Column E Explanation

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

2. Number **8** of animals used in this study.

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

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

   Rabbits were exposed, intravenously and percutaneously, to lethal concentrations of new military grade chemical agents. These compounds caused toxic signs such as respiratory distress and convulsions in eight (8) 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 were not used because their use could potentially mask or eliminate some of the toxic signs that must be observed to fully understand the toxicological aspects of the new compounds. Toxic signs and their severity, as well as time to death, had to be documented. The data had to be collected as accurately as possible to analyze the ECt_{50} (time to effects data), ED_{50} (dose to effects data), and LD_{50} (dose lethal to 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):

   Agency _____ CFR _____

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

2. Number **9** of animals used in this study.

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

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

Rabbits were exposed, via nose-only and whole-body inhalation, to lethal concentrations of military grade chemical agents. These compounds caused toxic signs such as gasping and bronchoconstriction in nine (9) 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 lethal or sub-lethal results through pre-exposure enhancement of liver metabolism. This potential metabolic enhancement is likely to shift the sub-lethal or NOAEL results and render the study potentially 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 **8** of animals used in this study.

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

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

Rabbits were exposed, via nose-only inhalation, to military grade chemical agents. These compounds caused toxic signs such as salivation, miosis, and rhinorrhea in eight (8) 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 were not used because their use could potentially mask or eliminate some of the toxic signs that must be observed to fully understand the toxicological aspects of the tested compounds. Toxic signs and their severity had to be documented. The data had to be collected as accurately as possible to analyze the ECt_{50} (time to effects data).

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 **104** of animals used in this study.

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

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

   Rabbits were exposed to a military grade chemical agent, 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 (time to effects data) or each exposure duration. The compound caused toxic signs such as gasping and bronchoconstriction in one hundred four (104) 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 render the study potentially 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} (time to effects data).

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 **8** of animals used in this study.

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

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

Rabbits were implanted with telemetric devices and exposed, via nose-only inhalation, to military grade chemical agents. 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 eight (8) 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 is likely to shift the sub-lethal or NOAEL results and render the study potentially 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 **4** of animals used in this study.

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

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

Minipigs were exposed to military grade chemical agents, via whole-body inhalation, to attain LCt_{50}'s (lethal to 50% of the animals tested) and ECt_{50}'s (time to effects data) for each exposure duration. The compounds caused toxic signs such as gasping and bronchoconstriction in four (4) 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 **238** of animals used in this study.

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

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

Guinea pigs were exposed, intravenously, to military grade chemical agents to estimate the 24-hour median lethal dose (LD_{50}; dose that is lethal to 50% of the animals tested) of the individual stereoisomers of various compounds. The compounds caused toxic signs such as gasping, bronchoconstriction, and convulsions in two hundred thirty-eight (238) 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 interfere or potentially block observable toxic signs, especially convulsions, to be used as endpoints.

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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