(1) 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: 52-R-0007

2. Number of animals used in this study: 23

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

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

   Twenty three rhesus macaques were used as part of our program to evaluate drug dependence liability of new compounds. These rhesus macaques are physically dependent on morphine. Periodically, they are allowed to go into spontaneous withdrawal, or withdrawal is precipitated by opioid antagonists. During this short period of time they experience moderate to severe stress. Once in withdrawal, the macaques are injected with the investigational compound, or morphine, or saline and then observed to assess their withdrawal state. On completion of the observation period, all withdrawal signs and symptoms are relieved by an injection of morphine. It is important to note that while these experiments are repetitive in nature, a new investigational chemical is being tested on each occasion. Many of these chemicals are opioid in nature and quickly relieve the withdrawal signs and symptoms.

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 the test results. (For federally mandated testing, see Item 6 below).

   The distress produced by this procedure is the experimental end-point being measured. As indicated above, many of the compounds being tested quickly relieve these symptoms. The experiment is terminated immediately after the observation period by the administration of morphine which also relieves the withdrawal signs.

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)

   The results from our testing are used by both the FDA and the DEA in making decisions concerning the Controlled Substances Act.

(2) Column E Explanation

1. Registration Number: 52-R-0007

2. Number of animals used in this study: 5

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

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

Five rhesus macaques were used to evaluate the drug dependence liability of a new formulation of an opioid analgesic with a potential for decreased production of physical dependence. Subjects were administered either the original formulation or the novel formulation at clinically relevant doses for approximately 21 days total. After seven days of repeated dosing, the subjects were evaluated for 30 min for precipitated withdrawal signs following administration of an antagonist drug. During this short period of time they experience mild to moderate stress. On completion of the observation period, all withdrawal signs and symptoms are relieved by an injection of the maintenance drug. If the withdrawal signs are severe, the observation is terminated before the full 30 min by re-administration of the maintenance drug. Dosing continued for an additional 7 days at which time animals underwent evaluation of spontaneous withdrawal following cessation of drug administration. Animals were observed for 30 min every 2 to 6 hours for 24 hours. At the end of the 24 hours, or if withdrawal signs were severe, drug dosing was reinstituted to relieve withdrawal. Because of the moderate doses used, withdrawal signs were generally mild (spontaneous) to moderate (precipitated). At the completion of each dosing regimen that was shown to produce dependence, the animals were slowly tapered off the drug to prevent producing spontaneous withdrawal as drug administration was discontinued.

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 the test results. (For federally mandated testing, see Item 6 below).

The distress produced by this procedure is the end-point being measured. The use of anesthetics, sedatives, and analgesics would interfere with evaluation of the withdrawal signs. As indicated above, the novel formulation may have diminished dependence; and, therefore, resulted in more mild withdrawal. Regardless, the experiment is terminated immediately after the observation period by re-administration of the maintenance drug or, if severe withdrawal is observed, prior to the end of the 30 min observation.

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)

The results from our testing may be used by the FDA.

(3) Column E Explanation

1. Registration Number: 52-R-0007

2. Number of animals used in this study: 1

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

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

   One rabbit was involved in a survival surgery followed by hemorrhage in non – anesthetized (conscious) condition. After 30 minutes recovery from surgery, the rabbit underwent moderate to severe hemorrhage (as evidenced by arterial lactate levels at 10 mM) via withdrawal of set volumes (~ 40% shed blood volume removal) with pressure control (30-35 mm Hg mean arterial pressure). At the hemorrhage experimental endpoint (sympathetic withdrawal as evidenced by sudden dramatic drop in blood pressure and heart rate), the rabbit was rapidly anesthetized with Alfaxan to harvest the contralateral epigastric artery. The surgical recovery is painful and hemorrhage resulted in mild momentary distress.

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 the test results. (For federally mandated testing, see Item 6 below).

   Both anesthetics and analgesics have been shown to attenuate sympathetic outflow, which is a cause of modest (as opposed to severe) vascular smooth muscle down-regulation. Hemorrhage without anesthesia or analgesia more closely mimic the clinical scenario and may cause more severe vascular smooth muscle down-regulation. After sufficient blood removal, animals are lethargic and less responsive to pain. Lidocaine (2% gel) was applied at the surgical sites to reduce pain intensity from moderate to mild, and the rate of blood removal is controlled (3 ml/kg/min progressively down to 1 ml/kg/min) to ensure that the animal did not suffer arterial hypoxia which would cause further distress.

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)

   NA

(4) Column E Explanation

1. Registration Number: 52-R-0007

2. Number of animals used in this study: 24

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

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

Twenty-four rabbits are used in the procedure of cardiac ischemia in conscious condition. One week after the initial surgery (implantation of the balloon), the rabbits consciously undergo a sequence of six 4-minute coronary occlusions interspersed with 4 minutes of reperfusion or administration of a pharmacological preconditioning drug prior to sustained ischemia for 30 min. The performance of successful coronary occlusions are verified by observing the development of ST-segment elevation, changes in the QRS complex on the ECG, and a drop in arterial blood pressure monitored from the ear dorsal artery. Before the induction of ischemia, the conscious rabbits receive Valium (4 mg/kg; IM) to minimize discomfort caused by the brief intermittent episodes of ischemia followed by sustained prolonged ischemia for 30 minutes. After ischemia, rabbits are allowed 72 hrs for recovery before their hearts are harvested for analysis of infarct size and protein expression.

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 the test results. (For federally mandated testing, see Item 6 below).

The investigator's justification indicates that the use of opiate analgesics, such as morphine, would interfere with results due to its preconditioning effects in the heart, which are well documented in rats (Schultz et al. Circ. Res. 78: 1100-1104, 1996) and rabbits (Miki et al. Mol. Cell. Biochem. 186: 3-12, 1998; Okubo et al. Am. J. Physiol. 287: H1786-H1791, 2004). Similar confounding effects may also be caused by non-steroidal anti-inflammatory agent (NSAID), since a number of studies showed the infarct size reducing effects of ibuprofen in dogs (Romson et al. Circulation 66: 1002-1011, 1982), cats (Flynn et al. Inflammation 8: 33-44, 1984) and rats (Gross et al. J. Pharmacol. Exp. Ther. 310: 185-191, 2004). Furthermore, the objective of this conscious model is to simulate an episode of heart attack where patients are not uniformly administered with analgesics or NSAIDs prior to a heart attack. For these reasons, the investigator only uses a high dose of Valium which causes muscle relaxation, and decreases anxiety and discomfort in the animals. Also, ketoprofen (3.0 mg/kg; s.c.) is used 2 hrs. prior to ischemia and every 12 hrs. up to 24 hrs. following myocardial infarction to alleviate pain in the conscious state.

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)

NA