This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 93-R-0280 **CUSTOMER NO.** 1117

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

ELAN PHARMACEUTICALS, INC.
800 GATEWAY BLVD.
SO.SAN FRANCISCO, CA 94080

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

ELAN PHARMACEUTICALS, INC.
SO.SAN FRANCISCO, CA 94080

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs		331	231	555	1117
7. Hamsters					
8. Rabbits					
9. Non-Human Primates					
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(B)(6) (B)(7)(c)		10/15/2008

APHIS FORM 7023 **(AUG 91)** **(Replaces VS FORM 18-23 (Oct 88), which is obsolete**

PART 1 - HEADQUARTERS

1. Registration Number: 93-R-0280 / 1117

2/3. Species (common name) & Number of animals used in this study:

Guinea Pigs (555)

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 8cm in length) and wiped with betadine or alcohol swab. Each animal is

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)

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 anti-inflammatory 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 that recapitulate 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 (Hall, Jagher et al. 1996), which is mechanistically similar to EAE induction. Buprenorphine has also been shown to possess anti-inflammatory properties in another inflammatory model of arthritis (Volker, Bate et al. 2000). The immuno-modulatory effects of opioid-receptor antagonists 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 (Makman, Dvorkin et al. 1995) where opiate receptors regulate phagocytosis (Szabo, Rojavin et al. 1993), T-cells where opiate receptors function in development and migration (McCarthy, Szabo et al. 2001), and B-cells where opiate receptors alter antibody formation (Lefkowitz and Chiang 1975; Bhargava, Thomas et al. 1994) and the mitogenic response to bacterial lipopolysaccharide (Bryant, Bernton et al. 1988). 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 of 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 (Panerai, Radulovic et al. 1994). An excellent review of opoid modulation affecting phagocyte and lymphocyte function can be found in the following article (Eisenstein and Hilburger 1998). 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. Added to possible unknown drug-drug interactions between pain modifying drugs and novel Elan compounds, multiple drugs/compounds are 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. Bhargava, H. N., P. T. Thomas, et al. (1994). "Effects of morphine tolerance and abstinence on cellular immune function." Brain Res 642(1-2): 1-10. Bryant, H. U., E. W. Bernton, et al. (1988). "Morphine pellet-induced immunomodulation in mice: temporal relationships." J Pharmacol Exp Ther 245(3): 913-20. Eisenstein, T. K. and M. E. Hilburger (1998). "Opioid modulation of immune responses: effects on phagocyte and lymphoid cell populations." J Neuroimmunol 83(1-2): 36-44. Hall, T. J., B. Jagher, et al. (1996). "The analgesic drug buprenorphine inhibits osteoclastic bone resorption in vitro, but is proinflammatory in rat adjuvant arthritis." Inflamm Res 45(6): 299-302. Lefkowitz, S. S. and C. Y. Chiang (1975). "Effects of certain abused drug s on hemolysin forming cells." Life Sci 17(12): 1763-7. Makman, M. H., B. Dvorkin, et al. (1995). "Murine macrophage cell lines contain mu 3-opiate receptors." Eur J Pharmacol 273(3): R5-6. McCarthy, L., I. Szabo, et al. (2001). "Expression of functional mu-opioid receptors during T cell development." J Neuroimmunol 114(1-2): 173-80. Panerai, A. E., J. Radulovic, et al. (1994). "Beta-endorphin concentrations in brain areas and peritoneal macrophages in rats susceptible and resistant to experimental allergic encephalomyelitis: a possible relationship between tumor necrosis factor alpha and opioids in the disease." J Neuroimmunol 51(2): 169-76. Szabo, I., M. Rojavin, et al. (1993). "Suppression of peritoneal macrophage phagocytosis of Candida albicans by opioids." J Pharmacol Exp Ther 267(2): 703-6. Volker, D., M. Bate, et al. (2000). "Oral buprenorphine is anti-inflammatory and modulates the pathogenesis of streptococcal cell wall polymer-induced arthritis in the Lew/SSN rat." Lab Anim 34(4): 423-9.

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:

Approval Status:
Approved/Disapproved By:
Date:

APHIS Form 7023 Column E Explanation

This form is intended as an aid to completing the APHIS Form 7023 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.

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1. Registration Number: 93-R-0280

2/3. Species (common name) & Number of animals used in this study:

Guinea Pigs (555)

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 8cm in length) and wiped with betadine or alcohol swab. Each animal is immunized by 5 intradermal injections of 120ul in shaved area with the immunogen emulsion. Each animal receives 75-150mg GPBSC / 1-3mg MT / 0.6ml. The immunized 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 in the study animals. The guinea pigs may experience some pain after the immunization. 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 relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

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 anti-inflammatory 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 that recapitulate human multiple sclerosis.

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: