USDA Category E Justification by species: Period covered from October 1, 2019 to September 30, 2020

Registration number: 43-R-0009

1. Hamsters (n=66)
    a. AUS 20-33 (project 111015.02.006.02)
    b. Title of work: Evaluating medical countermeasures against SARS-CoV-2 infection in a Syrian Golden Hamster mode
    c. Objective: This study generated data to compare a homogenous (good) stock, with little to no relevant mutations, to a variable (bad) stock, with mutations in loci that affect virulence and pathogenesis of SARS-CoV-2. The in vivo behavior of the two strains were evaluated when animals had already been immunized with SARS-CoV-2 Spike protein. The information from this study is critical for standardizing the hamster model to be able to generate reliable data for medical countermeasures before they are used in humans.
    d. Animals were immunized and then exposed to SARS-CoV-2. Blood collection and clinical observations were made. Hamsters have been shown to be the best sentient model for SARS-CoV-2 and therefore the reason for choosing this species for this study. The animals were not given tranquilizers or analgesics after exposure because the use of analgesics during the course of the study to alleviate pain might modify the host's response to infectious challenge and could compromise the results of the study or alter the clinical expression of the disease. Narcotic analgesics can cause respiratory depression which could cause death in a compromised animal that might otherwise survive exposure. Narcotics also stimulate the production of a number of immunomodulatory effects in both laboratory animals and humans. Steroidal and non-steroidal anti-inflammatory drugs interfere with inflammatory processes, which are critical in the pathogenesis of the infectious disease process, and therefore the scientific value of this study.
        i. Animals were offered hydrogel in attempts to maintain hydration. If an animal was moribund or not likely to survive to the next observation point, then the animal was euthanized.
2. Guinea pigs n=26
    a. AUS 20-09 (project 111139.01.001)
    b. Title of work: Shielded Oxime Nanoreactor for Wound Decontamination
    c. Objective: There are currently no medical countermeasures approved for the decontamination of open wounds exposed to organophosphates and chemical warfare agents (CWA, nerve agents). To address this urgent medical need, the overall aim of this study was to develop novel dendrimer polymers entitled "shielded oxime nanoreactors (SONs)" for wound decontamination and protection against OP poisoning. The primary objective of this proposed work aims to demonstrate that two lead SONs are efficacious for protecting animals exposed to OPs (paraoxon and VX).

d. The initial studies first were to confirm an LD50 for paraoxon (POX) and VX nerve agents. This was achieved by creating either a cutaneous wound or dermal abrasion wound with either sharp full thickness incision or dermabrader, respectively, under general anesthesia. The nerve agent was then applied to these wounds. Animals were observed for 24 hours post-challenge then euthanized. Animals were offered hydrogel to encourage and maintain hydration.
e. Animals experiencing irreconcilable pain were humanely euthanized. The animals were not given tranquilizers or analgesics after exposure because the use of analgesics during the course of the study to alleviate pain could compromise the results of the study or alter the clinical expression of the exposures. Narcotic analgesics can cause respiratory depression which could cause death in a compromised animal that might otherwise survive exposure. Narcotics also stimulate the production of a number of immunomodulatory effects in both laboratory animals and humans.

3. Guinea pigs (n=10)
    a. AUS 20-23 (project 111129.01.001.01)
    b. Title of work: Dose Range Finding Study for Soman Challenge Concentration in Guinea pig model
    c. The objective of this study was to determine the challenge dose of soman to use in a guinea pig model with co-administration of pyridostigmine bromide (PB), atropine, 2-PAM, and/or diazepam to ensure that animals have a seizure post-soman administration but do not die for 7 days following the challenge. These results will be used to set the challenge dose for soman in the study for AUS 20-11, where animals need to seize and survive for up to 7 days post-challenge in order to evaluate brain injury with and without the therapeutic being tested in that study.
    d. Animals were challenged with Soman in order to identify an appropriate dose for upcoming studies. Animals received the standard of care therapeutics and were offered hydrogel in attempts to maintain normal hydration.
    e. In these studies, early euthanasia would prevent nerve agent challenge concentration and nerve agent efficacy study evaluations of real mortality/survival rates. The purpose of the study is to properly assess the challenge concentration of soman to use in the nerve agent model. There are times in nerve agent studies where an animal (particularly a mouse, rat, or guinea pig) can appear to be moribund, but will recover overnight and have no unusual signs the following day based on our previous experience. Therefore, we did want to euthanize these animals early if they are actually going to survive as this will skew the evaluation of the challenge dose of soman in this model.
        i. Additionally, administration of analgesics or any medications other than those specified would interfere with clinical and potentially histopathological assessments of the compared treatments and the outcome of the study. Nerve agents affect the body's autonomic (involuntary) processes which are responsible for respiratory rate, heart rate, digestion

and salivation. Loss of consciousness can also occur. Administration of opioids can cause these symptoms to worsen. Specifically, pure mu-opioid receptor agonists like morphine can worsen the nerve agent mediated bronchoconstriction due to histamine release. Blood pressure can also elevate. Administration of NSAIDs could cause deleterious effects to the GI tract and also result in renal compromise if blood pressure effects were significant as a result of the nerve agent. Nerve agent toxicity is not considered a painful process and therefore, pain medications specifically would not be indicated and in most cases would be contraindicated. Respiratory discomfort would be treated with oxygenation and ventilation and is not practical in a rodent study and could also skew the results of the efficacy of the therapeutics being tested. The outcomes that could be affected by administration of opioids or NSAIDs would be the efficacy of the therapeutic; animals may succumb more quickly as a result of administration of these analgesics and/or anti-inflammatory.

4. Guinea pig (n=21)
    a. AUS 20-11 (project 111129.01.001.03)
    b. Title of work: Development of RAP-103 Peptide as a Chemical Nerve Agent Therapeutic
    c. The objective of this study is to evaluate the efficacy of the RAP-103 peptide in guinea pigs as part of a series of proof-of-concept studies using organophosphate (OP) chemical nerve agents with varying concentrations of RAP-103 peptide with and without standard of care (i.e., atropine sulfide, pralidoxime chloride (2-PAM), and diazepam).
    d. Animals were challenged with Soman and also given standard of care therapeutics, and in some groups, the addition of RAP-103. Challenged animals were also given hydrogel to maintain normal hydration. Guinea pigs were selected as they are an ideal species for nerve agent studies in their response to these agents.
    e. In these studies, early euthanasia would prevent nerve agent challenge concentration and nerve agent efficacy study evaluations of real mortality/survival rates. The purpose of the study is to properly assess the client's therapeutic in the nerve agent model. There are times in nerve agent studies where an animal (particularly a mouse, rat, or guinea pig) can appear to be moribund, but will recover overnight and have no unusual signs the following day based on our previous experience. Therefore, we did want to euthanize these animals early if they are actually going to survive as this will skew the evaluation of the efficacy of the test compound (RAP-103).
        i. Additionally, administration of analgesics or any medications other than those specified would interfere with clinical and potentially histopathological assessments of the compared treatments and the outcome of the study. Nerve agents affect the body's autonomic (involuntary) processes which are responsible for respiratory rate, heart rate, digestion

and salivation. Loss of consciousness can also occur. Administration of opioids can cause these symptoms to worsen. Specifically, pure mu agonists like morphine can worsen the nerve agent mediated bronchoconstriction due to histamine release. Blood pressure can also elevate. Administration of NSAIDs could cause deleterious effects to the GI tract and also result in renal compromise if blood pressure effects were significant as a result of the nerve agent. Nerve agent toxicity is not considered a painful process and therefore, pain medications specifically would not be indicated and in most cases would be contraindicated. Respiratory discomfort would be treated with oxygenation and ventilation and is not practical in a rodent study and could also skew the results of the efficacy of the therapeutics being tested. The outcomes that could be affected by administration of opioids or NSAIDs would be the efficacy of the therapeutic; animals may succumb more quickly as a result of administration of these analgesics and/or anti-inflammatory.

5. **No studies conducted on swine, feline, rabbits or swine were category E**

6. **NHPs (n=16)**

   a. AUS number 18-33 (project 110977.04.001)
   b. Title of work: Duration and Onset of Immunity of ΔclpB tularemia vaccine in Cynomolgus Macaques
   c. The objective of this study was to determine the duration and onset of immunity of ΔclpB tularemia vaccine in Cynomolgus macaques
   d. This study evaluated animals at 30, 90, or 365 days post-vaccination to assess whether the vaccine was protective against Tularemia. Animals were vaccinated and observed and maintained at the facility until the time of their challenge (28, 90, or 365 days). Animals received an aerosol challenge of Tularemia and were observed and had blood collection time points. Animals had temperature devices implanted to monitor temperatures remotely. At least the control animals (and possibly the vaccinated animals although the vaccine is expected to alleviate tularemia symptoms) experienced unalleviated symptoms of tularemia. Lethargy, hunched posture, and fever are expected. Oftentimes immediately prior to death, animals will experience hypothermia, i.e. body temperature lower than 35 °C. These animals were euthanized if their body temperature was lower than 35 °C.
   e. The animals were not given tranquilizers or analgesics after exposure because the use of analgesics during the course of the study to alleviate pain might modify the host's response to infectious challenge and could compromise the results of the study, alter the clinical expression of the disease, or cause CNS-related signs and symptoms. Narcotic analgesics can cause respiratory depression which could cause death in a compromised animal that might otherwise survive exposure. Narcotics also stimulate the production of a number of immunomodulatory effects in both laboratory animals and humans. Steroidal and non-steroidal anti-inflammatory drugs interfere with inflammatory processes, which are critical in

the pathogenesis of the infectious disease process, and therefore the scientific value of this study.

7. **Dogs project number 311580.01.003.02 AUS number 20-27 n=27**
   a. Title of work: Pivotal Efficacy Study of KIND-030 as a Prophylactic Treatment in dogs with Canine Parvovirus 2 (CPV-2)
   b. The purpose of this study was to confirm the virulence of inoculation doses of canine parvovirus type 2 (CPV-2) to produce morbidity and mortality in purpose-bred, unvaccinated, healthy dogs and evaluate the effectiveness of the test article, Anti-Canine Parvovirus Neutralizing Antibody, as a prophylactic treatment in dogs to prevent clinical signs of CPV-2 infection. This study also served as a proof of concept for efficacy of this test article.
   c. Dogs were exposed to canine parvovirus intranasallly and either treated post-challenge, pre-challenge, or no treatment (control animals) with the test article. Animals were expected to develop vomiting, diarrhea, and lethargy as a result of infection. If severe dehydration or moribundity developed, consultation with the veterinarians and study director would occur in order to then remove an animal from study and perform euthanasia if indicated. Animals were not provided supportive care as this could skew the results of the study outcomes.

8. **Dogs project number 311580.01.002.02 AUS number 20-20 n=30**
   a. Title of work: Pivotal Efficacy Study of KIND-030 for the Treatment of Canine Parvovirus (CPV) in Purpose-Bred Dogs
   b. The purpose of this study was to confirm the virulence of inoculation doses of canine parvovirus type 2 (CPV-2) to produce morbidity and mortality in purpose-bred, unvaccinated, healthy dogs and evaluate the effectiveness of the test article, Anti-Canine Parvovirus Neutralizing Antibody, as a treatment in dogs to stop clinical signs of CPV-2 infection.
   c. Dogs were exposed to canine parvovirus intranasallly and treated with the test article or control on the day a positive Parvo test was identified. Animals were expected to develop vomiting, diarrhea, and lethargy as a result of infection. If severe dehydration or moribundity developed, consultation with the veterinarians and study director would occur in order to then remove an animal from study and perform euthanasia if indicated. Animals were not provided supportive care, aside from canned gastrointestinal diet as long as there was no vomiting; supportive care could skew the results of the study outcomes.