Column E Explanation

Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part of in full), the animals room numbers, grant information, veterinary care programs, and the like. A Column E explanation must be written so as to be understood by lay person as well as scientists.

1. **Registration Number:** 51-F-0001
2. **Customer Number:** 432
3. **Facility Business Address:** 4301 Jones Bridge Road, Bethesda, MD 20814
4. **Telephone:** (301) 295 – 3303

5. **Number of animals categorized as column E used in this study**: 24

6. **Species (common name) of animals used in the study**: Ferret (Mustela putariusfuro)

7. **Explain the procedure producing pain and/or distress.**

   One part of the study includes how the ferrets respond to stress, thus the nature of the study is to create unalleviated distress. They were exposed to a randomized set of stressors over multiple days and then tested (via cortisol measurement) to determine the level of stress.

8. **Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For federally mandated testing, see Item 6 below).**

   Ferrets received analgesics/anesthetics for all surgical procedures. However, no analgesics were given after the stressors, as the purpose of the study was to determine the level of stress created and analgesics would affect the outcome.

9. **What, if any, federal regulation require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):**
   If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document.

   Agency: None CFR: None

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5. **Number of animals categorized as column E used in this study:** 9

6. **Species (common name) of animals used in the study:** Pig

7. **Explain the procedure producing pain and/or distress**.

Irradiation is not a painful procedure in itself. Animals are sedated prior to being moved to the irradiation chamber to reduce stress, and are recovered in their home cages or in procedures rooms equipped with thermal support (for instance, Bair huggers). Although irradiation in itself does not cause significant stress, sequelae of radiation exposure may lead to suppression of the bone marrow and changes in function and structure of the gastrointestinal (GI) system, potentially leading to infections, fever, lethargy, anorexia, bleeding, vomiting, constipation and diarrhea which can cause pain and distress in the animal, if supportive therapy is not provided.

Antibiotics, analgesics, anti-emetics, anti-diarrheal, gastrointestinal protectants, food supplements, probiotics, fiber supplements and oral electrolyte supplements were used in these studies for minimal symptomatic supportive care. Around 21-28 days post-irradiation, the animal's immune system, bone marrow and gastrointestinal system typically starts recovering toward normal status. If the animals did not recover or reached a point where pain and distress could not be medically managed, they were humanely euthanized. Animals were closely monitored post irradiation multiple times a day until they completely recovered from acute radiation syndrome (ARS).

8. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Depending upon the irradiation dose, irradiated animals succumb to death due to compromised immune responses, opportunistic infections and damage to the GI. The percentage of surviving animals is the indicator of the efficacy of a countermeasure.

We use a non-steroidal anti-inflammatory drug to relieve pain and inflammation associated with radiation injury. This drug is given three days post radiation exposure and continued until 30 days post-irradiation when the animal is well out of the critical period (period during which clinical symptoms are manifested). We also use inhalational and/or injectable anesthesia at the time of moderately invasive procedures such as blood collection and euthanasia. Use of supportive care in the form of antibiotics, analgesics, gastrointestinal protectants, food supplements, probiotics, fiber supplements and oral electrolyte supplements is expected to mitigate the pain and stress associated to the sequelae of irradiation.

9. **What, if any, federal regulation require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document.**

Agency: None CFR: None

with the Food and Drug Administration (FDA) *Animal Rule*. We have selected swine as a species currently acceptable by the FDA for drug testing.

9. **What, if any, federal regulation require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document.**

Agency	Food and Drug Administration
CFR	21 CFR 314.600 (drugs) or 21 CFR 601.90 (biological products) commonly referred to as the *Animal Rule*

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5. **Number of animals categorized as column E used in this study.**

   8

6. **Species (common name) of animals used in this study**.

   Swine (strain Gottingen minipig)

7. **Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.**

   Animals will be exposed to total total body irradiation. For this procedure, animals will be anesthetized, and then placed in a panepinto sling, and exposed to ionizing radiation for a period of 5-6 1/2 minutes. Following exposure to radiation, animals exhibit low red and white blood cell count, which can cause lethargy and reduced ability to fight infections. Animals may also exhibit inappetance, dehydration, GI distress (constipation or diarrhea), and ataxia.

8. **Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For federally mandated testing, see Item 6 below).**

   Anesthetics will be used for brief periods of restraint in the panepinto sling.

   However, analgesics will not be administered following radiation exposure. Anesthetic agents are known to interact with the immune system (see references in Jacobsen, K. O., V. Villa, V. L. Miner, and M. H. Whitnall. 2004. Effects of anesthesia and vehicle injection on circulating blood elements in C3H/HeN male mice. Contemp Top Lab Anim Sci 43:8-12.). As a result, this will skew our results and would not be productive. Alternatively, we are providing supportive care in terms of fluids and nutritional support to increase survival. Use of supportive care is expected to help to mitigate the pain associated to the sequelae of irradiation. For many years, we have used tissue culture models of radiation effects, but such models to not recapitulate the complexity of the mammalian biological systems and their interactions, for instance, the gastrointestinal system and the hematopoietic system. Although we have utilized mice for most of our studies a to provide preclinical information about countermeasures against the toxic effects of radiation, in order to develop a radiation countermeasure for future use for humans, efficacy of the drug must be demonstrated in an additional non-rodent species, in compliance