THESIS

DOSE RECONSTRUCTION IN THE LARGE JAPANESE FIELD MOUSE USING ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY OF TOOTH ENAMEL

Submitted by

Mariah Davis

Department of Environmental and Radiological Health Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2020

Master's Committee:

Advisor: Thomas Johnson

Alexander Brandl
Martin Gelfand

ABSTRACT

DOSE RECONSTRUCTION IN THE LARGE JAPANESE FIELD MOUSE USING ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY OF TOOTH ENAMEL

Electron spin resonance (ESR) analysis of tooth enamel is recognized as a reliable method for lifetime dose reconstruction, particularly in human tooth enamel. While the use of ESR to reconstruct dose is well understood for human tooth enamel, the reliability and usefulness of dose reconstruction using ESR in mouse tooth enamel has not been as thoroughly studied. This paper aims to resolve this gap in knowledge concerning the use of the tooth enamel from the Large Japanese Field Mouse as acting dosimeter using EPR spectroscopy. Methods of tooth preparation were analyzed to find a preparation method that resolved a baseline shift or slope in output signals of preliminary samples. Use of purity EDTA (ethylenedinitrilotetraacetic acid, disodium salt dihydrate) was initially found to reduce an observed baseline shift and slope in the output spectrum. Subsequent samples treated with EDTA, however, again saw baseline shifts. More needs to be done to analyze appropriate methodology to reduce the baseline shift, and to further determine the suitability of mouse teeth for ESR spectroscopy for reconstruction of lifetime dose.

ACKNOWLEDGEMENTS

Thank you to my advisor, Dr. Thomas Johnson for his support, encouragement, and help (all of which cannot be overstated) throughout my studies and throughout this research; and thank you to my other committee members for the investment of their time and support throughout my studies: Dr. Alexander Brandl and Dr. Martin Gelfand (and to Dr. Gelfand specifically for helping me to find this program in the first place!).

Thanks to Joshua Hayes, who provided extensive help and insight for this research project, as well as to Justin Bell, who helped with the irradiation of teeth for this research and thoughtfully wrote up information regarding the irradiation procedure. General thanks also to CSU's ERHS department: Dr. Ralf Sudowe for helping with some chemistry-related questions; administration that provided various kinds of support for my studies and research; and my classmates, who have provided support for this research in various ways either directly (Rebecca Mueller and Samantha Labb), or indirectly.

Thank you as well to Japan's Fukushima University, particularly Dr. Hiroko Ishiniwa for thoughtfully investing much, invaluable time and expertise to collaborating on this research, and showing me the ropes. Thank you to the staff at Fukushima University's Institute for Environmental Radioactivity for their administrative aid, as well as the welcome and kindness they showed to my compatriots and me during our research. Thanks to Yuki Odagiri for his collaboration on mouse preparation and capture, and to Donnovan Anderson for various forms of assistance.

Thank you is also extended to Tohoku University in Sendai Japan, particularly to Dr. Toshitaka Oka, who provided extensive insight and experience into the process of ESR

dosimetry, gave much of his time and energy to collaboration on the research, and who provided access to Tohoku University's ESR spectrometer. Thank you to Dr. Hisashi Shinoda for his advice regarding tooth morphology, and to Yusuke Mitsuyasu of Dr. Oka's graduate students, who generously dedicated his time to helping take some ESR spectra.

Finally, none of this would have been possible without the generous support from Japan's Environmental Radioactivity Research Network Center, Colorado's Mountain & Plains Education and Research Center, CSU alumni, and Mr. and Mrs. Oka (who generously agreed to house me during the field work in Japan, going above and beyond simply providing a place for me to sleep).

TABLE OF CONTENTS

Chapter 1 – Introduction

Background

Following either chronic radiation accumulation, or acute radiation events (be they large scale accidents like the nuclear power plant accidents, or smaller scale situations, such as dosing in a medical facility), it can become important to have an understanding of doses that humans, and non-human biota, could have acquired. Dose reconstruction can be challenging if there are no pre-placed dosimeters to draw measurements from (such as in accident situations or when looking at chronic doses), or in the case that it is difficult or impossible to get a dosimeter to an area. In such situations, alternative methods of dose reconstruction need to be used to gather information and assess dose.[5] One method of retrospective dose reconstruction makes use of the ability to analyze free electrons, generated via ionizing radiation, that have been trapped in inorganic substances. This methodology is called electron spin resonance (ESR) spectroscopy (also called electron paramagnetic resonance (EPR) spectroscopy).

Tooth enamel is an inorganic substance that can be reliably analyzed under ESR which lends itself to dosimetry purposes, as there are various benefits to using tooth enamel for dose reconstruction. One benefit is that teeth are always on one's person, ensuring teeth can act as a reliable personal dosimeter. A second benefit lies in the fact that teeth can be found by default in areas where a dosimeter may not be able to be easily placed due to local animal populations. A third benefit is the stability of the dosimetric signal induced in tooth enamel; the crystalline structure of the hydroxyapatite of tooth enamel ensures signal stability for approximately 10^7 years.[7]

The use of ESR for human dose reconstruction has been used for multiple events, and the area of dose reconstruction using tooth enamel was studied with increasing interest since its first use in 1955. [3] However, outside of our knowledge of ESR using teeth from human subjects, much less is known about the processes involved in, and the signal response at various levels of exposure of ESR using various non-human animal teeth. Animal teeth have been shown to have different responses and sensitivities under ESR spectroscopy than human teeth, leading to remaining questions regarding the feasibility of using animal teeth for ESR dose reconstruction. Some animal teeth may not lend themselves to analysis under ESR spectroscopy for various reasons: some animals may be more or less difficult to find, and more or less difficult to harvest teeth from than others; the process necessary for tooth preparation for ESR spectroscopy may be unreliable or particularly difficult; some animals have different tooth morphology that makes their teeth unusable for dose reconstruction with ESR.

Because it may be difficult, problematic, or ethically unsound to harvest teeth from victims involved in a radiation event or accident, it may be preferable to use animal teeth instead of human teeth to act as a surrogate dosimeter. It may also be useful to use animal teeth as dosimeters so that effects of radiation on areas not inhabited by humans may be understood, and doses to non-human populations and areas may be known.

For these reasons, this study initially aims to add to the growing level of knowledge regarding dose reconstruction in animal tooth enamel using ESR, with a particular focus on mouse teeth. The initial goal was to utilize mice captured from areas of high background dose rates in the wild to show the realistic applications of retrospective dosimetry using ESR and mouse teeth. With a dearth of mice in areas with high dose rates, and due to unforeseen problems with the output ESR spectra when using mice teeth, the aims of this study have shifted slightly;

results of this study can not effectively be used to determine usability of ESR to reconstruct lifetime dose in wild mice in areas of high dose rates. The study will focus instead on effects of methodology of sample preparation on ESR output, and will attempt to make more general conclusions concerning feasibility of dose reconstruction using mice based on sample processing steps and results from ESR spectra.

Electron Spin Resonance – Overview

ESR spectroscopy is a method of identifying free electrons in a sample material. ESR measures the transition of these free electrons between states (spin up ($+1/2$) or spin down ($-1/2$)). The change in state is measured by comparing the energy being applied to the system to the energy output of the system. The insight gained concerning the quantized states of electrons in experimental samples makes ESR a useful technique across a variety of fields, providing understanding of sample composition on a molecular level.

Modern ESR spectrometers work by sweeping across a set magnetic field range while applying a particular modulating frequency (a cavity resonant frequency). The overarching basis for how ESR works is described by an equation relating energy (E), the magnetic field (B), a quantity that represents electron spin, called the Bohr magneton (μ_B), and a dimensionless factor called the g-factor that describes magnetic moment and angular momentum for various particles (g). This relationship is defined as,

$$\Delta E = h\nu = g\mu_B B.$$

With a set cavity resonant frequency for a spectrometer, at a particular magnetic field, electrons will absorb energy being inputted into the system, allowing them to transition between states. This energy absorption yields a measurable energy difference; the derivative of the energy

difference between states may be graphed against magnetic field to provide an ESR spectrum. For ESR in tooth enamel, there are various g-factors that can define the signal of interest, depending on what radical is being measured. The CO_2^- radical is the radical most commonly used for ESR in tooth enamel, due to its prevalence in tooth enamel hydroxyapatite. The CO_2^- radical has a few g-factors that define points of interest in the radiation-induced signal: 2.003, 2.0015, and 1.997. [3] An example of a very simple ESR spectrum output may be seen in Fig. 1.

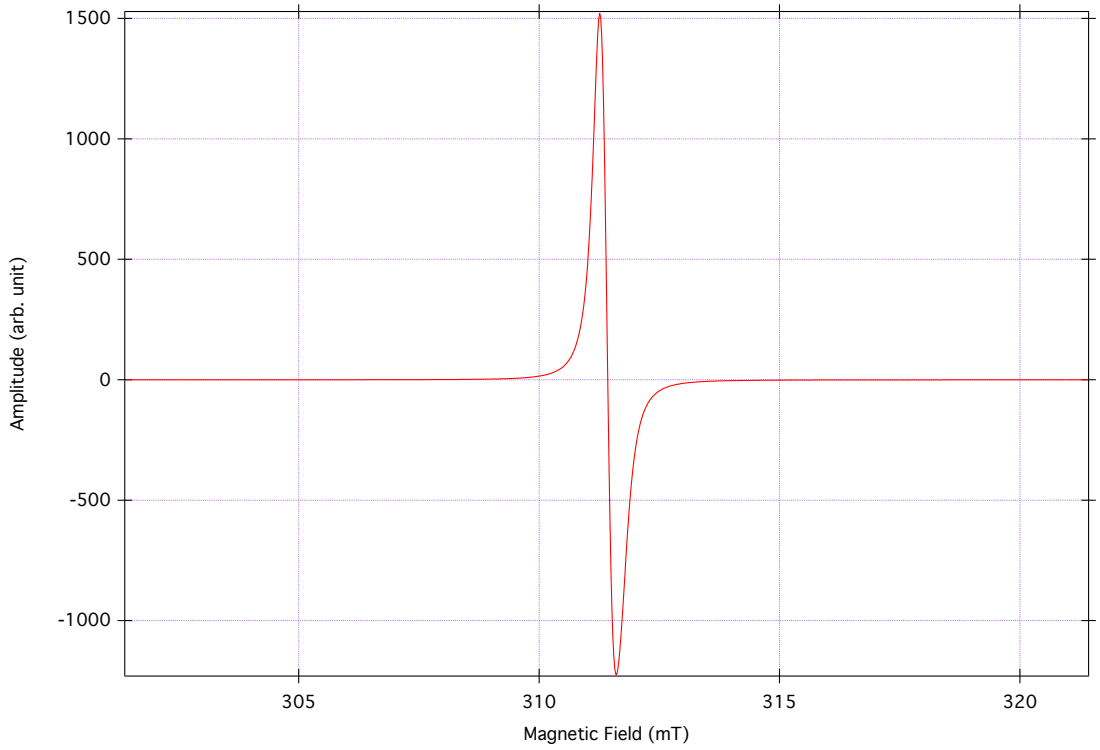


Figure 1: Spectrum of DPPH sample, used for calibration due to its well-known g-factor (2.0036). Spectrum illustrates a simple ESR spectrum output showing a transition in spin state. Abscissa is magnetic field strength and the ordinate is a dimensionless measure of the derivative of microwave energy absorption.

ESR Dosimetry

When used for radiation dosimetry, the spectral output differs from the typical output of Fig 1. As ionizing radiation interacts with a sample, electrons are removed from their shells. In inorganic materials, such as the hydroxyapatite of tooth enamel, ionizing radiation induced free

electrons become trapped in the crystalline structure. The resulting free electrons will create a radiation induced signal (RIS) in the default ESR output for a particular sample material. The peak to peak amplitude of the RIS will vary depending on the amount of radiation that interacts with the material, and is used to extrapolate dose. Higher dose in a material corresponds to a larger peak to peak amplitude of the RIS, and vice versa for lower dose. In tooth hydroxyapatite, dosimetric signal landmarks appear at 3 separate g-values: 2.0030, 1.9970, and 2.0015.

A dose reconstruction curve, or dose response curve, is then created from the RIS so that dose may be correlated with RIS peak-to-peak amplitude; dose reconstruction is done by comparing the measured RIS to a known calibration curve, which is created by applying known doses to the sample material and measuring the peak-to-peak amplitude of the RIS in the known doses. The dose response curve is linear over a set range, depending on the sample material, and radical being analyzed.[3]

ESR Dosimetry History – Human and Non-Human Usage

Use of ESR to detect radicals formed by irradiation stems back to 1955, where skull bone that had been irradiated with x-rays was analyzed. In 1963, ESR was used to measure radicals formed in human teeth, which was quickly followed by the suggestion, in 1968, of the usability of tooth enamel for retrospective dosimetry. [3, p. 2036] This rise in ESR dosimetry using tooth enamel of humans led to the eventual consideration of animal teeth for ESR dosimetry, given some question about the morality and practicality of using human teeth for ESR.

Since this time, teeth of more than 13 mammalian species have been studied in the context of dose reconstruction using EPR, and, where repeated, these studies have had various results in terms of the dose threshold for detection of RIS in teeth. [4] Studies have also seen

variation in terms of sensitivity of response, and range of linear dose response.[4] Though a field with some seeming growing popularity (as evidenced by increased studies on the topic, as well as multiple global intercomparisons) [3, p. 2036], there is still much more to know with respect to the effective use of various animals, due to the variation of tooth morphology between animals, variations in signal response caused by sample preparation techniques, and variation in linear dose response and sensitivity between species.

Overview of Tooth Morphology, and Considerations When Using Mouse Teeth

For use of teeth in dose reconstruction, some understanding of tooth anatomy and morphology should be acquired. There are two primary types of tooth dentition to be aware of: brachidont and ipsodont dentition.

While mouse molar teeth are brachidont teeth (which is the dentition that characterizes human teeth, as well as all carnivorous and omnivorous mammals), mouse incisors are ipsodont teeth – they are of a different morphological composition than the molars. Ipsodont teeth are characterized by continual growth (and continual wear by the animal). Ipsodont teeth do not exhibit the distinct separation between enamel, dentin, and cementum that is seen brachidont teeth; instead, the outer portion of the tooth consists of a combination of enamel, dentin, and cementum.[3, p. 2041] The continual growth means that mouse incisors do not reliably retain dose, and the different composition means that the teeth do not follow the same native response to ESR as the molars. On top of these issues with ESR response and ion retention, the incisors are exposed to sunlight due to their projection from the mouth, which can add spurious signal in ESR analysis. As a result, mouse incisors cannot be used for an accurate lifetime dose

assessment. In practice, then, each mouse has twelve useable molars for the purpose of ESR spectroscopy.

Determining the age of the mouse teeth may be done by looking at the tooth wear stages. Because mouse molars do not grow continuously, daily use causes them to be worn down; stages of wear are associated with mouse aging, and are used to determine the age of the mouse. The primary benefit of knowing mouse age when using mouse teeth to reconstruct dose using ESR is to group mice by age in order to avoid large differences in natural background dose received.

There are a few reasons why mouse teeth may be of interest for use as surrogate dosimeter. Mice are frequently found in the same areas as settled human populations.[5] It may be easier to collect mice in large numbers due to their lifespan and reproductive behavior. Mice are also relatively easy to capture, handle, and transport compared to larger animals.

With these benefits to using mouse teeth as surrogate dosimeters, there are also some drawbacks. One drawback of mice is the size of their teeth; ESR spectroscopy requires a particular amount of material for analysis – this minimum mass requirement is not met by the tooth enamel from one mouse. By comparison, there are many other animals where the teeth from a single individual can provide adequate mass for ESR. With only twelve useable teeth for dose reconstruction per mouse, multiple mice are required per aliquot. Results of one aliquot of mouse tooth enamel analyzed via ESR spectroscopy is necessarily an average over multiple individuals, rather than a reflection of the dose received by a single individual. Use of multiple individuals will be a known source of error in results aiming to reconstruct individual doses.

A second drawback to the use of mouse teeth for ESR dose reconstruction is the difference in sensitivity by mouse teeth when analyzed via ESR compared to human teeth. Response to radiation by mouse teeth yields a 25% smaller ESR signal than human teeth.[14]

This lower sensitivity indicates an increased difficulty of measuring lifetime doses in mice. Higher doses are needed in mouse teeth in order to perform ESR measurements for lifetime dose in mice, where doses between 0.8 Gy to 5.5 Gy have been found to exhibit linear response.[4]

Overview of the Fukushima Daiichi Nuclear Accident

The eastern coast of Japan, in Fukushima prefecture, has played host to two nuclear reactor sites since the 1970s. The first site is known as the Fukushima Daiichi Nuclear Power Station, which is comprised of reactor units 1-4 and is located in the town of Okuma. The second site, known as the Fukushima Daini Nuclear Power Station, is comprised of reactor units 5-6 and is located approximately 11 km away from Daiichi, in the town of Futaba. Both reactors are operated by Tokyo Electric Power Company, with Unit 1 being built in 1971 and subsequent units being brought online until Unit 6 in 1979.[2]

On March 11, 2011, the eastern coast of Japan was hit by the 2011 Great East Japan Earthquake, a 9.0 magnitude earthquake[9], which caused a subsequent 15 meter tsunami to hit the coast approximately 51 minutes later. Following the earthquake, external power was lost, but backup generators remained operational, allowing for safe automated shutdown to be initiated. Upon being hit by the tsunami that followed, however, generators were flooded, initiating a loss of generator power at units 3-6, and loss of generator and battery power at units 1 and 2. The loss of power led the failure of intermittent condenser systems operated in order to cool the fuel. The loss of normal operation by the intermittent condenser systems subsequently led to a reduction in water levels on Unit 1, and resulted in the eventual exposure of the top of the active fuel 2 hours and 45 min following the earthquake, or 1 hour and 54 minutes following the tsunami. Once the fuel was uncovered, heat and pressure in the reactor vessel increased, allowing for core

meltdown 6 hours and 37 minutes after the tsunami, hydrogen buildup, and two subsequent hydrogen explosions – the first, 1 day and 50 minutes following the earthquake, and the second, 3 days, 15 hours, and 27 minutes after the earthquake.[2]

Primary concern regarding doses acquired by people in the surrounding areas revolved around ^{134}Cs, ^{137}Cs, and ^{131}I, however there were multiple releases of primary concern following the accident [5, p. 106] The releases of primary concern included atmospheric releases of noble gasses (^{89}Kr and ^{133}Xe among the large contributors to external dose), as well as atmospheric releases, followed by deposition, of ^{134}Cs, ^{137}Cs, and ^{131}I. [5, p. 107] Estimates indicate that releases were approximately one tenth of those from the Chernobyl accident. [5, p. 107] I-131 has a relatively short half life of 8.02 days, however it can be easily taken up by the thyroid gland. Releases of ^{134}Cs ($T_{1/2}$ = 2.06 years) were another source of primary exposure concern, as was ^{137}Cs ($T_{1/2}$ = 30.08 years). For this study ^{137}Cs was the radionuclide of primary interest based on its long half life. Cs-137 is the largest remaining source of external exposure to mice in affected areas of Fukushima prefecture 8 years after the accident. Any mice with measurable doses that this study aimed to reconstruct would have received dose primarily due to Cs-137 exposure.

Following the accident, people within a 20 km radius of the plant were ordered to evacuate. [5, p. 77] General population and plant workers did not receive large enough doses to suffer from any deterministic effects due to the accident. [5, p. 130] An increased incidence of cancer among the population exposed due to the accident is possible, however the largest reported negative health effects from the accident to date are the social and psychological traumas due to the victims' experiences with the multiple disasters, as well as displacement and social stigma. [5, p. 131] The effects of the aftermath of the Fukushima Daiichi accident have been

far reaching; they can be found not only in Fukushima prefecture were remediation is ongoing, but also through the rest of Japan and worldwide, inside and outside of the academic community.

Chapter 2 – Materials and Methods

All animal procedures were either approved for exemption from oversight from the CSU IACUC, or were approved under protocol 19-8954A. The exemption and the approval protocol may be found in Appendix A and Appendix B respectively. A shortened overview of sample preparation procedure may be found in Appendix D, and a list of individual identification numbers for the Large Japanese Field Mice used and the samples they were used in may be found in Appendix E.

Preparation of Samples

CSU 1

Preliminary work to define a sample preparation procedure adequate for mouse tooth ESR analysis was completed at Colorado State University (CSU). A total of 69 previously expired lab mice (species *Mus mus*), yielded 10 samples of adequate size for ESR analysis. The size of the mouse was used as a rough metric to group 10 mice together per sample. This methodology was chosen to better sort the mice by age in order to reduce possible effects of age on differences in sample response. Skulls of specimen were cleaned and boiled. Teeth were removed using tweezers and mass was recorded.

The teeth were then irradiated to determine if lifetime doses less than 0.8 Gy provided sufficient ESR signal for measurement of radiation dose. Samples were assigned doses between 0.025 Gy and 2 Gy, with smaller doses assigned to samples with larger mass. This decision was made in order to attempt correct for the low amplitude response expected with lower doses and with smaller sample masses. Teeth were irradiated at CSU by the irradiator in room 004 of the

Molecular and Radiological Biosciences building following measurements with an ionization chamber radiation detector to determine required exposure times to get the desired doses. A write-up by Justin Bell, who exposed the teeth in this project, may be found in Appendix C. Four of the samples were exposed in an iterative process in order to make the exposure process more efficient.

Following irradiation, roots of the teeth for each sample were then removed using surgical scissors, and samples were soaked in a 15% wt. NaOH solution for 3 hours, in a 60˚ C sonic bath. For this procedure, NaOH was chosen due to its reported reduction in signal noise at lower concentrations compared to KOH.[14]

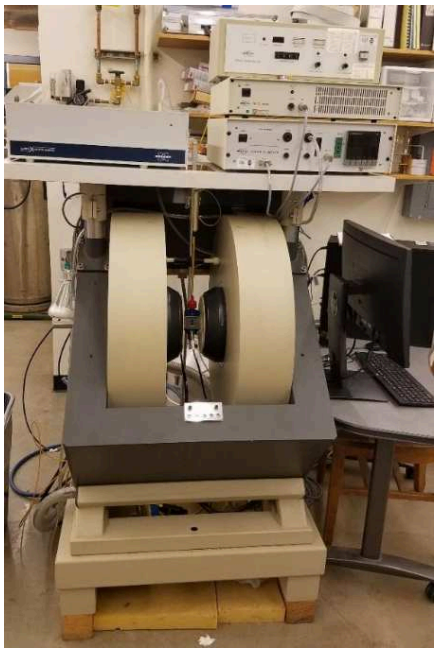


Figure 2: CSU's ESR spectrometry set up – Bruker ESR-300 Spectrometer located in the Central Instrument Facility.

Once teeth appeared to have enough dentin removed (via visual inspection – dentin appears more yellow and chalky compared to enamel, which tends to appear whiter, semi-transparent, and shiner), teeth were decanted of NaOH, rinsed in deionized water, placed in an oven between 40˚ C-60˚ C for 4.5 hours to dry, and then were weighed once more. Samples lost 47%-66% of their initial sample mass following processing.

Japan 1

Preliminary work in Japan at Fukushima University involved much of the same process as at CSU, with the addition of a step of washing teeth in acetone prior to processing. The procedures in Japan also were able to use a stereo microscope for visual inspection of teeth to determine if enough dentin had been removed, or if further treatment with NaOH was necessary.

Molar teeth from 6 mice previously collected in a low background area of Japan, Aizu, were removed from cleaned and boiled mouse specimens. These teeth were rinsed in acetone and subsequently rinsed in deionized water.[2] The initial mass of the sample was 176.2 mg. In order to expose dentin for chemical treatment, the roots were cut off from each tooth using dissection scissors. Following root removal, sample weight was approximately 80 mg (Fig. 3-4).

Teeth were then placed in a container with 15% by weight NaOH solution. The sample remained in the solution for 1 hour and 44 minutes at room temperature, and was then placed in a sonic bath at 60° C for 2 hours. Following this treatment, visual inspection indicated that enough dentin was removed from the teeth, and teeth appeared "shell-like" (Figures 7-9). Teeth were decanted of NaOH, then rinsed with deionized water and decanted, 5 times. Teeth were dried in an oven at 90-95° C for 2 hours. Once samples were dried, they were crushed in a mortar and pestle until uniform grain size had been obtained in order to reduce signal anisotropy. Graduated sieves, between 1 mm and 0.1 mm in diameter were used to achieve uniform enamel size, and appropriate ESR aliquot grain size. [7]

Once crushed, samples were weighed to ensure appropriate sample size for ESR analysis. Prior to spectroscopy, samples were dried overnight (or approximately 10 hours) in an oven at 50-60° C to anneal the sample and remove any spurious signal. [7]

Japan 2

Secondary work followed much the same procedure as Japan 1, except with the addition of treatment of samples with 0.1 M, EDTA (and subsequent rinsing treatments) following treatment in the NaOH sonic bath. 20 mice previously collected from two areas of Japan (10 from low background Aizu, and 10 from high background Takase) were used to create 2 samples. Following the NaOH treatment step, sample preparation proceeded as follows: teeth were rinsed and decanted with DI water. Samples were then placed in a 0.1 M EDTA solution in a sonic bath for 15 minutes. Following treatment with EDTA, teeth were rinsed for 5 minutes in DI water in a sonic bath. Samples were decanted, then placed in 70% ethanol in a sonic bath for 5 minutes. Finally, teeth were placed in DI water in a sonic bath for 5 minutes. Samples were rinsed and decanted, and teeth were dried in an oven at 90-95° C for 2 hours. Once samples were dried, they were crushed in a mortar and pestle until uniform grain size had been obtained using graduated sieves between 1 mm and 0.1 mm in diameter. One of the samples, ("Aizu" in Fig. 18), was prepared before EDTA was acquired, and so was treated with 0.1 M EDTA following NaOH treatment.

Japan 3

Mice from four different areas of Japan (Soma, Tadami, Yamakia, and Takase) were used to create multiple samples. 30 mice collected during research from a low background area of Japan called Tadami were used to create three samples for a dose reconstruction curve, - enough mass remained for one small "leftover" sample. 30 mice from a lower background dose area in Japan, Yamakia, were used to create two samples for age comparison. Finally, 18 mice were from two different areas in Japan were used to create two samples for background dose comparison (mice from low background Soma, and mice from high background Takase). For mice from Yamakia and mice from Tadami, sample preparation procedure was the same as that

in Japan 2. The other two samples (8 mice previously collected from a high background area of Japan, Takase, and 10 mice previously collected from a low background area of Japan, Soma) were put through a DI water wash twice, leading to excessive mass loss, and rendering the samples unusable for ESR.

CSU 2

One more round of sample preparation was necessitated following the third Japan sample analysis. Teeth from 24 mice (12 mice per sample), which were previously collected in low dose areas in Japan, were sent from Japan to CSU for final treatment.

Teeth were going to be separated by mouse age, but the mass of the teeth before beginning the sample preparation was too small; given the significant mass loss from teeth of older mice treated in the Japan 3 procedure, it was possible that too much mass would be lost from the older mouse teeth, and that the resulting sample would have been too small for ESR analysis. To avoid the possibility of too much sample mass loss, teeth were instead separated as an average across the mouse ages. Ages were determined and samples assigned based on tooth wear stage (which corresponds to mouse age); each sample had an average wear stage of 4.7, corresponding to an age of 5-14 months.[6] Samples were prepared following the same methodology as Japan 2, with EDTA treatment occurring before all other treatments. Samples were observed under a stereo microscope at CSU, and adequate amounts of dentin were deemed to be removed (Fig. 10). A few contaminants could be seen (such as hair or lint in samples), which was removed by hand before samples were placed in tubes for ESR analysis.

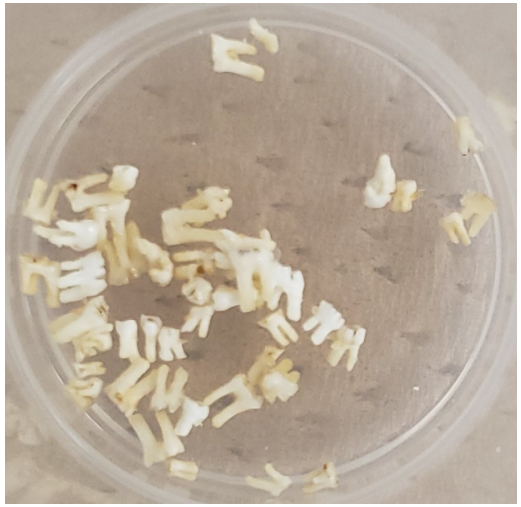


Figure 3: Teeth after acetone wash, prior to root removal.

Figure 4: Teeth following root removal.

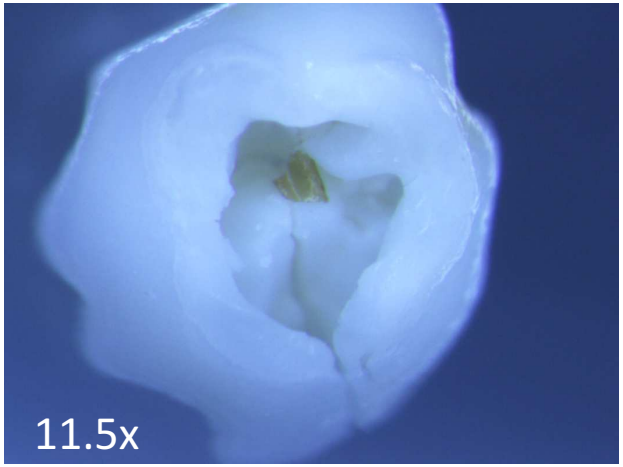


Figure 5: Figure 3: Though significant dentin/pulp removal seems to have occurred, some foreign materials are still seen.

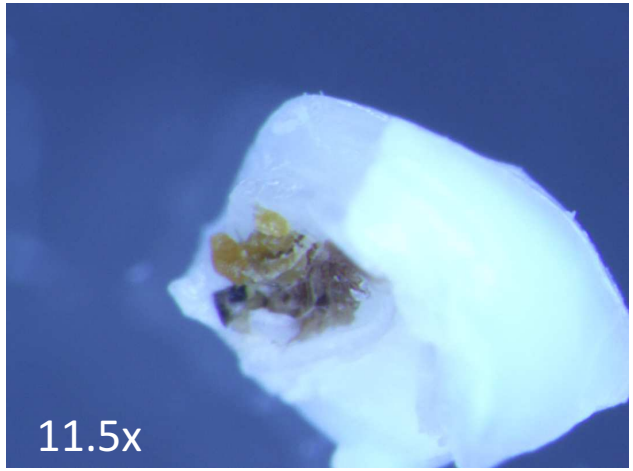


Figure 6: Foreign material impacted in teeth following 2 hours of 60◦ C sonic bath. Further treatment deemed necessary.

Figure 7: Teeth following 30 more minutes in 60◦ C NaOH sonic bath. More certainty of dentin removal, and no foreign particulate matter seen.

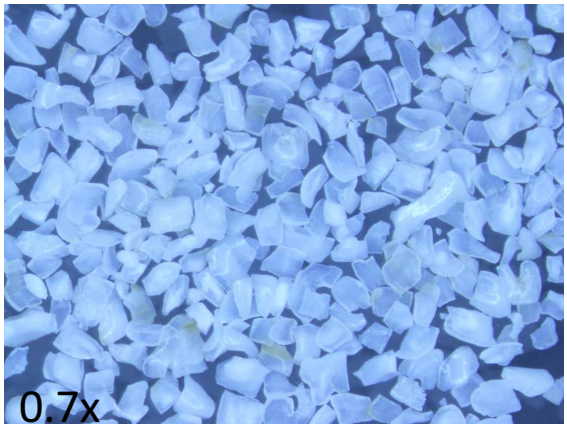
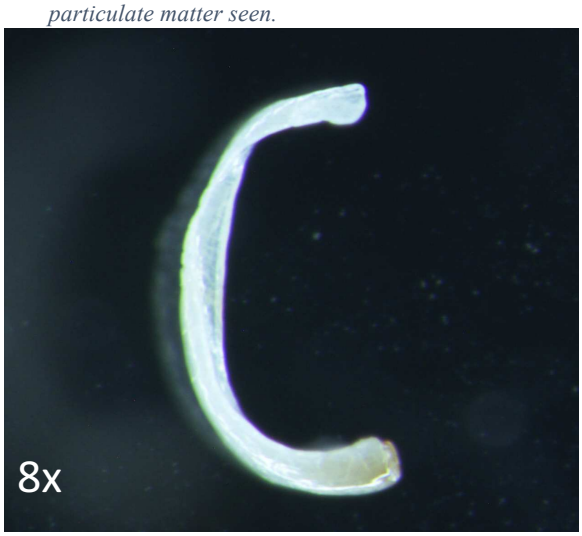


Figure 8:Teeth following crushing and sieving. Teeth are generally uniform in size and no foreign material can be seen (with the exception of stained enamel).



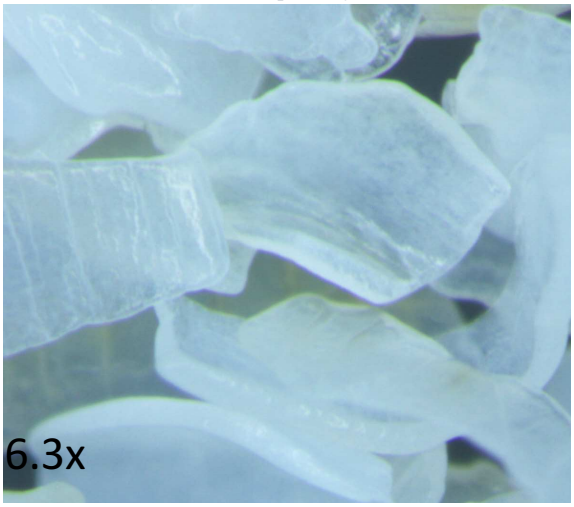


Figure 9: Prepared sample with addition of Titriplex and 70% ethanol rinses. Teeth appear more translucent and shell-like, with more dentin removed.

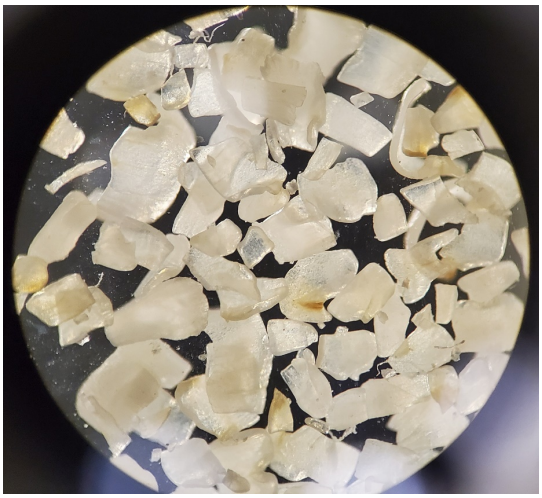


Figure 10:Image of CSU 2 sample following treatment through stereo microscope eyepiece at CSU's MRB building.

CSU ESR Spectroscopy Settings

Spectroscopy was done at CSU following some recommended settings on CSU's Bruker ESR-300 spectrometer. Spectrometry settings included a modulation amplitude of 0.2 mT, a microwave frequency of 9.85 GHz, microwave power of 0.635 mW and a sweep width of 10 mT. Sweeps were centered so that the CO^{2-} signal could be seen; a g-factor of 2.003 was the parameter that defined the center-field of the sweeps. Twenty scans, each of twenty seconds in duration, were taken for each measurement.

Because modulation amplitude can affect the width and appearance of the output spectrum, some sweeps were taken using the highest modulation amplitude possible on the spectrometer (1.92 mT). Initial CSU 1 spectrometry was taken with this large modulation amplitude; spectra for CSU 1 samples were retaken at the same time as the CSU 2 spectra were taken, however this time with the low, recommended modulation amplitude. Reducing the modulation amplitude yielded spectra with no significant baseline shift – this is the opposite result of sweeps taken with large modulation amplitudes. Because high modulation amplitude can significantly distort the output signal, the spectra taken with high modulation amplitude (and resulting large baseline shifts) are unreliable reflections of the signals of interest (the CO^{2-}).

Chapter 3 – Results and Discussion

Baseline Shifts and Dose Reconstruction

CSU 1

Initial work used a Cs-137 radiation source to deliver doses to mice teeth to test the ESR signal response. Measurements were initially taken with a large spectrometer modulation amplitude rather than the small modulation amplitude recommended for the spectrometer. For the spectra taken with a large modulation amplitude Doses below 1 Gy did not exhibit any clear ESR signal; they had low amplitudes leading to excessive noise in the spectra. The 1 Gy and 2 Gy doses resulted in a clear increase in signal amplitude compared to the 0.025 Gy and 0.5 Gy doses, but were accompanied by the same issues with the baseline on either side of the signal of interest, as well as noise. An attempt to create a linear dose reconstruction curve from these data result in large uncertainties, and no clear relationship between dose and signal peak-to-peak amplitude.

Spectra for these samples were re-taken a year later at the same time as CSU 2 samples and using the same spectrometry parameters, including the low modulation amplitude recommended for CSU's spectrometer (0.2 mT, or 2 G); when CSU 1 sample spectra were taken with a low modulation amplitude, there was little to no baseline shift – baseline shift was between 2% and 4.1% of the peak to peak signal amplitudes for the six samples that were analyzed (0.25 Gy, 0.5 Gy, 0.75 Gy, 0.8 Gy, 1 Gy, 2 Gy). This is likely because too high of a modulation amplitude can distort spectra for dose reconstruction with ESR. Appropriately low modulation amplitude is necessary for an undistorted output. Though the baseline shift was much smaller with the low modulation amplitude, using these data to create a linear dose

reconstruction curve from the RIS did not result in a linear relationship between amplitude and dose.

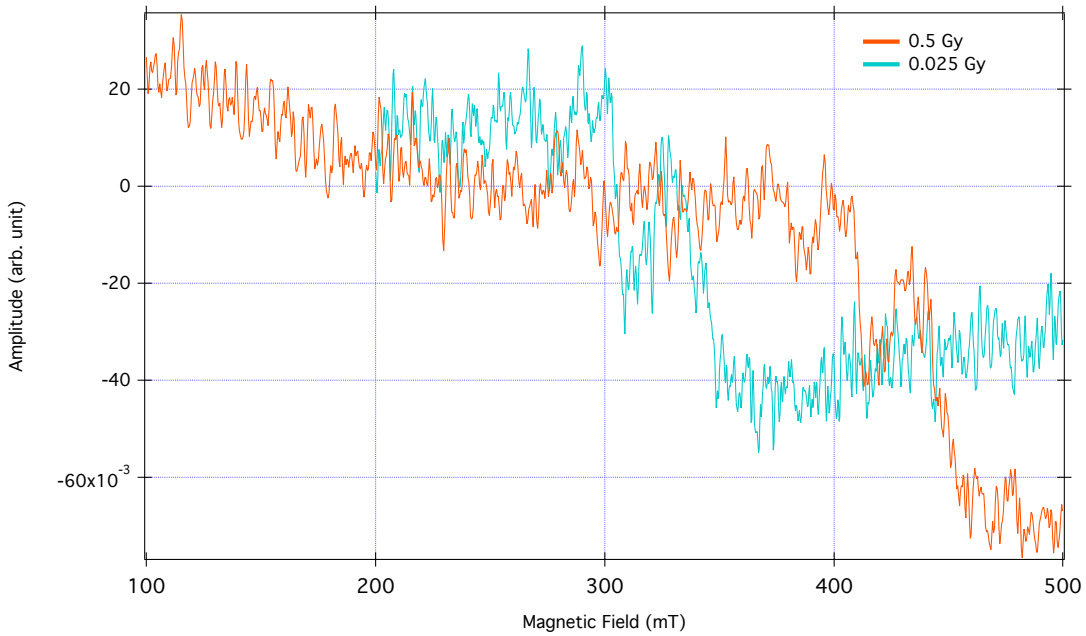


Figure 11: Graph of 0.025 Gy and 0.5 Gy sample spectra taken with large (1.92 mT) modulation amplitude. Some possible signals may appear, but amplitude is small, and there is no level baseline. Signal consists mostly of noise.

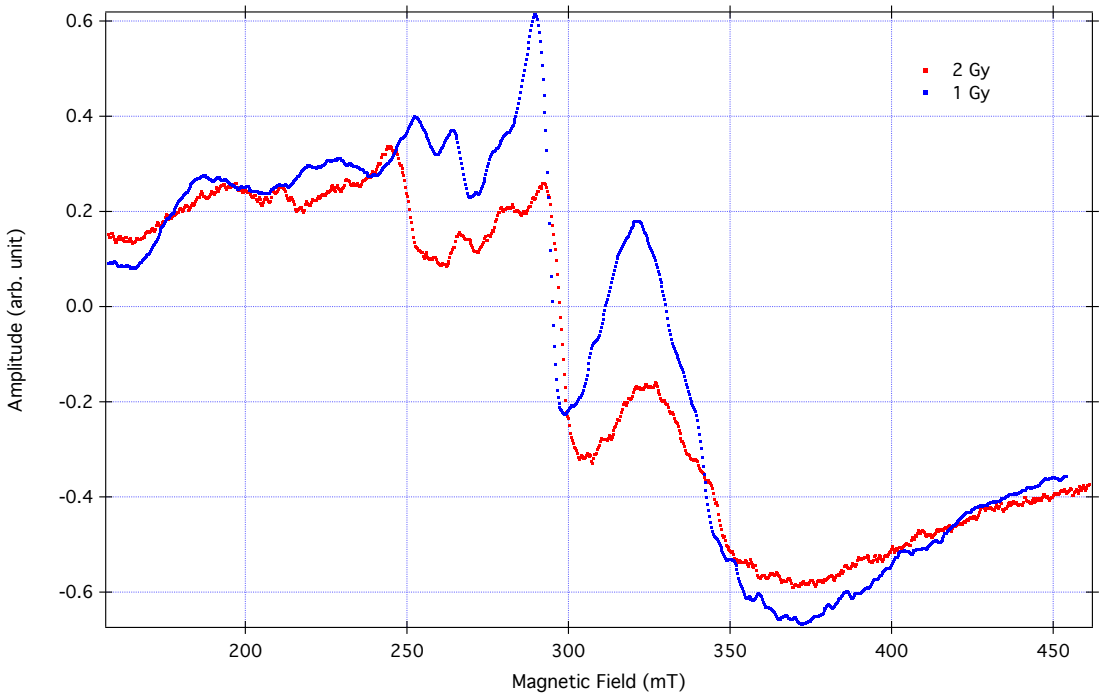


Figure 12: Graph of 1 Gy and 2 Gy sample spectra taken with large (1.92 mT) modulation amplitude. Signals have higher amplitude response than 0.025 Gy and 0.5 Gy spectra, and are distinguishable from noise, however large baseline shift interferes with interpretation of results, and the large modulation amplitude results in distortion of the signal.

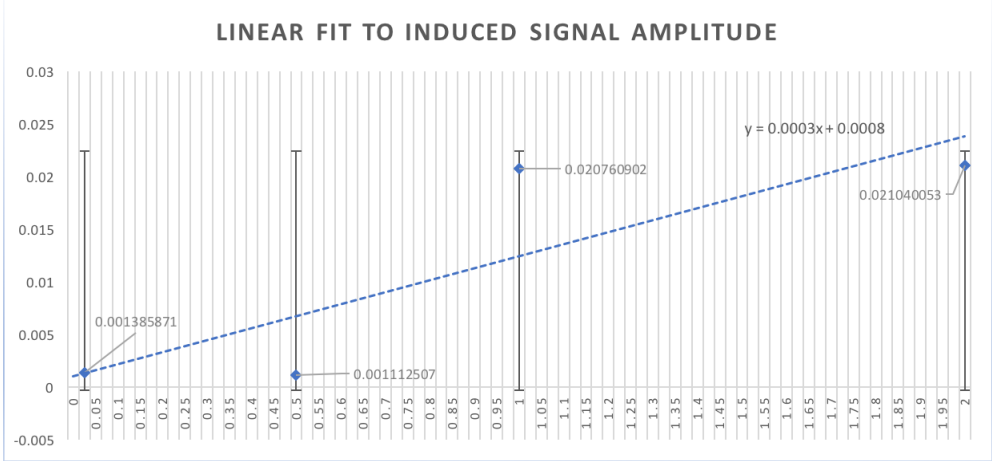


Figure 13: Attempted linear dose reconstruction curve. Sample RIS amplitudes are not linearly proportional to dose.

A second linear dose reconstruction curve from these preliminary spectra was created, however the samples were not normalized by mass, which is necessary in order to ensure that amplitudes of RIS are comparable and reduce uncertainty. This is one difficulty with the use of mice teeth, and one reason why it is necessary to use at least ten mice per sample – more mice are needed to ensure that, despite any variation in sample mass loss, samples will have enough mass for ESR spectroscopy and can also be normalized by mass.

Because they were not normalized by mass, these spectra and preliminary results cannot accurately represent the usability of mice teeth for dose reconstruction. Other studies have shown linear response at doses between 0.8-5.5 Gy.[4] These results need mass normalization in order to be useable for redefining or confirming the current doses for which dose reconstruction using mice teeth shows linear response.

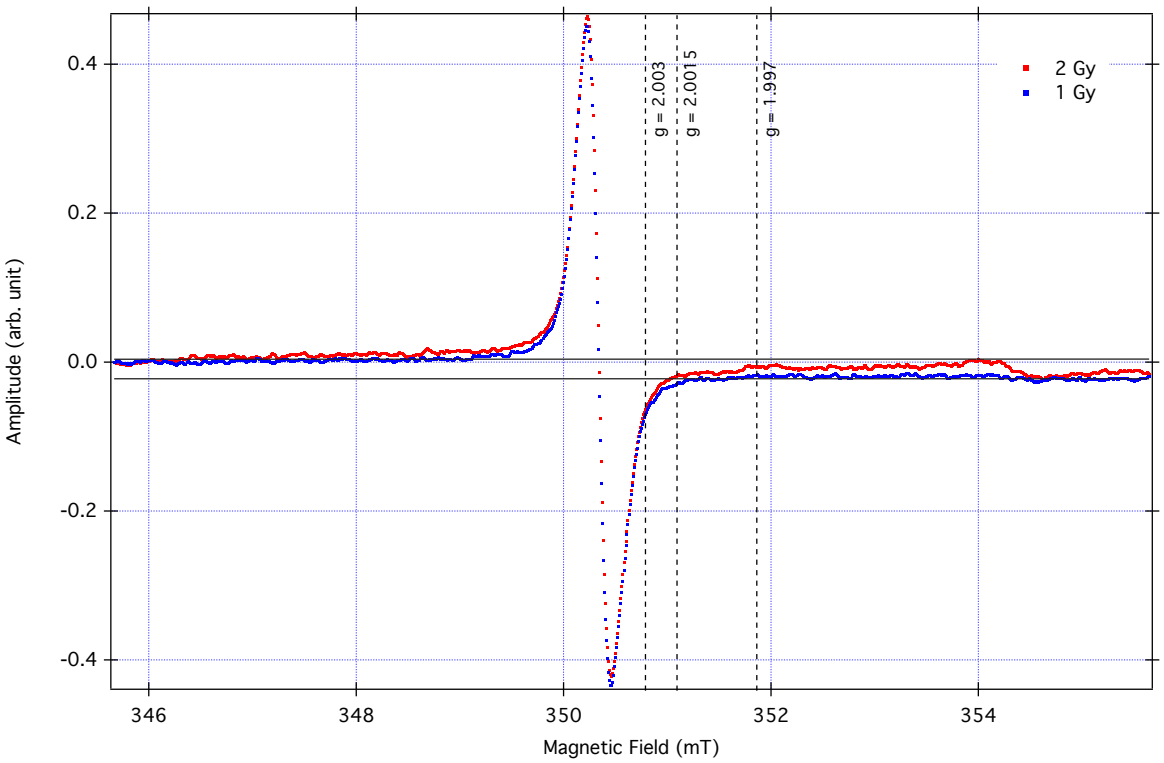


Figure 14: 1 Gy and 2 Gy CSU 1 sample spectra, retaken a year later. Baseline is not as prominent due in part to the small modulation amplitude setting (0.2 mT).

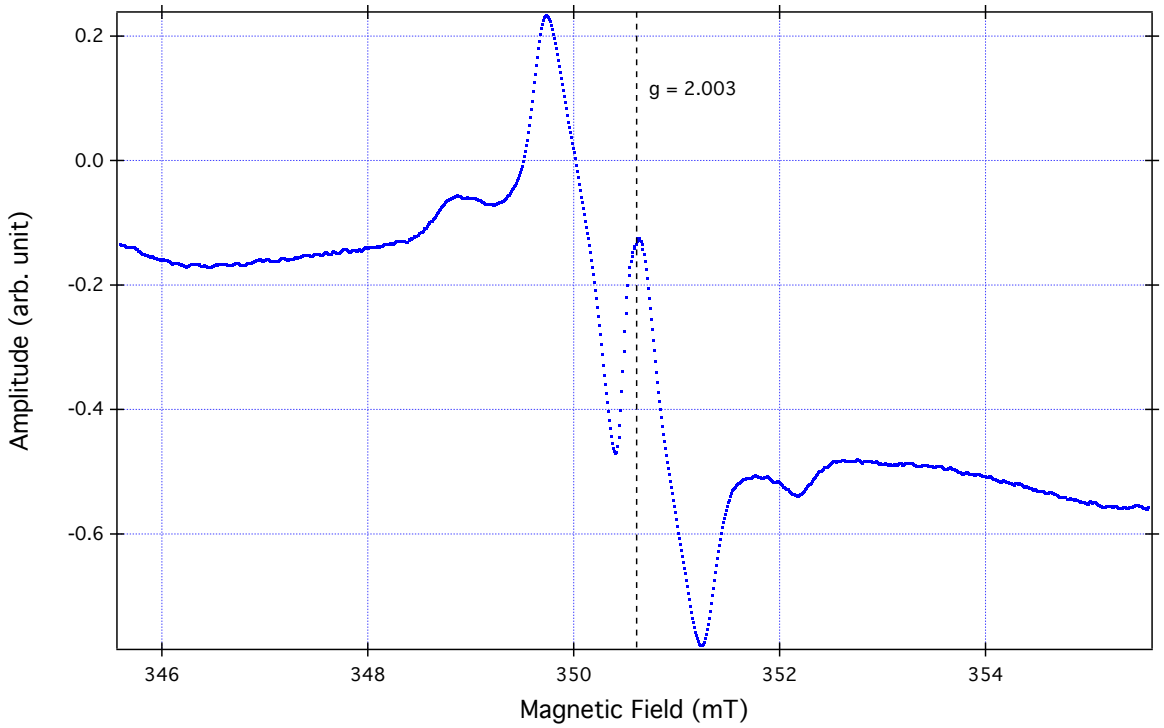


Figure 15:1 Gy CSU 1 sample spectrum, retaken a year later. Signal distortion caused by the large, 1.92 mT, modulation amplitude can be seen in comparison to the spectrum of the 1 Gy sample with a low modulation amplitude in Fig. 14.

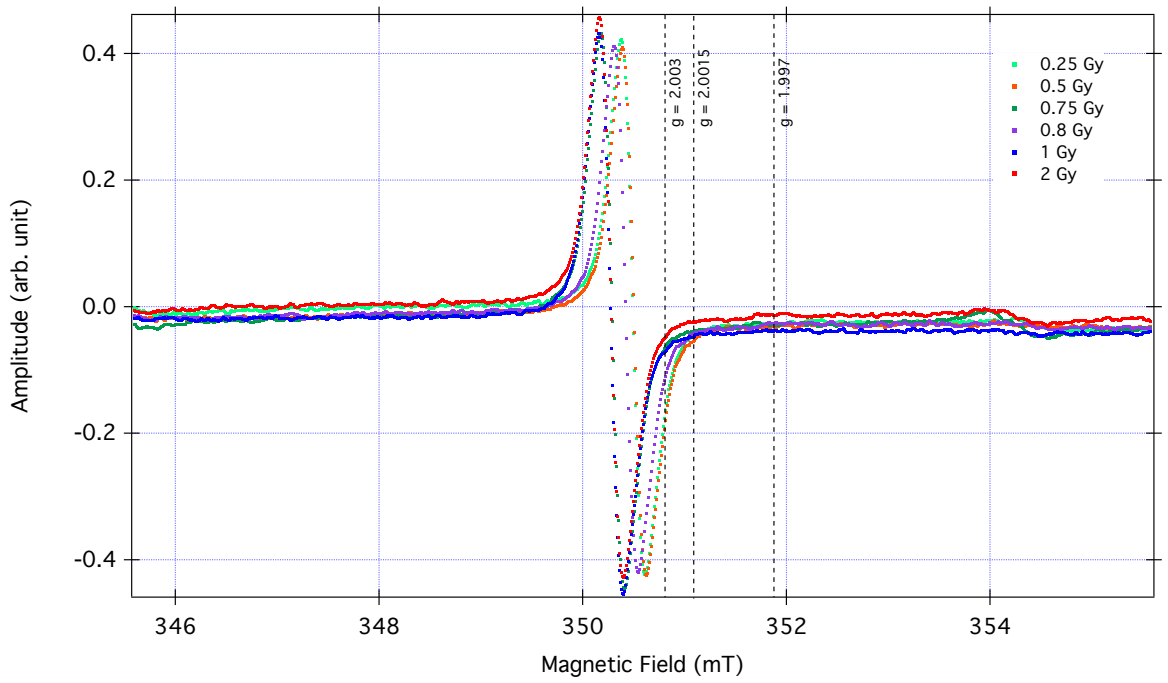


Figure 16: Comparison of amplitudes of CSU 1 sample spectra re-taken a year after their initial preparation. Samples with doses of 0.25 Gy, 0.5 Gy, 0.75 Gy, 0.8 Gy,1 Gy, and 2 Gy are compared. Sample signal peak to peak amplitudes do not seem to vary with dose.

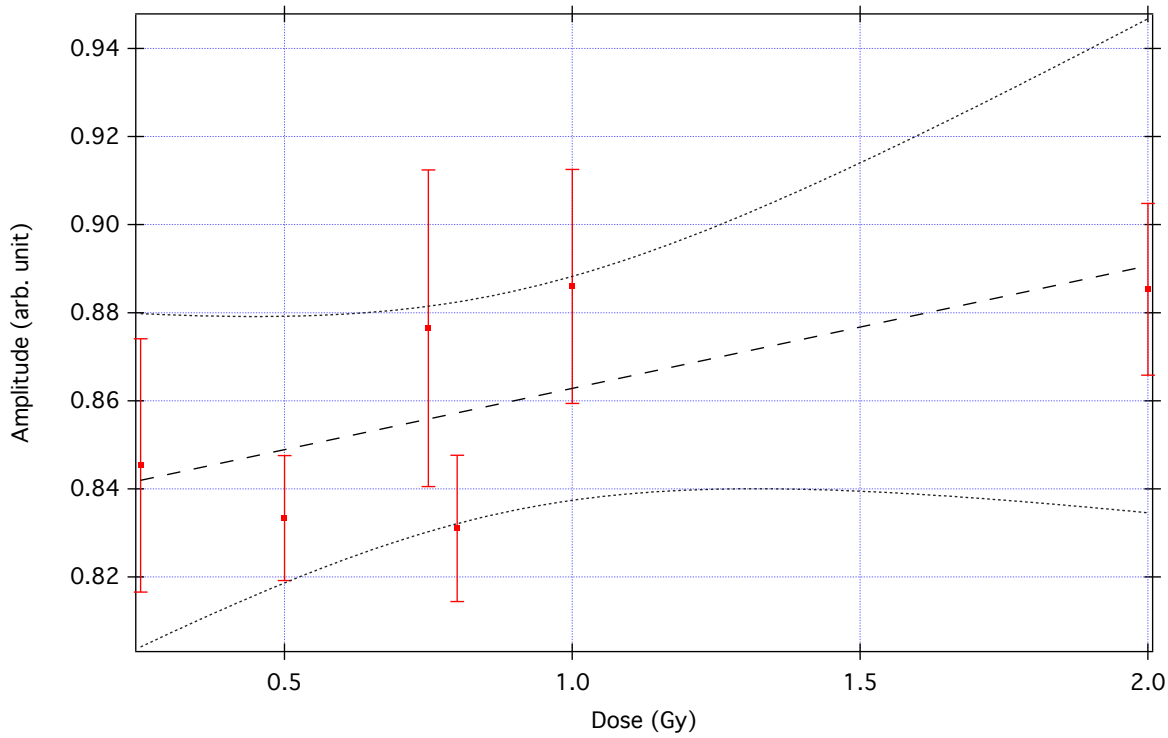


Figure 17: Attempted linear dose reconstruction curve created from re-measured CSU1 spectra. Sample RIS amplitudes are not linearly proportional to dose. Though baseline shift was small, it still contributes to error in the signal amplitude.

Because the large baseline shift in the spectra taken with large modulation amplitude was resolved when taken a year later with low modulation amplitude, it is unlikely that significant baseline shift seen in initial CSU 1 spectra is a result of lack of treatment with EDTA. It is possible that *Mus mus*, the species of mouse used for these samples, do not have equivalent iron composition in their teeth, hence the a small baseline shift despite a lack of EDTA treatment.

Japan 1

Mice that were initially measured in Sendai showed a shift in baseline. The initial spectral data are unavailable for graphing. It was hypothesized that spurious signal and baseline shifting was caused by possible iron atoms in the mouse teeth. Changes to the sample preparation procedure were made in an attempt to correct the baseline shift; one of the primary changes was the addition of a 0.1 M EDTA treatment in sample preparation procedure, which would help resolve spurious signal that was hypothesized to be due to interference of iron components in the teeth.

Japan 2

The second samples measured in Japan were the start of an important sample preparation step – the inclusion of EDTA. The spectra from these results had no distinct baseline shift – the baseline was estimated to be 0% of the signal amplitude (Fig 18). These results indicate that washing teeth with EDTA successfully removes spurious signal in output spectrum. The resolved baseline shift allowed for comparison of the lifetime doses acquired by mice living in two areas in Japan – one sample was from an area in Japan with higher levels of natural background radiation (Takase), and one sample was from an area with lower levels of natural background radiation (Aizu). As expected, mice from areas with higher background dose had a larger amplitude RIS, while mice from areas with lower background dose had a smaller amplitude RIS.

As noted in the methodology section for the Japan 2 samples, one of these samples was treated with EDTA following treatment with NaOH. Because the baseline of both samples was resolved despite different treatment order, these results indicate that samples may be treated with EDTA either before or after treatment with NaOH with no effect on the baseline of the output spectra.

These samples were also analyzed under an SEM to determine whether any dentin was left in the sample, as leftover dentin could cause spurious signal. Two leftover pieces of tooth from these samples were coated with a thin layer of gold, and then examined under an SEM (Figures 19-23).

The first piece of tooth analyzed had consistent, recurring holes throughout; it was determined that this piece of tooth was largely composed of dentin that was not completely removed during sample preparation. The consistent holes are called dental tubules, and are characteristic remnants of the cells that allow for formation of dentin: odontoblasts. Dental tubules extend all the way from the pulp of a tooth, to the dentin-enamel interface. The image from the second piece of tooth presented an orderly, columnar arrangement in structure; this structural arrangement of the sample was determined to be characteristic of the enamel portion of tooth. It was determined that no dentin remained in this second piece of tooth.

The different structure of these two pieces of tooth indicate that chemical methods of tooth preparation for dose reconstruction with ESR may not be completely adequate for removing dentin from enamel. Any remaining dentin can be a cause of noise and distortion of signal when taking ESR spectra due to the different radicals that contribute to signals of interest. The remaining dentin could be a reason for baseline shift if not adequately removed. These

samples indicate that, though some dentin likely remained in the samples, it was not a significant contributor to baseline shift.

Leftover pieces of tooth were also analyzed using EDX. This analysis was attempted to determine if any iron molecules in the teeth could be contributing to signal noise and baseline shift. Analysis indicated that there was no iron in the sample, implying that the inclusion of EDTA treatment may have removed metal ions that could cause spurious signal.

Samples were created to analyze mice from areas with two different dose rates. Distinct amplitudes can be seen, presumably depending on the background dose rate.

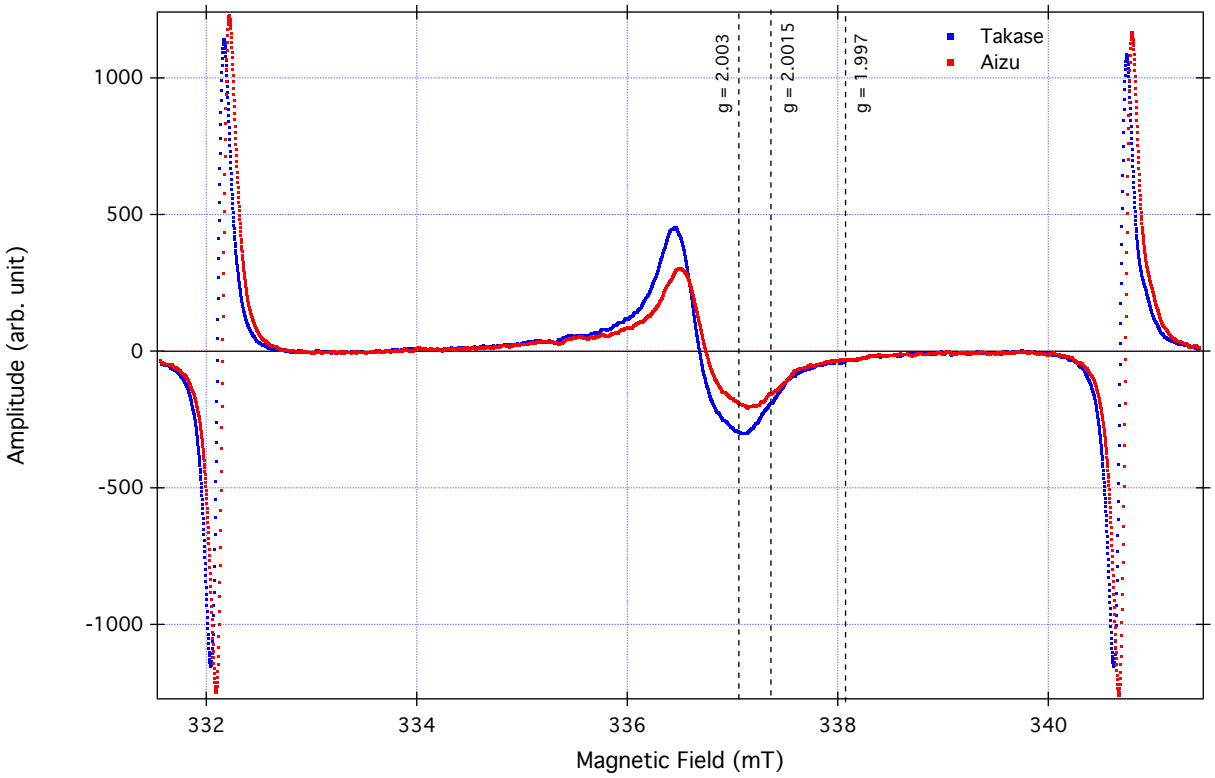


Figure 18: : Comparison of mice receiving low lifetime doses (Aizu), and higher lifetime doses (Takase) from Japan 2 samples.

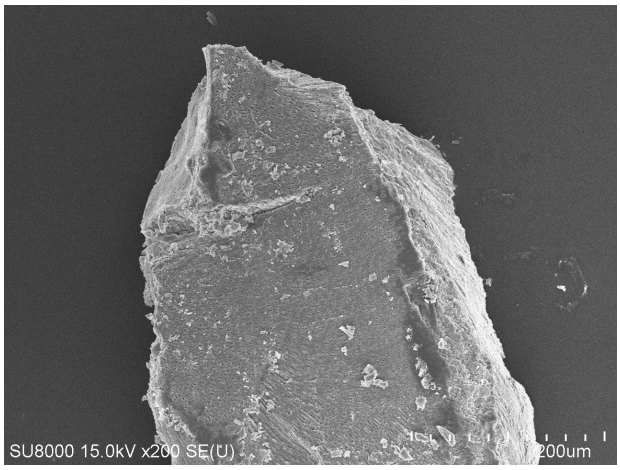


Figure 19: First tooth fragment from Control2190627 - zoomed out to 200 µm scale.

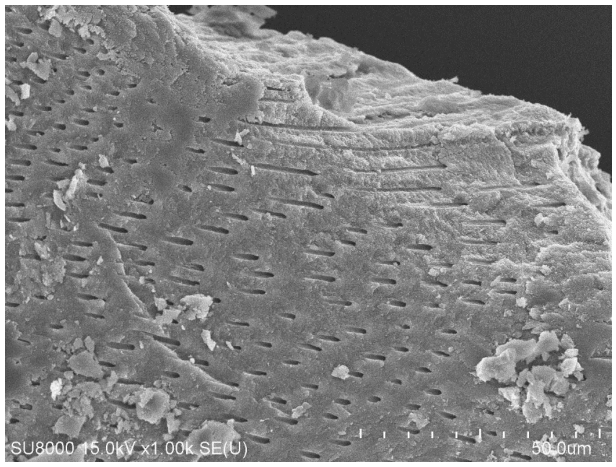


Figure 20:First tooth fragment zoomed in to 50 µm scale - holes characteristic of odontoblasts within dentin.

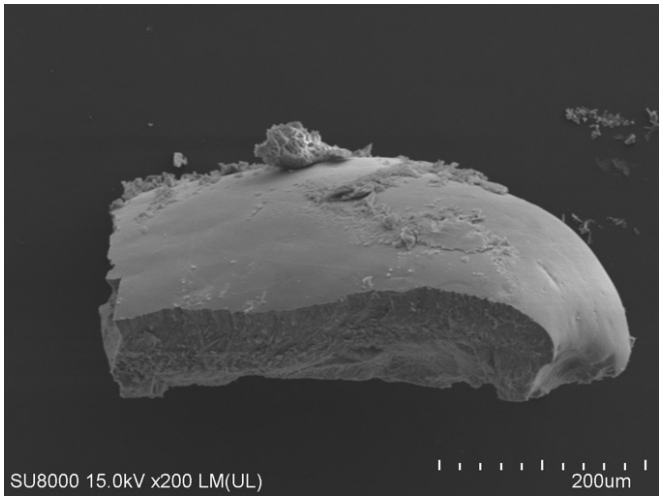


Figure 21: Second tooth fragment from Control2190627 - zoomed out to 200 µm scale.

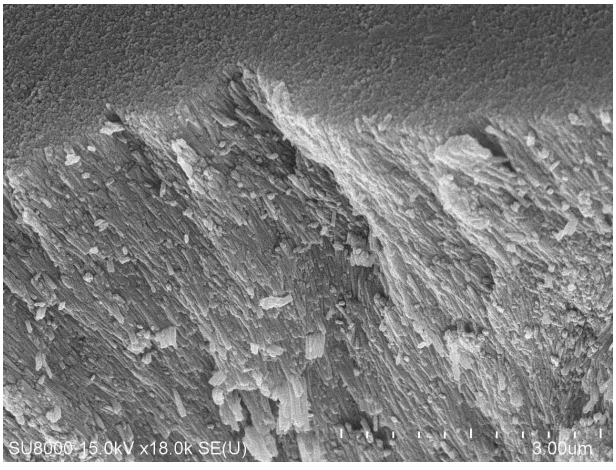


Figure 22: Second tooth fragment sample zoomed in to 3 µm scale - columnar alignment, indicating presence of only enamel.

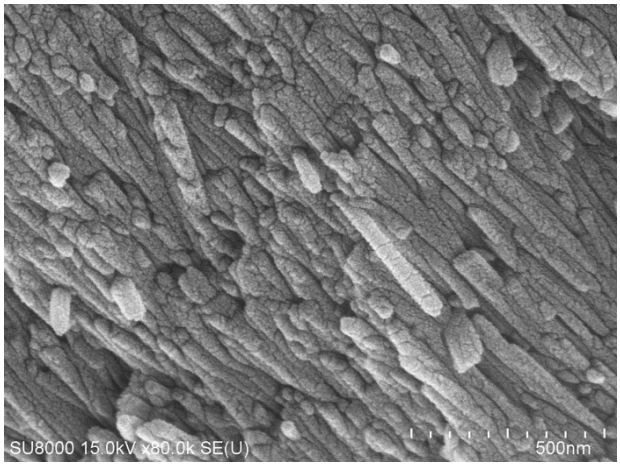


Figure 23: Second tooth fragment sample zoomed in 500 nm scale. Closer image of enamel arrangement.

Japan 3

Japan 3 samples were collected from low background areas of Japan, and were prepared with the intent of irradiating them to analyze the range at which mouse teeth will show a linear response to radiation. The spectra of the unirradiated Japan 3 samples, however, once again had baseline shifts; baseline shift was 61.9% of the peak to peak signal amplitude. These results indicate that the change to sample preparation methodology did not consistently remove the problematic baseline shift in the samples; treatment of the teeth with EDTA may not affect whatever causes spurious signal.

The baseline shift seen in these samples could have been partially a result of the order of sample preparation steps, since the samples were treated with EDTA following treatment with NaOH. However, because one of the samples from Japan 2 was also treated with EDTA following NaOH treatment and had a level baseline, order may not be the cause of baseline shift. More should be done to understand the effects of changing the order of certain sample preparation steps.

Two of the final samples, which were made from mice from Yamakia, were separated by age, and experienced a distinct difference in the amount of mass lost from sample preparation. The teeth of the mice that were one or more years old (as determined by the wear stage of 5 or more that characterized their teeth) saw seemed to in some way less robust than the teeth of mice that were 1 month to 14 months old (wear stage of 1-4 determined for their teeth).[6] Because mice have brachidont molars, older mice with higher levels of wear also had less tooth enamel to work with. This makes it difficult to work with a population of older mice rather than a population with mixed ages.

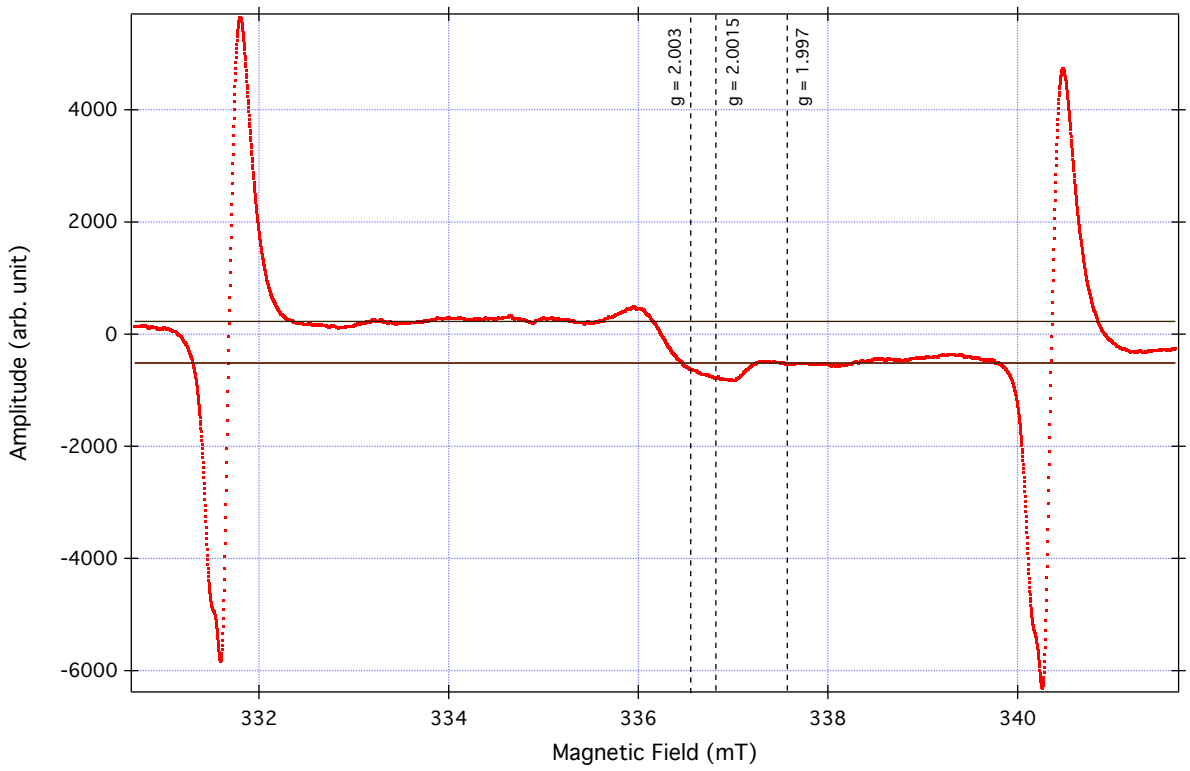


Figure 24:One of the final control samples prepared in Japan (Control 5 190729). Baseline shift is evident, at 68.1% of the peak to peak amplitude, despite EDTA treatment.

CSU 2

Spectrometry results of these final, unirradiated samples showed that the baseline shift was generally resolved once more. There is a small baseline shift – baseline is shift 1% of signal peak to peak amplitude.

The disappearance of the baseline shift, followed by the reappearance when changing spectrometer modulation amplitude indicated a few things. First, EDTA may be important for removing spurious signal in mice teeth, as the baseline shift was largely resolved in these samples. It may be important to treat the teeth with the EDTA in a specific order (before treatment with NaOH); samples that were untreated with EDTA, or that were treated with EDTA following treatment with NaOH are the samples that exhibited significant baseline shifts. The Japan 2 samples, however, did not show an effect to the baseline regardless of the order of

treatment with EDTA. Second, refining software settings can make a significant difference in the output spectra; changes to modulation amplitude can result in differences in spectra appearance, which may contribute to a baseline shift.

One possible reason for the issues with noise and baseline shift could have to do with the enamel structure of mouse tooth enamel compared to human tooth enamel. Mouse tooth enamel exhibits a complex interlocking pattern of columnar enamel structure, where-as human tooth enamel is similar, but generally more consistent in its structural layout.[11] Because ESR dosimetery relies on adequate alignment of enamel structure, the complexity of mouse tooth enamel could cause some problems with signal.

Some of the samples taken at CSU were noisy and unresolved, and required shifting samples in the spectrometer, or tapping the tubes to settle samples towards the bottom. Since shifting the samples has a large effect on the resulting spectrum, mouse enamel structure may be the reason for some of the variation in signal usefulness that was seen in this study.

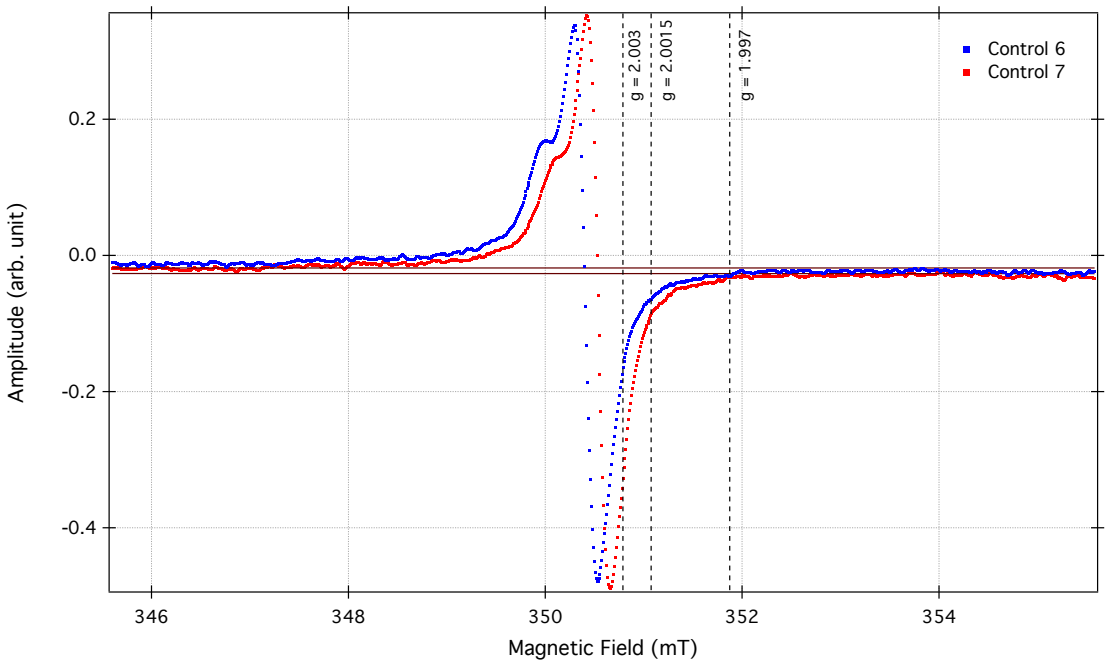


Figure 25: CSU 2 spectra. Native signals of the mouse teeth are able to be seen clearly, and baseline shift is only 3% of peak to peak amplitude. Spectra were taken with 0.2 mT modulation amplitude

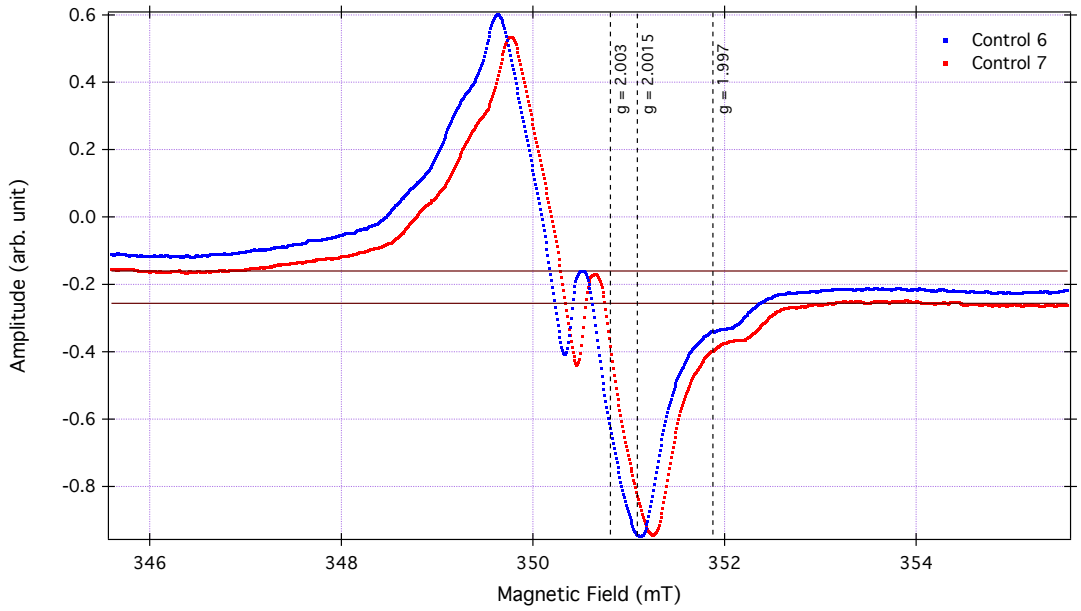


Figure 26: Native signals of the CSU 2 spectra using a 1.92 mT modulation amplitude. Large modulation amplitude results in distortion of signal is distorted compared to the 0.2 mT modulation amplitude of the spectra in Fig. 25.

Feasibility Issues

High Dose

One difficulty with using mice for lifetime dose reconstruction is the high total dose that may be necessary for linear response of the RIS with dose, and for detection of the RIS compared to noise in the spectrum. Previous studies have indicated that doses from 0.8 to 5.5 Gy are required for linear dose reconstruction. This was unable to be effectively tested in this study. Mice may not be an ideal species to use for dose reconstruction with ESR, unless they're being used in the aftermath of a radiation accident involving large, acute doses.

Number of Individuals

Another difficulty with using mice teeth is the necessity to use multiple individuals to generate a sample with enough mass for ESR spectroscopy. For this study, at least six mice were needed for each ESR aliquot analyzed, though sometimes samples that had more than six mice were not large enough to proceed with ESR. Anywhere from 68%-94% of the initial tooth mass

was lost during the preparation for the teeth for ESR. This estimate varied depending on the treatment procedure, and the age of mice used, but it may be a helpful for determining whether a sample mass will meet the minimum mass requirement for ESR analysis; it does not account for two samples that lost 97% of their initial mass, because these two samples were erroneously put through a redundant procedure, causing them to be in a sonic bath 1-2 hours longer than other samples. This estimate also does not include the 47%-66% estimate from the CSU 1 samples, which did not go through the sample preparation procedure in the same way as the other samples. When not separated by age, and when put through the sample preparation procedure with the determined, appropriate steps (Japan 2-CSU 2), sample mass loss ranged from 68%-92% of the initial sample mass.

Because multiple mice are required for each sample, samples automatically represent an average dose among individuals. This unavoidable consequence of mouse tooth size can result in increased result uncertainties. One benefit to using more mice per sample is that it could produce results with higher amplitude, and better low dose response – larger mass used for spectroscopy can result in more clear output.

The large number of mice required per sample also increase the logistical difficulty of finding and capturing enough mice in an area. The area mice are collected from should ideally be restricted to reduce uncertainty in the doses that the sample represents. Although mice do not present the logistical difficulties or handling dangers of capture and transport that larger animals can present, it can be difficult to trap large enough numbers of mice to generate samples representative of a single area. For example, in this study, mice could not be captured from areas with high levels of background radiation; an increase in environmental disturbances due to decontamination activity was thought to reduce mouse populations in those areas.

Dentin and Enamel Separation

Small tooth size can make separation of dentin from enamel more difficult. Manual methods of dentin removal that are used on other animals and humans cannot be used on teeth as small as mouse teeth. Chemical methods for dissolution of dentin may not reliably separate the dentin from enamel. Despite the appearance of adequate dentin removal when visually inspecting samples using a stereo microscope, SEM analysis of one of the Japan 2 samples indicated that dentin was not completely removed. The small teeth cannot be easily split open to expose dentin to chemical processes, and each chemical processing step (particularly those that make use of the sonic bath) can wear away at not only the dentin, but also the valuable enamel component of the teeth.

Practical Inconveniences

Because it is also important to consider the practicalities of a study, the logistical considerations of handling small teeth will also be discussed. Though mice are more manageable to collect and process compared to larger animals, their small teeth are more difficult to see and handle than the teeth from larger animals. One handling difficulty experienced in this study, for example, is the large effect of static electricity on the small mouse teeth. There are ways to help reduce the effects from static electricity (such as using a dryer sheet on an ESR tube when filling it), but in general, lower humidity labs may find this to be more of an issue than labs with higher humidity.

Care also needs to be made when pulling teeth, cutting off roots, or generally holding a tooth with tweezers; even before treatments to remove dentin, teeth are difficult to find if they're propelled from being pinched too tightly, or dropped from being held too delicately. This is primarily worth mentioning because of the importance of each tooth for meeting required sample

mass for ESR spectroscopy. These practical difficulties are not reasons to avoid using mouse teeth with ESR analysis when considered alone, but they are valuable to keep in mind when considering practicalities of sample preparation (such as time, effort, or sample preparation environment), and the consideration to use mouse teeth, teeth from other animals, or other inorganic substances altogether for ESR analysis.

Chapter 4 – Conclusion

Preliminary work intended to analyze the use of mouse teeth, particularly those of the Large Japanese Field Mouse (*Apodemus speciosus*) as a means of reconstructing lifetime radiation dose. Initial samples that were analyzed showed a problematic shift in the baseline signal of the ESR output, which introduces uncertainty in subsequent dose reconstruction, however, these baseline shifts were resolved when taking spectra a year later with different spectrometer settings. By this, it has been determined that care needs to be taken when choosing spectrometer settings, particularly modulation amplitude. High modulation amplitude can distort signal, but can make signal more apparent; low modulation amplitude reduces distortion of the spectral output, but can reduce signal intensity.

Additional attempts to resolve the baseline shift included changes to the sample preparation procedure (addition of a wash step using EDTA to remove possible iron components in teeth), and investigation of samples under SEM and with EDX. Some samples treated with EDTA showed a resolved baseline shift (a level baseline), while others did not, possibly depending on the order in which they were treated; specific procedural order may affect spectra baseline, though it did not consistently do so in this research. Because EDTA treatment did not consistently resolve baseline shift, iron components in teeth may not be the cause of the baseline shift issues seen. Analysis of teeth treated with EDTA using EDX indicated that no iron could be found in the sample as a possible contributor to the signal baseline shift.

SEM analysis indicated that chemical treatment of mice teeth may not completely remove dentin, which can cause competing signal. Incomplete dentin removal is an issue that should be looked further into –unfortunately in this study, SEM analysis was only completed for one of the

samples. While the inconsistency of the baseline shift resolution could be explained by possible inconsistencies in the amount of dentin removed, because the baseline shift of the CSU 1 samples was resolved when they were re-analyzed, dentin may also not be the cause of the baseline shift.

When the first CSU 1 sample spectra were taken, these samples were not dried in an oven at 52° C for at least nine hours. This is an important step for removing water from samples that can contribute to spurious signal. It is assumed that the samples were dried before spectra were taken in Japan, however whether or not they were dried is unknown – samples were dried after being completed, but it is more important to dry the samples prior to taking spectra in case any water returns to the sample. The CSU 1 samples were dried overnight at 52° C prior to having spectra re-taken a year later. It is possible that the inclusion of this step is a part of why the sample baseline was smaller, despite the CSU 1 samples not having been treated with EDTA. It is worth looking further into the sample response via ESR when dried compared to when not dried overnight.[3]

Due to the low response of mice teeth under ESR analysis (both on their own, and compared to human teeth) in combination with the uncertainty introduced by the baseline signal shift (which was unable to be reliably resolved), mice teeth may not be an ideal option in lieu of other animals for dose reconstruction – either as surrogates for human teeth, or on their own for understanding of dose to an environment.

Multiple mice must be used for ESR of mouse tooth enamel due to the inability for one mouse to provide enough mass for ESR analysis, causing ESR of small animals like mice to be an average across multiple individuals, rather than representative of individual dose. Samples lose anywhere from 68%-97% of the initial tooth mass from the beginning (prior to root

removal) to the end of the sample preparation process. This should be kept in mind when considering the amount of mice that will contribute teeth to a sample. More mass is lost from older mice due to the worn down state of their enamel, and due to the effects of sample preparation on mouse teeth. It has been hypothesized that animals with carious teeth have more signal noise – this could be a contributor to both sample baseline shifts seen in this study, as well as to the greater mass lost by older mouse teeth during sample treatments.

Neither sample preparation steps, nor spectroscopy settings are consistent in literature. This is perhaps partially due to the lack of understanding of dose reconstruction using ESR in non-human teeth. Therefore, this research has shown that, in order to determine suitability of various non-human teeth for dose reconstruction using ESR, more needs to be done to define consistent procedural steps for reliable dose reconstruction, as well as to set consistent and optimal measurement settings for spectroscopy.

References

[1] Arana-Chavez, V.E., Massa, L.F. Odontoblasts: the cells forming and maintaining dentine. The International Journal of Biochemistry & Cell Biology 36: 1367–1373; 2004. DOI:10.1016/j.biocel.2004.01.006

[2] Atomic Energy Society of Japan, The Fukushima Daiichi Nuclear Accident: Final Report of the AESJ Investigation Committee. Maruzen Publishing Co., Ltd., Tokyo; 2014. DOI: 10.1007/978-4-431-55160-7

[3] Fattibene, P., Callens., F. EPR dosimetry with tooth enamel: A review. Appl. Radiat. Isot. 68: 2033-2116; 2010. DOI: 10.1016/j.apradiso.2010.05.016

[4] Harshman, A., Johnson, T. A Brief Review—EPR Dosimetry and the Use of Animal Teeth as Dosimeters. Health Physics 115(5): 600-607; 2018. DOI: 10.1097/HP.0000000000000899

[5] Harshman, A., Toyoda, S., Johnson, T. (2018) Suitability of Japanese wild boar tooth enamel for use as an Electron Spin Resonance dosimeter. Radiat. Meas. 116: 46-50; 2018. DOI: 10.1016/j.radmeas.2018.07.001.

[6] Hikida T., Murakami, O. Age determination of Japanese wood mouse, *Apodemus speciosus*. Jap. J. Ecol 30: 109-116; 1980.

[7] IAEA. The Fukushima Daiichi Accident. Report by the Director General. PUB 1710. Vienna. 2002; ISBN: 978-92-0-107015-9

[8] IAEA. Use of electron paramagnetic resonance dosimetry with tooth enamel for retrospective dose assessment. Report of a coordinated research project. IAEA-TECDOC- 1331. Vienna. 2002

[9] JAPAN METEOROLOGICAL AGENCY, Information on the 2011 off the Pacific Coast of Tohoku Earthquake; 2015 http://www.jma.go.jp/jma/en/2011_Earthquake/Information_on_2011_Earthquake.html

[10] Joannes-Boyau, R. Detailed Protocol for an Accurate Non-destructive Direct Dating of Tooth Enamel Fragment Using Electron Spin Resonance. Geochronometria 40: 322-333; 2013.

[11] Møinichen, C.B., Lyngstadaas, S.P., Risnes, S. Morphological characteristics of mouse incisor enamel J. Anat. 189: ,pp.325-333; 1996.

[12] Pandya, M., Diekwisch, T.G.H. Enamel biomimetics—fiction or future of dentistry. Int J Oral Sci 11(8):1-9; 2019. DOI: 10.1038/s41368-018-0038-6

[13] Romanyukha, A. A., Hayes, R. B., Haskell, E. H., Kenner, G. H. Geographic Variations in the EPR Spectrum of Tooth Enamel. Radiat. Prot. Dosim. 84(14): 445-449; 1999.

[14] Toyoda, S., Imata, H., Romanyukha, A. A., Hoshi, M. Toward High Sensitivity ESR Dosimetry of Mammal Teeth: The Effect of Chemical Treatment. J. Radiat. Res. 47: A71-A74; 2006.

Appendix A: IACUC Exemption for CSU 1 portion of research

Research Integrity & Compliance Review Office
Office of Vice President for Research
200 University Services Center
2011 Campus Delivery
Fort Collins, Colorado 80523-2011
TEL: (970) 491-1553
FAX: (970) 491-2293
https://vpr.colostate.edu/RICRO/

To: Mariah Davis, Tom Johnson

From: Research Integrity and Compliance Review Office (RICRO)

Date: March 25, 2019

RE: IACUC Input on "Dose Reconstruction and Dose Threshold Assessment in Mice Teeth Using Electron Spin Resonance"

This is to inform you that your project, "Dose Reconstruction and Dose Threshold Assessment in Mice Teeth Using Electron Spin Resonance" has been reviewed by RICRO and the Attending Veterinarian (or his delegate) and is exempt from IACUC oversight. Therefore, an IACUC protocol does not need to be submitted for these activities (**IACUC Exemption #2019-082-ERHS).**

If there are any changes to this project in regards to animal activities, please submit changes via the IACUC Exemption Form to ensure that this exemption is still valid prior to implementation

Thank you for your diligence in the care and use of animals at CSU. Good luck with your project.

Sincerely,
Research Integrity and Compliance Review Office (RICRO)

Cc: Mark Zabel, IACUC Chair
Lon Kendall, CSU Attending Veterinarian
Karen Dobos, RICRO Director

Appendix B: IACUC Protocol 19-854A for Japan 1-3 and CSU 2 portion of research

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Personnel Information * * *

COLORADO STATE UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE ANIMAL USE APPLICATION

IACUC approval of this completed form is necessary prior to animals being obtained, housed or manipulated for research, testing or teaching purposes; performed at CSU or by CSU at other locations.

When you have completed all applicable sections of the protocol, you must also complete the certifications section and then click "Submit Form" link on the left-hand column.

All individuals listed on the protocol must have certified completion of the online CSU Animal Care and Use Training. Additionally, a "Training Record" should be uploaded in the Attachments section for the PI, Co-PI, and each person who will handle animals as a part of this study. Also, all individuals working with animals must be enrolled in the CSU Occupational Health and Safety Program (OHSP) via annual submission of a Risk Assessment Form to the OHSP.

Please contact an IACUC Coordinator if you have any questions.

Principal Investigator*

Name	Title	
Johnson, Thomas	Professor	
Email	**EID**	**Phone**
Thomas.E.Johnson@ColoState.EDU		(970) 491-0563
Department	**Mail Code**	
1681 Env & Rad Health Sciences		
Will PI work with animals as part of this project?	N	

Department Head

Name of Department Head	Degree	Title
Nickoloff, Jac		Professor
Email	**Phone**	**Fax**
J.NICKOLOFF@colostate.edu	(970) 491-6674	
Department Name	**Campus Delivery Code**	
1681 Env & Rad Health Sciences		

Will the Department Head work with animals as a part of this project? N

If this person will work with animals as a part of this protocol, upload a "Training Record" for this individual under the "Attachments" section of this

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

protocol.

Other Submitter

Name	Title	
Davis, Mariah		
Email	**EID**	**Phone**
Mariah.Davis@rams.colostate.edu	829347380	(913) 952-1091
Department	**Mail Code**	
1681 Env & Rad Health Sciences		
Will this person be working with animals as a part of this project?	Y	

* * * Species * * *

Species to be Used

Common Name	Field Mouse			
Scientific Name	Field Mouse			
Animal Sex	Male or Female			
Age Range	6	-	36	Month(s)
Weight Range	10	-	40	gm(s)
Strain/Breed/Subline	Any strain			
Housing Location	Other wildlife			
Room Number	N/A			
Maximum number of animals for three year project period	150			

USDA Pain Category (Choose all that will apply)

	Pain Category B	
X	**Pain Category C**	150
	Pain Category D	
	Pain Category E	

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Pain Categories
Category B: Animals bred, conditioned or maintained for use in teaching, testing, or research, but not yet used for such purposes.
Category C: Animal use subjects them to no more than momentary or slight pain or distress and they do not receive pain-relieving drugs. Example : euthanasia prior to tissue collection; observation under normal conditions; positive rewards; routine injections (not Freund's adjuvant); tattooing; blood sampling.
Category D: Animal use subjects them to procedures where pain or distress is appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress which would otherwise be more than slight or momentary. Example: Needle biopsy non-survival or survival surgeries, terminal cardiac blood collection under terminal anesthesia; exposure of blood vessels for catheter implantation; induced infections or antibody production. PROCEDURES AT PAIN D REQUIRE VETERINARY CONSULTATION WITH THE UNIVERSITY VETERINARIAN OR DESIGNEE.
Category E: Animal use in which they must be subjected to unrelieved pain or distress for scientific reasons. Examples: toxicological or microbial testing or infectious disease research that requires continuation until severe clinical symptoms are evident or death occurs; application of noxious stimuli from which the animal cannot escape; prolonged restraint; use of paralyzing drugs for restraint of conscious animal; infliction of burns or trauma. PAIN E PROCEDURES REQUIRE CONSULTATION WITH THE UNIVERSITY VETERINARIAN OR DESIGNEE, AND MUST BE SCIENTIFICALLY JUSTIFIED IN THE PROTOCOL.

Source of Animals

Please indicate the source of the animals that will be used in the protocol. Be as specific as possible:
Outside Vendor (indicate whether purchased through LAR or by the investigator/department);
Transferred from another approved protocol (indicate protocol number);
Free-ranging Wildlife;
Faculty/Staff/Student-Owned ;
Client-Owned;
Other (please explain).

NOTE: If this is a study using Client Owned animals, you must provide a copy of the Informed Owner Consent Form along with approval from VMC Director in the Attachments section.

Free-ranging Wildlife

* * * Are You Using? * * *

Please indicate if you propose to use any of the following so the IACUC may better assess your protocol.

1. **Will you be using live animals for teaching?** **N**

What are the goals of the course(s) and who is the intended audience(s)?

Please describe the preparation the students will have prior to handling live animals (e.g. lecture, demonstrations, anatomical model use, videos)

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

2. Will you be using euthanized animals for teaching purposes? N

What will be the source of the animals (LAR or Vendor) and what is the disposal plan?

What are the goals of the course(s) and who is the intended audience(s)?

3. Will you be collaborating with another institution(s)? Y

Institution(s)

Institution Name	Other
Other (please specify)	Institute for Environmental Radioactivity at Fukushima University
PHS Assurance #	
USDA Registration #	
Collaboration institution personnel	

Briefly explain how the collaboration or subcontract is structured

The IER at Fukushima University is the base operation for this project. They are intimately involved with sample collection and processing

Please summarize if animals will be purchased by, housed, or have procedures performed by CSU personnel at this other institution.

4. Will you be using biohazardous agents?

a) Recombinant DNA (rDNA), human fluids or human tissues N

b) Infectious Agents? N
If you indicated "Yes" to 4a. or b. above, please provide IBC protocol "PARF" number, or indicate "Submitted" or "Submission Pending," as appropriate.

c) Will this protocol involve the generation of new transgenic or knockout lines using rDNA? N

d) If using an infectious agent or toxin, is it on the USDA or CDC Select Agent List (see Select Agents for the two lists of agents)? N

5. Will studies be performed under Good Laboratory, Good Clinical, or Good Manufacturing Practices (GLP/GCP/GMP)? Such studies are regulated by the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA). Please contact the CSU Quality Assurance Manager for additional review and approval of GLP/GCP/GMP documentation. N

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

If yes, please provide the name of the individual who will be the Study Monitor, and briefly describe how the project involves GLP/GCP/GMP or preliminary product testing.

6. Will you be using controlled drugs?
Will controlled drugs (including HCG and Ketamine) be used? Y
If yes, list whose CSU "drug cabinet" will be accessed. Japan

7. Will carcinogenic or chemical substances that are hazardous to humans or animals be used? N

Toxic Agent(s)

8. Will you be using radiological agents N

Isotope(s)

9. Will this be a field study (i.e. conducted on free-living wild animals in their natural habitat)? In addition to IACUC approval, the investigator is responsible for obtaining all necessary federal/state or other government permits for wildlife studies. Y

Field Study or Wildlife Study

Are state, federal or local permit(s) required?	Y
For which species or circumstances are permits required?	Field Mouse
Do any of these species carry a zoonotic disease (e.g., rabies, hantavirus, bird flu)?	N
Do you need additional information on protective measures for personnel?	
Are any species involved in this research under endangered or protected categories? (State, Federal or IUCN listed species)	N

Indicate which species and explain why these species must be used for research

Other pertinent information regarding wildlife or fish studies that may help the IACUC review this protocol

If this research is conducted in the field, note the person responsible for, and storage location of records detailing sedation and/or other materials administered to the animals in this study

Person Responsible	Thomas Johnson
Storage Location	Molecular and Radiological Biosciences Building

If voucher specimens are collected, list the institution(s) where they will be deposited.

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** Funding Sources ***

Funding Checklist

Funding - Grants/Contracts

Funding - Other

Dept. Funding

Department Name	**1681 Env & Rad Health Sciences**
Account Number	12-60410

Other Funding

This protocol is funded (in whole or in part) with funding from an agency in the U.S. Department of Defense (DoD)? This includes direct grant/contract funding or subcontract work that is flow-through of funding from DoD. N

If DoD funding is involved, the PI will be responsible for obtaining approval from the DoD Animal Care and Use Research Office (ACURO) for all new protocols and amendments to existing protocols prior to initiation of the work/change to the protocol.

Check here if this project is self-funded (No aspect of this work will have charges to a sponsored project, departmental account, other CSU-related account associated with it.)

e-Protocol PROTOCOL IACUC Protocol # 19-8954A Date Printed: April 16, 2020

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

NOTE: Applicable Federal Grant Application, including competing renewals must be attached. Applicable investigator's brochure and sponsor's protocol must be attached for all industry sponsored clinical trials. You will be prompted for these in the Attachments section.

Has this protocol received other internal reviews (check all that apply):

Reviewed for CRC Funding	**Yes**	**No** X
Reviewed by VTH/Clinical Sciences Clinical Research Review Board:	**Yes**	**No** X
I assure that the activities described with in this document submitted for IACUC review are consistent with those described in any related grant, contract, or subcontract that has been submitted or awarded.	**YES** X	**NO**

*** Rationale ***

1. PROJECT INFORMATION

a) Protocol title

Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel

b) Application type

Note: If you are editing a previously approved protocol for an Amendment or Continuing Review, please leave the answer to the questions under b. below as they were in the originally approved protocol.

This project is a: (check only one)

X **New project**

4th year renewal (please enter number of protocol that you are renewing below)

If this is a 4th year renewal, please indicate the number of the protocol it is renewing.

2. LAY SUMMARY

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

a) **What is the overall goal or purpose of this animal use?**
Provide a brief description which would convey to a lay audience the purpose for the proposed use of animals. Use language understandable to a layperson. Avoid overly technical terms and define acronyms. The readability should be similar to a newspaper article. For example, the goal of a study could be expressed as follows: "Disease XYZ is a serious threat to the health of…. This project will seek to test the efficacy of treatment ABC." Or, "This project seeks to understand the cellular mechanisms that influence X through in vitro analysis utilizing tissues harvested from the proposed species Y."
Note: A section from your grant application using highly technical terms is not acceptable.

Electron paramagnetic resonance (EPR) of tooth enamel is a well known method of reconstructing radiation dose in humans following a radiation event. Less is known about dose measurement using EPR in other animals. A reliable ability to reconstruct dose following a radiation event can provide critical insight to the effects of the radiation event on the local ecology and/or local human populations, and allow for future risk assessment study. Dose reconstruction using EPR also allows for reconstruction of individual dose, rather than just dose to an area, giving a more thorough understanding of how a radiation event may directly affect individuals. This project seeks to determine usability of mouse teeth to reconstruct dose using EPR, for an understanding of both personal dose, as well as dose to an area following a radiation event.

b) **What will the impact of the use of live animals in this project be for human OR animal health, the advancement of knowledge, or the good of society?**
Regulations and ethical standards require that procedures involving the use of animals in research or teaching be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge or the good of society. Provide a brief description which would convey to a lay audience the impact the proposed research will have for one or more of the above considerations. For example, "1 million people are estimated to contract disease XYZ each year. The proposed project will further the cause of developing effective treatments for the disease." Or "The cellular mechanisms X have previously been studied, but no studies have looked at aspect C of this mechanism. This study will advance the scientific understanding of X by exploring aspect C."
Note: Projects are not required to have application for human health to receive IACUC approval.

In the case of a radiation event, a thorough understanding of the effects due to radiation in an area and on local animal (human and non-human) populations is necessary. Dosimeters may not always be in an area that experiences a radiation event or accident, however (such as the Fukushima Daiichi nuclear reactor accident). Understanding the response of mouse tooth enamel to EPR is beneficial in the case where personal dosimeters are not around following a radiation event. It is additionally beneficial in the case where human tooth enamel is unavailable or impractical to collect following a radiation event. Animal tooth enamel as acting dosimeter can also provide insight to the effects of a radiation event to animal populations and ecology where humans don't inhabit, and where dosimeters are impractical or unable to be taken and used. Mice are of particular interest due to their ability to be found in areas near humans and other animals, and the similarity of dose response by mouse tooth enamel to human tooth enamel.

3. JUSTIFICATION FOR USE OF ANIMALS

e-PROTOCOL PROTOCOL IACUC Protocol # 19-8954A Date Printed: April 16, 2020

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

For parts a. and b. below, please answer "Yes" or "No" for each question.
There should be a Yes/No answer in all questions a)i. through a)vii. and b)i. through b)v.

a) **Living animals are required for this project because:**
(You should select either Y or N for each query.)

i)	N	**Complexity of the processes studied cannot be duplicated/modeled using in vitro models**
ii)	N	**Not enough information known about processes being studied to design non-living models**
iii)	N	**Pre-clinical studies in living animals are necessary prior to human testing**
iv)	N	**This study requires tissue harvested from animals prior to in vitro testing**
v)	N	**Currently this is the best method to accomplish the required teaching**
vi)	Y	**Populations are being studied in natural or semi-natural environments**
vii)	N	**Animal behavior is being studied**
viii)		**Other (please specify):**

b) **This species has been selected because:**
(You should select either Y or N for each query.)

i)	N	**Anatomy, physiology, behavior or agent susceptibility of species uniquely suited to the study**
ii)	N	**Lowest phylogenetic species providing adequate size, tissue, or anatomy for proposed study**
iii)	N	**This species provides a particularly good model for the human or other animal disease or process**
iv)	Y	**Previous studies which form the background for this project used this species**

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

v) N The objective of this study is to provide information about the target species

vi) Other (please specify):

4. JUSTIFICATION FOR NUMBER OF ANIMALS TO BE USED

The IACUC requires justification of proposed animal use numbers. A power calculation, confidence interval width, or an explanation why a power calculation is not feasible for this project should be provided. Complete one or more of the following (as appropriate) to justify the number of animals you will use (you may refer to Russ Lenth's U. Iowa stats website for statistical calculations). For experimental designs with multiple groups/treatments, it is suggested that a table of animal numbers per group be provided in the Attachments section. In addition make sure the animal numbers justified here agree with those mentioned in other sections of the application.

Answer N/A for any question (a-i) that is not applicable. There should be an answer or N/A in all boxes a-i.

a) This is an exploratory or pilot study. Describe how the proposed number of animals needed was determined. Note: A total of more than 12 animals indicates to the IACUC that the project may not be a pilot:

Particularly when conducting wild capture to acquire samples, teeth are not guaranteed to be in good enough condition to use for accurate analysis with EPR. Aiming for 15 samples total (including controls), from no more than 150 mice should ensure the study can successfully acquire necessary sample mass to yield a meaningful result. A sample size of 15 is estimated based off of similar studies and procedures concerning EPR on animal tooth enamel.

At least two control samples will be needed (20 mice out of the aforementioned 150 mice total, for 2 control samples out of 15 total samples) in order to have a baseline for background radiation exposure. This baseline is necessary for comparison between the mice exposed to the environmental contamination in the Fukushima exclusion zone to unexposed mice. A comparison allows for a determination of whether or not significant difference between exposed and unexposed mice may be measured in their lifetime. Such a comparison could also have implications for usefulness of EPR in studies where low lifetime dose is being considered.

b) The group size was determined using a statistical package. Specify the statistical package used, effect size(s), estimate of variation used, and power level expected. (If multiple response variables are to be measured, the power calculation should be based on the most critical measures. When the objective is not to test but to estimate differences between mean or proportions, sample size may be justified based on confidence interval width criteria.):

N/A

c) This is a teaching protocol. Specify student-to-animal ratio, and explain how that was determined. There should be a clear correlation between the teaching objective and the number of animals per student:

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

N/A

d) This study involves tissue or cells harvested from animals for in vitro studies. Explain the number of animals requested for the amount of tissue needed to obtain a specified level of precision desired, or if an experiment involving the tissue samples will be conducted as part of this protocol, provide power calculations as described in b above. Cleary show the relationship between the number of animals requested and the number needed for the in vitro work:

N/A

e) This study involves breeding animals for later use in research, testing, or teaching. List the number of breeding males and females to be used/number of offspring produced each year, and describe how the animals are expected to be allocated to the subsequent experiment(s). If only a portion of the offspring will be usable in experiments, please indicate the number and reason for this:

N/A

f) This is a study of feral or wild animals where animals will be captured and released attempting to maximize sample size within logistical constraints. Describe and suggest a level of precision necessary to obtain useful information and the sample size required to obtain this precision:

N/A

g) This is an observational, non-manipulative study in which animals will only be observed and animal numbers cannot be predicted. The animals will not be captured nor will their behavior be manipulated:

N/A

h) Sample size is government driven or agency mandated. Provide appropriate references documenting this requirement (e.g. product safety testing as mandated by FDA regulations):

N/A

i) Other. Please describe in detail:

N/A

*** Procedures ***

Live capture

Procedure Type: Live capture

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Procedure Title: Live Capture by Sherman Trap
Species: Field Mouse (wildlife) **Pain/Distress Category:** C
Approximate number of animals to be used in this procedure: 0
All D and E studies require date of consultation with the University Veterinarian; or, the name of other vet who was consulted:

Use Location (Campus) Fukushima, Japan **Building Name:** N/A
Room Number: 241

*** Procedure Description ***

Procedure Description

Procedure Description. Provide a brief description of how the procedure will be conducted. For blood/fluid collections include the route(s) of collection, volume, and frequency. For drug/compound dosing include route(s) of administration, volume, and frequency. For inoculations, include agent/vaccine information, route(s) of administration, volume, frequency, and dose. For procedures requiring administration of anesthesia, analgesia, provide the doses/route of administration; and for procedures requiring aseptic preparation, briefly describe animal, surgeon, and instrument preparation. Please DO NOT simply cut-and-paste from laboratory SOPs with superfluous or overly general information in them.

Under the supervision of a trained mouse biologist associated with Fukushima University, mice will be caught during single-day sessions by Sherman traps. Traps will be baited with peanut butter and seeds. Traps will be set out in the morning and checked at 2 hour intervals. Once trapped, mice will be immediately sedated and euthanized, as described elsewhere in the protocol. All traps will be collected by the end of the day so no mice are left in traps overnight.

Average temperature in Fukushima does not exceed 80 degrees Fahrenheit or fall below 50 degrees Fahrenheit in June and July. Traps will be set in shaded areas of the forest to avoid direct sunlight, as well as to provide some protection in case of unforeseen rainstorms. Traps will have bedding material placed in them, as well as the bait material, for the comfort of the animal.

Please list any clinical effects or changes from normal health and behavior which may occur as a result of this procedure. This should include both short and longer-term effects of the procedure, as applicable.

Mice may be initially stressed within the trap. However, Sherman traps have been thoroughly evaluated for use with wild small rodents, and are agreed upon as an appropriate means of live capture.

Describe post procedure monitoring that will be performed. This should clearly indicate the frequency of monitoring, who will conduct it, and address the short- and longer-term complications that may result from the procedure.

N/A

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be given rescue analgesia, other clinical treatments, or euthanasia. Please include any scoring system that will be used to determine when humane intervention will be triggered in the Attachments section or provide the scoring criteria below, as applicable.

N/A

Euthanasia and harvest of tissues

Procedure Type: Euthanasia and harvest of tissues
Procedure Title: Wild mice euthanasia by sedative overdose and cervical dislocation
Species: Field Mouse (wildlife) **Pain/Distress Category:** C
Approximate number of animals to be used in this procedure: 0
All D and E studies require date of consultation with the University Veterinarian; or, the name of other vet who was consulted:

Use Location (Campus) Fukushima, Japan **Building Name:** Off-Campus
Room Number: 241

*** Procedure Description ***

Procedure Description

Procedure Description. Provide a brief description of how the procedure will be conducted. For blood/fluid collections include the route(s) of collection, volume, and frequency. For drug/compound dosing include route(s) of administration, volume, and frequency. For inoculations, include agent/vaccine information, route(s) of administration, volume, frequency, and dose. For procedures requiring administration of anesthesia, analgesia, provide the doses/route of administration; and for procedures requiring aseptic preparation, briefly describe animal, surgeon, and instrument preparation. Please DO NOT simply cut-and-paste from laboratory SOPs with superfluous or overly general information in them.

Under the supervision and instruction of a trained mouse biologist associated with Fukushima University, mice will be removed from Sherman traps and heavily sedated with 20mg/kg of intraperitoneal Xylazine (100mg/ml). Following complete sedation, mice will be euthanized via cervical dislocation. Death will be confirmed by a lack of heart beat and corneal reflex.

Please list any clinical effects or changes from normal health and behavior which may occur as a result of this procedure. This should include both short and longer-term effects of the procedure, as

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

applicable.

In order to minimize stress to the animal, sedative overdose will be preformed prior to euthanasia. All tissue evaluation and collection will occur post-mortem.

Describe post procedure monitoring that will be performed. This should clearly indicate the frequency of monitoring, who will conduct it, and address the short- and longer-term complications that may result from the procedure.

N/A

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be given rescue analgesia, other clinical treatments, or euthanasia. Please include any scoring system that will be used to determine when humane intervention will be triggered in the Attachments section or provide the scoring criteria below, as applicable.

N/A - subjects will all be immediately sedated, followed by euthanasia once in an appropriate sedated plane (approximately 10 minutes following injection).

* * * Anesthetic Regimen * * *

Anesthetic Regimen

Note: Documentation of training is not required if you are using VMC or LAR services

Anesthetists

Parameters monitored during surgery:

Anesthetic Agents

Paralytic Agents

Other premedications not already listed above

* * * Alternative Search * * *

Alternatives Search

Federal regulations require that the fewest number of live animals necessary are used for research, testing, or teaching, and that investigators document that they have given all due consideration to

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

reducing or eliminating the use of potentially painful or distressful procedures (Pain Category D or E). The USDA considers automated literature searches the most effective and efficient method for demonstrating compliance with the above requirements.

For ALL projects, regardless of pain categorization, please conduct a literature search utilizing terms that would allow you to demonstrate that the proposed research or other animal use is not unnecessarily duplicative of previously documented work. Please enter the appropriate Search Data (click the "Add" button) and answer Question 1 below.

If the proposed project involves procedures at Pain Categories D and/or E, documentation of a literature search which demonstrates that the fewest number of the lowest order of animals will be used to obtain valid results, and alternatives to EACH potentially painful/distressful procedure proposed have been sought. Therefore please enter the appropriate Search Data and answer Questions 2 & 3 below. See USDA Policies #11 and 12).

For assistance with alternatives searches, please consult the CSU Libraries IACUC Alternatives Search Help page, see the Alternatives to Painful or Distressful Procedures document (prepared by the University Veterinarian), or contact an IACUC Coordinator.

Click the "Add" button below to enter information pertinent to your search(es). Please then address question 1 and, as appropriate to the procedures to be conducted, address, questions 2-3.

Search Data

Search Range From: 1980
To: 2019
Search Date: 05/11/2019

Search Terms

Please provide the Keywords and the Boolean terms such as AND, OR used to relate keywords (e.g. term#1 [AND] term#2) for searches for each of the three components of the Alternatives Search indicated above:

wild mouse sedation; Wild [AND] Mouse [AND] sedation; (((mouse) [AND] anesthesia) [AND] wild); (((mouse) [AND] anesthesia) [AND] wild) [OR] telazol; (((mouse) [AND] anesthesia) [AND] wild) [AND] telazol; mouse capture sedation; wild mouse capture sedation; wild mouse capture xylazine; wild mouse capture; wild mouse capture immobilization; ((wild) [AND] mice) [AND] immobilization [AND] chemical; ((wild) [AND] mice) [AND] immobilization; ((wild) [AND] mice) [AND] sedation

Databases Searched (you must search at least 2 databases):

Agricola Data Base	Google Scholar
ALTBIB - Bibliography on Alternatives to Animal Testing	HSVMA Alternatives in Education Database

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

SCIRUS		Lab Animal
AnimAlt-ZEBET		Lab. Animals Journal
ATLA (FRAME--Alternatives to Laboratory Animal Journal)	X	Medline / PubMed
BioOne (access from CSU Libraries website)		NORINA
BIOSIS (Note: CSU Libraries does not subscribe to this database)		TOXLINE
CAB Abstracts (access from CSU Libraries website)	X	Web of Science (access from CSU Libraries Website)
		Other, please specify:

Search Range From: 1980
To: 2019
Search Date: 05/11/2019
Search Terms

Please provide the Keywords and the Boolean terms such as AND, OR used to relate keywords (e.g. term#1 [AND] term#2) for searches for each of the three components of the Alternatives Search indicated above:

((mice) AND tooth enamel) AND (EPR OR Electron paramagnetic resonance OR ESR OR Electron spin resonance); ((Apodemus speciosus) AND teeth) AND (EPR OR Electron paramagnetic resonance OR ESR OR Electron spin resonance); (mouse) AND teeth AND (EPR OR Electron paramagnetic resonance OR ESR OR Electron spin resonance) AND dose; "~Japanese field ~mouse" OR "Apodemus speciosus" AND (~teeth OR enamel) AND (EPR OR Electron paramagnetic resonance OR ESR OR Electron spin resonance) AND (~dose);(((~mouse) AND "~tooth ~enamel") AND ~dose) AND "electron paramagnetic resonance"

Databases Searched (you must search at least 2 databases):

Agricola Data Base	X	Google Scholar
ALTBIB - Bibliography on Alternatives to Animal Testing		HSVMA Alternatives in Education Database
SCIRUS		Lab Animal
AnimAlt-ZEBET		Lab. Animals Journal
ATLA (FRAME--Alternatives to Laboratory Animal Journal)	X	Medline / PubMed
BioOne (access from CSU Libraries website)		NORINA
BIOSIS (Note: CSU Libraries does not subscribe to this database)		TOXLINE
CAB Abstracts (access from CSU Libraries website)	X	Web of Science (access from CSU Libraries Website)
		Other, please specify:

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

1. N Did the search reveal that your project is duplicative of previously documented work?

a) Please provide the number of hits and an overview of the results.

3 publications were found on EPR analysis of mouse teeth, but studies were of either artificially applied doses, or involved methodology for finding a dose that is inapplicable to the current study

b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project anyway.

CITATIONS:

- Khan RF, Rink W, Boreham D. Biophysical dose measurement using electron paramagnetic resonance in rodent teeth. Applied Radiat Isotopes 59:189–196; 2003. DOI: 10.1016/S0969- 8043(03)00166-0.
- Toyoda S, Tanizawa H, Romanyukha AA, Miyazawa C, Hoshi M, Ueda Y, Nitta Y. Gamma-ray dose response of ESR signals in tooth enamel of cows and mice in comparison with human teeth. Radiat Meas 37:341–346; 2003. DOI: 10.1016/S1350- 4487(03)00059-3

DETERMINATION:
Studies applied artificial doses, while current study intends to measure doses acquired over a mouse's natural lifespan to determine suitability of mouse teeth as dosimeters, and as representative reflection of dose to individuals in some local ecology.

CITATION:

- Kitaya, T et al. 2016. "Attempts of Radiation Dose Measurement in the Teeth of Mice Living around the Nuclear Power Plant in Fukushima Using Electron Spin Resonance Spectroscopy" Radiation Environment and Medicine 2017 Vol.6, No.1 1–5

DETERMINATION:
Study didn't remove dentin from tooth enamel, a necessary process to remove spurious signal. This indicates that the non-conclusive results acquired may be a result of methodology rather than applicability of mouse tooth enamel for dose measurement with EPR

2. N Did the search reveal any possible reductions or replacements that would allow the use of fewer animals or animals of a lower order?

a) Please provide the number of hits and an overview of the results.

One study used fewer mice per sample, but did so as a result of inclusion of extraneous material and thus causing spurious signal

b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project as proposed.

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Kitaya, T et al. 2016. "Attempts of Radiation Dose Measurement in the Teeth of Mice Living around the Nuclear Power Plant in Fukushima Using Electron Spin Resonance Spectroscopy" Radiation Environment and Medicine 2017 Vol.6, No.1 1–5

- included dentin with tooth enamel, causing a non-conclusive signal.

3. N/A **Did the search reveal any possible refinements that would allow the use of alternative procedures to those that will potentially cause pain and/or distress for the animals (Protocols utilizing procedures at pain category D and/or E)?**

a) Please provide the number of hits and an overview of the results.

N/A

b) If "Yes," please provide a list of the relevant citations and a discussion of how you determined that it is necessary to conduct the project as proposed.

N/A

Teaching Protocols

1. If this is a teaching protocol, please specify why there are no alternatives to using live animals.

N/A

Protocols Involving Unrelieved Pain or Distress

1. For Pain Category E procedures, explain why drugs or other ameliorative treatments cannot be used to fully alleviate pain/distress. Please provide citations to the relevant literature.

Other Means of Determining Non-Duplication and Alternatives

The Animal Welfare Act allows other means of determining whether your project is duplicative AND whether it can be refined to decrease the animal number or order, AND to determine if alternatives to a potentially painful/distressful procedure can be used. For example, under some circumstances, colloquia, subject expert consultants, or other sources may provide relevant and up-to-date information regarding alternatives. When other sources are the primary means of considering alternatives, sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult should be provided. If you used an alternative search strategy, provide information on the strategy, methods, sources, and relevant findings.

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

N/A

*** * * Project Overview * * ***

Project Overview

Provide a clear and concise sequential description of the procedures the animals will undergo. The description should include information on the experimental groups and the study endpoints. It should allow the reader to see the timing and relationship of all procedures that will be conducted with the animals. For lengthy or complex experiments with many groups and/or procedures, a table or flowchart showing the experimental manipulations by group should also be uploaded into the Attachments section. A response here is required.

All under the supervision of a trained mouse biologist associated with Fukushima University, Sherman traps will be set in various sites in Japan in order to catch wild mice. Most mice will be caught within the Fukushima Daiichi exclusion zone, in areas where background radiation levels are high enough to provide a measurable signal (no more than 130 mice for no more than 13 samples). Mice will also be collected from areas not contaminated with radioactivity from the Fukushima nuclear reactor in order to provide a control sample (20 mice for no more than 2 samples). This means there will be 150 mice in total, for 15 total samples, collected primarily from areas exposed to radiation, with a small percent collected outside of irradiated areas to serve as a control.

Traps will be baited with peanut butter and seeds. Mice will spend no more than 2hrs in traps before the traps are checked by study personnel. The mice will then be sedated, and subsequently euthanized via cervical dislocation.

Once mice are determined to be dead by lack of heart beat and corneal reflex, skulls of euthanized mice will be removed and cleaned using boiling and manual flesh removal methods as necessary for clean tooth removal. Molar teeth (12 molars from each mouse) will be removed using dental tweezers and processed for EPR spectroscopy. Processing for EPR includes dentin removal by mechanical and chemical methodology, followed by crushing the enamel into grains appropriate for aliquots for EPR. (15 samples total)

Multiple Major Survival Surgery(MMSS) Description:

Describe why it is necessary to perform multiple major surgical procedures on the same animal.

*** * * Husbandry * * ***

Animal Care/Husbandry

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Emergency Contact Information

List all individuals/phone numbers that are to be notified by veterinary staff or others in the event of an emergency:
Thomas Johnson - (301) 213-6785
Mariah Davis - (913) 952-1091

Will Lab Animal Resources provide the daily care N
If "No," specify who will provide the daily care:
N/A

If "No," justify why LAR will not be providing animal care:
Mice will be field-animals in Fukushima, Japan to be euthanized upon catching; daily care not needed.

What veterinarian will provide medical care to animals? Other
If "Other" specify who:
Hiroki Sawada

Contact information:
(024) 597-8880

If "Other" justify why LAR will not be providing medical care:
Mice will be field-animals in Fukushima, Japan to be euthanized upon catching.

Location of medical records (indicate building/room or other applicable information):
N/A

Special Husbandry or Care

List any special or unusual requirements for care of the animals and who will provide this care (e.g. special diet, altered light cycle, variation from standard enrichment, etc.):
N/A

Non-standard Experimental Requirements (Procedures requiring Exemptions from the Guide).

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Social Housing

If you are using a social species there are mandatory housing requirements. CSU considers social housing to include compatible housing with conspecifics, as well as housing in the same secondary containment with visual, auditory, olfactory or tactile contact with conspecifics. See the "Policy on Social Management of Animals" on the IACUC Policies and Guidelines Page.

Please indicate which of the following is true:

1. Animals will be provided with social housing (unless an animal has individual incompatibility or vet care concerns, or due to cohort attrition).
X 2. Animals will not be housed at CSU.
3. Animals will be housed singly because that is appropriate for this species (including hamsters, rabbits, male mice, tom cats, and livestock in stalls).
4. Animals will be housed singly because such housing is necessary for research, testing or teaching goals.

If you will be housing animals singly for research, testing or teaching purposes (#4 above), you must provide a written justification which indicates the experimental constraints that make the housing necessary:

Food or Fluid restriction (other than up to 12 hours prior to surgery/general anesthesia) X None

Food or Fluid restriction

Species	Food Restriction	Length of Restriction	Fluid Restriction	Length of Restriction	Reason for Restriction
Field Mouse (wildlife)					

Description

Restraint of Conscious Animals (other than momentary restraint for routine procedrues, e.g. blood collections, injections, and such) X None

Restraint of Conscious Animals

Species	Type restraint (manual, commercial, manual and commercial)	Please describe Acclimation to restraint	Length of restraint
Field Mouse (wildlife)			

Description

Non-standard housing requirements X None

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Non-standard housing requirements

Species	Cage/Pen size	Cage Sanitation Interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from exercise (dogs only)
Field Mouse (wildlife)					

Description

*** Disposition of Animals ***

Please provide the information requested below regarding what will happen to animals at study end. (Check all that apply)

Animals will be adopted (Note, PI is required to follow the IACUC "Policy on Animal Adoptions" which is located on the page IACUC Policies and Guidelines Page.
Sold at auction (hoof stock only)
Released into home territory (wildlife studies)
Returned to client
Transferred to other studies (please specify below)

X Animals will be euthanized (Please add method below)

If using CO2 as the method of euthanasia for mice and rats, please be aware that the IACUC requires use of the "Directions for CO2 Euthanasia of Rodents" (available on the IACUC Policies and Guidelines Page) unless the protocol provides scientific justification why that procedure cannot be used.

Euthanasia Method

Species Field Mouse (wildlife)
Method of Euthanasia Primary Cervical dislocation

Will the animal be anesthetized or sedated? Y
Agent Name Ketamine/xylazine

Route of Administration Intraperitoneal injection (IP)

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Method of Euthanasia Secondary

Dosage (in mg/kg if possible) 20mg/kg Xylazine (no ketamine)
Justification for not using sedation

Please briefly describe what will happen with the animals at the conclusion of the study in the text box below:

Animal bodies will be used to determine radiocesium concentration

*** Attachments ***

PLEASE ATTACH ANY RELEVANT DOCUMENTS, INCLUDING:

Grant applications to any PHS agency, NSF, and USDA related to this activity
Training Records for all personnel on this protocol
Any scientific literature or articles relevant to the review of this project.

Please upload training records for the PI, Co-PI, and all individuals who will be working with animals as a part of this protocol. Click here to obtain the template for the Training Record.

Document Type Training Record
Attachment JohnsonT_Training Record
Document Name JohnsonT_Training Record

Document Type Training Record
Attachment Mariah_Davis_Training_Record
Document Name Mariah_Davis_Training_Record

*** Guidelines ***

Guidelines

The CSU IACUC Policies and Guidelines page can assist you and your staff in the protocol development and animal study process.

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

animal study process.

* * * Certifications * * *

I understand that changes in the approved protocol must be submitted in writing to the IACUC as a protocol amendment and approved by the IACUC prior to implementation. Such changes include, but are not limited to: species, animal numbers, animal-related procedures, animal restraint, food/water deprivation, euthanasia, PI, research staff, and the like. Minor changes can be reviewed by the IACUC via the designated member review process throughout the month; significant changes (e.g. a large increase in animal numbers, adding an invasive procedure) usually require a new protocol be submitted for review by the IACUC at its next regularly scheduled meeting.

Please contact an IACUC Coordinator if you have any questions about preparing new protocol applications, amendment requests, or continuing reviews.

Certification Test

By submitting this protocol to the CSU Institutional Animal Care and Use Committee (IACUC), the Principal Investigator certifies the following:

1) I assure that myself and all students, staff, and faculty on this project are familiar with the Animal Welfare Act (AWA) and AWA Regulations and the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Guide for the Care and Use of Agricultural Animals in Research and Teaching, as applicable, and all recognize their responsibility in strictly adhering to approved protocols.

2) I assure that all individuals listed on this project are qualified through education and/or training to conduct procedures involving animals under this proposal and have taken the online CSU Animal Care and Use Training, which includes information on the regulatory responsibilities of the institution, the IACUC, and investigators, as well as the concepts of research or testing methods that limit the use of animals or minimize distress, and the methods for reporting animal welfare concerns. Additionally, as applicable to their work with animals, all individuals on the protocol have received training in the biology, handling, and care of the species to be used; aseptic surgical methods and techniques; and the proper use of anesthetics, analgesics, and tranquilizers.

3) I assure that all procedures will be conducted in accordance with all applicable Colorado State University IACUC policies as well as Occupational and Biosafety requirements, including those pertaining to the use of personal protective equipment.

4) I assure that all individuals working on this proposed protocol are participating in the Occupational Health and Safety Program (OHSP).

5) I assure that ANY change in the care and use of animals involved in this protocol will be promptly

e-PROTOCOL PROTOCOL IACUC Protocol # 19-8954A Date Printed: April 16, 2020

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel
Protocol Type: IACUC
Date Submitted: 05/11/2019
Approval Period: Draft
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

forwarded to the IACUC for review. Such changes will not be implemented until approval is obtained from the IACUC. Animals will not be transferred between investigators without prior approval.

6) I assure that I have reviewed the pertinent scientific literature and the sources and/or databases and have found no valid alternative to any procedures described herein which may cause more than momentary or slight pain, distress, or generalized discomfort to animals, whether it is relieved or not.

7) I assure that every effort has been made to minimize the number of animals used and reduce the amount of pain, distress, and/or discomfort these animals must experience.

8) I assure that the activities described in this document submitted for IACUC review are consistent with those described in any related grant, contract, or subcontract that has been submitted or awarded.

9) I assure that the information contained in this application for animal use is accurate to the best of my knowledge.

10) I understand that this application and/or my animal use privileges may be revoked by the IACUC if I violate any of the aforementioned assurance statements.

X The Principal Investigator has read and agrees to abide by the above assurances

* * * Event History * * *

Event History

Date	Status	View Attachments	Letters
05/06/2019	NEW FORM CREATED		
05/11/2019	NEW FORM SUBMITTED	Y	
05/13/2019	NEW FORM PANEL ASSIGNED		
05/13/2019	NEW FORM REVIEWER(S) ASSIGNED		
05/20/2019	NEW FORM PANEL REASSIGNED		
05/28/2019	NEW FORM REVIEWER(S) ASSIGNED		
06/12/2019	NEW FORM APPROVED	Y	Y
03/31/2020	FINAL FORM CREATED		

Protocol Title: Reconstruction of Lifetime Dose to the Large Japanese Field Mouse Using Electron Paramagnetic Resonance Analysis of Tooth Enamel

Protocol Type: IACUC

Date Submitted: 05/11/2019

Approval Period: Draft

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

03/31/2020	FINAL FORM SUBMITTED	Y	
04/03/2020	FINAL FORM APPROVED	Y	N
04/03/2020	CLOSED		

Appendix C: CSU 1 Tooth Exposure Procedure, by Justin Bell

1/22/2018 Dosimetry for Mariah Davis samples

The Ion chamber and electrometer provided by Dr. Leary was calibrated with the LINAC to get a conversation factor of 2319 pC per Gy. Measurements were then conducted in Room 004 of MRB. Shown in Figure 1. The ion chamber was placed on the ground in the center of the small x located on the floor under the irradiator in room 004 of MRB. Measurements were recorded in the first sheet of the excel workbook titled 1.22.2018Room004-IonChamber.
30 second background measurements were acquired according to the electrometer's timer before being multiplied by the dose conversion factor and averaged to get the average background per second in room 004 at the specified location. The source transition was assessed by setting the irradiators control timer to 0.01 and the electrometer's timer to 30 seconds. The average background value was then subtracted from the source transition value to get the dose associated with the transition of the source.

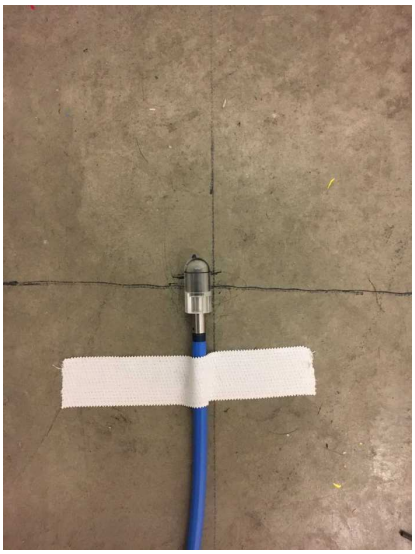


Figure 27: Ion chamber location on small x under the irradiator in Room004

The source was exposed on the manual setting from the control box. 30 second and 100 second acquisition times were taken according to the electrometers timer. The corresponding values were multiplied by the dose conversion factor before subtracting the background dose rate. The corresponding values were averaged to get an averaged dose rate at the specified location shown in Figure 1.
On Sheet 3 of the excel workbook titled 1.22.2018Room004-IonChamber exposure durations and control box settings for room 004 were calculated according to Mariah's requested doses. The dose rate used was corrected from the date of dosimetry, 1/22/2018, to the date of radiation exposure, 5/7/2019. These results are shown as well in Table 1.

Table 1. Dosimetry Results

Mariah Davis samples all at center floor location						
Rack #	Sample #	Dose Requested (Gy)	Source tranisiton dose	Ave. Dose rate @ center floor	exposure duration (sec)	Set control room timer to
1	RGG9190323	0.025	3.79474E-05	0.000599022	41.67133191	0.694522199
11	RGG4190323	0.05	3.79474E-05	0.000599022	83.40601272	1.390100212
21	LGG7190323	0.075	3.79474E-05	0.000599022	125.1406935	2.085678225
31	SMG2190323	0.1	3.79474E-05	0.000599022	166.8753743	2.781256239
41	RGG10190323	0.25	3.79474E-05	0.000599022	417.2834591	6.954724319
51	SMG3190323	0.5	3.79474E-05	0.000599022	834.6302672	13.91050445
61	LGG6190323	0.75	3.79474E-05	0.000599022	1251.977075	20.86628459
71	SMG1190323	0.8	3.79474E-05	0.000599022	1335.446437	22.25744061
79	LGG5190323	1	3.79474E-05	0.000599022	1669.323883	27.82206472
77	RGG8190323	2	3.79474E-05	0.000599022	3338.711115	55.64518526

Some pitfalls with this experiment were that the electrometer was only set to a -300 V bias, and all measurements are only relative to the ion chamber position. Additional film measurements could be taken concurrently while samples are exposure to assess any shadowing. It is recommended that samples be placed as close to small x on the ground as possible.
The sample chamber in room 004 was left in the closed position for this experiment. It was estimated that limited scattering and attenuation would occur due to the chamber dimensions. It was not recorded during previous dosimetry on the floor of room 004 if the sample chamber was left open or closed. Additionally, the particle board shelf was not removed because it was likewise estimated that its presence would cause limited attenuation and scatter.

Figure 28:Sample positioning for LGG6, SMG1, LGG5, RGG8

To limit experimental duration, samples LGG6, SMG1, LGG5, RGG8 were positioned as shown in Figure 2 and exposed to radiation at the same time. After an exposure duration of 1251.97 seconds, sample LGG6 was removed. The remaining 3 samples therefore had a prior dose of 0.75 Gy. This dose was subtracted from the requested total dose for each sample and before each subsequent radiation exposure occurred the dose associated with any additional source transitions was corrected for. For example, following the initial exposure, sample SMG1 was exposed for an additional 83.406 seconds to receive a total dose of 0.8 Gy. To determine this exposure duration, the dose associated with 2 source transactions was subtracted from the total requested dose. Similarly, the exposure durations for sample LGG5 and RGG8 had the radiation dose associated with 3 and 4 source transitions included in the sample's respective exposure calculation.
Samples were returned to the freezer in 319 MRB following radiation exposure.

Appendix D: Outline of Preparation Procedure Steps

Related section	Sample name(s)	Step	Chemical used (name or formula)	Concentration (percent or molarity)	Sonic bath (Y/N)	Temperature of treatment	Treatment time	Treatment description
CSU 1		1	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		2	NaOH	15% wt.	Y	60° C to 66° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 4 times with DI water
		3	-	-	N	40° C to 50° C	2 hours	Placed in oven to dry
		4	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		*5	-	-	N	19° C	11 hours 58 min.	Left to anneal overnight
		*6	-	-	N	~52° C	9 hours	Left to anneal for 9 more hours, since original annealing process was not at the correct temperature
		*7	-	-	N	Room temp.	-	Transferred to ESR tubes as needed
								*Took place at same time as CSU 2 samples
Japan 1	Control 1 190611	1	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		2	NaOH	15% wt.	N	Room temp.	1 hour 44 min.	Treated with NaOH for 1 hour 44 min. at room temperature while sonic bath heated
		3	NaOH	15% wt.	Y	60° C to 66° C	4 hours	Treated with NaOH for 4 hours in sonic bath, with alternating rinsing steps, due to remaining dentin and foreign material, then rinsed and decanted 4 times with DI water
		4	Water (DI)	-	Y	59° C to 64° C	2 hours	Rinsed with DI for 2 hours in sonic bath, with a rinsing step with fresh DI water after 1 hour elapsed, then rinsed and decanted 4 times with fresh DI water
		5	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		6	-	-	N	90° C	2 hours	Placed in oven to anneal
		7	-	-	N	~52° C	15 hours 6 min.	Left to dry overnight
		8	-	-	N	Room temp.	-	Transferred to ESR tube

Related section	Sample name(s)	Step	Chemical used (name or formula)	Concentration (percent or molarity)	Sonic bath (Y/N)	Temperature of treatment	Treatment time	Treatment description
Japan 2	Control 2 190627	1	Acetone	-	N	Room temp.	-	Washed (via container agitation and/or rinsing) in acetone and decanted, then rinsed and decanted 4 times with DI water
		2	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		3	NaOH	15% wt.	Y	60° C to 67° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 4 times with DI water
		4	Water (DI)	-	Y	58° C to 61° C	2 hours	Rinsed with DI for 2 hours in sonic bath, then rinsed and decanted 4 times with fresh DI water
		5	NaOH	15% wt.	Y	60° C to 67° C	30 min.	Treated with NaOH again due to visual inspection appearing to have remaining dentin, then rinsed and decanted 4 times with DI water
		6	Water (DI)	-	Y	60° C	2 hours	Rinsed with DI for 2 hours in sonic bath, then rinsed and decanted 4 times with fresh DI water
		7	-	-	N	90° C	2 hours	Placed in oven to anneal
		8	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		9	EDTA	0.1 M	Y	Room temp.	15 min.	Treated with EDTA for 15 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		10	Water (DI)	-	Y	Room temp.	5 min.	Rinsed in DI water for 5 minutes in sonic bath, then rinsed and decanted 4 times with fresh DI water
		11	Ethanol	70%	Y	Room temp.	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		12	-	-	N	90° C	2 hours	Placed in oven to anneal
		13	-	-	N	~52° C	15 hours 26 min.	Left to dry overnight
		14	-	-	N	Room temp.	-	Transferred to ESR tube

Related section	Sample name(s)	Step	Chemical used (name or formula)	Concentration (percent or molarity)	Sonic bath (Y/N)	Temperature of treatment	Treatment time	Treatment description
Japan 2 (cont.)	Control 3 190712	1	Acetone	-	N	Room temp.	-	Washed (via container agitation and/or rinsing) in acetone and decanted, then rinsed and decanted 4
		2	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		3	EDTA	0.1 M	Y	27° C to 30° C	15 min.	Treated with EDTA for 15 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		4	Water (DI)	-	Y	31° C	5 min.	Rinsed in DI water for 5 minutes in sonic bath, then rinsed and decanted 4 times with fresh DI water
		5	NaOH	15% wt.	Y	60° C to 63° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 4 times with DI water
		6	Ethanol	70%	Y	60° C	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		7	Water (DI)	-	Y	60° C to 63° C	2 hours	Rinsed with DI for 2 hours in sonic bath, then rinsed and decanted 2 times with fresh DI water
		8	-	-	N	90° C	2 hours	Placed in oven to anneal
		9	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		10	-	-	N	~52° C	11 hours 20 min.	Left to dry overnight
		11	-	-	N	Room temp.	-	Transferred to ESR tube
Japan 3	YamakiaO 190730,A; YamakiaY 190730,B; Control 5 190729	1	Acetone	-	N	Room temp.	-	Washed (via container agitation and/or rinsing) in acetone and decanted, then rinsed and decanted 5 times with DI water
		2	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		3	NaOH	15% wt.	Y	~60° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 5 times with DI water
		4	Ethanol	70%	Y	59° C to 60° C	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 5 times with DI water

Related section	Sample name(s)	Step	Chemical used (name or formula)	Concentration (percent or molarity)	Sonic bath (Y/N)	Temperature of treatment	Treatment time	Treatment description
Japan 3 (cont.)	YamakiaO 190730,A; YamakiaY 190730,B; Control 5 190729	5	Water (DI)	-	Y	55° C	5 min.	Rinsed in DI water for 5 minutes in sonic bath, then rinsed and decanted 5 times with fresh DI water
		6	EDTA	0.1 M	Y	50° C	15 min.	Treated with EDTA for 15 minutes in sonic bath, then rinsed and decanted 5 times with DI water
		7	Water (DI)	-	Y	~60° C	2 hours	Rinsed in DI water for 2 hours in sonic bath, then rinsed and decanted 2 times with fresh DI water
		8	-	-	N	90° C	2 hours	Placed in oven to anneal
		9	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		10	-	-	N	~52° C	10 hours 8 min.	Left to dry overnight
		11	-	-	N	Room temp.	-	Transferred to ESR tubes
CSU 2	Control 6 200309; Control 7 200309	1	Acetone	-	N	Room temp.	-	Washed (via container agitation and/or rinsing) in acetone and decanted, then rinsed and decanted 4 times with DI water
		2	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		3	EDTA	0.1 M	Y	22° C to 29° C	15 min.	Treated with EDTA for 15 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		4	Water (DI)	-	Y	28° C	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 2 times with DI water
		5	NaOH	15% wt.	Y	60° C to 66° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 4 times with DI water
		6	Ethanol	70%	Y	59° C to 60° C	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 4 times with DI water
		7	Water (DI)	-	Y	59° C to 64° C	2 hours	Rinsed in DI water for 2 hours in sonic bath, then rinsed and decanted 2 times with fresh DI water
		8	-	-	N	90° C	2 hours	Placed in oven to anneal

Related section	Sample name(s)	Step	Chemical used (name or formula)	Concentration (percent or molarity)	Sonic bath (Y/N)	Temperature of treatment	Treatment time	Treatment description
CSU 2 (cont.)	Control 6 200309; Control 7 200309	9	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		10	-	-	N	19° C	11 hours 58 min.	Left to dry overnight
		11	-	-	N	~52° C	9 hours	Left to dry for 9 more hours, since original annealing process was not at the correct temperature
		12	-	-	N	Room temp.	-	Any contaminants seen were removed, then teeth were transferred to ESR tubes
Japan 3 (unable to be used)	Control 4 190727' and 'Takase 190729'	1	Acetone	-	N	Room temp.	-	Washed (via container agitation and/or rinsing) in acetone and decanted, then rinsed and decanted 5 times with DI water
		2	-	-	N	Room temp.	-	Roots cut from teeth using surgical scissors
		3	NaOH	15% wt.	Y	~60° C	3 hours	Treated with NaOH for 3 hours in sonic bath, then rinsed and decanted 5 times with DI water
		4	Ethanol	70%	Y	59° C to 60° C	5 min.	Treated with ethanol for 5 minutes in sonic bath, then rinsed and decanted 5 times with DI water
		5	Water (DI)	-	Y	~60° C	2 hours	Rinsed in DI water for 2 hours in sonic bath, then rinsed and decanted 5 times with fresh DI water
		6	EDTA	0.1 M	Y	50° C	15 min.	Treated with EDTA for 15 minutes in sonic bath, then rinsed and decanted 5 times with DI water
		7	Water (DI)	-	Y	~60° C	2 hours	Rinsed in DI water for 2 hours in sonic bath, then rinsed and decanted 2 times with fresh DI water
		8	-	-	N	90° C	2 hours	Placed in oven to anneal
		9	-	-	N	Room temp.	-	Crushed as necessary, and sieved
		10	-	-	N	~52° C	10 hours 8 min.	Left to dry overnight
		11	-	-	N	Room temp.	-	Transferred to ESR tubes

Appendix E: Mouse Sample Numbers and Associated Individual Identification Numbers for *Apodemus speciosus*

Sample Name	Sample IDs Used	Number of mice	Sample wear stage	Initial mass (mg)	Final mass (mg)	Percent of initial mass lost
Control 1 190611	F17-041az/M170721; F17-042az/M170721; F17-043az/M170721; F17-021az/M170712; F17-022az/M170712; F17-023az/M170712	6	-	176.2	33.8	80.82
Control 2 190627 (Aizu)	F17-016ier/Ma170703; F17-024az/Md170712; F17-025az/Me170712; F17-027az/Mg170712; F17-028az/Mh170712; F17-044az/M170712; F17-045az/M170721; F17-046az/M170721; F17-047az/M170721; F17-048az/M170721	10	-	258.3	32	87.61
Control 3 190712 (Takase)	M181017F1; M181023T1(1of2); M181023T1(2of2); M181024T1; M181024T2; M181025T1; M181025T2; M181025T3; M181025T4; M181027T1	10	-	414.2 (wet)	31.8	92.32
Control 4 190727	M181103S1; M181108S1; M181110S1; M181110S2; M181110S3; M181110S4; M181110S5; M181111S3; M181112S2; M181112S3	10	-	257.6	8.2	96.82
Takase 190729	M181104T1; M181109T1; M181110T2; M181111T1; "M181112S3 or M181112T1" (determined to be M181112T1); M181030T1; M181107T1; M181106T1	8	-	218.9	5.8	97.35
Control 5 190729A **Control 5 190729B** **Control 5 190729C** **Control 5 190729D**	F19-002; F19-003; F19-004; F19-005; F19-006; F19-007; F19-008; F19-009; F19-010; F19-011; F19-013; F19-014; F19-015; F19-016; F19-017; F19-018; F19-020; F19-021; F19-022; F19-023; F19-024; F19-025; F19-026; F19-027; F19-028; F19-029; F19-030; F19-031; F19-032; F19-033	30 (4 total samples)	-	703.2	23.1 66.8 66.6 67.1	68.20

Sample Name	Sample IDs Used	Number of mice	Sample wear stage	Initial mass (mg)	Final mass (mg)	Percent of initial mass lost
YamakiaO 190730,A	F18-001; F18-002; F18-003; F18-006; F18-008; F18-009; F18-011; F18-012; F18-015; F18-018; F18-019; F18-021; F18-027; F18-028; F18-029	15	All over 5 (1+ year old)	389.6	24	93.84
YamakiaY 190730,B	F18-004; F18-005; F18-007; F18-010; F18-013; F18-014; F18-016; F18-017; F18-020; F18-022; F18-023; F18-024; F18-025; F18-026; F18-030	15	All under 5 (1 month to 14 months)	429.9	67	84.41
Control 6 200309	F18-031; F18-032; F18-033; F18-034; F18-035; F18-036; F18-037; F18-038; F18-040; F18-041; F18-042; F18-043	12	4.67 average (wear stage range: 2-8)	225.1	56	75.12
Control 7 200309	F18-044; F18-046; F18-047; F18-048; F18-049; F18-050; F18-051; F18-052; F18-053; F18-054; F18-056; F18-057	12	4.67 average (wear stage range: 2-8)	224.3	46.9	79.09