Protocol 1

1. A total of _46_ column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Animals are exposed to organophosphorus nerve agents and sulfur mustard. The toxicity of nerve agents includes fasciculations, tremors, salivation, lacrimation, bronchoconstriction, dyspnea, broncho-secretions, seizures, motor convulsions and respiratory paralysis. Agent exposure is thought to cause some pain and/or distress from the intense physiological changes produced by these toxicants. Sulfur mustard exposure induces skin lesions and systemic toxicity which are considered to cause pain/distress.

3. Justification:

Agent exposure potentially causes some pain and/or distress as a result of the intense physiological changes produced by these toxicants. Subjecting animals to levels of nerve agent exposure that reliably elicit these toxic effects is essential for the goals of this protocol. The use of anesthetics or analgesics would obscure the results, making it impossible to assess the efficacy of the candidate medical countermeasures.

4. No federal regulations mandate this procedure.

Protocol 9

1. A total of 29 column "E" nonhuman primates were utilized in this study.

2. Painful procedure:

Agent Exposure

3. Justification:

This protocol requires exposure to potentially lethal doses of chemical agents in unanesthetized NHPs to be able to monitor behavioral performance. Agent exposure is thought to cause some pain and/or distress because of the intense physiological changes produced by these toxicants. We cannot pre-treat the animals with any medication as this would compromise the results of the study in determining the dose of the chemical agent that leads to behavioral deficits.

4. No federal regulations mandate this procedure.

Protocol 8

1. A total of 48 column "E" nonhuman primates were utilized in this study.

2. Painful procedure:

Agent Exposure

3. Justification:

This protocol requires exposure to potentially lethal doses of chemical agents in unanesthetized NHPs to be able to monitor behavioral performance. Agent exposure is thought to cause some pain and/or distress because of the intense physiological changes produced by these toxicants. We cannot pre-treat the animals with any medication as this would compromise the results of the study in determining the dose of the chemical agent that leads to behavioral deficits.

4. No federal regulations mandate this procedure.

Protocol 6

1. A total of 192 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Nerve agent intoxication sufficient to elicit a toxic response.

3. Justification:

Nerve agents are by definition toxic compounds that elicit a variety of toxic responses that are considered distressful. The goal of this protocol is to determine the medical benefit of adding alternative anticholinergic drugs to the standard immediate therapeutic medical countermeasure regimen. Thus, subjecting the animals to levels of nerve agent intoxication that reliably elicit the toxic responses is essential to the goals of this protocol.

4. No federal regulations mandate this procedure.

Protocol 5

1. A total of 41 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Chemical exposures may lead to convulsions which can be followed by pain or distress.

3. Justification:

Chemicals that cause seizures lead to unconsciousness and no pain is experienced at this time; however, there is thought that some pain or distress may follow seizures as a result of the intense physical activity (convulsions). Anesthetics and analgesics have profound effects on brain function that can interact with the drugs being evaluated (Marshall and Wollman, 1985) and/or the toxicity of the chemical being evaluated (Clement and Copeman, 1984). Anesthetics may have pro-convulsive or anticonvulsive effects which would complicate interpretation of the anticonvulsant effects of experimental treatments.

4. No federal regulations mandate this procedure.

Protocol 4

1. A total of 94 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Animals are exposed to organophosphorus nerve agents and sulfur mustard. The toxicity of nerve agents includes fasciculations, tremors, salivation, lacrimation, bronchoconstriction, dyspnea, broncho-secretions, seizures, motor convulsions and respiratory paralysis. Agent exposure is thought to cause some pain and/or distress from the intense physiological changes produced by these toxicants. Sulfur mustard exposure induces skin lesions and systemic toxicity which are considered to cause pain/distress.

3. Justification:

Agent exposure potentially causes some pain and/or distress as a result of the intense physiological changes produced by these toxicants. Subjecting animals to levels of nerve agent exposure that reliably elicit these toxic effects is essential for the goals of this protocol. The use of anesthetics or analgesics would obscure the results, making it impossible to assess the efficacy of the candidate medical countermeasures.

4. No federal regulations mandate this procedure.

Protocol 3

1. A total of _3__ column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Initially, all animals were assigned to C Category. The experiment requires 3 successive injections of PCB-OPH-YT and OPH-YT with a 2 week-gap between each injection. After the second injection of proteins, the 3 animals injected with OPH-YT were sick and one animal died due to anaphylactic shock. Based on this experience, we predicted that the surviving animals may go into anaphylactic shock after the third injection. Therefore we submitted an amendment changing the pain category from C to E which was approved by the IACUC. However, none of the animals went into anaphylactic shock, showed only mild signs of lethargy and recovered fully after an hour. Therefore, we placed the 3 animals with OPH-YT in pain category E and the rest in pain category C.

3. Justification:

The animals were not subjected to any painful procedures. The proteins were given as subcutaneous injections at a dose of 10 mg/Kg body weight. The reasons for the death of one animal and the other two animals showing signs of sickness after the protein injections were unclear. Subsequently, a third injection of the same protein was given and the animals were fine.

4. No federal regulations mandate this procedure.

Protocol 2

1. A total of 54 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Animals are challenged with either the nerve agent sarin or VX at a dose that elicits electrographic seizure activity.

3. Justification:

The goal of the study is to utilize a guinea pig model to evaluate brain AChE activity and oxime therapy following nerve agent-induced exposure. Thus, subjecting the animals to nerve agent intoxication is essential to the goals of this protocol. EEG monitoring is essential to evaluate whether electrographical seizure activity is controlled by centrally active oxime therapy and how this relates to AChE activity. Anesthetics such as isoflurane, sevoflurane, halothane, flurothyl, propofol, or ketamine are known to interfere with seizure activity, causing either pro- or anti-convulsant effects. Analgesics can potentially interact with the toxicity of the agents because of their ability to enhance respiratory depression and thus complicate the interpretation of the results.

4. No federal regulations mandate this procedure.

Protocol 12

1. A total of 84 column "E" pigs were utilized in this study.

2. Painful procedure:

Nerve agent exposure

3. Justification:

This protocol requires exposure to otherwise convulsive or lethal doses of nerve agents in unanesthetized swine. Agent exposure is thought to cause some pain and/or distress because of the intense physiological changes produced by these toxicants. We cannot pretreat the animals with any medication as this would compromise the results of the study. The administration of anesthetics or analgesics to relieve pain or distress would lead to an erroneous evaluation of the toxicity of these agents and the efficacy of pretreatment, treatment, and decontamination procedures. Use of these anesthetics or analgesics would undermine the purpose of these experiments and may increase the number of animals needed to obtain statistical significance.

4. No federal regulations mandate this procedure.

Protocol 11

1. A total of _6___ column "E" nonhuman primates were utilized in this study.

2. Painful procedure:

Agent Exposure

3. Justification:

This protocol requires exposure to potentially lethal doses of chemical agents in unanesthetized NHPs to evaluate the toxicity of these agents and the efficacy of pretreatment, treatment, decontamination procedures. In addition it allows monitoring of behavioral performance after exposure, pre-treatment, and treatment. Agent exposure is thought to cause some pain and/or distress because of the intense physiological changes produced by these toxicants. We cannot pre-treat the animals with any analgesic medication as this would compromise the results of the study in determining the dose of the chemical agent that leads to behavioral deficits.

4. No federal regulations mandate this procedure.

Protocol 7

1. A total of 34 column "E" rabbits were utilized in this study.

2. Painful procedure:

Animals are anesthetized and exposed by inhalation to a toxic dose of sulfur mustard. The study is a chronic (28 day) study and the mustard injury gets more severe over time.

3. Justification:

The procedures in this protocol that could cause pain or distress, i.e., the intubation, inhalation exposure, and blood sampling, will be conducted under anesthesia. An estimated 83% of rabbits used may have distress brought on by congestion, wheezing, and difficulty breathing following SM exposure with no alleviation. Due to the nature of this study we cannot interfere with the natural progression of injury in this model since we are investigating treatments.

4. No federal regulations mandate this procedure.

Protocol 10

1. A total of 23 column "E" nonhuman primates were utilized in this study.

2. Painful procedure:

Exposure to botulinum neurotoxin.

3. Justification:

Botulinum neurotoxin causes profound physical weakness without directly affecting brain. We cannot administer any sedatives because they are respiratory depressants, and thus may compound the effects of botulism. Thus we cannot mitigate animal distress.

4. No federal regulations mandate this procedure.
