Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay person as well as scientists.

1. Registration Number: **51-F-0019 (CCDC Chemical Biological Center)**

2. Number of animals used in this study: **24**

3. Species (common name) of animals used in the study: **guinea pigs**

4. Explain the procedure producing pain and/or distress.

   Twenty-four (24) guinea pigs were exposed to a military grade compound intra-venously to determine 24-hour LD_{50}'s (lethal to 50% of the animals tested). The compound caused toxic signs such as lethargy, difficulty breathing, and ataxia.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see item 6 below.)

   Non-steroidal anti-inflammatory analgesics are contraindicated in this study as the compound under investigation plays a significant role in inflammatory responses throughout the body. Opioid analgesics are contraindicated because the compound under investigation is expressed in nearly all tissues of the body and is proposed to have many, so far unidentified, regulatory roles which may include a role in the opioid system. The same applied for other mechanisms of pain relief such as alpha adrenergic agonists.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency _____ CFR _____

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1. Registration Number: **51-F-0019 (CCDC Chemical Biological Center)**

2. Number of animals used in this study: **21**

3. Species (common name) of animals used in the study: **rabbits**

4. Explain the procedure producing pain and/or distress.

   Thirteen (13) rabbits were implanted with telemetric devices and exposed via nose-only inhalation to military grade compounds. Doses ranged from a no observable adverse effects level (NOAEL) to a 100% blood acetylcholinesterase inhibition level. The compounds caused toxic signs such as ataxia and respiratory stress.

   Eight (8) rabbits were exposed via nose-only inhalation to a military grade compound to estimate the 24-hour LD_{50} (lethal to 50% of the animals tested) values. The compound caused toxic signs such as convulsions and respiratory failure.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see item 6 below.)

   Pre-treating the thirteen (13) rabbits with analgesics was not an option because it could potentially affect the sub-lethal results through pre-exposure enhancement of liver metabolism. This potential metabolic enhancement was likely to shift the sub-lethal or NOAEL results and potentially render the study invalid.

   Anesthetics and analgesics cause respiratory depression and immobilization so their use would have interfered with the observation of overt toxic signs and assessment of the condition of the eight (8) rabbits.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency _____ CFR _____

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1. Registration Number: **51-F-0019 (CCDC Chemical Biological Center)**

2. Number of animals used in this study: **59**

3. Species (common name) of animals used in the study: **pigs**

4. Explain the procedure producing pain and/or distress.

   Fifty-nine (59) pigs were exposed to military grade compounds via whole-body inhalation to attain LCt_{50}'s (lethal to 50% of the animals tested) and ECt_{50}'s (concentration that causes an effect in 50% of the animals tested) for each exposure duration. The compounds caused toxic signs such as gasping and broncho-constriction in twenty-one (21) pigs, and salivation, miosis, tremors, and convulsions in thirty-eight (38) pigs.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see item 6 below.)

   Pre-treating the animals with analgesics was not an option because it would interfere or potentially block clinical signs to be used as endpoints. Additionally, administering analgesics would result in respiratory depression which could alter the respiratory minute volume during the aerosol exposure and exacerbate the expected signs and symptoms following the exposure, thereby confounding the intent of the experiments.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency _____ CFR _____