Citizens for Alternatives to Animals Research & Experimentation
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July 22, 2016

Institutional Care & Use Committee
Medical College of Wisconsin
Via email: IACUCAdmin@mcw.edu

Dear IACUC Members:

I am writing on behalf of Citizens for Alternatives to Animal Research (CAARE), a national non-profit organization dedicated to promoting research without using animals.

CAARE was very disturbed to read about radiation experiments on animals conducted at the Medical College of Wisconsin and approved by your committee. These experiments manifest some of the worst cruelty to animals and fail to offer a clear justification.

It is the responsibility of the IACUC to determine that appropriate criteria for humane endpoints are in place and that animals do not suffer extreme distress, and also that reasonable alternatives to animals should be used where available. CAARE believes that the Institutional Animal Care and Use Committee for MCW failed in its obligation to uphold proper standards for animal research when approving these experiments.

Under the Public Health Service, all animal research receiving federal funds must comply with the policies under the Office of Laboratory Animal Welfare. As excerpted from these guidelines:

IV. Implementation by Institutions
C. Review of PHS-Conducted or Supported Research Projects

The IACUC shall determine that the research project conforms with the institution's Assurance and meets the following requirements:

> a. Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.

b. Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator.

c. Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

CAARE has reviewed a number of publications that detail radiation studies on rats at MCW and believes that these fail to comply with the guidelines under the PHS.

One study (Whole-thorax irradiation induces hypoxic respiratory failure, pleural effusions and cardiac remodeling), irradiated rats with a single lethal dose (15 Gy) of thoracic radiation.

Funded under an NIH grant (AI107305) which claims to seek drugs to mitigate radiation's side effect, this study did not explore radiation antidotes or even discuss them. Its sole aim was to irradiate animals and watch them die, recording their progressive organ dysfunction as they slowly suffered.

It is already know that intense radiation to the thoracic organs causes devastating effects, leading to death. CAARE fails to see the justification for this experiment and how it aligns with the stated goals of the NIH grant. Many prior studies have documented death from thoracic radiation, including a long list that can be obtained from the references section of the study. Avoiding duplication of research is part of the IACUC's role in approving experiments, as well as consideration of alternatives to animal use.

As detailed in the Guide for the Care and Use of Laboratory Animals

Program Oversight/ The Role of the IACUC/ Protocol Review

The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

- availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, Alternatives)
- unnecessary duplication of experiments
- impact of the proposed procedures on the animals' well-being
- appropriate sedation, analgesia, and anesthesia (indices of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, Anesthesia, Pain, and Surgery)

The IACUC criteria for euthanasia in this study, and in others CAARE reviewed, were extreme, entailing intense and sustained suffering.

As stated in the MCW publication:

Based upon direction from the IACUC, rats were designated as morbid and euthanized if they met specified veterinarian's criteria. Rats were considered morbid if they showed 20% loss in body weight or met at least three of the following specified criteria:
(i) greater than or equal to 10% loss in body weight;
(ii) inactivity for one day, defined as no movement unless actively stimulated;
(iii) lack of grooming that became worse after 24 h;
(iv) breathing rates of less than 50 or greater than 250 breaths per minute or demonstration of open-mouth breathing or increased effort for breathing;
(v) hunched posture on two consecutive days.

Any one of these criteria placed animals in extreme distress, let alone requiring that animals meet three out of five. Hunched posture indicates great suffering in small animals like rats. A subsequent publication by MCW refers to it as "death pose." Requiring animals to remain in this condition for two days entails needless, sustained suffering. A respiratory rate of 250 breaths per minute is 3 times higher than the normal respiratory rate of 80 breaths per minute for a rat, and would be equivalent to a human breathing at 60 breaths per minute, which is indicative of great respiratory distress due to severe hypoxia.

Another experiment employed euthanasia criteria that were unnecessarily severe. (Enhanced survival from radiation pneumonitis by combined irradiation to the skin). Rats received skin irradiation in varying strengths (12.5Gy – 30 Gy)to 10% of the total body surface to determine its effect when coupled with radiation to the thorax. The aim of the study was to assess the ability of the ACE inhibitor captopril to mitigate radiation injury.

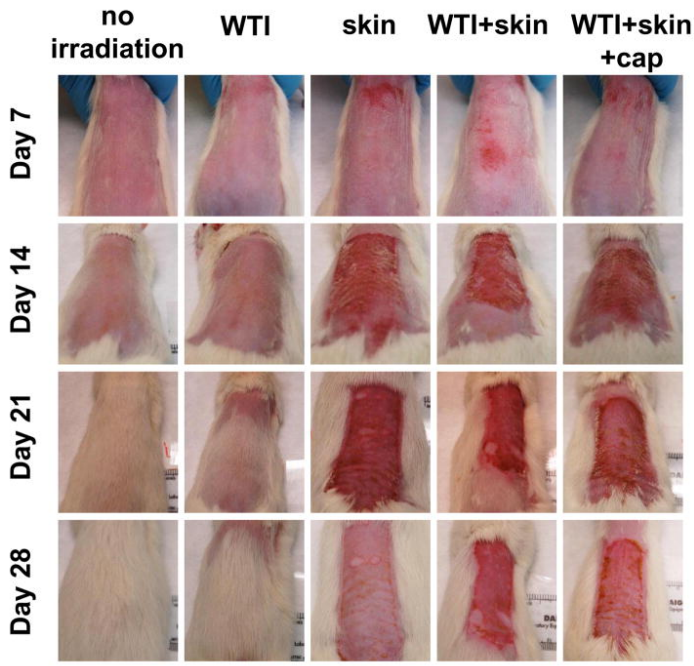
The euthanasia criteria, as detailed in the publication, were as follows:

…rats were considered morbid and were euthanized if they met specified veterinarian's criteria. These included at least 3 of the following:
(1) animals are emaciated with prominent skeletal structures;
(2) inactivity on 2 consecutive days, as no movement unless actively stimulated;
(3) lack of grooming that becomes worse after 24 h;
(4) breathing rates of less than 60 or greater than 250 breaths per minute; and
(5) hunched posture on 2 consecutive days.

Again, these criteria are excessive. Weight loss should be evaluated in terms of a designated percent loss, not pushed to the level of "emaciation with prominent skeletal structures." This is also a subjective measure regarding what constitutes "prominent," which may differ between observers.

Given the extent of the radiation burns experienced by the test animals, analgesia should have been administered. Photos from the publication indicate that these were extensive and undoubtedly caused a

high degree of prolonged suffering. Yet nowhere in the publication is there indication that any form of analgesia was administered at any time, or even considered. This is unacceptable.



get permission from Dr. Thulin, Director TBRC

Similarly extreme euthanasia criteria were used for another radiation study on rats to assess the use of ACE inhibitors for radiation injury. The 2014 publication, Model development and use of ACE inhibitors for preclinical mitigation of radiation-induced injury to multiple organs, carried out total body irradiation of rats followed by bone marrow transplant to test four doses of radiation injury.

Once again, the criteria for euthanasia included the following:

Following IACUC directives, the rats were considered morbid and euthanized if they met specific veterinarian's criteria that included at least three of the following:
1. greater than 20% loss in body weight;
2. inactivity on two consecutive days, defined as no movement unless actively stimulated;
3. lack of grooming that became worse after 24 h;
4. breathing rates of less than 60 or greater than 250 breaths per minute; and
5. hunched posture, death pose on 2 consecutive days.

As part of the procedure the irradiated rats received a bone marrow transplant a few hours after irradiation. The addition of bone marrow transplant demonstrates how this experiment fails to be applicable to human biomedical application. In fact, the researchers acknowledge this point, writing: *"One limitation of our model was the use of a bone marrow transplant to protect the rats from acute*

death by hematopoietic depletion, since such a procedure would not be practical after a mass casualty event."

They go on to note that, despite an increase in survival time with ACE inhibitors in this study "*Very few countermeasures mentioned above have demonstrated efficacy in irradiated humans in trials on cancer patients receiving radiotherapy.*" Those countermeasures include antioxidants, the tyrosine kinase inhibitor genistein, statins, oxidized glutathione variants, and nutraceuticals such as triptolide and flaxseed lignin, and steroids.

Despite years of animal radiation experiments, we are still lacking antidotes to mitigate damage from radiation release in the event of a mass casualty. This is why it is incumbent upon us all to seek alternative methods of research to using animals.

Alternatives to using animals

Increasingly, top scientists are turning to methods other than animals to boost efficiency and more human-relevant results than are possible with animal tests. This shift has been especially notable in chemical and drug testing, where high failures from animal tests – averaging greater than 90% – have forced scientists to confront the shortcomings of animal studies.

At the University of Virginia, School of Medicine, researchers in the pharmacology department are seeking antidotes to radiation poisoning by utilizing state-of-the-art, non-animal methods to identify potential compounds that could serve as a first-line antidote to radiation exposure. (A small molecule screen exposes mTOR signaling pathway involvement in radiation-induced apoptosis.)

This research utilizes human pluripotent stem cells exposed to ionizing radiation to screen for compounds that mitigate the toxic effects of radiation. Using high-throughput screening, the research team screened more than 3,400 existing drugs, vitamins and other compounds to identify ones with potential to counteract the effects of radiation exposure.

They next created 3D computer models of the promising compounds for closer analysis of their chemical structures. That analysis identified a number of other compounds that shared similar chemical structures, pointing to qualities to look for to expand the search for more promising candidates.

Testing one chemical at a time on animals sickened with radiation poisoning is crude, cruel, costly and ineffective. This is why the National Academy of Sciences issued its report in 2007 on Toxicity Testing in the 21st Century: A Vision and a Strategy which outlined a plan that would "dramatically reduce the need for animal testing because the new tests would be based on human cells and cell components," and further that "the result will be a more efficient, informative and less costly system for assessing the hazards posed by industrial chemicals and pesticides."

One year later, the EPA announced its collaboration with NIH to utilize the principles outlined by the NAS report, stating its intent "to rely less on animal studies and more on in vitro tests using human cells and cellular components to identify chemicals with toxic effects."

The EPA-NIH announcement noted the benefit of using

> "…high-speed, automated screening robots to test suspected toxic compounds using cells and isolated molecular targets instead of laboratory animals. This new, trans-agency collaboration is ***anticipated to generate data more relevant to humans; expand the number of chemicals that are tested; and reduce the time, money and number of animals involved in testing."*** [emphasis added]

This paradigm shift was the basis for the recent passage of federal legislation to update the Toxic Substances Control Act, after standard reliance on animal toxicity tests resulted in only five chemicals regulated or banned in 40 years, and an estimated 80,000 chemicals remain in use without adequate testing or data. (See here, here and here).

Though the radiation experiments on animals at MCW are not for chemical testing, the principles are the same. The goal is to assess the biological response of human cells and tissues to compounds to ascertain which demonstrate the ability to mitigate damage from ionizing radiation.

The method employed by the team at University of Virginia to test for radiation countermeasures aligns with this 21st century method for chemical screening and safety testing, utilizing a high throughput assay and the value of computational chemistry. MCW could certainly do this as well.

In addition, MCW could replace irradiating rats with the use of organs-on-chips to assess the ability of a universe of compounds (as opposed to a handful) to ameliorate damage from ionizing radiation, and with better information specific to human biology.

Indeed, the U.S. Department of Defense invested $24 million into organ-chip technology in 2013, specifically for the purpose of developing countermeasures against biological and chemical warfare.

Since 2013, organ-chip technology has only expanded with much promising research arising from its use. Current research at Lawrence Livermore Laboratory in California is being carried out to develop bioterror countermeasures and antidotes to toxins by using networks of organs-on-chips. These research areas are closely analogous to developing radiation antidotes.

There are now companies that make organs-on-chips commercially available, such as Emulate, Inc. in Cambridge, Massachusetts. MCW could make use of these for less than the cost of utilizing animal experiments and with greater benefit for human medicine.

CAARE is aware that the information contained in this letter is based on research described in earlier publications. Since the grant is ongoing, we have no reason to believe that the research has not continued as described. If it has, we would like to be informed about that and/or any modifications.

In addition, CAARE requests to see the justifications in writing presented to the IACUC to circumvent the need for analgesia and timely euthanasia. We will file through your Freedom of Information Office if you require it.

In closing, we feel compelled to point out that these experiments were alarming in their degree of animal suffering. Our team regularly reads a great deal pertaining to animal experiments and we have developed a high threshold of exposure to unpleasant procedures on animals. These experiments stood out as exceedingly and excessively cruel. The IACUC must closely re-evaluate these experiments and consider suspending this line of research.

Society has an obligation to animals used in research, backed by a federal mandate, to assure that their suffering is not excessive and that their lives are not taken recklessly for research that shows weak promise for human translation. The IACUC's failure in this obligation, as outlined in PHS policy, is a clear dereliction of its duties.

Thank you for your consideration. I look forward to your reply.

Sincerely,

Barbara Stagno, RN
President, CAARE

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