UNIVERSITY OF WAIKATO ANIMAL ETHICS COMMITTEE

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Protocol Number: 917

APPLICATION COVER SHEET

Project Details (Do not use acronyms)
Full Protocol Title: Near-miss effect in hens **Name of Primary Applicant**: Kiyoteru Takagi **Faculty/School/Department:** School of Psychology **Expected start date**: 20 May 2014 **Expected completion date**: 13 Aug 2015 **Animals species:** Hens (common name) **Number to be used over entire project:** 6 **Impact Level:** Little impact (E.g. No impact, Little impact, Moderate impact. See Q 6 Animal Use Statistics Form – Appendix 1):

Type of Application (Can tick more than one box):	☑ Research ☑ Part of research thesis (Part-time thesis) ☐ Teaching ☐ Other (Specify)
Standard Operating Procedures:	☑ No ☐ Yes: SOP Number/ Title: Was the application approved ☐ No ☐ Yes
Other AEC approval:	Has this application been submitted any other AEC for approval ☑ No ☐ Yes (Specify Committee) Details:
Funding support:	Is this research part of a funding grant either received or pending ☑ No ☐ Yes (Specify funding source) Details:

OFFICE USE ONLY This proposal is approved for the period:	***Protocol Number:***
From: 21 May 2014	**To:** 13 August 2015
Signature AEC Chair:	**Date:** 16/05/14

All research involving the use of animals must comply with the *Animal Welfare Act (1999)* and the University Of Waikato Code Of Ethical Conduct for the Use of Animals in Teaching and Research.

Please submit this form to the Animal Ethics Committee, Research Office, B Block, University of Waikato or email animal.ethics@waikato.ac.nz

APPLICATION

Section1: Personnel Information

1.1 PRIMARY APPLICANT (Researcher or student undertaking thesis)	
Title, first name, last name: Kiyoteru Takagi	
Qualifications: BA (Waikato) Undergraduate diploma in Psychology , PGDip (Psychology)	
Institutional mailing address: School of Psychology University of Waikato	
Email: kiyoterut@gmail.com	**Phone**: 0273220115
Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.	
Previous experience working with hens in PSYC314 in 2012. Training from other Masters/PhD students with the hens.	

1.2 CHIEF SUPERVISOR (WHEN APPLICABLE) TO BE COMPLETED BY THE STUDENT'S SUPERVISOR (IT IS EXPECTED THAT THE SUPERVISOR WILL ASSIST THE STUDENT WITH THE DEVELOPMENT OF THIS APPLICATION)	
Title, first name, last name: Dr Lewis Bizo	
Qualifications: BSc, PGDipSci, PhD(Otago)	
Mailing address: School of Psychology, University of Waikato, Private Bag 3105, Hamilton 3240	
Email: lbizo@waikato.ac.nz	**Phone**: +64 7 838 4466 extn. 6401
What is your Role in this project?	
Supervisor – I will assist the student in planning her research project, data analysis, and in the preparation of her thesis. I will also monitor the student progress and the health of animals, and state of her experiment during weekly meetings. I will not be assisting with the day-to-day handling of animals or data collection.	
Please detail the relevant experience you have (including the number of years) in the procedures/techniques to be used in this project.	
Associate Professor Bizo has over 24 years' experience conducting research with non-human animals, rats, pigeons, hens, and possums specifically. In the past 3 years he has supervised 3 Masters research projects that have used the peak procedure specifically to investigate the timing perception abilities of both hens and possums.	

1.3 OTHER PERSONNEL CONTACT DETAILS MUST ALWAYS BE PROVIDED
(Indicate which personnel are handling and which are watching)

Title, First Name, Last Name	Qualification	Contact details	Role in Project
Ali Cullum	BSc (hons), BVM&S, MACVSc (Avian Health)	027 288 3068	Veterinarian to the Project
Jenny Chandler	BSc, MAScTA	07 838 5568	Animal technician
PhD and Masters students	Completing PhDs and Masters thesis	07 838 5568	Running the experiment/ animal husbandry (feeding and weighing the possums).

Section 2: Project Description

2.1 LAY SUMMARY OF OVERALL PROJECT (one paragraph)
(To be written in terms that people with a non-scientific background will understand)

This project examines the near-miss effect using an experimental preparation with animals. When humans gamble, it has been suggested that near-misses promote higher rates of gambling. The near-miss appears to function as a conditioned reinforcer. In my experiment, I intend to use a technique common in classical conditioning. Hens will be conditioned to associate a stimulus with food. The hens will then be reinforced on two schedules of reinforcement where the reinforcer is usually food. In some conditions, the hens will also receive conditioned reinforcers as well. The conditioned reinforcer will be the stimulus previously conditioned to food. If the conditioned reinforcers function like near-misses, then the hens should respond at higher rate when they receive food and conditioned reinforcers compared to when they just received the food reinforcers.

2.2 AIM OF THE PROJECT
(Brief and written in terms that people with a non-scientific background will understand)

The aim of the project is to investigate whether conditioned reinforcers increase rates of responding on concurrent variable interval (VI VI) schedules of reinforcement.

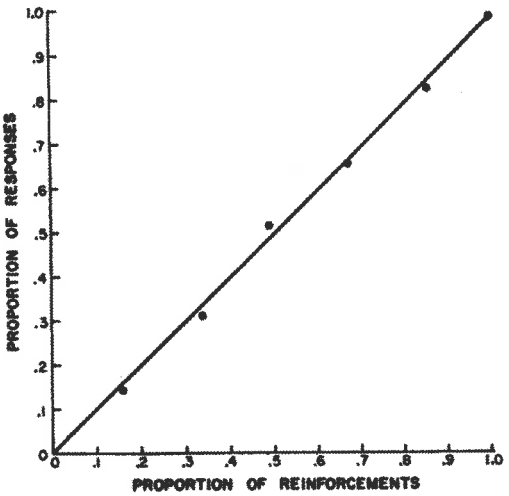
2.3. BACKGROUND
(Include a short review of previous relevant studies in this area and references where appropriate)

Peters, Hunt and Harper (2010) found that the rats responded more at near-miss trials than in losing trials and suggested that this was caused by the near-miss effect. As discussed above, the near-miss effect is the effect by which conditioned reinforcement without primary reinforcement following has an effect on increasing response rates.

Harris and Carpenter (2011) found that the conditioned reinforcement rate matches with the reinforcement rate under the matching law. They studied hens' choice behaviour under concurrent schedules and my study will use the similar procedure.

Similarly, Shahan, Podlesnik, and Jimenez-Gomez (2006) found that the conditioned reinforcement rate correlated with the reinforcement rate while the primary reinforcement rate and value of the conditioned reinforcement remained constant. Hence, these studies suggest that the response rate can be increased when the conditioned reinforcement rate is increased regardless of the rate of primary reinforcement. This supports the idea that the near-miss trials can increase the response rate.

One of the problems with previous research is that the effects have been weak and it has not been clear if the animals really have treated the stimuli associated with near-misses as conditioned reinforcers. The design used by Shahan et al. will be modified to make conditioning of the stimulus associated with near-misses more obvious. I intend to use concurrent VI VI schedules because they generate stable rates of responding. The pattern of responding they generated by concurrent VI VI schedule has previously analysed using the matching law (Herrnstein, 1961). Animal's rate of responding to the two schedules tends to be in the same proportion as the rates of reinforcement. Figure 1 shows the pattern of the results generated by these schedules.



When you vary the rates of reinforcement, such as by adding conditioned reinforcers that should alter the proportion of responding to the two alternatives. If the conditioned reinforcers are not treated by the animals as the reinforcers, response rates should remain unchanged.

References

Harris, J. A., & Carpenter, J. S. (2011). Response rate and reinforcement rate in Pavlovian conditioning. *J Exp Psychol Anim Behav Process, 37*(4), 375-384. doi: 10.1037/a0024554

Herrnstein R. J. (1961). Relative and absolute strength of response as a function of frequency of reinforcement. *Journal of the Experimental Analysis of Behavior, 4,* 267-272.

Peters, H., Hunt, M., & Harper, D. (2010). An animal model of slot machine gambling: the effect of structural characteristics on response latency and persistence. *J Gambl Stud, 26*(4), 521-531. doi: 10.1007/s10899-010-9183-3

Shahan, T. A., Podlesnik, C. A., & Jimenez-Gomez, C. (2006). Matching and Conditioned Reinforcement Rate. *Journal of the Experimental Analysis of Behavior, 85*(2), 167-180. doi: 10.1901/jeab.2006.34-05

2.4 JUSTIFICATION FOR THE PROJECT

a. What are the potential benefits of the research – to humans, animals, or the environment?
The potential benefits of the project would be that it contributes to the wider field of knowledge in behavioural psychology. The findings may help build on previous findings in the study of gambling behaviour.

b. How will the results of this work be disseminated?
The results of this experiment will be used as part of a Masters Thesis. The results may be published in journal articles or presented at conferences.

2.5 DESCRIPTION OF PROCEDURES

(Detailed description of all procedures)

What will happen to the live animals? Give a step-by-step description of all procedures to be carried out on each group of animals. The use of your own flowchart, table or "research design" figure is recommended for complex experiments.

The hens will be housed individually in cages and fed commercial pellets daily to be maintained at 80% of their free feeding body weight. Each hen will be weighed before every experimental session. When required, the hen will be placed in an operant chamber. On one side of the chamber there will be two response keys and a hole underneath to allow access for food via a raised magazine. Hens will initially be taught to peck both of the keys. They will then receive conditioning trials where a condition stimulus (CS), a light, will be paired with food, the unconditioned stimulus (US).
The hens will be trained to respond for food on concurrent VI VI schedule where key pecks are reinforced after some average number of seconds. Table 1 provides an indication of the VI pairs but not in order that they will be run. In some conditions, the hens will only receive food reinforcers, a

couple of seconds of access to wheat. In other conditions, the hens will receive food reinforcers as well as condition reinforcers, where the CS will be presented but not with food. I anticipate that the portion of food to CS reinforcers will be 50:50, however the exact proportion needs to be piloted.

	Left	Right
1	VI 60s	VI 30s
2	VI 45s	VI 45s
3	VI 30s	VI 60s
4	VI 30s	VI 30s
5	VI 60s (half food and half CS)	VI 30s (half food and half CS)
6	VI 45s (half food and half CS)	VI 45s (half food and half CS)
7	VI 30s (half food and half CS)	VI 30s (half food and half CS)

Experimental sessions will last for 1 hour and animals will be fed any supplementary food after experimental sessions.

2.6 DATA COLLECTED AND PROPOSED STATISTICAL ANALYSIS

(Give a clear description of the design of the experiment. Describe the statistical approach that will be used and evidence that the approach can yield answers to the proposed research question.)

This study will be a repeated measures design with the main dependent variables being the rate of responding and the proportion of choice to the two alternatives. This will be analysed using appropriate inferential and descriptive statistics.

2.7 ADDRESSING THE THREE Rs

Replacement, Reduction and Refinement (or the "Three Rs") are the cornerstone for ethical use of animals in research, testing and teaching.

(Please complete all three sections – one to two sentences per section)

2.7.1 Replacement (what alternatives to animal use have you considered e.g. computer modelling)

The nature of this project requires hens or other animal species to be used. There are no possible alternatives for this particular project.

2.7.2 Reduction (what ways do you propose to minimise the use of animals while still keeping the results meaningful)

A minimal number of hens are going to be used in the experiment and the hens will only be working in the operant chamber when necessary, for short periods of time each day, while the experiment is running.

2.7.3 Refinement (how have the procedures been refined to decrease the negative impacts these procedures have on animals e.g. analgesic use, appropriate housing, the skill of those involved in the use and care of the animals).

The condition and general health of the hens is monitored closely on a daily basis. The hens are housed individually with adequate space. There is very limited negative impact from the procedure relating to the project. The students handling the hens are instructed on how to properly care for and handle the hens so that their welfare needs are met.

2.8 ANIMALS TO BE USED IN TEACHING (I.E. UNDERGRADUATE LABS)

If no, proceed section 3

2.8.1	Detail preparation of students for animal use	NA

2.8.2	Detail supervision of students	NA
2.8.3	Detail overall relevant experience of students	NA
2.8.4	Copy of laboratory handout is attached	NA

Section 3: Animals Used and Welfare

3.1 ANIMAL SUMMARY (Please also complete Appendix 1) Species scientific & Common name ONE SPECIES PER LINE		**Strain** (type of animal)	**Species Code (see Q1 of Animal Use Statistics Form Appendix 1)**	**Total number required** (over the life of the project)	**Grading of manipulation (see Q6 of Animal Use Statistics Form Appendix 1)**
1.	Gallus gallus domesticus, hen	Mixed	1t	6	B
2.					
3.					

3.2 WILL ANIMALS BE HOUSED OR HELD (SHORT-TERM OR LONG-TERM)? If no, proceed to 3.3		
3.2.1	Where will the animals be housed?	During the project the hens will be housed individually in cages at The Learning Behaviour Welfare Unit (No. 3 Dairy, Ruakura Rd).
3.2.2	Describe container (dimensions of cages / pens)	The cages are industry standard size and made of durable, strong wire. Each cage allows the hen free access to water via a water tube that runs along the top of the cage.
3.2.3	How many animals per container / enclosure?	One hen per cage, 36 cages per room.
3.2.4	What will be the duration of housing?	The hens will be housed for the duration of the project, approximately 52 weeks.
3.2.5	Who will be responsible for the care of the animals?	Masters and Doctoral students are responsible for attending to the immediate needs of the animals. This includes daily feeding and monitoring. Jenny Chandler, as the animal technician, has overall responsibility for the welfare of the animals housed at Dairy No. 3 and is kept informed about concerns that may arise regarding animal care.

3.3 PRIOR HISTORY OF THE ANIMALS
(If animals are to be used from another project a summary of the type of project, its protocol number, and other information such as the amount of time between projects etc. is to be stated)

The hens being used in this procedure may have previously been used in undergraduate research in PSYC314, protocol 832.

3.4 MANAGEMENT OF ADVERSE EVENTS
(Describe any possible adverse events and how you might manage these. For example, proposed methods of

prevention or control such as regular inspection, analgesic regimes and specified humane end points)
No adverse events are expected during the experiment. Hens will be monitored by PhD and masters students as well as Jenny. If anything adverse happens to the hens then the animal technician will be informed and the vet called.

3.5 FATE OF THE ANIMALS *(What will happen to the animals at the end of the experiment?)*
The hens will remain at No. 3 dairy for future experimental use.

Section 4: Specific Procedures

4.1 INSTITUTIONAL DRUG ADMINISTRATION ORDER **(See Appendix 2)**
Is there an operational procedure required for the use of a product (drug /chemical) in the experiments? If 'yes' this will require an Institutional Drug Administration Order. Name of Product: NA

4.2. USE OF ANAESTHIC **If 'Yes' complete the table below**		
4.2.1	Name of anaesthetic	NA
4.2.2	Local or general	NA
4.2.3	Method of restraint	NA
4.2.4	Will animals have to recover from anaesthetic? How long is the recovery period?	NA
4.2.5	How will you deal with post-operative pain and/or discomfort?	NA

Section 5: Declaration

5.1 PERMITS AND APPROVALS		
5.1.1	Has an application been made to another Committee e.g. Ruakura?	No
5.1.2	Are any DOC permits required?	No
5.1.3	Are any Iwi approvals required?	No
5.1.4	Are any other approvals / permits required?	No

5.2 DECLARATION		CHECK
5.2.1	I have read and understand the conditions outlined in the Code of Ethical Conduct for the Use of Animals for Teaching and Research. http://www.waikato.ac.nz/research/unilink/ethics/animal_ethics.shtml	√
5.2.2	I have read the Good Practice Guide for the Use of Animals in Research, Testing and Teaching http://www.biosecurity.govt.nz/files/regs/animal-welfare/pubs/naeac/guide-for-animals-use.pdf	√
5.2.3	If this application is approved, I will inform the Committee of any changes in the project or unexpected outcomes affecting animal welfare, and any event (beyond any approved manipulation) impacting adversely on animal welfare.	√
5.2.4	I will submit a complete Animal Use Statistics Form by the specified date.	√
5.2.5	I will report as required to the Animal Ethics Committee.	√

Signed by the applicant:

_____ **Date:** 8/5/14

I accept responsibility for this project's compliance with the University's Code of Ethical Conduct for the Use of Animals for Teaching and Research.

Signed by the Chief Supervisor (if applicable):

_____ **Date:** 8/5/14

I accept responsibility for this project's compliance with the University's Code of Ethical Conduct for the Use of Animals for Teaching and Research.

Appendix 1

Animal Use Statistics Form– one species per sheet

Note: Fill in the YELLOW areas now with the number of animals you propose using (this is part of the application form). The BLUE areas are to be filled in after the research has been completed, and a SIGNED hard copy of this form only is to be submitted to the AEC Coordinator by the completion date indicated on page 1 of this application form.

P = Planned to Use AU = Actually Used

1. Animal Type:
Hens 1p

2. Source of Animals:	P	AU
Breeding unit	6	
Commercial		
Farm		
Born during project		
Captured		
Imported into New Zealand		
Public sources		

3. Status of Animals:	P	AU
Normal/conventional	6	
SPF/germ free		
Diseased		
Transgenic/chimera		
Protected species		
Unborn/prehatched		
Other		

4. Purpose:	P	AU
Teaching		
Species conservation		
Environmental management		
Animal husbandry		
Basic biological research	6	
Medical research		
Veterinary research		
Production of biological agents		
Development of alternatives		
Other		

5. Re-use:	P	AU
No prior use	6	
Previously used		

6. Grading:		P	AU
No impact	A		
Little impact	B	6	
Moderate impact	C		
High impact	D		
Very high impact	E		

(see attached grading form)

7. Alive:	P	AU
Retained [by your institution]	6	
Returned [to owner]		
Released [to the wild]		
Disposed of [eg to works or rehomed]		
Total Alive		

8. Dead:	P	AU
Killed for dissection, sampling		
Died/destroyed in the course of manipulation/use		
Euthanased after manipulation or use		
Died/destroyed for reasons not associated with manipulation/use		

Completed by:	Kiyoteru Takagi
Signature:	
Date:	08/05/2014
Protocol No.	

ANIMAL TYPE CODES:

Type of animal used. No distinctions on basis of sex, age, breed, strain or physiological condition.

BOX 1	CODE LETTERS				CODE LETTERS		
Rodents	1	a	Mice	Birds	1	p	Fowls, Chickens
	1	b	Rats		1	q	Pigeons
	1	c	Guinea Pigs		1	r	Other Birds
	1	d	Hamsters	Miscellaneous	1	s	Marine Mammals
Rabbits	1	e	Rabbits		1	t	Possums
Farm Animals	1	f	Sheep		1	u	Reptiles
	1	g	Cattle		1	w	Amphibia
	1	h	Goats		1	x	Fish
	1	j	Deer	Other	1	y	Other Species (°name)
	1	k	Pigs				
Other Domestic	1	m	Horses				
Mammals	1	n	Dogs				
	1	o	Cats				

Grading of Manipulation Examples

Grade A – "No impact or virtually no impact"

Examples:

Mental state: Field observations of grazing behaviour on farms, or benign handling of tame and trained animals that are familiar with all personnel and procedures and with the place where the procedures are conducted.

Food/water: Animals kept outdoors eating their usual food in appropriate amounts; grazing trials on treated pastures; offering supplements to naturally available food; provision of complete, balanced rations to meet all nutritional requirements of animals maintained indoors.

Environmental challenge: Exposure to ambient conditions that are within the thermoneutral range; reduced barometric pressures which do not cause increases in red blood cell production.

Disease/injury/functional impairment: Studies of healthy uninjured animals that are kept in physical conditions which do not themselves lead to injuries such as lameness or compression sores; studies to establish normal characteristics of healthy animals.

Behaviour: Studies of wild or undomesticated animals in their natural habitats; field studies of domesticated animals.

Grade B – "Little impact"

Manipulations of minor impact and short duration

Examples:

Mental state: Experiments on completely anaesthetised animals that do not regain consciousness; simple venipuncture or venisection; injection of non-toxic substances; skin tests which cause low- level irritation without ulceration/erosion; feeding trained animals by orogastric tube; movement of free-range domesticated animals to unfamiliar housing; minor restrictions of water and/or feed intake beyond the normal period of satiation.

Food/water: Water priming for kidney function tests; short-term overall food intake restrictions or excesses that are within usual tolerance levels for the species; short-term changes in dietary composition that cause no clinical signs of deficiency or toxicity, but which would cause such symptoms in the longer term.

Environmental challenge: Exposure to levels of cold or heat that are outside the thermoneutral range, or barometric pressures that increase red blood cell production, but which remain within the capacity of the animals to adapt and do not lead to debility in the long term.

Disease/injury/functional impairment: Studies of vaccines using killed pathogens; tuberculosis tests; induction of mild fever without other debilitating effects; induction of subclinical parasitism; healing of minor superficial incisions, cuts or wounds; minor surgical and/or pharmacological modification of homeostatic capacity (for example, creation of non-obstructive gut fistulae; splenectomy; endocrine gland removal with complete and permanent hormone replacement therapy); physical conditions which cause transient lameness of low intensity, mild compression sores or abrasions.

Behaviour: Mild and short-term physical restraint; keeping free-range domesticated animals in a yard; movement of free-range domesticated livestock to unfamiliar housing; operant conditioning with positive reinforcement in barren laboratory environments; benign preference tests in unnatural surroundings.

Grade C – "Moderate impact"

Manipulations of minor impact and long duration or moderate impact and short duration

Examples:

Mental state: Recovery from major surgeries like thoracotomy, orthopaedic procedures, hysterectomy or gall bladder removal with effective use of analgesics; surgical procedures on conscious animals but with the use of local anaesthesia and systemic analgesic; movement of excitable free-range domesticated livestock to unfamiliar housing; short term capture, handling and restraint of wild or semi-domesticated animals that exhibit marked flight responses; moderate restrictions of water and/or feed intake beyond the normal period of satiation.

Food/water: Simulation of usual overall intake restrictions often experienced by pregnant/lactating ruminants during cold winters or drought; dietary induction of milk fever in cattle; induction of mild deficiency or toxicity signs by feeding diets containing inadequate or excessive amounts of essential nutrients.

Environmental challenge: Short-term exposure to severe extremes of cold or heat which would lead to collapse if prolonged.

Disease/injury/functional impairment: Studies of live vaccines; induction of clinical parasitism; induction of mild reversible infectious diarrhoea; moderate surgical and/or pharmacological modification to homeostatic capacity (for example, limited gut resection; endocrine gland removal with delayed or incomplete hormone replacement therapy); physical conditions that cause minor chronic lameness or other injuries; studies of the effects of infectious or toxic agents that cause rapid death without distress.

Behaviour: Medium-term restrictions of instinctive behaviour; medium-term holding of ruminants in a metabolism crate; long-term restraint leading to the development of reversible stereotypies; changing social group composition.

Grade D – "High impact"

Manipulations of moderate impact and long duration or high impact and short duration

Examples:

Mental state: Recovery from major surgery under anaesthesia without the use of postoperative analgesics; marked social or environmental deprivation; longer term capture, handling, restraint or housing, without the use of tranquilisers, of wild or semi-domesticated animals that exhibit marked flight responses.

Food/water: Dietary induction of advanced pregnancy toxaemia in sheep or ketosis in dairy cattle; dietary induction of advanced signs of nutrient deficiency or excess; severe deleterious effects of dietary toxins; severe restrictions of water and/or feed intake beyond the normal period of satiation.

Environmental challenge: Prolonged exposure to severe cold or heat that would lead to failure of thermoregulation and collapse, but the exposure is terminated just before those outcomes.

Disease/injury/functional impairment: Studies of severe facial eczema; induction of severe diarrhoea or severe infectious pneumonia; protracted or irreversible pharmacological modification of homeostatic capacity (for example, chemical induction of diabetes mellitus without replacement therapy); marked surgical modification of homeostatic capacity (for example, extensive gut resection; cutting of sensory or motor nerves serving large areas of the body from which no self-mutilation injury results; precise lesioning of limited areas of the brain but with intervention before collapse); physical conditions that cause moderate chronic lameness or other injuries; studies of the effects of infectious and toxic agents that cause either a protracted death with minor distress or a rapid death with moderate distress.

Behaviour: Application of marked and repeated noxious stimuli from which escape is impossible; prolonged periods (several hours or more) of close physical restraint; marked alterations to the perceptual or motor functions of animals to test consequent behaviour.

Grade E – "Very high impact"

Manipulations of high impact and long duration

Examples:

Mental state: Conducting major surgeries without the use of anaesthesia on control animals in assessing efficacy of analgesics; testing the efficacy of analgesics in animals with severe induced pain.

Food/water: Experiments that cause animals to die from poisoning by toxins in the diet; protracted and severe restrictions on water and/or feed intake.

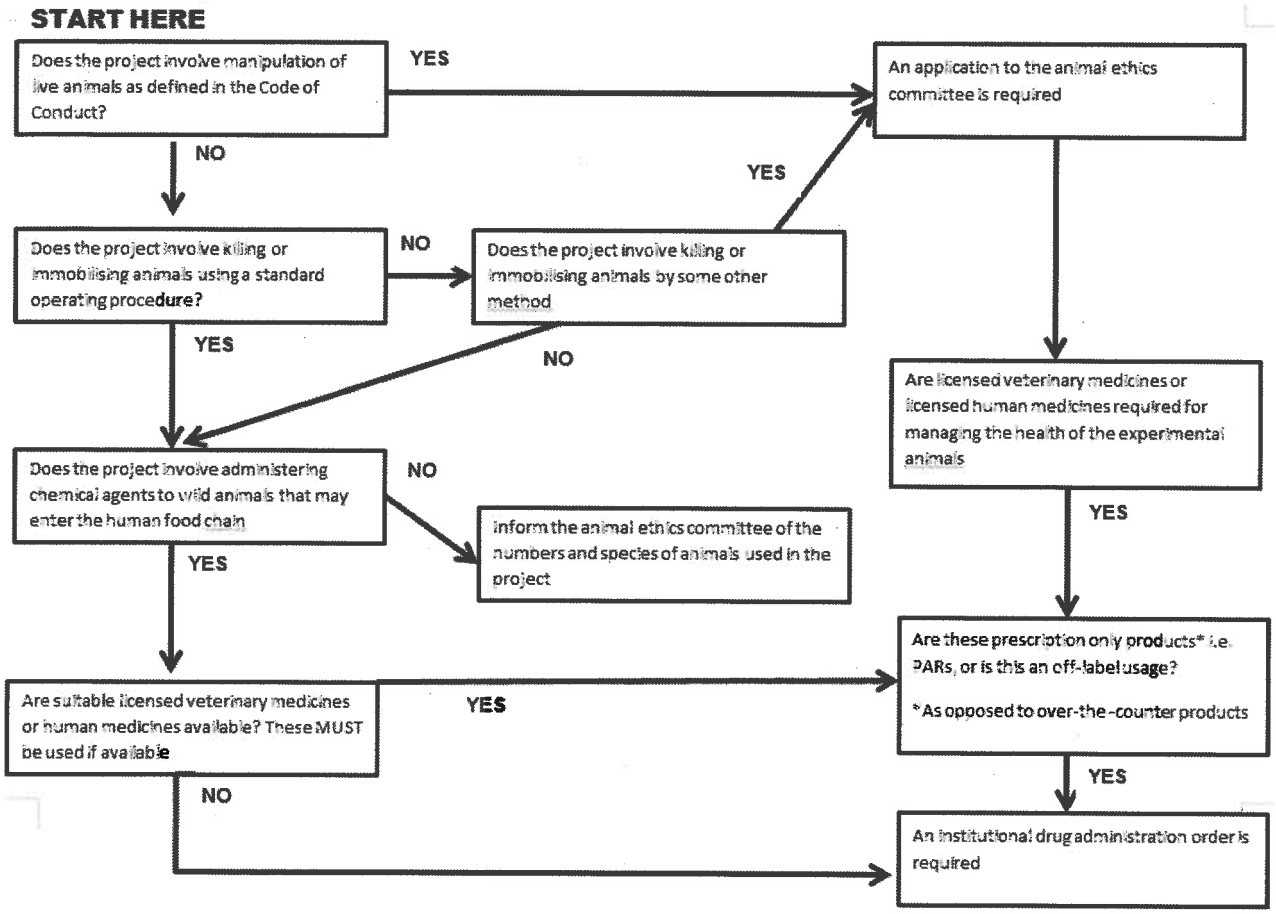
Environmental challenge: Purposeful exposure of conscious animals to lethal extremes of cold, heat or barometric pressure which duplicate naturally occurring conditions.

Disease/injury/functional impairment: Studies of methods for killing pest animals; cutting of sensory or motor nerves serving large areas of the body from which self-mutilation injury results; evaluation of vaccines

where death is the measure of failure to protect; studies of the effects of infectious or toxic agents which cause either a protracted death with marked distress or a rapid death with severe distress.
Behaviour: Application of marked and repeated extremely noxious stimuli from which escape is impossible; prolonged periods (several hours or more) of close physical restraint.

Appendix 2

Is an Institutional Drug Administration Order (IDAO) Required?



If a decision remains unspecified then no further action is required.