aragen

November 23, 2022

To,
The Regional Director,
Animal Care USDA-APHIS Western Regional Office
USDA/APHIS/AC
2150 Centre Ave.
Building B, Mailstop 3W11
Fort Collins, CO 80526-8117

Subject: Annual Report of Research Facility from October 1st 2021 through September 30th 2022 USDA Registration #: 93-R-0504

Dear Regional Office Director:

We have attached our Annual Report (form 7023) pertaining to research activities at Aragen Biosciences, Inc. The use of covered animal species reported in USDA Category E (animal pain and/or distress without alleviation) is explained below.

The model in which there were covered species reported in USDA Category E is the *Clostridioides difficile (C. Difficile),* post-antibiotic colitis model in hamsters [also called C difficile associated disease (CDAD)].

Hamsters are a well-accepted animal model for CDAD and are commonly used in studies evaluating new therapeutics for CDAD. The complexity of this infectious disease cannot be duplicated or modeled in a non-living system. The hamster is the lowest vertebrate in which a full spectrum of symptoms including diarrhea (recognized as wet tail) and gross pathological changes to the colon following C. difficile infection are seen. Hamsters infected with C. difficile display many of the pathophysiological features seen in humans. Use of this model is seen as a final step before testing novel therapies in humans.

This year the hamster model for CDAD was used in six studies under one protocol. The studies were designed optimize the relapse CDAD model. Hamsters were given a single dose of the clindamycin, which disrupts the normal intestinal flora, followed by infection with C. difficile.. A typical control is the use of Vancomycin, a standard of care for C. difficile infections. Like the uninfected controls, hamsters treated early and with sufficient levels of Vancomycin did not display CDAD and survived to the end of the study at which time they were humanely euthanized were placed in Category C. When vancomycin treatment is delayed and reduced, some hamsters will succumb to disease once the vancomycin treatment is stopped. There were 114 hamsters that fell into Category C. Animals that were infected with C. Difficle and in the sham treated or using vancomycin treatment regimen that results in relaypse that developed disease were all categorized as E. There were 74 hamsters that fell into Category E. All study animals were monitored at least twice daily during the acute phase for signs of disease including diarrhea, weight loss, piloerection, lethargy, and ambulatory discomfort. Animals that appeared moribund during observation were immediately humanely euthanized.

The C. difficile studies are classified as pain category E since the animals will display symptoms of diarrhea and become moribund prior to death or euthanasia. Other than supportive care, no anesthetics or analgesics (non-steroidal or opioid) were given. The effect of analgesics may cause a delay of gastric clearance and therefore increase the disease severity and decrease time to death in the control group. Also, since there is the potential for analgesics to impact the pharmacokinetic properties of the test compounds, the impact of analgesics could result in false data.

(b) (6), (b) (7)(C) [redacted]

Sincerely,
Malavika Ghosh, PhD
Vice President, IO
Aragen Biosciences, Inc

380 Woodview Avenue, Morgan Hill, CA 95037. Tel# 408-779-1700, Fax: 408-779-1711