

THESIS

UTILIZING ELECTRON SPIN RESONANCE ON TEETH TO DETERMINE EXTERNAL  
LIFETIME DOSE TO WILD BOAR NEAR FUKUSHIMA DAIICHI NUCLEAR POWER  
PLANT

Submitted by

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## ABSTRACT

### UTILIZING ELECTRON SPIN RESONANCE ON TEETH TO DETERMINE EXTERNAL LIFETIME DOSE TO WILD BOAR NEAR FUKUSHIMA DAIICHI NUCLEAR POWER PLANT

Following the 2011 nuclear accident at the Fukushima Daiichi Nuclear Power Plant, the flora and fauna surrounding the affected area were exposed to radioactive contamination. The purpose of this project is to determine if the external radiation dose to the wild boar living in Fukushima Prefecture can be determined using Electron Spin Resonance. Electron spin resonance (ESR), sometimes referred to as electron paramagnetic resonance, is a spectroscopic technique for studying materials with unpaired electrons to ascertain radiation dose. ESR was performed on wild boar teeth to ascertain if a signal was present that could be used to quantify external radiation dose. Teeth can function as an integrating dosimeter which records the accumulated dose to an animal. The results of this experiment demonstrated that an ESR signal exists in wild boar teeth that appears to be proportional to dose. This pilot study will assist in developing methods to enhance our understanding and verifying the external radiation dose received by the wildlife in the Fukushima area.

## ACKNOWLEDGMENTS

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## TABLE OF CONTENTS

ABSTRACT .....	ii
ACKNOWLEDGMENTS .....	iii
INTRODUCTION.....	1
2.1 The Area .....	1
2.2 The Boar.....	2
2.3 Electron Spin Resonance .....	4
LITERATURE REVIEW.....	8
MATERIALS AND METHODS.....	10
3.1 Removal and Disinfection .....	10
3.2 Sample Preparation .....	12
3.3 Sample Measurement .....	15
RESULTS .....	18
DISCUSSION .....	22
CONCLUSION .....	24
REFERENCES .....	25
APPENDIX 1: Permit Documentation .....	29
APPENDIX 2: Copyright Information .....	35

# INTRODUCTION

## 2.1 The Area

On March 11, 2011 the Fukushima Daiichi nuclear disaster occurred. The accident was the result of a magnitude 9 undersea earthquake followed by a tsunami (Reilly, 2011). The earthquake and subsequent tsunami damaged the facilities of the Fukushima Daiichi Nuclear Power Station (1FNPS) and the Fukushima Daini Nuclear Power Station (2FNPS) of Tokyo Electric Power Co., Inc. The damage led to an unprecedented complex nuclear accident that resulted in the release of substantial amounts of radioactive materials into the atmosphere (Ministry of the Environment, 2015). The following day, cities located within 20 kilometers of the nuclear power plant were evacuated (World Nuclear Association, 2017). Figure 1 is an overhead view of the affected area.

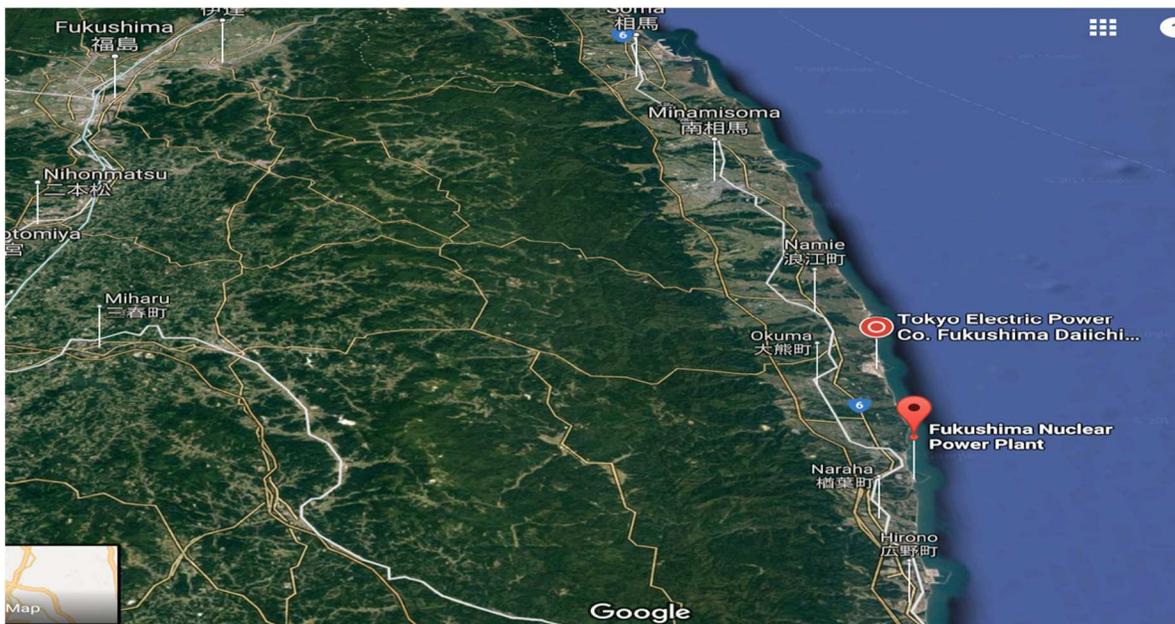


Figure 1: Map of the Area

## 2.2 The Boar

Due to the abandonment of various areas, the wild boar population within the evacuation zone began to increase steadily. The wild boar of this area (*Sus scrofa leucomystax*) (Figure 2) are located throughout all of Japan save for Hokkaido and the Ryukyu Islands (Watanobe, Okumura, & Ishiguro, 1999). The wild boar is an omnivore known for a diet comprising of roots, leaves, bark, insects, rodent, fish, and human garbage among other things. Wild boar are medium-sized animals reaching to 2 m in length, 45 to 300 kg, and an average life span of up to 10 years in the wild (Soft Schools, 2005).

Although the boars' diet could possibly result in an internal dose from the ingestion of contaminated flora and fauna, an evaluation of internal dose contribution to the ESR signal in teeth was beyond the scope of this study. Due to the complexity involved in calculating the internal dose contribution to tooth dose, all dose was assumed to be external. Separation of internal and external dose contribution to the ESR signal in teeth should be a subject of future research. Teeth were evaluated for incorporated radionuclide contribution to dose, and only one measurable example of incorporated radionuclide(s) was found. External dose to the wild boar is generally from the radiocesium present in the landscape (Ministry of the Environment, 2015). The wild boar examined for this study were located in the exclusion zones of the Fukushima Prefecture. The tooth samples were procured from boar captured in Namie, Okuma Town, and Fukushima City. (Figure 3)



Figure 2: Image of Wild Boar

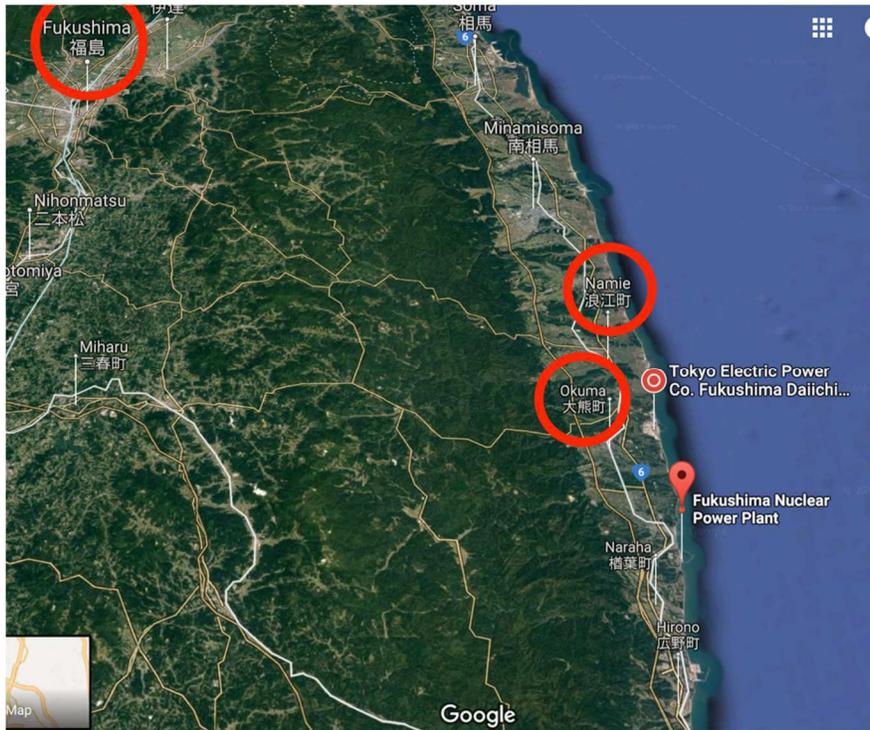


Figure 3: Image of cities where samples were procured.

## 2.3 Electron Spin Resonance

Electron spin resonance, sometimes referred to as electron paramagnetic resonance, is a spectroscopic technique for studying materials with unpaired electrons. Teeth function as an integrating dosimeter via creation of quasi-free unpaired electrons by ionizing radiation interacting with the tooth (Bougai, Desrosiers, & Romanyukha, 2002). Irradiation of teeth creates unpaired electrons which are then trapped in the hydroxyapatite, the crystalline component of teeth. The population of trapped unpaired electrons is hypothesized to be proportional to the radiation dose to the tooth and may be measured in excised tooth samples using ESR (Assenheim, 1967).

ESR is a technique that was created over time with the effort of many individuals. A key concept known as the “Zeeman effect” was observed and named after physicist Pieter Zeeman around 1896. The next stage of development was attributed to physicists Otto Stern and Walther Gerlach who contributed to the discovery of spin quantization with the Stern-Gerlach experiment in 1922. In 1924 theoretical physicist Wolfgang Pauli introduced the “two-valued quantum degree of freedom”. Early in 1925 physicist Ralph Kronig suggested that this “degree of freedom” was due to the self-rotation of the electron or “spin”.

Later the same year physicists George Uhlenbeck and Samuel Goudsmit published the theory of “electron spin” (Commins, 2012). Experimental and theoretical physicist, CJ Gorter, discovered paramagnetic relaxation in 1936. The theory was finally brought to fruition by physicist Yevgeny Zavoisky, who in 1944 observed ESR signals (Atherton 1993). Electron spin resonance references the condition where photons of a certain frequency,  $f$ , are absorbed or released during conversions (Commins, 2012) between the  $E_2-E_1$  (Eq. 1.1). Electrons exist within the electron cloud surrounding the nucleus of an atom. These electrons possess orbital

angular momentum and as they circle the nucleus they are also being deflected by magnetic fields exhibiting “angular momentum”. Sometimes referred to as a “magnet”, the charge can be related to a rotating magnetic dipole. When placed inside an external magnetic field, the magnetic dipole will begin to orientate itself in discrete directions.

A visual example of a stable state would be a compass with the head of the needle pointing north and the tail positioned southward. This position is equivalent to the lowest energy position. An unstable state would be the equivalent of the compass needle pointing perpendicularly east to west or vice versa and this equates to a higher energy position. During the process of transitioning from the lower energy level to the higher energy level, energy is absorbed. The technique of Electron Spin Resonance is simply measuring the transition from the lower level to the higher level. The equation and the quantities involved are shown in eq. 1.1:

$$E_2 - E_1 = h\nu \quad (\text{Eq.1.1})$$

Where:

$E_2$  = Higher energy level

$E_1$  = Lower energy level

$\nu$  = frequency

$h$  = Planck's constant  $(4.135667662(25) \times 10^{-15} \text{ eV/s})$  or  $(6.626070040(81) \times 10^{-34} \text{ J/s})$

If the two energy levels are denoted as  $E_2$  and  $E_1$ , then the energy required to produce one transition from the lower level to the higher level is  $h\nu$  (Assenheim, 1967).

Due to the Zeeman Effect, the state energy difference of an electron with  $s = \frac{1}{2}$  in a magnetic field may be determined by employing:

$$\Delta E = g\beta B \quad (\text{Eq.1.2})$$

where:

$$\Delta E = h\nu$$

$\beta$  = Bohr magneton

$g$  = constant of proportionality

$B$  = magnetic field

The energy absorbed by the electron should be equivalent to the state energy difference  $\Delta E$  or  $E_2 - E_1$  (as denoted above). Equation 1.2 may then be simplified to:

$$h\nu = g\beta B \quad (\text{Eq.1.3})$$

where:

$\nu$  = microwave frequency

The magnetic field and microwave frequency can be manipulated to suit one's purpose. The variable,  $g$ , is known as the constant of proportionality, whose corresponding value is the property of the electron in a definite environment. The magnetic field used for the resonance is not a unique "fingerprint" aiding in the identification of a compound because spectra may be acquired at numerous microwave frequencies.

The “fingerprint” of the molecule is denoted as  $\Delta g$  in equation:

$$h\nu = (g_e + \Delta g)\beta B \quad (\text{Eq.1.4})$$

where:

$g_e$  = g value of a free electron in a vacuum

$\Delta g$  = change in constant of proportionality (fingerprint of the molecule)

The change in the constant of proportionality contains the chemical information needed for molecule identification. The chemical information of a molecule may be found in the interaction between the electron and the electronic structure of the molecule. The value of the equation  $g = g_e + \Delta g$  denotes the “fingerprint” of the molecule. When measuring organic radicals, the g value is very close to the  $g_e$ . For transition metal complexes, the g value may vary depending on the spin-orbit coupling and zero-field splitting. The center of the signal is used to determine the g value (Kaur, Oyala, & Guo, 2017).

## LITERATURE REVIEW

The purpose of this project was to evaluate Electron Spin Resonance Spectroscopy (ESR) of wild boar teeth as a means to measure external radiation dose in Fukushima Prefecture. ESR on teeth has been used on humans and other animals as a means to measure external radiation dose, but there are many differences between human teeth and those of other mammalian species. Various animals' teeth have been tested to determine if they are suitable for radiation dose estimation. Cow, goat, walrus, and some rodent teeth have been evaluated using ESR and found to be useful estimators of external radiation dose (Jiao, Toyoda, & Hayes 1998). According to one study, the radiation sensitivity of cow tooth enamel was found to be close to that of human tooth enamel (Jiao L. , et al., 2014). This same study reported that mouse teeth were found to exhibit a sensitivity of about 25% lower compared to human teeth (Toyoda S. , et al., 2003). Another study reported that mouse tooth enamel when compared to human tooth enamel was found to be half as sensitive when exposed to gamma radiation (Khan, Rink, & Boreham, 2003).

This project utilized enamel samples collected from Japanese wild boar, *Sus scrofa leucomystax* (Watanobe T. , et al., 1999). No other ESR studies have previously been conducted on the tooth enamel of Japanese wild boar. The wild boar is considered a sentinel animal, based on its trophic level, size, and diet. After the 2011 Fukushima reactor accident, the area near the reactor has become over-run with wild boar, making it an excellent sentinel animal of opportunity. All ESR measurements took place at Okayama University of Science under the guidance of Shin Toyoda and his graduate student Mika Murahashi. Upon review of the ESR

signal results, it was determined that the enamel of the Japanese wild boar produced a detectable signal, and that ESR could be a technique suitable for wild boar enamel measurements.

## MATERIALS AND METHOD OF ANALYSIS

This project was approved by the Colorado State University Institutional Animal Care and Use Committee as an exempt protocol (Appendix 1).

### 3.1 Removal and Disinfection

Each jaw was removed from the boar, bagged, labeled, and frozen until time of tooth extraction. A chart with all of the boar data was kept on file (Figure 4). Prior to extraction, each jaw was removed from the freezer and thawed overnight. An outline of each jaw was drawn on paper to mimic the position of the molars and to facilitate labeling after all the molars were removed (Figure 5). First, second, and third molars (if available) were extracted from each jaw using dental forceps and a 20 lb. (9.1 kg) sledgehammer. The lower mandible was struck with the hammer to loosen the roots. After the roots were detached, forceps were used to remove each tooth from the socket.

Once all teeth were removed, each tooth was photographed on the jawline map and then placed in a labeled container. Per IAEA-TECDOC-1331 (Bougai, Desrosiers, & Romanyukha, 2002), each tooth was placed in a labeled container (Figure 6) and submerged in a disinfectant solution, 90% tap water and 10% chlorine and sodium hydroxide, and cycled through an ultrasonic cleansing system for forty-eight hours. After forty-eight hours, each container was emptied and all the teeth were rinsed with water, placed back into a container, and stored at room temperature until being transported to Okoyama University of Science.

ID	Capture date	Capture location			Sex	Age (weeks)	Weight (kg)	Germanium	Imaging	ESR
		Place name	Lat.	Lon.						
160617-1	Jun. 17, 2016	Fukushima	Yamada-Onbou	37.704008	140.401985	Female	57-61	51.6		
160726-1	Jul. 26, 2016	Okuma	Otozawa-Chuodai	37.415958	141.012303	Female	87	38.7		
160603-E	Jun. 3, 2016	Nome	Tatsuno	37.523055	140.931445	Female	127			
160621-2	Jun. 21, 2016	Nome	Minami-Tsushima-Shimohiyada	37.549639	140.796942	Female	33-39	23.3		
160603-1	Jun. 3, 2016	Nome	Kazawoe-Minamosaka	37.491431	140.978599	Male	57-61			

Figure 4: Chart of sample information, location, and analysis.

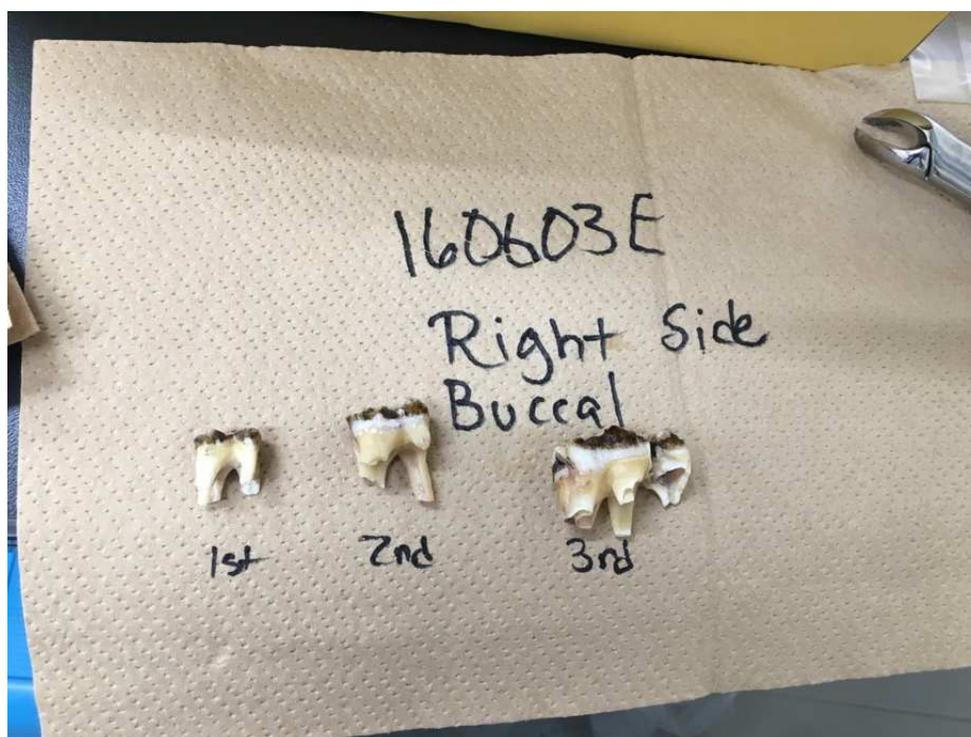


Figure 5: Example of image and labeling system used for samples.

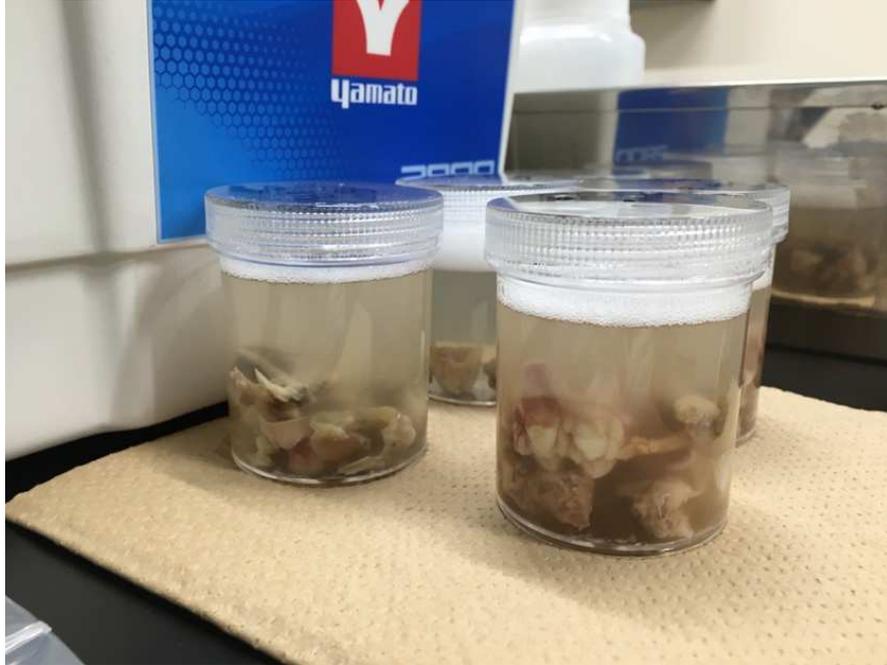


Figure 6: Labeled container containing samples submerged in the disinfectant solution.

### 3.2 Sample Preparation

Following the IAEA TECDOC (Bougai A. , et al., 2002) recommendations, each tooth was cut in half for proper dentin removal. Each tooth was prepared for cutting in the sample room located in the Earth Science department of Okayama University of Science. Nikka Seiko brand wax from the skywax series resin patching sticks were melted until completely liquid in a crucible. Each tooth was dipped in resin and fixed to a 75 by 26 mm microscope slide and allowed to dry for approximately ten minutes (Figure 7).

After the resin was completely dry, the glass was affixed to a table saw (Figure 7). A metal holder was used to attach the plated tooth to the saw. The microscope slide was placed inside a holder, and a screw was secured on each end of the holder to ensure the slide containing the tooth would not move. The blade was adjusted so that it lined up with the center of the tooth to ensure it evenly separated the lingual side of the tooth from the buccal side. To assure no

signal was induced by the process, the saw was set to low speed (90 rotations/minute) and the blade was passed through a cool oil bath during each rotation.

Once all of the teeth were completely sawn in half they were each placed into individually labeled containers. At this stage the dentin was still intact and the teeth were only physically cut in half. Two large teeth were selected for a preliminary measurement, 160603-1 and 160603 E. A chemical separation method was chosen for the first step in dentin removal. Each tooth was placed in a glass beaker and submerged in a 150 mL 20% KHO solution. The beakers were placed inside a heated ultra-sonic cleansing system with a water temperature of 30 °C. An hour later, the water temperature was measured again and recorded at 40°C.



Figure 7: The plated tooth sample affixed with resin. The table saw is in the background.

After recording the temperature, the heater was turned off and the samples remained in the solution overnight. Sixteen hours later (the next morning) the temperature of the water was measured and recorded at 40 °C. The heater was turned on in the cleaning system and warm water was added to reach a steady temperature of 60 °C. The teeth were removed from the beaker, rinsed, and re-submerged in a new beaker containing 150 mL of 20% KHO solution. After 10 hours, the heater was turned off and the teeth remained in the solution overnight.

Thirteen and one half hours later (following morning), each tooth was removed and rinsed thoroughly with deionized water and placed on a paper towel. The entire chemical separation process took forty-one hours (from the time the teeth were submerged in the KHO solution until the teeth were removed from the KHO solution). Due to the presence of dentin found in sample 160603- E, a dremmel tool was employed to mechanically remove the remaining dentin. A fine pointed grinding attachment made of a hard-alloy was used to grind away the remaining dentin. Upon the removal of all visible dentin, each tooth was then rinsed with deionized water followed by ethanol, and placed in a glass beaker. The beakers were placed inside a drying oven set to 40 °C for four hours.

After the four hour drying period, each tooth was removed from the oven, and then crushed using a mortar and pestle. The finely ground enamel powder was poured through a sieve to reach a desired diameter of 250 micro meters (Figure 8). The 250 micro meter powder was weighed and 0.1 gram of each sample was placed into ESR measuring tubes.



Figure 8: Crushed sample being poured through the sieve.

### 3.3 Sample Measurement

Sample 160617-1 (left upper second molar of a 61-week old female boar) underwent analysis utilizing a Canberra High Purity Germanium detector model (GC1520). Analysis via Gamma Spectroscopy was chosen to determine and quantify the presence of  $^{137}\text{Cs}$  incorporated into the teeth. If incorporated radioactive materials are present in the teeth, it will cause an increase in the free radicals measured, thus deeming the tooth an undesirable external dosimetry candidate.

In order to determine the presence of incorporated beta emitting radionuclides, random samples were chosen for analysis via film imaging. This method of measurement was performed prior to ESR analysis to determine if any radionuclides were incorporated into the teeth. Any superficially incorporated beta or alpha emitters can be detected by this technique. Incorporated radionuclides can generate an ESR signal that cannot be distinguished from signals generated by

external irradiation. The presence of incorporated radionuclides would increase the ESR signal in a manner not proportional to the external radiation dose. .

Samples 160617-1 (right upper second molar of a 61-week old female boar), 160726-1 (right upper first molar of an 87-week old female boar), 160603E (right upper second molar of a 127-week old female boar), 160621-2 (right upper third molar of a 33-39-week old female boar), and 160603-1 (right upper second molar of a 57-61-week old male boar) were placed on an imaging plate (Figure 9) with a potassium chloride, KCl, standard for beta imaging. Samples 160603-1 and 160603E were then analyzed on a JEOL Electron Spin Resonance Detector model (PX2300). (Figure 10) Although 105 tooth samples were collected in the Fukushima Prefecture, only two were analyzed at Okoyama University of Science. The remaining samples were scheduled to be measured in the Chemistry Department located at Colorado State University, but the ESR machine was taken out of service for maintenance for an indefinite period of time. Due to this set back only the results received from Okoyama are presented here.



Figure 9: Samples for KCl Film imaging being placed in shielded covering.



Figure 10: ESR sample measurement.

## RESULTS

Three methods of analysis were utilized, gamma spectroscopy, KCl Film imaging, and ESR analysis. Gamma spectroscopy and KCl Film imaging were needed to ascertain if any incorporated radionuclides were present in the teeth that would interfere with the ESR measurement. Upon receiving the results of the gamma spectroscopy it was determined that sample 160617-1 contained 0.00786 Bq/g of  $^{137}\text{Cs}$  (Figure 11).

		160617-1Rights7-61wk		JSAC0473 Std		日本分析化学会 土壤認証標準物質	
		面積 <i>count</i>	誤差 <i>error</i>	面積 <i>count</i>	誤差 <i>error</i>	JSAC0473(30g)	
Ge測定		74.75	16.90	4317.12	103.62		
Cs-137							
	面積(h)			86400		Cs-137(Bq/kg	115
測定時間	57600			30		面積	4317.12
<i>t:mg</i> (cs)	質量 <i>mass</i>	11.4				誤差	103.62
	Bq	0.090	0.02	3.450	0.12	測定時間	86400
	Bq/g	0.00786	0.00178703	0.115	0.00390357	Bq	3.45

Figure 11: Cesium Analysis results for sample 160617-1. Results received at Okoyama University of Science. Boxed results show the incorporated  $^{137}\text{Cs}$  concentration.

Only one of the samples chosen for KCl Film (Figure 12) imaging displayed any visible results. A small highlighted spot is visible on the upper right buccal side of sample 160726-1. This coloring indicates that there is some form of background radiation present in the sample likely due to a beta emitting radionuclide incorporated into the tooth's structure.

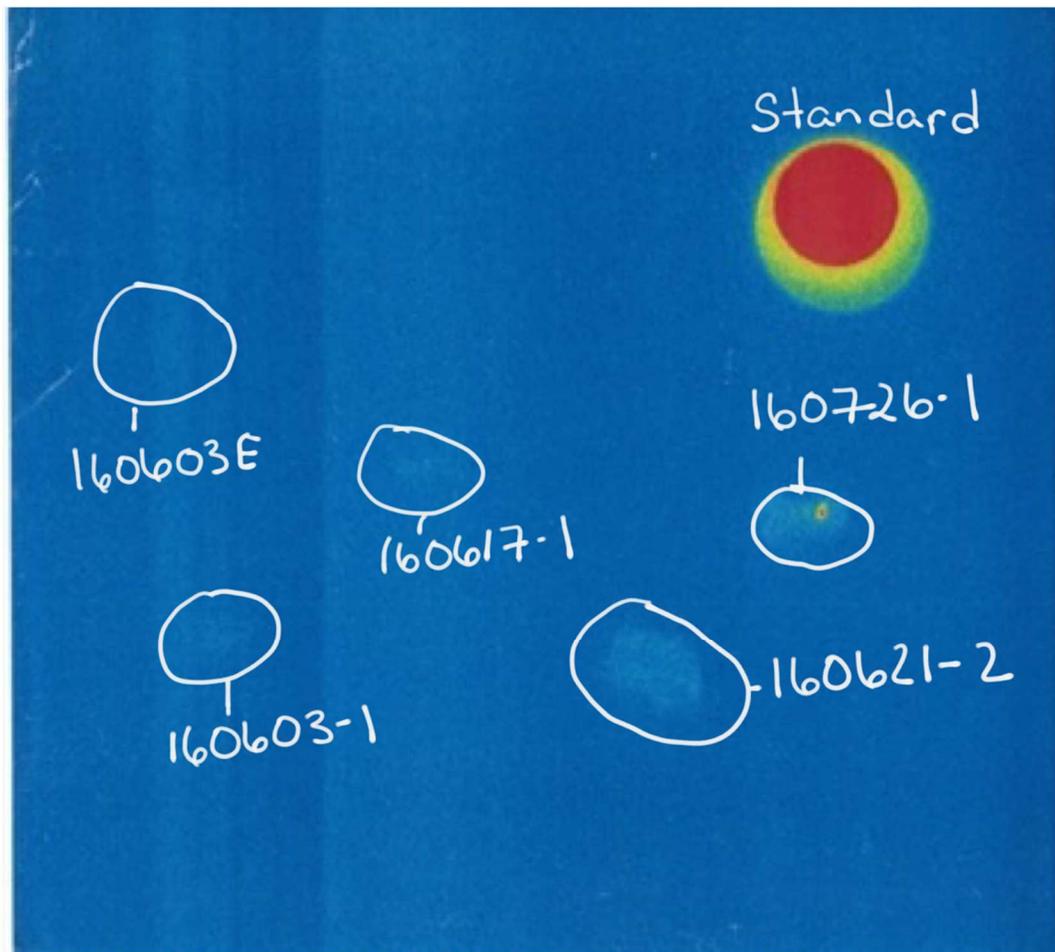


Figure 12: KCl Film results displaying samples. Samples 160617-1, 160726-1, 160603E, 160621-2, and 160603-1.

Samples 160603-E and 160603-1 ESR results (Figure 13 and 14) both display a distinguishable downward concavity in the g2 position. The deflection will ultimately, with further calculation, be equated to a dose in Gray and the molecule ( $\Delta g$ ) that caused the deflection determined in future work.

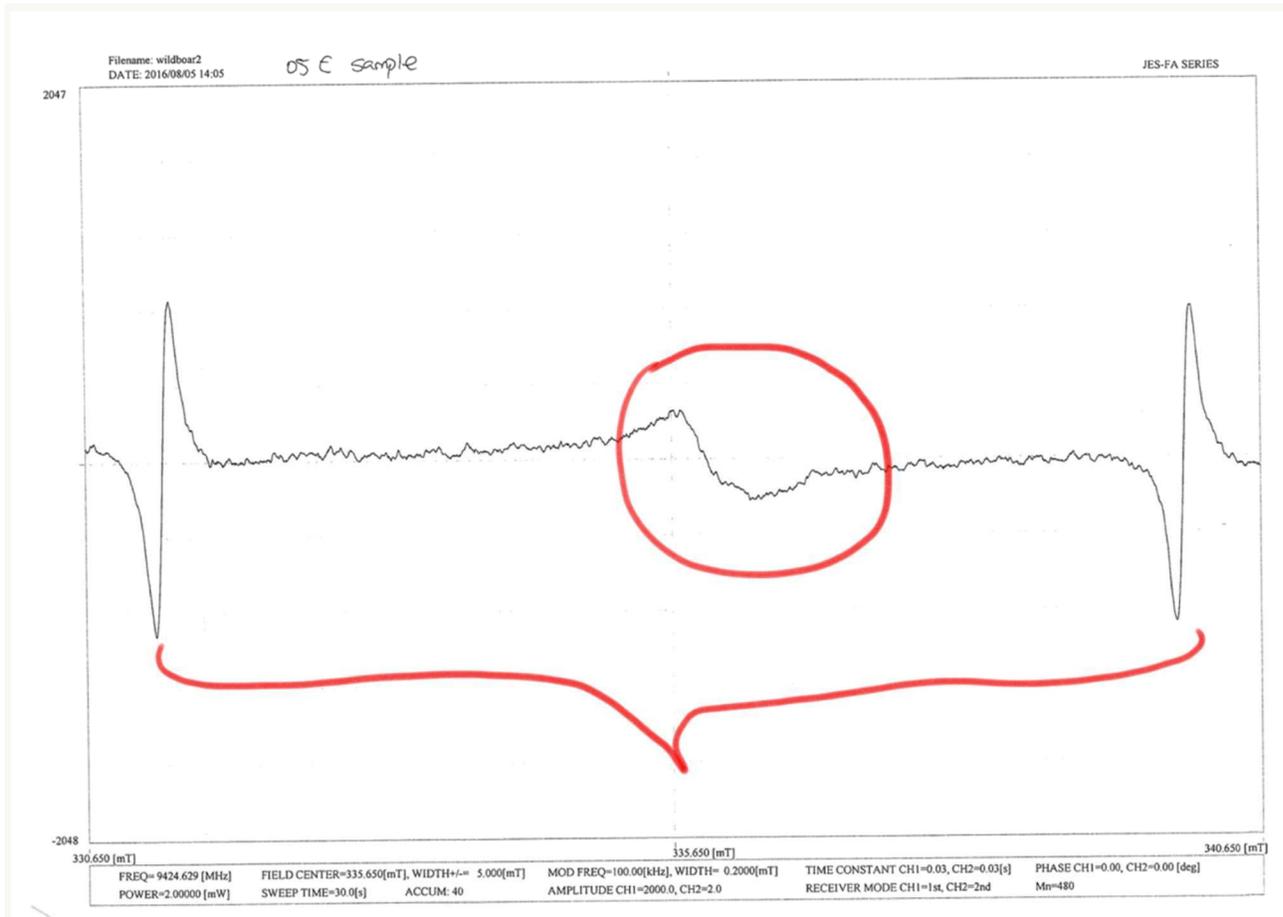


Figure 13: ESR results of sample 160603- E (127-week old female). Note the features at the arrows that indicate a dose response. This sample was collected in Tatsuno (dose rate of area:  $1\mu\text{Sv}$  per/hour or 0.1 mrem/hour)

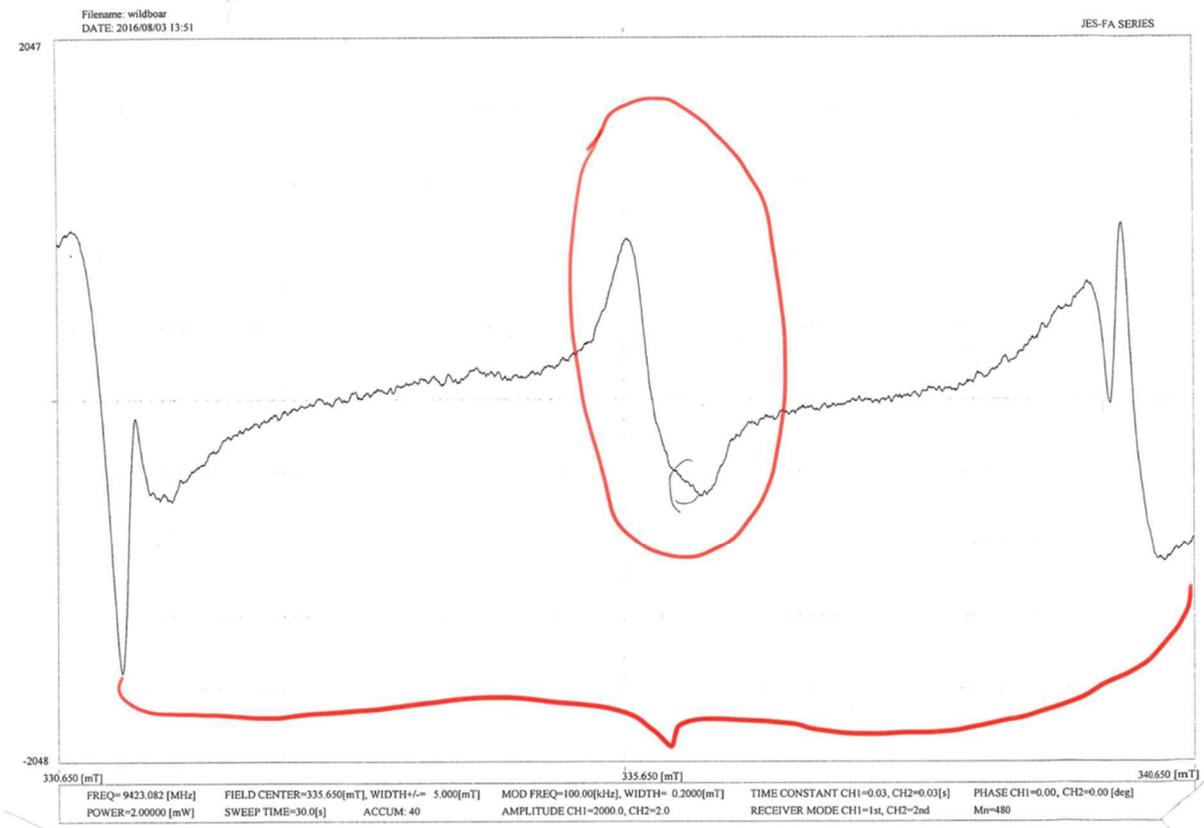


Figure 14: ESR results for sample 160603-1. Note the features at the arrows that indicate a larger dose response than in Figure 13. This sample was collected in Kazawoe-Minamiosaka (dose rate of area:  $8\mu\text{Sv}/\text{hour}$  or  $0.8\text{ mrem}/\text{hour}$ )

## DISCUSSION

Teeth have been proven to be excellent integrated lifetime dosimeters for external doses in humans. The mineral matrix, hydroxyapatite, crystallizes and becomes a fixed cage. Over time this structure may change due to growth or demineralization in bones. Fortunately, this process has not been observed in tooth enamel (Baffa, Kinoshita, & Abrego, 2002). Teeth may also be used as a comparable sample to biological dosimetry methods. Electron Spin Resonance on teeth is a proven method of external dose determination in humans, and is reliable and repeatable (Bougai A. , et al., 2002).

One question that arose during this study was the lower limit of detection (below 1 centiGy). Until recently, the lowest detectable dose for electron spin resonance was thought to be 29 mGy. According to one study, certain ESR spectrometers now have a detection limit as low as 100 mGy (Guilarte, Trompier, & Duval, 2016).

A hypothetical dose estimate was calculated for samples 160603-E (127-week old female) and 160603-1 (57-61-week old male). After receiving a dose of  $1\mu\text{Sv}$  per/hour or 0.1 mrem/hour for 127 weeks it was estimated that boar sample 160603-E external lifetime dose was approximately 0.021 Sv (2.1 rem). Sample 160603-1 was collected in a dose field of  $8\mu\text{Sv}$  per/hour (0.8 mrem/hour) for 57 weeks and received an estimated lifetime external dose of 0.076 Sv (7.66 rem).

Although a numerical value for dose was not determined, a visible dose response appeared to be present when comparing the low to high dose wild boar teeth. Other methods of analysis were employed to determine whether or not any radionuclides were incorporated in the tooth matrix. Cesium-134/137 analysis was performed before Electron Spin Resonance

measurement in order to determine the presence of incorporated radionuclides. If radioactive materials were measured in the tooth, its use as an external dosimeter would not be possible. Potassium chloride plates were utilized to measure the presence of and ensure that no beta emitting radiation (strontium, cesium, or any other nuclide) was incorporated in the tooth's structure. The presence of incorporated radionuclides would interfere with the ESR results and would render the measurement invalid, as incorporated radionuclides would cause the external dose to be erroneously high.

During the process of analyzing the collected teeth, the ESR machine located at Colorado State University was placed out of service. Unfortunately, only two samples at that time had undergone analysis. The two samples ,160603-E (127-week old female) and 160603-1 (57-61-week old male), yielded valuable information about the wildlife of the area. For assurance/consistency it is preferable that all the samples were analyzed with ESR, but it was not possible at the time.

## CONCLUSION

The objective of this study was to determine if the external radiation dose to the wild boar living in Fukushima Prefecture could be determined using Electron Spin Resonance. Teeth have been proven to function as an integrating dosimeter that can record the accumulated dose received by the animal. For this study, wild boar were collected from various locations near the Fukushima Daichii Nuclear Power Plant and surrounding cities and towns. The teeth of these boar were collected and processed for ESR analysis, gamma spectroscopy, and KCl film imaging. The gamma spectroscopy and KCl imaging analyses were chosen to rule out the presence of incorporated radionuclides in the samples. Upon reviewing the results of the two methods, the presence of incorporated radionuclides was deemed non-existent (or negligible). The ESR results of the two samples chosen, 160603-1 and 160603-E, display definite distinguishable concavity which indicate a dose response. Sample 160603-1 was collected in Kawawoe-Minamiosaka ( $8\mu\text{Sv/h}$ ) from a 57-61-week old male boar. Sample 160603-E was collected in Tatsuno ( $1\mu\text{Sv per/h}$ ) from a 127-week old female boar. Upon reviewing the ESR results, it was determined that the sample found in the higher dose area (160603-1) produced a higher signal than the sample found in the lower dose area (160603-E). The results of this experiment demonstrate that ESR, a technique that has been proven to be reliable and replicable, may be suitable to measure external dose to wild boar living in the Fukushima Prefecture. This pilot study will provide a basis for future studies on understanding and verifying the external radiation dose received by the wildlife in the Fukushima area.

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# APPENDIX 1



INSTITUTE OF  
ENVIRONMENTAL  
RADIOACTIVITY

環境放射能研究所  
INSTITUTE OF ENVIRONMENTAL RADIOACTIVITY

11 January 2017

To Whom It May Concern:

This shipment EJ 214982715 JP contains teeth of porcine origin from Japan.

We hereby state the following:

- There are 127 samples contained in this shipment. All samples are of porcine origin. Samples are contained in a 15ml or 50ml conical tube. All samples are identified by their unique animal ID written on the tube.
- All samples are derived from pigs that originated in and are housed in Japan.
- Prior to export, all samples were subjected to the following treatment (as required by permit 132546):
  - Treated using a solution of bleach in an ultrasonic cleansing system
- The exported materials have not been exposed to or commingled with any other animal origin material.
- The exported materials have a commercial value of \$0 USD.

Thomas Hinton  
Professor  
Institute of Environmental Radioactivity  
Fukushima University  
1 Kanayagawa, Fukushima 960-1296, Japan  
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INSTITUTE OF  
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環境放射能研究所  
INSTITUTE OF ENVIRONMENTAL RADIOACTIVITY

平成 29 年 1 月 10 日

ご担当者様

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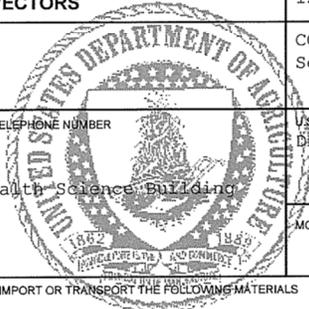


この積荷 EJ 214982715 JP には、日本原産  
のブタから採取した菌が含まれています。

つきましては、以下の通り、申し述べます。

- 積荷には総数 127 の試料が収容されています。すべての試料はブタから採取されたもので、15ml または 50ml のコニカルチューブに収容されています。全試料は、チューブ記載の固有の動物 ID によって識別されます。
- 全試料は、日本原産で日本国内に生息するブタから採取されたものです。
- 輸出に先立ち、全試料に対し以下の処理を行いました。（許可証 (permit) 132546 の要求通り）
  - 超音波洗浄システムに入った漂白剤溶液によって処理
- 輸出品は、他の動物由来の物質と接触したり混合されたりしていません。
- 輸出品の商業的価値は 0 (ゼロ) US ドルです。

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE VETERINARY SERVICES RIVERDALE, MARYLAND 20737 <small>file:///D:/inetpub/wwwroot/Epemits/images/</small>		PERMIT NUMBER 132546 Research	
<b>UNITED STATES VETERINARY PERMIT FOR IMPORTATION          AND TRANSPORTATION OF CONTROLLED MATERIALS AND          ORGANISMS AND VECTORS</b>		DATE ISSUED 12/12/2016	DATE EXPIRES 12/12/2017
		NAME AND ADDRESS OF SHIPPER(S) Various shippers within... Japan	
NAME AND ADDRESS OF PERMITTEE INCLUDING ZIP CODE AND TELEPHONE NUMBER Prof. Thomas E. Johnson Colorado State University Environmental and Radiological Health Science Building 1618 Campus Delivery Fort Collins, Colorado 80523 970-491-0563		U.S. PORT(S) OF ARRIVAL DENVER, CO	
AS REQUESTED IN YOUR APPLICATION, YOU ARE AUTHORIZED TO IMPORT OR TRANSPORT THE FOLLOWING MATERIALS		MODE OF TRANSPORTATION AIR	



Teeth, hair, and/or blood samples (fixed and/or on slides) from wild boar (porcine origin)

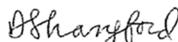
**RESTRICTIONS AND PRECAUTIONS FOR TRANSPORTING AND HANDLING MATERIALS AND ALL DERIVATIVES**

THIS PERMIT IS ISSUED UNDER AUTHORITY CONTAINED IN 9 CFR CHAPTER 1, PARTS 94.95 AND 122. THE AUTHORIZED MATERIALS OR THEIR DERIVATIVES SHALL BE USED ONLY IN ACCORDANCE WITH THE RESTRICTIONS AND PRECAUTIONS SPECIFIED BELOW (ALTERATIONS OF RESTRICTIONS CAN BE MADE ONLY WHEN AUTHORIZED BY USDA, APHIS, VS).

- o Adequate safety precautions shall be maintained during shipment and handling to prevent dissemination of disease.
- o With the use of this permit I, Prof. Thomas E. Johnson, Permittee, acknowledge that the regulated material(s) will be imported/transported within the United States in accordance with the terms and conditions as are specified in the permit. The Permittee is the legal importer/recipient [as applicable] of regulated article(s) and is responsible for complying with the permit conditions. The Permittee must be at least 18 years of age and have and maintain an address in the United States that is specified on the permit; or if another legal entity, maintain an address or business office in the United States with a designated individual for service of process; and serve as the contact for the purpose of communications associated with the import, transit, or transport of the regulated article(s). \*\*Note: Import/Permit requirements are subject to change at any time during the duration of this permit.
- o \*\*\*Each shipment shall be accompanied by an ORIGINAL signed document from the producer/manufacture confirming that: 1) the exported material was derived from wild boar (porcine) that originated in Japan; 2) prior to export to the United States, the exported materials were treated as follows: (a) the teeth were treated using a solution of bleach in an ultrasonic cleansing system, (b) the hair samples were washed with isopropanol and air dried, (c) the slides contain blood fixed with methanol and acetic acid then sealed with a fixative and coverslip, and ...[continued on page 2]...

*continued on subsequent page(s).....*

TO EXPEDITE CLEARANCES AT THE PORT OF ENTRY, BILL OF LADING, AIRBILL OR OTHER DOCUMENTS ACCOMPANYING THE SHIPMENT SHALL BEAR THE PERMIT NUMBER

SIGNATURE Deborah Langford 	TITLE Staff Veterinarian National Import Export Services	NO. LABELS
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U.S. DEPARTMENT OF AGRICULTURE  
APHIS / VETERINARY SERVICES, RIVERDALE, MARYLAND 20737.  
ATTACH TO U.S. VETERINARY PERMIT - 132546

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RESTRICTIONS AND PRECAUTIONS: (continued from Permit Form VS 16-6)

- o ... [continued from page 1] ... (d) the blood samples were fixed with methanol and acetic acid plus glutaraldehyde to achieve a final concentration of 0.2% glutaraldehyde; and 3) the exported material was not exposed to or commingled with any other animal origin material.  
[This certification must CLEARLY correspond to the shipment by means of an invoice number or shipping marks or lot number or other identification method. An English translation must be provided.]
- o COMMERCIAL DISTRIBUTION OF THE IMPORTED MATERIAL IS PROHIBITED.
- o This permit DOES NOT authorize direct or indirect exposure of or inoculation into laboratory and domestic livestock (including but not limited to: birds/poultry, cattle, sheep, goats, swine, and/or horses). Work shall be limited to *in vitro* uses only. No extraction of nucleic acids is to be performed on imported material.
- o Packaging, containers, and all equipment in contact with these materials shall be sterilized or considered a biohazard and be disposed of accordingly.
- o THIS PERMIT IS VALID ONLY FOR WORK CONDUCTED OR DIRECTED BY YOU OR YOUR DESIGNEE IN YOUR PRESENT U.S. FACILITY OR APPROPRIATELY INSPECTED LABORATORY. THE AUTHORIZED IMPORTED MATERIAL(S) MUST BE SHIPPED/CONSIGNEE DIRECTLY TO THE ADDRESS OF THE PERMITTEE OR TO THE ADDRESS OF THE ADDITIONAL PERMITTEE(S) AS IDENTIFIED ON THIS PERMIT. (MATERIALS SHALL NOT BE MOVED TO ANOTHER U.S. LOCATION, OR DISTRIBUTED WITHIN THE U.S., WITHOUT USDA, APHIS, VS, NIES AUTHORIZATION.)
- o On completion of your work, all permitted materials and all derivatives therefrom shall be destroyed.
- o This permit does not exempt the permittee from responsibility for compliance with any other applicable federal, state, or local laws and regulations.
- o Imported material may be subject to regulations enforced by the United States Department of Interior, Fish and Wildlife Service (FWS). Importer must contact FWS, information is available at web pages <http://www.FWS.gov/permits/> and/or <http://www.FWS.gov/le/travelers.html>
- o The restrictions on this permit remain in force as long as the material is in the United States.

U.S. DEPARTMENT OF AGRICULTURE  
APHIS / VETERINARY SERVICES, RIVERDALE, MARYLAND 20737.  
ATTACH TO U.S. VETERINARY PERMIT - 132546

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RESTRICTIONS AND PRECAUTIONS: (continued from Permit Form VS 16-6)

- o Any person who VIOLATES the terms and conditions of permits, and/or who forge, counterfeit, or deface permits may be subject to criminal and civil penalties in accordance with applicable law. In addition, all current permits may be cancelled and future permit applications denied.
  - o A copy of this permit must be included with the shipping documents. For imported materials, these documents must be presented to CBP Agricultural Specialists upon arrival at the U.S. port of arrival.
-



Research Integrity & Compliance Review Office  
Office of Vice President for Research  
208 University Services Center  
2011 Campus Delivery  
Fort Collins, Colorado 80523-2011  
TEL: (970) 491-1553  
FAX: (970) 491-2293  
<http://ricro.research.colostate.edu>

To: Nhung Nguyen, Thomas Johnson

From: Research Integrity and Compliance Review Office (RICRO)

Date: May 5, 2016

RE: IACUC Exemption of "The Potential Use of EPR on Wild Boar Teeth to Measure Radiation Doses in Fukushima Prefecture"

This is to inform you that your IACUC Exemption request for "The Potential Use of EPR on Wild Boar Teeth to Measure Radiation Doses in Fukushima Prefecture" 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.

**If there are any changes in this project, 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: Terry Engle, PhD, IACUC Chair  
Lon Kendall, DVM, PhD, CSU Attending Veterinarian  
Karen Dobos, PhD, RICRO Director

## APPENDIX 2

Figure 1 and Figure 3 are maps created using custom location markers in Google Earth.

Copyright information can be found in the link below:

<https://www.google.com/permissions/geoguidelines.html>

Per Fair Use Section:

“Apart from any license granted to you by Google, your use of the Content may be acceptable under principles of "fair use." Fair use is a concept under copyright law in the U.S. that, generally speaking, permits you to use a copyrighted work in certain ways without obtaining a license from the copyright holder.”