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This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 215

See attached form for additional information.

Interagency Report Control #:

| | | |
|---|---|--|
| UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE ANNUAL REPORT OF RESEARCH FACILITY (Type or Print) | 1. Certificate Number: 51-F-0006 Customer Number: 437 US Army Med Research Inst of Chemical Defense (b)(2)High, (b)(7)f 3100 Ricketts Point Road Aberdeen Prov Grnd, MD 21010 Telephone: (410) 436-3804 | Form Approved OMB NO. 0579-0036 NOV 30 2009 |
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3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites)

(b)(2)High, (b)(7)f

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or USE APHIS Form 7023A)

| A. Animals Covered By the Animal Welfare Regulations | B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes | C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs. | D. Number of animals upon which experiments research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used. | E. Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilization drugs would have adversely affected the procedures, research or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reason such drugs were not used must be attached to the report. | F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E) |
|--|--|---|--|---|--|
| 4. Dogs | 0 | 0 | 0 | 0 | 0 |
| 5. Cats | 0 | 0 | 0 | 0 | 0 |
| 6. Guinea Pigs | 0 | 775 | 301 | 6,547 | 7,623 |
| 7. Hamsters | 0 | 0 | 0 | 0 | 0 |
| 8. Rabbits | 0 | 7 | 100 | 0 | 107 |
| 9. Non-human Primates | 57 | 17 | 73 | 93 | 183 |
| 10. Sheep | 0 | 0 | 0 | 0 | 0 |
| 11. Pigs | 0 | 0 | 16 | 0 | 16 |
| 12. Other Farm Animals: | N/A | | | | |
| 13. Other Animals: | | | | | |

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and is has required the exceptions to the standards and regulations be specified and explained by the principal investigator and approving Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

| | | |
|---|--|-------------|
| CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL (Chief Executive Officer or Legally Responsible Institutional Official) | | |
| SIGNATURE C.E.O. OR INSTITUTIONAL OFFICIAL | NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print) | DATE SIGNED |
| (b)(6), (b)(7)c | | 23 NOV 09 |

EG 12-4-09

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Protocol 1

1. A total of 908 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Animals were injected subcutaneously with organophosphorus anticholinesterase nerve agents (up to $2 \times LD_{50}$), which produced EEG seizures, motor convulsions and other cholinergic toxicities that may cause pain and distress.
3. Justification:

Anesthetics or analgesics will affect the brain functions and will also interact with the actions of anticonvulsant/anti-seizure/neuroprotectant drugs that are under investigation.
4. No federal regulations mandate this procedure.

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 2

1. A total of 389 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Animals were injected subcutaneously with organophosphorus anticholinesterase nerve agents (up to 1 x LD₁₀₀ dose), which produced EEG seizures, motor convulsions and other severe cholinergic toxicities that may cause pain and distress.
3. Justification:

Anesthetics or analgesics will affect the functions of the nervous systems and will also interact with the actions of drugs that are under investigation. This project also studies the mortality/survivability of treatment drugs following nerve agent exposure and administration of anesthetics or analgesics will confound the experimental outcomes.
4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 3

1. A total of 479 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Exposure to nerve agent.
3. Justification:

Most of the animals will receive a potentially convulsive dose of the nerve agent to be evaluated. Death from nerve agent intoxication is the primary endpoint of this protocol. Since we cannot make an accurate assessment of numbers of animals experiencing pain or distress under these conditions, we can only assume the worst-case scenario. Death from nerve agent exposure is aesthetically unpleasant and probably painful to the animals due to the intense physical activity caused by the seizures. However, quantitative evaluation of nerve agent toxicity and the efficacy of therapeutic countermeasures require exposure to lethal doses of a nerve agent in conscious animals. The administration of anesthetics or analgesics to relieve pain would lead to an erroneous evaluation of the toxicity of these agents and the efficacy of pretreatment, treatment, and decontamination procedures. Since nerve agents are anticholinesterases (Decandole et al. 1953) and anesthetics/analgesics (Trevor and Miller 1992) are respiratory depressants, their combined use may enhance the toxicity of the nerve agent compounds and, therefore, affect the MLD values. The short-term anesthesia employed in this protocol, using ketamine and xylazine, is for the safety of the operator (reference section V.C.1.b.i.). The potential interaction between these anesthetic agents and cutaneously applied nerve agents is fully discussed in Skvorak et al. 2000A. Briefly, the combined effect of ketamine and xylazine may be a delay in systemic absorption of the applied agent, but the short duration of anesthesia suggests that the net cardiovascular effects would be minimal.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 4

1. A total of 318 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Eliciting a seizure state.
3. Justification:

Reduction/cessation of seizure activity with novel drug treatments is the goal of this research. Thus, it is necessary to use nerve agents to trigger seizures to study potential beneficial drug effects.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 5

1. A total of 550 column "E" guinea pigs were utilized in this study.
2. Painful procedure:

Subcutaneous injection of lethal VX or soman between the shoulder blades.

Intramuscular injection of test compounds (Galantamine, Atropine, pralidoxime chloride, and others) as inhibitors of nerve agent toxicity.

3. Justification:

Presently, there are no computer models that can substitute for animals in the study of Galantamine neuroprotection from nerve agent intoxication. The purpose of the study is to evaluate the efficacy of compounds as therapeutics and prophylaxis to counteract the harmful toxicity of nerve agent. Scoring of unalleviated, toxic clinical signs after nerve agent is paramount to the survival, behavioral, neuromuscular, and electroencephalographic components in this protocol. Even therapeutics provided for harmful procedures (ketamine) have a demonstrated benefit as neuroprotectants against nerve agents.

4. No federal regulations mandate this procedure.

NOV 30 2009

Protocol 6

1. A total of 572 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Anesthesia/Analgesia/ Tranquilization: CWA agent exposure by Microinstillation, protective drug administration, BAL and exsanguinations and blood collection at the end of each experiment will be done under anesthesia using Telazol (60mg/kg, im)/ medetomidine (1 mg/kg, sc)
3. Justification:

Sixteen animals in Experiment 1, 64 animals in Experiment 2 and 16 animals in Experiment 3 will receive saline that may not cause any pain. The pain due to exsanguinations will be alleviated by anesthesia using Telazol (60 mg/kg, im)/Medetomidine (1 mg/kg, sc). Sixty-four animals in Experiment 1 will receive 0.1LC₅₀ or 0.2LC₅₀ or 0.3LC₅₀ or 0.4LC₅₀ GB under anesthesia, which will reduce the pain. In Experiment 2, 192 animals will receive 0.2LC₅₀ or 0.3LC₅₀ or 0.4LC₅₀ GB under anesthesia, which will reduce the pain. The pain due to exsanguination will be alleviated by anesthesia using Telazol (60 mg/kg, im)/Medetomidine (1mg/kg, sc). Sixty-four animals in Experiment 3 will receive a minimum dose of GB to produce significant inhalation toxicity under anesthesia will reduce the pain, and 224 animals in Experiment 4 will receive a minimum dose of GB to produce significant inhalation toxicity under anesthesia, which will reduce the pain. In Experiment 4, 208 animals will receive different antidotes that may attenuate the inhalation toxicity and reduce the pain. In Experiment 5 192 animals will receive GB under anesthesia, which will reduce pain. The pain due to exsanguinations will be alleviated by anesthesia using Telazol (60 mg/kg, im)/Medetomidine (1mg/kg, sc). In Experiment 6, 224 animals will receive a minimum dose of GB to produce significant inhalation toxicity under anesthesia, which will reduce the pain. The pain due to exsanguinations will be alleviated by anesthesia using Telazol (60 mg/kg, im)/Medetomidine (1mg/kg, sc). The overall goal of the task area and this study is to investigate the inhalation toxicity of GB exposure to establish a dose-response relationship, identify the toxic effects and the mechanism of toxicity and study the protective effect of novel antidotes on the inhalation toxicity. Accurate results of the study are critically important for extrapolating the data to other species or humans, to establish a dose-response relationship for inhalation toxicity of GB exposure and to determine the efficient therapeutic strategy for war fighter and world-wide civilian exposure to organophosphates. Using anesthetics/analgesics or other pharmacological agents during recovery is expected to interfere and complicate the results and will be used at the beginning and end to improve the integrity and experimental outcome.
4. No federal regulations mandate this procedure.

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 7

1. A total of 521 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Agent-injection, subcutaneous, in the absence of the any pre- or post-exposure therapy other than administration of Bioscavenger proteins.
3. Justification:

LD₅₀ determination in the absence of bioscavenger pretreatment can only be conducted in the absence of supporting drugs, analgesics, or other compounds that might alter the pharmacology of either the experimental protein or the organophosphorus nerve agent.
4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 8

1. A total of 225 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Injection of chemical agents (sc) and therapeutic compounds (im or ip). All injectables will be delivered through a 27-Ga hypodermic needle. The dose volume for subcutaneous, intra-muscular and intra-peritoneal (ip) injections will be 0.5, 0.25 and 0.5 ml/kg respectively.

3. Justification:

Literature searches showed that there is no alternative technique(s) that would permit parenteral administration of chemical agents and therapeutic compounds without incurring brief and mild degree of nociception in animals.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 9

1. A total of 1,288 column "E" guinea pigs were utilized in this study.
2. Painful procedure:

Guinea will be exposed to a dose of an organophosphorus nerve agent, which may cause some pain and/or distress due to excess cholinergic function. The guinea pigs will receive a convulsive dose of a nerve agent, which is thought (but not documented) to cause some pain and/or distress due to the intense physical activity caused by the seizure. This may be relieved to some extent by administration of one of the test drugs that successfully terminates the seizure, improves survival or reduces incapacitation

3. Justification:

Anesthetics and analgesics are known to have profound effects on brain function that can interact with the drugs of interest the synthesis and release of brain and/or the toxicity of the nerve agent and thus complicate interpretation of the results. This is especially so since the purpose of the study is to model the pharmacological intervention a human nerve agent casualty would receive.

4. No federal regulations mandate this procedure.

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 10

1. A total of 898 column "E" guinea pigs were utilized in this study.
2. Painful procedure: This protocol involves injecting animals with near lethal and lethal doses of chemical agents which result in numerous toxic signs to include fasciculations, tremors, salivation, lacrimation, bronchoconstriction, dyspnea, broncho-secretions, seizures, motor convulsions and respiratory paralysis. These signs are not thought to be painful *per se*. However, animals may experience distress during and after the end of the toxic crisis.

3. Justification:

Anesthetics and analgesics including non-steroidal anti-inflammatory drugs (NSAIDS) could not be used in any of the experiments because of the possibility of interactions with the agents and/or the other drugs used in this protocol as pretreatment or post challenge treatments. Anesthetics and many analgesics produce respiratory depression or have other pharmacologic effects that could interfere with the response of the animals to agents and the medical countermeasures. Respiratory depression is a principal sequelae of chemical agent intoxication and conducting these experiments under anesthesia or analgesia could lead to faulty interpretation of the toxicity data and/or the effectiveness of the countermeasures because of the synergistic respiratory depressant effects of these drugs with the agents.

4. No federal regulations mandate this procedure.

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 11

1. A total of 55 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Exposure to Sarin.
3. Justification:

This is an experimental animal model for testing therapeutics for protection and recovery from nerve agent exposure.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 12

1. A total of 226 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Potential distress following nerve agent-induced seizures.
3. Justification:

Toxic levels of nerve agents induce seizures, which, although not considered painful, may lead to discomfort associated with muscle fatigue following convulsions/tremors. There are currently no alternatives to pain induced by nerve agent toxicity. Use of analgesics would be a confound when assessing kinetics of nerve agent exposure and therefore cannot be used in these studies.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 13

1. A total of 104 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Induction of seizures by nerve agent.
3. Justification:

Convulsive seizures induced by GD and the associated intense muscular contraction must be assumed to produce some pain, discomfort and/or distress. Analgesics may have anticonvulsive properties; therefore, the use of analgesics to relieve the possible pain/discomfort/distress caused by nerve agent-induced convulsions and seizures would decrease the severity of brain injury, as brain damage is strongly associated with the intensity and duration of seizures. In addition, the test article promethazine is expected to reduce seizures and brain injury, so the use of analgesics, which may have anticonvulsive properties, may interfere with the interpretation of the results generated from promethazine administration.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 14

1. A total of 14 column "E" guinea pigs were utilized in this study.
2. Painful procedure: Animals were injected subcutaneously with organophosphorus anticholinesterase nerve agents (up to 1 x LD₁₀₀ dose), which produced EEG seizures, motor convulsions and other severe cholinergic toxicities that may cause pain and distress.

3. Justification:

Anesthetics or analgesics will affect the functions of the nervous systems and will also interact with the actions of drugs that are under investigation. This project also studies the mortality/survivability of treatment drugs following nerve agent exposure and administration of anesthetics or analgesics will confound the experimental outcomes.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 15

1. A total of 17 column "E" primates were utilized in this study.
2. Painful procedure: Exposure to lethal levels of chemical agents.
3. Justification:

Evaluation of medical countermeasures.

4. No federal regulations mandate this procedure.

NOV 3 0 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 16

1. A total of 39 column "E" nonhuman primates were utilized in this study.
2. Painful procedure: Nerve agent exposure
3. Justification:

Agent exposure is thought to cause some pain and/or distress due to the intense physiological changes produced by these toxicants. Anesthetics and analgesics for relief of pain or distress are known to have profound effects on brain function that can interact with the drugs of interest and/or the toxicity of nerve agents and thus complicate the interpretation of the results.

4. No federal regulations mandate this procedure.

NOV 30 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 17

1. A total of 24 column "E" primates were utilized in this study.
2. Painful procedure: Severe seizures and violent convulsions will be produced in marmosets after injection of soman. This response is expected to be painful and can cause distress. Because these observations form the most important part of the protocol, drugs to alleviate pain or distress may interfere with the action of soman, PB, atropine, 2-PAM and will not be used. Anesthetics and analgesics could not be used in any of the procedures involving OP administration and/or pretreatment and treatment with any of the medical countermeasures. These procedures require exposure to near lethal and lethal doses of OP compounds in live unanesthetized animals.
3. Justification:

One of the principle effects of OP intoxication is respiratory paralysis, which also happens to be a major side effect of anesthetics and analgesics (Goodman & Gilman, 1975). Conducting these experiments under anesthesia or analgesia could lead to faulty interpretation of the toxicity data and/or the effectiveness of the countermeasures because of the synergistic respiratory depressant effects of these drugs with OPs.
4. No federal regulations mandate this procedure.

NOV 3 11 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 18

1. A total of 9 column "E" nonhuman primates were utilized in this study.
2. Painful procedure: Nerve Agent Exposure
3. Justification:

We do NOT anticipate severe pain or distress in this study because we will utilize a sublethal dose of soman (0.8 LD50) in the positive control group that is not expected to cause seizures or long-lasting discomfort. Animals pretreated with HuBChE are expected to experience no discomfort from the treatment compound or from the 2 LD50 dose of soman. To err on the side of caution, however, all of these animals have been listed in Category E. Anesthetics and analgesics are known to have profound effects on brain function that can interact with the drug of interest (Marshall and Woilman, 1985), the synthesis and release of brain neurotransmitters (Beleslin and Polak, 1965; Ngai et al., 1978; Hanin, 1978), and/or the toxicity of the nerve agent (Clement, 1984) and thus complicate interpretation of the results.

4. No federal regulations mandate this procedure.

NOV 30 2009

Column E Explanation Form
USAMRICD – FY 09
Registration Number: 51-F-0006

Protocol 19

1. A total of 4 column "E" nonhuman primates were utilized in this study.

2. Painful procedure: Nerve agent exposure.

3. Justification:

Agent exposure is thought to cause some pain and/or distress due to the intense physiological changes produced by these toxicants. Anesthetics and analgesics for relief of pain or distress are known to have profound effects on brain function that can interact with the drugs of interest and/or the toxicity of nerve agents and thus complicate the interpretation of the results. Indeed, the experimental drug of interest falls into this general class of compounds.

4. No federal regulations mandate this procedure.

NOV 3 0 2009