ABSTRACT

Although monthly preventative flea medications are highly effective with minimal side effects in most dogs, it is well known that in some dogs label doses cause life-threatening complications. Recently, the FDA issued a warning that spinosad, the active ingredient in the popular monthly flea medication Comfortis®, can cause neurologic toxicity when used in combination with ivermectin. The mechanism of this toxicity has not been elucidated, but the reported signs resemble those noted in ABCB1-1∆ polymorphism dogs treated with ivermectin. ABCB1-1∆ polymorphism dogs have impaired function of the membrane transporter P-glycoprotein and show signs of ivermectin toxicity at doses within the established therapeutic range for normal dogs. P-glycoprotein plays a critical role in maintenance of the blood brain barrier and in transport of substrates into the bile for elimination from the body.

Given P-glycoprotein’s critical role in both the distribution and elimination of ivermectin within the body, we hypothesize that the toxicity observed with the co-administration of spinosad and ivermectin results from inhibition of P-glycoprotein by spinosad. We will use the radiolabeled P-glycoprotein substrate ⁹⁹mTc-sestamibi as a marker of P-glycoprotein function. The brain and gallbladder of six healthy dogs will be imaged using nuclear scintigraphy before and after administration of spinosad, and uptake of ⁹⁹mTc-sestamibi in the brain and gallbladder will be measured and compared. Administration of spinosad is expected to (i) increase brain accumulation and (ii) decrease biliary excretion of ⁹⁹mTc-sestamibi. This outcome would show that spinosad suppresses the function of P-glycoprotein, resulting in increased susceptibility to the toxic effects of ivermectin.

Understanding the mechanism of the toxic interaction between spinosad and ivermectin will help prevent other potentially fatal drug-drug interactions involving spinosad. Many commonly used veterinary drugs such as vincristine, doxorubicin and digoxin are substrates of P-glycoprotein, have low therapeutic indexes and have potentially fatal side effects. Given that alternative flea preventatives exist, if our hypothesis is true then dogs requiring any of the drugs known to be substrates of P-glycoprotein can simply be switched to other flea medications that do not suppress P-glycoprotein.
SPECIFIC AIMS

We propose to investigate the potential role of P-glycoprotein suppression in the recently reported drug-drug interaction involving spinosad and ivermectin. Ivermectin is a known substrate of P-glycoprotein, and dogs with impaired P-glycoprotein function are at increased risk for the toxic effects of ivermectin. Determining if spinosad suppresses P-glycoprotein is necessary so that other potentially life-threatening drug interactions with spinosad can be anticipated and prevented.

We hypothesize that the toxicity observed with the co-administration of spinosad and ivermectin results from the extrinsic inhibition of P-glycoprotein by spinosad. Impaired P-glycoprotein function leads to accumulation of ivermectin within the CNS and subsequent toxicity.

Using a gamma camera, the concentration of $^{99m}$Tc-sestamibi, a radiolabeled substrate of P-glycoprotein, can be measured in the CNS and gallbladder of dogs, assessing P-glycoprotein function.

The specific aims of the proposed research are to:

I - Using a gamma camera, compare dog brain activity of intravenously administered $^{99m}$Tc-sestamibi before and after treatment with spinosad.

II - Using a gamma camera, compare dog gallbladder activity of intravenously administered $^{99m}$Tc-sestamibi before and after treatment with spinosad.

We expect that there will be (i) increased uptake of $^{99m}$Tc-sestamibi within the CNS after treatment with spinosad and (ii) decreased uptake of $^{99m}$Tc-sestamibi within the gallbladder after treatment with spinosad. These findings will show that the mechanism of toxic interaction between spinosad and ivermectin is through suppression of P-glycoprotein function. With normal P-glycoprotein function, $^{99m}$Tc-sestamibi that diffuses into the brain is pumped out and unable to accumulate, and $^{99m}$Tc-sestamibi in the circulation is removed, in part, through transport and accumulation in the gallbladder. If $^{99m}$Tc-sestamibi activity in the brain and gallbladder is unchanged after treatment with spinosad, then the mechanism of the drug interaction between spinosad and ivermectin does not involve P-glycoprotein and further studies will be necessary to determine the mechanism.
BACKGROUND AND SIGNIFICANCE

Monthly flea preventative medications, both oral and topical, are a common component of a balanced preventative medical plan for many canine patients. Because they are regarded as having a high margin of safety, many can even be purchased over-the-counter without a veterinary prescription. That veterinarians understand potential unwanted effects of these preventative medications so that potentially life-threatening drug-drug interactions can be avoided is important. Adverse drug interactions, including drug-drug interactions, are well documented in the human literature. An estimated 7,000 people die annually in the United States as a result of adverse drug reactions at a cost of $136 billion dollars in additional health care costs\(^1,2\). Similar studies of adverse veterinary drug reaction costs have not been performed, but the occurrence of veterinary drug-drug interactions has been reported. Recently, the FDA released a warning regarding a drug-drug interaction between ivermectin and spinosad that causes neurologic toxicity in normal dogs\(^3\). Knowledge of the mechanism of interaction between spinosad and ivermectin will help to prevent similar adverse drug-drug interactions between spinosad and other veterinary medications.

Ivermectin induces tonic paralysis in invertebrate organisms by potentiating glutamate-gated and/or gabba amino butyric acid (GABA)–gated chloride channels of the peripheral nervous system\(^4\). In most mammals, including dogs, GABA receptors are limited to the central nervous system\(^5\). Ivermectin is normally excluded from the CNS of mammals through the action of the membrane transporter P-glycoprotein, thus neurologic signs are not seen at recommended doses\(^6\). However, dogs that have deficient P-glycoprotein function accumulate markedly increased concentrations of ivermectin within their CNS tissues, causing toxic effects when treated with label doses of ivermectin\(^6\).

The importance of P-glycoprotein as a membrane transporter is well documented\(^7,8\). In the dog, P-glycoprotein is found in the liver, kidneys, adrenal glands, colon and brain capillary endothelial cells\(^9\). Specifically, P-glycoprotein's presence in the CNS and liver is of particular importance in regards to drug disposition. A variety of common veterinary pharmaceuticals are P-glycoprotein substrates. Examples include vincristine, vinblastine, cyclosoprin A, digoxin, ivermectin, loperamide and others\(^9,10\). Many of these drugs have low therapeutic indexes, meaning that small increases in concentration or in duration of action can cause life-threatening complications. For example, when dogs with the ABCB1-1\(\Delta\) mutation receive vincristine at recommended doses, they are significantly more likely to develop myelosuppression than dogs with the wild-type ABCB1 genotype (functional P-glycoprotein)\(^11\).

P-glycoprotein dysfunction can result from both intrinsic and extrinsic factors. Intrinsic dysfunction is seen in dogs with the ABCB1-1\(\Delta\) polymorphism that results in a truncated, non-functional
Extrinsic dysfunction can be induced by administration of certain medications such as ketoconazole, which act to suppress the function of P-glycoprotein. Co-administration of ketoconazole, a commonly used antifungal medication, and ivermectin dramatically alters the disposition of ivermectin, leading to increased systemic exposure to ivermectin. The neurologic toxicity described by the FDA in dogs receiving both spinosad and ivermectin is similar to that reported in dogs receiving ivermectin and ketoconazole. Thus, hypothesizing that spinosad interacts with P-glycoprotein in a manner similar to ketoconazole is reasonable.

99mTc-sestamibi is a radiolabeled pharmaceutical that is used primarily in myocardial perfusion imaging in humans. 99mTc-sestamibi is a P-glycoprotein substrate and has been shown to be a sensitive probe of P-glycoprotein function. 99mTc-sestamibi has been used successfully in studies assessing intrinsic and extrinsic impairment of P-glycoprotein. A recent study comparing brain uptake of 99mTc-sestamibi between ABCB1-1Δ polymorphism dogs and ABCB1 wildtype dogs is an example of the use of 99mTc-sestamibi to assess intrinsic P-glycoprotein impairment. ABCB-1Δ dogs had significantly greater 99mTc-sestamibi uptake in the brain, demonstrating P-glycoprotein’s role in maintenance of the blood brain barrier. Even more recently, 99mTc-sestamibi was used as a marker to assess extrinsic impairment of P-glycoprotein function in dogs treated with ketoconazole. 99mTc-sestamibi uptake in the gallbladder was measured in 6 ABCB1 wildtype dogs before and after administration of ketoconazole. After treatment with ketoconazole, subjects had significantly less gallbladder accumulation of 99mTc-sestamibi, showing that ketoconazole inhibits P-glycoprotein-mediated biliary excretion, and successfully demonstrating the utility of 99mTc-sestamibi as a marker for detection of extrinsic impairment of P-glycoprotein. The proposed study design is primarily modeled after this study by Coelho et al, with spinosad replacing ketoconazole as the hypothesized P-glycoprotein suppressant.

More than ever, animal owners are electing to treat their pets diagnosed with serious diseases such as cancer and heart failure. Given that many drugs used to treat these conditions (i) are substrates for P-glycoprotein and (ii) have low therapeutic indexes, if our hypothesis is correct, the potential for adverse drug reactions between these medications and spinosad is great. It is for this simple fact that this study is so important. The information derived from this study will allow veterinarians to predict, and therefore prevent, potentially life threatening drug interactions involving spinosad, a drug commonly used for flea prevention.
CURRENT RESEARCH/ PRELIMINARY DATA

Two of the investigators (XXX, XX) have recently used the proposed methods to provide \textit{in vivo} evidence of ketoconazole’s ability to inhibit P-glycoprotein in the dog\textsuperscript{15}.

Figure 1 - Ventral images of the abdomen acquired at 120 minutes after intravenous injection of \textsuperscript{99m}Tc-sestamibi to an ABCB1 wild/wild dog (a) and to an ABCB1 mutant/mutant dog (b). Intense gallbladder \textsuperscript{99m}Tc-sestamibi uptake (arrow head) is present in (a). A void of activity in the location of the gallbladder (arrow) is present in (b). Similar images were obtained before [similar to (a)] and after [similar to (b)] ketoconazole treatment.

Figure 2 - Time-activity curves of gallbladder to liver activity ratios (using mean counts per pixel per ROI) for ABCB1 wildtype dogs before (○: mean G/L ratio + SD, n=6) and after (■: mean G/L ratio - SD, n=6) administration of ketoconazole.
Treatment with ketoconazole resulted in significantly less gallbladder accumulation of the radiolabeled P-glycoprotein substrate $^{99m}$Tc-sestamibi, indicating that ketoconazole inhibits P-glycoprotein-mediated biliary excretion. Clearance of drugs such as vincristine and doxorubicin that rely on biliary excretion will be compromised in patients receiving a drug that inhibits P-glycoprotein, potentially resulting in adverse drug events.

The investigators propose to examine spinosad in a similar manner. The figures shown above demonstrate that the investigators have the experience to conduct the proposed study, and that the experimental design will adequately address the hypothesis.

**RESEARCH DESIGN AND METHODS**

**Design:** The proposed study will be conducted in a prospective, masked, randomized crossover design with each animal serving as its own control. The hypothesis to be tested is:

*Spinosad treated dogs have a greater concentration of the P-glycoprotein substrate $^{99m}$Tc-sestamibi in their brain and lower concentrations in their gallbladder compared to pre-treatment values.*

**Methods:**

**Sample size**—The study population will include 6 ABCB1 wild-type dogs. In a recent study performed by two of the investigators (KLM, JM)\textsuperscript{15}, $^{99m}$Tc-sestamibi activity in the gallbladder of dogs was compared before and after treatment with ketoconazole. A study size of 6 dogs was successful in showing a statistically significant decrease (p<0.05) in gall bladder activity of $^{99m}$Tc-sestamibi after treatment with ketoconazole, a known P-glycoprotein inhibitor. We hypothesize that spinosad has a similar mechanism of P-glycoprotein suppression as ketoconazole, and therefore, by using similar methods, a sample size of 6 dogs will be sufficient to show statistical significance. A study population of only 6 dogs allows us to limit animal use, limit costs and still achieve statistical significance.

**Screening of dogs for inclusion in study**—To be enrolled, dogs must not be receiving medication known to interact with P-glycoprotein\textsuperscript{9} within 30 days of initiating the nuclear imaging phase of the study. The dogs must be healthy based on physical examination, complete blood count, serum biochemistry profile, total serum bilirubin and urinalysis. Additionally, dogs must have the ABCB1 normal/normal genotype and have no abnormalities of the biliary tract as determined by abdominal ultrasound.
Treatment Randomization and Masking—After 6 dogs are identified for inclusion in the study, each will be assigned a number. Using the Microsoft Excel® randomization function; 3 dogs will be randomly assigned to receive spinosad (Comfortis®) 3 days prior to their first nuclear scintigraphic imaging session and the other 3 dogs will be assigned to receive spinosad 3 days prior to their second nuclear scintigraphic imaging session. One investigator (KLM) will administer the oral spinosad preparation (3 days prior to imaging) while the other two investigators (CSM, JM) will perform the imaging and data analysis (masked as to when each dog received spinosad). Spinosad will be dosed based on the manufacturer’s label recommendations. There will be a 60-day washout period for each subject between the two imaging studies.

Nuclear Scintigraphy—Image acquisition using the gamma camera requires that the dogs remain in an immobile position for 2-4 minutes in both dorsal recumbency (for brain scans) and ventral recumbency (for gall bladder scans). This can be accomplished with sedation (dexmedetomidine and hydromorphone). A cephalic catheter will be placed prior to sedation for immediate intravenous (IV) access in the event of an emergency as well as for administration of the radionuclide (99mTc-sestamibi). Three scans will be performed on each dog: (i) immediately after, (ii) 60 minutes after, and (iii) 120 minutes after IV injection of 99mTc-sestamibi (10 mCi per dog). The gamma camera will be fitted with a low-energy, high-resolution, parallel hole collimator. Energy discrimination will be accomplished using a 20% window centered at the 140 keV photopeak of technetium-99m. A 256 x 256 matrix image size will be used. Static acquisition images of the abdomen, including the entire liver and gallbladder (for gallbladder images) and of the entire head and neck (for brain images) will be obtained at time 0, 60 and 120 minutes. These methods were used previously by two of the investigators (KLM, JM).

Per radiation safety protocol, following the scintigraphy studies, dogs will stay in isolated housing until their body radioactivity, measured with a Geiger-Muller survey meter, is less than or equal to 0.2mR/hr at 1m. It is expected that the dogs will be released the day following the procedure, but 2 nights of hospitalization is possible.

Data analysis and statistics—Change in liver and gallbladder activity over time will be determined using standard nuclear scintigraphy protocols. Regions of interest (ROI) around the liver and the gallbladder and around the brain and neck musculature will be drawn during the initial time point and used for subsequent time points. The automated image analysis software program included in the gamma camera computer (Starcam, General Electric Medical Systems, Milwaukee, WI) will be used to measure the activity in the ROIs at 0, 60 and 120 minutes. A gallbladder to liver activity (G/L) ratio and brain to muscle (B/M) ratio will be calculated for each of the time points using the mean counts per pixel of each
ROI. Mixed one-way ANOVA repeated measures in time using G/L and B/M ratios will be used to compare pre-treatment and post-treatment values. A value of $p<0.05$ will be considered significant.

We predict that there will be (i) increased uptake of $^{99m}$Tc-sestamibi within the CNS and (ii) decreased uptake of $^{99m}$Tc-sestamibi within the gallbladder will occur after treatment with spinosad. Given $^{99m}$Tc-sestamibi's known role as a sensitive marker for P-glycoprotein function, these results will demonstrate suppression of P-glycoprotein by spinosad. If pre- and post-treatment values of $^{99m}$Tc-sestamibi are not significantly different, more research will be necessary to elucidate the mechanism of the reported toxicity in dogs treated concurrently with spinosad and ivermectin.

This research will be performed over a 3 - 4 month period starting as soon as funding is secured. During the first 1 to 2 months, candidate dogs will be screened for inclusion into the study. Once the six subjects are identified, they will undergo their first nuclear imaging study. Approximately 2 weeks will be required to perform studies on all six subjects. After a 60 day washout period the subjects will undergo their second imaging study, concluding the data collection portion of the study.

**VERTEBRATE ANIMALS**

Our study protocol has been approved by the Washington State University Institutional Animal Care and Use Committee (Animal Subjects Protocol #03936-001). See Appendix B.

Animals for inclusion in the study will be client owned animals. The students, house officers, faculty and staff of the WSU College of Veterinary Medicine will be solicited initially for participation in the study. If adequate participation is not achieved, clients of the WSU College of Veterinary Medicine Teaching Hospital will be solicited for participation.

The owners of dogs that complete the 2 nuclear scintigraphic studies will be awarded a $200.00 credit towards services at the Washington State University Veterinary Teaching Hospital as incentive for participation.

A sample of the release form that will be given to study participants is included in Appendix A.
## Budget

### Clinical Pathology

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<th>Test</th>
<th>Per dog</th>
<th>Number of Dogs*</th>
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<tr>
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### Pharmacy:

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* Note-We anticipate having to disqualify 1 dog in the screening process, thus we are asking for funding for 6 dogs + 1 that that may be disqualified.

** TOTAL: $9,802**

** An extramural grant is pending with the Morris Animal Foundation that would cover the $2,802.00 not covered by the maximum of $7000.00 through intramural funding. If the grant is not approved, the $2,802.00 deficiency will be paid through proceeds from the Veterinary Clinical Pharmacology Laboratory at Washington State University.

*** In addition to the above costs there will be a Financial Incentive to Enhance Study Participation: $200 to each owner after their dog has completed both scans x 6 = $1200

** this will be paid by proceeds from the Veterinary Clinical Pharmacology Laboratory at Washington State University**
REFERENCES


Appendix A

WASHINGTON STATE UNIVERSITY

Informed Consent Form for Animal Owners

Some research projects in Veterinary Teaching Hospital involve client-owned animals. WSU Institutional Animal Care and Use Committee (IACUC) does not control the inclusion of specific animals in the research. As a part of the approval process for the research, IACUC requires that owners are informed and consent for research is freely granted by reviewing and signing the form below. This step should be completed before an animal is used in any research activity.

Researcher: XXXX XXXXXX

24- hour Emergency number: 509-595-XXXXX (Dr. XXXXXX) primary; 509-338-XXXX (Dr. XXXXXX) secondary

Animal Number__________________

Case Number_________________________

Owner or Custodian_______________________________________

Purpose of this form

We would like to include your dog in an on-going research study designed to determine if the flea preventive spinosad interacts with the drug transporter P-glycoprotein. This form is to provide information to help you decide whether you want your dog to be in the study or not. Please read this form carefully.

You can ask questions about the purpose of the study, the possible risks and benefits, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want your dog to be in the study or not.

Purpose of this Study

This study is being done to determine whether or not the flea preventive spinosad (Comfortis®) interacts with the drug transporter P-glycoprotein. The FDA has recently reported that the combination of spinosad and ivermectin (antiparasitic drug) results in neurological toxicity. We believe that the drug spinosad interacts with the drug transporter P-glycoprotein, and inhibits its ability to transport ivermectin out of the brain into the blood stream and from the blood stream into the gall bladder. By determining the mechanism of the drug interaction, we can predict (and therefore prevent) other drug interactions that may occur with patients receiving spinosad.

Background of Study Procedure

To determine if spinosad inhibits the drug transporter P-glycoprotein, we will use a method that was developed and used successfully in a previous experiment by one of the researchers (KLM). This method involves using a radio-labeled probe (99mTc-sestamibi) that is known to be pumped by P-glycoprotein. When P-glycoprotein is functioning normally, this probe is pumped out of brain tissue and into the gall bladder for excretion from the body (shown in previous experiments). A gamma camera is used to detect radioactivity in brain and gall bladder at time 0 (immediately after injection), and at 60 minutes and 120 minutes after injection of the probe. When P-glycoprotein is not functioning properly, this probe can be detected in brain tissue but is not detected in gall bladder. This probe is routinely used in humans and veterinary patients for diagnostic procedures including bone scans, to assess kidney
function, cardiac function and thyroid function. Its radioactivity decays quickly such that it is not detectable roughly 48 hours after it is synthesized. Additionally, dogs excrete the probe through the bladder and feces, so the duration of exposure to radioactivity is relatively short (roughly 24-48 hours).

Study Procedure

A. Screening your dog for participation in the study-In order to determine if your dog qualifies for the study, we must determine if your dog is healthy. The first step will be to determine if your dog has a common mutation of the MDR1 gene (the gene that codes for P-glycoprotein). This will be accomplished by obtaining a DNA sample from your dog using a cheek swab. If your dog does not have a mutation in the MDR1 gene, we will next perform a physical examination (checking the respiratory system, cardiovascular system and abdomen for abnormalities). Once this has been performed, we will collect blood for a complete blood count, a biochemistry profile, a total serum bilirubin concentration, and collect urine for a urinalysis. Once the dog has ‘passed’ these tests, an abdominal ultrasound will be performed to ensure that no abnormalities are visible in the liver or gall bladder.

B. Study procedure-Six dogs will participate in this study. Dogs participating in the study will be scanned on 2 separate occasions spaced at least 2 weeks apart. One scan will occur after your dog has received spinosad (Comfortis®) flea preventive at the manufacturer’s recommended dose and the other study will occur without prior drug dosing. The order of the scans will be determined randomly (i.e., 3 dogs will receive spinosad before their first scan and the other 3 dogs will receive spinosad before their 2nd scan).

Day of scan-An intravenous (IV) catheter will be placed in a cephalic (forelimb) vein for injection of 99mTc-sestamibi as well as to have immediate IV access in the event of an emergency. Dogs will be sedated (not anesthetized) in order to make sure that the dog remains immobile during imaging procedure. In order for the gamma camera to collect an image, the dog must be immobile for 3-5 minutes while lying on its back (for the brain image) and another 3-5 minutes while lying on its stomach (for the gall bladder image). After the dog is sedated, 99mTc-sestamibi will be injected through the IV catheter, and the dog will be positioned for imaging the brain and then the gall bladder (these are the time 0 scans). The dog will be allowed to rest on a padded surface while monitored by at least one of the veterinarians between the time 0 scan, the time 60 (minutes) scan, and the time 120 (minute) scan. After the scan at 120 minutes, the dog will receive an injection of a drug that reverses the effects of the sedative. The dog will be housed in a special VTH dog run for the next 24-48 hours depending on your dog’s radiation level as required by the WSU radiation safety office. While it is true that people receiving 99mTc-sestamibi are allowed to leave the hospital immediately following a scan, radiation safety officials have determined that it is best for dogs (whose elimination habits differ from people) be housed until their radiation level reaches a pre-determined level. Designated personnel will monitor your dog’s radiation levels and determine when your dog has reached acceptable levels for release from the hospital. This is generally 24 to 48 hours after 99mTc-sestamibi administration.

How does this study benefit my dog?

Direct benefits from having your dog participate in this study: There are 2 direct benefits for having your dog participate in this study. 1. Owners of dogs will receive a $200 credit at the WSU VTH on completion of the study (both scans). 2. Your dog will receive a physical examination by a veterinarian, a complete blood count, biochemistry profile, total serum bilirubin, and complete urinalysis, and abdominal ultrasound. The total cost of these laboratory procedures at WSU VTH would be $76.75; the cost of an abdominal ultrasound is $256. Additionally, the cost of MDR1 genotyping is $70. Thus, your dog would receive a health screening worth more than $400.

Indirect benefits from having your dog participate in this study: By identifying the mechanism of the spinosad interaction, it is possible that an adverse drug-drug interaction may be prevented in your dog.

Withdrawal from the study

Enrolling your dog in this study is voluntary and you can withdraw permission and your dog from the study at any time. It is also important to understand that your dog can be withdrawn from the study by the investigators if
necessary (for example if certain abnormalities on blood work or abdominal ultrasound are detected). If your dog is withdrawn from the study for any reason, data already collected may continue to be used for research purposes. If your dog is withdrawn from the study for any reason prior to completion of both scans, you are no longer eligible for the $200 credit at WSU VTH.

Your dog will not be treated differently by any veterinarian at Washington State University Veterinary Teaching Hospital if you decline to participate in the study. However, results from blood tests or abdominal ultrasounds performed on your animal may not be made available to you unless your dog completes the study. Your decision to participate, not participate, or withdraw your dog from the study will not affect your relationship with WSU or any other treatment your dog is receiving.

Name of PI ________________________________Signature __________________________Date:___________

Subject's Statement

This study has been explained to me. I agree that my dog can take part in this research. I have had a chance to ask general questions about the research, with one of the researcher listed above. If I have additional concerns, I can call the WSU Institutional Animal Care and Use Committee (IACUC) at (509) 335-7951. This study has been reviewed and approved by the WSU IACUC for using client owned animals for research. I will receive a copy of this consent form.

I certify that I am the legal owner or custodian of this dog (name__________________________________) and have the authority to consent medical treatment for this dog.

Name of the subject___________________________ Signature ________________________Date:_____________
Appendix B

From: iacuc@wsu.edu [mailto:iacuc@wsu.edu]
Sent: Friday, November 13, 2009 9:39 AM
To: XXXXXX
Subject: Approval by IACUC # XXXXXX
Importance: High

MEMORANDUM

TO: XXXXXX

FROM: Rani Muthukrishnan for Thomas E. Besser, Chair, The Institutional Animal Care and Use Committee, IACUC

DATE: 11/13/2009

TITLE: Effect of spinosad on brain and biliary distribution of 99mTc-sestamibi

NEXT REVIEW DATE: 11/12/2010
NEXT REVIEW TYPE: Annual

The Animal Subjects Protocol, #03936-001 has been approved on 11/13/2009. Please note this protocol will require an Annual Renewal Form to be completed each year listing any changes to the protocol.

If you do have any changes before the protocol's annual review is due, please inform the IACUC by submitting an amendment to the IACUC Program Coordinator at the Office of Research Assurances (campus zip 3005).

All IACUC approved protocols are subject to Post Approval Review and monitoring. Please contact Gary Turner immediately (335-8043) to schedule one before submitting your renewal.

If you have any questions please contact the Program Coordinator listed below.

Thank you,

Rani Muthukrishnan, PhD.
IACUC Program Coordinator
Institutional Animal Care and Use Committee (IACUC)
Office of Research Assurances
Washington State University
Pullman, WA 99164
Phone: 509-335-7951
Fax: 509-335-6410
Email: rani_m@wsu.edu

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