

UNITED STATES DEPARTMENT OF AGRICULTURE  
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. 85-R-0003	CUSTOMER NO. 1072	FORM APPROVED OMB NO. 0579-0036
2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include Zip Code)		
LOVELACE RESPIRATORY RESEARCH INSTITUTE 2425 RIDEGECREST SE ALBUQUERQUE, NM 87108 (505) 348-9400		

**ANNUAL REPORT OF RESEARCH FACILITY**  
(TYPE OR PRINT)

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)

See Attached Listing

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	85	6	152	0	158
5. Cats	0	0	0	0	0
6. Guinea Pigs	15	0	4	0	4
7. Hamsters	6	0	48	0	48
8. Rabbits	0	194	0	2	196
9. Non-Human Primates	59	1	152	142	295
10. Sheep	0	0	0	0	0
11. Pigs	0	0	0	0	0
12. Other Farm Animals	0	0	0	0	0
13. Other Animals					
Ferrets	30	0	11	17	28

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 CEO OR INSTITUTIONAL OFFICIAL	DATE SIGNED
(b)(6), (b)(7)c	11-27-07

## Category E explanations

This Institute had 13 studies with USDA covered species in Category E during the reporting period of 10/1/06 – 9/30/07.

**Study A: 11 nonhuman primates (*Cynomolgus macaques*)**

The purpose of this study is to evaluate the efficacy of an anti-viral therapeutic to prevent infection with an infectious agent (the pharmacokinetic portion where monkeys are not exposed to the agent is not classified as Category E). The painful part of the study is the infection from the pathogen. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a dose of the pathogen and follow the disease process up to death. The administration of pain or stress relieving agents is contraindicated because in a real world scenario of a terrorist attack or other mass release of the pathogen, the majority of humans will not have the benefit of anesthetics, analgesics, or tranquilizers. The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators used an established murine model of endotoxemia and showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases

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in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).”

**Study B: 2 rabbits (New Zealand White)**

The purpose of this study is to investigate the efficacy of a vaccine in the NZW rabbit model for infectious agents. The painful part of the study is the infection from the pathogen. The results of this study may be used to support future submissions to the FDA under the “Animal Rule” amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA’s new drug and biological products regulations. The “Animal Rule” applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the rabbits with a lethal dose of agent and follow the disease process up to death. The giving of pain or stress relieving agents is contraindicated because in a real world scenario of a terrorist attack or other mass release of the pathogen, the majority of humans will not have the benefit of anesthetics, analgesics, or tranquilizers. Furthermore, there is a need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some rabbits may not survive to the next observation period.

**Study C: 19 nonhuman primates (Cynomolgus macaques)**

The purpose of this study is to look at the efficacy of a vaccine in the Cynomolgus macaque model of an infectious agent. The painful part of the study is the infection from the pathogen. The results of this study will support future submissions to the FDA under the “Animal Efficacy Rule” amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA’s new drug and biological products regulations. The “Animal Efficacy Rule” applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death in the untreated animals in order to determine the efficacy of the vaccine. The giving of pain or stress relieving agents is contraindicated because it may interfere in determining the efficacy of the therapeutic. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of the infectious agent. The animals are observed and the telemetry allows for continuous monitoring. If they exhibit signs that they will not survive to the next observation period they are humanely euthanized.

The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease.

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Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process, or with the vaccine treatment whose efficacy is being assessed. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

**Study D:** 4 nonhuman primates (*Cynomolgus* macaques)

This is a pilot study. The data will be used to validate the telemetry system that will be used to monitor the progression of a pathogenic infection in the *Cynomolgus* model. The painful part of the study is the infection from the pathogen. The results of this study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death. The giving of pain or stress relieving agents is contraindicated because we need to study the natural progression of the disease process in the absence of therapeutic agents in order to relate the disease process to the telemetry measurements of a pathogen-infected monkey. The animals are observed and the telemetry allows for

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continuous monitoring. If they exhibit signs that they will not survive to the next observation period they are humanely euthanized.

The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in the telemetry system. It is likely the use of analgesics will interfere with the accurate measurements of the disease process, or with the vaccine treatment whose efficacy is being assessed. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

**Study E: 10 nonhuman primates (African Green)**

The purpose of this study is to develop a model for a pathogenic infection and test therapeutic interventions that could be used in humans. The painful portions of this study are the telemetry implant surgery and sequele of the disease itself (proper surgical anesthesia and analgesia is used to minimize pain from the telemetry surgery). We are trying to develop a model using the telemetry data that will allow us to better predict the progression of the disease and to develop earlier, more humane euthanasia endpoints for future studies. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. We need to study the natural progression of the disease process in the absence of therapeutic agents in order to relate the disease process to the telemetry measurements of a pathogen-

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infected monkey. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized.

**Study F: 8 nonhuman primates (Cynomolgus macaques)**

The purpose of this study is to look at the efficacy of an antiserum in the cynomolgus monkey model for an infectious agent. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death. The giving of pain or stress relieving agents is contraindicated because in a real world scenario of a terrorist attack or other mass release of the pathogen the majority of humans will not have the benefit of anesthetics, analgesics, or tranquilizers. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of the infection.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the pathogen must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating

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levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

**Study G: 16 nonhuman primates (Cynomolgus macaques)**

The purpose of this study is to look at the efficacy of a vaccine in a cynomolgus monkey model of pathogenic infection. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death. The giving of pain or stress relieving agents is contraindicated because in a real world scenario of a terrorist attack or other mass release of the pathogen the majority of humans will not have the benefit of anesthetics, analgesics, or tranquilizers. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et. al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify

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the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

**Study H: 30 nonhuman primates (Cynomolgus macaques)**

The purpose of this study is to use data from the telemetry system to monitor the progress of pathogenic infection in the cynomolgus monkey model. Data from this study will be used to plan future studies that will support submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to the endpoints in some of the animals that indicates morbidity.

The giving of pain or stress relieving agents is contraindicated because we need to study the natural progression of the disease process in the absence of therapeutic agents in order to relate the disease process to the telemetry measurements of a pathogen-infected monkey. These measurements are taken continuously and can be monitored from outside the ABSL3 facility and will allow us to be able to better predict that death is eminent. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and

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airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, J Immunol 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, J. Immunol 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

**Study I: 16 nonhuman primates (Macaca mulatta)**

The purpose of this study is to determine the LD<sub>50</sub> of an infectious agent toxin following IV administration. The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., J. Toxicol Environ Health 2:577-582, 1977; Hung, C. Y. et. al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124:249-252, 2001; Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) and respiratory depression (Soma, L.R., Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, J Immunol 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, J. Immunol 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases

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in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

**Study J: 14 nonhuman primates (*Cynomolgus macaques*)**

This is a preliminary study to determine which factors are important in the development of infectious disease. The disease process in primates instilled with a mutant agent lacking a particular factor (lethal factor, edema factor or protective antigen) will be compared to pathogenesis of the disease following bronchial instillation of the agent. If a mutant does not cause disease, then it will be eliminated from future studies to determine the ED<sub>50</sub>, but may still be considered for further pathology studies. This is a pilot study, so we need to follow the disease process without interference in order to understand the pathogenesis of the disease induced by the various strains of the agent.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

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The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

**Study K: 17 ferrets**

This is a preliminary study to determine the natural history and pathogenesis of an infectious agent the ferret. The disease process in ferrets instilled with various doses of agent will be followed to determine the LD50. This is a pilot study, so we need to follow the disease process without interference in order to understand the pathogenesis of the disease induced in ferrets.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et. al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some ferrets may not survive to the next observation period.

**Study L: 12 nonhuman primates (Cynomolgus macaques)**

The purpose of this study is to look at the efficacy of a vaccine in the cynomolgus monkey model for an infectious agent. This study will support future submissions to the

## Category E explanations

FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death. The giving of pain or stress relieving agents is contraindicated because in a real world scenario of a terrorist attack or other mass release of the pathogen the majority of humans will not have the benefit of anesthetics, analgesics, or tranquilizers. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection.

The painful part of the study is the infection from the pathogen. The illness experienced by animals exposed to the agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanism(s) responsible for interferon production (Geher, W.F. et al., *J. Toxicol Environ Health* 2:577-582, 1977; Hung, C. Y. et. al. *Proc Soc Exp Biol Med* 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al., *Brain Behav Immun* 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al *Int Arch Allergy Immunol* 124:249-252, 2001; Stellato, C., and Marone, G., *Chem Immunol* 62: 108-131, 1995) and respiratory depression (Soma, L.R., *Ann NY Acad Sci* 406: 32-47, 1995). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A., et al, *J Immunol* 170: 2269-2273, 1999), inhibit interferon alpha release from dendritic cells (Marone, G., et al., *Int Arch Allergy Immunol*, 124, 249-252, 2001) and increase the synthesis and release of IL-10 from human macrophages (Sirois, J., et al, *J. Immunol* 164:2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (*Lab Anim* 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

The animals are observed and when they exhibit signs that they will not survive to the next observation period they are humanely euthanized however, some monkeys may not survive to the next observation period.

## Category E explanations

**Study M: 2 nonhuman primates (Macaca mulatta)**

This is a pilot study to confirm the high dose selected for Study FYXX-XXX is lethal as reported in 1967 and to provide training for the staff who will participate in FYXX-XXX in the early recognition and scoring for severity of the symptoms of agent intoxication in monkeys.

The illness from the pathogenic agent is the procedure which will cause the discomfort and distress in the animals. The illness experienced by animals exposed to the agent must not be treated. Treatment of humans with this disease consists of supportive care (fluid and nutritional support, assisted ventilation, treatment of complications, perhaps antibiotics if secondary infections are present) and passive immunization with equine antitoxin (Arnon, et al. *JAMA*. **85** 1059-1070, 2001). This type of treatment is not appropriate for these study animals as the goal of the project is to develop skill in scoring the symptoms and to follow the progression of the disease so that euthanasia scoring which can lead to refined euthanasia criteria can be performed. The information learned from this study and the technical skills developed in scoring the disease symptoms will be used in the follow-on LD50 study and in studies testing treatments with antitoxin. Treatment would interfere with the goals of this study.