Column E Explanation

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

2. Number of animals used in this study: **25**

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

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

   Minipigs 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 bronchoconstriction in twenty-five (25) minipigs.

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):

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

2. Number of animals used in this study: **100**

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

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

   Rabbits were exposed to a military grade compound via whole-body inhalation for 20 and 60 minutes, to attain ECt_{50}'s (concentration that causes an effect in 50% of the animals tested) for each exposure duration. The compound caused toxic signs (gasping, bronchoconstriction, involuntary movement, collapse) in one hundred (100) rabbits.

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 could potentially affect the lethality results through pre-exposure enhancement of liver metabolism. This potential metabolic enhancement is likely to shift the lethality or no observable adverse effects levels (NOAEL) results and potentially render the study invalid. Toxic signs and their severity had to be documented.

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):

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

2. Number of animals used in this study: **60**

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

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

Rabbits were exposed intravenously 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, tetanus/flaccid paralysis, and respiratory failure in sixty (60) rabbits.

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.)

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 test subjects.

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):

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

2. Number of animals used in this study: **46**

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

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

   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 gasping and bronchoconstriction in forty-six (46) rabbits.

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 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.

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):

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

2. Number of animals used in this study: **258**

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

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

   Guinea pigs were exposed to a military grade compound via whole-body inhalation for 20 and 60 minutes 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 compound caused toxic signs (gasping, bronchoconstriction, miosis, collapse) in two hundred fifty-eight (258) guinea 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 could potentially affect the lethality results through pre-exposure enhancement of liver metabolism. This potential metabolic enhancement was likely to shift the lethality or no observable adverse effects levels (NOAEL) results and potentially render the study invalid. Toxic signs and their severity had to be documented. The data had to be collected as accurately as possible to analyze the ECt_{50} (concentration that causes an effect in 50% of the animals tested).

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):

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

2. Number of animals used in this study: **40**

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

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

Ten (10) guinea pigs were exposed intravenously to a military grade compound in an up-and-down procedure to estimate the 24-hour LD_{50} (lethal to 50% of the animals tested) values.

Ten (10) guinea pigs were exposed intravenously to a military grade compound in an up-and-down procedure to estimate an ED_{50} (effective dose for 50% of the animals tested) value for moderate toxic signs (salivation, fasciculations, and/or tremors).

Twenty (20) guinea pigs were exposed intravenously to a low or high dose of a military grade compound, then serial blood samples were collected at 10 time points to identify biomarkers.

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.)

Anesthetics and analgesics could not be used for the LD_{50} study because it was necessary to gather as much toxic sign data as possible from the animals to help guide dose selection for the ED_{50} and biomarkers studies. Data collection included the onset and duration of each toxic sign as well as the time of death.

Anesthetics and analgesics could not be used for the ED_{50} study because their use could have potentially masked or eliminated some of the toxic signs that were being observed and documented for their onset and severity. The data had to be collected as accurately as possible.

To prevent confounding the study of biomarkers, the pain/distress associated with exposure to the compound under investigation could not be alleviated with anesthetics and analgesics.

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):

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

2. Number of animals used in this study: **12**

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

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

   Guinea pigs were exposed to a military grade compound intravenously to determine 24-hour LD_{50}'s (lethal to 50% of the animals tested). The compound caused toxic signs (gasping and bronchoconstriction) in twelve (12) guinea 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.)

   NSAID 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):

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