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-10!

2. Number of animals used in the study (s). 2 Dogs, 21 Guinea Pigs, 5 Rabbits. 1 Pig

3. Species (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 relieved would interfere with test results. (For Federally mandated testing, see question 6 below)

Studies conducted at Toxicol 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, Toxicol does employ a step approach, exposing one or two animals at a time, thus minimizing the total number of animals needed. Toxicol'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 133.102).

Based upon the following standards:

Dog:


Guinea Pig:


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


Rabbit:


Swine:

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


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

**FACILITY IDENTIFICATION (CONT'D)**

- **FACILITY NAME:** 510 VA Medical Center
  - **Address:** 200 Springs Rd.
  - **City, State, ZIP:** Nashua, NH 03060

**REPORT OF ANIMALS (USDA) UNDER CONTROL OF RESEARCH FACILITY (MARCH 1, 2003 TO FEBRUARY 28, 2004)**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Animals Controlled</th>
<th>No. of Animals Held</th>
<th>No. of Animals Derived</th>
<th>No. of Animals Remaining</th>
<th>No. of Animals Transferred Out</th>
<th>No. of Animals Transferred In</th>
<th>No. of Animals Slaughtered</th>
<th>No. of Animals Sacrificed</th>
<th>No. of Animals Given Away</th>
<th>No. of Animals Lost</th>
<th>No. of Animals Died</th>
<th>No. of Animals Other Reasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Farm Animals</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Animals</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TOTAL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ANIMAL HEALTH STATEMENTS**

1. **Physiologically acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetics, sedatives, and tranquilizers, will be followed by the research facility.**

2. **The person responsible for animal health and welfare for the research facility, Dr. J. A. C. M. V. J. A. C. M. V., has attended the appropriate veterinary course on the humane care and use of animals in research.**

3. **The facility is in compliance with the regulations of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service.**

**CERTIFICATION BY HEADQUARTER RESEARCH FACILITY OFFICIAL (Chief Executive Officer) OR Legally Responsible Institutional Official**

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL: [Signature]

DATE: [Date]

APRIL FORM 7023 (Replaces 16-73) (Oct 88), which is for

PART 3 - SECTOR OFFICE
**United States Department of Agriculture**

**Animal and Plant Health Inspection Service**

**ANNUAL REPORT OF RESEARCH FACILITY**

**TYPE OR PRINT**

| NOV 26 2003 |

1. **CERTIFICATE NUMBER:** 14-F-0009
2. **CUSTOMER NUMBER:** 463
4. **711 Washington Street**
5. **Boston, MA 02111**
6. **Telephone:** (617) -555-3200

**FACILITY LOCATIONS**

- **REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY**
  
  **A. Number of Animals Covered By the Animal Welfare Regulations**
  
  **B. Number of Animals Being Housed or Used in Actual Research, Testing, or Experimentation.**
  
  **C. Number of Animals Being Experimentally Treated, Conditioned, or Held for these Purposes.**
  
  **D. Number of Animals Upon which Teaching, Research, Experiments, or Tests were Conducted Involving No Pain or Distress, or Use of Pain-Relieving Drugs.**
  
  **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 Appropriate Anesthetic, Analgesthetic, or Tranquilizing Drugs Were Used.**
  
  **F. TOTAL NUMBER OF ANIMALS**

<table>
<thead>
<tr>
<th><strong>Animals</strong></th>
<th><strong>Number of Animals</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>37</td>
</tr>
<tr>
<td>Cats</td>
<td>2</td>
</tr>
<tr>
<td>Guinea Pigs</td>
<td>37</td>
</tr>
<tr>
<td>Hamsters</td>
<td>492</td>
</tr>
<tr>
<td>Rabbits</td>
<td>529</td>
</tr>
<tr>
<td>Non-Human Primates</td>
<td>2</td>
</tr>
<tr>
<td>Sheep</td>
<td>2</td>
</tr>
<tr>
<td>Pigs</td>
<td>37</td>
</tr>
<tr>
<td>Other Farm Animals</td>
<td>429</td>
</tr>
<tr>
<td>Mice</td>
<td>3,363</td>
</tr>
<tr>
<td>Rats</td>
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<tr>
<td>Ferrets</td>
<td>4,957</td>
</tr>
<tr>
<td>Other Animals</td>
<td>4,917</td>
</tr>
</tbody>
</table>

**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, 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 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 is 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**

<table>
<thead>
<tr>
<th>SIGNED</th>
<th>NAME &amp; TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)</th>
<th>DATE SIGNED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11-10-03</td>
</tr>
</tbody>
</table>

**APRIS FORM 7023A**

(Replaces V5 FORM 18-23 (OCT 81), which is obsolete.)

(AUG 91)
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.
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-(methylatrosino)-1-(3-pyridyl)-1-butane (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.
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, i.e., 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-1 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

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.