Animal Ethics Office
The University of Western Australia
35 Stirling Highway
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ANIMAL ETHICS COMMITTEE RESEARCH APPLICATION

OFFICE USE ONLY	**FILE REF: RA/3/100/1087**
This proposal is approved for the period:	
From:15.03.2012	**To:15.12.2015**
Signature AEC Chair:	Date: 15/03/2012

PROTOCOL DETAILS	
Protocol Title: *(full title)*	**The effects of desferroximine as iron chelator, anti-oxidant and cyto-protection inducer on rat liver graft function and liver transplant survival after extended cold preservation**
Chief Investigator: (full name)	Gary Jeffrey
School: (Centre/Dept)	Medicine and Pharmacology
Please indicate if this is an	**INITIAL APPLICATION x ☐** or **RENEWAL ☐** *Application can only be approved for the period of funding and may not exceed 5 years. At the conclusion of the funding period a Renewal application will be required.*
Proposed starting date: (dd/mm/yy) 5 February 2012	**Expected completion date:** (dd/mm/yy) 15 December 2015
Commercial in confidence:	**Yes ☐ No x ☐**
NHMRC Classification Category (Compulsory): ***http://www.research.uwa.edu.au/staff/forms***	1b and 3b

All research involving the use of animals must comply with the *Animal Welfare Act (2002)* and the requirements of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th Edition 2004) *'The Code'. Please see*

www.nhmrc.gov.au/health_ethics/animal/issues.htm ***Code of Practice***
www.austlii.edu.au/au/legis/wa/consol_act/awa2002128/ ***Animal Welfare Act* (2002)**

Applications received after 4pm on the submission closing date will be held over for consideration at the next available meeting. For Submission deadlines and AEC meeting dates - *Please refer* www.research.uwa.edu.au/staff/animals/ethics-committee

The Animal Welfare Veterinary Advisors (AWVAs) and the Animal Welfare Officer (AWO) are available for advice on specific aspects of your application. *Please consult*

Animal Welfare Veterinary Advisors - 6488 4549 / 4700 - vets-research@uwa.edu.au *or*
Animal Welfare Officer – 6488 7882 - awo@admin.uwa.edu.au

This application must be discussed with Manager Animal Care Services or Curator	**Signature of MACS/Curator**
Animal Care Service (ACS) Manager - 6488 6616 - malcolm.lawson@uwa.edu.au or **facility curator for non ACS or non UWA sites**	

As indicated in the application: The following documents are attached to support this application		✓
Permission to Use Animals (PUAs) for each investigator (Xianwa Niu)	**Section 2 & 3**	x
Permits eg Transport / AQIS / Department of Environment and Conservation (DEC) etc (NB: Provide a copy of the application to DEC for permit)	**Section 10C**	
Risk Assessment	**Section 11**	
Genetically Modified Organisms: OGTR assessment / Phenotype Reports	**Section 12B**	
Flow Diagram/s	**Section 13A**	x
Monitoring Sheets		x

Glossary of Terms	

1. Sponsors - Source of Fund and Status of Outcome

Source of Funding:
ID Number (if approved):

Approved? Pending [ ] **Yes** [x] **No** [ ]

1. McCusker Foundation Award 2012.
2. Internal funding from School of Medicine and Pharmacology UWA (Research grant name: Working account – G Jeffrey and code: 63104119).
3. We are applying for a NHMRC project grant (2013 – 2015) to support this project.

Other Approval (if applicable)

Does this project involve more than one AEC? **Yes** [ ] **No** [x]
*If **Yes**, an Inter-Institutional Agreement is required. Please arrange this with the Animal Ethics Office, complete the following details and attach a copy of the approved application.*

Name of the Institution and approval number:

Outcome of the application: **Approved** [ ] **Not Approved** [ ] **Under consideration** [ ]

Previous Approval (if applicable)

Protocol title: Effects of iron chelator, antioxidant and ex vivo hypothermic perfusion on rat liver graft function after extended cold preservation

AEC Approval no: 100/735

Unexpected deaths or adverse events: nil

Please attach list of publications arising:
1. Desferrioxamine protects rat livers from ischemia/perfusion injury aggravated by cold storage (Hepatology Research, under revision)

2. CHIEF INVESTIGATOR

Title, first name, middle, last name	**Staff / Student number (UWA)**
Gary P Jeffrey	

Qualifications: MBBS, MD, FRACP, FRCP

Work mailing address /school (Include UWA MBDP):
M503
School of Medicine and Pharmacology
4th Floor, G Block,
Sir Charles Gairdner Hospital

Email: gary.jeffrey@uwa.edu.au	**Phone:** 9346 2098	**Mobile:** 0408828887

Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.

Dr Jeffrey has 7 years of collaboration with Dr Wen Hua Huang in rat liver transplant project, but does not perform the surgery

Animal Competency and Experience *AEO checked* ☐

Have you obtained Permission to Use Animals (PUA) within the last 5 years? **Yes** [ ] **No** [x]
*If **No**, Please download a PUA form available from our website and attach to this application.*
http://www.research.uwa.edu.au/staff/forms

3. CO-INVESTIGATOR/S (copy and paste additional tables as required for each co-investigator)

Title, first name, middle, last name Wen Hua Huang		**Staff /Student number (UWA)**
Qualifications: MBBS, PhD (Surgery)		
Work mailing address /school(include UWA MBDP): M503 WA Liver and kidney transplantation Surgical Services/School of Medicine and Pharmacology 4th Floor,G-Block Sir Charles Gairdner Hospital		
Email: whuang@cyllene.uwa.edu.au	**Phone:** 9346 2593	**Mobile:** 0422226775

What is your Role in this project?

1. Perform animal surgery
2. Perform post operation observation and sampling

Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.

Dr Wen Hua Huang had established the rat liver transplant technique in her PhD study and has more than ten years experience with the surgical skill and currently works at the Western Australian Liver and kidney transplantation Surgical Services. Dr Huang has performed more than 150 rat liver transplant surgeries (under AEC approval numbers 100/817 and 100/735) during the last 3 years

Animal Competency and Experience *AEO checked* ☐

Have you obtained Permission to Use Animals (PUA) within the last 5 years? **Yes** [ ] **No** [x]
If **No,** *Please download a PUA form available from our website and attach to this application.*
http://www.research.uwa.edu.au/staff/forms

3. CO-INVESTIGATOR/S **(copy and paste additional tables as required for each co- investigator)**

Title, first name, middle, last name Xianwa Niu		**Staff /Student number (UWA)**
Qualifications: PhD (Biochemistry)		
Work mailing address /school(include UWA MBDP): M503 School of Medicine and Pharmacology 4th Floor, G Block, Sir Charles Gairdner Hospital		
Email: xianwa@cyllene.uwa.edu.au	**Phone:** 9287 6061	**Mobile:** 0403853940

What is your Role in this project?

1. Assist animal surgery – very experienced
2. Perform organ ex vivo experiment - very experienced
3. Perform post operation observation and sampling - experienced

Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.

Animal (rat) handling – 7 years
Anaesthesia – 7 years
Minor procedures (liver perfusion, autopsy) - 7 years
Euthanasia - 7 years
Post-surgery monitoring - 6 years
Injections - 7 years
Blood taken from rat tail and heart - 6 years

Animal Competency and Experience *AEO checked* ☐

Have you obtained Permission to Use Animals (PUA) within the last 5 years? **Yes** [x] **No** [ ]
If **No,** *Please download a PUA form available from our website and attach to this application.*

http://www.research.uwa.edu.au/staff/forms

3. CO-INVESTIGATOR/S **(copy and paste additional tables as required for each co- investigator)**

Title, first name, middle, last name		**Staff /Student number (UWA)**
Ling-Jun Mou		
Qualifications: MBBS MS		
Work mailing address /school(include UWA MBDP): WA Liver & Kidney Surgical Transplant Service 6th Floor, G Block Sir Charles Gairdner Hospital		
Email: Lingjun.Mou@health.wa.gov.au	**Phone:** 9346 4055	**Mobile:** 0413237051

What is your Role in this project?

Perform animal surgery (liver transplant) – experienced

Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.

Dr Mou has been doing General Surgery and liver transplant in human for more than 12 years, with more than two year's experience in animal microsurgery, especially rat liver transplantation, and currently is a Transplant Fellow at the Western Australia Liver and Kidney Surgical Transplant Service. Dr Mou as one of the co-investigators under AEC approval number 100/735 has performed more than 15 rat liver transplant surgeries in 2011

Animal Competency and Experience ***AEO checked*** ☐

Have you obtained Permission to Use Animals (PUA) within the last 5 years? **Yes [x]** **No [ ]**

If ***No,*** *Please download a PUA form available from our website and attach to this application.*
http://www.research.uwa.edu.au/staff/forms

4. EMERGENCY CONTACT PERSONNEL - DURING STUDY

Name: Xianwa Niu

What is their role in this project? Co-ordinating the project, assisting surgery and monitoring post-procedure animals

Email: xianwa@cyllene.uwa.edu.au **Phone:** 9287 6061 **Mobile:** 0403853940

After hours/emergency contact number: 0403853940

5. ANIMAL MONITOR - DURING STUDY - ANAESTHESIA, SURGERY & POST- OP RECOVERY PERIOD

Name: Xianwa Niu and Wen Hua Huang

Details of their relevant experience: six years experience in animal post-surgery monitoring (XN)
> 10 years experience in animal post-surgery monitoring (WH)

Email: xianwa@cyllene.uwa.edu.au **Phone:** 9287 6061 **Mobile:** 0403853940
whuang@cyllene.uwa.edu.au 0422226775

After hours/emergency contact number: 0403853940

6. PERSONNEL RESPONSIBLE FOR EUTHANASIA

Name: Xianwa Niu and Wen Hua Huang

Details of their relevant experience: seven years experience in euthanasia of sick animals

Email: xianwa@cyllene.uwa.edu.au **Phone:** 9287 6061 **Mobile:** 0403853940
whuang@cyllene.uwa.edu.au 0422226775

After hours/emergency contact number: 0403853940

7. REVIEWER – INDEPENDENT EXTERNAL (non UWA staff)
Please note this person may be asked to review aspects of this application

Title and name: Alex Bishop

Position /Business address / contact details:
A W Morrow Gastroenterology and Liver Centre
D06 - Blackburn Building
The University of Sydney
NSW 2006 Australia

Email: a.bishop@med.usyd.edu.au **Phone:** 02 9351 6131 **Mobile:**

8. OVERVIEW AND GENERAL AIM OF THE PROJECT
Must be written in lay language (i.e. as if it was a press release) and must not exceed one A4 page.

Cold donor organ preservation techniques were developed to reduce cellular metabolic activity and maintain cellular viability in donor organs. Currently most donor livers are preserved in University of Wisconsin solution (UWS) at 4°C before being transplanted. Improved preservation techniques allow early resumption of normal cellular metabolism after reperfusion of the donor organ in the recipient, however, after extended preservation times poor graft function still occurs (Strasberg *et al.* 1994, Huet *et al.* 2004) and results in increased recipient mortality and morbidity rates following liver transplantation. Therefore livers preserved in UWS beyond 12 hours are generally considered unsuitable for transplantation in Australia. Rarely organs with cold preservation times up to 24 hours have been used in a medical emergency however graft function and patient survival have been compromised. Our previous studies using an isolated rat hepatocyte model and a perfused rat liver model showed that iron chelator desferroximine reduced cell injury and improve liver graft function after extended cold preservation and warm reperfusion (Arthur *et al.* 2008, Niu *et al* 2010). This study will examine the effects of desferroximine as an iron chelator, an anti-oxidant and an inducer of cyto-protection gene (Halliwell, 1989, Loor and Schumacker, 2008) on liver cold preservation and reperfusion injury using a rat liver transplant model. As cold preservation-reperfusion injury is a major cause of primary graft non-function and subsequent morbidity and mortality in liver transplant recipients, the outcome of this study may help us to improve the cold preservation conditions and extend preservation period for human liver transplant.

These important studies have implications in increasing the limited pool of donor organs to Western Australian recipients by potentially allowing the use of donor livers from the Eastern States of Australia and New Zealand with long cold preservation times. In addition these techniques may also improve donor organ preservation of livers obtained from non-heart beating donors, a group of potential donors that to date has been under- utilised.

The project will be carried out in three stages

Stage one: we will examine if desferroximine protects livers from non-heart beating donors after cold storage using a perfused rat liver model. Desferroximine will be added before non-heart-beating warm ischemia and/or during cold storage (5 hours). The liver injury will be examined after ex vivo liver warm perfusion.

Stage two: we will examine if desferroximine protects livers from non-heart beating donors after cold storage using a rat liver transplant model. Desferroximine will be added before non-heart-beating warm ischemia and/or during cold storage. The liver injury will be examined after liver transplant. The data from the Stages one and two will help us improve donor organ preservation of livers obtained from non-heart beating donors and increase the limited pool of donor organs.

Stage three: we will examine if desferroximine protects livers from heart-beating donors which are currently practiced in Australia after extended cold storage (20 hours) using a rat liver transplant model. This study is a continuation of previous study using an isolated rat hepatocyte model and a perfused rat liver model.

Glossary

Ex vivo: outside of living body

Graft: a tissue or an organ taken from a site or a person/animal and inserted into a new site or person/animal

Hypothermia: low temperature

Ischemia: an insufficient supply of blood to an organ

References

Arthur PG, Niu X, Rigby P, Steer JH, Jeffrey GP. (2008) Oxidative stress causes a decline in lysosomal integrity during hypothermic incubation of rat hepatocytes. *Free Radic. Biol. Med.* **44**: 24-33.

Halliwell B. (1989) Protection against tissue damage in vivo by desferrioxamine: what is its mechanism of action? *Free Radic. Biol. Med.* **7**: 645-51.

Huet PM, Nagaoka MR, Desbiens G, *et al.* (2004) Sinusoidal endothelial cell and hepatocyte death following cold ischemia-warm reperfusion of the rat liver. Hepatology **39**:1110-1119.

Loor, G and Schumacker PT. (2008) Role of hypoxia-inducible factor in cell survival during myocardial ischemia-reperfusion. Cell death and differentiation **15**:686-90

Niu X, Arthur PG, Jeffrey, GP. (2010) Iron and oxidative stress in cold-initiated necrotic death of rat hepatocyte. Transplant Proc. **42**:1563-8

Strasberg SM, Howard TK, Molmenti EP, Hertl M. (1994) Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. Hepatology **20**: 829-38.

9. ANIMAL SUMMARY (add additional lines as required)

	Species scientific & Common name ONE SPECIES PER LINE	Strain	NHMRC Animal Species Code	Dietary Modifications Yes/No	Total number required (over the life of the project)	Vendor / Source (ARC/Jackson labs/ Wandalup/Murdoch)
1.	Rat	PVG	120	No	565	ARC
2.						
3.						
4.						

Justification of Number of Animals Requested (ie number in each experimental group)

Has a biostatistician been consulted? **Yes** [ ] provide contact details below **No [x]**

Please see the attached flow diagram and Section 14B for the details on how we calculate the number of the animals requested.

DOES THE PROTOCOL INVOLVE ANY OF THE FOLLOWING? **Yes [x]** **No [ ]**

If **Yes**, please complete the details below

	Species	Strain	Total number required
Creation of hybridoma			
In vivo (ascites) production of monoclonal antibodies			
Genetically modified mice			
Other genetically modified animals			
Transplantation			
• autograft			
• allograft	Rat	PVG	565
• xenograft			
- cells			
- tissue			
- organs			

10. HOUSING AND LOCATION OF ANIMALS

If using UWA facilities (listed below) the signature of the Manager Animal Facilities is required.

UWA:
- **QEII:** M Block
- **Shenton Park:** BRF, NAF, SRF
- **Crawley Campus:** PCF, LAF

OTHER UWA FACILITIES:
- **Fremantle Hospital** – Z Block
- **Allandale Farm / Ridgefield Farm**
- **Animal Biology**

Other (please specify) - Royal Perth Hospital					
Facility room number/ zone	Species (and/or strain if applicable)	Gender	Age or initial weight	Reproductive status	Manager Animal Facilities Signature and Date
QEII: M Block	Rat/PVG	M	250 g	No	
QEII: M Block	Rat/PVG	M	270 g	No	

A. WHAT IS THE MAXIMUM LENGTH OF HOLDING IN WEEKS?

4 weeks

B. WHAT ADDITIONAL ENRICHMENT WILL BE PROVIDED FOR ANIMALS WHICH WILL BE HELD LONGER THAN 3 MONTHS?

N/A

C. PLEASE DETAIL THE METHOD OF TRANSPORT AND ANY ANIMAL WELFARE IMPLICATIONS:

N/A

[ ] I have attached copies of any necessary transport and acquisition permits/approvals.

11. SAFETY AND HEALTH RISKS

Does project involve use of:	Y/N	Approval Y/N	If YES, explain risks involved	Precautions to protect staff and/or animals
Teratogens or carcinogens	N			
S7, 8 ,9 drugs *(a copy of the permit will be required)*	Y	Y (see attached)	No risk with the drug except for needle injury	PPE worn Good practice in handling sharps
Radioisotopes or x-rays	N			
Other potentially infectious or hazardous (chemical/physical / biological) agents which may pose a health risk to staff or animals	N			

IDENTIFY POTENTIAL RISKS TO STAFF AND HOW THEY WILL BE MANAGED AND MINIMISED

[ ] I have attached a Risk Assessment

12. GENE TECHNOLOGY / BIOLOGICAL SAFETY

A. PLEASE PROVIDE DETAILS OF ANY BIOLOGICAL SAFETY CONCERNS TO STAFF, AND HOW THEY WILL BE MANAGED

N/A

B. ARE YOU DEALING WITH GENETICALLY MODIFIED ORGANISMS? **Yes [ ] No [x]**

If Yes, please provide details of phenotypic expression.

[ ] Established Phenotype report attached?

[ ] I have attached copies of required permits/approvals.

Dealing Type: **EXEMPT DEALING[ ]** **NLRD [ ]** **DNIR [ ]** **DIR [ ]**

Exempt Dealings - Requires IBC assessment
Notifiable Low Risk Dealings (NLRD) - requires IBC assessment and notification to OGTR
Dealings Not Involving an Intentional Release of a GMO into the Environment (DNIR) - Requires IBC Assessment & application to OGTR for a licence.
Dealings Involving an Intentional Release of a GMO into the Environment (DIR) - Requires IBC Assessment and application to OGTR for a licence.

IBC Approval Number: **Approval Status: Pending [ ] Yes [ ] No [ ]**
(if approved)

IF USING GMOS, HAVE ALL INVESTIGATORS UNDERTAKEN THE (COMPULSORY) GENE TECHNOLOGY REGULATION TRAINING COURSE? **Enrolled [ ] Yes [ ] NO [ ]**

If *NO*, or Enrolled, please specify which investigator/s have not completed the course:

[ ]AQIS Permit is attached (please supply 1 photocopy)
[ ] I have attached copies of all required permit/approvals.

13. EXPERIMENTAL PROPOSAL – Cost Benefit Analysis

A. FLOW DIAGRAM OUTLINING THE PROJECT AND THE PROCEDURES – Can be attached as separate sheet/s. Must include a time line and list of procedures to clearly illustrate what will happen to each group of animals from acquisition to disposal.

[x] **Attached is a separate flow diagram**

B. THE "COST" OF THE RESEARCH TO THE ANIMALS – Please describe any potential harms, pain, distress, that may arise from the procedures being performed.

NOTE: IF YOU STATE THAT THERE IS NO LIKELY WLEFARE IMPACT, THIS MUST BE FULLY SUPPORTED BY APPROPRIATE EVIDENCE.
If using genetically modified animals or mutant, please attach phenotype reports.

The donors will not suffer from any pain and discomfort because the procedure is the end point of the experiments. The recipients will experience some pain and discomfort after the procedures. This will be addressed by using analgesia during and after the procedure and closely monitoring the animals. The recipient will receive analgesics buprenorphine (0.01 mg/kg Sub Cut) at the anesthesic induction and after 12 h and 24 h. The recipient will also receive one dose of non-sterioidal anti-inflammatory drug Meloxicam (1 mg/kg sub cut) before awaken and oral buprenorphine jelly (0.5 mg/kg) for the following 3 days. We will monitor the post-procedural animals closely. If the signs of pain and discomfort persist, the animal will be euthanased (please see attached Post-monitoring sheet). The ultimate cost to the animals is death/euthanasia.

The detailed animal welfare costs and the measures to minimize those costs are

(1). Transportation: the animals will be transported according to the Animal Care Services Standard Operating Procedure

(2). Housing of animals: the animals will be housed for 7 – 14 days before the procedures for (i). acclimatision of the animals, and (ii) obtaining the desired experimental weights, and for ≤ 5 days after the procedures in a temperature (26°C) and humidity controlled cabinet

(3). General anesthetic using isoflurane (2%) with oxygen (400 – 500 ml/min): we will closely monitor animals' respiration and response to stimulation during procedure to avoid over- or under-anesthesia

(4). Surgical procedures: only skilled and experienced micro surgeons will perform the procedures.

(5). Post-procedure: the recipients will experience pain (last 2 days) and unwellness due to compromised liver functions (last ≤5 days). We will closely monitor the pain and discomfort of animals and give analgesics promptly. If the signs persist, the animals will be euthanised.

(6). Death: all donors will die after the liver removal and all recipients will be sacrificed under general anesthesia 3 h, 24 h or 5 days after the procedures. We have designed our experiments carefully to use the minimal number of animals and take care during the procedures to avoid any un-necessary death.

C. POTENTIAL "BENEFITS" OF THE RESEARCH – to humans, animals, or the environment.

Currently the donor liver is kept only for up to 12 hours before transplantation. We have identified several factors which may affect the period of the hypothermic preservation. The outcome of this study may help us to improve the cold preservation conditions and extend preservation period for human liver transplant, therefore improve the liver transplantation services in Western Australia.

14. ADDRESSING THE THREE R'S – ALL 3 SECTIONS MUST BE COMPLETED
If Genetically modified animals are to be used also provide justification for the strain

A. REPLACEMENT – describe the alternatives to animal use that you have considered and/or adopted. **See the Australian Code of Practice Section 1.8**

Animal models are used extensively as the basis for organ preservation studies, especially in rat models, well-defined strains are available and immunological rejection problems can be avoided by the use of a single inbred strain for donors and recipients. Moreover, rats and humans are both omnivorous mammals and have the same major organs performing the same functions. In the both instances the organs have similar structures both macroscopically and microscopically and have similar blood supplies. Therefore, the rat allograft model is a powerful and reliable tool to study the basis for organ preservation.

B. REDUCTION – describe the ways that you propose to minimise the use of animals.
See the Australian Code of Practice Section 1.9 – 1.13

Total of 565 rats are proposed to be used between 2012 and 2015 for this project including any losses due to surgical complications and errors. The number of animals we propose to use is the minimum required for performing these experiments. Please see attached sheet (Flow diagram) for detailed experiments.

In the stage one (perfusion model), we propose to do 5 liver perfusion experiments per group and expect the results to be statistically valid as the proposed numbers of animals used per group are based on our data from the previous isolated liver perfusion model (Arthur *et al*, submitted).

In the stages two and three (transplant model), we propose to do 8 liver transplants per group and expect the results to be statistically valid as the proposed numbers of animals used per group are based on the data from other studies using a similar liver transplant procedure and protocol (Howden and Jablonski, 2000, Kato, H, Amersi, F, Buelow, R, *et al* 2001).

References:

Arthur, P, Niu, X, Huang WH, *et al*. The presence of desferrioxamine in ischemic and perfusion media prevents liver injury aggravated by cold storage, submitted

Howden, B. O, Jablonski, P. Liver preservation: a comparison of celsior to colloid-free University of Wisconsin solution. (2000) Transplantation **70**:1140-2

Kato, H, Amersi, F, Buelow, R, *et al*. Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. (2001) Am. J Transpl. **1**: 121-8

C. REFINEMENT – Also complete the tables at Section 15 & 16.
Step-by-step description of procedures. What will happen to animals and their tissues identifying the actions taken at each step to minimise suffering and distress
See the Australia Code of Practice Section 1.14 – 1.28.

There will be no impact on the donors as they will die under general anaesthetics when the livers are removed. There will be impact on the recipients (please see below) and several steps will be taken to minimise the impact (please also see Section 8D)

Procedures	**Expected impacts**	**Steps taken to minimise impacts**
1. Anaesthesia of rats	potential overdose/under dose	monitor vital signs during surgery for over/under dose
2. After major surgery	A. distress and pain	1. give analgesics (injection and oral) during and after procedure for 3 days 2. closely monitor any signs for pain and distress If the signs develop, euthanese animal immediately
	B. infections	1. disinfect/autoclave all instrument 2. give antibiotic after surgery Ampicyn 10 mg IM
	C. haemorrhaging	1. careful microsurgery 2. minimising handling after surgery

15. NON SURGICAL PROCEDURES

FULL DESCRIPTION OF ALL NON SURGICAL PROCEDURES. ***Please also include details in the table below.***
If substances are being administered to animals please include details of route, volumes, frequency, intervals and duration.

Six drugs will be used in this study. All injections will be administrated using an insulin needle

Note: Only the title of the procedure should be listed in column one of table. Full details of the procedures must be completed in the relevant space below each table.

Type of non surgical procedure to be carried out	Expected impacts of the procedure	Expected frequency of adverse impacts	Refinement taken to minimise impacts
e.g. gavage	*Minor discomfort rarely substance enters airway or oesophagus is damaged*	*Some discomfort on each occasion. Substance in airway or oesophageal damage in less than 1 in 1000 administrations.*	*Good handling to minimise discomfort and observation after dosing with humane killing of any animal showing signs of mis-dosing or damage.*
1. Iron chelator desferroximine (IV) left hindlimb femal vein	Under general anaesthetics, no impact	20 mg/kg body weight (255 µl/300 g rat) Once before surgery	N/A
2. Analgesia buprenorphine (SC) At back of neck	Under general anaesthetics, no impact	0.01 mg/kg body weight (200 µl/300 g rat) At anaesthetic induction	N/A
	Minor discomfort	Twice daily for 3 days Some discomfort	Good handling
3. Anticoagulant Heparin (IV) In penis vein	Under general anaesthetics, no impact	200 U (200 µl) Once before liver removal	N/A
4. Antibiotic Ampicyn (IM) at left hindlimb	Under general anaesthetics, no impact	10 mg (100 µl/300 g rat) Once after surgery	N/A
5. Analgesia Meloxicam (SC) at right hindlimb	Under general anaesthetics, no impact	1 mg/kg body weight (60 µl/300 g rat) Once after surgery	N/A
6. Buffered-electrolytes Normocarb (IP)	Under general anaesthetics, no impact	10 ml/kg body weight (3 ml/300 g rat) Once after surgery	N/A

Which investigator as detailed in Section 3 or 4 will perform these procedures?

Applications 1 – 6 (application 2: 1st dose): Wen Hua Huang and Ling-Jun Mou, application 2 (2nd and 3rd doses and application 7: Xianwa Niu

16. SURGICAL PROCEDURES

A. FULL DESCRIPTION OF ALL SURGICAL PROCEDURES. ***Please also include details in the tables below.***

All animals will be anaesthetised by inhaling isoflurane with oxygen. The animal is taken out of the cage and placed in an anesthetic induction chamber to be anaesthetized with isoflurane (5%)

mixed with oxygen (500 ml/min). When the animal is fully anaesthetized (in recumbent position and eyes closed), it is weighed and moved onto the surgical table and anaesthetized through a nose cone with isoflurane (2%) mixed with oxygen (400 - 500 ml/min). During the an-hepatic period (< 30 min) the dose will be reduced to isoflurane (0.25 - 0.5%) with oxygen (400 - 500 ml/min). We will check the anesthetic system before use to ensure adequate amounts of supply oxygen and isoflurane for duration of the procedure.

The surgical room is located in M-Block Animal Care Services, Sir Charles Gairdner Hospital and is classified as a PC2 facility. All personnel are required to wear sterile surgery gown, cap, surgical mask and shoes, and spray gloves with 70% ethanol before entry.

Before each surgery, the surgical table will be wiped with 70% ethanol and all surgical instruments will be soaked in 70% ethanol for 20 min and then rinsed with sterile physiological saline. All reusable containers will be autoclaved. All solutions (organ preservation medium containing antibiotic gentamicyn, saline and injection water) will be prepared under sterile conditions.

Before the surgery, the animal is taken out of the cage, put into a clean cage in a laminar flow hood and then is taken to the surgical room. The animal is secured on a heated (37°C) dissection board under general anesthesia. The abdomen skin is disinfected with 70% ethanol after shaving before the operation.

Donor rats

(1). Liver isolation (the same procedure for heart-beating and non-heart-beating donor)

Rat is placed on a heating board (37°C) and under general anaesthetics. A full length midline incision is made and the xiphoid cartilage is grasped with Allis forceps, drawn over the rat's head and secured to the board. The two retractors are placed under the ribcage and drawn up- and outwards. The underside of the liver and the viscera are cover in saline-soaked gauze. The viscera are drawn to the rat's left and the liver, portal vein, inferior vena cava, coeliac artery and bile duct are exposed. The bile duct is cannulated using a teflon stent. The anticoagulant heparin (200 U) is injected (IV) before the liver is flushed with 20 ml of Hartmann's solution (+ 100 U heparin) via the aorta just before excision and the blood is washed out. The superhepatic vena cava, portal vein, and infrahepatic vena cava are divided, and then the liver is removed, flushed with 4°C UWS and placed in a 4°C University Wisconsin solution (UWS) bath for 20 hours before being transplanted into the recipient rats. An essential feature of the technique requires the preparation of cuffed, externally stented vessels to simplify and accelerate the re-implantation anastomoses of the portal vein and infrahepatic inferior vena cava before the liver is removed (Kamada and Calne, 1979).

(2). Induction of non-heart-beating warm ischemia

For non-heart-beating donor, an extra procedure is performed to induce cardiac arrest. After open the abdomen, the non-heart-beating warm ischemia is induced by incision of the diaphragm. Five minutes after apnea, the subcardial aorta was ligated and the non-heart-beating warm ischemia starts from this point (Dutkowski *et al* 2006). During the warm ischemia, the liver is covered in saline-soaked gauze. After 60 minutes, the liver is flushed with cold Ross solution with heparin (10 U/ml) through aorta at 40 ml/min and the liver is removed.

Recipient rats

(1). Liver removal

At the induction of anesthesia, analgesia buprenorphine (0.01 mg/kg body weight) is given (Sub Cut). The right renal artery is dissected and any tributaries including adrenal artery is cauterised and divided. A right nephrectomy is performed following ligation of the right renal vein and ureter. The infrahapatic vena cava and portal vein are cross-clamped and then divided. The recipient's livers are taken out and discarded.

(2). Liver implantation

The donor liver is removed from the cold UWS bath, flushed thoroughly with 4°C Hartmann's solution and placed in the orthotopic position. The suprahepatic inferior vena cava is sutured end-to-end. Portal vein and infrahapatic vena cava are cannulated using the cuff techniques. Bile ducts are joined by a teflon stent. Hepatic arterial circulation is restored by anastomosing recipient right renal and donor coeliac arteries. The anhepatic phase in the recipients is limited to less than 25 minutes.

Post procedure care

After transplantation the rats will be given antibiotic (10 mg Ampicyn) and non-steroidal anti-inflammatory drug Meloxicam (1 mg/kg body weight) immediately. The analgesia buprenorphine (0.01 mg/kg body weight Sub Cut) is repeated 12 hourly x 2 from the induction of general anesthestic followed by oral analgesic buprenorphine for 3 days. The rats will be caged separately

and placed in a temperature (26°C) and humidity-controlled cabinet for 3 days. The rats will be monitored for distress and weight loss during the study.

References

Dutkowski, P, Furrer K, Tian Y, Graf R amd Clavien PA (2006). Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg. **244**: 968-77

Kamada, N, Calne, RY. (1979). Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation **28**:47-50.

Type of surgical procedure to be carried out	**Expected impacts of the procedure**	**Expected frequency of adverse impacts**	**Refinement taken to minimise impacts**
e.g. insertion of catheter	*Pain*	*Always*	*Analgesia*
Liver removal	N/A	N/A	N/A
Liver transplant	Pain	Constant pain lasts for a few days	Administration of analgesics after operation
	Hemorrhaging	First 2 h after operation	Careful surgery and Minimising handling after operation
	Infection	Lasts for a few days	Administration of antibiotic (Ampicyn) after operation
	Impaired liver functions	Lasts ≤ 5 days	Result of procedure N/A
			Euthanasia of animals which show distress which is not relived by analgesia

Which investigator as detailed in Section 3 or 4 will perform these procedures?

Wen Hua Huang, Ling-Jun Mou and Xianwa Niu

B. ANAESTHETICS AND NEUROMUSCULAR BLOCKADE

Species	**Agent (s)**	**Dose**	**Route**	**Frequency**	**Duration**
e.g. Rat	*Ketamine and Xylazine*	*80 mg/kg 10 mg/kg*	*ip ip*	*Once only*	*Single injection is sufficient for the 10 minute procedure*
Rat	isoflurane	2 % with O_2 400 - 500 ml/min	Inhale from a nose cone	Once only	Liver isolation: < 30 min Liver transplantation: < 90 min

Which investigator as detailed in Section 3 or 4 will perform these procedures?

Wen Hua Huang and Ling-Jun Mou

C. ANALGESICS - Analgesics to be used intra- and post-operatively

Species	**Agent**	**Dose**	**Route**	**Frequency & Duration**
e.g. Mouse	*buprenorphine*	*0.05-0.1mg/kg*	*Sub cut*	*Initial dose given at induction of anaesthesia then continued every 8 hours for 3 days post-op*
Rat	Buprenorphine	0.01 mg/kg	Sub Cut	12 hourly x3 From anesthetic induction
Rat	Meloxicam	1 mg/kg	Sub cut	Once prior to the recipient rat awakening

Rat	Buprenorphine jelly	0.5 mg/kg	Oral	daily, day 1 - 3

Which investigator as detailed in Section 3 or 4 will perform these procedures?

Wen Hua Huang, Ling-Jun Mou and Xianwa Niu

17. ANIMAL WELL-BEING

A. POST-PROCEDURAL PAIN AND DISTRESS – How will pain and distress be monitored, scored and treated.

Please see attached post-transplant monitoring sheets

B. MONITORING SCHEDULE – provide a "Post-Procedural Monitoring Sheet" and /or "Long Term Monitoring Sheet"

1. Immediately after surgery monitoring

The rats will be monitored continuously from the completion of the surgery until they regain conscious (usually happens within 10 min), in sternal recumbency with a swallow reflex present (drinking water usually happens within 30 min), and then will be monitored according to the Postoperative Monitoring Schedule.

2. long term monitoring (day 1 to day 5)

The rats will be monitored twice a day from day 1 until the end of experiment on day 5 according to the Long Term Postoperative Monitoring Schedule.

C. CRITERIA FOR EUTHANASIA - How will animals be assessed for euthanasia.

A. **Potential welfare problems** - identify potential animal welfare concerns and how they will be addressed

Potential animal welfare: Pain, distress, Hemorrhaging and infection after the procedure will be reduced by

1. Injecting analgesics buprenorphine and Meloxicam during and after the procedure. The oral buprenorphine made in jelly will be given on day one, two and three.
2. Injecting antibiotic Ampicyn after the procedure
3. Minimising handling the animals

B. **Post-procedural pain and distress** - detail how pain and distress will be monitored and scored

Scores (please see the details in the post-procedure monitoring sheet cover)

0. perfect normal appearance and behaviour, no intervention required
1. Animal shows slightly or intermittently deviated from normal,and weight loss < 10%, require close monitoring
2. Animal demonstrates moderate deviation from normal and weight loss 10 – <15%, require closer and more careful monitoring and give analgesic buprenorphine (0.1 mg/kg body weight SC). and reaccess after 1 h
3. Animal demonstrates moderate to severe deviated from normal and weight loss = 15%, assess for euthanasia and seek expert advice
4. Animal demonstrates significant deviation from normal or is obviously unwell and/or distressed, immediate euthanasia and contact AWO

D. WHAT % OF ANIMALS DO YOU EXPECT TO DIE OR REQUIRE INTERVENTION EUTHANASIA DURING THIS PROJECT? PLEASE EXPLAIN LIKELY REASONS FOR THE ANTICIPATED LOSS RATE.

We expect that approximately 46% of animals will survive after the procedures in this study, including 0% of donors and 92% of recipients (~ 8% death)

Potential cause of death or euthanasia	Impact on welfare	Steps taken to minimise impact	Percentage of animals affected
e.g. vessel rupture	*irreversible haemorrhage*	*haemorrhage apparent to surgeon, animal would be euthanased whilst still under general anaesthesia*	*<1%*
Blood vessel damage during surgery	severe blood loss; animal unable to use further and euthanased immediately	Careful microsurgery by experienced microsurgery personnel	≤ 3%
Unable to restore circulation once graft is in place	animal unable to use further; euthanased immediately	Proper handling the graft before surgery; work quickly to prevent blood clotting during surgery	≤ 2%
Severe internal bleeding after surgery	animal unable to use further and euthanased immediately	confirm no further bleeding before closing incision Minimise handling animals after surgery	≤ 2%
Infection	Unwell	disinfect instrument and work area give antibiotic after surgery	≤ 1%

PLEASE NOTE THAT ALL UNEXPECTED /UNPLANNED DEATH/S MUST BE REPORTED PROMPTLY TO THE AEC.

18. COMPLETION OF EXPERIMENT – Fate of the animals at the end of the experiment?

Researchers are expected to share animal tissue where possible via Ethitex: **www.ethitex.com.au**
To register please contact the Animal Ethics Office at aeo@admin.uwa.edu.au

A. WHAT ARRANGEMENTS HAVE YOU USED TO SHARE TISSUE?

We have registered to share tissues

B. ARE ALL THE ANIMALS EUTHANASED AT THE END OF THE EXPERIMENT? **Yes [x] No [ ]**

If *No*, What is the fate of non-euthanased animals?

If *Yes*, please complete the table below.

METHOD OF EUTHANASIA – provide details of the generic constituents (not the trade name), the dose rate as mg/kg, and the route of administration.

Species	Agent	Dose	Route
e.g. Rat	*Pentobarbitone*	*>160mg/kg*	*Intraperitoneal injection*
Rat			

C. DETAIL HOW DEATH WILL BE CONFIRMED:

To euthanize the animals: they will be given a terminal cardiac puncture to collect blood and their livers will be removed under general anesthesia (2% isoflurane mixed with oxygen at 500 ml/min) through a nose cone

D. METHOD OF DECONTAMINATION /DISPOSAL OF GMOS.

N/A

E. METHOD AND DETAILS OF CARCASS DISPOSAL

The dead rat will be a placed in plastic bag labelled with all details (date and time of death, AEC approved number, chief investigator, etc.) and stored in an Animal Care Unit freezer

19. DECLARATION

I/we, the undersigned:

(i) have read and agree to abide by the conditions and constraints of the WA Animal Welfare Act 2002 and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004);

(ii) acknowledge that the information contained in this form is a true and accurate record;

(iii) understand any non-compliance with the Code of Practice must be reported immediately to the AEC and may result in the withdrawal of project approval and possible disciplinary action;

(iv) understand that in keeping with AEC and Animal Facility policy, all animals are to be monitored as detailed in the application. The Animal Welfare Officer (AWO) has the authority to euthanase distressed animals. Every attempt will be made to inform the CI before any action is taken;

(v) understand It is the responsibility of the CI to maintain animal records annually to the AEC on animal usage;

(vi) understand that in the event of an animal death, or an unplanned euthanasia, we will immediately report the death to the AEO, complete a Notification of UNEXPECTED DEATH FORM and email to AEO within 48 hours, and arrange for an autopsy to be carried out and the results of the autopsy report to be sent to the AEO;

(vii) will ensure that the qualifications and/or experience of all listed personnel are appropriate to the procedures to be performed;

(viii) certify that the resources in the school or department, including housing and personnel, are appropriate for the welfare of the animals and the satisfactory completion of the project.

[x] **I agree to all of the above**

CHIEF INVESTIGATOR - It is the responsibility of the CI to obtain all required signature/s on the application form.

PRINT NAME Gary Jeffrey	SIGNATURE	DATE 27/10/2011
CO-INVESTIGATOR		
PRINT NAME Wen Hua Huang	SIGNATURE	DATE 27/10/2011
CO-INVESTIGATOR		
PRINT NAME Xianwa Niu	SIGNATURE Xianwa Niu	DATE 1/11/11
CO-INVESTIGATOR		
PRINT NAME Ling-Jun Mou	SIGNATURE	DATE
CO-INVESTIGATOR		
PRINT NAME	SIGNATURE	DATE

As Head of the School (or Acting), I acknowledge that I have reviewed this application and I confirm that sufficient financial and other resources are available to enable the research to occur in compliance with the Regulations above.

I understand that when I am named on an application as an investigator, the Dean of the Faculty will be required to sign below.

[ ] **A peer review of this application has taken place within the school / faculty.**

HEAD OF SCHOOL/(*DEAN OF FACULTY) **Acting** ✓

PRINT NAME ~~Fiona Lake~~ BRENDAN McQUILLAN SIGNATURE DATE 31/10/11.

The AEC will not consider incomplete, unsigned or inadequate applications. Incomplete applications will be returned to the Chief Investigator for completion and may miss submission deadlines.

SUBMISSION OF APPLICATION

Please lodge **one original FULLY SIGNED application (including all attachments) to: Animal Ethics Office – M459, Main Administration Building (NWW) room 2.05.**

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CO-INVESTIGATOR		
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CO-INVESTIGATOR		
PRINT NAME Ling-Jun Mou	SIGNATURE	DATE 3/10/11
CO-INVESTIGATOR		
PRINT NAME	SIGNATURE	DATE

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HEAD OF SCHOOL/(*DEAN OF FACULTY) **Acting** [ ]

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Rat liver Transplants (AEC Approval No:)

Postoperative Monitor Schedule

File Name: **Animal ID#:** **Date of Surgery:** **Time of Recovery:** **Weigh after Recovery (gm)**

Date	Post surgery Time	Time 00:00-24:00	Awake (Y/N)	Hunched (Y/N)	Lethargic (Y/N)	Bleeding (Y/N)	Gasping (Y/N)	BP jelly (Y/N)	COMMENTS	Observer's Initial
	15 min									
	30 min									
	45 min									
	1 hour									
	1 hr 30 min									
	2 hours									
	2 hrs 30 min									
	3 hours									

Assessment of animals: After surgery, the animal will be in a cage on a heating pad. The rats will be monitored continuously from the completion of the surgery until they regain conscious, in sternal recumbency with a swallow reflex present (drinking water or taking buprenorphine jelly), and then will be monitored according to the Postoperative Monitoring Schedule. Normally, this should occur within the first two hours. Once animals awaken, vital signs should be monitored closely. Animals which are Hunched or Lethargic (general signs of pain and discomfort) should be reassessed during the following 60 min. If the animal's signs of pain and discomfort have not been relieved, it should be euthanased. Animals which are cyanotic, bleeding, gasping, or emitting sounds of stridor should be euthanased immediately. We will be monitoring for signs of internal bleeding as listed below. (1) Pale limbs and ears of rat (2) Increased respiratory rate (normal rate 80 – 110 per min). It will be monitored before, during and after liver transplant (3) Distended or bloated abdomen (4) Unwilling to walk/lethargic/not returning to sternal recumbency If any signs of internal breeding are evident, the animal will be euthanased immediately. A post-mortem examination should be performed on all euthanased animals to determine the cause of their condition. Once fully recovered, animals should be housed in the recovery cabinet for further 3 days. Then the animals will be kept in the holding room until the end of experiments – 5 days after liver transplant.

BP: buprenorphine

Long Term Postoperative Monitoring Schedule (day 1 - day 5)

For euthanasia contact Xianwa Niu Tel: 0415715897; Dr. Wen Hua Huang Tel: 0422226775

File Name: **Animal ID#:** **Date of Surgery:** **Weight after Recovery (gm):** **Weigh loss: 10%:** **20%:** **30%:**

Date	Weight (g)	Post –Op Day	Hunched (Y/N)	Lethargic (Y/N)	Ruffled (Y/N)	Normal Faeces (Y/N)	Incision site OK? (Y/N)	Discharge from Orifices (Y/N)	Other comments and Pain and Distress Scores	Observer's Initial
		D1 am								
		D1 pm								
		D2 am								
		D2 pm								
		D3 am								
		D3 pm								
		D4 am								
		D4 pm								
		D5 am								
		D5 pm								

Assessment of animals: Animals which are hunched, lethargic or ruffled (general signs of pain and discomfort) should be given buprenorphine analgesic subcutaneously (0.1 mg/kg body weight), and reassessed after one hour. If the animal's signs of pain and discomfort have not been relieved, it should be euthanased. Animals which are cyanotic, jaundiced, or emitting sounds of stridor should be euthanized immediately. The animal's weight should be determined daily (am). Animals which exhibit ≥10% weight loss compared to the weight after recovery baseline should be inspected for other signs of pain, discomfort, or life-threatening complications, and either administered analgesia or euthanased. A post-mortem exam should be performed on all euthanased animals to determine the cause.

Post-procedure pain and distress scores:

1. Perfect normal appearance and behaviour
2. Lack of self-grooming, slightly ruffle
3. Hunched, loss of appetite, reduced responsiveness or voluntary movement or any other abnormal behaviour – consider euthanasia
4. Weight loss >15%, little voluntary movement, non-responsive - euthanasia
5. Weight loss > 20%, unable to right itself, non-responsive to prodding - euthanasia

Government of **Western Australia**
Department of **Health**

PERMIT 7926

Valid From: 01 July 2010
Valid Until: 30 June 2013

MR GEOFFREY BILLIEWICZ

on behalf of UNIVERSITY OF WESTERN AUSTRALIA - ANIMAL CARE SERVICES Is authorised to purchase:

Buprenorphine in Schedule 8
Butorphanol in Schedule 8
Fentanyl in Schedule 8
Ketamine in Schedule 8
Methadone in Schedule 8
Morphine in Schedule 8
Poisons included in Schedules 2,3 & 4

And Store at:

PREMISES LISTED BELOW

POISONS ACT 1964

The holder of this Permit/Licence shall comply with all relevant areas of the Poisons Act 1964,Poisons Regulations 1965 and subject to any conditions shown below.

* Poison/s listed on this permit may be purchased for research purposes in a tertiary institution.

All usage of Schedule 4 and Schedule 8 drugs to be recorded.

Schedule 8 drugs to be entered in a Register of Drugs of Addiction on receipt, use and disposal.

Schedule 8 drugs to be stored in compliance with the Poisons Regulations. An inventory of each drug of addiction must be recorded in the register at least monthly.

Schedule 8 drugs to only be disposed of in compliance with the Poisons Regulations 1965.

Premises to which this permit applies:

UWA-ANIMAL CARE SERVICES LARGE ANIMAL FACILITY	CRAWLEY	6009
UWA-BIOMEDICAL RESEARCH-2ND FLR M BLOCK, QE11	NEDLANDS	6009
UWA-BIOMEDICAL RESEARCH FACILITY-SHENTON PK CAMPU	SHENTON PARK	6008
ROYAL PERTH HOSP RESEARCH CTR (ANIMAL FACILITY)	PERTH	6000

delegate of CHIEF EXECUTIVE OFFICER

Issue Date: 15 July 2010