Updated By: [redacted] @ 16-Jan-2018 01:16:33 PM

Application Form AE / Bio-Safety v4

Animal Ethics Application Form

1. Before Applying:
In accordance with Part 6 of the Animal Welfare Act 1999, persons using animals or animal tissues for research or teaching purposes need to acquire approval from the University's Animal Ethics Committee (AEC). Any person using animals, or animal tissues, is required to read the regulations governing these activities, as detailed below. Approval may only be granted to persons who can assure the committee that they are familiar with these documents.

2. User Guide:
Please ensure you read the **User Guide** to help guide you through the submission process.

3. Prior to completing the application form:

* Read the 'Before applying for animal ethics approval' and the 'Apply to use animals for research or teaching' sections on the Animal Ethics web page.

* Determine whether approval by the Animal Ethics Committee (AEC) is required using the flowchart (also available on the web page under 'When applying'). If in doubt, contact the Animal Ethics Administrator.

* **All personnel named on the application** must read the following documents (all of which are available on the web page) to understand the legislation with which the University and all investigators must comply.

- The Animal Welfare Act 1999 - Part 6

- The University of Auckland Code of Ethical Conduct

- The NAEAC Good Practice Guide for the Use of Animals in Research, Testing and Teaching

4. What information is required for approval by the Animal Ethics Committee?

The task of the AEC is to ensure the use of animals in research and teaching at the University is justifiable and compliant with the relevant legislation. The AEC members are required to take into account the requirements of the legislation and that consideration has been given to the Three Rs (Refinement, Reduction and Replacement) when animals are manipulated. These requirements acknowledge the fundamental importance of these concepts in the humane use of animals in scientific investigation.

To be able to determine whether the use of animals in your project is both justifiable and compliant with legislation, the AEC needs to understand what the research and/or teaching project comprises of. The AEC therefore requires the following information to be supplied:

* Why the requested type and numbers of animals applied for represents not only the minimum number, but also a large enough number to obtain meaningful results or to meet the proposed research or educational objectives.

* What will be done to the animal before, during and after the project, specifically: -

- What harm or distress will be felt by the animal?

- How can the harm or distress be minimised?

- What measures are to be taken to ensure the general health and welfare of the animals?

* Why this research, or the use of animals in the laboratory classes, is required and why the manipulations to be used are relevant to the objectives of the project?

* Will the research or educational outcomes of the project justify the use of animals?

* Who will perform the experiments and are they able to do this correctly and responsibly?

* Any other matters that are considered by the AEC to be relevant.

Notes:
* It is the **protocol as a whole** that is approved. Approval does not merely represent permission to use a particular manipulation or a certain number of animals.

* All personnel named in the application are required to sign the Animal Ethics Application 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments' of this eform.

Where research is proposed using funds other than those granted following an application round, and the protocol has not been subjected to peer-review and scientific assessment, the committee may need additional information.

5. When filling out the application form:

* Please read all questions and instructions carefully and refer to the User Guide throughout.

* Answer all questions as completely and as clearly as possible. This will help the AEC understand your project and determine whether it can be approved. If the information you provide is incomplete or not clear, your approval might be delayed.

* Please check spelling, grammar and check all sections have been completed.

* Ensure that all relevant appendices (e.g. monitoring sheets) have been attached.

* To prevent any loss, please save your work regularly by clicking on the "save" icon located on the top left corner of this form.

Notes:
* If your response to a question exceeds 4000 characters (including spaces), please (a) write "Refer to attachment" as a response to the question and (b) upload a word document of your response in 'Section G: Attachments'.

* Applicants for whom English is a second language are strongly advised to have their application reviewed by someone whose first language is English.

6. Submitting the application

Applications need to reach the Research Office by 5.00 pm on the closing date advertised on the AEC web page. After you have clicked the 'submit' icon, the application automatically gets routed to the Head of Department. Please allow time for the HOD to view and sign-off before the closing time.

Applications will be pre-screened. This procedure allows the identification of any issues in the application that may prevent or delay its approval by the committee, and will facilitate the approval process. We therefore encourage the early submission of applications.

If you have not received a letter of receipt after 3 days from submitting your application, please contact the Animal Ethics Administrator

Assistance with questions within the application form can be emailed to the Animal Ethics Administrator or by phone on 09 923 6356 (DDI) or ext: 86356 (internal).

General Information

Completion of Application

Research Type:

Select the purpose for this Application: *1. Research

General:

Protocol Number:	001986
Project Title:	Investigations on the role of adenosine receptors in cochlear injury
Responsible Investigator:	[redacted]
Department:	*Audiology

Project Funding - Research:

?☐

1: Please indicate how the project is funded:

Public good or academic research:	100	%
Commercially funded contract:		%
=	100	%[must equal 100]

***2a: Has the proposed work been peer reviewed as part of a successful funding application?**

No

Note: for NIH funding applications our Animal Welfare Assurance Renewal number is #A5014-01.

***2e: The following (University of Auckland) colleague(s) - not named as personnel in this application - could provide local expert comment, if required:**

[redacted]

If the proposed work has not been peer reviewed as a successful funding application, please provide the name of a colleague (who is not involved in the proposed work), so that the AEC can contact him/her if they would like to access any expert views on the proposed work.

Other:

* **3a: Will any Genetically Modified Organisms (animals, cells, bacteria etc.) be used during the manipulations described in this application?**

No

Please indicate from which other bodies approvals or permits for this project are required:

?☐

- ☐ The University of Auckland - Biological Safety Committee
- ☐ The University of Auckland - Human Ethics Committee
- ☐ Another Animal Ethics Committee
- ☐ Department of Conservation
- ☐ Other (please specify)

Section A: UoA Personnel

Personnel - Review

(Add **Personnel - Review**
)

Personnel - Review

Name of UoA Personnel [redacted]

RI	Start Date	End Date	Role	E-Mail:
☑	02-Oct-2017		PI	[redacted]

Certification Begin End
- - Certifications

No response is required for Start Date, End Date and Certifications

***Qualifications:** ?
Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

Yes *Module 2a - Handling, sexing and euthanasia of rodents

Yes *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

***Experience:** ?
[redacted] has 30 years experience in auditory research using guinea pigs, mice and rats. He has experience in the anaesthesia of small rodents, surgical techniques for exposing the cochlea and the assessment of auditory evoked potentials as described in the application. He will oversee the experiments on guinea pigs.

Categories of procedures to be performed:

☑ Manipulation

☑ Monitoring

☑ Euthanasia

Note: All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Personnel - Review

Name of UoA Personnel [redacted]

RI	Start Date	End Date	Role	E-Mail:
☐				[redacted]

Certification Begin End
- - Certifications

No response is required for Start Date, End Date and Certifications

***Qualifications:** ?
Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

Yes *Module 2a - Handling, sexing and euthanasia of rodents

Yes *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

***Experience:** ?
[redacted] has experience with rodent anaesthesia and animal injections and euthansia. She will be involved in euthanising animals and removing tissue for histological analysis.

Categories of procedures to be performed:

☑ Manipulation

☑ Monitoring

☑ Euthanasia

Note: All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Personnel - Review

Name of UoA Personnel [redacted]

RI	Start Date	End Date	Role	E-Mail:
☐				[redacted]

Certification Begin End
- - Certifications

No response is required for Start Date, End Date and Certifications

***Qualifications:** ?
Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

Yes *Module 2a - Handling, sexing and euthanasia of rodents

Yes *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

***Experience:** ?

[redacted] has substantial experience with euthanasia of rats and mice and extraction of tissues for in vitro analysis, having recently completed her PhD in this area. She will be involved in euthanising mice and removing tissue for histological analysis.

Categories of procedures to be performed:

☑ Manipulation

☑ Monitoring

☑ Euthanasia

Note: All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Personnel - Review

Name of UoA Personnel [redacted]

RI	Start Date	End Date	Role	E-Mail:
☐				[redacted]

Certification Begin End	
- -	Certifications

No response is required for Start Date, End Date and Certifications

***Qualifications:** ?
Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

Yes *Module 2a - Handling, sexing and euthanasia of rodents

Yes *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

***Experience:** ?

[redacted] is a pharmacologist. He has substantial experience with small animal surgery and anaesthesia (guinea pigs, rats and mice), animal injections and euthanasia. He has worked for several years with the auditory group. He has undertaken surgeries to insert cochlear implants into guinea pigs, and has conducted the same procedures for noise exposures and auditory assessments as described in this application.

Categories of procedures to be performed:

☑ Manipulation

☑ Monitoring

☑ Euthanasia

Note: All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Personnel - Review

Name of UoA Personnel [redacted]

RI	Start Date	End Date	Role	E-Mail:
☐			Co-Inv	[redacted]

Certification Begin End	
- -	Certifications

No response is required for Start Date, End Date and Certifications

***Qualifications:** ?
Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

Yes *Module 2a - Handling, sexing and euthanasia of rodents

Yes *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

***Experience:** ?

[redacted] is the co-PI. He has 23 years of experience in many of the proposed techniques. He has experience in small animal anesthesia and euthanasia, and in the assessment of hearing using evoked potentials. He will co-supervise the studies and particularly oversee the mouse and rat studies described in the application

Categories of procedures to be performed:

☑ Manipulation

☑ Monitoring

☑ Euthanasia

Note: All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Section A: non-UoA Personnel

Instructions:

Please use this section only for the addition of UoA Personnel not found in the HR List or for Personnel outside of the University:
1. Please click on the yellow + icon
2. Complete all the requirements for that person

3. The 3 checkboxes prefixed by the # icon is in reference to "Categories of procedures performed" and is to be interpreted as follows:

#Man = Manipulation; #Mon = Monitoring; #Eut = Euthanasia

4. The 3 questions prefixed by the * icon is in reference to completion of training and is to be interpreted as follows:

*Mod 1 = Module 1 - Legislation
*Mod 2a = Module 2a - Handling, sexing and euthanasia of rodents
*Mod 2b = Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs
In the event that the response to the applicable questions above is "no", please refer to the Animal Ethics website for details of how to register for these Modules.

5. Under 'Experience', please describe the experience this person has with the technique/s described in the approved protocol. If this person has no experience, nominate the Supervisor who will provide the training. Where there is no appropriate supervision available, the Animal Welfare Officer will arrange training and supervision.

6. All personnel named in the application are required to sign the 'Details of Personnel' form. This can be downloaded here. Once all the personnel have read and signed this form, the document needs to be uploaded into 'Section G: Attachments'.

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form

UoA Personnel not found in the HR List or addition of non UoA Personnel:

First & Last Name:	Email:	Role:	Qualifications:	Experience:	#Man	#Mon	#Eut	*Mod 1	*Mod 2a	*Mod 2b
					☐	☐	☐			

Section B: Description

B.1: Protocol Number:
001986

***B.2: Project Title:**
Investigations on the role of adenosine receptors in cochlear injury

***B.3: Lay Summary:**
These are a series of studies around the ongoing injury which develops in the cochlea of the inner ear following a number of insults, such as noise exposure, ototoxic drugs and insertion of a cochlear implant. They particularly aim to understand how a naturally occurring compound in the ear, adenosine, may be a regulator of injury. Some studies will be undertaken to look at how adenosine may regulate injury at the nerve synapse (connection with the sensory cell) and death of the sensory cells. Another set of studies will focus on how to reduce the effect of surgical trauma and ongoing tissue reaction to the presence of a cochlear implant in the inner ear and as a result how to protect the inner ear during cochlear implant surgery. A cochlear implant is a wonderful invention that has brought hearing to over 400,000 people worldwide. It is a device that is placed in the cochlea to electrically stimulate auditory nerves after the sensory cells have died. However, during surgery the ear becomes inflamed and thus causes more damage which can result in further injury and death of nerve fibres because of scar formation around the electrode. This can limit the benefits of the implant. In an attempt to reduce the injury during surgery we will investigate the nature, timing and molecular basis of the inflammatory response. We will then look at whether promising protective, anti-inflammatory drugs delivered by microscopic particles and inserted into the cochlea during the implant surgery can prevent or reduce the inflammation and the ongoing cell death. These studies collectively will give us new information about mechanisms of neural cell death in the cochlea and ways to protect the ear and thereby enhance the outcomes for cochlear implant patients, and, potentially, various cochlear diseases.

***B.4: Describe why animals are needed for this project:**
These studies include in vitro and in vivo experiments. Some can be undertaken using extracted ear tissues but those using cochlear implants need to be undertaken in an intact animal system that mimics the human clinical scenario, so that the inflammatory and neurodegenerative processes as well as the neuroprotective potential of drugs can be assessed. We need to be able to follow the inflammatory and degenerative and protective processes over time and this can be only undertaken in an animal. We will use these data to inform clinical studies, which would enable them to be transferred to human subjects at some stage.

***B.5: Describe how the experimental findings will be used, promoted or published at the conclusion of the study:**
Findings obtained from this study will be disseminated through publications in international scientific and clinical journals along with presentations at national and international
scientific conferences and local departmental seminars and talks.

***B.6a: Are the experiments an extension of previous animal studies from your laboratory?**
Yes

***B.6b: Please provide the AEC reference number of the previously approved study:**
AEC 1376: Expires Oct 2017, no final report yet
AEC 871: Final report submitted
AEC 1339: No final report yet

***B.6c: Has an End of Approval report of the previous study been submitted to the AEC yet?**
Yes

***B.7a: Do these experiments repeat work performed by you or others?**
No

Section C: Animal Use

Species and Strain

Manipulations
The term manipulation is defined in the Animal Welfare Act 1999 as follows: '...manipulation, in relation to an animal, means, (...) interfering with the normal physiological, behavioural or anatomical integrity of the animal...'. This includes dietary manipulations, catching and handling wild animals, observational studies which interfere with the normal behaviour, etc. For the complete definition, please read the relevant sections of the Act.

Tissue Collection only:
Animals used for tissue collection only will not undergo any type of manipulation, but are killed directly in order to undertake research or teaching on the dead animal or on the tissue of the animal.

Note that animals that have been manipulated and then had tissues taken, should be included in the 'manipulation' section only - **not** under tissue collection.

One of the duties of the Animal Ethics Committee is to determine that the research is completed with the minimum number of animals being used, while still enabling meaningful outcome to be gained. This should be considered when filling out the application form.

Consideration should also be given to adding a number of extra animals as a contingency for developing the techniques to be used, unforeseen deaths, etc.

If required, state an age range, rather than a specific age, to prevent limitation of the experiments (i.e. if you specify you will use 10 week old rats, you will need to request an amendment if the rats are 11 weeks old).

State a weight range, rather than a specific weight, to prevent limitation of the experiments (i.e. if you specify you will use 350 grams rats, you will need to request an amendment if the rats are 340 grams).

Note: For your response to the "Species and Strains to be used" question below, please note the following:
- 'Tissue Collection' is used when describing animals that are being used for no other reason than 'tissue collection'
- Manipulation and Tissue Collection (Tissues to be collected at a later stage) is to be described as 'Manipulation'
- 'Both' is to be selected when 'some' animals are being manipulated and 'some' are being used for tissue collection.
- If you are using a capturing method, please enter a 0 (ZERO) under the 'Tot. No. req'd for Project' field below, and enter a 0 (ZERO) in the appropriate boxes of the Animal Usage Statistics page (later in this eForm).

Species and Strains to be used:
IMPORTANT: Please click on the yellow '+' icon to add a species or strain. Fill out the details for each species and/or strain.

Species:	Strain:	Usage:	Tot. No. req'd for Project	Sex:	Age Range:	Weight Range:
1c - Guinea pigs	Duncan Hartley	Both	102	Either	4-6wks	300-500g
1b - Rats	Wistar	Tissue Collection only	85	Either	P3-P6	
1a - Mice	C57BL/6	Tissue Collection only	25	Either	P3-P6	

Please justify species selection:

For each of the species and strains listed, please explain why you need to use this specific species and strain of animal i.e. explain why this animal is appropriate on scientific, technical, humanitarian and/or educational grounds for the procedures proposed.
The guinea-pig is the animal generally used for the cochlear implant studies because it has a large middle ear and a large and accessible cochlea enabling the surgery for cochlear implant insertion. It is possible to measure cochlear electrical potentials as an index of function during insertion and to measure the physiological responses from the cochlear implant. Also our collaborators in Australia use this as the animal model of choice for their implant studies that will be the basis of our studies.

Rat pups will be used in our tissue culture studies to determine the effect of adenosine receptor agonists on the development of excitotoxic injury in cochlear explants. We are using the NMDA/kainate model of excitotoxicity developed in rats.

Mice will be used in our tissue culture studies to determine the effect of adenosine receptor agonists on cochlear survival after ototoxic injury. We have developed ototoxic model in mice using an aminoglycoside antibiotic neomycin.

If your response to this question exceeds 4000 characters (including spaces), please (a) write "Refer to attachment" in the field above and (b) upload a word document of your response in 'Section G: Attachments'.

AFM Approval

* **If the animals are being obtained from, or are being housed in an animal facility, has the facility manager confirmed in writing that the proposal can be accommodated within the resources of the facility?**
Before submitting this application, please obtain confirmation from the facility manager, as this is a requirement of the AEC. The AEC will not approve an application unless written confirmation has been received. Please upload the written confirmation into section G.

Yes

Section C con't:
Animal Usage Statistics

Animals used for Manipulations/Tissue Collection
- Instructions

Complete all nine sub-sections by writing the **number** of animals in the appropriate category (e.g. if you use ten rats from the animal facility unit and ten imported rats, you would write a 10 in the 'Breeding unit' and also in the 'Imported' box, which makes the total for sub-section 'Source of animals' 20). If the rats are all normal / conventional, write a 20 in that box for 'Status of animals', etc. Choose only one category for each animal (e.g. all twenty animals are used for 'Species conservation' or for 'Basic biological research' you cannot fill out 20 in both categories).

Make sure the total number per sub-section is the same.

Please make an overall estimate of the severity or invasiveness of the manipulation that is used on each animal. Take into account the effect of any anaesthetic, analgesic, euthanasia technique, and/or any other strategy or practice that is applied or used, and/or any other step taken to avoid or alleviate the stress or pain caused to the animal. Select which of the five grades (A, B, C, D, E) best describes the severity of the proposed manipulation, using the examples below:

Grade:	**Welfare Impact:**	**Expected maximum impact of manipulations(s) - Pain/Distress**
A	No Impact or virtually no impact	None
B	Low	Low, brief
C	Moderate	Alleviated
D	High	High
E	Very high	Severe

These examples are not exhaustive or definitive

* **Box 7 and 8** Please fill in the status of the animal at the end of the project, according to the following guidelines:

- In the animals will be alive, fill in box 7, indicating what their fate will be.
- If the animals will be dead, fill out box 8 only.
- The sum of box 7 and 8 should always be the same as the total in box 9.

Note:
Grading the manipulation(s) clearly requires a value judgement to be made by the applicant. If in doubt, please contact the Animal Welfare Officer to discuss the manipulations and their grading. The selected grading will be verified, or may be amended, by the AEC. The experience of the investigator, and the quality of the environment in which the manipulations are carried out, may alter the grading that is selected; this needs to be kept in mind.

The National Animal Ethics Advisory Committee (NAEAC) understands that some inconsistencies may occur when those judgements are made. What is expected is that an honest assessment will be made.

If some animals of a species are being used for manipulation (with or without additional tissue collection) and some are being used for tissue collection **only**, two Animal Usage Statistics Forms need to be completed. In this case, please click on the yellow '+' icon twice (where instructed) to add the two Usage Types for that Species.

☐ **Please check this box for additional instructions on the requirements and use of the Animal Usage Statistics Forms**

Animal Usage Statistics

Species section(Add Species section)

Species section

1c - Guinea pigs

☐
☐
☐

Note: Admin use only! Please ignore the fields above.

*** 1. Please select the Usage Type for this Species:** Manipulation

2. Source of Animals:	Number:
Breeding Unit	102
Commercial	
Farm	
Born during project	
Captured	
Imported into NZ	
Public sources	
TOTAL:	*102

3. Status of animals:	Number
Normal/Conventional	102
SPF/germ free	
Diseased	
Transgenic/Chimera	
Protected Species	
Unborn/prehatched*	
Other	

4. Purpose:	Number:
Teaching	
Species conservation	
Environmental management	
Animal husbandry	
Basic biological research	
Medical research	102
Veterinary research	
Testing	
Production of biological agents	
Development of alternatives	
Other	

5. Re-Use:	Number:
No prior use	102
Previously used	

6. Grading:	Number:
No impact - A	
Little impact - B	
Moderate impact - C	102
High impact - D	
Very high impact - E	

7. Alive:	Number:
Retained [by institution]	
Returned [to owner]	
Released [to the wild]	
Disposed [to works or rehomed]	
Total Alive:	*0

8. Dead:	Number:
Total Dead	*102

9. Total manipulated/used:	*102

Notes:

* The Animal Welfare Act (1999) describes pre-natal stages as 'any mammalian foetus, or any avian or reptilian pre-hatched young, that is in the last half of its period of gestation or development; the definition includes any marsupial pouch young'. This means that the mothers and young are required to be added as separate groups in Table 3. The young will have the status 'Unborn/prehatched' box, and the mothers whichever status is appropriate with reference from the AWA to the stages which are specifically excluded e.g. larval stages.

IMPORTANT:

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Species section

1b - Rats

☐
☐
☐

Note: Admin use only! Please ignore the fields above.

*** 1. Please select the Usage Type for this Species:** Tissue Collection Only

2. Source of Animals:	Number:
Breeding Unit	85
Commercial	
Farm	
Born during project	
Captured	
Imported into NZ	
Public sources	
TOTAL:	*85

3. Status of animals:	Number
Normal/Conventional	85

5. Re-Use:	Number:
No prior use	85
Previously used	

6. Grading:	Number:
No impact - A	
Little impact - B	85
Moderate impact - C	
High impact - D	
Very high impact - E	

7. Alive:	Number:

SPF/germ free		Retained [by institution]	
Diseased		Returned [to owner]	
Transgenic/Chimera		Released [to the wild]	
Protected Species		Disposed [to works or rehomed]	
Unborn/prehatched*		Total Alive:	*0
Other			

4. Purpose:	Number:	**8. Dead:**	Number:
Teaching		Total Dead	*85
Species conservation			
Environmental management		**9. Total manipulated/used:**	*85
Animal husbandry			
Basic biological research			
Medical research	85		
Veterinary research			
Testing			
Production of biological agents			
Development of alternatives			
Other			

Notes:

* The Animal Welfare Act (1999) describes pre-natal stages as 'any mammalian foetus, or any avian or reptilian pre-hatched young, that is in the last half of its period of gestation or development; the definition includes any marsupial pouch young'. This means that the mothers and young are required to be added as separate groups in Table 3. The young will have the status 'Unborn/prehatched' box, and the mothers whichever status is appropriate with reference from the AWA to the stages which are specifically excluded e.g. larval stages.

IMPORTANT:

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Species section

1a - Mice

☐
☐
☐

Note: Admin use only! Please ignore the fields above.

*** 1. Please select the Usage Type for this Species:** Tissue Collection Only

2. Source of Animals:	Number:	**5. Re-Use:**	Number:
Breeding Unit	25	No prior use	25
Commercial		Previously used	
Farm			
Born during project		**6. Grading:**	Number:
Captured		No impact - A	
Imported into NZ		Little impact - B	25
Public sources		Moderate impact - C	
TOTAL:	*25	High impact - D	
		Very high impact - E	

3. Status of animals:	Number	**7. Alive:**	Number:
Normal/Conventional	25	Retained [by institution]	
SPF/germ free		Returned [to owner]	
Diseased		Released [to the wild]	
Transgenic/Chimera		Disposed [to works or rehomed]	
Protected Species		Total Alive:	*0
Unborn/prehatched*			
Other			

4. Purpose:	Number:	**8. Dead:**	Number:
Teaching		Total Dead	*25
Species conservation			
Environmental management		**9. Total manipulated/used:**	*25
Animal husbandry			
Basic biological research			
Medical research	25		
Veterinary research			
Testing			
Production of biological agents			
Development of alternatives			
Other			

Notes:

* The Animal Welfare Act (1999) describes pre-natal stages as 'any mammalian foetus, or any avian or reptilian pre-hatched young, that is in the last half of its period of gestation or development; the definition includes any marsupial pouch young'. This means that the mothers and young are required to be added as separate groups in Table 3. The young will have the

status 'Unborn/prehatched' box, and the mothers whichever status is appropriate with reference from the AWA to the stages which are specifically excluded e.g. larval stages.

IMPORTANT:

Note: To prevent any loss, please save your work regularly by clicking on the 'save' icon located at the top left corner of this application form!

Section D: Scientific description of the project

***D.1: The aim of the experiments:**

This is a programme of 3 studies on the role of adenosine receptors in cochlear neural and sensory cell injury that underpins hearing loss, and the development of pharmacological therapies. These will be undertaken in different animal models (guinea pigs, rats and mice) because of needs for in vitro (rats and mice) and in vivo (guinea pig) preparations.

In Study 1 we will look at the role of A1 receptors on the survival of sensory cells after aminoglycoside antibiotic injury. Our previous work has shown that adenosine has a profound protective effect on sensory cell degeneration (in vitro) after neomycin insult. We speculate this is due to an action on A1 receptors and here we will pharmacologically enhance A1R activity [redacted]) or block A1R or A2AR activity [redacted] respectively), to test this hypothesis in neonatal mouse cochlear explants.

In Study 2 we will specifically look at the effect of adenosine receptors on the development of synaptopathy and excitotoxic injury in rat cochlear explants. Conditions like noise exposure cause excess glutamate release at cochlear afferent synapses and given A1R are expressed at the synapse, and in the brain adenosine regulates glutamate release, we postulate that manipulation of A1R may protect the synapse from glutamate excitotoxicity. Rat pups will be used as this glutamate excitotoxicity model has been developed in rats previously (Wang, 2011). Here we will induce excitotoxicity in cochlear explants using kainic acid, as previously described, and test the effect on synapse morphology of different adenosine receptor agonists [redacted]) and antagonists [redacted]) on the development of excitotoxic injury in cochlear explants.

The third study (Study 3) builds on a series of studies (undertaken under AEC1376) which demonstrated that an Adenosine A2A receptor agonist ([redacted]), introduced within nanoparticles inserted into the cochlea during surgery, improves viability of cochlear function following cochlear implantation in our guinea pig model. Cochlear implants have previously only been used with profoundly deaf subjects but are now available for those with severe forms of deafness where there is still some residual hearing. However, implantation causes degeneration of these residual sensory cells and neurons and methods need to be developed to protect the cochlear tissues during the surgery. Our studies are promising as they show some degree of protection to hair cells during surgery. We postulate that the compounds are affecting the course of inflammation and reducing the extent and effect of the tissue foreign body response and the degree of fibrosis that occurs around the electrode. Here we intend to undertake a series of studies to further investigate the inflammatory response.

Aim 1: we will use nanoparticles (that form supraparticles ~400µm), engineered to bind and slowly release otoprotective compounds into the cochlea. We will examine the dose response of these compounds that can reduce the injury and improve the long-term survival of sensory hair cells and spiral ganglion neurons in the deafened cochlea after insertion of a dummy (non-functional) cochlear implant and thereby preserving residual hearing. The primary compound of interest is the A2A receptor agonist [redacted], but we will also look at combining this with an A1 receptor agonist ([redacted] or [redacted] which enhances A1R activity to endogenous adenosine) compounds are Adenosine Receptor (A1 and A2) agonists (eg [redacted],) we have shown to be otoprotective and neurotrophic factors (BDNF) to enhance recovery of neural injury.

Aim 2: here we will investigate the inflammatory response and development of fibrosis in the cochlea using MRI and µCT and the effect of the anti-inflammatory effect of the A2A receptor agonist [redacted]) on the extent of fibrosis and the cochlea inflammation.

[sections 9(2)(b)(ii), 9(2)(i)]

*** D.2: The design of the experiments: (max 4000 characters)**

See supplementary attachment for full details and explanations

Study 1: Effect of adenosine receptor agonists and antagonists on cochlear survival after ototoxic injury: Exposure to neomycin causes sensory cell death and here we will further investigate the role of adenosine receptor manipulation on this injury. Mouse pups (P3-P6) will be killed by decapitation. Cochlear explants will then be pre-incubated for 19 hours with [redacted] or [redacted] and exposed to neomycin (1 mM) for 3 h at 37°C. Explants will be incubated for further 19 hours and then fixed with 4% PFA. Quantitative histology will determine the survival rate of hair cells in whole mounts of the organ of Corti. Number of mice required = 25 (Grade B, tissue collection). The number of animals required determined by number of compounds (4) + vehicle

control x 10 cochleae per group = 50 cochleae (25 mice).

Study 2: Effects of adenosine receptor agonists [redacted]) and antagonists [redacted]) on excitotoxic injury in cochlear explants: In all studies we will use organotypic tissue cultures of Wistar rat cochlea at postnatal day 3-6 (P3-P6) as described previously (Wang, 2011). Cochlear explants will be exposed to glutamate receptor agonists (NMDA and kainic acid) for 2 hrs to mimic glutamate excitotoxic neuronal injury. After treatment with agonists and antagonists tissue will be fixed and the effect on neuronal death measured histologically. The number of animals required for this study is 80 rats (Grade B, tissue collection), based on four experimental groups (3 drug-treated and a vehicle-treated) x 20 animals/group. Additional 5 rat pups are required to establish the NK model of excitotoxicity in our lab. This number has been determined from our previous experience in cochlear organotypic cultures.

[sections 9(2)(b)(ii), 9(2)(i)]

Study 3: Stemming inflammation and fibrosis after cochlear implantation
Aim 1. Protection of hearing: Guinea pigs will be unilaterally implanted with a dummy implant along with supraparticles containing different concentrations of [redacted] 2 weeks after deafening (noise exposure, 16kHz, 2hr, 120dBSPL). Function will be measured before and up to 8 weeks later and histology will determine survival of hair cells and neurons at 8 weeks. The opposite will serve as a non implanted control. Another group of animals will be implanted along with empty nanoparticles as a drug delivery control. Numbers: 6 in each group x 4 groups (3 concentrations and 1 control): 24 animals. The A2A agonist (at concentration with maximum effect from above) will be used alone or in combination with a neurotrophin (BDNF) or another compound ([redacted]). After noise exposure deafening procedure, animals will be bilaterally implanted with supraparticles and one ear will also receive a dummy implant. Function will be measured each week for 8 weeks, and histology will determine survival of hair cells and neurons at 8 weeks. Numbers: 6 in each group x 4 groups (3 combinations and 1 control): 24 animals
Aim 2. Effect on Fibrosis: Guinea pigs will be unilaterally implanted with a dummy implant along with supraparticles containing [redacted](3 different concentrations as above) 2 weeks after deafening. Two separate measures will determine the anti-inflammatory action of the [redacted] on the cochlea. (1) Vascular permeability will be assessed by Dynamic Contrast MRI to determine timepoints of the inflammatory response. (2) Fibrosis in the cochlea will be quantified and characterised using µCT and histology. Animals used for MRI will be euthanised at 14 or 28 days after the introduction of the cochlear implant. Tissue will be taken for histology (n=6 at each interval) or µCT (n=6 at each interval). Six additional animals are requested in case of surgical mishaps during the implantation procedure. Numbers: 54

Note: If required, please attach any supplementary information on the experimental design, including any tables, flowcharts, pictures, etc which would assist the AEC in assessing this application, and upload to Section G.

***D.3a: Will the animals be captured in the wild?**
No

Manipulation:

***D.4a: Please describe the following for each surgical or non-surgical manipulation that the animals will undergo: i.e. drug in drinking water, injection or dietary supplementation (max 4000 characters) :** ?☐
Procedures for Study 3. See Appendix (Additional information) for details on manipulations
Cochlear function: Auditory Brainstem Responses (ABRs) will be obtained by placing 3 fine subdermal needle electrodes in the scalp under anaesthesia (see IDAO). Sound (tone-pips at 4 – 40 kHz) will be delivered to the ear at varying intensities and the threshold of the response will be assessed. Distortion product otoacoustic emissions (DPOAE) analysis (2f1-f2) measures the activity of the outer hair cells. Two pure tone stimuli (f1 and f2) will be delivered to the auditory canal via paired electrostatic ear probe speakers and the threshold of the emitted cubic disotortion product (2f1 – f2), detected via a microphone ear probe will be determined. The duration of these procedures is 45 – 60 minutes and the animals recover quickly afterward.

Noise exposure:
These will be carried out in a custom-built acoustic chamber with internal speakers and external electronic controls. Guinea-pigs will be anaesthetised (see IDAO) and exposed unilaterally or bilaterally to a pure tone (16 kHz) for 30 min at 120 dB SPL in a closed-field sound delivery system. We have developed this model in our laboratory and together with our collaborators at the Bionics Institute, Melbourne achieves symmetrical, high frequency hearing loss as assessed by ABR.

Cochlear Implantation and insertion of the supraparticles:

Guinea pigs will be anesthetised (see IDAO) and placed on a heating blanket. An ABR will be recorded to check the level of hearing loss before surgery to implant the electrode and supraparticles. Surgery will be performed under aseptic conditions, supplemental doses of anaesthesia administered during surgery at a level sufficient to maintain the animal in an areflexic state and temperature will be maintained at 37°C using a heating pad. A small incision will be made in the skin overlying the dorsal region of the auditory bulla to expose the middle ear and cochlea. The cochlea round window and basal turn will be visualised and a small hole (cochleostomy) will be made in the bone of the cochlea posterior to the round window and the sterile dummy electrode array inserted approximately 5-6 mm into the scala tympani of the cochlea. The cochleostomy will be sealed with muscle tissue, and the protruding part of the electrode array fixed to the bone using dental cement. The surgical hole in the auditory bulla will then be closed with the piece of bone removed to make the opening and dental cement, and the skin wound sutured. Each animal will be given sterile saline solution (15ml/kg/hr sc/ip) and allowed to recover from the anaesthetic and monitored during recovery. They will be treated with analgesics and broad-spectrum antibiotics (see IDAO). Surgery will be performed on one or both ears, with one side acting as the experimental ear and the other the deafened control (untreated or with an implant but no supraparticles).

Magnetic resonance imaging (used in Aim 2)
Guinea-pigs will be anaesthetised (see IDAO) and body temperature maintained at 37°C by circulating warm air through the magnet bore. The guinea-pigs' heart and respiration rate, and body temperature will be monitored throughout the scan which takes approximately one hour and the animal will then be allowed to recover. Repeated scans will be undertaken over a period of several weeks after the electrode and supraparticle insertion.

* **D.4b: The extent and duration of the manipulation(s):** ?☐
See above

* **D.4c: The extent to which the animals may experience pain or distress during or after any of the manipulations, and which signs may be seen:** ?☐
From our experience, guinea-pigs recover soon from the noise exposures within 24 hrs.
Animals recover well from the cochlear surgery and show signs of pain associated with the surgery which can be mitigated by appropriate pain management (see below) and observation over the first 24 hrs of recovery.

* **D.4d: Please explain why this extent of pain or distress is unavoidable:**
It is essential to implant the electrode into the cochlea using surgical techniques that mimic those used in human surgery. We need to also produce a hearing loss which produces cochlear injury similar to the situation in humans and the use of high intensity exposures has been shown to deliver the appropriate amount to high frequency hearing loss in guinea-pigs.

* **D.4e: Describe the pain management plan that has been developed for the alleviation of pain:** ?☐
The animals will be given an analgesic (see IDAO) before and after the surgery and will be given analgesia for 3 days following the procedure. An assessment will also be made at this time to see if further analgesia is required

* **D.4f: Detail the post manipulation care and/or any special housing needs:**
Animal behaviour will be carefully monitored during all manipulations and will recover from anaesthesia in a warm environment and observed until they are fully awake (2-3 hours). They will be administered sterile saline (sc/ip) after the surgery to reduce the effects of dehydration. No special housing is required.

* **D.4g: Explain the monitoring procedures and contingencies that will be in place to detect and limit signs of pain or distress:** ?☐
Animal welfare will be monitored in the days following all manipulations as per our standard welfare monitoring sheet. Any animal showing signs of distress, including dehydration and weight loss exceeding 15% body weight of starting weight, will be removed from the study and euthanised. We will treat animals for pain by analgesics as per IDAO and subcutaneous injections of saline solution for dehydration as described earlier.

* **D.4h: Describe the humane endpoints that will be applied if applicable i.e. specific clinical signs being shown by an animal that will require its immediate euthanasia.** ?☐
The animals are weighed before any manipulation (i.e. ABR, CI surgery, MRI expt) and then weighed daily for next 10 days if it has been operated on (i.e. CI and MRI expt). Any animal showing signs of distress and weight loss exceeding 15% body weight from the starting weight will be removed from the study and euthanised. We have a welfare monitoring sheet for each such animal and observations noted.

* **D.5a: Will the animals undergo any new manipulations not described in previous applications you have submitted to the Committee?**
No

* **D.6a: Will neuromuscular blockade be used?**
No

*** D.7a: Will the animals be killed at any stage during the experiment?**
Yes

D.7b: Please select the technique/s used:

☑ Anaesthetic overdose	☐ Blunt trauma	☐ Captive Bolt
☐ Cervical dislocation	☑ Decapitation	☐ Exsanguination
☐ Injection	☐ Pithing	☐ Poisoning
☐ Sharp trauma	☐ Snap traps	☐ Chilling in ice water
☐ Intracardiac injection of potassium citrate	☐ CO2	☐ Other

Methods of euthanasia should be selected with reference to the ANZCAART Guidelines. A copy of this is also available on the Animal Ethics web page. If a prescription drug is to be used, please state that "animals will be euthanized as per the IDAO", complete an IDAO form and attach it to 'Section G: Attachments'.

*** D.8: For the animals that are killed for tissue collection only, have other researchers that may be interested in the other tissues been notified?**
No

*** D.9: Describe any other animal welfare or ethical implications of this project:**
We are not aware of any welfare or ethical implications, other than those stated previously in this application.

***D.10: Describe why the nominated number of animals is needed:**
The animal numbers for Studies 1 and 2 are calculated to achieve a statistically significant result with sensory cell loss ($p<0.05$) on the basis of our previous studies using in vitro cultures and neomycin antibiotics. For study 3 we have calculated the numbers of animals based on our previous experience to achieve a 15dB improvement (a clinically acceptable result) in hearing thresholds with [redacted]. We have included a small contingency (~10%) because of potential issues with post-surgical complications and repeat anaesthesia.

[sections 9(2)(b)(ii), 9(2)(i)]

D.11: Detail where the experiments will be conducted:

	Building:	Room Number:
a. before experimentation	[redacted]	[redacted]
b. during manipulation		
c. after manipulation		
d. disposal		

*** D.12: Estimated period of housing per animal:**
The animals will be housed for 3 days to 10 weeks.

D.13: Describe how this study has taken into account the purpose of the Animal Welfare Act 1999 to promote the principles of Refinement, Reduction and Replacement that govern the use of animals in research, testing and teaching (the 3 R's). In particular, describe the extent to which you have:

(i) assessed the possibility of using non-sentient or non-living alternatives in the project; and
(ii) replaced animals as subjects with suitable non-sentient or non-living alternatives where possible; and
(iii) identify the sources you have used to make the assessment under (i), and the methods you have used to consider any replacement under (ii) - e.g. Animal Welfare Information Centre, www.nc3rs.org.uk or internet search engines.

***a. Refinement:**
The effect of cochlear implantation and resulting inflammatory processes can only be studied in living animals, which mimic the acute and chronic time course of the inflammatory processes that occur in humans, as well as the systemic reaction to the presence of a foreign body tissue response. We utilize appropriate animal models, that are consistent with those used by other groups and our interventions are based on identified cellular and molecular targets. Non-sentient animal models are not suitable for these intervention studies for a variety of reasons. The inner ear structures are very different anatomically and the range of frequencies and mechanisms of hearing are very different to mammals. Sensory cells and neurons generally regenerate after injury unlike mammalian species, and so they recover auditory function more spontaneously after any trauma. It is possible as the technology for developing inner ear organoids (Koehler et al., Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells Nature Biotechnology 35, 518–520 (2017) doi:10.1038/nbt.3899), that studies of the localised tissue responses and mechanisms of injury can be undertaken and interventions trialed before in vivo intervention experiments. To ensure limited stress on animals, we have developed euthanasia, anaesthetic and analgesic procedures that are effective and all staff and students are trained to handle animals and recognize animals that may be in distress.

***b. Reduction:**
We are constantly looking to reduce the number of animals, beyond ensuring statistical power of the experiments. For example, in these studies we will utilize both cochlea for the in vitro studies, performing different experiments on each. In our in vivo studies on the inflammation with cochlear implants we have put a lot of effort into developing non-invasive measures of inflammation (vascular permeability and invasion of inflammatory cells; eg Le Floc'h et al., 2014) using MRI, thus enabling 2-3 sequential measures on each animal and thereby reducing the number of animals required at each time point. Furthermore we can then take tissues for microCT for quantification of fibrosis, followed by histology thus reducing the number of experiments that would be required for each of these procedures separately.

***c. Replacement:**

We are very interested in replacement approaches (especially for Study 1 and 2) and have assessed, through a search of the literature (PubMed and Medline), the possibility of using non-sentient or less sentient (zebrafish) animals and in silico or immortal cell line approaches for this work. Zebrafish are used substantially to screen for ototoxic drugs (eg Ou et al., Drug Discov Today, 15(7-8): 265–271. doi:10.1016/j.drudis.2010.01.001) but we have not yet assessed whether these would be suitable as alternatives to study otoprotective mechanisms and it is often required to repeat these studies in mammalian species as a proof-of-principle. There are several immortal cochlear sensory hair cell lines (Rivolta and Holley, 2002 J Neurobiol. 53:306-18), which have been used to look at ototoxicity metabolic mechanisms. But these lack the integrity of the sensory epithelium necessary to look at the interaction of supporting and sensory tissues, and needed for this study (the supporting cells are considered to be involved in organising sensory cell death). Furthermore, some of the aminoglycoside ototoxic pathways in immortal cochlear sensory hair cell lines are different to those seen in vivo (Chen et al., 2012, Hearing Research 284:33-41). However, we have developed systems for partial replacement and are using organotypic cultures of the inner ear where possible, such as in the first two studies described in this proposal. These are taken from neonatal (P3-P6 mice) and do not involve any experimental manipulation of the animal. The studies of cochlear implantation (Study 3) require in vivo experiments in order to assess the natural immune response and formation of the fibrosis as it occurs in human surgical implantation. It is not possible to use cell lines for these particular research questions as these do not mimic the complex relationship between the different sensory, neural and secretory tissues involved in the cochlear response to injury. However, we are investigating ways to model the local changes in the inner ear following surgery (ie those not involving a systemic response or are confined to signal transduction pathways expressed in cell lines) to evaluate the impact of treatments using cell culture or in vitro systems in a similar way to previous studies (eg Bas et al., 2015, Frontiers of Cellular Neuroscience doi: 10.3389/fncel.2015.00303, and our previous studies Vlajkovic et al. (1998) Hear Res.117:71-80). The technology for developing inner ear organoids (Koehler et al., Nature Biotechnology 35, 518–520 (2017) doi:10.1038/nbt.3899), may eventually allow study of localised tissue responses and mechanisms of injury.

Section E: Brief Synopsis of current work

Please list all current University of Auckland Animal Ethics Committee approved protocols. Please detail the species and number of animals used/manipulated/observed for each, to date. Then add a one or two sentence summary of scientific progress to date.

AEC Number:1439

Species & No. of animals approved
Guinea pigs: 100

Species & No. of animals used
Guinea pigs: 69

Summary of progress to date
This work is mostly completed. Two papers have been published and a PhD thesis [redacted] is nearing completion.

[redacted]

AEC Number:1584

Species & No. of animals approved
Rats: 315

Species & No. of animals used
Rats: 101

Summary of progress to date
Studies have been completed on the changes in the auditory pathways and cochlea following extreme hypoxia. This has resulted in three Masters theses and one more is currently in progress

AEC Number:

Species & No. of animals approved

Species & No. of animals used

Summary of progress to date

AEC Number:

Species & No. of animals approved

Species & No. of animals used

Summary of progress to date

AEC Number:

Species & No. of animals used

Species & No. of animals used

Summary of progress to date

Section F: References

List a reasonable number of references (5 - 10), either by the investigators or others that the committee would find helpful in assessing your application:

Shepherd, RK, Cocoa, A, Eppa, SB (2008). Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hearing Research, 242: 100–109

Wang Q, Green SH. (2011). Functional role of neurotrophin-3 in synapse regeneration by spiral ganglion neurons on inner hair cells after excitotoxic trauma in vitro. J. Neurosci. 25:31(21):7938-49

Le Floc'h J, Tan W, Telang RS, Vlajkovic SM, Nuttall AL, Rooney WR, Pontré B and Thorne PR (2013) Markers of cochlear inflammation using magnetic resonance imaging. Journal of Magnetic Resonance Imaging doi: 10.1002/jmri.24144

Wise AK and Gillespie LN (2012) Drug delivery to the inner ear J. Neural Eng. 9 065002 doi:10.1088/1741-2560/9/6/065002

Vlajkovic S.M., Housley G.D., Thorne P.R. (2009) Adenosine and the auditory system. Current Neuropharmacology, 2009, 7:246-56

Ann E. Hickox AE, M. Charles Liberman MC (2014) Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? Journal of Neurophysiology, 111: 552-564 DOI: 10.1152/jn.00184.2013

Seyyedi M and Nadol JB Jr. (2014). Intracochlear Inflammatory Response to Cochlear Implant Electrodes in the Human. Otol Neurotol; 35(9): 1545–1551. doi:10.1097/MAO.0000000000000540

Bas E, Goncalves S, Adams M, Dinh CT Bas JM, VanDeWater TR and Eshraghi AA (2016) Spiral ganglion cells and macrophages initiate neuro-inflammation and scarring following cochlear implantation. Frontiers of Cellular Neuroscience doi: 10.3389/fncel.2015.00303

Section G: Attachments

Document Name:	Document Version:	In reference to Question No.?	
AEC 1986 Supplementary details on the design and methods	02	D2	
1986 Personnel	01	Section A	

Animal monitoring sheet	01	Section D	
VJU guinea pig	01	Section C	
VJU mouse	01	section C	
VJU rat	01	Section C	
Timeline of experiments	01	Section D	
IDAOs for the drugs to be used in the project	01	Section D	
Memo with responses to committee	02	overall	
Additional Information	02	Section D	

Feedback

Please help us to improve this system by providing feedback on your experience with creating this eForm application: include all your positive and negative experiences as well as what improvements you would like to see in using this application.

*Is this Application now complete and ready for submission?
No

Please change the response above to "Yes" once this application is complete and prior to ticking the 'Complete' checkbox (located at the top right corner of the application). This is to hide the majority of the instruction text on the form.

Appendix 1

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: AEC 1986 Supplementary details on the design and methods.docx

Supplementary details on the design and methods.

Study 1: Determine the effect of adenosine receptor agonists and antagonists on cochlear survival after ototoxic injury

In this study, we will determine the effect of adenosine receptor agonists ( ) and antagonists ( ) on hair cell survival in tissue cultures treated with ototoxic aminoglycoside antibiotic neomycin which selectively depletes outer sensory hair cells. Exposure to neomycin is used as a model of metabolic stress leading to hair cell death in this study. Mouse pups (P3-P6) will be killed by decapitation, and cochlear tissues collected for tissue culture studies. Cochlear explants will be pre-incubated for 19 hours with and then exposed to neomycin (1 mM) for 3 h at 37°C. Cochlear explants will be incubated for further 19 hours in culture medium and then fixed with 4% PFA. Quantitative histology will determine the survival rate of hair cells in whole mounts of the organ of Corti. **Number of mice required = 25 (Grade A, tissue collection).** The number of animals required for this study is determined by the number of compounds (4) + vehicle control x 10 cochleae per group = 50 cochleae (25 mice).

Study 2: Determine the effects of adenosine receptor agonists ( ) and antagonists ( ) on the development of excitotoxic injury in cochlear explants

In all studies we will use organotypic tissue cultures of the Wistar rat cochlea at postnatal day 3-6 (P3-P6) as described previously. Briefly, rat pups will be decapitated and auditory bullae removed. The cochleae will be decapsulated in ice-cold dissection solution and the membraneous labyrinth will be separated from the modiolus, and the organ of Corti and spiral ganglion neurons (SGN) will be kept intact. Cochlear explants will be cultured in Dulbecco's Modified Eagle Medium and Earle's Balanced Salt Solution (Thermo Fisher) with addition of fetal bovine serum and penicillin G. Cochlear explants will be transferred to a cell culture incubator and maintained at 37°C with 5% CO_2 for 24 hours (pre-incubation). To cause excitotoxic injury, the explants will be exposed to glutamate receptor agonist (NMDA, 0.5 mM) and kainic acid (0.5 mM) for 2 hours ("NK" treatment). This treatment results in loss of IHC afferent synapses and degeneration of SGN peripheral axons, mimicking excitotoxic damage caused by noise. The explants will be maintained for further 48 hours in control culture medium or in culture medium containing , or . The explants of the organ of Corti will be then fixed in 4% PFA and prepared for immunofluorescence and histology. The number of animals required for this study is **80 rats (Grade A, tissue collection),** based on four experimental groups (3 drug-treated and a vehicle-treated) x 20 animals/group. Additional 5 rat pups are required to establish the NK model of excitotoxicity in our lab. The number of animals (n = 20/group) is required to obtain 40 cochleae for quantitative analysis of SGN, hair cells and afferent synapses, semi-quantitative analysis of synaptic swelling and the loss of peripheral axons, and characterisation of A_1 and A_{2A} receptor distribution at the afferent synapses and SGN. This number has been determined from our previous experience in organotypic culture studies in the cochlea.

Study 3: Stemming inflammation and fibrosis in the cochlea after cochlear implantation

Aim 1: Protection of hearing

1.1. **Protecting residual hearing**: Guinea pigs will be unilaterally implanted with a dummy implant along with supraparticles containing different concentrations of 2 weeks after deafening with noise exposure (16kHz, 2hr, 120dBSPL, closed field). Function will be measured before and weekly up to 8 weeks later and histology will determine survival of hair cells and neurons at 8 weeks. One ear will be implanted and the opposite will serve as a non implanted control. Another group of animals will be implanted

along with empty nanoparticles as a drug delivery control. **Numbers 6 in each group x 4 groups (3 concentrations and 1 control): 24 animals**

1.2. **Combination therapy:** The A2A agonist (at concentration with maximum effect from Aim 1.1) will be used alone or in combination with a neurotrophin (BDNF) or another compound ([redacted]) which enhances endogenous adenosine signalling through A1 receptors. After noise exposure deafening procedure, animals will be bilaterally implanted with supraparticles and one ear will also receive a dummy implant. Function will be measured each week for 8 weeks, and histology will determine survival of hair cells and neurons at 8 weeks. **Numbers 6 in each group x 4 groups (3 combinations and 1 control): 24 animals**

2. **Aim 2**

Effect on Fibrosis: Guinea pigs will be unilaterally implanted with a dummy implant along with supraparticles containing [redacted] (3 different concentrations as above) 2 weeks after deafening with noise exposure (16kHz, 2hr, 120dBSPL). Two separate measures will be undertaken to determine the anti-inflammatory action of the [redacted] on the cochlea:

The cochlear vascular permeability will be assessed using Dynamic Contrast MRI (DCE-MRI). This technique measures the uptake of Gadolinium-based contrast agent (GBCA) into the cochlea from the circulation, using MRI. The rate of uptake can be used to determine the vascular permeability in the cochlea, which is a surrogate measure of the inflammatory response in this tissue. Measurements can be made on the same animal using MRI at 3 different intervals (intervals limited by the need to inject GCBA into the femoral or jugular vein each time). The fibrosis formed in the cochlea will be quantified and characterised using µCT and histology. Animals used for MRI (2.1) will be euthanised at 14 or 28 days after the introduction of the cochlear implant. Tissue will be taken for histology (n=6 at each interval) or µCT (n=6 at each interval). Histology, using immunohistochemistry for inflammatory cytokines will indicate the influence of the drugs on inflammatory signalling and µCT will be used for 3D reconstruction of the electrode and cochlear tissues to assess the amount of fibrotic tissue around the electrode using our developed techniques for soft-tissue rendering with µCT. Six additional animals are requested in case of death or surgical mishaps during the implantation procedure.

Explanation of animal numbers for Aim 2:

As we can only do three MRI timepoints on each animal and one has to be a baseline for comparison and the last one is terminal because we cannot keep repeating vascular injections on the same vein we will have 12 experiments running for 14 days and 12 experiments running for 28 days for histology and the same for µCT.(6 animals each time with [redacted] and 6 without). 6 additional animals for contingencies. **Total 54 animals.** 3 days and 14 day timepoints have been selected for MRI as these are the times of maximum inflammation (Bas et al., 2014) and 28 days is selected as the final timepoint to determine the effect on the fibrotic changes.

Procedure	Baseline MRI	Day 3 MRI	Day 14	Day 28
uCT				
Reg	X	X		6
Reg	X		6	
Control	X	X		6
Control	X		6	
Histology				
Reg	X	X		6
Reg	X		6	
Control	X	X		6
Control	X		6	

Appendix 2

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: 1986_personnel.pdf

Animal Ethics Application - Details of Personnel **Ref No: _001986____**

Every person named in the Personnel section of this application (other than the RI and HoD) shall complete and sign the following declaration:

1. I have read the University of Auckland Code of Ethical Conduct available at www.auckland.ac.nz/ae.
2. I have read this application and approve the approach to the study, with particular reference to the ethics of experimentation and the welfare of the animals being used.
3. I agree that I will not deviate from the conditions in the approved application.
4. I have read and agree to abide by the University of Auckland Institutional Operating Plan for the Direct Management of Animals.
5. I have read and I agree to abide by any Institutional Drug Administration Orders (IDAOs) linked to this ethics approval.
6. In accordance with Part 6, Section 80, Paragraph 2 of the Animal Welfare Act 1999, I will ensure that:
   (i) in relation to animals used in research, testing, and teaching, all reasonable steps are taken to ensure that the physical, health, and behavioural needs of those animals are met in accordance with both good practice and scientific knowledge;
   (ii) where animals used in research, testing, and teaching are ill or injured, they receive, where practicable, treatment that alleviates any unreasonable or unnecessary pain or distress;
   (iii) where, because of the nature of the research, testing or teaching, the needs referred to in subparagraph (i) cannot be fully met or the treatment referred to in subparagraph (ii) cannot be provided, any degree of pain or distress is reduced to the minimum possible in the circumstances.

	Personnel	[redacted]	Date	Contact No.
1.	[redacted]		4/10/17	[redacted]
2.			4/10/17	
3.			4/10/17	
4.			04.10.17	
5.			4 Oct. 2017	

Please nominate two of these named individuals who may be contacted 24 hours 7 days if any animal health or welfare issues arise outside the normal working hours of the facility in which you will carry out the manipulations in this protocol.

If the work proposed in this application will take place in the VJU - FM&HS, only these two investigators (*please provide Access card numbers) will be granted 24/7 access to the VJU facility. The others will receive access from 0800 until 1800, 7 days a week.

Name:	Mobile No:	Work No:	Home No:	*Access Card No:
[redacted]				

Appendix 3

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: Monitoring sheet.xlsx

Animal V

Species Guinea Pig **Experiment:**

Animal # **Surgeon:**

Experiment day	Day 1	Day 2	Day 3
Date			
Day			
Time			
ON OBSERVATION			
Activity (normal/active/alert/inactive/lethargic)			
Posture (normal/hunched)			
Coat (normal/rough)			
Breathing (normal/regular/rapid/laboured/shallow)			
Skin turgor			
ON HANDLING			
Inquisition			
Diarrhoea			
Vocalisation			
Signs of dehydration			
CNS signs (seisures/convulsions)			
BODY WEIGHT			
Measured weight			
Weight loss (gms)			
Weight loss (%)			
FOOD AND WATER			
Food intake ok			
Weight of full bottle			
Weight of bottle today			
Fluid intake			
WOUND SITE			
Okay/clean			
Bleeding			
Other discahrges/infection			
Suture/clips Ok			
POST-OP SUPPORT			
Name of analgesic			
Dose			
Other			
Signature			

Comments			

Humane endpoints	Perform euthanasia if weight loss is 15% of start

Velfare Record Sheet

Cochlear implant

[redacted]

Date of expt:

AEC #

Day 4	Day 5	Day 6	Day 7	Day 8	Day 9

t body weight or greater.

Day 10

Animal Welfare Monitoring Record (no abnormalities found)

Species:		Date of treatment/surgery:		AEC #:	

Number of animals:	
Animal identifications:	

Researcher name:		A/h contact:	
RI name:		A/h contact:	

Instructions for animal technicians:	

Date	Time	No abnormalities	Initials	Date	Time	No abnormalities	Initials
		√				√	

Appendix 4

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: [redacted]

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

[redacted]

Dear Researcher/AEC Secretary,

This is to confirm that the [redacted] is able to supply the following animals

Species: Guinea Pig

Strain: Tricolour/Duncan Hartley

Sex: Either

Age or weight required: Adult

Total Number: 102

As detailed in the Ethics application for

Responsible Investigator Name: [redacted]

Department / External Institution: Physiology

Ethics Project Title (section B2 of application): Investigation on the role of adenosine receptors in cochlear injury

[redacted]

Date 6th October 2017

Appendix 5

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: [redacted]

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

[redacted]

Dear Researcher/AEC Secretary,

This is to confirm that the [redacted] is able to supply the following animals

Species: mouse

Strain: C57/Bl6

Sex: Either

Age or weight required: Post-natal day 3-6

Total Number: 25

As detailed in the Ethics application for

Responsible Investigator Name: [redacted]

Department / External Institution: Physiology

Ethics Project Title (section B2 of application): Investigations on the role of adenosine receptors in cochlear injury

[redacted]

Date 6th October 2017

Appendix 6

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: [redacted]

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

[redacted]

Dear Researcher/AEC Secretary,

This is to confirm that the [redacted] is able to supply the following animals

Species: Rat

Strain: Wistar

Sex: Either

Age or weight required: Post-natal day 3-6

Total Number: 85

As detailed in the Ethics application for

Responsible Investigator Name: [redacted]

Department / External Institution: Physiology

Ethics Project Title (section B2 of application): Investigations on the role of adenosine receptors in cochlear injury

[redacted]

Date 6th October 2017

Appendix 7

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: 001986_Animal Experimental Timeline.docx

Animal Experimental Timeline (AEC# 001986)

The following is the timeline for the experiments described

Study 3 – Aim 1a – Protection of hearing – determining optimal drug dose
Animal numbers: 24 [4 groups (3 drug concentrations and 1 control). 6 animal per group.]
Timeline: (for a representative animal in one group)

Day 1 - Baseline Auditory Brainstem Response (ABR) and noise exposure to both ears

Day 14 - Anaesthetise animals and perform ABR to confirm permanent hearing loss followed by cochlear implant surgery on one ear along with supraparticles with or without drugs. The other ear will not be operated and serve as noise control.

Day 28 - ABRs on both ears (2 weeks post implantation)

Day 42 - ABRs on both ears (4 weeks post implantation)

Day 56 - ABRs on both ears (6 weeks post implantation)

Day 70 - ABRs on both ears (8 weeks post implantation) and euthanise the animal, collect tissues for histology.

Study 3 – Aim 1b – Protection of hearing – drug combination
Animal numbers: 24 [4 groups (3 drug combinations and 1 control). 6 animal per group.]
Timeline: (for a representative animal in one group)

Day 1 - Baseline ABR on and noise exposure to both ears

Day 14 - ABR to confirm permanent hearing loss followed by cochlear implant surgery along with supraparticles with or without drugs.

.
Day 28 - ABRs on both ears (2 weeks post implantation)

Day 42 - ABRs on both ears (4 weeks post implantation)

Day 56 - ABRs on both ears (6 weeks post implantation)

Day 70 - ABRs on both ears (8 weeks post implantation) and euthanise the animal, collect tissues for histology.

Study 3 – Aim 2 – Effect of fibrosis
Animal numbers: 54 [8 groups (2 timelines of 3 drug concentration and 1 control). 6 animal per group, plus 6 additional animal for contingency.]
Timeline: (for a representative animal in one group)

Day 1 - Baseline MRI on guinea pig

Day 3 - Baseline ABR and noise exposure to one ear

Day 17 - ABR to confirm permanent hearing loss followed by cochlear implant surgery along with supraparticles with or without drugs. The other ear will not be operated and serve as control.

Day 31 - MRI on guinea pig (2 weeks post implantation). The animal will be euthanised and tissue collected for µCT and histology. This will end the expts on one group with 14 day timeline.

Day 45 - MRI on G.pig (4 weeks post implantation). The animal will be euthanised and tissue collected for µCT and histology. This will end the expts on one group with 28 day timeline.

Appendix 8

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: IDAOs for AEC 001986.pdf

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: A. Surgical plane of anaesthesia B. Sedation for procedures that only require minimal restrain

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	A Ketamine B Xylazine
Active ingredient	A Ketamine B Xylazine
Strength	A 100mg/ml B 20mg/ml
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	Yes
Product Type	Anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	0.75 ml ketamine and 0.5ml xylazine, 0.75 ml physiological saline. The drugs are put into sterile eppendorf tube.
Dosage (e.g. mg/Kg)	ketamine 75 mg/kg, xylazine 10 mg/kg
Dose (e.g. mL/Kg)	Using mixed diluted solution: 0.2ml per 100g of body weight
Frequency of dosing	to effect administer initial dose and top up as required to prolong surgery. Initial dose provides 30-40 minutes of anesthesia.
Site	intraperitoneal
Equipment	syringe with 27G needle
Technique used	Gently restrain and lift abdominal skin of anesthetised animal. Inject into peritoneal cavity after withdrawing on the plunger to check that it is not in a blood vessel.

EFFECTS AND OUTCOMES

Expected treatment outcome	Surgical level of anesthesia (loss of righting reflex, loss of reflex on hind paw)
Unexpected outcomes	too deep a plane of anesthesia (deep jerky irregular breathing) or too light an anesthesia (stirring and panting during surgical stimulation)
Possible adverse events	respiratory depression, bradycardia
Measures to be taken to correct adverse effects or unexpected outcomes	Too deep: allow Guinea pig to recover on oxygen or air only until breathing and reflexes indicate suitable level of anaesthesia. Too light, top up with a dose of 1/4 or 1/3 of original dose.
Actions to be taken in case of inadvertent self administration	Injected or inhaled: treat symptomatically Swallowed: DO NOT induce vomiting, contact a doctor or the NPC immediately. Eye: flush thoroughly. Skin: remove contaiminated clothing and wash skin thoroughly.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton campus, 502-424
How will **un-used** product be disposed of?	Flush down sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Anaesthesia while performing MRI measurement.

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Isoflurane
Active ingredient	Isoflurane
Strength	100%
Formula type	Liquid
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	No
Product Type	Anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	None
Dosage (e.g. mg/Kg)	1-5% as required
Dose (e.g. mL/Kg)	1-5% as required
Frequency of dosing	to effect
Site	gaseous
Equipment	Open circuit anaesthetic machine with scavenging to fume cabinet
Technique used	Induction and maintenance both via facemask

EFFECTS AND OUTCOMES	
Expected treatment outcome	Surgical plane of anaesthesia as judged by negative ear pinch reflex.
Unexpected outcomes	Too light a plane of anaesthesia (start to react to stimuli) and too deep a plane of anaesthesia (irregular jerky breathing movements.
Possible adverse events	Moderate to severe respiratory depression (very slow/stop breathing) and possibly death
Measures to be taken to correct adverse effects or unexpected outcomes	If guinea pig stops breathing - check head and neck are extended, administer 100% oxygen (flush circuit to get rid of

	residual circuitry gases), and compress the chest gently to stimulate breathing. Too deep: turn off vapouriser, consider ventilation with air + supplementary oxygen in case of overdose and if experimentally appropriate consider euthanasia. Too light: stop surgery, incrementally turn up vapouriser to higher % and recheck reflexes (at each increment) until an adequate plane of anaesthesia is reached.
Actions to be taken in case of inadvertent self administration	Skin: Wash splashes from skin with soap and water. Eyes: Wash contaminated eyes with water for 15mins and go to hospital without delay, leave contacts in place Oral: Contact Poisons information centre 0800 764 766. Do not induce vomiting. Give water to rinse out mouth, then provide liquid slowly and as much as casualty can comfortably drink. Avoid giving milk, oils or alcohol. Seek medical advice. Inhalation: Remove person from source of volatile anaesthetic to well ventilated area, symptomatic therapy as required.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	MRI facility in the basement.
How will un-used product be disposed of?	As per manufacturer instructions.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Sedation/anti-anxiety medication, prior to general aneasthesia.

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	54
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Midazolam
Active ingredient	Midazolam Hydrochloride
Strength	1 mg/mL
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	Yes
Product Type	Anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	None
Dosage (e.g. mg/Kg)	0.5 mg/kg
Dose (e.g. mL/Kg)	0.05 mL/100g
Frequency of dosing	once
Site	subcutaneous
Equipment	1ml syringe and small needle
Technique used	Injection into the subcutanous tissue of the neck/shoulder area

EFFECTS AND OUTCOMES	
Expected treatment outcome	Calm behaviour, and possibly mild to moderate sedation
Unexpected outcomes	No effect or excitation
Possible adverse events	Moderate respiratory depression (very slow)
Measures to be taken to correct adverse effects or unexpected outcomes	If guinea pig breathing slows significantly - check head and neck are extended, administer 100% oxygen. If excitation occurs, leave guinea pig in a dark quiet area for 15 minutes and re-check. Do not use on that animal again. Animal should calm down within 1 hour.

Actions to be taken in case of inadvertent self administration	Skin: Wash splashes from skin immediately with soap and water. Eyes: Wash contaminated eyes with water for 15mins and seek medical attention. Ingestion: contact Poisons information centre 0800 764 766. Do not induce vomiting. Give water to rinse out mouth, then provide liquid slowly and as much as casualty can comfortably drink. Avoid giving milk, oils or alcohol. Seek medical advice.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Flush down the sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Post operative analgesia

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Temgesic
Active ingredient	Buprenorphine
Strength	300 µg/ml
Formula type	Injectable
Prescription Status	Human Prescription Medicine
Is the medicine a controlled drug?	Yes
Product Type	Anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	None required
Dosage (e.g. mg/Kg)	0.05 mg/kg
Dose (e.g. mL/Kg)	0.17 ml/kg
Frequency of dosing	to effect
Site	subcutaneous
Equipment	syringe with 27G needle
Technique used	Injection while under anaesthesia prior to surgery or in conscious animal with gentle manual restraint

EFFECTS AND OUTCOMES	
Expected treatment outcome	Adequate intraoperative analgesia Adequate post operative analgesia - normal post op movement, respiration, posture, exploratory behaviour, restoration of eating and drinking, no staggering or skin twitching
Unexpected outcomes	Inappropriate level of post operative analgesia indicated by abnormal observations of the above
Possible adverse events	Excess analgesia leading to reduced consciousness
Measures to be taken to correct adverse effects or unexpected outcomes	Insufficient analgesia: administer further doses of buprenorphine to upper limit of dosage range above

	Overdose: supportive measures e.g. oxygen, keep airway open (posture) or euthanasia if not recovering or not able to continue in experiment
Actions to be taken in case of inadvertent self administration	Injected or inhaled: treat symptomatically Swallowed: DO NOT induce vomiting, contact a doctor or the NPC immediately. Eye: flush thoroughly. Skin: remove contaiminated clothing and wash skin thoroughly.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton campus, 502-424
How will **un-used** product be disposed of?	Flush down sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Inflammation and pain management

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Rimadyl
Active ingredient	Carprofen
Strength	50 mg/mL
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	No
Product Type	Analgesic and antiinflammatory

ADMINISTRATION DETAILS	
Preparation (if required)	Dilute 1 in 2 in normal saline
Dosage (e.g. mg/Kg)	5 mg/kg
Dose (e.g. mL/Kg)	0.1 mL/kg
Frequency of dosing	once pre-op then once daily for upt to 5 days post-op
Site	subcutaneous
Equipment	Syringe and needle
Technique used	Subcutaneous either while animal is under anaesthesia, or subcutaneous in restrained conscious animal.

EFFECTS AND OUTCOMES	
Expected treatment outcome	Adequate post operative analgesia: as evidenced by appropriate post-op indices such as normal respiration, normal posture, normal exploratory behaviour, restoration of eating or drinking, no staggering or repeated skin twitching.
Unexpected outcomes	Inappropriate level of post operative analgesia indicated by reduced movement, postures that favour the wound, failure to eat or drink, staggering gait, repeated skin twitching.
Possible adverse events	Excessive bleeding around the injection site Gastrointestinal bleeding in post-operative period

Measures to be taken to correct adverse effects or unexpected outcomes	Insufficient analgesia: adminster further dose(s) of carprofen. Look for reversal of the signs of insufficient analgesia as state above. Excessive bleeding from injection site: local pressure for 3-5 mins if it has not spontaneously subsided over initial 10 mins of observation. Gastro-intestinal bleeding: stop drug. If excessive, then euthanasia.
Actions to be taken in case of inadvertent self administration	Swallow: contact poisons centre, induce vomiting with fingers only if conscious. Eyes: wash with water, contact lens removed by skilled personel. Skin: wash with soap and water. Nil action required unless allergic readion then standard resuscitative support. IV or IM: monitor cardia and respiratory function and support as required depending on dose. If concerned contact Poisons Information Centre 0800 764 766.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Flush down the sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Antibiotic prophylaxis

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Baytril 2.5%
Active ingredient	Enrofloxacin
Strength	25 mg/ml
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	No
Product Type	Antibiotic

ADMINISTRATION DETAILS	
Preparation (if required)	Ready to use formulation, dilute 1:1 with saline if given subcutaneously
Dosage (e.g. mg/Kg)	5 mg/kg
Dose (e.g. mL/Kg)	0.2 ml/kg
Frequency of dosing	once
Site	subcutaneous or intravenous, 30 mins prior to surgery and up to 3 days post-op
Equipment	Syringe, catheter if iv, fine needle for sc
Technique used	Manual restraint of animal

EFFECTS AND OUTCOMES	
Expected treatment outcome	Prenvetion of wound infection
Unexpected outcomes	Abscess or infection at site of surgery, septicemia (animal sick, off-food, lethargic, possibly diarrhoea and fever)
Possible adverse events	Possible transient reaction at injecetion site
Measures to be taken to correct adverse effects or unexpected outcomes	Sick animal - call the Vet.
Actions to be taken in case of inadvertent self administration	Eye: flush with running water Swallowed: do not induce vomiting, seek medical advice. Skin: wash with soap and water

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Flush down the sink with running water or into Mediwaster container for incineration.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Local anaesthesia and vasoconstriction prior to skin incisions.

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Lignocaine / Xylocaine with adrenaline
Active ingredient	Lignocaine hydrochloride Adrenaline
Strength	Lignocaine: 100 mg/10 ml Adrenaline: 0.05 mg/10 ml
Formula type	Injectable
Prescription Status	Human Prescription Medicine
Is the medicine a controlled drug?	No
Product Type	Anaesthetic Local anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	None
Dosage (e.g. mg/Kg)	Lignocaine 1-2 mg/kg
Dose (e.g. mL/Kg)	0.1-0.2 ml/kg
Frequency of dosing	once at the site of surgical incision
Site	subcutaneous
Equipment	Appropriate graduated syringe and fine needle
Technique used	Inject along the line of intended incision or skin entry point prior to incising

EFFECTS AND OUTCOMES	
Expected treatment outcome	Adequate local anaesthesia: as evidenced by lack of response to surgical stimulation or suturing
Unexpected outcomes	Inadequate local anaesthesia: as evidenced by response to surgical stimulation or suturing
Possible adverse events	Bleeding around injection site Toxic effects from overdose leading to hypotension and reduced consciousness

Measures to be taken to correct adverse effects or unexpected outcomes	Insufficient dosage: administer further dose(s) of lignocaine. Recheck for signs of adequate local anaesthesia Bleeding: local pressure for 3-5 min Overdose: supportive measures such as oxygen and posture to keep airway open, and consider euthanasia if deteriorating and no longer useful in experiment.
Actions to be taken in case of inadvertent self administration	Injection: if low dose no action required. If larger dose, effects will be seen in 1-3 mins but peak plasma level is in about 30 mins. Look for tremors, parasthesiae, seizures, hypotension, bradycardia. Administer supportive procesures to maintian airway and seek medical advice. Contact Poisons Centre if necessary 0800 764 766 Skin: wash with soap and water. Oral: no fluids as gag response may be lost Eyes: irrigate with water copiously, medical attention if pain develops. Seek medical advice.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Flush down the sink with running water or into Mediwaster container for incineration.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Sedation, Anaesthesia

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Domitor
Active ingredient	Medetomidine
Strength	1 mg/ml
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	No
Product Type	Anaesthetic

ADMINISTRATION DETAILS	
Preparation (if required)	None
Dosage (e.g. mg/Kg)	0.3 mg/kg
Dose (e.g. mL/Kg)	0.3 ml/kg
Frequency of dosing	once
Site	subcutaneous
Equipment	syringe with fine needle
Technique used	Manual restraint

EFFECTS AND OUTCOMES	
Expected treatment outcome	Sedation
Unexpected outcomes	No sedation
Possible adverse events	Reduction incardiac output, respiratory depression
Measures to be taken to correct adverse effects or unexpected outcomes	Administer top up dose of 0.1 ml/kg
Actions to be taken in case of inadvertent self administration	Injected: treat symptomatically Swallowed: DO NOT induce vomiting, seek medical advice Eye: flush thoroughly with water Skin: wash with soap and water

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton campus, 502-424
How will un-used product be disposed of?	Flush down sink with running water or into Mediwaste container for incineration.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no. Replaces IDAO no. AEC approval commencement date AEC/IDAO approval end date Cancellation date if replaced Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Reversal of medetomidine (Domitor) anaesthesia

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Antisedan
Active ingredient	Atipamezole hydrochloride
Strength	5 mg/ml
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	No
Product Type	Reversal agent

ADMINISTRATION DETAILS	
Preparation (if required)	None
Dosage (e.g. mg/Kg)	1.5 mg/kg
Dose (e.g. mL/Kg)	0.3 ml/kg
Frequency of dosing	once
Site	subcutaneous on completion of surgery
Equipment	syringe with fine needle
Technique used	Manual restraint

EFFECTS AND OUTCOMES	
Expected treatment outcome	Reversal of medetomidine induced anaesthesia
Unexpected outcomes	Partial or no reversal of anaesthesia
Possible adverse events	If overdosed can cause tachycardia for 10 mins after administering
Measures to be taken to correct adverse effects or unexpected outcomes	Monitor recovery of animal, prevent from self damage if abrupt reversal occurs (ataxia likely)
Actions to be taken in case of inadvertent self administration	Injected: treat symptomatically, seek medical advice if necessary Swallowed: DO NOT induce vomiting, seek medical advice Eye: flush thoroughly with water Skin: wash with soap and water

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton campus, 502-424
How will **un-used** product be disposed of?	Flush down sink with running water or into Mediwaste container for incineration.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Euthanasia.

ANIMALS	
Species/Breed	Guinea Pig
Gender	Male and Female
Age	4-16 weeks
Weight	300-600g
Method of identification	Coat colour/markings
Number	102
Reproductive status	Not important

MEDICINES INFORMATION	
Name (trade or generic)	Pentobarb 300
Active ingredient	sodium pentobarbitone
Strength	300 mg/mL
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	Yes
Product Type	Euthanetic

ADMINISTRATION DETAILS	
Preparation (if required)	Dilute to 90 mg/ml with normal saline
Dosage (e.g. mg/Kg)	90 mg/kg
Dose (e.g. mL/Kg)	0.1 mL/100 g
Frequency of dosing	once
Site	intraperitoneal
Equipment	1ml syringe and 27G needle
Technique used	Gently restrain and lift abdominal skin of anesthetised animal. Inject into peritoneal cavity.

EFFECTS AND OUTCOMES	
Expected treatment outcome	Euthanasia - cessation of breathing and heartbeat
Unexpected outcomes	unconscious but not dead
Possible adverse events	none
Measures to be taken to correct adverse effects or unexpected outcomes	administer second dose
Actions to be taken in case of inadvertent self administration	Injected or inhaled: contact a doctor or the National Poisons Centre (0800 POISON or 0800 764 766) immediately. In case of respiratory depression or cyanosis use resuscitative measures if necessary, oxygen.

	Swallowed: DO NOT induce vomiting, contact a doctor or the NPC immediately. Eye: flush thoroughly. Skin: remove contaiminated clothing and wash skin thoroughly.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Non- diluted (300mg/ml): Bring to Vernon Jansen Jnit for proper disposal as controlled drug Diluted (90mg/ml) : Flush down sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

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AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Euthanasia.

ANIMALS	
Species/Breed	Wistar
Gender	Male and Female
Age	P1 and older
Weight	5g +
Method of identification	Permanent skin marker, nail clip/ear punch
Number	85
Reproductive status	Non pregnant

MEDICINES INFORMATION	
Name (trade or generic)	Pentobarb 300
Active ingredient	sodium pentobarbitone
Strength	300 mg/mL
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	Yes
Product Type	Euthanetic

ADMINISTRATION DETAILS	
Preparation (if required)	Dilute to 90 mg/ml with normal saline
Dosage (e.g. mg/Kg)	90 mg/kg
Dose (e.g. mL/Kg)	0.01 mL/10 g
Frequency of dosing	once
Site	intraperitoneal
Equipment	1ml syringe and 27G needle
Technique used	Gently restrain and lift abdominal skin of anesthetised animal. Inject into peritoneal cavity.

EFFECTS AND OUTCOMES	
Expected treatment outcome	Euthanasia - cessation of breathing and heartbeat
Unexpected outcomes	unconscious but not dead
Possible adverse events	none
Measures to be taken to correct adverse effects or unexpected outcomes	administer second dose
Actions to be taken in case of inadvertent self administration	Injected or inhaled: contact a doctor or the National Poisons Centre (0800 POISON or 0800 764 766) immediately. In case of respiratory depression or cyanosis use resuscitative measures if necessary, oxygen.

	Swallowed: DO NOT induce vomiting, contact a doctor or the NPC immediately. Eye: flush thoroughly with water Skin: remove contaiminated clothing and wash skin thoroughly.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Non- diluted (300mg/ml): Bring to Vernon Jansen Jnit for proper disposal as controlled drug Diluted (90mg/ml) : Flush down sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Institutional Drug Administration Order

This form applies to use of AEC approved prescription medicines (human or animal) and/or medicines for the direct management of the animals, such as anaesthetics, analgesics and prophylactic antibiotics.

AEC OFFICE USE ONLY
IDAO no.
Replaces IDAO no.
AEC approval commencement date
AEC/IDAO approval end date
Cancellation date if replaced
Replaced by IDAO no.

Reason for issue of IDAO (excessive detail is not required or expected)
Summary of aim of trial: Title: Investigations on the role of adenosine receptors in cochlear injury.
Reason for involvement of medicines: Euthanasia.

ANIMALS	
Species/Breed	C57/Bl6 and CBA
Gender	Male and Female
Age	P1 and older
Weight	2g +
Method of identification	Permanent marker, nail clip/ear punch
Number	25
Reproductive status	Non pregnant

MEDICINES INFORMATION	
Name (trade or generic)	Pentobarb 300
Active ingredient	sodium pentobarbitone
Strength	300 mg/mL
Formula type	Injectable
Prescription Status	Restricted Veterinary Medicine
Is the medicine a controlled drug?	Yes
Product Type	Euthanetic

ADMINISTRATION DETAILS	
Preparation (if required)	Dilute to 90 mg/ml with normal saline
Dosage (e.g. mg/Kg)	90 mg/kg
Dose (e.g. mL/Kg)	0.01 mL/10 g
Frequency of dosing	once
Site	intraperitoneal
Equipment	1ml syringe and 27G needle
Technique used	Gently restrain and lift abdominal skin of anesthetised animal. Inject into peritoneal cavity.

EFFECTS AND OUTCOMES	
Expected treatment outcome	Euthanasia - cessation of breathing and heartbeat
Unexpected outcomes	unconscious but not dead
Possible adverse events	none
Measures to be taken to correct adverse effects or unexpected outcomes	administer second dose
Actions to be taken in case of inadvertent self administration	Injected or inhaled: contact a doctor or the National Poisons Centre (0800 POISON or 0800 764 766) immediately. In case of respiratory depression or cyanosis use resuscitative measures if necessary, oxygen.

	Swallowed: DO NOT induce vomiting, contact a doctor or the NPC immediately. Eye: flush thoroughly with water Skin: remove contaiminated clothing and wash skin thoroughly.

VET COMMENTS	
If the issuing veterinarian has any specific comments regarding this usage please enter here	

FOOD SAFETY	
Will products from these animals enter the food chain of any other animal (i.e. human or animal)?	No
Does use of the medicine pose any threat to agricultural security?	No
If the answer to either of the above questions is 'yes', please provide detail	

STORAGE AND DISPOSAL	
Where will the medicine be stored?	Grafton Campus Building 502 Room 424
How will **un-used** product be disposed of?	Non- diluted (300mg/ml): Bring to Vernon Jansen Jnit for proper disposal as controlled drug Diluted (90mg/ml) : Flush down sink with running water.

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]			

*Signature confirms acceptance of this statement: I have read, and agree to abide by, the Auckland University "Institutional Operating Plan for the Direct management of Animals".

Authorisation by prescribing veterinarian

Date:..... Signed:.....

Name:.....

Appendix 9

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: 001986 MEMO_2.docx

List of additional corrections and responses to the AEC reviewers' comments for AEC 001986

1. Please ensure [redacted] and [redacted] contact [redacted] to complete module 2a and 2b or be signed off as competent before they handle any animals.

We have been unable to contact [redacted] as he is on leave and will come back next month. We have been in touch with [redacted] and have emailed him with a request to check records whether we have to undergo this training. If required he will enrol us both on the training in next slot but these are not held very frequently (and are often oversubscribed). We are very concerned that the project (and student progress) will be held up until the training is complete. [redacted] is not involved in working with animals and is happy to agree not to be involved with any animal work, or to remove himself from the ethics approval completely until the training is complete, so the work can proceed. [redacted] has been involved in the same experimental animal research for many years under previous ethics approvals and we ask that he be allowed to continue to do this work while waiting for his records to be assessed and for him to be signed off by [redacted] as competent or to complete the modules.

2. You state you will give 15 ml/kg during anaesthetic after surgery, but the SOP attachment says "Before the scanning procedure, saline solution will be injected subcutaneously (20 ml/kg) to avoid dehydration during the scanning procedure". Please amend this or clarify why you have different dose rates.

This saline dose has been amended in the SOP (now referred to as "additional information", see next question).

3. You cannot have a document that is called an SOP unless it has been approved by the AEC. Please either completely remove attachment 7 (AEC SOP) and ensure this information is included within the text of the application, or rename this attachment e.g. 'Additional Information'. Please keep in mind that attachments should add value or additional information to what is already in the application.

This SOP file is now referred to as "additional information". The reference to this file in D.4a first line is amended to "See appendix (additional information) for details…". We have included this to provide details on procedures which we cannot provide in the space provided for the text (and were asked for this after the application screening)

4. Please do not mention the name of drugs in your application or within attachments (particularly attachment 7). You should refer to IDAO's for the use of drugs (For example, "anaesthesia as per IDAO").

The attachment 7 now referred as "Additional information" has been amended so does not mention any drug name, except for Gadolinium-DPTA which is a standard MRI contrast agent.

5. Section A: As per provision 20 of the previous outcome letter, please remove the sentence "one of our investigators is a veterinary surgeon". All people using the title veterinarian in New Zealand must be registered with the Veterinary Council of NZ and hold a current practicing certificate.

We cannot find reference to this anywhere in the documents.

6. D13. Replacement: Please add that you assessed the possibility of using non-sentient or non-living alternatives using your sources (insert your sources) and there were no alternatives.

We have provided additional information in D13c as below:

Replacement: We are very interested in replacement approaches (especially for Study 1 and 2) and have assessed, through a search of the literature (PubMed and Medline), the possibility of using non-sentient or less sentient (zebrafish) animals and *in silico* or immortal cell line approaches for this work. Zebrafish are used substantially to screen for ototoxic drugs (eg Ou et al., Drug Discov Today, 15(7-8): 265–271. doi:10.1016/j.drudis.2010.01.001) but we have not yet assessed whether these would be suitable as alternatives to study otoprotective mechanisms and it is often required to repeat these studies in mammalian species as a proof-of-principle. There are several immortal cochlear sensory hair cell lines (Rivolta and Holley, 2002 J Neurobiol. 53:306-18), which have been used to look at ototoxicity metabolic mechanisms. But these lack the integrity of the sensory epithelium necessary to look at the interaction of supporting and sensory tissues, and needed for this study (the supporting cells are considered to be involved in organising sensory cell death). Furthermore, some of the aminoglycoside ototoxic pathways in immortal cochlear sensory hair cell lines are different to those seen *in vivo* (Chen et al., 2012, Hearing Research 284:33-41). However, we have developed systems for partial replacement and are using organotypic cultures of the inner ear where possible, such as in the first two studies described in this proposal. These are taken from neonatal

(P3-P6 mice) and do not involve any experimental manipulation of the animal. The studies of cochlear implantation (Study 3) require *in vivo* experiments in order to assess the natural immune response and formation of the fibrosis as it occurs in human surgical implantation. It is not possible to use cell lines for these particular research questions as these do not mimic the complex relationship between the different sensory, neural and secretory tissues involved in the cochlear response to injury. However, we are investigating ways to model the local changes in the inner ear following surgery (ie those not involving a systemic response or are confined to signal transduction pathways expressed in cell lines) to evaluate the impact of treatments using cell culture or in vitro systems in a similar way to previous studies (eg Bas et al., 2015, Frontiers of Cellular Neuroscience doi: 10.3389/fncel.2015.00303, and our previous studies Vlajkovic et al. (1998) Hear Res.117:71-80). The technology for developing inner ear organoids (Koehler et al., Nature Biotechnology 35, 518–520 (2017) doi:10.1038/nbt.3899), may eventually allow study of localised tissue responses and mechanisms of injury.

In the attachments – the attachment no 1 (AEC 1986 supplementary details on design and methods) seems to be wrong. It contains the information on personnel instead.

The wrong file was uploaded by mistake. Thank you. This has been changed. The file includes details on the procedures for the experiments that could not be included in the text space and give more detailed information to the committee..

Appendix 10

EForm Name: AE and Bio-Safety Form v4

Page:

Section: **Section G: Attachments**
Please list all attachments appended in support of this application:

Question:

File Name: AEC 1986 Additional information.docx

Additional information for operating procedures

Preparation of tissues for organotypic cultures

Mouse and rat pups (P3-P6) will be euthanised by decapitation, and cochlear tissues will be collected for tissue culture studies.

Procedures for in vivo experiments on guinea pigs, including measurements of auditory brain response (ABR), surgery of cochlear implantation (CI) and post-operation monitoring, and MRI scanning.

1. Procedures of ABR measurements

Animals will be anaesthetised as per IDAO, and then placed onto a heating pad to maintain body temperature at 37°C. ABRs were obtained by placing fine platinum electrodes subdermally at the mastoid region of the ear of interest (active electrode), scalp vertex (reference) and mastoid region of the opposite ear (ground electrode). The acoustic stimuli for ABR were produced and the responses recorded using a Tucker Davis Technologies auditory physiology System 3 workstation (Alachua, FL, USA). A series of pure tone pips (5 ms duration, 1.5 ms rise and fall times, 1-32 kHz) are presented at varying intensity (10-90 dB SPL).

Animals will be allowed to recover in a dimmed area with warming blanket prior to returning to VJU housing. The duration of each ABR measurement is around 40 minutes. The same animal will have a maximum of three ABR measurements in total with intervals of 2-4 weeks according to allocated study groups.

2. Procedures of CI surgery and post-operation monitoring

The Guinea pigs will be anesthetized as per IDAO. The animal will also receive analgesic as per IDAO. The surgical area is shaved and aseptically prepared with 70% ethanol and povidone-iodine. Local anesthetic will be injected along the intended incisions, as per IDAO. The subjects will be placed on a heating pad and body temperature maintained around 37°C. The eyes will be instilled with lubricating gel/liquid.

Surgery will be performed as follows under a surgical stereo microscope. Through a left post-auricular incision, the left bulla will be exposed and opened using a 2 mm steel burr and bone forceps to gain access to the round window niche. The basal turn is cleared off any connective tissue and underneath bone exposed. A cochleostomy will be made just inferior and anterior to the round window using a 0.5 mm steel burr. Through the cochleostomy, the CI electrode array is inserted into the scala tympani until slight resistance was felt. The cochleostomy will be sealed with muscle plugs. The bulla opening will then be sealed with bone pieces and dental cement. The skin will be closed with surgical sutures and skin adhesive.

Animals will be monitored daily post-operation until Day 10 using the customised ‘monitoring sheet’ and then will be monitored weekly until the end of each experiment (up to 8 weeks according to the study plan).

3. Procedures of animal preparation for MRI scanning

The Guinea pigs will be sedated as per IDAO and transferred to inhalant anesthetic chamber. The anesthesia will be induced as per IDAO. The animal will then be transferred to HFMRI (Varian 4.7T) coil chamber and anesthesia maintained using an appropriate face mask throughout the MRI procedure for a maximum of 2 hrs period.

If the animal shows sign of irritation or distress, it will be alternatively anesthetized with injectable anaesthetics as per IDAO. Supplemental doses of anaesthetics may be given during the procedure as necessary.

Before the scanning procedure, saline solution will be injected subcutaneously as per protocol to avoid dehydration during the scanning procedure. Throughout the MRI scanning procedure core body temperature was monitored with a rectal probe and maintained with warm air and heart rate/respiration was monitored continuously. The eyes will be instilled with lubricating gel/liquid.

For injecting the contrast agent the protocol will be as follows:
The animal will receive analgesic as per IDAO. The surgical area, inner thigh region of hind legs will be shaved and aseptically prepared. The subjects will be placed on a heating pad and body temperature maintained around 37°C.

An incision, about 1cm long, will be made just over the femoral region and vein exposed. Animal will then be slowly injected with warm contrast agent Gadolinium DTPA (1.5 mmol/kg, @ 1ml/min) using a fine 30G needle. The wound will be sutured and sealed with skin adhesive.

The animal will then be placed back into the HFMRI chamber and recordings done.
Some animals will recover at the end of the scans for re-imaging one or more weeks later (as per experimental methods) following the same method to investigate the chronic changes in cochlea permeability in same animal. A maximum of three scanning procedures will be performed for each animal. After completion of the final MRI scan, the animals will be euthanized and tissues harvested for histochemistry procedures.