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 21

NOV 2 8 2008

See attached form for additional information.

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 14-R-0082
CUSTOMER NUMBER: 140

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

Tufts- New England Medical Center, Inc.
171 Harrison Avenue, Nemc #112
Boston, MA 02111

Telephone: (617) -636-5615

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

FACILITY LOCATIONS (Sites) - See Atached 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 animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o 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 an 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 wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	0	0	0	0
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	0	12	0	12
7. Hamsters	0	0	30	0	30
8. Rabbits	0	0	7	56	63
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	0	0	0	0
11. Pigs	0	0	68	0	68
12. Other Farm Animals	0	0	0	0	0
13. Other Animals	0	0	0	0	0

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese 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 ap Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary inc 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)

DATE SIGNED
11/26/08

(b)(6), (b)(7)c

NP

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: **14-R-0082**

2. Number **56** of animals used in this study.

3. Species (common name) **rabbits** of animals used in this study.

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

Infant rabbits are fed Shiga toxin, ricin, or control intragastrically which will cause inflammation of the intestines and diarrhea. Experimental groups will be given doses of MAPKinase inhibitors or inhibitor vehicle. All animals are housed with their mother and will be sacrificed between 48-72 hours after inoculation. Animals will be monitored for diarrhea three times per day until sacrifice. At these times, animals will be assessed for: diarrhea, shallow breathing, scruffy fur, poor color, lethargy, decreased muscle tone, and failure to nurse. Diarrhea will be graded according to I: no diarrhea, II: mild to moderate diarrhea (feces stuck to perineum and/or legs), or III: severe diarrhea (feces stuck to hind legs, wet tail, and prolapse of rectum). Animals will be euthanized immediately they appear ill or develop severe, bloody diarrhea.

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).

The infant rabbit is the only species that responds to Shiga toxins in the same way as humans do. Feeding Shiga toxins 2 (Stx2) to rabbits that are 2-3 days old results in the kind of damage that is observed in humans exposed to Shiga toxins. We aim to determine if the gene activating effects of Shiga toxin that we observe in human intestinal studies epithelial cells *in vitro* are occurring in the infant rabbit model. Because we are studying how these toxins affect the whole intestine, we must utilize a live animal model and the effects of the inoculation in the *in vivo* system. We will be assessing how certain infection-fighting cells called "neutrophils" are called from the blood circulating through the intestinal blood vessels in response to these toxins and how they migrate into the deeper layers of the organ. We will analyze these effects in two ways, by clinical observations of the rabbits and the amount of diarrhea caused and by histopathological evaluations of neutrophil infiltration, edema/swelling of the tissue, and any amount of hemorrhage. Therefore, we need the animals to be infected without any therapeutic intervention to be able to assess our data accurately.

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