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 persons as well as scientists.

1. Registration Number: 54-G-0001

2. Number of animals used in this study: 270

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

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

   Fish were infected with *Edwardsiella piscicida*, delivered under anesthesia (80 mg/L of MS-222) using a 27G1/2 inch needle. During the 28-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

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):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 203

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

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

   Fish were infected with *Weissella ceti*, delivered under anesthesia (80 mg/L of MS-222) using a 27G1/2 inch needle. During the 28-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, discoloration, exophthalmia, and lesions at the injection site. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

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):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 603

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

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

   Juvenile fish were infected with the *Yersinia ruckeri*, the causative agent of enteric redmouth disease. The pathogen was introduced by intraperitoneal injection of 100 µL of the bacterial preparation suspended in PBS using a 26-guage × ½-inch needle while fish were in an anesthetized state. During the 28-day post-challenge observation period, fish experienced typical disease signs including inappetance, altered ventilation and swimming, loss of equilibrium, discoloration, and either succumbed to the disease naturally or were identified as being moribund and immediately euthanized via immersion in 250 mg/L of tricaine methanesulfonate for a minimum of 10 minutes following cessation of respiratory movements.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

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):

   N/A

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Column E Explanation

1. Registration Number: 54-G-0001

2. Number of animals used in this study: 1,244

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

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

   Juvenile fish were infected with *Flavobacterium columnare*, the causative agent of columnaris disease, by immersing fish in water containing the active bacteria. During the 21-day post-challenge observation period, some fish experienced typical signs of disease including, decreased appetite, altered ventilation and swimming, loss of equilibrium, and discoloration. Most fish were humanely euthanized prior to the development of severe disease, although some died before euthanasia could be performed. We are reporting those fish that experienced more than momentary discomfort in Column E.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

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):

   N/A

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1. Registration Number: 54-G-0001

2. Number of animals used in this study: 9,357

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

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

   Juvenile fish were infected with the *Flavobacterium psychrophilum*, the causative agent of bacterial cold water disease. The pathogen was introduced by intramuscular or intraperitoneal injection of the bacterial preparation in a volume of 10 µL, 50 µL or 100 µL dependent on fish size, and delivered with either a 27- or 26-guage × ½-inch needle while fish were in an anesthetized state. During the 21 or 28-day post-challenge observation period, fish experienced typical disease signs including inappetance, altered ventilation and swimming, loss of equilibrium, discoloration, injection-site lesions, and either succumbed to the disease naturally or were identified as being moribund and immediately euthanized via immersion in 250 mg/L of tricaine methanesulfonate for a minimum of 10 minutes following cessation of respiratory movements.

5. Attach or include an explanation with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Accomplishment of the research goals requires the disease to progress naturally without interference, resulting in relatively high rates of mortality due to natural causes and/or euthanasia of moribund animals. Few data are available regarding the use of analgesics in fish, and protocols tested to date only pertain only to alleviation of acute pain and distress (Sneddon, 2012, J. Exotic Pet Medicine, 21:32-43). Furthermore, the effects of anesthetics, analgesics, and tranquilizers on disease resistance mechanisms are unresolved and may have interfered with the normal course of disease progression and host response, and thus could not be used in these studies for long-term periods. Tricaine is currently the only anesthetic/analgesic agent approved for use in fish in the U.S., and its long term use for the duration of this study was not feasible.

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):

   N/A

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CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY
EXPLANATION FOR APHIS FORM 7023 COLUMN E ENTRIES

FY 2020
CUSTOMER NUMBER: 334026
REGISTRATION NUMBER: 71-G-0001

Species: ARS Fish (Hybrid Striped Bass)
Number: 2130
Fish were used to determine the effect of 1) the administration of therapeutics, 2) immunization, 3) genetic strains of dietary feed grains, 4) full/half-sib genetic families, and/or 5) second generation backcross hybrid fish on their mortality during use of a bacterial disease model. The proposed studies can only be accomplished using live fish as an in vivo response to infection is to be evaluated. During the bacterial disease model some fish may experience distress from exposure to bacteria which may result in the obstruction of airways (gills) or in the osmoregulation process (skin). Drug intervention (beyond the testing paradigm) to eliminate distress is contraindicated due to the scientific need to test the efficacy of therapeutics or immunization strategies in the disease model. Fish that are identified as moribund or show visual signs of swimming incoordination, lack of proper buoyancy, general lethargy, or otherwise displaying abnormal behavior will be euthanized.

Note: No exceptions to the regulations and standards were requested by the PI or approved by the IACUC.

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