| Animal Experimentation | |
|---|---|
| **Protocol No.:** | **12-13** |
| **Category of Pain:** | **C** |

# NEW ANIMAL EXPERIMENTATION PROTOCOL
## Standing Committee on the Use of Animals in Research and Teaching
## HARVARD UNIVERSITY/Faculty of Arts and Sciences

**This is to certify that I accept responsibility to assure that the project/laboratory exercise described on the following pages, and within the research proposal entitled:**

➔ Experimental Determination of Tooth Mineralization Patterns in Ungulates for Application to Paleoseasonality Reconstruction

**(1) meets the *Guidelines for the Use of Vertebrate Animals in Research and Teaching* of the Faculty of Arts and Sciences of Harvard University; (2) follows recommendations included in the NIH *Guide for the Care and Use of Laboratory Animals*; and (3) is in accordance with existing Federal (9 CFR Parts 1,2&3), state and city laws and regulations governing the use of animals in research and teaching.**

*Signature of Daniel Green (Sponsor: Tanya Smith)*

09/13/12
*Date*

| | |
|---|---|
| **Name of Principal Investigator** | Tanya Smith |
| **Harvard Appointment Title** *(if none, provide name & title of sponsor, and sponsor's co-signature)* | Associate Professor |
| **Department** | Human Evolutionary Biology |
| **Telephone Number(s)** | **Home:** 16177145979 **FAS:** 16174968259 **Fax:** 16174968041 |
| **Investigator's Harvard Address** | 11 Divinity Avenue |
| **E-Mail Address** | tsmith@fas.harvard.edu |
| **Funding Source** | National Science Foundation Doctoral Dissertation Improvement Grant |
| **Next Funding Application Due Date** | 10 June 2012 |
| **Type of Funding Application:** (check one) | ☒ Grant ☐ Fellowship ☐ FAS Course ☐ Lab exercise ☐ Pilot Project ☐ Other (specify) ➔ |

Approved on behalf of the Committee on the Use of Animals in Research/Teaching:

Please note that this signed face page of the protocol is not to be offered as certification that the IACUC has reviewed and approved the animal-related activities described on any grant application associated with this protocol. If an IACUC approval letter is required by a funding agency, IACUC administration will provide the letter upon request following completion of the protocol/funding application vetting process.

**Part One - Species Information**

Please duplicate this page as many times as necessary; use a separate Page 2 for EACH SPECIES.

**A.1. Name of Species and STRAIN(S) ➔** *Ovis aries* (DORSET)

**A.2. Total No. of Animals to be Used Per Year ➔** 10

**B.1. State source of animals ➔** Earle Parsons & Sons, Inc., Hadley MA

**B.2. Indicate if the animals have undergone procedures prior to being used on this protocol ➔** No

**C. Procedures:** Please check all appropriate procedures below for the SPECIES listed in A.1.

| **CATEGORY B - Teaching, research experiments, or tests that involve ONLY breeding or housing of animals.** | | | |
|---|---|---|---|
| ✓ | **Procedure** | ✓ | **Procedure** |
| ☐ | Breeding Colony (no genotyping) | ☐ | Observation only (no physical contact with animals) |

| **CATEGORY C - Teaching, research experiments, or tests conducted that involve NO PAIN, DISTRESS, OR USE OF PAIN RELIEVING DRUGS.** | | | |
|---|---|---|---|
| ✓ | **Procedure** | ✓ | **Procedure** |
| ☐ | Alert animals (behavioral observation, behavioral control, brief restraint) | ☐ | Microbiological agents |
| ☐ | Administration of toxic substances/ agents | ☒ | Non-surgical collection of body fluids (blood, urine, etc.) |
| ☒ | Change in the environmental parameters (water) | ☐ | Radioisotopes |
| ☒ | Euthanasia followed by tissue/organ harvest | ☐ | Use as parasitic host |
| ☐ | Food or water deprivation | ☐ | Forced exercise |
| ☐ | Other (provide details): Administration of calcein and oxy-tetracycline labels | | |

| **CATEGORY D - Teaching, research experiments, surgery or tests involving PAIN OR DISTRESS and for which appropriate ANESTHETIC, ANALGESIC OR TRANQUILIZING DRUGS will be used.** | | | |
|---|---|---|---|
| ✓ | **Procedure** | ✓ | **Procedure** |
| ☐ | Anesthetize and release (i.e., for blood sampling) | ☐ | Multiple survival surgery |
| ☐ | Antibody production: polyclonal (non-ascites, NO footpad) | ☐ | Survival surgery |
| ☐ | Antibody production: monoclonal (ascites/hosting hybridoma cells) | ☐ | Non-survival surgery |
| ☐ | Electric shock | ☐ | Physical trauma |
| ☐ | Footpad injections (antibody production or microorganism injections) | ☐ | Transgenic animal production |
| ☐ | Gavage | ☐ | Transgenic animal maintenance including genotyping |
| ☐ | Induction of illness (including the administration of toxic substances) | ☐ | Tumor induction or implantation |
| ☐ | Lavage | ☐ | Unusual restraint |
| ☐ | Other (provide details): | | |

| **Category E - Teaching, research experiments, surgery, or tests involving PAIN OR DISTRESS and for which appropriate anesthetic, analgesic, or tranquilizing drugs would adversely affect the procedures, results, or interpretation of data (ANESTHETIC, ANALGESIC OR PAIN RELIEVING DRUGS ARE NOT USED).** | | | |
|---|---|---|---|
| ✓ | **Procedure** | ✓ | **Procedure** |
| ☐ | Death as an endpoint | ☐ | Noxious stimuli form which there is no escape |
| ☐ | LD studies | ☐ | Pain study |
| ☐ | Other (provide details): | | |

**D. Use of Animals**

**D.1 Number of Animals Per Procedure(s)**

*Keeping in mind the TOTAL NUMBER of animals indicated in A.2., please specify how many animals will be used for what procedure(s).*

5 sheep will be used for "change in environmental parameters" **AND** "administration of calcein and oxy-tetracycline labels **AND** "nonsurgical collection of body fluids" **AND** "euthanasia followed by tissue/organ harvest."
5 sheep will be used for "administration of calcein and oxy-tetracycline labels **AND** "nonsurgical collection of body fluids" **AND** "euthanasia followed by tissue/organ harvest."
TOTAL = 10 sheep used per year

**D.2. Will you be donating or sharing TISSUE, ORGANS, FLUIDS, CELLS, etc. [tissue] to a project not covered in your protocol?** **YES ☐** **NO ☒**

*If you don't know the answer to this question at the time of form completion, this information can be added any time via e-mail communication to the Protocol Research Officer (spragens@fas.harvard.edu).*

| | | |
|---|---|---|
| **Name of PI receiving the tissue:** | N/A | |
| **If the receiving PI has an approved Harvard protocol, please list protocol number:** | | |
| **Species:** | | |
| **Type of tissue you are sharing / donating:** | | |
| **Number of animals from which you will harvest this tissue:** | | |

**E. What methods of euthanasia will be used for each species listed in Section A.? List each species and method/dosage separately.**

*(Even if euthanasia is not part of the experimental procedure(s), please list a method to be used in the event of illness or injury. Please note that cervical dislocation and guillotining without anesthesia are no longer acceptable methods of euthanasia unless scientifically justified. Any deviation from the recommendations of the 2007 American Veterinary Medical Association (AVMA) Guidelines on Euthanasia must be justified below. A copy of these guidelines is available at http://www.avma.org/issues/animal_welfare/euthanasia.pdf*

**E.1. Method** (include justification, if necessary):
Sedation with xylazine/ketamine (IM) followed by Intravenous lethal injection by sodium pentobarbital.(jugular IV), following. Death will be confirmed with exsanguination at the jugular.

**E.2. If drugs will be employed as euthanizing agent, what is the:**

| | |
|---|---|
| **Species:** | *Ovis aries* |
| **Drug Name:** | Sedation with Xylazine+ketamine followed by anesthetic Overdose of sodium pentobarbitol |
| **Drug Dose (in mg/kg or % rate):** | 0.05mg/kg (X) + 10mg/kg (K), 180mg/kg (SP) |
| **Route of Administration:** | Intramuscular injection (X+K), Intravenous injection (SP) |

**F. Location of Animal Housing and Animal Use:**

**F.1. Permanent Housing:**

*Please indicate within the table below he OAR managed housing facility or IACUC approved housing satellite in which your animals will be permanently housed*.

| Species | Building | Room # |
|---|---|---|
| **a. *Ovis aries*** | ☐ Bio Labs (basement), ☐ BRI , ☐ NWL, ☐ Fairchild (basement), ☒ CFS, ☐ Other (Specify: ) | Outside field, TBD |
| **b.** | ☐ Bio Labs (basement), ☐ BRI , ☐ NWL, ☐ Fairchild (basement), ☐ CFS, ☐ Other (Specify: ) | |
| **c.** | ☐ Bio Labs (basement), ☐ BRI , ☐ NWL, ☐ Fairchild (basement), ☐ CFS, ☐ Other (Specify: ) | |

| Species | Building | Room # |
|---|---|---|
| **d.** | ☐ Bio Labs (basement), ☐ BRI , ☐ NWL,<br>☐ Fairchild (basement), ☐ CFS, ☐ Other (Specify: ) | |

**F.2. Location of Animal Use:**

*Please state within the table below the location (building and room) where all procedures will be performed on animals. Clarify what procedures will be performed in each area AND the amount of time the animals will spend in each area per day.*

*PLEASE NOTE:*

*Animals may not remain in a lab or use area for more than 12 hours without express permission from the IACUC. Animals removed from barrier housing facilities may only be returned to that facility via quarantine.*

| Species | Building | Room # | Planned Procedures in this Area | Time in this Area |
|---|---|---|---|---|
| **a) *Ovis aries*** | CFS | TBD | Drinking of water ad libitum | Unrestricted |
| **b) *Ovis aries*** | CFS | TBD | Administration of calcein, oxy-tetracycline | 5 minutes |
| **c) *Ovis aries*** | CFS | TBD | Collection of blood or breath samples | 5 minutes |
| **d) *Ovis aries*** | CFS | TBD | Euthanasia and tissue harvest | 5-10 minutes |

**G. Animal Identification**

Will you utilize a means of identification for your animals?

☒ **YES**
☐ **NO**

*If "YES," indicate below the manner in which you plan to identify each species used and provide the details of the procedure in Sections G.1. through G.3. ** PLEASE NOTE: If you plan to use toe clipping, you must also answer Section G.4.*

| G.1. Species | Identification Method |
|---|---|
| ***Ovis aries*** | ☐ Ear notching/clipping ☒ Ear tagging ☐ Tattooing ☐ Banding<br>☐ USDA-placed tag or tattoo ☐ Toe Clipping** ☐ Micro-chipping<br>☐ Other (please specify): |
| | ☐ Ear notching/clipping ☐ Ear tagging ☐ Tattooing ☐ Banding<br>☐ USDA-placed tag or tattoo ☐ Toe Clipping** ☐ Micro-chipping<br>☐ Other (please specify): |
| | ☐ Ear notching/clipping ☐ Ear tagging ☐ Tattooing ☐ Banding<br>☐ USDA-placed tag or tattoo ☐ Toe Clipping** ☐ Micro-chipping<br>☐ Other (please specify): |
| | ☐ Ear notching/clipping ☐ Ear tagging ☐ Tattooing ☐ Banding<br>☐ USDA-placed tag or tattoo ☐ Toe Clipping** ☐ Micro-chipping<br>☐ Other (please specify): |

**G.2. Describe each procedure used for each animal identification method indicated:**

Each sheep will receive an ear tag.

**G.3. How old are the animals when the above described identification method is utilized?**

One month old.

**G.4. ** Scientific Justification for the Use of Toe Clipping**

*Per NIH guidelines and institutional policy, toe clipping may only be utilized if you can prove to the IACUC in writing that alternative means of identification have been first considered that they have been found to be scientifically unsatisfactory. Please detail your argument below:*

N/A

**Part Two - Personnel Information**

**H. Will the principal investigator perform the procedure(s)?** ☐ **YES** ☒ **NO**

*Please provide below the (1) name, (2) Harvard title, (3) qualifications (i.e., M.D., Ph.D., and the number of years of experience working with species listed on this assurance), and (4) the date of completion of the FAS Course on the Humane Care and Use of Laboratory Animals of ALL the personnel (including the PI, students, postdoctoral fellows, and visiting faculty) who will perform procedures involving animals. All new personnel must complete a Credentials Form (available from the Animal Research Studies Coordinator, 617-496-2063 or majkut@fas.harvard.edu.)*

**PLEASE NOTE: All NEW personnel must complete a Credentials Form and fax it to 617-496-7400.**

| H.1. Name | H.2. Title | H.3. Qualifications, Work Experience, or Previous Training in procedures or with species | H.4. "Course" Attendance Date |
|---|---|---|---|
| Dr. Meir Barak | Post-doctoral fellow | Doctor of Veterinary Medicine, 7 years experience | Next available |
| Daniel Green | Doctoral candidate | Will be trained by Dr. Barak | Next available |
| | | | |
| | | | |

Notes:

**I. Have all the individuals listed in H. been informed of the "OCCUPATIONAL HEALTH PROGRAM FOR PERSONNEL HANDLING ANIMALS"?** ☒**Yes** ☐ **NO**

*Please list all personnel who are enrolled in the occupational health program.*

Barak and Green are in the process of being assessed and enrolled in the FAS Occupational Health program

**Part Three - Experimental Procedures**

**J. Describe the (1) procedures you will perform as well as the (2) aims and significance of your experiment(s).** *(Please use language that can be readily understood by biomedical investigators not working in your specific field of research and by lay persons.) State the time schedule for the procedure(s), and provide details regarding the ultimate fate of the animals.*

**J.1. PROCEDURE(S)**

**J.1.a. Name of Procedures:**

Procedure 1) Water switch
Procedure 2) Calcein and oxy-tetracycline administration
Procedure 3) Blood and breath sampling
Procedure 4) Euthanasia

**J.1.b. Description of Procedure:**

Procedure 1) Water switch

At eight months of age, five sheep will freely drink from high altitude melt water that is isotopically depleted (contains fewer heavy oxygen isotopes) compared to Boston water for a period of two weeks. This water will replace ordinary drinking water, will be labeled, and will be checked as frequently as regular drinking water. Switch water will be approximately 10 $\delta^{18}O‰$ lighter than CFS water, which should result in a depletion of 0-3 $\delta^{18}O‰$ in some portions of the enamel of switch sheep. After the switch (2 weeks) they will return to regular water provided by the CFS. The five other sheep, a control group, will continue to drink CFS water throughout this period. The oxygen isotopic value of drinking water will be monitored every two weeks.

Procedure 2) Calcein and oxy-tetracycline administration

All sheep will receive two subcutaenous oxy-tetracycline injections (50mg/kg): one at seven and one at ten months of age. Both injections will be delivered via 16 guage needles, and Dr. Moira Sheehan will assist with procedures. All sheep will also receive two subcutaneous calcein injections (20mg/kg): one at eight and one at eight-and-a-half months of age, delivered with 16 gauge long needles at the scruff. Sheep will be brought to receive injections in pairs in order to comfort them. Sheep will be inspected for swelling, redness or heat at the site of the injection, and monitored for obvious signs of discomfort including scratching of injection site. In the event of an adverse reaction including some or all of the above symptoms, topical antiseptic or benedril may be applied to alleviate pain. These injections will result in fluorescent labeling of enamel prior to and following the water switch, because both compounds are incorporated into enamel during tooth formation, and fluoresce under UV light. Viewing these labels in the second molars with histological sections will demonstrate where in the enamel depleted oxygen isotopes will most likely be found.

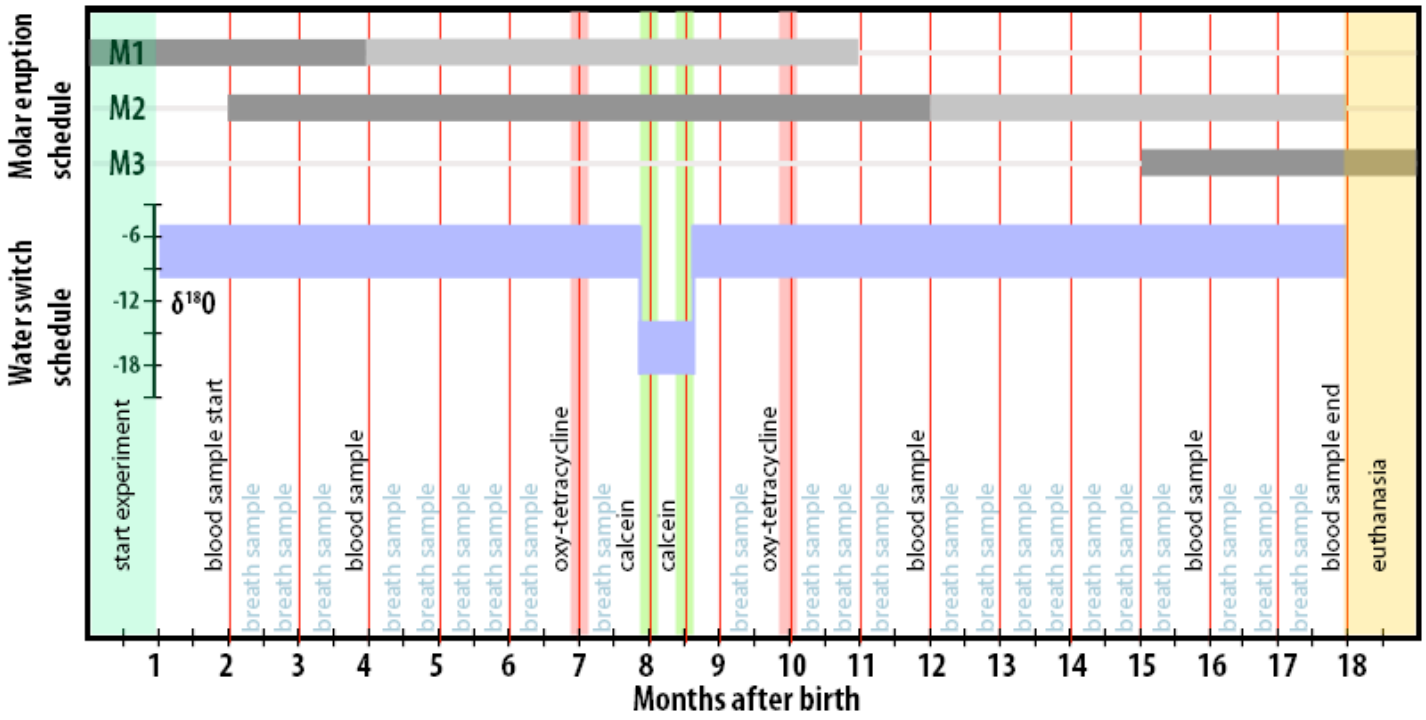
Procedure 3) Blood and breath samples

Each sheep will have 2mL blood drawn using a 21 gauge long medical syringe on 18 occasions: once each month beginning at two months of age, and twice in their eighth month. Blood will be drawn at the jugular vein, and in the event of infection or discomfort topical antiseptic will be applied. Sheep will also have breath samples collected every two weeks, except between eight and nine-and-a-half months of age, during which time breath samples will be collected more regularly: at 8 months of age sheep will have breath samples collected daily for three weeks, after which they will have breath samples collected every three days for an additional three weeks. Breath samples will be collected by placing fitted masks made from halved 2L coke bottles and foam, connected to Douglas bags, over sheep snouts for approximately five seconds. Sheep will be acclimated to breath sampling by fitting masks over their snouts for ten seconds every three days for three weeks following their arrival at Concord Field Station. Breath samples in Douglas bags will be transferred to air-tite brand vials with a syringe, $CO_2$ isolated from water using a $CO_2$ slurry, and $CO_2$ will be injected into a gas chromatograph (GC) column for isotope ratio mass spectrometry (IRMS). All breath and blood will be collected from animals brought from their field in pairs, to comfort them during the procedures.

Procedure 4) Euthanasia

All sheep will be humanely sacrificed at 18 months of age, and their teeth harvested for histology and isotope analysis. The sheep will be sedated using xylazine and ketamine (0.05 mg/kg and 10 mg/kg, respectively, using IM delivery) and euthanized using intravenous administration of sodium pentobarbitol (180 mg/kg) at the jugular vein. Animals will then be exsanguinated at the jugular to confirm death.

### J.1.c. Flow Chart




## J.2. AIMS & SIGNIFICANCE

This experiment is part of a project testing the relationship between environmental water chemistry, meteorology and climate on the one hand, and mammalian blood and tooth chemistry on the other. Enamel in teeth is chemically stable over geological timeframes, and grows in sequential mineralized bands that are in isotopic equilibrium with blood and ultimately drinking water. Oxygen isotope ratios in enamel therefore reflect environmental oxygen isotope ratios during tooth formation; analysis of archaeological or fossil teeth can reveal meteorological and climatic patterns of the distant past. This inference however requires an understanding of the rate at which oxygen isotopes in the blood reach equilibrium with drinking water, and the pattern of blood oxygen isotope incorporation into enamel. It also requires an understanding of normal physiological variation in enamel oxygen isotope values given a consistent and known drinking source.

In this experiment, normal physiological variation in blood oxygen isotopes will be derived from repeated measurements of blood and breath, which are in an equilibrium that will be measured here. The rate of blood oxygen isotope equilibration with drinking water will be derived from the breath samples of animals given a water switch. Lastly, the pattern of oxygen isotope incorporation into enamel for animals with known drinking water and blood oxygen isotope ratios will test existing theories of enamel mineralization patterns, and our ability to reconstruct changes in environmental water chemistry using enamel oxygen isotope ratios. Oxy-tetracycline and calcein labels will be used to locate distinct locations in the teeth where enamel was forming during the water switch administered to half the sheep.

**K. What are the potential benefits to human/basic knowledge which justify the above use of experimental animals?**

Blood oxygen isotope turnover and equilibration rates are as yet unknown in large animals including humans, and will be revealed here for sheep. More importantly, this project will test and possibly improve a method of reconstructing past human environments that relies upon the analysis of oxygen isotope ratios in ungulate teeth. Ultimately, accurately reconstructing environmental water isotope ratios from enamel will allow us to infer the timing, magnitude, and variation of precipitation patterns during periods of interest in human history. These periods include the emergence of agriculture, repeated dispersals from Africa into Eurasia, or even early instances of stone tool production and use between two and three million years ago.

**L. Will the animals be immunized to raise antibodies?** ☐ **YES** ☒ **NO**

*If "NO", proceed to question M.*

**L.1.Are you using adjuvants?** ☐ **YES** ☒ **NO**

*If "YES", specify the following:*

L.1.a. - Type of adjuvant:

L.1.b. - Dose (in mg/kg) of adjuvant for 1st Injection:

L.1.c. - Dose (in mg/kg) of adjuvant for subsequent injections:

L.1.d. - Route of application:

L.1.e. - Location of injections site(s):

L.1.f. - Number of immunizations:

**L.2.Are you using footpad injections?** ☐ **YES** ☒ **NO**

*If "YES", justify the use of footpad injections instead of other methods of immunization. If your experiments require multiple footpad injections, you **must** justify the use of more than one footpad, and you must provide assurance that the animals will be housed on soft bedding.*

**M. Will the animal(s) be used to host hybridoma cells?** ☐ **YES** ☒ **NO**

*If "NO", proceed to N.*

*If "YES", please complete the following sections M.1. through M.5.*

The Office for Laboratory Animal Welfare (OLAW/NIH) has concluded that there is evidence that the monoclonal antibody production of ascites in mice does cause discomfort, distress, and pain. Practical *in vitro* methods exist which can replace the employment of mice in the production of ascites in many experimental applications without compromising the aims of the study. If yours is NOT such an experiment, you must convince the Committee that:

**M.1.** The proposed use of ascites production in mice is scientifically justified (include references) and;

**M.2.** *In vitro* methods have been considered and found unsuitable ("cost" is NOT considered to be a suitable

reason for exclusion of *in vitro* methods).

**Please comment on 1. and 2. Here:**

**M.3.** specify where the animals will be housed:

**M.4.** who will check the animals:

**M.5.** how often the animals will be checked (please note that animals MUST be checked at least daily when producing ascites AND may not be tapped more than three times with the 3rd tap being terminal):

**N. Will your study involve transgenic, knockout/in, or chimeric animals?** ☐ **YES** ☒ **NO**

If "NO", proceed to Question O.

If "YES", you must answer **ALL** of the following questions carefully.

**N.1.** **List SPECIES, STRAINS, and PHENOTYPES** (if any) ➔

**N.2.** **Will the animals experience any pain or distress in association with the phenotype expressed?**

**N.3** **Please describe the phenotype(s) and your proposed procedure(s) to avoid or alleviate pain?**

**N.4.** **Who will check the animals if pain or distress is anticipated?**

**N.5.** **What is the duration of survival after expression of the phenotype?**

**N.6.** **If you are generating transgenic or knockout animals and do not know the phenotype, please discuss the criteria you will use to determine pain and distress in the animals.**

**N.7.** **The construction of transgenic animals must be approved by the Committee on Microbiological Safety (COMS).** Contact Environmental Health and Safety at 495-2060 for appropriate registration.

Please provide COMS Registration #:

**N.8.** **If receiving animals from an outside source, please state the origin of the animals.**
**If the animals are coming from the Harvard Medical Area, please state their COMS Number.**

Origin: COMS #:

**O. Will you administer any of the following?** ☒ **YES** ☐ **NO**

If "NO", proceed to Question P.

| Check all that apply and provide the information requested: | | |
|---|---|---|
| **YES** | **NO** | **Specifics:** |
| ☐ | ☐ | Chemical carcinogen.<br>Specify name of carcinogen ➔ |

| Check all that apply and provide the information requested: | | |
|---|---|---|
| **YES** | **NO** | **Specifics:** |
| ☐ | ☐ | Toxic substance.<br>Specify name of substance ➔ |
| ☐ | ☐ | DEA controlled substance (exclusive of veterinary anesthetics, analgesics, and tranquilizing agents).<br>Specify name of substance ➔<br>If you are unsure if the substance you wish to use is controlled, please refer to<br>http://www.deadiversion.usdoj.gov/schedules/alpha/alphabetical.htm |
| ☐ | ☐ | Radioisotope.<br>• Specify name of isotope ➔<br>• The use of radioactive substances must be permitted through the Radiation Safety Office of Harvard University's Department of Environmental Health & Safety at<br>• http://www.uos.harvard.edu/ehs/radsafety/authorizations.shtml<br>• If previously approved for the use of this isotope, please provide Radioactive Use Permit # ➔ |
| ☐ | ☐ | Microbiological agent.<br>• Specify name of agent ➔<br>• The use of microbiological agents must be approved by the Committee on Microbiological Safety (COMS). Information and instructions can be found at http://www.uos.harvard.edu/ehs/bio.shtml<br>• If previously approved for the use of this agent, please provide the COMS registration # ➔ |
| ☐ | ☐ | Animal tissue, tumor, or primary cell line (including monoclonal antibodies and serum derived products).<br>• Specify type of tissue, tumor or primary cell line ➔<br>• Specify species of origin ➔<br>• The introduction of any animal tissue, tumors or primary cell lines into animals must be registered with the Committee on Microbiological Safety (COMS). Reference the EH&S Biosafety Office for forms and instructions http://www.hms.harvard.edu/orsp/coms/Forms/FAS-Forms/FAS-forms.htm<br>• If previously approved, please provide the COMS registration # ➔<br>• All animal derived tissue, tumors, primary cells lines must be tested for biological contaminants prior to use in animals and prior to entry into any animal housing/use area and should be re-tested every year. Please contact the Office of Animal Resources (617-496-9989; jorgenson@mcb.harvard.edu) for instructions and paperwork. |
| ☐ | ☐ | Human tissue or cell line.<br>• Specify type of tissue, tumor or primary cell line ➔<br>• The introduction of any human tissue or primary cell lines into animals must be registered with the Committee on Microbiological Safety (COMS). Reference the EH&S Biosafety Office for forms and instructions http://www.hms.harvard.edu/orsp/coms/Forms/FAS-Forms/FAS-forms.htm<br>• If previously approved, please provide the COMS registration # ➔<br>• All human-derived cell lines must be tested for biological contaminants prior to use in animals and prior to entry into any animal housing/use area and should be re-tested every year. Please contact the Office of Animal Resources (617-496-9989; jorgenson@mcb.harvard.edu) for testing instructions and the paperwork. |
| ☐ | ☐ | Human Embryonic Stem Cells (hESC) or Induced Pluripotent Stem (iPS) Cells. Specify ➔<br>• Animal experimentation protocols utilizing human embryonic stem cells or human induced pluripotent stem cells may require review and approval by the Harvard University Embryonic Stem Cell Research Oversight (ESCRO) Committee prior to commencement of the experiments. Please contact ESCRO administration (617-496-0123; escro@harvard.edu ) for a determination.<br>If already approved, please provide ESCRO Committee approval number ➔ |
| ☐ | ☐ | Other *(i.e.*, hormones, novel antibiotics, cytokines, etc.) Specify ➔ **Calcein, oxy-tetracycline** |

*For EACH of the agents/substances listed above in Section O, you **must** complete sections O.1. through O.9. and if applicable, O.10., below (please duplicate the ENTIRE BOX if more than 3 agents/substances are being utilized):*

| | | | | |
|---|---|---|---|---|
| **NAME of substance/agent/ tissue/ cell, etc.➔** | | Calcein | Oxy-tetracycline | |
| **Used in (NAME OF SPECIES) ➔** | | Ovis aries | Ovis aries | |
| **O.1.** | **Route of administration:** | SC injection | SC injection | |
| **O.2.** | **Site and number of injections (if applicable):** | Subcutaenous, 2 | Subcutaneous, 2 | |
| **O.3.** | **Doses or amounts for each administration (in mg/kg):** | 20 | 50 | |
| **O.4.** | **If injecting, state volume of injection:** | No more than 5 cc | No more than 5 cc | |
| **O.5.** | **Effects of the agent or substance(s):** | Label teeth | Label teeth | |
| **O.6.** | **Whether the animal(s) will experience pain as a result of the treatment:** | None | None | |
| **O.7.** | **Whether the animal(s) will be involved in any other procedure(s):** | Water switch, human sacrifice | Water switch, human sacrifice | |
| **O.8.** | **Length of survival time after administration:** | 9 months | 9 months | |
| **O.9.** | **Names of personnel who will be using the substance:** | Daniel Green will administer | Daniel Green will administer | |

**O.9. Use of Non-Pharmaceutical Grade Compounds**

*Investigators are expected to use pharmaceutical-grade substances and medications whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds may only be used in animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in animals.*

**Please detail below which of the agents listed in this section are not of pharmaceutical grade. Explain how sterility of the agent will be maintained or why it is impossible to do so:**

Oxy-tetracycline is pharmaceutical grade and a widely used antibiotic. Ultra-pure grade calcein commonly used as a tracer is not available as a pharmaceutical grade compound, but will be maintained in a sealed container that will be stored in Ziploc baggies and refridgerated. Solutions will be mixed in sterile containers with sterile saline immediately prior to injection. We have consulted with Dr. Dan Lieberman and on the basis of his experience with calcein injections will keep volumes below 5 cc's.

**O.10. Are you creating chimeras?** ☐ **YES** ☒ **NO**

*If "YES", please complete the following:*

**O.10.a. Describe what species are involved:**

**O.10.b. Is the chimera human-animal?** ☐ **YES** ☒ **NO**

*If "YES", please address the follow question:*

**Breeding of chimeras**

*NAS guidelines state that "no animal into which hESCs have been introduced at any stage of development should be allowed to breed." Therefore, PIs are required to take reasonable measure to prevent breeding and euthanize offspring if breeding does occur.*

**Please detail the procedures you will take to prohibit the breeding of chimeras or the euthanasia measures to prevent offspring from reaching sexual maturity:**

**O.10.c. Are you creating teratomas?** ☐ **YES** ☒ **NO**

*If "YES", please detail your monitoring procedures:*

**P. Will you perform surgery?** ☐ **YES** ☒ **NO**

*(If "NO", proceed to Question R.)*
*If "YES", you must answer **ALL** of the following questions carefully.*

**P.1. Will you fast your animals?** ☐ **YES** ☒ **NO**

If "yes", please provide the details of the fasting:

**P.2. Surgical Procedures**

**P.2.a. Name of Procedure(s) and Surgeon(s):**

| Name of Surgical Procedure | Name of Surgeon(s) |
|---|---|
| **N/A** | |
| | |
| | |
| | |
| | |

**P.2.b. Details of Surgical Procedure(s)** *(for each surgical procedure, include methods of animal restraint and anesthesia induction, aseptic animal preparation, draping, maintenance of aseptic surgical field, type of incision, steps of surgical procedure, suture materials, removal of wound clips or sutures (if survival), use of prophylactic antibiotics, use of pre-emptive analgesia, if more than one surgical procedure is to be performed then detail the amount of recovery time between procedures, etc.)***:**

**N/A**

**P.3. Anesthesia Regimen(s)**

State below the anesthetic agents and dosages to be used for each species. Provide justification if you do not intend to use anesthesia.

| Species | Name of Surgical Procedure | Anesthetic Agent | Dose (in mg/kg or % rate) | Route | If injecting, state the volume of the injection |
|---|---|---|---|---|---|
| **N/A** | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**OR justification for not using anesthetics:**

**POST-OPERATIVE CARE AND ANALYSIS**

**Q Are the animals to recover from surgery?** ☐ **YES** ☐ **NO**

If "NO", proceed to R.
If "YES", answer Q.1. through Q.7.:

**Q.1. Why is it necessary for the animals to recover from surgery?**

**Q.2. Who will check the animals during recovery and how often?** (Name the specific individuals who will do the checking).

**Q.3. What impairment is caused by the surgery, and will the animal experience pain as a result of the surgery?**

**Q.4. Postoperative Care**:

**(a) What is the duration of the survival after the surgery?**

**(b) How will the animal(s) be monitored immediately postoperatively?**

**(c) State below the post-operative analgesics and dosages to be used for each species. Provide justification if you do not intend to use analgesics.**
*Please be advised that per institutional policy you are obliged to administer analgesia for a minimum of 48 hours after major surgical procedures and for a minimum of 24 hours after minor surgical procedures or explain/scientifically justify why this cannot be done. All animals must be monitored for 96 hours following surgery regardless of when analgesic administration ceased.*

- *A major surgical procedure is that which penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions. Examples: laparoscopy, thoracotomy, craniotomy, joint replacement, limb amputation.*
- *A minor surgical procedure is does not expose a body cavity and causes little or no physical impairment. Examples: wound suturing and peripheral vessel cannulation*

| Species/Name of Surgical Procedure | Name of Analgesic Agent | Dose (in mg/kg) | Route | If injecting, state the volume of the injection | Frequency of Administration (i.e., 1x /12 hrs for 48 hours) |
|---|---|---|---|---|---|
| **N/A** | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**<u>OR</u> justification for <u>not</u> using analgesics:**

**(d) List any expected or potential postoperative complication and plans to handle them.**

**Q.5. If you are performing survival surgery (including rodents), you must observe proper sterile technique.**

(a) I agree to use sterile instruments at all times. ☐ **YES** ☐ **NO**
(b) I agree to maintain sterile conditions/field during surgery. ☐ **YES** ☐ **NO**
(c) I agree to prep animals for surgery by clipping and disinfecting ☐ **YES** ☐ **NO**
The skin surface using suitable materials such as Betadine or Nolvasan solution.

If you answered "NO" to any of the above, please scientifically justify the variations in compliance:

**Q.6. Location of surgery (for each species utilized in the protocol, complete the following section):**

| Name of Species: | Building | Room |
|---|---|---|
| **Where will you perform surgery?** | **N/A** | |
| **Where will the animals be housed during recovery?** | | |
| **Where will the animals be housed after recovery?** | | |

| Name of Species: | Building | Room |
|---|---|---|
| **Where will you perform surgery?** | | |
| **Where will the animals be housed during recovery?** | | |
| **Where will the animals be housed after recovery?** | | |

**Q.7. Will the animals undergo more than one surgical procedure?** ☐ **YES** ☒ **NO**

*If "NO", proceed to R.*
*If "YES", you **must** justify below why it is scientifically necessary to do so.*
*Assure to indicate the time between surgical procedures.*

**N/A**

**R.Will your study be concerned with the effects of trauma or burns?** ☐ **YES** ☒ **NO**
**Will you be studying pain or using electric shock?** ☐ **YES** ☒ **NO**

*If "YES", please describe the details of the procedure and assure to provide:*

- *the means to alleviate pain and distress*
- *the names of anesthetic or analgesics used, their dose and route, and time interval between administrations or provide a scientific justification as to why pain relieving drugs cannot be used*
- *the monitoring parameters and how the animals will be observed during the procedure*
- *the names of those who will monitor the animals after the procedure and how often*
- *details of any impairments from the procedure and what care will be provided to compensate for the impairments*
- *the duration of survival after the procedure*

**S. Will your study involve experiments:**

S.1.on alert animals ☐ **YES** ☒ **NO**
S.2.or involve behavioral studies ☐ **YES** ☒ **NO**
S.3.and/or training, control, restraint, ☐ **YES** ☒ **NO**
S.4.or analysis of behavior? ☐ **YES** ☒ **NO**

*If "YES" to any, please provide the details of the experiment and assure to include:*

- *any means or training to achieve behavioral control;*
- *any use of food deprivation;*
- *any use of psychotropic drugs and their withdrawal symptoms;*
- *any use of paralytic [a] agents or perform procedures which result in paralysis;*
- *and the means employed to ensure the welfare of the animal.*

N/A

**T. Will you study the effects of diet or environmental changes?** ☒ **YES** ☐ **NO**

*If "NO", proceed to Question U.*
*If "YES", check all that apply and provide the details below:*

[a] *Instructions If Using a Paralytic Agent or Performing Procedures Which Will Result in Paralysis: If you will be using paralytic agents or performing paralytic procedures, in Section U, (1) describe the use of the agent in detail; (2) provide scientific justification for the use of the agent; (3) describe any impairment expected from the procedure; (4) describe your proposed procedures to monitor pain and distress levels and how you will alleviate any detected pain and/or distress; (5) state whether the animals will be able to urinate/defecate properly or reach food and water, and if not, what supportive care will be provided; (6) identify who will monitor the animals and the monitoring schedule; and (7) state the duration of survival after the paralytic procedure.*

T.1. Food deprivation? ☐ **YES** ☒ **NO**
T.2.Water deprivation? ☐ **YES** ☒ **NO**
T.3.Temperature changes? ☐ **YES** ☒ **NO**
T.4.Changes in the light cycle? ☐ **YES** ☒ **NO**
T.5. Special diet? ☒ **YES** ☐ **NO**

*Provide below the details of the experiments, including the effects of the protocol on the animal's health, and the duration of the experiment.*

Five sheep will receive Idaho water that is isotopically light compared to Boston water for two weeks, *ad libitum*, when they are 8 months of age. After this time they will be returned to ordinary CFS water. Isotopically light water has no adverse side affects and is virtually indistinguishable from water that is isotopically enriched.

**U. Will you be performing field studies or field observation?** ☐ **YES** ☒ **NO**

*If "NO", proceed to Question V.*
*If "YES", please provide the details of the study and assure to include:*

- *the location to which you are traveling;*
- *the name of the research institution with which the location is affiliated (if any); and*
- *the details of the precautions you will be taking while in the field (i.e., types of personnel protective equipment which will be worn such as gloves, goggles, face masks, etc.).*

N/A

## Part Four - Federal Assurances

**YOU ARE REQUIRED TO PROVIDE THE ASSURANCES REQUESTED IN QUESTIONS "S" THROUGH "V" BY THE USDA. FAILURE TO DO SO WILL RESULT IN AUTOMATIC DISAPPROVAL.**

**V. The USDA requires that you justify the following to reduce, replace and refine the numbers of animals to be used:**

**V.1. Why are animals needed for this study?** *(For example: Could the same information be obtained by experiments using tissue culture or computer models? If you are generating antibodies, why are you doing in vivo immunization instead of in vitro?)*

Oxygen isotope equilibrium rates in blood, normal physiological variation in blood and enamel, and patterns of incorporation into enamel are not currently known in large animals such as sheep (mineralization patterns are poorly understood in all mammals). Multiple, competing models regarding mineralization in enamel have been proposed, and cannot be evaluated without an experimental study.

**V.2. Justify <u>each</u> choice of species:**

*Ovis aries* is an ideal species in this project because its physiology and pattern of tooth formation is broadly similar to many ungulate species, which are abundant in archaeological and fossil records and whose teeth can be used in climatic reconstructions. It is furthermore ideal because its larger size can help expand our understanding of isotope ecology to larger mammals.

**V.3. Justify the number of animals to be used in the experiment(s).** *(You must convince the Committee that the number of animals is appropriate to the work being proposed. For example: There may be a minimum number required for statistical significance but you must explain how you arrived at that number.)*

Oxygen isotope measurements from blood, air or enamel, analyzed using mass spectrometry (GC/TCEA-IRMS), typically have standard errors of .2-.4 $\delta^{18}O‰$. For a comparison of means between the enamel oxygen isotope ratios of water switch and control sheep, expected to vary from 0-3 $\delta^{18}O‰$ depending upon where samples are taken from enamel, the 95% confidence interval for the difference in means requires n=4 per treatment group. Acceptable uncertainty in this case is 0.5 $\delta^{18}O‰$. An additional individual is used for both switch and control animals in the event that an animal should become sick or

otherwise unable to contribute to the experiment.

**W. Alleviation of Potential Pain or Distress** (check C, D, or E):

| | |
|---|---|
| ☒ | **Categories B or C:** Procedures cause No pain or distress OR any pain or distress will be slight or momentary; no pain relieving drugs are necessary. (*i.e.*, breeding only, housing only, simple injections, imaging, anything listed in Categories B or C on Page 2 of this document, etc.). |
| ☐ | **Category D:** Procedures have the potential to cause more than slight or momentary pain and/or distress and appropriate anesthetic, analgesics or tranquilizing drugs will be used (*i.e.*, surgical procedures, anything listed in Category D on Page 2 of this document, etc.) |
| ☐ | **Category E:** No method is available for completely alleviating the pain or distress caused by this procedure OR anesthetic, analgesic or tranquilizing drugs would adversely affect the procedures, results, or interpretation of data. (*i.e.*, Anything listed in Category E on Page 2 of this document.) |

**If you checked C, proceed to Question X at the bottom of the next page.**

**If you checked D or E**, then you must explain below why you cannot use alternative procedures that might cause less pain or distress. Check one and follow the instructions carefully.:

☐ **Alternatives DO NOT exist (complete Section W.1.a. through W.1.d.)**

☐ **Alternatives exist and are not being used (complete Section W.2.a. through W.2.d.)**

**W.1. The Animal Welfare Act requires that you provide the methods and sources by which you determined that alternatives to potentially painful procedures do not exist.**

**W.1.a.** MEETINGS and CONFERENCES attended:

| Title of Meeting or Conference | Attendance Dates |
|---|---|
| **N/A** | |
| | |
| | |
| | |

**W.1.b.** Names of PERIODICALS read on a regular basis:

| Titles of Periodicals | | |
|---|---|---|
| **N/A** | | |
| | | |
| | | |
| | | |

**W.1.c.** Consultation with COLLEAGUES:

| Name of Colleague | Colleagues Credentials | Colleagues Field of Expertise | Topic of Consultation | Date of Consultation |
|---|---|---|---|---|
| **N/A** | | | | |
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**W.1.d.** Literature search(es): For **EACH** potentially painful procedure used in this experiment, please indicate the name of the procedure, the database searched, the key words used and the date the search was conducted.

| Name of Procedure | Date of Search | Years Covered by Search | Name of Database Searched | Key Words Used |
|---|---|---|---|---|
| **N/A** | | | | |
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**W.2. You must explain below why you cannot use alternative procedures that might cause less pain or distress.**

- Please explain why alternative experiments are unsatisfactory.

  N/A
- Detail the steps you will take to assure that you will cause no more discomfort than absolutely necessary.

  N/A
- Assure the Committee that the duration of the discomfort will be as short as possible.

  N/A
- Describe your plans to monitor and correct problems (*i.e.* by euthanasia).

  N/A

**X. The USDA requires that you provide a description of procedures designed to assure that discomfort, distress or pain to the animals will be limited to that which is unavoidable for the conduct of scientifically valuable research.**

*Please summarize your procedures to avoid or minimize **ANY** discomfort, pain or distress the animals may experience. Include:*

**X.1. observation schedule (performed by research staff);**

Animals will be observed at least once per day in the field.

**X.2. criteria for determining whether the animal is undergoing pain and distress;** and

Distress during blood and breath collection, or during calcein or oxy-tetracycline injection, will be determined by frantic efforts to escape treatment. Sites of injection and collection on the animal's body will be inspected within two days of each procedure to insure there is no infection, swelling or unusual response.

**X.3. subsequent actions to be taken if pain and/or distress are encountered.**

Sheep in distress will be taken from the location of sampling or injection and returned to other sheep; effort will be made to complete procedure later that day. If sheep show signs of infection or other problems, the veterinarian will be consulted immediately.

**X.4.** Summarize the **names, dosages, and routes of ALL anesthetics, analgesics, tranquilizing, euthanasia** drugs and **antibiotics** to be utilized for each species in this project (even if this information appears elsewhere in the protocol it must be summarized below.).

| Species | Use (analgesia, anesthesia, euthanasia, antibiotics, etc.) | Drug Name | Drug Dose (in mg/kg or % rate) | Frequency of Administration | Drug Route | If injecting, state the volume of the injection |
|---|---|---|---|---|---|---|
| Ovis aries | Anesthesia | Xylaxine | 0.05 mg/kg | One time | IM | Based on weight of animal |

| Species | Use (analgesia, anesthesia, euthanasia, antibiotics, etc.) | Drug Name | Drug Dose (in mg/kg or % rate) | Frequency of Administration | Drug Route | If injecting, state the volume of the injection |
|---|---|---|---|---|---|---|
| Ovis aries | Anesthesia | Ketamine | 10 mg/kg | One time | IM | Based on weight of animal |
| Ovis aries | Euthanasia | Sodium pentobarbitol | 180 mg/kg, to effect | One time | IV | Based on weight of animal |
| | | | | | | |
| | | | | | | |

**Y. Duplication of results.**

*In accordance with USDA Regulations (9 CFR Parts 1-3) and the Animal Welfare Act, the FAS Standing Committee on the Use of Animals in Research and Teaching is required by Federal Law to obtain the following assurances from you:*

**Do these activities unnecessarily duplicate previous experiments whether your own or another investigator's experiments?** ☐ **YES** ☒ **NO**

If "no", indicate below how you determined that these activities do not unnecessarily duplicate previous experiments. Please check and complete each method and source that applies.

| | | |
|---|---|---|
| MEETINGS and CONFERENCES attended: | **Title of Meeting or Conference** | **Attendance Dates** |
| | **Society for Integrative Biology - DVM** | **Oct 2009, Oct 2010** |
| | **American Association of Physical Anthropology** | **Apr 2008, 2009, 2010, 2011** |
| | **Did Climate Change Shape Human Evolution (Columbia)** | **May 2012** |
| | | |

| | | | |
|---|---|---|---|
| Names of PERIODICALS read on a regular basis: | **Titles of Periodicals** | | |
| | Science | Am. J. Phys. Anthropology | |
| | Nature | Earth and Planetary Science letters | |
| | PNAS | J. Hum. Evolution | |
| | Geo. et Cosmochemica Acta | Paleogeography, Paleoclimatology, Paleoecology | |

| | | | | | |
|---|---|---|---|---|---|
| Consultation with COLLEAGUES: | **Name of Colleague** | **Colleagues Credentials** | **Colleagues Field of Expertise** | **Topic of Consultation** | **Date of Consultation** |
| | Thure Cerling | PhD | Bio, Chem | Isotopes | April 2012 |
| | Meir Barak | PhD | Biology | Animal protocol | May 2012 |
| | | | | | |
| | | | | | |

| | | | | |
|---|---|---|---|---|
| Literature search(es) for duplicative studies: | **Key Words Used** | **Years Covered by Search** | **Name of Database Searched** | **Date of Search** |
| | **Reaction progress variable, oxygen isotopes, sequential sampling, sheep, mineralization, seasonality** | **All dates** | **Google Scholar** | **Latest search: June 5th 2012** |