactions. At Epistem, animals are considered moribund if they have $>$20% weight loss that doesn't rebound in 8 hours and exhibit at least one other sign such as hypothermia, hunched posture, rough coat, abnormal respiration, little peer interaction, or listlessness when handled. Discussions with the IACUC are encouraged to minimize animal suffering and to determine additional surrogate endpoints for euthanasia criteria. Upon euthanasia, tissue histology and blood plasma biomarkers can further define endpoints that are useful for euthanasia criteria.

*Other Factors to Consider.* In humans, DEARE following GI-ARS is characterized by intermittent episodes of diarrhea and constipation. Humans develop obstructions, fistulas, sepsis, increased vascular permeability with persistent local inflammation and fibrosis, vascular sclerosis, and local hypoxia (*30, 31*); however, mice do not develop these episodes of diarrhea and constipation, but they do lose weight and develop intestinal fibrosis. GI-ARS causes a reduction in intestinal barrier function as measured by electrical resistance and increased bacterial translocation. Different sizes of fluorescently-tagged dextran administered in mice by oral gavage, which only crosses leaky intestinal epithelial barriers, have shown both time and radiation dose-dependent progressive loss of intestinal barrier function (*32, 33*). Additionally, at Epistem, lipopolysaccharide (LPS) levels, an indicator of bacterial translocation and systemic infection, showed an increase in plasma and tissue lysates from various locations in the GI tract over time and with increasing doses of radiation. Blood was evaluated for specific bacterial species; *Escherichia coli*, *Streptococcus faecalis*, and *Streptococcus faecium* were identified, indicating that the circulating bacteria are derived from the gut. Organs were found to contain the same bacterial species as well, indicating bacteremia which can lead to sepsis as has been seen in humans at comparable timeframes post-irradiation (*13*).

Differences in radiosensitivity, immune tolerances, and gut permeability have been noted across mouse strains as well as strain variants, including knock-out or knock-in variants for investigating the involvement of certain key genes. Age and sex differences in irradiated mice have not been extensively studied at Epistem; however, age-related trends have been observed, with 6–8-week-old mice being more radiosensitive than the typically used 10–12-week-old mice, and 16–20-week-old mice being more radioresistant. Researchers have also noted that the proliferation of intestinal stem cells appears to be influenced by the estrous cycle, though they have not evaluated sex-matched dose-response curves in PBI GI-ARS mouse models.

Other factors that may impact survival in PBI models include co-housing with littermates, as lone animals become moribund faster (especially when their littermates are euthanized), due to social stress and loss of littermate warmth. Epistem has not attempted re-housing for fear of in-fighting to establish new social hierarchies. The impact of circadian rhythm and GI severity was also addressed, in that researchers have observed that different GI sensitivity depends on the time of day that animals are irradiated. Animals are more radiosensitive just after their GI cells are stimulated to proliferate. Since mice are nocturnal and feed at night, care should be taken not to irradiate animals during this time.

The PBI model can also be used to study lung-DEARE, but strain differences in pathology and severity of lung

damage have been noted. C57BL/6 mice are more radioresistant than C3H/HeN or BALB/c strains. C57BL/6 mice, often used in H-ARS and GI-ARS studies, develop terminal pleural effusions, a treatable condition in humans (*34*). C57L/J, C3H/HeN, and CBA mouse strains develop pneumonitis, which may be more representative of lung radiation damage in humans (*35*). In addition, sex differences in pneumonitis response in the WAG/RijCmcr rat lung-DEARE model have also been noted with male rats displaying more radiosensitivity and higher mortality due to lung-DEARE (*36*). Importantly, inbred rodents with standardized age, weight, housing conditions, etc., which are typically used for animal models do not represent the diversity seen in an affected human population. To have a successful study outcome, it is critical to have a well-developed and well-characterized model whose natural history is known.

*Topic 4: Partial-Body Irradiation in Large Animal Models (NHP)*

*Natural History of the Models.* The overall goal of preclinical laboratory models is to build an interspecies bridge linking the human response to radiation exposure and subsequent treatment to that of animals in controlled radiation and treatment studies. To achieve this goal, animal models require development, refinement, and validation, which requires an understanding of the relationship between radiation physics and radiobiology in the model species and humans. Thomas MacVittie presented data from studies performed at the University of Maryland School of Medicine (UMSOM) in a PBI, BM-sparing model in the NHP, focusing on linking ARS with DEARE and multiorgan injury in NHPs. When planning NHP work, one should first explore/determine: 1. the purpose of the animal model, 2. the condition being modeled, 3. key elements of animal care (e.g., medical management, study design, choice of animal model, sex, age), and 4. whether the natural history of the animal model reflects radiation exposure in humans, including whether treatment effects are similar between the animal model and humans.

The NHP model has been used to establish radiation dose-response curves for GI-, H-ARS, delayed lung, and multiple organ injury effects (*37*). The model is intended to assist in the determination of longitudinal effects of radiation including latency, incidence, severity, progression, and duration of concomitant ARS and DEARE organ-specific effects as well as the impact of medical management including supportive care, on these acute and delayed effects. Information was presented on the dose-response curves, comparing mortality in various irradiation protocols that included TBI, PBI/BM, and whole thorax lung irradiation (WTLI) (*38–41*). These curves suggest that ARS does not impact the development of lung injury in the PBI/BM-sparing model (*39*).

Researchers have tried to compare the effects of irradiation in NHPs and humans; however, significant gaps remain in the field (*23, 42–54*). A better understanding of latency, incidence, severity, progression, resolution/durability of effects, and whether key signs of morbidity can predict clinical outcomes in terms of mortality is needed. The challenge is to determine optimal, validated models that define the early and delayed effects of acute radiation exposure. This can identify potential links between these effects and MCM efficacy, to be predictive of the human response to acute irradiation and treatment.

Depending on the observation time for multi-organ injury within the ARS and DEARE sub-syndromes, different calculations for $LD_{50}$ values based on the time when each phase manifests itself can be derived from the same NHP PBI/BM5 model. For example, for GI-ARS, the $LD_{50/15}$ is 12.01 Gy, for H-ARS, the $LD_{50/60}$ is 10.88 Gy, and for lung DEARE, the $LD_{50/180}$ is 9.94 Gy (as compared to WTLI, which is 10.24 Gy) (*37*). A reduction from 5.0% to 2.5% in BM-sparing increased mortality from 28% to 58% due to H-ARS, suggesting a major impact of bone marrow shielding.

*Irradiation Setup.* Factors again include the radiation source, dose rate, best practice for dose validation, radiation setup and geometry. At UMSOM, male rhesus macaques studied under the PBI/BM2.5 protocol, received a dose of 10 Gy to a point at midline tissue using 6-MV linear accelerator photons at a rate of 0.80 Gy/min. Point and organ doses were calculated for each NHP from computed tomography (CT) scans using heterogeneous density data. Three-dimensional reconstruction of the CT images can provide valuable information about the variations in body shape and size. Density corrections can be made for each of the tissue types, bone, water, muscle, and air, to determine differences in dose to the NHP (*41*). PBI/BM-sparing models are unique because they allow for a multi-organ injury-based approach rather than a limited injury as with TBI and other irradiation models such as whole thoracic lung injury (WTLI) (*55, 56*), TBI plus WTLI (i.e., ''top-up'' model) (*57*), and/or total-abdominal irradiation (*58, 59*), among others. PBI/BM sparing, at either ~5.0% or 2.5%, uses a high threshold dose of near TBI with marginal bone marrow sparing, to permit dose- and time-dependent survival through the ARS to delayed multiple organ injury.

*Supportive Care: Prophylactic and Trigger to Treat.* Medical management of NHPs at the UMSOM is based on triggers-to-treat and stop through the ARS and DEARE periods. Treatment triggers are based on measurements, such as blood counts or body weight, or clinical signs, such as dehydration. For GI- and H-ARS, clinical signs, and blood parameters such as neutrophil and platelet counts, as well as diarrhea and hydration, can be assessed. Histopathologic changes to intestinal crypts, villus architecture, and mucosal integrity of small and large intestines can be assessed at autopsy. For DEARE, clinical signs such as non-sedated respiratory rate, SpO2, and arterial blood gases can be evaluated for changes over time. CT scans and

parameters like blood urea nitrogen can also be used to assess lung or kidney damage.

For H-ARS models at UMSOM, NHPs receive an antibiotic regimen when febrile neutropenia [39.4°C and absolute neutrophil count (ANC) <500/μL] is observed. Whole blood transfusions are administered upon triggers related to hematocrit and platelet counts. During the DEARE period, NHPs are given dexamethasone intramuscularly if the non-sedated respiration rate is ≥80 breaths per min, then tapered for 13 days and repeated if the NHP meets trigger-to-treat criteria again. This regimen permits the animals to be maintained through euthanasia criteria to allow for assessment of MCM interventions at later time points. Other supportive care based on trigger-to-treat in the PBI/BM-sparing model includes antipyretics, fluids, antidiarrheals, antiemetics, nutritional support, analgesics, and diuretics. Given the multi-organ injury of ARS/DEARE, it may be of interest to investigate polypharmacy approaches, particularly the use of MCMs of interest concomitant with Neupogen, Neulasta, Leukine, and/or Nplate (Romiplostim), in the context of comorbidities and impact on DEARE.

*Euthanasia Criteria*. Criteria in place at UMSOM that could trigger euthanasia in NHPs include inactivity and non-responsiveness, self-mutilation, seizure, hemorrhage from an orifice, rectal temperature ≥41.1°C or <35.5°C for more than 6 h, SpO2 ≤88% in the room air, weight loss ≥25% of baseline for three consecutive days, or observation of severe injury or condition. NHPs are also euthanized if they experience any two of the following criteria: 1. respiratory distress, 2. abnormal activity, 3. severe dehydration, 4. weight loss >20% of baseline weight for more than three consecutive days, or 5. abnormal appearance. Endpoints and euthanasia criteria in model development, and MCM studies should reflect the primary goal of translating what is learned to the human condition.

*Other Factors to Consider*. Notable gaps in knowledge for the PBI NHP model include limited data on 1. sex differences due to a predominance of young male animals in studies over the past several years, although recent efforts to include both sexes in NIAID-funded studies is addressing this gap, 2. certain age subsets for ARS or DEARE, 3. ARS and DEARE outcomes with mixed-field, neutron/gamma, radiation exposure, and 4. effects from prompt, high dose rate, non-uniform, unilateral, exposure. Additionally, study durations are generally limited to 180 days, thus limiting knowledge of the longer-term duration of dose-dependent radiation effects, as well as the duration of efficacy of organ-specific MCMs (*60, 61*).

While there are significant gaps in knowledge relating the NHP model to humans as mentioned above, NHP and murine models can provide the most information for H-and GI-ARS, with the NHP model having the highest predictive validity. Varying euthanasia criteria can impact the harmonization of animal models; therefore, scientists should work closely with veterinarians who interface with the IACUC to provide any needed information that can facilitate decision-making. It was also noted that strictly numerical euthanasia criteria could be problematic, thus clinical observations and conditions should also be considered.

## SESSION I: DISCUSSION

The various elements of animal care as discussed by the four speakers in Session I showed similarities in care, but also some differences due to species, nature of injury, or facility differences.; therefore, animal care harmonization is attractive. In keeping with this goal, key areas of concern and ways to ensure harmonization and consistency across institutions were discussed. While harmonization may help provide reliable reproducible data that can help bridge the animal-to-human gap, it may not be possible to harmonize all the different (national and international) IACUCs involved in radiation exposure studies. In addition, harmonization should focus on specific elements that will increase the quality and reproducibility of the data, such as radiation dosimetry based on standards, statistical analysis, and animal-use protocols for each species and model (TBI/PBI). Some institutions adopt core protocols for each species and model, and once established, the IACUC need only consider small deviations, making the process of review more efficient. This effort ensures closer harmonization and minimizes IACUC review variability. Statistical considerations that can impact study design should be discussed with the IACUC, and any type of inter-institutional data that can be used to support appropriate endpoints should be provided.

Given that individual IACUC decisions can have an impact on study designs, it was suggested that investigators should interact with their veterinarians first, to address issues before a protocol is drafted. The implementation of Standard Operating Procedures (SOPs) with proper controls and conditions can provide reassurances to all parties. Overall, good communication and planning between researchers, veterinarians, and the IACUC will not only strengthen the working relationship but also enhance the quality and safety of the studies. In addition, since funding agencies have a significant role in establishing study and animal model expectations, conversations with government agencies can help harmonize IACUC-approved animal-use protocols. Most importantly, funding agencies have access to cross-institutional information that may help support certain metrics; for example, data that may support euthanasia or weight-based criteria in similar models at other institutions.

When assessing NHP protocols, the IACUC conversation is even more demanding, given that the IACUC is especially concerned about pain and suffering in NHPs, so communication is key. Harmonization has been attempted in terms of using the same strain, food, water, climatization, etc. (*13*). Although it would be easier to compare data between institutions, different animal models serve different

purposes, so by completely harmonizing all models, serendipitous findings and welcome variability may be lost. Moreover, a single model at all sites may not address all scientific and regulatory questions regarding specific MCM mechanisms of action. Finally, every institution would have to be stringent in the execution of its models to maintain inter-institutional consistency.

Harmonization could look very different depending on whether data are derived from basic research labs conducting mechanistic studies or contract research organizations (CRO) employing Good Laboratory Practices (GLP) with established SOPs. During the early stages of product development, differences may be more acceptable, but as products become more advanced, well-controlled and characterized animal models are essential to determine the effectiveness or usefulness of the product. Furthermore, harmonization could look very different for mice vs. NHPs, since NHP animal availability is limited, and preliminary studies may not be statistically well-powered. However, it is important to publish these research and model development studies with detailed methods (e.g., animal handling, radiation pie/jig setup, dosimetry, etc.) so that the community can learn from one another.

The NHP model for H-ARS that was used for the FDA licensure of several H-ARS MCMs was originally established at the UMSOM (*40*). Fine-tuning the details down to the dosimetry was essential to the transfer of the NHP model to other institutions (*62, 63*). Radiation dose delivery to the target (at midline tissue) can change with each animal due to animal size (small and large), tissue depth, and radiation quality. Furthermore, dose differentials can alter the outcome of the model dramatically, so it is important to have a radiation physicist on the team. It is imperative that NHP models are well-controlled and understood so that even if complete harmonization is not possible, controls are in place to understand the impact of an intervention. Regardless of the dose or dose rate, the goal is to obtain a certain biological response or outcome, which can differ by the radiation source and animal model being developed.

Another area of consideration for harmonizing animal models is the standard of care or supportive care used in these models. For instance, the use of dexamethasone in NHPs can increase decedent mean survival time but not overall survival, and its use in animal models and the clinic has been controversial. The UMSOM IACUC required the use of dexamethasone for NHP studies, but some studies suggest that dexamethasone can increase mortality in mouse animal models (unpublished). In addition, dexamethasone use requires a trigger-to-treat (e.g., NSRR $\geq$ 80 bpm) and taper (*64*), making it difficult to administer properly in small animal models. The idea of ''standard of care'' is complicated since some or none of the approved MCMs may or may not be available in a mass casualty, low-resource situation. Moreover, as more drugs are approved, studies will become very complex if all drugs need to be considered with any new MCM development. A new drug should be developed on its own to ensure it is useful for the indication being sought. Any combination studies should be planned in consultation with the FDA to ensure proper and necessary studies are being conducted. The poly-pharmacy approach is complicated and has been discussed in detail since understanding contraindications and synergy between drugs are important to the field (*65*). Ultimately, each model and situation require careful thought and should be modeled to best fit the human condition as this is the goal of the Animal Rule.

As drugs advance toward FDA licensure, harmonization of pivotal studies makes it easier for government agencies to review the data and consider approval of a product. Therefore, a well-developed animal model that aligns with the criteria of the FDA's Animal Rule can help bridge the gaps from an animal model to the human condition. The FDA has established an Animal Model Qualification Program, as a means of providing a ''product-independent, resource-conserving approach to advance the development of animal models for use in Animal Rule applications''.[3] Finally, funding agencies must also be part of the conversation since they have a broad view of all studies in the program portfolio and can provide guidance on the animal models and MCM studies. The scope of studies conducted along the non-clinical product development pathway differs between the funding agencies. NIAID supports early through advanced-stage MCM and biodosimetry development for radiation-induced injuries, while BARDA is primarily focused on later development, including FDA licensure and procurement. Other U.S. government agencies, such as the Department of Defense and the National Aeronautics and Space Administration also have programs that fund the development of approaches to assess dose and protect/mitigate/treat radiation-exposed individuals, consistent with their mandates. Regardless, acceptable studies funded by these agencies must be well-controlled, well-powered, and the models used should be well-characterized.

## SESSION II: BASELINE ANIMAL CARE IN RADIATION RESEARCH

Several models are being developed to determine the efficacy of MCMs via the FDA's Animal Rule (*1*). As with any model, the baseline of animal care in radiation research can affect the model outcome. While similarities exist between models, some are very specific to the species or model of choice. To harmonize research and laboratory practices across institutions, similarities, and differences in animal care between species as well as select institutions were discussed in this session, with a focus on the following topic areas: 1. animal housing and handling, 2. infection control, 3. hydration and diet, and 4. euthanasia criteria.

[3] https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/animal-model-qualification-program-amqp.

**TABLE 4**
**Animal Housing and Handling at NIAID-supported Institutions**

| Sub-topics | Rodents (IU) | Minipig (AFRRI) | NHP (SRI/CRL) |
|---|---|---|---|
| Social housing/interaction, enrichment strategies | Housing: group housing. Enrichment: nesting materials | Housing: single housing. Enrichment: food, species/strain, age, gender, animal-specific. Toys, challenging, time-consuming; no sharp objects | |
| Biological variables | Macro environment (Air, humidity, temperature, lighting, noise, vibration, etc.) Facility design, Guide for the Care and Use of Laboratory Animals Interaction with staff Good knowledge of animal behavior (recognize and report abnormal behaviors) Acclimation to procedures | | |
| | Bedding: estrogen-free, paper, non-scented, low-dust | Bedding: towels, blankets, heating pads for inactive, sick animals | |
| Sex and age | Housing, temperature, feeding depend on age, gender, weight Separation by sex | | |
| Animal transport | Sensitive to transport and temperature changes | Sedated at the time of transport | Acclimation necessary |

Given the potential impact of these experimental design variables, the goal of this session was to explore institutional animal care variability, implement standard procedures and minimize biological variables.

*Panel Discussion Topic 1: Animal housing and handling*

Comparisons were made regarding the management of these areas across institutions and species including rodents, MPs, and NHPs. The macroenvironment and facility design, which is based on the *National Research Council Guide for the Care and Use of Laboratory Animals,* is consistent among facilities (*66*). Similarly, staff training and acclimation to procedures designed to foster good knowledge of animal behavior, and to recognize and report abnormal activities, were deemed critical to successful animal husbandry of any species. Certain institutional standards were found to be species-specific.

Institutional standards for animal care can have a major impact on animal model development. Sources of variability in animal housing and handling include (Table 4): 1) macroenvironment and facility design (e.g., air, humidity, temperature, lighting, noise, vibration), 2) staff knowledge, training, and interaction with animals, 3) social housing/interaction (e.g., group vs. single housing), 4) enrichment strategies (e.g., food, toys), 5) choice of bedding, 6) sex and age of animals, and 7) animal transport. A summary of what was discussed beyond what has already been described In Session I for animal housing and handling is provided.

*Mice.* Since mice are traditionally group-housed, issues arise either when animals are euthanized or die because the loss of littermates results in loss of body warmth. To address this, researchers often pool animals. However, the pooling of mice can be problematic for males, due to fighting for cage dominance, which can result in injuries and deaths, whereas females can usually be successfully pooled, when necessary, to prevent isolation and loss of body warmth. In most instances, increased warming of singly housed mice is impractical or impossible, since other cages containing multiple animals in the cage racks are likely to become overheated. An additional point of consideration for group housing of mice is their coprophagic nature. During MCM testing with animals randomized in cages across study arms, control animals may ingest excreted test articles. This could result in control group animals being exposed to low levels of the MCM, skewing results.

Lifespan differences and radiosensitivity have been noted to differ because of the husbandry practices at different facilities. For example, geriatric C57BL/6 mice (24 months old), raised at the NIA, NIH, demonstrate increased radiosensitivity as compared to C57BL/6J mice housed at IU. After radiation exposure, stress should be minimized, since it can harm the survival of irradiated mice. Frequent handling from multiple dosing has been correlated with increased lethality (*12*) and frequent bleeding of mice has also been associated with an increased morbidity and mortality profile (*11*). Furthermore, rodents were very sensitive to animal transport and temperature changes. Extended restraint in irradiation pie jigs leads to increased levels of stress cytokines as well as hyperthermia. Hence, pre-loading of pie jigs with mice is not recommended. Therefore, while these factors cannot be eliminated, they should be optimized and controlled. In addition, pediatric mice require small restrainers (e.g., pie jig or single jig), while geriatric mice, who gain weight over time, require larger restrainers often used for rats. In addition, to limit pheromone effects, separate radiation restrainers should be used to irradiate males and females (C. Orschell).

*Minipigs.* Consideration of the MP environment can help minimize variables and enhance reproducibility. MPs are group-housed when quarantined, but once in the study, are kept single-housed in large metal cages that allow safe visual, touch, and smell cues between animals. Although no pheromone data has been collected to date, males and females are generally housed separately. In some institu-

tions, hay or bedding is not included in cages, to not conceal initial traces of bleeding. Other institutions find that bleeding is not concealed by pine shaving bedding, so that is used. These differences can also be due to the type of caging used; if cages have raised flooring, bedding is not necessary, but with MPs housed on a hard surface the bedding can help to minimize contact with water and urine contaminated with feces.

Given that procedures may require animal transport, care should be taken not to cause undue stress to animals; therefore, it is important to allow some time for acclimation to their new environment (i.e., 1–2 weeks) before their introduction to research conditions. To minimize this stress, MPs are generally sedated for transport and are kept in a covered cage with a maximum of two animals per cage. Furthermore, animals also require acclimation to a sling made from elastic and soft material, which is usually custom-made to accommodate different weights and sizes. Once MPs are exposed to experimental procedures, they are returned to a separate housing quarter, minimizing the exposure of naïve animals to unnecessary stress.

Biological variables such as sex, age, and weight must be considered for MPs as well. For example, male MPs are usually more aggressive than females, while females gain weight faster than males. Moreover, pediatric MPs require a special diet and housing conditions; therefore, it is important to communicate with the vendors to maintain the diet criteria. As for enrichment, it is important to adhere to the amount and type of vendor-recommended nutrition and treats, because pigs can gain weight very easily. For example, any food that is co-administered with medications should be subtracted from the total daily food ration. (M. Moroni)

*NHPs.* NHPs were not offered bedding except for inactive/sick animals, which could be offered towels, blankets, and heating pads. For NHPs, social interactions and visuals are very important; therefore, cage and room positioning of dominant males vs. females or younger animals should be considered. Blocking screens can be used to provide a refuge for less-dominant animals, thereby diminishing stress. NHPs are singly housed like MPs, but unlike MPs, both NHP sexes are housed in the same room, because it is important for visual and olfactory cues. Facility temperature and relative humidity are kept constant and monitored closely. Lights and noise are controlled to maintain 12-h cycles of light and dark. In addition, since night observations can cause undue stress, infra-red camera systems are often used.

Enrichment for NHPs is critical and includes: 1. foraging toys that challenge and engage the animals, 2. mirrors that limit adverse behaviors by providing visual stimulus, and 3. food/treats consisting of natural soft foods (e.g., fruits) and a high-fiber diet (e.g., nuts), but with care to maintain the appropriate caloric content and ensure animal hydration. It is also important to consider drug and food contraindications as one would for humans.

As with MPs, acclimation periods for NHPs are essential to minimize stress. Staff should be introduced one at a time so that the animals can become familiar with them. All procedures should be introduced slowly, in an individualized step-by-step process. In addition, all procedures should be done in an anteroom, out of sight from other NHPs in the study. If NHPs are to be transported, anesthesia is not necessary, but an acclimation period is needed. NHPs should be acclimated by being transported in a temperature-controlled vehicle on more than one occasion, before any procedure. Furthermore, certified NHP treats and/or juice should be provided before and after transport as a reward and to support nutrition and hydration, limiting the effect of transport.

When using NHPs for radiation studies, age and size matter, especially when considering blood budgets. The amount of blood to be withdrawn will be dependent on the size of the animals and the number of fluids or blood products administered; regardless, it should not exceed 7.5–10% of blood volume collected weekly to prevent hypovolemia and anemia. For this reason, it is recommended that for both sexes, animals should be between the ages of 3–5 years old and weigh between 3.5–6 kg at the start of treatment. Radiation dose responses that have kept to these sex and weight guidelines have reported no significant differences to date. (P. Chang)

*Other Considerations.* Nocturnal monitoring varied substantially among institutions; some chose not to monitor at all, others used flashlights or room lights during night checks, and some used infrared cameras as previously discussed. To ensure the socialization of larger animal model studies such as rabbits, MPs, and NHPs, which are kept in single cages but grouped in holding rooms, it was recommended to move cages from empty rooms to rooms with other members of the same species to the extent possible within a particular study. Overall, good communication of the detailed procedures used in animal housing, husbandry, transport, and irradiation conditions was considered critical to robust and repeatable experiments. In keeping with this idea, the consensus was to encourage the publication and description of the detailed aspects of the models and experiments for reproducibility and harmonization purposes.

### *Panel Discussion Topic 2: Infection Control*

Uncontrolled infections can lead to bacteremia, which is the presence of bacteria in the blood, or sepsis, an illness that results due to the inflammation caused by the persistence of microorganisms or toxins in the bloodstream (*67*). At some study sites, researchers indicated that sepsis, particularly in MPs, was either rare or not observed, and clinical symptoms of sepsis did not present in the animals; however, bacteremia was detectable in the animals. At other sites, clinical signs of sepsis and positive blood cultures were obtained in some studies with NHPs and MPs. Clear

histopathological evidence of sepsis was demonstrated upon euthanasia. One outcome of these discussions was the recognition that definitions of sepsis in animal studies are not harmonized among study sites, and that achieving a common definition is important. Currently, clinical definitions of sepsis used in animal studies are not aligned with the histopathological definitions being used at other institutions. Controlling infection is critical when handling immunocompromised animals, such as those that have been irradiated. Antibiotic selection and schedule are important, as the outcome of a study can change whether antibiotics are used prophylactically, therapeutically, as a trigger-to-treat, or not at all depending on the animal model (Table 3).

*Rodents.* Mouse models do not usually include antibiotics, or only incorporate them day 1 postirradiation to clear the gut bacteria, before leakiness of the GI epithelium increases. Antibiotics used in mice include ciprofloxacin, enrofloxacin, and amoxicillin, at time points ranging from one to four days, or up to 21 days postirradiation. When given early, a marked improvement in survival has been observed, with a start time of 1 day postirradiation being most effective. Rats are typically treated with enrofloxacin in the drinking water, with a SC bolus of saline for hydration (*68, 69*).

*Large Animals.* Antibiotic use for larger animals can be more complex and differ based on species; therefore, tissue bacteriology of the liver, lung, spleen, and kidney is often conducted to determine the level of bacterial translocation for larger species. For rabbits, bacterial translocation is seen in all tissues after irradiation, with *Pseudomonas aeruginosa* being the major source of bacteria present during culture. The current standard of care calls for the daily administration of sulfamethoxazole and trimethoprim (Bactrim) for rabbits; however, this antibiotic is not useful in the treatment of *Pseudomonas* infections, so better antibiotic strategies are still needed. Multiple bacterial strains have been detected in MP tissues; therefore, the best strategy has been to control common bacteria such as *Staphylococcus* and *Streptococcus*, with the administration of amoxicillin twice daily and/or gentamicin once daily.

The level of supportive care used to treat NHPs after irradiation is by far the most complex and can include antibiotics, hydration, blood transfusions, and nutritional support. Nasal swabs and tissue samples have been used to probe the bacterial load present, to determine the best antibiotic approach, although optimum timing of antibiotic administration is the most effective strategy. The use and effectiveness of enrofloxacin are based on the model; the full-supportive care model uses a trigger-to-treat approach, whereas the minimal supportive care model institutes antibiotic use at set time points for all animals. A comparison of therapeutic strategies for NHP care showed that subject-based care (i.e., trigger-to-treat) led to a better survival outcome as compared to population-based care (*62, 70*). For population-based care, NHPs received enrofloxacin earlier (day 5) regardless of symptoms, whereas enrofloxacin was administered at a later time point (day 8.2 $\pm$ 2.2) during subject-based care (*70*). Overall, antibiotic administration during the neutropenic phase after irradiation in the subject-based group was thought to be critical in minimizing infection-related mortalities. It is important to note that while enrofloxacin helps improve overall survival, after irradiation antibiotic resistance of *Staphylococcus* and *Streptococcus* is still an issue in NHPs. (M. Lindeblad)

Trigger-to-treat antibiotic schedules are normally prompted by an ANC of $<$500/μL (severe neutropenia) that triggers the administration of enrofloxacin [5 mg/kg IM or IV, once daily (QD)]. If a breakthrough fever occurs (39.4°C), antibiotics can be changed to a 2-day gentamicin regimen (5 mg/kg IM/IV, QD), and blood cultures are conducted immediately to determine if antibiotic resistance is at fault. Subsequent antibiotic selections will be dependent on the results of blood culture, and any one of the following antibiotics is an option: 1. cefotaxime [Claforan 50 mg/kg IM, twice a day (BID)], 2. ceftriaxone (Rocephin; 50 mg/kg, IM, QD), or 3. Ertapenem (Invanz; 15 mg/kg, IM, BID). During this time, an extended drop in ANC of $<$100/μL is possible and can last for up to 7 days before improving. The antibiotic regimen can be stopped once the fever has stopped and ANC is greater than 500/μL for 2-consecutive days (*26, 37*).

The direct effects of antibiotic administration on NHP survival were first examined in 1964, in a study where NHPs were exposed to a lethal dose of radiation (*71*) and were then treated prophylactically and after irradiation with tetracycline. Cohort-based prophylactic use of antibiotics after irradiation reduced lethality from 100% to 72%. The effects of antibiotics alone were also assessed in a recent NHP pilot study (Farese et al., unpublished), where NHPs were irradiated at an $LD_{50/30}$ dose. One cohort received antibiotics via the trigger-to-treat schedule described above, leading to 62.5% lethality. A second NHP cohort was administered antibiotics on the first day of febrile neutropenia (ANC $<$500/μL and body temperature $\geq$39.4° C), which resulted in 100% lethality (*26*). These studies show an improvement in NHP survival with trigger-to-treat medical management including IV fluids, prophylactic antibiotics, blood transfusions, anti-diarrheal drugs, analgesics, and nutrition. (T. MacVittie)

Among irradiated NHPs, moribund animals present with a high prevalence of sepsis (58%), hemorrhage (3%), both sepsis and hemorrhage (37%), or other (3%), as determined by bacteriology (organ and hemocultures), clinical examinations, hematology, and pathology (gross exams and histopathology) (*72*). Given that microbial flora is very diverse in NHPs, and not all NHPs come from the same source, the staff must minimize cross-contamination and implement procedures to help control the spread of new bacterial strains. Using hemoculture and organ culture, bacterial infections are caused mostly by gram-positive of predominantly cutaneous (43.5%) origin, gram-negative of GI origin (45.3%), or both (11.2%) bacterial species. The

**TABLE 5**
**Infection Control Procedures in Irradiated Animal Models, Survey Findings by Participating Subject Matter Experts**

| Mouse | Rat | Rabbit | Minipig | NHP |
|---|---|---|---|---|
| Housed in microisolator cages and single housed | Housed in microisolator cages | Cages changed twice weekly | Cages rotated daily | Separate treatment room for blood collection and treatments |
| Autoclaved, acidified water | All cage components sterilized before use | Cages sanitized and surveyed with RODAC plates then wrapped in plastic until use | Cages completely sanitized between use and surveyed with RODAC plates | All tables where animals are placed are disinfected between animals |
| | Cages changed weekly | High touch surfaces cleaned with disinfectant daily | Minipigs trained for targeting to move around room | Room entry guidelines and restrictions |
| | Reverse osmosis, hyperchlorinated water | Restrainers cleaned between animals | Neutropenic animals housed in separate room | Twice weekly cage sanitization with RODAC survey |
| | Room entry order and guidelines followed, including PPE | Neutropenic animals housed in separate room | All animals isolated from colony from arrival until termination | |
| | | | Fresh bedding for sick animals | |

origin of these bacteria can impact the susceptibility to different antibiotics and when they are administered (i.e., prophylactic vs. health status initiated). If resistance is encountered, antibiotics need to be adjusted; however, data needed to make that decision are often received too late to control the infection. It is important to note that some readily used agents such as azithromycin (65%), enrofloxacin (70%), and gentamicin (50%) exhibit high rates of antibiotic resistance (*73*), while others, including ceftazidime, amoxicillin/K clavulanate, and imipenem-cilastatin have shown no or limited antibiotic resistance. (S. Authier)

*Surface Cleaning*. Given that the type of antibiotic and the frequency of use can impact survival and lead to antibiotic resistance, strategies that help minimize cross-contamination must also be considered in infection control (Table 5 Table 6). At the University of Illinois at Chicago, mice are housed in microisolator cages and water is autoclaved and acidified. For larger animals, changing the cages often is recommended. Rabbit cages should be changed twice weekly, and MPs and NHP cages should be changed often (daily preferred). All cages should be sanitized and surveyed with Replicate Organism Detection and Counting (RODAC) plates to ensure proper cleaning and disinfection have been accomplished. In addition, it is important to institute daily sanitization of high-touch areas and floors. Animal restrainers and tabletops used for animal handling should be disinfected daily after use. Technical staff should be well trained on entry/exit guidelines, and staff PPE should be changed between animals to limit cross-contamination. If possible, neutropenic animals should be housed in a separate room from normal healthy animals. Finally, all animals should undergo a thorough infection survey and quarantine upon arrival. The end goal is to create

**TABLE 6**
**Hydration and Diet for TBI and PBI Animal Models**

| | Mouse | Rabbit | Minipig | Non-human primate |
|---|---|---|---|---|
| Adequate feeding | TBI/PBI: Gel packs due to anticipated tooth decay/loss<br>WTLI: standard chow only | Fruits and vegetables | Fruits and vegetables | Fruits, vegetables, high-protein diet in MCT oil for $\geq$10% BW loss<br>Citrus (acidity) is avoided |
| Adequate hydration | TBI/PBI: Hyperchlorinated $H_2O$ (10 ppm); Hydropac system | Treated water via self-watering system (in-cage lixit) | | |
| | Gel packs include large $H_2O$ component | Pedialyte, fruit juice or water may be provided | | |
| | WTLI: Hyperchlorinated $H_2O$ | Trigger to treat hydration: fluids provided IV or oral gavage based on dehydration<br>Less dehydration in rabbits/minipigs | | |
| Best route of administration | Oral, SC, IV or OG | Multiple routes depending on severity of dehydration | | |

a clean environment similar to a hospital setting (M. Lindeblad).

*Panel Discussion Topic 3: Hydration and Diet*

Hydration and diet can have an impact on the reproducibility of animal models. The best strategies for routes of administration, the influence of dietary components such as isoflavones (antioxidants) on experimental endpoints (survival, blood counts), and their influence on the diurnal cycle differ based on the animal model under consideration (Table 6) (IL Jackson).

*Rodents.* Mice are normally fed standard chow. Anecdotally, no difference in survival of mice with the isoflavone-rich diet compared to the normal chow has been documented, but survival differences have been noted in rats (*74*). In TBI and PBI rodent models, where the head is included in the irradiation field, gel packs are introduced on the day of exposure in anticipation of tooth decay and loss several days/weeks after exposure, leading to an inability to bite hard food pellets. This is not typically done for the WTLI model. Therefore, rodents are provided with chlorinated water (10 parts per million), and if nutrition is included in gel packs, the water content is increased so that the TBI/PBI mice have both food and hydration. WTLI mice receive hyper-chlorinated water in the regular watering system (bottles).

At the Medical College of Wisconsin (MCW), the rat PBI model (~8% bone marrow shielding) is used in DEARE studies that typically last for 3–6 months (*69, 75, 76*) and animals are provided powdered food on days 35–70, where tooth loss is observed (M. Medhora). In cases where oral gavage is needed such as in longer studies, MCW developed a syringe feeding approach wherein a measured amount of drug is fed to the rats in pudding to avoid daily gavage that can aggravate esophageal radiation damage. MCW has shown that a lower isoflavone diet started before irradiation increases the severity of pneumonitis in irradiated male rats; however, this effect is not observed in C57L/J mice at UMSOM. Interestingly, a change from a normal to a refined diet (a chow that contains no antioxidants or isoflavones) after exposure mitigated radiation-induced nephropathy (*74*). In the same study, it was noted that the protein source (soy vs. casein) did not impact survival; however, a high protein diet is likely to exacerbate kidney injury.

For hydration, PBI/BM-shielded rats are provided water ad libitum that has been purified by reverse osmosis and then hyperchlorinated. If the radiation dose is above 13 Gy, rats are also provided antibiotics in drinking water, starting at days 1 or 2, up to 14 days postirradiation (*75, 76*). There was no advantage to longer antibiotic administration. The main limitation to providing drugs in drinking water is the uncertainty regarding the drug dose to the rats, and whether the intake is continuous or sporadic, especially during GI-ARS, which is assumed but difficult to confirm. Regarding routes of administration for hydration, MCW veterinary staff recommend SC administration of saline (4% of body weight) during GI-ARS, which typically occurs at or after day 2 and up to day 10 postirradiation, by which time most rats start to gain weight. Supportive care that includes hydration and antibiotics is routine for rats exposed to radiation doses of 13 Gy and above.

Epistem uses PBI/BM-shielded mice to study GI-ARS, and irradiated mice are fed wetted chow 4–8 days postirradiation, before the onset of diarrhea (C. Booth). For H-ARS, the wetted chow is administered up to 10 days after TBI. The chow is wet with acidified water, or if the water contains MCM or antibiotics, that water/drug solution is used to wet the chow. The use of wetted chow is triggered when the mice experience tooth loss. Gel packs are used only in instances of oral ulceration. As with the earlier speakers, the preferred hydration administration route was SC, preferably once a day or as needed. Since handling can negate the benefit of nutritional supplements, this strategy is more effective following lower radiation doses ($LD_{50}$ or lower). To ensure handling stress is considered, proper naïve controls are needed. While a high-fat diet can increase $Lgr5^+$ stem cell proliferation following irradiation, it can also lead to a higher risk of tumorigenicity (*77*). Another strategy is to manipulate the GI microbiome, indirectly or via nutritional approaches, to mitigate radiation injury. Nutritional status plays a role in radiation sensitivity since fasting overnight makes mice more resistant to irradiation presumably because fasting induces GI proliferation, hence reducing tissue radiosensitivity (*78*).

The use of gel packs or wetted chow as the source of nutrition for irradiated rodents was discussed in detail. Some facilities began wetted chow 1–2 days postirradiation and others used tooth loss as a trigger to introduce wetted chow. In some cases, medicated water (containing MCM and antibiotics) was used to moisten the chow and enable the rodents to acquire nutrition and medication simultaneously. While some investigators found gel packs to be effective and well accepted by the animals, others had difficulty getting their animals to take anything from a gel pack, and this impacted the study outcome. A study at the Armed Forces Radiobiology Research Institute (AFRRI) which used gel packs shifted the survival curve to the left in a TBI study, with 20–30% more mortality than regular bottled water arm (*79*). This phenomenon was also noted in a study conducted by BCN Biosciences (A. Norris). Additional concerns were raised about the reduced nutritional content of gel packs in comparison to wetted chow and the likelihood of fecal contamination in wetted chow, which could be offset by changing the wetted chow daily. Although the discussion on gel packs vs. wetted chow did not result in a consensus or determination of benefit outweighing detriment, it was clear that good results are achievable once an effective approach is established.

*Large Animals.* As previously described, rabbits, MPs, and NHPs are provided with fresh fruits (no citrus) and

**TABLE 7**
**Comparison of Euthanasia Criteria in Animal Models Across Subset of Representative Institutes**

| | Large animal | | | Small animal | | |
|---|---|---|---|---|---|---|
| | LBRI (NHP/swine) | CRL (NHP/Swine) | UMSOM (NHP) | AFRRI (Mice) | MCW (Rat) | UMSOM (Rabbits) |
| Excessive bleeding | X | X | X | | | X |
| Severe dehydration | X | X | X | | X | |
| Respiratory distress | X | X | X | X | X | X |
| Body weight loss | X | X | X | X | | X |
| Decreased food/water | X | X | | | | |
| Lethargy/weakness | X | X | X | X | X | X |
| Seizure activity | | X | X | | X | X |
| Loose bloody stool | X | | | X | | |
| Pain[a] | X | X | | | | X |
| Self-mutilation | | | X | | | |
| Rectal temperature ≥106°F (41.1°C) or <96°F (35.6°C) for >6 h | | | X | | | |

[a] Animals in pain receive analgesics, and unrelenting pain in the face of analgesic treatment would be a de facto euthanasia criterion.

*Abbreviations*. Lovelace Biomedical Research Institute (LBRI); Charles River Laval (CRL); University of Maryland School of Medicine (UMSOM); Armed Forces Radiobiology Research Institute (AFRRI); Medical College of Wisconsin (MCW).

vegetables. Rabbits are provided with hay after irradiation to stimulate GI mobility. All animals are provided water ad libitum via a self-watering system. Juice or Pedialyte can also be administered, which provides an additional means to deliver MCMs. In cases of dehydration, rabbits and MPs can be administered fluids intravenously or by oral gavage. It was noted that dehydration in rabbits and MPs is uncommon but is frequently observed in irradiated NHPs.

In NHPs, oral rehydration is often associated with better outcomes than parenteral administration; however, oral rehydration solutions (ORS) may be problematic since they generally have a bitter taste. Accordingly, the use of fruit juice or ORS in a palatable form may be more effective. In larger animals such as MPs and NHPs, identifying foods left behind after feeding can be useful to better select which foods to incorporate into the diet to overcome inappetence; however, other causes should also be investigated, such as oral ulcerations.

When NHP bodyweight loss exceeds 10% of baseline they will receive a high protein diet rich in medium-chain triglycerides oil, but even with nutritional supplements, moribund animals tend to reach euthanasia criteria. In one study, a feeding protocol was invoked when animals ate less than 50% of the unirradiated normal intake. Efforts were made to enhance food attractiveness by moistening food with juice or offering preferred fruits and vegetables, but regardless of efforts to supplement caloric uptake, survival in this group was not improved. Cachexia, a wasting syndrome marked by severe weight and muscle loss, is another after irradiation complication observed in NHPs that can be difficult to manage and is demanding on staff resources (*80*).

*Other Considerations*. Bulk chow can have varying levels of alfalfa and other components based on seasonal variability, which could affect study outcomes if feed changes occur within or between studies. The impact that fasting might have on study outcomes should also be considered. In the few studies where a feeding and fasting schedule was employed with mice, differences in MCM efficacy were not observed. However, caloric restriction is known to impact the insulin-like growth factor (IGF) pathway in the lungs of humans, and in Göttingen MPs, where the IGF-1 signaling is not normal and radiation-induced inhibition of IGF-1 is observed (*81*) Consequently, caloric restriction may have an impact on radiation sensitivity, possibly via the endocrine system.

### *Panel Discussion Topic 4: Euthanasia Criteria*

An overview of euthanasia criteria across five select institutes and several animal models is provided in Table 7. The fact that not all blocks are checked indicates that euthanasia criteria are not harmonized across institutes. According to American Veterinary Medical Association (AVMA) guidelines,[4] the goal of having euthanasia criteria is to euthanize animals justifiably and humanely and not inflict undue pain and distress. Euthanasia criteria ensure that signs of moribundity are discovered early enough to prevent the animals from experiencing pain (W. Weber). Notwithstanding adherence to these criteria, some animals are still found dead following irradiation since the onset of overt symptoms can sometimes occur very close to mortality.

Euthanasia criteria are easier to monitor in large animal models since more endpoints are available (K. Wong). Newer technologies allow for the non-invasive gathering of information, increased frequency of monitoring, and overall stress reduction. CRL has similar criteria to Lovelace Biomedical; however, some events don't fit into these pre-described criteria, such as necrosis and fractures. At the end

[4] https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals.

of the day, euthanizing an animal still requires consideration of stated criteria and an evaluation by the veterinary staff.

At the Uniformed Services University of the Health Sciences (USUHS)/AFRRI, a mouse intervention scoring system was developed to better identify mice meeting the pre-set euthanasia criteria (S. Ghosh) (*82*). This scoring utilizes five main criteria:1. general appearance, 2. respiratory rate, 3. general behavior, 4. provoked behavior, and 5. weight loss. Animals exhibiting signs of pain and distress are scored on these criteria, and a cumulative score is assigned. Individual investigators have added additional IACUC-approved sub-criteria within this system as a means of further refinement because of the differences that occur between various strains. For example, subtle hints may be observed in a relatively radiosensitive strain such as C57BL/6, which might not exist in a more radioresistant strain such as CD2F1, or vice versa. Body weight is a strong indicator of survival in a murine model of radiation exposure; therefore, an accurate assessment of this parameter is essential. Animals that have lost more than 35% of their body weight are not likely to survive and are euthanized accordingly. Identification of individual animals is also required to track the scores; animals need to be uniquely identified, meaning they must be permanently marked (i.e., tattooed), or possess other identifying features such as ear tags. The success of this system is reliant upon the inclusion of frequent health checks and accordingly, the USUHS IACUC has adopted an interval of checks that results in all animals being assessed a minimum of three times per 24-hour period. This assessment is typically done once in the morning, once in the afternoon, and once in the late evening, such that the time elapsed between each check is never longer than 10 h.

Using a criteria-based system to independently assess the health of animals has many potential benefits, most importantly it reduces the likelihood that an animal will experience prolonged pain and distress and ultimately succumb to the injury without the intervention of euthanasia. Additionally, the adoption of criteria such as these decreases inter-study variability and increases uniformity between personnel tasked with assessing the conditions of these animals which ultimately reduces bias when making euthanasia determinations.

In general, developing international or even inter-institutional harmonized euthanasia criteria is a challenge and may not be possible. IACUC-approved euthanasia criteria vary across institutions and protocols. While some harmonization of criteria for a species-specific model may be possible, at present, there is not a high degree of consensus for the criteria used even within a single model species. Although, in some cases, during the implementation of a new model and protocol at a site, coordination with other investigators using the same animal model at other sites can inform what criteria might be most useful. Discussion of select criteria with site veterinarians and IACUC members can help provide the information needed for acceptance of the proposed euthanasia criteria. For mice, some scoring systems currently in use at various sites include the assessment of weight loss, body temperature, posture, activity, eye appearance, and neurologic symptoms. Others use more subjective judgments based on the experience of the handlers, which can add another level of variability. In the case of larger animals, somewhat more harmonized criteria may be possible between sites and investigators due to greater SOP sharing, but even so, concerted efforts to harmonize criteria have not been fully implemented and can vary across facilities.

## CONCLUSIONS

As laboratory animals are used to simulate human responses to radiation exposure during a mass casualty incident, awareness, and minimization of animal care confounders discussed in this report are needed. All these factors will affect MCM development and determination of radiation-induced biomarkers, through the regulatory approval pathway. As such, to the extent possible, these considerations should be incorporated into the planning. Investigators should also provide details of these aspects of their protocols in publications to widely disseminate the criteria that are being used and help build consensus within models. It is imperative that current animal models and protocols are used, so efforts are not wasted and that ultimately FDA licensure can be achieved.

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