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: 93-R-0280

2. Number _______ of animals used in this study.

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

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

   see attached.

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)

   see attached

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 __________________________

   NOV 24 2010
4. Explain the procedure producing pain and/or distress.

Immunization of animals does produce slight pain and/or distress in the guinea pigs. First, animals are weighed, numbered, and shaved at the injection site (nuchal area approximately 8 cm in length) and wiped with betadine or alcohol swab. Each animal is immunized by 5 intradermal injections of 120 ul in the shaved area with the immunogen emulsion. Each animal receives 75-150 mg GPBSC / 1-3 mg MT / 0.6 ml. The animals are expected to develop hind limb paralysis approximately 10-17 days post immunization. This hind limb paralysis will be clinically scored from 0-7 (0 being baseline and 7 being moribund). Each assessed score defines the clinical symptoms expected in the progression of EAE (Experimental autoimmune encephalomyelitis) in the study animals. During the paralysis, the guinea pigs do not appear to experience acute or surgical type pain. They are active and do not show behavior typical of guinea pigs in pain, but they likely experience symptomatic distress resulting from the disease. The disease can cause dehydration, atonic bladder, fecal impaction and weight loss. These symptoms are each addressed individually in the “post immunization care” section of the protocol. We hope that our treatments will prevent the paralysis and the distress, so that only 30% of the guinea pigs (the negative control group) in a study may experience the temporary paralysis.

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 would interfere with test results.

Unfortunately, the use of analgesics in inflammatory mediated models such as EAE is contraindicated. Many such drugs, including the popular non-steroidal anti-inflammatory (NSAIDS), and opiate receptor agonists possess immune system modifying properties and interfere with the model in an unpredictable fashion. Possible side effects resulting from the use of analgesics in EAE include altering the immune response through inflammatory mediator and cytokine regulation. The development of a coordinated autoimmune response is critical for the progression of pathological changes in EAE recapitulating human multiple sclerosis.

Buprenorphine hydrochloride, a narcotic analgesic, is a partial mu-opioid receptor agonist and kappa-opioid receptor antagonist. While potentially an attractive alternative for the management of moderate to severe pain this drug has also been documented to interact with inflammatory mediated models of disease in the rodent. Buprenorphine has been demonstrated to exacerbate inflammation in a model of adjuvant induced arthritis [9], which is mechanistically similar to EAE induction. Buprenorphine has also been shown to possess anti-inflammatory properties in another inflammatory model of arthritis [10]. These two seemingly contradictory effects of buprenorphine demonstrate the variability of using the drug in animal models similar to EAE.

The immuno-modulatory effects of opioid-receptor agonist/antagonist drugs are both direct and indirect. It is well documented that cells of the immune system (critical for the induction of EAE) express and are regulated by opiate receptors. These cell populations include macrophages [11] where opiate receptors regulate phagocytosis [12], T-cells where opiate receptors function in development and migration [13], and B-cells where opiate receptors alter antibody formation [14, 15] and the mitogenic response to bacterial lipopolysaccharide [16].

A large body of work also exists demonstrating the direct connection between the nervous system and immune system. Agents acting on neuropeptide and opiate receptors can modulate the immune system despite their commonly accepted specificity for the nervous system. Beta endorphin concentrations and the pharmacologic antagonism of opiate receptors has been shown to have a direct effect on the immune system in EAE [17]. An excellent review of opioid
modulation affecting phagocyte and lymphocyte function can be found in the following article [18].

While the metabolic pathways for agents such as NSAIDS and Buprenorphine are well described (i.e. buprenorphine is metabolized via Hepatic; P450 CYP3A4; N-dealkylation and glucuronidation), the experimental compounds under evaluation in the present EAE model lack a complete metabolic characterization. Metabolic interactions added to unknown drug-drug interactions between pain modifying drugs and novel Elan compounds shows that concurrent dosing is contraindicated. When critically evaluating potential clinical candidate compounds produced by Elan in models of MS, the cleanest biological system, free of confounding variables is essential.

Alleviation of pain and distress in animals is not achieved solely by the use of analgesics. Experimental procedures offer many opportunities for enhancing the animals' well-being by the refinement of procedures to reduce the severity of injury or stress and by the provision of supportive care. By giving the appropriate level of post induction care, we will minimize the level of pain and distress the animals experience without the use of additional pharmacological agents.