Final Technical Report

Report Title: Investigation of a novel approach to forensic analysis using neutron imaging techniques
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Abstract
This proof-of-principle research focused on establishing the novel use of neutron radiography for forensic research and, more precisely, for the determination of post-mortem interval (PMI) by the measurement of changes in neutron attenuation in decaying canine skeletal muscle. This research answered two questions: (1) how to select the optimal tissues and how those tissues are best prepared for neutron radiography and (2) what is the effectiveness of using neutrons to measure changes in the decaying tissues as a function of time. Controlled (i.e. laboratory settings at the Oak Ridge National Laboratory) and uncontrolled (i.e. Anthropology Research Facility at the University of Tennessee, Knoxville) conditions were used to study the effects of different environmental conditions (mainly temperature and humidity) on the neutron transmission through the tissue samples. The primary hypothesis of this study is that by measuring the change in the hydrogen (H) content determined by neutron transmission, the state of canine skeletal muscle degradation can be predicted. Results demonstrated that the increase in neutron transmission that corresponded to a decrease in hydrogen content in the tissue was correlated with the time of decay of the tissue. Tissues depleted in hydrogen are brighter in the neutron transmission radiographs of skeletal muscles, lung, and bone, under controlled conditions. Over a period of 10 days, changes in neutron transmission through lung and muscle were found to be higher than bone by 8.3%, 7.0 %, and 2.0 %, respectively. In conclusion, neutron radiography can be used for detection of hydrogen changes in decaying tissues that are correlated with the post-mortem interval.

Disclaimer
Canine cadavers were obtained from the Tennessee (TN) Animal Control Agency after humane euthanasia due to the inability of the shelter to secure adequate long-term care or adoption. No animals were euthanized for this study.

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Executive Summary (2500-4000 words max)

One of the most difficult challenges in forensic research for criminal justice investigations is to determine objectively the post-mortem interval (PMI). The determination of PMI is often a critical piece of information at the crime scene. Most PMI techniques rely on gross observational changes of cadavers that are subjective and highly sensitive to environmental variables that can drastically alter the estimated PMI. Chemical analysis of volatile fatty acids (VFA) based on chemical elements rather than physical changes, can be used to create a more accurate timeline of decomposition. The further development of an objective method to estimate PMI can significantly impact forensic science and meet Daubert standards.

After death, tissue undergoes sequential organic and inorganic changes, as well as a gradual reduction of water content. Living organisms consist mainly of the six basic elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. These elements combine to form the four major classes of biological macromolecules found in living matter: proteins, nucleic acids, polysaccharides, and lipids. While carbon dominates the chemistry of biological tissues, hydrogen atoms are the most abundant element. Neutron imaging is based on the attenuation due to both scattering and absorption of a directional neutron beam by the matter through which it passes. Neutron imaging is complementary, rather than competitive with X-ray imaging. Since X-rays are scattered and absorbed by the electrons, the attenuation increases monotonically with atomic number. Neutrons interact with atomic nuclei in a complex manner so that neutron attenuation does not scale in a regular way with atomic number. Hydrogen nuclei scatter thermal and cold neutrons more strongly than any other atomic nucleus. Thus, unlike other imaging methods such as X-rays, neutrons are strongly attenuated by hydrogen, which is the predominant elemental constituent of biological materials and the attenuation manifests as contrast in an image. These contrast differences in neutron imaging can readily be applied in a forensic context to determine small changes in hydrogen amount. Since body decomposition starts just minutes after death, hydrogen concentration changes over time as well. This may create a timeline of a cadavers' decomposition.

The purpose of this research is to assess the capability of neutron radiography to detect hydrogen changes in decaying tissues and whether this innovative approach can be a useful and objective tool for estimating post-mortem interval. This report focuses on two sets of experiments performed on medium size dog cadavers with no apparent diseases. Several canine tissues were collected, fixed and imaged using neutrons. Neutron radiography of fixed tissues was compared to neutron radiographs of fresh tissues.

The first phase of this research was to optimize the choice of tissue samples, the method of extraction and preparation of the tissue samples that was compatible with neutron imaging. Several samples were measured including lung, heart, liver, skeletal muscle, kidney, skin, and bone. Three fixation techniques were employed: formalin, ethanol, and deuterated ethanol. Although deuterated ethanol fixation did not affect neutron transmission (no hydrogen was added to the tissue during fixation and deuterium has a low neutron cross-section and thus does not contribute to neutron transmission in a significant way), after 16 hours the tissues shrank by 50%. Ethanol and formalin fixation did alter neutron transmission and formalin had less shrinkage (only 4% after 16 hrs). Thus, formalin was selected as the optimum fixation for this forensic research.

Two sets of environmental conditions were used to measure the neutron transmission changes through time: controlled (laboratory setting) and uncontrolled (at the University of Tennessee Anthropology Research Facility). The team focused on skeletal muscles primarily, as these tissues can be removed using biopsy needles. Lung and bone were also studied in controlled environmental conditions as fast and slow decaying tissues, respectively.

The choice of tissue (lung, muscle, or bone) directly affected the rate of change in neutron transmission due the changes in H content in the decaying tissue. Depending on environmental

conditions, skeletal muscle will decay in a few days but this can extend to a few weeks, as observed at the UTARF during winter 2011. Finally, bones outlast all "soft" tissues. These different putrefaction rates may be an advantage for this technique. Depending on the stage of decomposition at the crime scene, one could select a short-lasting sample, such as lung and/or muscle, or a long-lasting sample, such as a bone.

Fixation of the tissue offers the option to "freeze" the sample in time, i.e. no further decomposition occurs, and transport it to a location where neutron imaging can be performed. As determined by this research, fixation does not alter the information but creates an offset in the transmission value that can be accounted for during data processing and analysis. Thus, tissues can easily be fixed in the field by taking the samples and placing them into a container full of formalin. After that time, the sample will not change for a very long time as far as neutron radiography is concerned. This is then a convenient, simple, cheap, and easy method to obtain a tissue sample that can be used to accurately determine the PMI.

While controlled conditions data provide linear response in neutron transmission, the uncontrolled measurements do not show the same linear increase as a function of time. The data are highly affected by environmental conditions. The choice of summer measurements at the UTARF seems a less than optimum time to collect muscle tissue samples to understand the science due to excessive insect activity. Dealing with such situations can be better addressed when the correlations have been modeled. In conclusion, the neutron radiography can be used for detection of hydrogen changes that can provide an objective determination of PMI.

I. Introduction

1. Statement of the problem

One of the most difficult challenges in forensic research for criminal justice investigations is to objectively determine the post-mortem interval (PMI). The determination of PMI is often a critical piece of information at the crime scene and subsequently at a trial. Most PMI techniques rely on gross observational changes of cadavers that are subjective and highly sensitive to environmental variables. Chemical analyses of volatile fatty acids (VFA) that have proven useful indicate that chemical elements rather than physical changes can create a better timeline of decomposition. The development of an objective method to estimate PMI would significantly impact forensic science and meet Daubert standards.

2. Literature citations and review

The Process of Decomposition - Human decomposition begins a few minutes after death. Decomposition is governed initially by a process called autolysis – or self-digestion. As cells of the body are deprived of oxygen, carbon dioxide increases, pH decreases, and metabolites accumulate, which further degrade normal cellular function. Concomitantly, cellular enzymes (lipases, proteases, etc.) begin to digest the cells from the inside out which eventually causes them to rupture and release nutrient-rich fluids. [1] Autolysis usually does not become visually apparent for a few days. It is first observed by the appearance of fluid filled blisters on the skin and skin slippage where large sheets of skin slough off the body. After enough cells have ruptured their contents of nutrient-rich fluids, the process of putrefaction begins.

Putrefaction is the destruction of the soft tissues of the body by the action of microorganisms (bacteria, fungi and protozoa which are present in and on the body naturally) and results in the catabolism of tissue into gases, liquids and simple molecules. Usually, the first visible sign of putrefaction is a greenish discoloration of the skin due to the formation of sulfhemoglobin in settled blood. This process progresses into distension of tissues due to the formation of various gases (hydrogen sulfide, carbon dioxide, methane, ammonia, sulfur dioxide, and hydrogen) that

is especially prominent in the bowels. Putrefaction is associated with anaerobic fermentation, primarily in the gut, releasing by-products rich in volatile fatty acids (primarily butyric and propionic acids). Shortly after the purging of gases due to putrefaction, active decay begins [1,2]. Muscles are composed of mostly proteins, which in general break down into amino acids by proteolysis and further bacterial decomposition yield to hydrogen sulphide gas, pyruvic acid, thiols, and ammonia. In addition to protein, muscle contains fat that is degraded into fatty acids and glycerol by lipases. Further free fatty acids are degraded in anaerobic conditions through hydrogenation by bacterial enzyme (hydrogen content is increased by transforming unsaturated bonds into single bonds) or in aerobic environmental condition by oxidation to produce peroxide linkages in fatty acids, eventually producing aldehydes and ketones. The different conditions of decomposition will affect the amount of measured hydrogen content.

The Post-mortem Interval - Post-mortem interval techniques can be estimated on cellular phone records, receipts, etc., which may not always be available as they strongly depend on the economical status and life style of the victim. There are currently few scientific methods based on chemical measurements that can be used to estimate the PMI. Typically, such information is gained through the cooperation of trained forensic scientists who provide information based on experience and opinion. For example, estimating the PMI prior to the onset of putrefaction (36-72 hrs) generally involves visual inspection of the body by observing the appearance (i.e. rigor and livor mortis), determining the core body temperature, and examining the gastric contents [3, 4]. During this time period, changes in blood and cerebrospinal fluid biochemistry are often determined but these measurements are subject to considerable error and are often unreliable for determining the PMI, as is measuring the vitreous humour potassium concentration [4]. To date, volatile fatty acids have been the only reliable biomarkers of PMI for preskeletonized remains during putrefaction [5]. The decay rate of DNA in ribs has also been investigated, but has yet to be field-tested [6]. The reported accuracy of the fatty acid determination method for PMI of pre-skeletonized remains is ± 2 days/month. Once a corpse is skeletonized, the concentrations of inorganic elements (K^+, Ca^{2+}, Mg^{2+}, etc.) that migrate into the surrounding soil have been used for determining PMI [5]. The reported accuracy of this method for PMI of skeletonized remains over a year old is ± 2 weeks. Forensic entomology is another useful tool that has, in the last several years, gained success in determining PMI [7-10] and, along with decomposition by-products, is currently one of the best means of determining the PMI. While these methods cover the entire breadth of human decomposition, from early (<12 hours) to very late (skeletonized >10 years), PMIs are still highly problematic due to their large error ranges.

3. Statement of hypothesis or rationale for the research

The purpose of this research was to assess the capability of neutron radiography to detect hydrogen (H) changes in decaying tissues and whether this innovative approach can be a useful and objective tool for estimating PMI.

The hypothesis was that neutron transmission through decaying tissues increases with time due to the decrease of H content in the tissues. Furthermore, the change in hydrogen content would be related to the PMI by some function of time. It was also predicted that environmental conditions would change the rate of hydrogen decrease in the tissues.

II. Methods

1. Experimental design

A prior report was submitted to the National Institute of Justice (NIJ) (submission date: January 31, 2011) and was focused on the project's first task: the optimization of choice and

preparation of the tissue samples prior to neutron radiography. The report determined the optimal preparation by comparing fixed and fresh tissues and by examining the effect of the thickness of different canine tissues.

This report focuses on two sets of experiments performed on medium size dog cadavers with no apparent diseases. Several canine tissues were collected, fixed and imaged using neutron imaging. The fixed tissues were compared to their equivalent fresh ones. The selection of the carcasses was independent of the sex, breed and estimated age of the dogs.

Controlled environmental conditions: Skeletal muscle samples of 2 cm x 2 cm x 2 mm size were dissected from canine cadavers, wrapped in aluminum (Al) foil, and stored in a 4 ºC refrigerator at the University of Tennessee College of Veterinary Medicine (UTCVM) before being transported on ice to the Oak Ridge National Laboratory (ORNL) High Flux Isotope Reactor (HFIR) for neutron radiography. Bones and lung tissues were also extracted for neutron radiography assessment. Tissues were kept under constant conditions (22 ºC +/- 2 ºC and ~ 50-60% humidity) at the neutron facility and were exposed for a few minutes to neutrons at regular time intervals over several days.

Uncontrolled environmental conditions: In collaboration with the University of Tennessee Anthropology Research Facility (UTARF), canine cadavers were positioned on the ground, in the shade of a tree and with a protective net to minimize scavenger activities during winter 2011 and summer 2012. Temperature and humidity information in Knoxville, TN was obtained from the Biosystems Engineering Department of the Institute of Agriculture at the University of Tennessee [11]. One cadaver was placed at the UTARF on the first day of the neutron radiography measurements for the winter uncontrolled environmental conditions studies (Day 0 or December 7, 2011). During the summer experiments, four dog cadavers were brought to the UTARF at six, four, and two weeks prior to the neutron measurements. The fourth cadaver was placed at the facility on the first day (Day 0 or June 18 2012) of the experimental neutron measurements. Photographs of the dog cadavers at the UTARF were collected to monitor decay changes. Three samples of decaying skeletal muscle tissues were extracted at each sampling interval using bone biopsy needles to obtain 2 and 5 mm thick samples. The use of biopsy needles was driven by the need to extract similar samples and reduce the risk of human error in tissue preparation. An example of tissue preparation is illustrated in Figure 1A (tissue removed using the biopsy needle) and Figure 1B (tissue ready to be wrapped in Aluminum foil). A representative gray scale and color neutron radiographs are illustrated in Figures 1D and 1E, respectively. The other two samples were fixed in formalin (24-hr fixation) for histological analysis and neutron radiography the next day, respectively. After extraction, the incision was covered with duct tape, and a new incision was used each time of sampling. New incisions were made along the anterior thigh until sampling could no longer continue due to either the absence of muscle tissue or excessive maggot activity during summer.

2. Neutron imaging facility

The ORNL Neutron Sciences Directorate has a beamline (CG-1D) at the High Flux Isotope Reactor (HFIR) that is used for neutron imaging. A pinhole-geometry aperture system is used at the entrance of a He-filled flight path to allow L/D variation from 400 to 800 (L is the distance between the aperture and the detector image, and D is the diameter of the aperture). An increase in L/D can increase spatial resolution at the cost of neutron intensity. Samples sit on a translation/rotation stage for alignment and tomography purposes. Detectors for the CG-1D beamline are (1) an ANDOR DW936 charge coupled device (CCD) camera with a field of view of approximately 7 cm x 7 cm with ~ 50 μm spatial resolution and 1 frame per second time resolution (with binning) and (2) a Micro-Channel Plate (MCP) detector with a 4 cm radius field

of view and a 40 μm spatial resolution. ^{6}LiF/ZnS scintillators of thickness varying from 50 to 200 μm are being used at this facility. The white beam flux at CG-1D is approximately 1 x 10^{7} neutrons/cm^{2}/s for an L/D of ~ 460. A schematic and a photograph of the facility are shown in Fig.1C.

Neutron imaging is based on the transmission of neutrons through an object. Neutron transmission is governed by the neutron interactions, scattering and absorption, with matter. Beer-Lambert law states that the transmission, *I*, of a homogeneous sample is defined as:

$$I = I_0 e^{-\mu x} \quad (1)$$

where I_0 is the intensity of the incident beam, μ is the attenuation coefficient, and *x* is the thickness of the sample. The attenuation coefficient can be calculated from neutron cross-sections.

3. Neutron radiography, data processing and analysis

Samples were positioned on a translation stage between the neutron beam flight tube and the charge coupled device (CCD) camera as shown in Figure 1C. Tissues wrapped in Al were exposed to the neutron beam for approximately 2 minutes. For these measurements, a spatial resolution of 50 μm was achieved. Three images of the same tissue were collected each time. Under controlled conditions (22 ºC +/- 2 ºC and ~ 50-60% humidity), the tissues were kept wrapped in aluminum (Al) and left to decay at the neutron facility. They were measured at regular intervals over 10 days. Samples from uncontrolled conditions were measured ~ 1 hr after extraction at the UTARF. Transmission values were obtained from the neutron radiographs using MATLAB® [12] after image normalization. Images were normalized using Equation (1), where $\text{Image}_{\text{normalized}}$ corresponds to the normalized image, $\text{Image}_{\text{raw}}$ is the raw image of the tissue, $\text{Image}_{\text{dark\_field}}$ is the electronic noise of the CCD, $\text{Image}_{\text{open\_beam}}$ is the image of the beam (without the tissue sample) and f_{cor} is the correction factor for beam fluctuation. A 4 x 4 median filter was also applied to remove the CCD hot/cold pixels and gammas strikes on the CCD chip.

$$Image_{normalized} = \frac{Image_{raw} - Image_{dark\_field}}{Image_{open\_beam} - Image_{dark\_field}} \times f_{cor} \quad (2)$$

An example of a gray scale and color enhanced neutron radiograph of a skeletal muscle is shown in Figures 1D and 1E, respectively. Neutron attenuation was estimated from five regions of interest that were away from the edge of the tissue for the 2 cm x 2 cm x 2 mm tissues (controlled conditions protocol). This approach was chosen to minimize the statistical variation between tissues. Moreover, segmentation of the tissue was used for the 2 and 5 mm diameter samples extracted using a biopsy needle (uncontrolled conditions protocol) such that neutron attenuation was estimated using the whole sample. Standard deviation was used to calculate statistical errors.

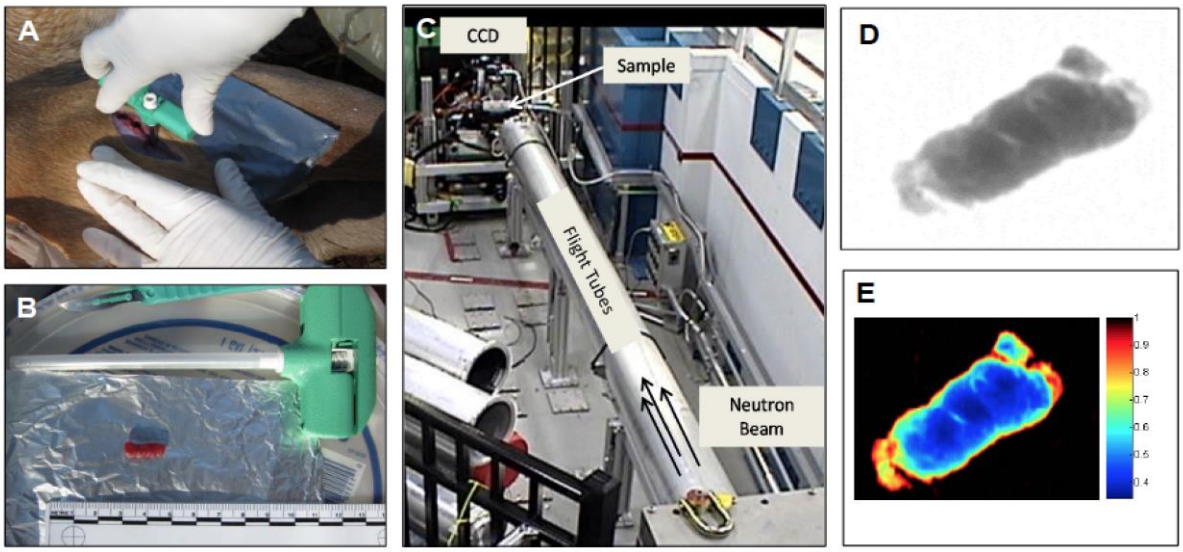

Figure 1: Sample collection for neutron radiography measurements. (A) 2- and 5-mm thick samples of decaying skeletal muscle tissues were extracted at different times using an 8-gauge bone biopsy needles. **(B)** Tissue samples were wrapped in aluminum foil and measured ~ 45 min after extraction. **(C)** Samples were positioned on a translation stage between the neutron beam flight tube and the CCD camera at the ORNL neutron imaging facility. Tissues were exposed to the neutron beam for ~ 2 minutes and radiographs were collected by a 2048 x 2048 CCD camera at a spatial resolution of approximately 50 µm. **(D)** Gray scale neutron radiograph and **(E)** color enhanced neutron radiograph of the skeletal muscle.

III. Results

1. External examination of decomposed canine cadavers during winter 2011 and summer 2012 in East Tennessee (uncontrolled conditions)

In order to visually assess the level of decay, a series of photographs were taken at the UTARF during winter 2011 and summer 2012. During the winter, the decomposition of the canine cadaver was not externally visible due to cold weather conditions (see Table 1; Day 0 corresponds to the day the cadaver was placed at the UTARF) and absence of insect and scavenger activities over the 10 days of the study. The average values of recorded environmental data over the duration of the study were relative low, i.e. average rain fall of 0.9 mm, 72.8 % humidity, air and soil temperatures of 6.4 ºC and 10 ºC, respectively.

During the summer experiments, four dog cadavers were brought to the UTARF six (Day -41 or May 8 2012), four (Day -25 or May 24 2012), and two (Day -14 or June 4 2012) weeks prior to the neutron measurements. In addition, a "fresh" cadaver was placed at the facility on the first day (Day 0 or June 18 2012) of the experimental neutron measurements. Due to high average air and soil temperatures, i.e. 22.4 ºC and 25.5 ºC, respectively (Table 2) and high insect activities, the decomposition of the cadavers that were left two and four weeks was advanced as compared to the freshly placed cadaver on Day 0. The cadaver placed six weeks prior to external examinations had reached a full-skeletonized stage.

Table 1. Recorded environmental conditions close to the burial site during winter 2011 [11]. Data comprise solar energy per surface area, rainfalls, humidity, air and soil temperatures, and high and low air temperatures.

Winter 2011							
Day	**Solar (MJ/m2)**	**Rain (mm)**	**Humidity (%)**	**AirTemp (ºC)**	**Soil Temp, 15 cm depth (ºC)**	**Air HighTemp (ºC)**	**Air LowTemp (ºC)**
Day 0	1.25	5.64	86.20	5.08	12.60	9.94	1.62
Day 1	10.51	0.63	71.50	2.48	9.96	9.27	-1.44
Day 2	10.02	0.31	74.60	3.71	9.63	11.06	-2.18
Day 3	6.84	0.00	64.22	3.03	9.31	7.27	0.29
Day 4	10.82	0.00	63.86	2.56	8.27	10.46	-2.31
Day 5	7.07	0.00	60.22	6.60	9.15	12.00	1.02
Day 6	3.96	0.00	75.10	9.50	10.91	13.79	7.15
Day 7	9.09	0.00	79.20	11.26	N.D.	19.57	6.35
Day 8	3.53	1.88	80.60	13.17	N.D.	18.52	6.82
Average over study	**7.01**	**0.94**	**72.83**	**6.38**	**9.98**	**12.43**	**1.92**

Table 2. Recorded environmental conditions close to the burial site during winter 2011 [12]. Data comprise solar energy per surface area, rainfalls, humidity, air and soil temperatures, and high and low air temperatures.

Summer 2012							
Day	**Solar (MJ/m2)**	**Rain (mm)**	**Humidity (%)**	**AirTemp (ºC)**	**Soil Temp, 15 cm depth (ºC)**	**Air HighTemp (ºC)**	**Air LowTemp (ºC)**
Day -10	27.96	0.00	51.00	22.23	25.57	29.48	15.41
Day -9	22.51	0.00	60.82	23.43	25.59	30.46	16.47
Day -8	13.22	0.31	71.30	22.77	26.29	27.81	20.05
Day -7	13.31	3.44	78.10	22.85	25.27	28.28	19.72
Day -6	16.73	2.51	75.60	24.32	25.65	29.15	20.72
Day -5	23.62	0.00	60.39	24.64	26.03	31.27	17.99
Day -4	22.10	0.00	61.67	24.23	26.22	33.33	18.33
Day -3	23.35	0.00	64.04	24.89	26.72	32.33	18.86
Day -2	18.36	3.13	67.83	24.11	27.18	29.22	20.12
Day -1	21.33	0.00	64.48	24.37	26.43	30.73	19.72
Day 0	27.10	0.00	58.19	25.83	27.15	32.66	18.73
Day 1	25.52	0.00	58.76	26.01	27.59	34.25	18.66
Day 2	24.87	0.00	57.00	27.35	28.57	34.19	20.65
Day 3	23.77	2.82	61.09	27.39	29.37	34.92	21.12
Day 4	23.83	0.31	62.79	27.23	29.46	35.32	21.65
Average over study	**20.91**	**2.13**	**65.61**	**22.44**	**25.52**	**29.31**	**16.95**

2. Neutron radiography of canine tissues under controlled environment

Three representative sections of 2 cm x 2 cm x 2 mm canine tissues (lung, skeletal muscle, and bone) were imaged at the ORNL High Flux Isotope Reactor (HFIR) using neutron radiography. The tissues were kept under controlled decay environment and the neutron radiography was performed daily over 10 days. Neutron transmission through the respective tissues was plotted as a function of time as shown in Figures 2 (lung), 3 (muscle) and 4 (bone). The transmission increased over time (16h). Over a period of 10 days, changes in neutron transmission through lung and muscle were higher than bone and were 8.3%, 7.0 % and 2.0 %, respectively. The largest change in transmission in lung and muscle may be attributed to their decay rate, thus they may outgas H a lot quicker than bones. The main contributor of H in bones is the bone marrow and blood. It is likely that its decay is slower due to minimal loss of H content.

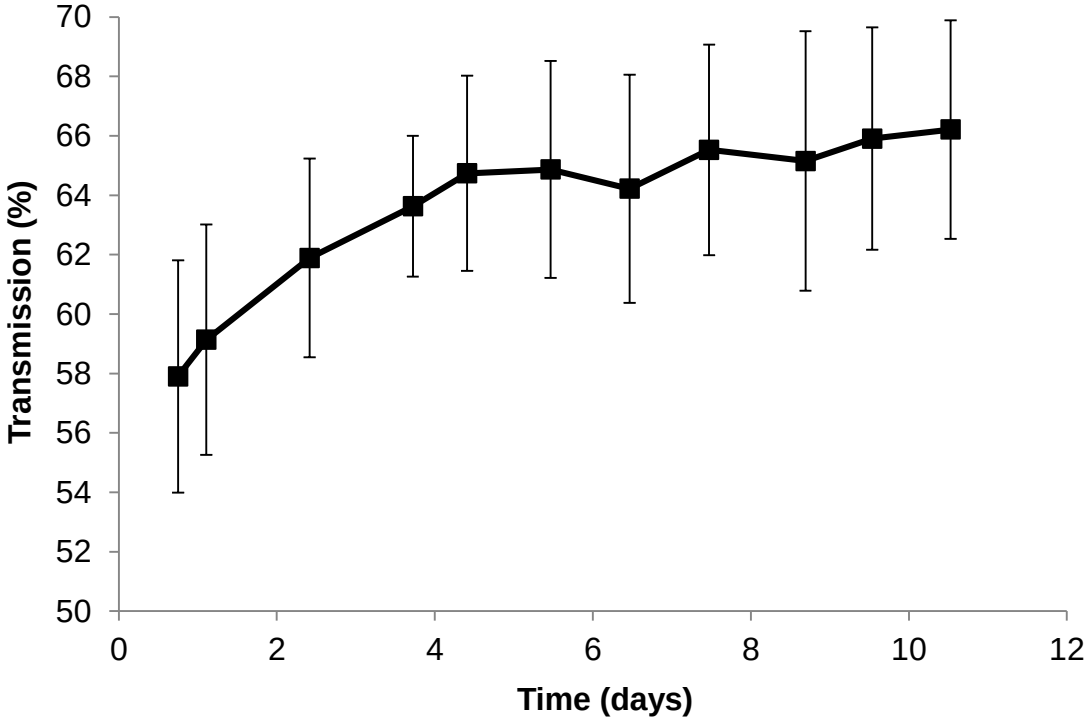

Figure 2. Lung tissue neutron transmission as a function of time over 10 days.

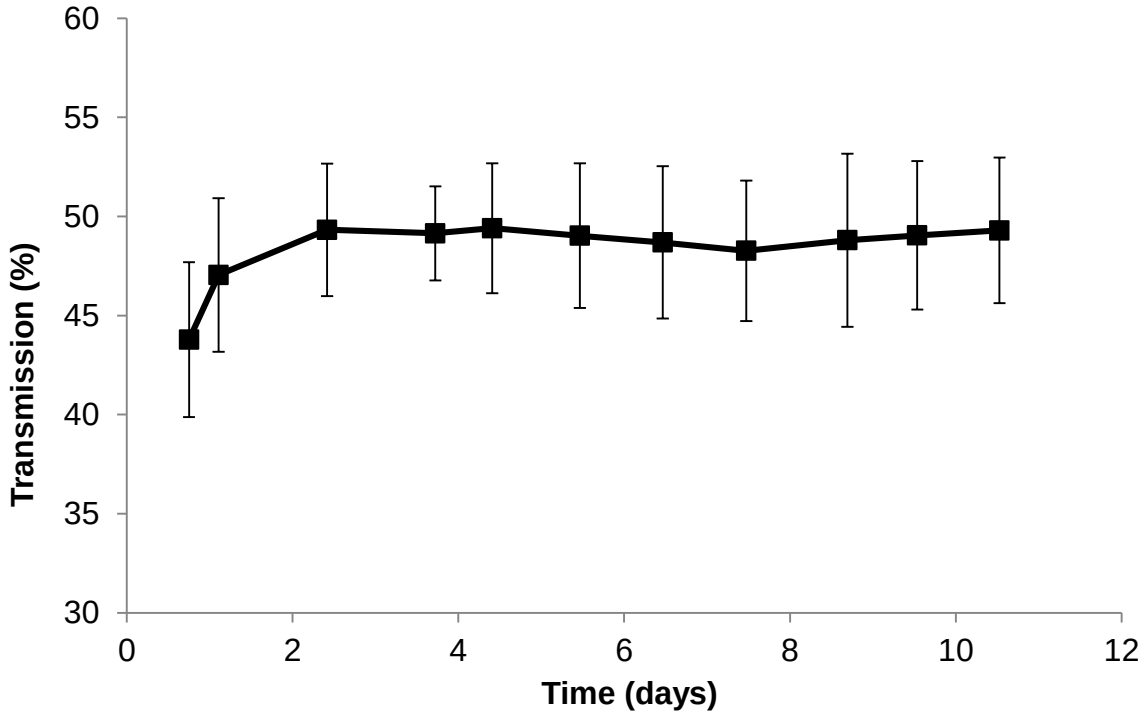

Figure 3. Skeletal muscle tissue neutron transmission as a function of time over 10 days.

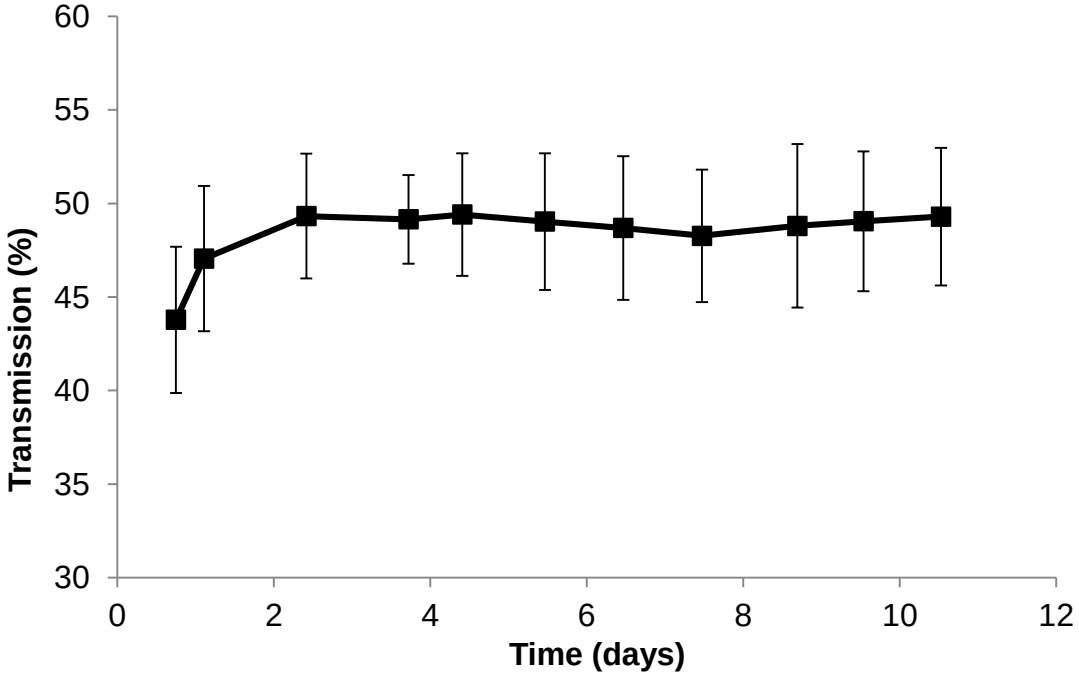

Figure 4. Bone neutron transmission as a function of time over 10 days.

3. Neutron radiography of canine skeletal muscle under uncontrolled conditions (winter 2011 and summer 2012 in East Tennessee)

Winter 2011 was mild with a few overnight freezes. Summer 2012 was hot and humid. These two different seasons were selected by the team to compare the two most opposite environmental conditions in Tennessee. There were no observable insect or rodent activities during winter 2011 (even after a month) whereas, during summer 2012, in just a few days the carcasses were overwhelmed with insect activities. Skeletal muscle samples were collected from canine cadavers placed at the UTARF during winter 2011. Biopsied samples of 2-mm and 5-mm were imaged over a period of 10 days during winter 2011. Changes in transmission over time were not significantly different, which corresponded to the absence of apparent decay of the carcass.

In contrast four canine cadavers placed at UTARF during summer of 2012 decomposed almost to bone within a few weeks due to high temperature (Table 2) and insect activity. The fourth canine cadaver placed at UTARF was used to obtain muscle biopsies to evaluate changes in skeletal muscle by neutron radiography over a period of 5 days. The samples of skeletal muscles from the fourth canine cadaver were collected using biopsy needle daily for 5 days during summer of 2012 as shown in Figure 5. One sample was fixed using formalin (24 hr. fixation in 10% BNF) as a means to stop further decay. Fixation of the sample tissue after extraction may help the forensic team on the field to transport the samples to a facility Our preliminary research had demonstrated that fixation of a tissue creates an offset in the neutron data that can be corrected during data processing. Changes in neutron transmission over time varied significantly in a 5-day period. The fresh tissue transmission decreased over time by 6.3% instead of the expected increase, which was likely due to the carcasse exposure to rainfall and humidity (over 50%, see Table 2). The fixed tissue transmission increased by 7.2% over 5 days with a peak that corresponded to an increase in humidity (but no rainfall, see Table 2).

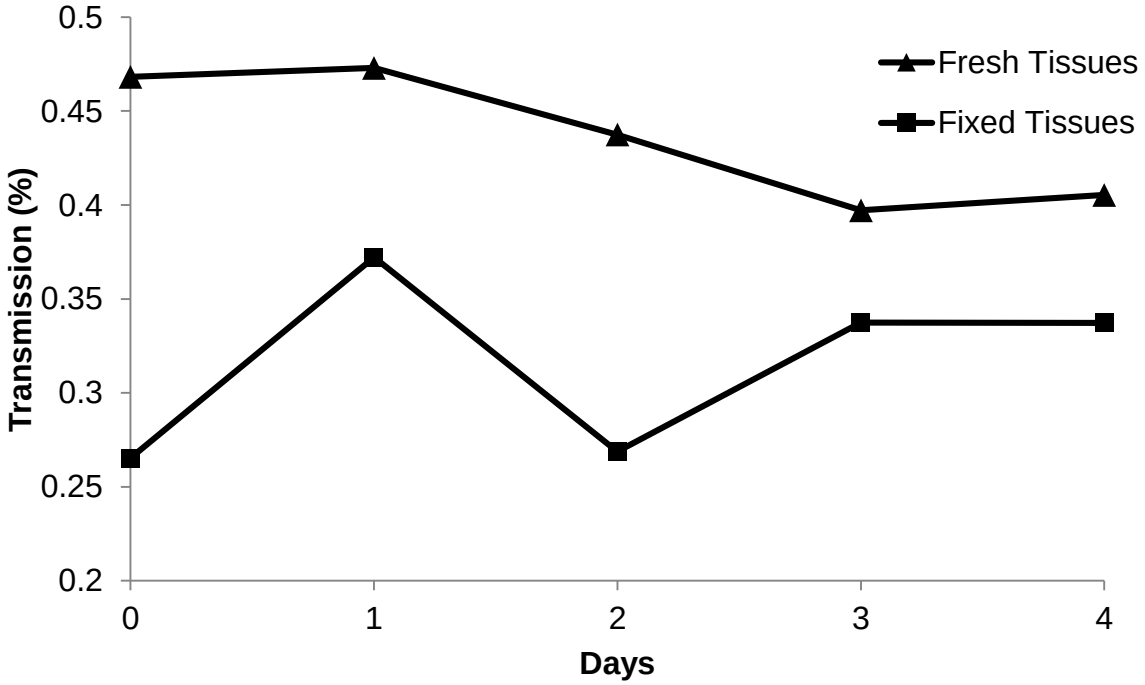

Figure 5. Fresh and fixed skeletal muscle tissue neutron transmission of as a function of time during summer 2012 (uncontrolled conditions).

IV. Conclusions

1. Discussion of findings

This proof-of-concept research has focused on establishing a relationship between neutron transmission and decay of tissues of forensic interest. Under controlled conditions, a linear increase in transmission was observed as a function of time. This linear relationship could potentially provide a novel way to estimate PMI by comparing tissues obtained at a crime scene with neutron transmission changes of known cadavers exposed to a set of environmental conditions that allow the appropriate adjustments for the weather.

The choice of tissue (lung, muscle or bone) directly affects the rate of change in neutron transmission, which means that the rate of change of the H content as a function of time in the decaying tissue is a function of the tissue type. It is most likely related to the different decay rates of different tissue types. Depending on environmental conditions, skeletal muscle will decay in a few days but this can extend to a few weeks as observed at the UTARF during winter 2011. Finally, bones outlast all soft tissues. These different putrefaction rates may be an advantage for this technique. Depending on the stage of decomposition at the crime scene, one could select a short-lasting sample such as lung and/or muscle or long-lasting samples such as a bone when the decomposition is advanced.

Fixation of the tissue offers the option to "freeze" the sample in time, and transport it to a location where neutron imaging can be performed. As measured during this research, fixation does not alter the information, and creates an offset in the neutron transmission value that can be accounted for during data processing and analysis.

While controlled conditions data provide linear response in neutron transmission, the uncontrolled measurements do not show the same linear increase in transmission through time. The data is highly affected by environmental conditions. The choice of summer measurements at the UTARF seems a less than optimum time to collect muscle tissue samples due to excessive insect activity during summer.

2. Implication for policy and practice

It is too soon to establish a policy and practice for this research. This research was limited to a proof-of-concept and further studies are necessary to establish this technique at the level where it can be trusted for PMI estimates.

3. Implications for further research

Further research should focus on using human rather than canine cadavers. One major issue with canine cadavers is that the team was not able to assess the effect of the chemicals used in humane euthanasia. Additionally, the use of human cadavers will be more representative of an actual crime scene set of remains. Among the next steps for this research would be to focus on establishing statistical models from neutron imaging data of human cadavers decomposing under different environmental conditions. Because several environmental factors play an "intertwined" role in the decomposition of a cadaver and thus the neutron transmission of the extracted tissue, it is likely that this research will require the establishment of a database or library that contains neutron transmission data of a selected tissue for a given body type, climate, state of decomposition, insect activity, etc. These factors will be essential in developing modeling PMI estimate from neutron transmission measurements.

With the advances of portable neutron generators development, the devices have become more affordable so that with appropriate algorithms based on neutron data and modeling, it is conceivable that a system could be installed in a law enforcement vehicle to be used at the crime scene to estimate PMI from measured hydrogen content (publication in preparation) and from that determination the PMI can be calculated. The environmental factors could be input into the

algorithms using current smart phone technology to access the local weather conditions for the previous several weeks or months. The adaptation of these portable devices for the determination of the PMI could provide a more reliable and on scene determination of the post-mortem interval

V. Acknowledgments

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VI. References

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[11] Weather information, http://bioengr.ag.utk.edu/weather/

[12] MATLAB® is a commercial software developed by The MathWorks Inc., Natick, MA, 2000.

VI. Dissemination of research findings

Presentations

The 2011 National Institute of Justice Conference, Arlington, VA, June 20-22, 2011
7th International Topical Meeting on Neutron Radiography, Kingston, Ontario, June 16-22, 2012

Publications

Hassina Z. Bilheux, Maria Cekanova, Trent L. Nichols, Sophie Voisin, Vincenzo Finocchiaro, Arpad A. Vass, Jean-Christophe M. Bilheux, Alfred M. Legendre, and Robert L. Donnell "Optimization of sample thickness and preparation for forensic and medical imaging using neutron imaging", *Journal of Medical Imaging*, to be resubmitted
H. Z. Bilheux, M. Cekanova, "Investigation of a novel approach to determine post-mortem interval using neutron radiography", *Forensic Science International*, to be submitted