

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 14-R-0101
CUSTOMER NUMBER: 147

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Toxikon Corporation
15 Wiggins Ave
Bedford, MA 01730

Telephone: (781)-275-3330

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 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 NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	48	100	2	150
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	13967	0	21	13988
7. Hamsters	0	99	0	0	99
8. Rabbits	0	846	1208	5	2059
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	0	0	0	0
11. Pigs	0	31	36	1	1
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 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 such exceptions is attached to this annual report, in addition to identifying the IACUC-approved exceptions, this summary in 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)

NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)
DATE SIGNED
1/30/03

C. K. ...

Optional Column E Explanation Form

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 laypersons as well as scientists.

1. **Registration Number:** 14-R-101
2. **Number of animals used in the study (s).** 2 Dogs, 21 Guinea Pigs, 5 Rabbits, 1 Pig
3. **Specie (common name) of animals used in this study (s).**

Dogs, Guinea Pigs, Rabbits, Pigs

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

Exposure to a test article (drug /chemical compounds).

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 question 6 below)

Studies conducted at Toxikon are performed for sponsors to obtain toxicity information on experimental materials, drugs or chemicals, or to ensure the safety of a new lot of material. Regulatory guidelines do not permit the use of analgesic or anesthetics during toxicity determination studies. However, Toxikon does employ a step approach, exposing one or two animals at a time, thus minimizing the total number of animals needed. Toxikon's IACUC approves and monitors all animal use protocols.

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):**

Based upon the following standards:

Dog:

Organization for Economic Co-operation and Development (OECD), Guidelines for Testing of Chemicals, "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-day Study," Section 407 (adopted 27 July 1995).

ICH Harmonized Tripartite Guideline. Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies, S3A, 1994. FDA: Published in the Federal Register, Vol. 60, No. 40, March 1, 1995, pages 11264-11268.

ICH Harmonized Tripartite Guideline. Dose Selection for Carcinogenicity Studies of Pharmaceuticals, 1997 (revised). FDA: First published in the Federal Register, Vol. 60, No.40, March 1, 1995, pages 11278- 11281, Revision published in the FR, Vol. 62, No. 233, December 4, 1997, page 64260.

Guinea Pig:

Organization for Economic Co-operation and Development (OECD), Guidelines for Testing of Chemicals, " Skin Sensitization" section 406, July 1992.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency, Health Effects Test Guidelines. OPPTS 870.2600, Skin Sensitization, March, 2003

United States Pharmacopoeia 26, National Formulary 21, 2003, <88> Biological Reactivity Tests, *In Vivo*.

21 CFR, Part 610.11, 2002.

Rabbit:

Organization for Economic Co-operation and Development (OECD), Guideline for Testing of Chemicals, Section 402, 1987.

Procedure and facilities complied with the requirements of Commission Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. National legislation, harmonising with this Directive, is defined in Decreto Legislativo No. 116 of 27th January 1992.

Swine:

Toxicokinetics: Guidance on the Assessment of Systemic Exposure in Toxicity Studies, International Conference on Harmonization/FDA/CDER/CBER.

Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents for the National Toxicology Program, 8/1992; Revised 10/1996.

CRC Handbook of Toxicology (eds: Derelanko MJ, Hollinger MA) CRC Press, 1995.

Ninth Addendum to the Organization for Economic Cooperation and Development Guidelines for the Testing of Chemicals, Neurotoxicity Study in Rodents, Section 424, adopted July 21, 1997.

Handbook of Pre-clinical Continuous Intravenous Infusion (eds. Healing, G., & Smith, D.) Taylor and Francis, 2000.

	Date 11/30/03
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DEC 02 2003

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

See according to the regulations can addi side for information.

See side for information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO.

14-V-0001

657 NO
652

FORM APPROVED
OMB NO. 0579-0036

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

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

Dept. of Veterans Affairs Central Office
810 Vermont Avenue NW
Washington DC 20420

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)

518 VA Medical Center
200 Springs Rd.
Bedford, MA 01730

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	0				
5. Cats	0				
6. Guinea Pigs	0	0	14	0	14
7. Hamsters	0				
8. Rabbits	0				
9. Non-human Primates	0				
10. Sheep	0				
11. Pigs	0				
12. Other Farm Animals	0				
13. Other Animals	0				
total	1112	6356	68	0	6424

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 AIA, 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 such 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.

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL

DATE SIGNED

11/3/03

APHIS FORM 7023
(AUG 91)

(Replaces VS FORM 18-23 (OCT 88), which is obsolete)



Jean Mayer
United States Department of Agriculture
Human Nutrition Research Center on Aging
At Tufts University

RO-17

Nutritional Immunology Laboratory

October 15, 2001

To:
Animal Care and Use Committee, HNRCA
From: *SNW*
Re: Category E animals in Amendment to Protocol MS-31

The major limiting factor in conducting our study is the large number of animals needed to collect sufficient number of macrophages for our experiments. This inherent difficulty can be overcome by intraperitoneal injection of thioglycollate (TG) which elicits recruitment of macrophages to peritoneal cavity. TG is a widely used stimulatory agent which induces non-infectious acute peritoneal inflammation in mice and rats. Administration of TG has been shown to increase the total number of macrophages up to four-fold, which will reduce the number of animals necessary for addressing our specific aims.

A number of recent studies have successfully demonstrated that TG-elicited macrophages can be used in the study of some gene expression and signal transduction. However, the feasibility of using TG-elicited macrophages to study COX-2 gene expression is not known.

To test this, we need to inject TG intraperitoneally to mice three days before they are euthanized by CO₂ asphyxiation for macrophage collection. Peritoneal injection will cause discomfort and moderate pain in mice, which unfortunately can not be alleviated. Thus we have classified the animals under category E.

711 Washington Street
Boston, Massachusetts 02111
FAX: (617) 556-3344

To: Animal Care and Use Committee

From: PI of WA-1 Protocol

RE: Justification of Category E in WA-1 Protocol: Effects of Combined Chemopreventive Agents (9-cis retinoic acid, celecoxib, and 1,25(OH)₂ vitamin D₃) Against NNK-induced Lung Carcinogenesis in AJ Mice

Protocol WA-1 will include USDA Category E research in which some experimental animal groups will experience pain and/or distress without alleviation. This letter will verify a lack of alternative methods and assure the committee that the proposed research does not unnecessarily duplicate previous experiments.

We propose to conduct an *in vivo* intervention study to investigate the effectiveness of 9-cis retinoic acid, 1,25(OH)₂ vitamin D₃, and a COX-2 inhibitor drug alone and in combination as anti-carcinogenic agents in the AJ mouse model of lung cancer. Lung tumors in strain AJ mice resemble human lung adenocarcinoma and have become the preferred test system to study this form of cancer. The target of chemoprevention is premalignant lung disease, making animal models essential for evaluating the efficacy of compounds and interactions in the suppression of tumor progression. Because symptoms rarely occur in the early stages of human lung cancer and many of these early cancers go undiagnosed, mice genetically predisposed to this form of cancer allow us to study lung cancer chemoprevention over the course of months and with fewer animals than similar studies with human subjects. The induction of lung tumors in AJ mice progresses through several distinct stages similar to the stages of human lung cancer. In both mice and humans, adenocarcinomas progress to adenomas and ultimately carcinomas. Further, tumor initiation by a tobacco-derived carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in AJ mice is characterized by premalignant lesions containing a gene alteration that is also present in some human cancers. This makes the AJ mouse an ideal model in which to study lung cancer chemopreventative agents that may be of benefit to the human population. Although we cannot alleviate tumor formation in the NNK-injected control group, the treatment group using combined chemopreventive agents should alleviate tumor formation/distress/animal pain.

While mechanistic hypotheses and data from cellular studies suggest that combinations of vitamins and anti-inflammatory drugs may be effective in lung cancer chemoprevention, there is a clear lack of *in vivo* work in this area. This will be the first study to examine vitamin A and vitamin D interactions in an animal model of lung cancer and the first study to combine these vitamins with a COX-2 inhibitor to examine synergistic effects. If successful, this study could lead to new approaches in cancer chemoprevention, utilizing combinations of chemopreventive vitamins and drugs in smaller and less toxic doses, thereby avoiding the side effects commonly seen in early clinical trials testing single agents. This research cannot be done using cell models as results cannot be applied to *in vivo* tumorigenesis.

TO: The HNRC Animal Care and Use Committee
FROM: NEPS Laboratory

RE: Justification of Category E in protocol RO-17, "Roles of TNF and interleukin-1 in stress-induced cachexia: Effects of age in transgenic mice"

Our protocol RO-17 addresses the question of whether the cytokines involved in cachexia are the same as sarcopenia (namely TNF, IL-1, and IL-6). This line of research pertains to the mission of the NEPS laboratory, ie, the understanding and alleviation of physiological or pathological processes leading to sarcopenia, wasting and cachexia.

In RO-17, turpentine will be delivered subcutaneously in one of the hind limbs of wild type and IGF-I transgenic mice. Unfortunately, turpentine injection, although not lethal, results in a sterile abscess that cause pain. This pain is comparable to that felt by humans with a thigh abscess. We anticipate the abscess to be maximal 16 days after injection, and to gradually shrink thereafter. Unfortunately, the pain will not be alleviated by pain killers, as these drugs may induce changes in the levels of muscle cytokines, one of the major endpoints of this study. Because sub-clinical inflammation is a recognized feature of human aging, the proposed experiments are germane to the issue of age-related changes in protein catabolism, inflammation, and immune responses.



November 10, 2003

Jean Mayer
United States Department of Agriculture
Human Nutrition Research Center on Aging
At Tufts University

NOV 26 2003

Elizabeth Goldentyer, D.V.M.
Regional Director - Animal Care
APHIS, Eastern Regional Office
920 Main Campus Drive, Suite 200
Raleigh, NC 27606-5213
Reference: USDA Annual Report (Registration No.: 14-F-0009)

Dear Dr. Goldentyer:

The enclosed documents represent the U.S.D.A. Human Nutrition Research Center on Aging at Tufts University's (HNRCA) "Annual Report of Research Facilities" for the Federal fiscal year, October 1, 2002 through September 30, 2003. Aspects of this report that require comment are:

1) Animals reported under Category E:

a) Mild non-infectious peritoneal inflammation was induced in sixty-one (61) mice by the intraperitoneal injection of thioglycollate to increase the total number of peritoneal macrophages available (which reduced the number of animals used) for peritoneal macrophage harvest. The letter of justification for category E research was submitted with the IACUC animal protocol and is attached.

b) Lung tumors were induced in one hundred one (101) mice to examine the combined synergistic effects of vitamin A, vitamin D and COX-2 inhibitors to evaluate their role in lung cancer chemoprevention. The letter of justification for category E research was submitted with the IACUC animal protocol and is attached.

c) Sarcopenia was induced in one hundred fifteen (115) mice by the subcutaneous injection of sterile turpentine into the hind limbs of the mice to evaluate if the cytokines involved in cachexia are the same as those of sarcopenia (namely TNF, IL-1 and IL-6) in an effort to understand and potentially alleviate the physiological or pathological processes leading to sarcopenia, wasting and cachexia. The letter of justification for category E research was submitted with the IACUC animal protocol and is attached.

Should you have any questions regarding the report, please do not hesitate to contact me.

711 Washington Street
Boston, Massachusetts 02111
FAX: (617) 556-3344