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: 3,784

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

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

   Juvenile fish were subjected to immersion challenge with *Flavobacterium columnare*, the causative agent of columnaris disease, in a static bath for 1 hour. During the 21-day post-challenge observation period, some fish experienced typical disease signs including inappetance, altered ventilation and swimming, loss of equilibrium, discoloration, and 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 or died acutely before euthanasia could be performed. We are reporting the subpopulation of fish that may experience 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)

   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 disrupted 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 is 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

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: 4,226

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 intraperitoneal injection of 100 µL of the bacterial preparation suspended in sterile PBS using a 26-gauge × ½-inch needle while fish were in an anesthetized state. During the 21-day post-challenge observation period, some fish experienced typical disease signs including inappetance, altered ventilation and swimming, loss of equilibrium, discoloration, and injection-site lesions, and 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 or died acutely before euthanasia could be performed. We are reporting the subpopulation of fish that may experience 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)

   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 disrupted 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 is 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

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: 197

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 *Lactococcus garvieae*, the causative agent of Lactococcosis. The pathogen was introduced by intraperitoneal injection of 100 µL of the bacterial preparation suspended in sterile PBS using a 27-gauge × ½-inch needle while fish were in an anesthetized state. During the 28-day post-challenge observation period, some fish experienced disease signs typical of this disease including reduced activity and appetite, altered swimming, loss of equilibrium, altered ventilation, melanosis, exophthalmia, and 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 or died acutely before euthanasia could be performed. We are reporting the subpopulation of fish that may experience 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)

   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 disrupted 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 is 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

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: 228

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

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

   Juvenile fish were subjected to immersion challenge with *Yersinia ruckeri*, the causative agent of enteric redmouth disease, in a static bath with aeration for 1 hour. During the 28-day post-challenge observation period, some fish experienced disease signs common to *Y. ruckeri*-caused disease. These signs included reduced activity, loss of appetite, melanosis, altered swimming and ventilation, and 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 or died acutely before euthanasia could be performed. We are reporting the subpopulation of fish that may experience 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)

   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 disrupted 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 is 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