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**Animal Activity Protocol Summary**  
University of Mississippi Medical Center

Protocol Number: 0801A	Date: 12/30/02	Classification: 2-D
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Project Title: Effects of Diet on the Initiation of Gingivitis

- Species:
- |  |  |
|--|--|
| <input type="checkbox"/> Rats            | <input type="checkbox"/> Mice              |
| <input type="checkbox"/> Hamsters        | <input type="checkbox"/> Rabbits           |
| <input checked="" type="checkbox"/> Dogs | <input type="checkbox"/> Cats              |
| <input type="checkbox"/> Swine           | <input type="checkbox"/> Nonhuman Primates |
| <input type="checkbox"/> Birds           | <input type="checkbox"/> Fish              |
| <input type="checkbox"/> Frogs           | <input type="checkbox"/> Other: _____      |

- Appendices:
- Appendix A: Environmental Enhancement/Enrichment
  - Appendix B: Breeding Programs
  - Appendix C: Surgery & Management of Surgical Pain and Distress
  - Appendix D: Collection of Biological Samples
  - Appendix E: Antibody Production
  - Appendix F: Administration of Drugs/Test Compounds
  - Appendix G: Prolonged Physical Restraint
  - Appendix H: Multiple Major Surgical Procedures
  - Appendix I: Food and/or Fluid Restriction
  - Appendix J: Animal Pain and/or Distress
  - Appendix K: Progress Report

Original IACUC Approval Date: <u>12/30/02</u>	Amendments: _____ _____ _____
Renewals: <u>12/16/03</u>	_____
3-yr Resubmit: _____	_____



8. A Anticipated start date of study: Study is ongoing  
 B Study duration: (maximum - 3 years)  #1 year  1-2 years  2-3 years

**All investigators MUST adhere to a federally mandated three-year cycle of full protocol review, even if a funding period exceeds three years in duration.**

***Animal Husbandry & Care***

9. Animal Requirements:

Species A: Canine

Strain/stock: Beagle

Sex: Female

Source: UMMC/IAMS Beagle colony & COVANCE

Total number of animals to be used: 21

Species B:

Strain/stock:

Sex:

Source:

Total number of animals to be used:

**Animal numbers MUST be calculated for a period not to exceed three (3) years from the start of the study. See 8-B.**

- 10 Will animals be involved in a breeding program at UMC?

No  
 Yes

Note: If yes, provide a specific description of the **type of breeding program** to be utilized (e.g. harem/monogamous, who is to be responsible for mating, who is to be responsible for weaning, how will genetic quality be ensured?).

- |     |  |                                     |                          |                                  |
|-----|--|-------------------------------------|--------------------------|----------------------------------|
| 11. | Potential hazards to personnel or other animals: | <u>NO</u>                           | <u>YES</u>               |                                  |
|     | A. Chemical toxins in bedding/cages/carcasses    | <input checked="" type="checkbox"/> | <input type="checkbox"/> |                                  |
|     | Reviewed by Risk Mgmt?                           | <input type="checkbox"/>            | <input type="checkbox"/> | <input type="checkbox"/> PENDING |
|     | B. Radioisotopes in bedding/cages/carcasses      | <input checked="" type="checkbox"/> | <input type="checkbox"/> |                                  |
|     | Reviewed by Radiation Safety?                    | <input type="checkbox"/>            | <input type="checkbox"/> | <input type="checkbox"/> PENDING |
|     | C. Infectious agents or recombinant DNA usage    | <input checked="" type="checkbox"/> | <input type="checkbox"/> |                                  |
|     | Reviewed by Biohazards Committee?                | <input type="checkbox"/>            | <input type="checkbox"/> | <input type="checkbox"/> PENDING |

Note: If yes, provide specific details of **specialized animal husbandry, care, cleaning, or decontamination procedures**, especially identifying responsible parties.

12.	Animal Husbandry:	<u>Standard</u>	<u>Nonstandard</u>
	Feeding	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Watering	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	Caging/housing	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Note: Provide complete explanation and justification for any **nonstandard animal husbandry**. Protocols listing non-standard cleaning/sanitation (e.g. metabolic caging, restraint chairs, transport devices) must provide complete details of the cleaning and sanitation, as well as validation methods.

Animals to be fed experimental diets supplied by IAMS

13. Will animals be housed outside of the LAF for greater than 12 hours?  
 No  
 Yes      Where?

Note: If yes, provide complete explanation and justification for any **decentralized animal housing**

**Experimental Procedures & Animal Manipulations: Items 14&15 MUST be written specifically for review by persons lacking scientific training (i.e. in non-technical/lay terminology). Assistance in preparation of this federally mandated requirement may be obtained by consultation with the UMC Office of Research, ext. 5-5000.**

14. In non-technical/lay terminology, what is the objective of the proposed Animal Activity Protocol?

During the past three years we have conducted several trials of dog food formulations, which were designed to inhibit the initiation and progression of gingivitis. These trials have resulted in the design and marketing of the "Dental Defense Diet" within the dry Eukanuba® dog foods. The effectiveness of the components of the "Dental Defense Diet" in soft dog foods is unknown.

The objective of these proposed trials is to refine the composition of the "Dental Defense Diet" and to determine whether its active ingredients would be effective in the other food matrices (both hard and soft diets) produced by IAMS Company

15. A. What is the rationale for using animals rather than using non-animal models?

There are no non-animal models available to test the effectiveness of food formulas on the whole animal.

- B. What is the rationale for using the particular animal species noted in #9?

The diets are marketed to owners of dogs. Thus, these animals must be used to test the

efficacy of the diets.

16. Will surgical procedures be a component of this Animal Activity Protocol?  
 NO       YES

Note: If answered YES, complete Appendix A.

17. Does this proposal include any of the following procedures?
- | <u>NO</u>                           | <u>YES</u>               |  |
|-------------------------------------|--------------------------|--|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Prolonged Physical Restraint   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Multiple Major Surgical Procedures   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Food and/or Fluid Restriction  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Animal Pain and/or Distress ( <i>other than momentary for injections, etc.</i> ) |

Note: If answered YES, complete Appendix B.

18. Does this proposal utilize non-human primates?       NO       YES

Note: If answered YES, complete Appendix C.

19. Provide a complete description of the animal procedures and experimental design **(exclusive of specific surgical procedures)**. (Detail drugs, dosages, post-procedure management, biologic sampling, nursing care of implants [catheters, acrylic, flow probes], etc. (Note, complete descriptions of surgical procedures must be provided in Appendix A.)

We will test two dietary formulas using two animal groups and utilizing a blinded, split-mouth design

**Baseline evaluation (Week 1-Week 4).** A breath sample will be made for analysis of volatile sulfur compounds using sulfide detector. For this procedure, a plastic tube will be placed near the maxillary 2<sup>nd</sup> molar tooth, kept in place for 30 seconds, and a sample will be delivered to the instrument and recorded. Animals will be weighed and administered acepromazine (0.1 mg/kg, IM) prior to the general anesthesia procedures. A blood sample (8 ml from the cephalic vein) will then be obtained. Animals will be administered thiopental (15 mg/kg to effect, IV) via the cephalic vein, intubated, and maintained on isoflurane (1-2%) for the remaining procedures. A baseline periodontal examination will then be performed. This examination includes obtaining ultraviolet digital images, photographs and periapical radiographs of the dentition; determination of a gingival bleeding index, plaque index, and calculus index on the facial surfaces of the teeth; and assessment of gingival sulcular depths at the mesial and distal line angles on the facial surfaces of the teeth. Teeth will be cleaned of plaque and calculus by scraping and the interdental papilla will be excised between the mandibular 1<sup>st</sup> and 2<sup>nd</sup> premolar teeth. Marcaine will be injected into the mental foramen and into the buccal fold to anesthetize the mental and buccal nerves for prevention of post-operative pain. Animals will be revived and returned to their quarters. "Group A" animals will be placed on a diet

containing a test ingredient (Diet #1) and "Group B" animals will be placed on a diet without the test ingredient (Diet #2) for the remainder of the experiment. The diets will be furnished by IAMS. The diets will meet the nutritional needs of the animals and the principal investigator will be blinded regarding the specific dietary formulation of each diet. Gingival biopsies will be solubilized and assayed for inflammatory biomarkers in the laboratory.

**Initiation of gingivitis.** On week 12, animals will weighed and pre-medicated with acepromazine and a breath sample obtained as previously described. A blood sample will be obtained and the animals will be anesthetized as previously described. Periodontal evaluations, radiography, photography and ultraviolet digital images will be performed as previously described. 4-0 silk suture material will be tied around the right mandibular 3<sup>rd</sup> and 4<sup>th</sup> premolar teeth and adjusted to contact the gingiva. This side will be "experimental". The mandibular left side will be sham operated and will serve as an untreated "control". The animals will be revived and returned to quarters.

14 days later (Week 14), animals will be weighed and pre-medicated with acepromazine, and a breath sample obtained as previously described. A blood sample will be obtained and animals will be anesthetized as previously described. Periodontal evaluations, radiography, photography and ultraviolet digital images will be performed as previously described (These parameters will be compared to baseline data and to the control sites). The suture materials will then be removed from the teeth. The interdental papilla between the 2<sup>nd</sup> and 3<sup>rd</sup> premolar teeth on the "experimental" and "control" sides will be excised. Plaque and calculus will be scraped from all teeth except the right 4<sup>th</sup> premolar and 1<sup>st</sup> molar teeth. Marcaine will be injected into the mental foramen and into the buccal fold to anesthetize the mental and buccal nerves to prevent postoperative pain. Animals will be revived and returned to their quarters and will be maintained on their assigned diets.

**Resolution of gingivitis.** At week 16, animals will be weighed, pre-medicated with acepromazine and a breath sample obtained as previously described. A blood sample will be obtained and then animals will be anesthetized as previously described. Assessments of gingival sulcular depth; plaque, calculus and bleeding indices; and photographs, ultraviolet digital images and periapical radiographs will be made (These parameters will be compared to previous data from "experimental" and "control" sites). The interdental papilla between the 4<sup>th</sup> premolar and 1<sup>st</sup> molar teeth will be excised on both "experimental" and "control" sides. Remaining plaque and calculus will be scraped from the right 4<sup>th</sup> premolar and 1<sup>st</sup> molar teeth. Marcaine will be injected into the mental foramen and into the buccal fold to anesthetize the mental and buccal nerves to prevent postoperative pain. Animals will be revived and returned to their quarters.

**Crossover experiments.** The experiment will be repeated using a cross-over model. The animals will be fed the opposite diet ("Diet" A will be switched to "Diet B" and *vice versa*). Data will be compared to that from experiment #1. At the end of the experiments, the animals will be sold and transferred to another research facility.

- 20 Explain and justify how the number of animals requested was determined  
(Flow diagrams/tables to define animal use are encouraged)

We have created two groups of animals based on weight and history of plaque accumulation. We have detected significant differences in clinical gingivitis parameters between the groups and will continue to use those groups in these studies.

21. Indicate room(s) where animal procedures (other than surgery) will be conducted

R822A

Studies involving animal transportation to locations other than the housing area must identify the animal transport device, the nature of shrouds used to cover the transport device, and describe the route of transport.

NA

22. A. At what point in the proposed protocol will animals normally be euthanized, (experimental end-points)? Or at what point will any individual animal be euthanized?

No animals will be euthanized.

- B. What criteria will be used to determine if an animal is to be euthanized prior to, rather than at, the anticipated end-point of an experiment?

Unforeseen health problems may warrant euthanasia during the experiments. These procedures will be performed as advised by the Laboratory Animal Facilities veterinarians.

Note: Contact the Office of Research, ext 5-5000, for recommendations on the assessment criteria.

23. What procedures will be used to euthanize the animals? Euthasol IV followed by tissue harvest

Note: Secondary methods are recommended to ensure death. (Consult Section VII of the LAF Training & Procedural Manual for appropriate methods of euthanasia.)

## Assurances

Have all personnel received a medical evaluation from UMC Student/Employee Health?

- No  
 Yes

Have all personnel become familiar with the *LAF Training & Procedural Manual*?

- No  
 Yes

Review of the available resources and previous experiments have determined that the proposed activity is not unnecessarily duplicative of previously reported activities.

- No  
 Yes

You are required by law to provide a written narrative of the sources you consulted to determine whether or not alternatives exist to procedures that may cause pain and distress. Minimal information should include: the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used to determine that no alternatives were available to the painful or distressful procedure. Reduction and refinement should be addressed in addition to replacement. [*AWA Section 13(a)(3)(b) and 9 CFR, part 2, Section 2.31 (d)(1)(ii)*] (See the Office of Research web site to read the regulations.)

Sources utilized:     Index Medicus     Toxline     other  
                           Medlar             AWIC  
                           MEDLINE             Agricola

Search date: October 31, 2002

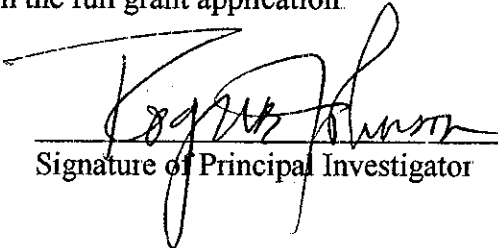
Covered years of search: 1966-2002

Number of "hits": 184

Key words:        (*To include in vitro, animal model, etc.*)  
periodontal disease, gingivitis, animal model, *in vitro*

**Certification of the Principal Investigator:**

Signature certifies that the Principal Investigator will conduct the project in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations, and UMC policies governing the use of live vertebrate animals for research and teaching purposes. The procedures involving animals will be conducted by trained or experienced personnel or under the direct supervision of trained or experienced persons. It is understood that IACUC approval is valid for a period of 12 months following the date of original approval and must be renewed annually for continued approval. I understand there is a 3-year requirement for full protocol rewrite. It is further understood that should this project be submitted for external funding, the information presented on the UMC Animal Activity Protocol form accurately reflects the animal use in the full grant application.

  
\_\_\_\_\_  
Signature of Principal Investigator

Date 12/30/02

Approval by the Attending Veterinarian:

  
\_\_\_\_\_  
Signature

Dec 30, 2002  
Date

Approval by the Institutional Animal Care and Use Committee:

  
\_\_\_\_\_  
Signature

12/30/02  
Date