The University of Mississippi Medical Center

Animal Activity Protocol

IACUC - Institutional Animal Care and Use Committee
Telephone 601 815-5006 / Facsimile 601 815-5010
iacuc@umc.edu

To be completed by IACUC

Protocol Number: 1431A	Date: 10/16/17	Classification: D

1. Principal Investigator

Name	**Hua Liu**		
	☐PhD ☒ MD ☐ Other:		
Title	**Assistant Professor**		
Dept.	**Pediatrics**		
Phone #	**984-5978**	**Office Location**	**R117B**
email	**hliu@umc.edu**	**Emergency #**	**601-214-0425**

Note: The emergency number should be a number at which the PI can be contacted on nights and weekends.

2. Other Personnel

All listed personnel must complete IACUC required training, including completion of Occupational Health forms and submit a Training Requirements Registration form prior to working with animals and receiving access into the Center for Comparative Research (CCR).

You may authorize personnel to submit modifications to this protocol by checking the box for signing privileges.

Name	Title	Ext/Cell	Email	Signing Privileges
Michael J. Nowicki	Professor	984-5323	mnowicki@umc.edu	☒
				☐

(Insert additional lines as needed)

3. Project Title:

Effects of cholestasis on the expression and function of organic anion transporter protein 2 (Oatp2) and P-glycoprotein (mdr1), and on digoxin clearance in rat.

4. Proposal is **3 year Full Submission Renewal (must attach Appendix K)**

5a. Outside Contracts

Will any components of this study involve live animals maintained at another institution?

☒ No

☐ Yes (if yes, provide information on the level of involvement)

5b. Animal Behavior Core

Will this study use the Animal Behavior Core (ABC)?

☒ No

☐ Yes – Requires review and approval by ABC Director. See Appendix L.

6. Funding Source

☐ Extramural/Intramural Funding

Title			
PI			
Funding Agency			
Status	☐ Submitted ☐Funded	Grant Number	
Covered Dates			

(Copy and paste table if project is funded by multiple grants.)

☒ Department – List Department: Pediatrics

☐ Other (*Example: Divisional funds which you have control over, start-up funds*)

Explain:

7. Dates of Study

Anticipated start date of study: 10/17/2017

All investigators must adhere to a federally mandated three-year cycle of full protocol review, even if a funding period exceeds three years in duration.

8. Source of Animals

Will any animals be obtained from non-commercial sources? ☒No ☐Yes

If Yes, list:

Note: Animals from non-commercial sources must have their health status evaluated by a CCR veterinarian prior to their arrival at UMMC. This question does not relate to the acquisition of animals from other UMMC investigators. If animals are transferred from a UMMC source, an Animal Transfer Form must be completed and approved for each transfer.

9. Animal Requirements

For **New** submissions complete **Table A.**

For **3 Year FSR** submissions complete **Table B**.

Animal numbers MUST be calculated for a period not to exceed three (3) years from the start of the study.

A. New:

Species	Strain/stock	Sex	Source	Total for 3 years	Average daily census

(Insert additional lines as needed)

Note: If using nonhuman primates, complete Appendix A.

B. 3 Year FSR: For a 3-year renewal, number of animals needed to complete the studies in this protocol. This must include the number of animals to be received plus the number of animals currently on campus to be carried over from the previous version of this protocol.

Example: You need 100 animals to complete your study and you have 20 animals currently in house to carry over to this is protocol.

Total Needed for 3 years		*Total Carried Over*		*Total Requested*
100	-	20	=	80

You will be approved for 100 animals to complete the study (number to be justified in question #17) of which you already have 20, so you will have 80 animals available to order.

Species	Strain/stock	Sex	Source	Total Needed For 3 years	Total Carried Over	Total Requested (Needed – Carried Over)	Average daily census
Rat	Sprauge-Dawley	Male	Harlan	24	4	20	6

(Insert additional lines as needed)

Note: The number of animals available for ordering will be the difference between total animals needed minus carryover animals.

C. List any unusual phenotypes or abnormalities associated with the animals (including sublines) listed above (i.e., prone to diarrhea, decreased appetite, patchy hair loss, increased sensitivity to pain, slow wound healing, etc.).

N/A

10. Breeding program

Will animals be involved in a breeding program at UMMC or will time-pregnant animals be used?

☒ No

☐ Yes (if yes, provide information in Appendix B)

11. Potential Hazards

		Yes	No	Pending
A	**Chemical toxins used in animals?**	☐	☒	
	Reviewed by Environmental Health & Safety?	☐	☒	☐
B	**Radioisotopes used in animals?**	☒	☐	
	Reviewed by Radiation Safety?	☒	☐	☐
C	**Use of laser, CT, x-ray, or fluoroscopy?**	☐	☒	
	Reviewed by Radiation Safety?	☐	☒	☐
D	**Biohazards used in animals?**	☐	☒	
	Reviewed by Institutional Biohazard Committee?	☐	☒	☐
E	**Human cells used in animals?**	☐	☒	
	Reviewed by Institutional Biohazard Committee?	☐	☒	☐

If YES, provide specific details of specialized animal husbandry, care, cleaning, or decontamination procedures**, especially identifying responsible parties**.

The contaminated animal carcasses will be placed in the 8th floor freezer at Research Wing once the tissue and blood have been collected. All sharps used during the procedure will be placed in a sharp container labeled with radioactive material tape, the date it was used, the isotope, activity and name of the principle investigator. Dry solid waste will be collected and placed in the collection containers in G039 for disposal. All work will be followed by decontamination and a swipe survey with use of a liquid scintillation counter.

12a. Animal Husbandry

	Standard	Nonstandard
Feeding	☒	☐
Watering	☒	☐
Caging	☒	☐
Room/Environment	☒	☐
Altered light cycle	☒	☐

Note: Provide complete explanation and justification for any **nonstandard animal husbandry** (e.g. metabolic caging, restraint chairs, transport devices, singly housed animals, altered light cycle). Protocols listing non-standard husbandry must provide complete details of the cleaning and sanitation, **especially identifying responsible parties**:

12b. Singly Housed Animals

Will animals be singly housed?

☒ No

☐ Yes – Please provide justification for single housing. **NOTE: If using non-human primates you must complete Appendix A.**

13. Housing

Will animals be housed outside of the LAF for greater than 12 hours?

☒ No

☐ Yes Where?

Note: If yes, provide complete explanation and justification for any **decentralized animal housing.**

14. Objectives in lay terminology

In **non-technical**/lay terminology, what is the **objective of the experiments** proposed in this Animal Activity Protocol? (i.e. ***Response should be written in non-scientific language, as though explaining the study to a high school student***.)

- In non-technical/**lay terminology**, what is the objective of the experiments proposed in this Animal Activity Protocol?
- Why are the experiments proposed?
- What knowledge do you hope to achieve?
- What is the potential relevance (e.g. benefits) of experimental findings to human or animal health, advancement of knowledge, and/or the good of society?

Generally, **single sentence explanations** for these types of questions will suffice.

Cholestasis is a condition where bile cannot flow from the liver to the intestine (duodenum). Obstructive cholestasis is a mechanical blockage in the bile duct system that can occur from a gallstone or malignancy. Clinical evidence revealed that obstructive cholestasis results in impaired excretion of digoxin, a mainstay in the treatment of congestive heart failure. The Objectives of the study are:
1. To look at the effect that blocking drainage of the liver has on levels of specific proteins that control the uptake of a commonly used heart medication (digoxin) in the liver, brain, kidney, and intestine.
2. To show how these proteins control removal of digoxin from the body.
3. To show how blockage of liver drainage affects levels of digoxin in the liver, brain, kidney, and intestine.
4. The information may help determine novel treatments to lower the degree of cholestasis in order to avoid digoxin toxicity.

15. Rationale

A. What is the rationale for using animals rather than using non-animal models?

Studies of pharmacokinetics and transporter proteins require an intact, living biological system which cannot be replaced by cell-culture work or computer-simulated biological systems.

B. What is the rationale for using the particular animal species and/or strain noted in Item 9?

Previous studies have shown that rats are a good model to study pharmacokinetics of digoxin in rats (Su SF, Huang JD. Drug Metabolism and Disposition 1996;24:142-7; Reguiga MB, et al. Pharmaceutical Research 2005;22:1829-36).

16. Brief Outline

Provide a general description of the animal procedures included in the experimental design.

- **Briefly outline** the proposed animal manipulations and provide a time-line of events.
- **Note that specific details about methods and procedures will be required in the appropriate appendix (see list below)**
- **Complete** only those **appendices that apply** to the animal manipulations in your experimental design.
- If possible, flow charts and/or time lines should be included to clarify the timing of procedures which are to be performed.

Verbatim descriptions from a grant submission are not acceptable and will not be reviewed.

Effects of cholestasis on expression of protein transporters and digoxin clearance.

1. Animals will be received and allowed to acclimate to their new environment for 1-2 days.
2. A pharmacokinetic (PK) study for digoxin clearance will be performed (pre-surgery pk study). A Catheter will be placed in the right jugular vein, then digoxin 0.02 mg/kg (about 1ml in volume) will be infused through the catheter. Blood samples (250ul each) will be drawn at 0, 2, 5, 10, 30, 60, 120, 240, and 360 minutes following administration of digoxin for the measurements of digoxin, liver function and bilirubin. The catheter will be flushed with saline following each blood draw. Blood loss (sample collectings) will be replaced cc for cc with normal saline. The catheter will be removed after the PK study. The PK study is outlined in more detail in Appendix C and D.
3. Two weeks after the pre-surgery PK study, the animals will be divided into 2 groups – 6 animals per group. One group will have common bile duct ligation (BDL) and the other group will have a sham operation (sham).
4. Animals will be returned to their cages where they will reside for 7-days with free access to food, water, and normal activity.
5. On post-surgical day 7, digoxin PK studies will be performed on all animals. A catheter will be placed in the left jugular vein for blood samplings and infusions as detailed in Appendix C and D. Then animals will be euthanized and tissue collected (brain, heart, liver, kidneys, and intestine).

Effect of cholestasis on tissue distribution of digoxin.

1. Animals will be received and allowed to acclimate to their new environment for 1-2 days.
2. Animals will be divided into 2 groups – 6 animals per group. One group will have common bile duct ligation (BDL) and the other group will have a sham operation (sham).
3. On post-surgical day 7-days, 3H-digoxin will be administered by injection into the jugular vein (see Appendix D). Animals will be sacrificed 2-hours following digoxin administration and digoxin levels determined for organs (brain, heart, liver, kidneys, and intestine) and serum.

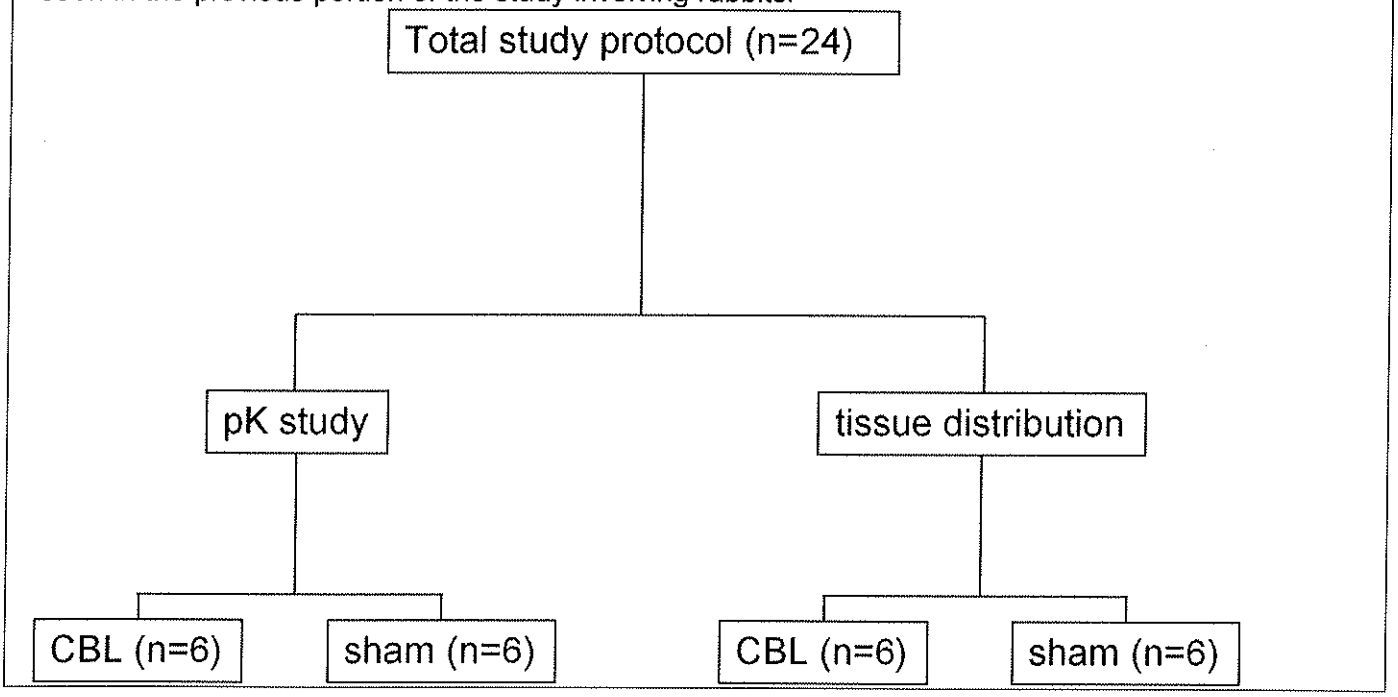
Appendix A	**Environmental Enhancement/Enrichment**
Appendix B	**Breeding Programs**
Appendix C	**Surgery & Management of Surgical Pain & Distress**

Appendix D	**Collection of Biological Samples**
Appendix E	**Antibody Production**
Appendix F	**Administration of Drugs/Test Compounds**
Appendix G	**Prolonged Physical Restraint**
Appendix H	**Multiple Survival Surgical Procedures**
Appendix I	**Food and /or Fluid Restriction**
Appendix J	**Animal Pain and/or Distress**
Appendix K	**Progress Report**
Appendix L	**Behavior Testing and Training**

17. Justification of animal number

Explain and **justify** how the number of animals requested was determined. (Flow diagrams/tables to define animal use are encouraged. **Statistical support should be included.** This number should support the request made in the *Total for 3 years* column in #9 and be consistent with the outline in #16).

1. To determine the effect of cholestasis on the expression of the protein transporters 6 animals will be used in each group. Two groups will be needed to compare sham operation with cholestasis. We believe that this small number of animals will allow us to determine statistical differences between the groups. Thus 12 animals are requested.
2. To determine the effect of cholestasis on tissue distribution of digoxin 6 animals will be used in each group. Two groups will be needed to compare sham operation with cholestasis. We believe that this small number of animals will allow us to determine statistical differences between the groups. Thus 12 animals are requested.
3. A total of 24 animals are requested, 4 have been used.
4. There was not a power analysis performed; the number was derived from the statistical differences seen in liver injury (transaminase level and bilirubin) and changes in the pharmokinetics of digoxin seen in the previous portion of the study involving rabbits.



18. Location & transportation

A. Indicate room(s) where animal procedures will be conducted.

Room Number	Procedures performed
LAF	Animal surgery / procedures / euthanasia will be performed in the laboratory animal facility.
R-117B, Res Wing	Injection of ^{3}H-digoxin.

(Insert additional lines as needed)

B. Studies involving animal transportation to locations other than the housing area must identify the animal transport device, the nature of the shrouds used to cover the transport device, and describe the route of transport. **Include transport within the LAF (e.g. IVIS, surgery room).**

N/A

19. Euthanasia

A. At what point in the proposed experiments will animals normally be euthanized, (experimental end-points)? Or at what point will any individual animal be euthanized?

1. In the first part of the protocol to determine the effect of cholestasis on protein transporter expression, animals will be euthanized on post-operative day 7.
2. In the second part of the protocol to determine the effect of cholestasis on tissue distribution of digoxin, animals will be euthanized on post-operative post-operative day 7.

B. What humane endpoints or criteria will be used to determine if an animal is to be euthanized prior to, rather than at, the anticipated end-point of an experiment? Note: Contact LAF, ext.4-1385, for recommendations on the assessment criteria.

If at any point the animal appears sick, such as loses the righting reflex, failure to groom/ruffling of fur, wound dehiscence, lethargy and abnormal posture, the veterinary staff will be consulted. The animal will be euthanized prior to the usual end point of the experiment.

C. Will natural death (or death due to manipulations) be used as an endpoint?

☒No ☐Yes – if "Yes", explain and justify.

20. Euthanasia Procedures

What procedures will be used to euthanize the animals? Note: Secondary methods are required to ensure death. (Consult the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition for appropriate methods of euthanasia or contact the LAF.)

The experimental animals will be euthanatized by deep anesthesia with isoflurane (3.5%) followed by tissue harvest (heart, liver, brain, kidney, and intestine) and exsanguination.

Assurances

1. Have all personnel received a medical evaluation from UMMC Student/Employee Health and updated Occupational Health Information annually?

   ☐No ☒Yes

2. Have all personnel listed on this protocol been informed and understand their role in the experiments?

☐No ☒Yes

3. Review of the available resources and previous experiments have determined that the proposed activity is not unnecessarily duplicative of previously reported activities.

☐No ☒Yes

USDA Policy #12, "Consideration of Alternative to Painful/Distressful Procedures": states the following: ***The Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions, and replacements.***

List each potentially painful or distressing procedure included in these protocol:

Bile duct ligation	
Catheterization	

To comply with Policy #12, investigators are required to conduct literature searches using **two different search engines (see below)** addressing each of the procedures listed above. Specific procedures listed may be utilized as key terms.

Additional assistance may be obtained by contacting the Rowland Medical Library reference desk at ext. 4-1231. See IACUC Guidance on Minimizing Pain and Distress in Animals and Searching for Alternatives.

Helpful Databases

(Please note: PubMed and Medline are the same and cannot both be used.)

☒Medline/PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
☒Toxnet (http://toxnet.nlm.nih.gov)
☐AWIC (http://awic.nal.usda.gov)
☐Agricola (http://agricola.nal.usda.gov)
☐Scopus (http://www.scopus.com/home.url)
☐Other (Click here to enter text.)

		Period of years	Potentially painful or		Indicate which mandate each search addressed

Name of the database	Date of search	covered by the search	distressing procedures addressed	Key words and/or search strategy used	Replacement of animals	Reduction in numbers of animals used	Refinement to minimize pain or distress	Lack of unnecessary duplication
Medline	08/20/17	1946-present	Yes	1. Catheterization, alternative, reduction, refinement, digoxin pharmacokinetics, and rat	☒	☒	☒	☒
				2. Bile duct ligation, cholestasis alternative, reduction, refinement, and rat	☒	☒	☒	☒
Toxnet	08/20/17	1965-present	Yes	1. Catheterization, alternative, reduction, refinement, Digoxin pharmacokinetics, and rat	☒	☒	☒	☒
				2. Bile duct ligation, cholestasis alternative, reduction, refinement, and rat	☒	☒	☒	☒

Narrative

Below, provide a brief summary of any articles that were identified in the search and how these studies relate to the current animal protocol. The narrative must discuss what efforts were made to REDUCE animal number and REFINE experimental procedures to reduce or eliminate pain and distress to the experimental animals, as well as whether there are alternatives that could REPLACE the use of animals. Interaction with peers and educational materials may be used to supplement discussion of literature searches.

Summary of articles:

Obstructive cholestasis is a mechanical blockage in the bile duct system that can occur from a

gallstone or malignancy. Clinic evidence showed that obstructive cholestasis results in impaired excretion of digoxin, a mainstay in the treatment of congestive heart failure. Our literature searches through Ovid medline/Pubmed showed that only 4 publications reported studies for the effect of cholestasis on digoxin pharmacokinetics in different animals. No similar study has been done in vitro. When search the alternative method for cholestasis and bile duct ligation, there was no result. Pharmacokinetic study of digoxin is proposed in the protocol. This animal experiment involves multiple blood samplings and possible fluid infusion to replace the blood loss during the procedure. There is no alternative method for collecting multiple blood samples and infusion for pharmacokinetic study in rat except catheterization.

Reductions in animal number:

The number of animals proposed in the study is a minimal requirement for reaching statistical differences in liver injury (transaminase level and bilirubin) and changes in the pharmokinetics of digoxin based on a previous portion of the study involving rabbits.

Refinements to methods to reduce distress:

Animals will be administrated with post-operative analgesics carpofen for three days as detailed in Appendix C. Refinements will be added based on our observations in the ongoing study and discussion/consultation with LAF veterinary staffs.

Animal **Replacement**:

Through the Medline and Toxnet search, there is no mathematical, physical or chemical model for examining the effect of cholestasis on digoxin pharmacokinetics. Digoxin excretion is a body process, and it cannot be replaced by in vitro system.

Training and Qualifications

- PI

  Name► Hua Liu
  Animal research experience ► 20 years

  Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this Protocol
Bile duct ligation (BDL)	Did BDL on rabbits and performed BDL on several rats.
Catheterization	Multiple experiences for rat venous catheterization

- Other research personnel (copy the lines below for each individual listed as personnel on protocol)

  Name►
  Animal research experience ►

  Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this Protocol

- **Training to be provided.** List here each procedure for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone, enter "N/A"

Certification of the Principal Investigator:

Signature certifies that the Principal Investigator will conduct the project in full accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations, and UMC policies governing the use of live vertebrate animals for research and teaching purposes. The procedures involving animals will be conducted by trained or experienced personnel or under the direct supervision of trained or experienced persons. It is understood that IACUC approval is valid for a period of 12 months following the date of original approval and must be renewed annually for continued approval. I understand there is a 3-year requirement for full protocol rewrite. It is further understood that should this project be submitted for external funding, the information presented on the UMMC Animal Activity Protocol form accurately reflects the animal use in the full grant application.

X _____

Signature of Principal Investigator (Paste digital copy of signature)

Approval by the Attending Veterinarian:

X _____

Signature of Attending Veterinarian or Designee

Approval by the Institutional Animal Care and Use Committee:

X _____

Signature of IACUC Chair or Designee

Appendix H Multiple Survival Surgical Procedures

A major surgical procedure is defined as a surgical intervention that penetrates or exposes a body cavity (peritoneal, thoracic, cranium), produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Guide, 2011). Multiple procedures are those whereby an animal will regain consciousness after each procedure. Procedures must be described in Appendix C. A surgery followed by a second procedure where the animal is euthanized is not considered multiple surgical procedures.

Surgeries performed on the animal prior to the animal's arrival at UMMC (e.g., ovariectomy procedure performed by vendor) must be considered. For additional information consult the IACUC's policy statement on Multiple Major Surgical Procedures.

1. **Justify** the need for multiple major surgical events in a single animal.

One of the goals of the study is to examine the effect of cholestasis, achieved by bile duct ligation (BDL), on digoxin clearance. The digoxin clearance is assessed by a PK study requiring multiple blood sampling with a jugular vein catheter. Between the pre- and post- surgery PK studies there 21 days (see time interval below). In order to prevent infection or other complications, the catheter will be withdrawn after pre-surgery PK study (detailed in App C).

2. What is the time interval between the surgical events?

The blood loss for the pk study is about 10% total volume of a rat. There will be a two week recovery time before the BDL surgery (The UFAW Handbook on the Care and Management of Laboratory Animals. 1999, Vol 1: 298). The post-surgery PK study will be performed 7 days after BDL then the experimental animals will be euthanatized by deep anesthesia with isoflurane followed by tissue harvest (heart, liver, brain, kidney, and intestine) and exsanguination

Appendix C Surgery & Management of Surgical Pain and Distress

1. **Complete description of surgical procedures** – List details for each surgical approach noted in question #16.

Surgical site preparation
Following induction of anesthesia the abdomen will be shaved with electric clippers and then prepped with betadine solution. The area will then be draped in a sterile fashion.
Surgical approach
At the day of PK study (2 days before BDL and 7 days after BDL), jugular vein catheterization will be performed for blood draws. Jugular vein catheterization: Flush the catheters with heparinized glycerol (250 IU heparin/1 ml glycerol) to ensure patency and avoid leakage → rat is anesthetized with isoflurane → shave fur from anterior and posterior areas of the neck → Gently scrub the surgical area 3 times alternating use of Betadine and 70% alcohol → restrain the legs →Make a 2 cm ventral cervical skin incision right of the midline of the neck →Using a hemostat, bluntly dissect the right jugular vein and isolate a 5 mm section of the vessel → Using 4-0 silk suture, place a loose tie on both cranial and caudal ends of the vessel → Using a micro surgical scissor make an incision large enough to pass the catheter, in line with the vessel between the two ligatures and tie the cranial ligature around the vessel →Insert the venous catheter into the vessel towards the heart → Use the ligatures at the cranial and caudal ends to secure the catheter to the vessel → Using a Straight Hemostat, Tunnel a 5 cm Tube back Subcutaneously behind the Ear →Exteriorize the Catheters through the Tube and Remove the Tube. The wound will be closed in a single layer with non-absorbable suture. The catheter will be removed after the PK study. The rat will be anesthetized with isoflurane. The incision will be reopened by cutting the suture. The suture that secures the catheter around the right jugular vein will be cut off and the catheter be withdrawn. Tie the caudal end of the vessel using 4-0 silk suture. The wound will be closed in a single layer with non-absorbable suture. Bile duct ligation: Anesthetize rat with inhalation of isoflurane → Shave the abdominal fur with an electric fur shaver →Place therat on a 37 °C heated hot plate → Sterilize the shaved abdominal skin with a gauze swab that is moistened with Betadine and 70% alcohol →Open the abdomen with a midline laparotomy of a length of approximately 2 cm by cutting the cutis plus fascia at the same time with an surgical scissor → Dissect the connective tissue on top of the peritoneum by using the scissor as a spreader → Cut the peritoneum along the linea alba to open the peritoneal cavity → Spread the operation area by inserting a Colibri retractor in the peritoneal cavity → Lift the liver with a moisturized (0.9% NaCl solution) cotton swab so that the ventral side of it sticks to the diaphragm and the hilum is clearly visible → Expose the bile duct by caudal movement of the gut → Separate carefully the bile duct from the flanking portal vein and hepatic artery using a micro-serrations forceps → Place the 5-0 suture around the bile duct and secure it with two surgical knots. When tying the knots increase the tractive force continuously to ensure effective obstruction without severing the bile

duct → Add a second cranial ligation in the same manner but do not dissect the bile duct in between (Otherwise there is a risk of bile leaks) → Cut the ends of the sutures, lower the sternum, and remove the retractor → Rinse the peritoneal cavity with 0.9% NaCl solution and replace the abdominal organs to the physiological positions → the wound will be closed in 2 layers with absorbable suture material (coated Vicryl). The muscle layer will be closed with interrupted sutures; the skin will be closed with a running, subcutaneous stitch followed by skin glue. The animal will be sacrificed post-surgery day 7 after a post-surgery PK study. Post-surgery PK study Left jugular vein catheterization will be performed for blood draws. Make a 2 cm ventral cervical skin incision left of the midline of the neck. The rest of the procedure is similar to pre-surgery PK study using right jugular vein catheterization.
Wound closure method, materials, and removal plan
For placement of the jugular vein catheter for blood draws, the wound will be closed in a single layer with non-absorbable suture. For bile duct ligation, the wound will be closed in 2 layers with absorbable suture material (coated Vicryl). The muscle layer will be closed with interrupted sutures; the skin will be closed with a running, subcutaneous stitch followed by skin glue.

2. Provide a complete formulary of medications related to surgical procedures:

	Agent	Dose	Route	Frequency/Duration	Pharmaceutical Grade
Pre-anesthetic					☐Yes ☐No
Pre-operative analgesics	isoflurane	2%-4%	inhaled	continuous	☒Yes ☐No
Post-operative analgesics	carpofen	4~5 mg/kg	Sub Q	pre-op and Q24H post surgery for 3 days	☒Yes ☐No
Anesthetics					☐Yes ☐No
Fluid/blood replacement					☐Yes ☐No
Antibiotics					☐Yes ☐No

For non-pharmaceutical-grade compounds:

a. **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

3. Anesthesia

a. Who will conduct the anesthesia procedure(s)?

Hua Liu.

b. Describe experience and training with anesthesia.

Dr. Liu have had experience with sedation, anesthesia, and surgical procedures in rabbits, rats and hamsters. Dr. Liu attended refresher anesthesia training by LAF staff 2 month ago.

c. What criteria will be used to assess anesthetic depth and how will this be monitored?

The level of anesthesia will be assessed by the response to a painful stimuli, i.e., ear pinch or squeezing the foot. Once a deep level of anesthesia is induced, the surgical procedure will be performed - the respiratory rate, heart rate, oxygen saturation (via pulse oximetry), and response to stimuli.

4. Aseptic Technique

a. What procedures will the surgeon use to prepare himself/herself for aseptic surgery?

The surgeon will wash his hands for 3 minutes at the OR sink, then dress in sterile gown, head cap, mask, and gloves.

b. How will the instruments be prepared for aseptic surgery? (Sterile instruments must be used for each animal.)

The instruments will be washed and dried following surgery; placed in a contained that can be sterilized; wrapped; then sterilized by autoclave.

5. Location of Procedures

Where will the surgical procedures be conducted?

LAF

6. Post-procedural Care

a. Who will conduct and document post-procedural animal care (post-op analgesia, nursing care, etc.)? *Documentation will be checked at IACUC semi-annual inspection.*

Hua Liu

b. Include a plan of monitoring frequency, duration and intervals of post-op analgesia, nursing care, etc.

The animals will be observed in the LAF operating until fully recovered and returned to their home cage. Animals will be assessed twice daily for the first 3-days, then daily. The incision site will be assessed at

least once daily. If there are signs of stress, pain, or complications - assessing appetite, urine/feces output, activity, posture - the frequency of assessment will be changed accordingly.

c. What is the expected time from end of procedure until animal(s) are returned to home environment?

2-3 hours.

7. Emergency Contacts

Provide emergency contact information (pager/phone number) for evenings or weekends concerning post-operative complications.

Liu: cell 214-0425; home 853-3818; laboratory 984-5978
Nowicki: cell 750-0885; home 853-9425; office 984-5232; pager 952-5321.

Appendix F Administration of Drugs/Test Compounds

For instructions for completing this form, click here.

All agents given to the animals **must** be listed in this section with the exception of veterinary pharmaceuticals (antibiotics for treatment, anesthetics, and analgesics for treatment). Those will be listed in Appendix C.

NOTE: A pharmaceutical-grade compound (PGC) is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy.[1]
The Food and Drug Administration (FDA) maintains a database listing of FDA approved commercial formulations for both FDA approved human drugs (the Orange Book) and veterinary drugs (the Green Book).

Provide the following information:

Agent	Dose	Volur	Vehicle	Route	Frequency	NDC or CAS#	Hazard?	Pharmaceutical Grade
Digoxin	0.02 mg/kg	1 ml	5% glucose solution containing 1.75% ethanol (V/V)	IV	once	20830-75-5	Highly toxic in concentrated form	☒Yes ☐No
^{3}H-Digoxin	0.02 mg/kg	1 ml	5% glucose solution containing 1.75% ethanol (V/V)	IV	once	10028-17-8	Radioactive materials	☒Yes ☐No
								☐Yes ☐No
								☐Yes ☐No
								☐Yes ☐No

NDC# is preferred over CAS#, if available. The NDC# will be on the bottle or box if the substance is a pharmaceutical. If there is no NDC# then include the CAS#. CAS# and hazard information can be obtained from the MSDS sheet through the UMMC Intranet (http://www.umc.edu/intranet/index.php). Choose the "MSDS On-Line" link under "Hot Spots".

1. Describe any potential adverse side effects that may result in the animal from the administration of this material. If agents are unknown or their potential side effects are not documented, provide a reasonable estimate of the effects of the general class of chemicals (e.g., compound may have sedative properties, compound will likely produce diarrhea, etc.).

In the small doses we will be giving to the test animals the risks are essentially nil. Reported effects seen with overdose in humans include: diarrhea, loss of appetite, nausea, vomiting, headache, visual disturbance, cardiac dysrhythmia. However, the only risks during the administration would be gastrointestinal upset (if accidently ingested) and becoming sensitized to the chemical if absorbed through the skin; there are no inhalation or skin contact risks (per MSDS).

2. For chemicals/compounds classified as hazardous, list special procedures and precautions that animal care personnel and veterinary caregivers need to assume when working with these animals or their bedding/cages?

None.
In the digoxin tissue distribution study radioactive 3H-digoxin will be used. On post-surgical day 7 (BDL or sham surgery), animals will be administered with 3H-digoxin; they will not be returned to their cages. Rather, after injection they will be kept asleep for 2-hours then euthanized. Liver, brain, kidney, intestine and blood will be collected to determine tissue distributions of digoxin in the organs. The researcher does NOT require respiratory protection; hand, skin, body, and eye protection is accomplished by wearing gloves, appropriate lab attire, and safety glasses while handling the chemical (per MSDS).

3. For hazardous materials, describe any potential adverse side effects or health risks to humans during preparation and administration.

Ingestion may lead to gastrointestinal irritation, nausea, vomiting, and/or diarrhea. The chemical can cause sensitization; there is not an inhalation risk or a skin contact risk (per MSDS)

4. For hazardous materials, describe decontamination procedures and means of disposal of contaminated animal carcasses and waste.

Carcasses will be disposed of as radioactive waste according per established recommendations.

5. For hazardous materials, what is the time frame that animals and/or caging are considered hazardous?

They will be euthanized and the carcass discarded after 2 hours of injection with 3H-digoxin.

6. For non-pharmaceutical-grade compounds:
   a. **Justify** the need to use the non-pharmaceutical-grade compound(s) (e.g., veterinary or human pharmaceutical-grade product is not available).

N/A

   b. Discuss steps taken to ensure the health and welfare of the animals. Examples may include grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, compatibility, storage, side effects, and pharmacokinetics of the compound(s).

N/A

Reference: UMMC Chemical Safety Manual
http://ehs.umc.edu/documents/ChemicalSafetyPolicy2010.pdf

Please remember that the use of any hazardous material in animal rooms requires that a sign be posted in that room and on the cages containing the hazard in accordance with the policy on Signage for Hazardous Studies.

[1] AAALAC Frequently asked questions about Non-Pharmaceutical Grade Compounds

Appendix D Collection of Biological Samples from the Live Animal

Biological samples include blood collection, urine collection, ascites, tail tips for DNA, cerebrospinal fluid, biopsy, etc. Appendix D is completed for all sample collections from live animals, including under terminal anesthesia. Appendix D is not required for samples taken after euthanasia.

1. Indicate the body fluid or material to be collected.

Blood collections for digoxin pharmacokinetic study.

2. Indicate the method and site of collection.

Catheter placed in the jugular vein at the day of PK study (2 days before BDL and 7 days after BDL or sham surgery). It will be tunneled out of the back where the rat cannot reach it. The catheter will be flushed with saline following each blood draw.

3. Indicate the volume of fluid or amount of material to be collected.

250 microliters / sample with 9 samples per animal = 2.25 ml.

4. Indicate the frequency of collection.

At 0, 2, 5, 10, 30, 60, 120, 240, and 360 minutes following administration of medication (Digoxin). Blood loss will be replaced cc for cc with normal saline.

5. Will the animal(s) be anesthetized or sedated during this procedure?

☐No ☒Yes

If No, describe restraint method. (Note: If methods require a prolonged period of restraint, Appendix G is required.)

If Yes, list agents used for anesthesia and anaglesia:

Agent	Dose	Route	Frequency/Duration	Pharmaceutical Grade
Isoflurane	2% - %4	inhaled	continuous	☒Yes ☐No
Carpofen	4~5mg /kg	Sub Q	pre-op	☒Yes ☐No
				☐Yes ☐No
				☐Yes ☐No

Any use of non-pharmaceutical grade compounds requires completion of Appendix M.

Appendix K Progress Report

1. Give a brief description of the work performed on these projects in the past 3 years. If progress did not occur or was less than expected, please give a brief explanation.

4 rats have been used for the study, 3 for sham surgery and one for BDL. Both pre- and post- surgery pk studies were performed. The BDL surgery was successful because there was a significant increase in serum bilirubin and jaundice in this rat. The progress is slow for the past 3 years because the PI has been tied up with another new research project for extramural funding.

2. List any publications, abstracts, and/or presentations coming directly from the work performed on these projects in the past 3 years.

No

3. Answer the following questions in regard to the last year of the previous version of this protocol.

I. Animals

1. Have any unanticipated (morbidity, mortality, inability to collect data) events occurred in the past year?
   ☐ Yes ☒ No
2. Has any mortality occurred prior to the anticipated end-point of an experiment or as a result of surgical manipulation?
   ☐ Yes ☒ No
3. Have any animals been euthanized prior to the anticipated end-point of an experiment?
   ☐ Yes ☒ No
4. Did any animals show signs of morbidity or sickness following experimental manipulation other than what was detailed in the protocol?
   ☐ Yes ☒ No

If yes to 1 -4, answer #5.

5. Describe any unanticipated events (morbidity, mortality, inability to collect data) and any identified contributing factors (e.g., recurring postoperative complications, excessive or unanticipated mortality rate, unplanned event that causes the removal of an animal(s) from an experiment for a period of time, loss of implant, etc.).

If the protocol involves breeding: No

Breeding: Animals born over the past year as part of this protocol

Species	Strain	# of pups born in last year	# of pups used in the last year for experiments

What was the final disposition of any pups not used for experiments?

II. Personnel

1. During the past year did any Occupational Health & Safety "incidents or accidents" *(needle sticks, animal bites, cuts, burns, etc.) occur that involved personnel participating* in the conduct of this study?

   ☐ Yes ☒ No

2. If yes, describe the event and identify any contributing factors:

3. What treatment measures were taken:

THE UNIVERSITY OF MISSISSIPPI
MEDICAL CENTER
EDUCATION • RESEARCH • HEALTHCARE

October 19, 2017

Dr. Hua Liu
Department of Pediatrics
University of Mississippi Medical Center
2500 North State Street
Jackson, MS 39216-4505

Dear Dr. Liu:

Thank you for providing the information requested for your three-year full submission protocol, *Effects of cholestasis on the expression and function of organic anion transporter protein 2 (Oatp2) and P-glycoprotein (mdr1), and on digoxin clearance in rat*, considered at the September 19, 2017 meeting of the Institutional Animal Care and Use Committee (IACUC). The protocol received IACUC approval **October 16, 2017** is assigned protocol number **1431A**. This protocol will remain valid until October 16, 2020 provided Annual Renewals are submitted as required.

Please update any exiting cage ID cards to reflect the current protocol number for this study. New cage cards are available upon request to the Center for Comparative Research main office, ext. 4-1385.

Approval of your animal protocol does not imply that the protocol is congruent with any grant since the IACUC does not review a corresponding grant or grant application during the review of an Animal Activity Protocol. Congruency verification between a protocol and a grant is conducted when a grant submission or transfer is routed from the Office of Sponsored Programs to the Office of Animal Welfare for assurance verification. At that time there is a side-by-side comparison of an application/proposal and the IACUC protocol. Should any inconsistencies exist, you will be contacted. Congruency verification is a requirement of the NIH Public Health Service that must be met in order to maintain UMMC's Institutional Animal Welfare Assurance.

The Animal Activity Protocol form is recognized as a binding agreement between the Principal Investigator and the institution (via the IACUC). This document is designed to address the unique information relative to the animal study, as required by the USDA's Animal Welfare Act and the NIH/ OLAW's Public Health Service Policy on Humane Care and Use of Laboratory Animals. The IACUC recognizes that over the life of an Animal Activity Protocol the experimental studies with animals may "drift" or take seemingly minor departures from their original, documented plan. These deviations from the original IACUC-approved protocol are

considered issues of noncompliance if they have not been previously covered by an amendment to the original protocol. All Principal Investigators and their staff are requested to review and become familiar with the IACUC policy that governs how instances of noncompliance are addressed by the IACUC. This policy, *Management of Suspected Protocol Noncompliance*, is available for review on the IACUC web site: http://www.umc.edu/uploadedFiles/UMC.edu/Content/Research/IACUC/ManageSuspectedProtNoncompl_001.pdf?n=398

Reference should be made to the protocol number when animal orders are placed or when inquiries are made about this protocol. Committee approval of your protocol does not assure timely availability of animal housing space. Animal housing availability is coordinated through the Center for Comparative Research, ext. 4-1385.

A copy of the approved protocol is included with this communication. Please save a copy of the electronic file with your protocol documents for future reference. The IACUC encourages you to make a copy of the protocol available electronically and/or in paper format to your laboratory staff for reference.

Sincerely,

Robert L. Hester

Robert L. Hester, PhD
Chair-Institutional Animal Care and Use Committee

RLH/amk