Protocol 1

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

2. Painful procedure:

Animals were injected subcutaneously with organophosphorus anticholinesterase nerve agents (up to 2.0 x LD50), 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.

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wp 1/19/12

Protocol 2

1. A total of 384 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Animals received a potentially convulsive dose of nerve agent.

3. Justification:

The animals receiving nerve agent exposure probably experienced pain and distress due to the intense physical activity caused by the seizures. Nevertheless, quantitative evaluation of nerve agent toxicity and the efficacy of therapeutic countermeasures required exposure to lethal doses of a nerve agent in conscious animals. The administration of anesthetics or analgesics to relieve pain would have led to an erroneous evaluation of the toxicity of these agents and the efficacy of pretreatment or decontamination procedures because the interaction between these pain relief medications and the chemical agents is unknown. In addition, since nerve agents are anticholinesterases and the most potent anesthetics/ analgesics are cardiac and respiratory depressants, their combined use may enhance the toxicity of the nerve agents compounds and, therefore, affect the LD50 values. The short-term anesthesia employed in this study, using ketamine and xylazine, was for the safety of the operator. The potential interaction between these anesthetic agents and percutaneously applied nerve agents was fully discussed in Skvorak et al. 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 suggested that the net cardiovascular effects would be minimal.

4. No federal regulations mandate this procedure.

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Protocol 3

1. A total of 782 column "E" guinea pigs were utilized in this study.

2. Painful procedure:

Subcutaneous exposure to organophosphorus nerve agents at doses near or above the median lethal dose

3. Justification:

Median lethal dose (LD50) determination in the presence 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.

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