Column E Explanation QA 2568, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the twelve (12) animals exposed, all were determined to have experienced more than momentary or slight pain or distress.

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

Domestic Pig

4. Explain procedure producing pain and/or distress:

An experimental toxicant bait containing sodium nitrite will be fed to domestic swine. Sodium nitrite is expected to induce mild discomfort in swine prior to unconsciousness, including incoordination and vomiting. Study QA 2376 revealed that death for feral swine from sodium nitrite toxicity mostly occurred within 4 hours and without signs of severe pain and/or distress. However, death from sodium nitrite has not been researched extensively enough to conclude that no individuals will experience more than momentary or slight pain or distress.

5. Provide scientific justification why pain or distress could not be relieved. State method or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see item 6 below).
Sodium nitrite is considered a humane toxicant because severe pain or distress has not been observed from related toxicity (Cowled et al. 2008). Typical behaviors observed in feral swine after ingestion of lethal doses of sodium nitrite include lethargy, dyspnea, and occasional mild vomiting (Cowled et al. 2008). Such observations will not be interpreted as signs of pain or distress warranting intervention. If any animal observed, in the opinion of research staff, experienced extended periods of pain or distress, they were examined and euthanized.

The effect of using sedative, analgesics, or anesthetics in conjunction with sodium nitrite is unknown but cannot be assumed to be negligible. Sodium nitrite produces methemoglobinemia, an inability of red blood cells to transfer oxygen to tissues. Using sedatives, analgesics, or anesthestics that would affect the respiratory system of animals could confound this study by increasing/decreasing the lethality of methemoglobinemia from sodium nitrite. In addition, any sedative, analgesics, or anesthetics leaving drug residues in the tissues of domestic swine will confound the examination of secondary toxicity for coyotes consuming those carcasses.

6. What, if any federal regulations require this procedure?

The EPA requires evaluation of secondary hazards for new toxicants to nontarget species.

Column E Explanation QA 2278, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 42 animals exposed, two (2) were determined to have experienced more than momentary or slight pain or distress.

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

Raccoons

4. Explain procedure producing pain and/or distress:

As part of a research project to evaluate a new rabies vaccine for raccoons, the animals were infected with rabies virus. Rabies infection is a severe disease and can lead to pain and distress in an animal.

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

There was no plan to withhold relief from pain or distress. Animals were closely monitored for the progression of signs of disease and assigned scores based on observations. Those scores were used to determine when animals were to be euthanized. Despite routine monitoring, occasionally an animal's condition deteriorated rapidly to a terminal state before euthanasia could be administered.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for vaccine licensing purposes.

Column E Explanation QA 2671, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 40 animals exposed, twenty-eight (28) were determined to have experienced more than momentary or slight pain or distress.

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

Richardson's Ground Squirrels

4. Explain procedure producing pain and/or distress:

Animals were exposed to an experimental toxicant as part of a project to develop new rodenticides for the agricultural industry.

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

Sedatives, analgesics, and/or anesthetics were withheld in the final testing because the animals must be functioning normally (metabolically and physiologically) when exposed so substance efficacy can be determined under more or less normal conditions. Other substances in the animals' systems could confound the results of the study and lead to a false determination of the efficacy of this compound. Euthanasia of animals was allowed by the protocol for animals determined to be experiencing more than momentary or slight pain or distress.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for registration purposes and the study was conducted in accordance with current guidance from regulatory authorities.

> *Agency: US Environmental Protection Agency*
>
> *CFR: 40 CFR Chapter 1, Part 158: Data Requirements for Registration of Pesticide Products.*
>
> *Also: U.S. EPA 2002 Humane practices for acute oral toxicity studies which include the recommendation to follow the guidelines published by the OECD (2000)*

Column E Explanation QA 2467, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 45 animals exposed, twenty-one (21) were determined to have experienced more than momentary or slight pain or distress.

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

House Mouse

4. Explain procedure producing pain and/or distress:

Animals were exposed to an experimental toxicant as part of a project to develop new rodenticides for the agricultural industry.

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

Sedatives, analgesics, and/or anesthetics were withheld in the final testing because the animals must be functioning normally (metabolically and physiologically) when exposed so substance efficacy can be determined under more or less normal conditions. Other substances in the animals' systems could confound the results of the study and lead to a false determination of the efficacy of this compound. Euthanasia of animals was allowed by the protocol for animals determined to be experiencing more than momentary or slight pain or distress.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for registration purposes and the study was conducted in accordance with current guidance from regulatory authorities.

Agency: US Environmental Protection Agency

CFR: 40 CFR Chapter 1, Part 158: Data Requirements for Registration of Pesticide Products.

Also: U.S. EPA 2002 Humane practices for acute oral toxicity studies which include the recommendation to follow the guidelines published by the OECD (2000)

Column E Explanation QA 2464, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 40 animals exposed, twelve (12) were determined to have experienced more than momentary or slight pain or distress.

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

House Mouse

4. Explain procedure producing pain and/or distress:

Animals were exposed to an experimental toxicant as part of a project to develop new rodenticides for the agricultural industry.

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

Sedatives, analgesics, and/or anesthetics were withheld in the final testing because the animals must be functioning normally (metabolically and physiologically) when exposed so substance efficacy can be determined under more or less normal conditions. Other substances in the animals' systems could confound the results of the study and lead to a false determination of the efficacy of this compound. Euthanasia of animals was allowed by the protocol for animals determined to be experiencing more than momentary or slight pain or distress.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for registration purposes and the study was conducted in accordance with current guidance from regulatory authorities.

Agency: US Environmental Protection Agency

CFR: 40 CFR Chapter 1, Part 158: Data Requirements for Registration of Pesticide Products.

Also: U.S. EPA 2002 Humane practices for acute oral toxicity studies which include the recommendation to follow the guidelines published by the OECD (2000)

Column E Explanation QA 2300, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 45 animals exposed, one (1) was determined to have experienced more than momentary or slight pain or distress.

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

Norway rats

4. Explain procedure producing pain and/or distress:

Animals were exposed to an experimental toxicant as part of a project to develop new rodenticides for the agricultural industry.

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

Sedatives, analgesics, and/or anesthetics were withheld in the final testing because the animals must be functioning normally (metabolically and physiologically) when exposed so substance efficacy can be determined under more or less normal conditions. Other substances in the animals' systems could confound the results of the study and lead to a false determination of the efficacy of this compound. Euthanasia of animals was allowed by the protocol for animals determined to be experiencing more than momentary or slight pain or distress.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for registration purposes and the study was conducted in accordance with current guidance from regulatory authorities.

Agency: US Environmental Protection Agency

CFR: 40 CFR Chapter 1, Part 158: Data Requirements for Registration of Pesticide Products.

Also: U.S. EPA 2002 Humane practices for acute oral toxicity studies which include the recommendation to follow the guidelines published by the OECD (2000)

Column E Explanation QA 2546, FY16

1. Registration Number: 84-F-0001

2. Number of animals used in this study during this reporting period:

Of the 51 Polynesian Rats and 52 House Mice exposed to toxic rodenticide baits, all were determined to have experienced more than momentary or slight pain or distress. The animals either died or some that showed signs of toxicity recovered.

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

Polynesian Rats and House Mouse

4. Explain procedure producing pain and/or distress:

The test substance will be offered to caged rats and mice over a 15 day period. Intoxication by anticoagulant may cause more than momentary pain or distress.

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

Relatively normal physiologic and metabolic processes must be occurring in order to evaluate the rodenticide. Pharmacologic intervention would affect these processes. Specific criteria were established and followed in order to provide euthanasia as a humane endpoint.

6. What, if any federal regulations require this procedure?

Although no regulations specifically required this study, the data could be used for registration purposes and the study was conducted in accordance with current guidance from regulatory authorities.

Agency: US Environmental Protection Agency

CFR: 40 CFR Chapter 1, Part 158: Data Requirements for Registration of Pesticide Products.

Also: U.S. EPA 2002 Humane practices for acute oral toxicity studies which include the recommendation to follow the guidelines published by the OECD (2000)