Updated By: [redacted] @ 28-Jul-2017 11:10:24 AM

Application Form AE / Bio-Safety v4

General Information

Completion of Application

Research Type:

Select the purpose for this Application: * 1. Research

General:

Protocol Number: 001933

Project Title: Needle-Free Injection of Nicotine: Pharmacokinetics and Tissue Effects - INDI

Responsible Investigator: [redacted]

Department: *UNISERVICES

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?
Yes

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

*2b: Is this project (partially) funded by Uniservices?
No

*2d: The following (other) granting body has allocated (additional) funds specifically for work covered by this proposal:
The Tobacco Control Turanga (Health Research Council)

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:
☑			PI	[redacted]

Certification Begin End

- - - Certifications

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

*Qualifications: ?☐

MSc(Tech), Ph.D.

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

No *Module 2a - Handling, sexing and euthanasia of rodents

No *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

Please refer to the Animal Ethics website for details of how to register for these Module(s).

*Experience: ?☐

No experience, A[redacted] will not be doing any direct procedures on the animals. However, if required will be supervised by [redacted] who has over 25 years of experience in animal husbandry, manipulation and surgical technique. [redacted] will be overseeing the project and be responsible for all the work done.

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:
☐	25-May-2017		Co-Inv	[redacted]

Certification Begin End

- - - Certifications

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

*Qualifications: ?☐

PhD

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

No *Module 2a - Handling, sexing and euthanasia of rodents

No *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

Please refer to the Animal Ethics website for details of how to register for these Module(s).

*Experience: ?☑

[redacted] has no experience with the technique described in the protocol. She will not be conducting any direct procedures on animals. However, if required, she will be supervised by [redacted] who has over 25 years of experience in animal husbandry, manipulation and surgical technique. [redacted] will be coordinating the project including drafting of study procedures, liaising with [redacted], and day-to-day management of the project.

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.

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'.

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Personnel - Review

Name of UoA Personnel: Madadkhahsalmassi, Bahareh

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

Certification Begin End Certifications

Certification	Begin	End
	-	-

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

*Qualifications:
Masters of Science in Chemical Engineering

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

No *Module 2a - Handling, sexing and euthanasia of rodents

No *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

Please refer to the Animal Ethics website for details of how to register for these Module(s).

*Experience:
No experience. Will be supervised by [redacted] who has over 25 years of experience in animal husbandry, manipulation and surgical technique.

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'.

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Personnel - Review

Name of UoA Personnel: [redacted]

RI	Start Date	End Date	Role	E-Mail:
☐			AEC Administrator	

Certification	Begin	End
	-	-

Certifications

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

*Qualifications:
Certificate in Animal Science and Technology – Veterinary Nursing
Certificate in Animal Technology and Nursing – Laboratory Animal Science

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

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

No *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

Please refer to the Animal Ethics website for details of how to register for these Module(s).

*Experience:
[redacted] has over 25 years experience in animal husbandry, manipulation and surgical technique.

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:
☐			AEC Administrator	[redacted]

Certification	Begin	End
	-	-

Certifications

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

*Qualifications:
BSc(Hons) in Medical Physics and Imaging Technology

Please confirm whether you have completed the following training modules:

Yes *Module 1 - Legislation

No *Module 2a - Handling, sexing and euthanasia of rodents

No *Module 2b - Handling, sexing and euthanasia of rabbits and guinea pigs

Please refer to the Animal Ethics website for details of how to register for these Module(s).

*Experience:

[redacted] has experience with using the needle-free injection device and will be able to provide the technical expertise in using it for nicotine delivery. She will be supervised by [redacted] who has over 25 years of experience in animal husbandry, manipulation and surgical technique.

Categories of procedures to be performed:

- [x] Manipulation
- [x] 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

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:
001933

*B.2: Project Title:
Needle-Free Injection of Nicotine: Pharmacokinetics and Tissue Effects - INDI

* B.3: Lay Summary:
There is an urgent need to improve the efficacy of stop-smoking treatments for highly dependent smokers. Traditional nicotine replacement therapy (NRT), such as transdermal patches, provides a slow delivery of nicotine and thus often insufficient for smokers to deal with sudden cravings. A new technology involving a needle-free nicotine injector device could deliver cigarette-equivalent dose of nicotine through the skin in a jet of fluid faster than traditional nicotine patches without requiring the use of a needle. This novel technology may allow flexible dosing and self-administration of efficacious nicotine concentrations with negligible pain, to relieve sudden urges to smoke and is therefore worth evaluating.

*B.4: Describe why animals are needed for this project:
The concentration of a drug in plasma, which is then available to cross into the brain to have a clinical effect, is a complex interplay of many processes, including diffusion from the site of administration, uptake into the circulation, distribution around the body (including crossing the blood-brain barrier) and elimination through generally hepatic metabolism and or renal elimination. Whilst many of these processes can be modelled based on existing data, a key factor in evaluating needle-free technology is whether sufficient dose can be delivered and then distributed to the blood circulation. Given the novelty of this device there are no data to model what will happen to nicotine under these circumstances. A simple pharmacokinetic approach in whole animals, as outlined herein will provide key data to ascertain whether this approach will have value in human application, not just for nicotine. Assessing potential damage at the injection site will also provide invaluable information as to the potential safety of such an approach in humans. The current work will contribute to the further development and refinement of these models. We will publish this work freely, meaning that our group is making a substantive progressive global contribution to reducing animal model research in nicotine delivery methods through this work.

*B.5: Describe how the experimental findings will be used, promoted or published at the conclusion of the study:
The key findings from this experiment will be to assess the viability of needle-free subcutaneous delivery of drugs, both in terms of pharmacokinetics and safety. Experimental findings will be presented at local (within and outside the University) and international conferences and will be published in peer-reviewed scientific journals.

*B.6a: Are the experiments an extension of previous animal studies from your laboratory?
No

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

Section C: Animal Use

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:
1k - Pigs	White cross-breed	Both	12	Both	12 weeks	35-50kg

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.

Pigs have similar anatomy and size to humans and provide a good model for our studies, prior to translating our techniques to human studies. Pig skin has previously been demonstrated to properties similar to that of human skin. It has important similarities in morphology, cellular composition, and immunoreacticity to human skin. Pig skin also has comparable vascular organization in the dermis albeit with a smaller blood supply than human.

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

Animal Usage Statistics

Species section(Add Species section)

Species section

1k - 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	
Commercial	12
Farm	
Born during project	
Captured	
Imported into NZ	
Public sources	
TOTAL:	*12

3. Status of animals:	Number
Normal/Conventional	12
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	12
Veterinary research	
Testing	
Production of biological agents	
Development of alternatives	
Other	

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

6. Grading:	Number:
No impact - A	
Little impact - B	12
Moderate impact - C	
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	*12

9. Total manipulated/used:	*12

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:

Part A:
1. To develop a needle free nicotine injector that can deliver nicotine subcutaneously;
2. To develop and validate an analytical method to quantify nicotine and cotinine in pig plasma;
3. To measure the pharmacokinetics of nicotine administered by needle free subcutaneous injection;
4. To investigate if there is any evidence of local tissue trauma at the injection site.

Part B:
1. To test methods for needle-free blood extraction

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

Pigs (35 – 50 kg) will be delivered to the [redacted] at Grafton campus, University of Auckland on the morning of the study. The studies will be conducted in the [redacted]. Anaesthesia will be induced and maintained as per SOP914. Both parts of the study will be carried out on fully anaesthetised animals.

Part A: Pharmacokinetic study:
This part of the study will allow us to address the aims stated above, including testing the nicotine injector device, validate analytical method, measure the pharmacokinetics of nicotine administered and investigate if there is any local tissue trauma. We will use a two group design, with 6 pigs assigned to either the needle-free condition, or the usual needle condition. A total of 12 pigs is required. The needle condition will act as a control group for the study.

Needle Free administration Usual Needle administration
Dose (25 mcg/kg) Group 1 (n =6) Group 2 (n =6)

1. Cannula inserted in ear vein for IV fluid administration, femoral artery for blood pressure measurement.
2. Twenty-five mcg of nicotine will be delivered into pigs using either needle free injector or subcutaneous needle injection.
3. Arterial blood sampling will be conducted at various time points over 2 hours. This include blood samples taken at 0 (before injection), 5, 10, 15, 30, 50 minutes then 1.5, 2 hours after the injection. 6mL of blood will be taken per sample. Total of 48mL blood. This will allow us to examine the absorption of nicotine.
4. Local tissues will be collected and the histology will be examined. This will include Haematoxylin and Eosin (H & E) staining. This is conducted to test the null hypothesis of no difference in local tissue trauma at the injection sites, therefore local tissues of pigs from both conditions would be required. It is anticipated that 2 pigs from each condition will be required, unless something is clearly visible that warrants further examination with additional pigs.

Analysis of plasma samples:
Plasma will be obtained from blood samples and stored at -80°C until batch tested for nicotine and cotinine concentrations. Preparation will involve deproteinisation of the sample followed by injection onto HPLC, with mass-spectrometry (available within the Faculty of Medical and Health Sciences) used for quantification.

Part B: Blood extraction:
This part of the study will allow us to test methods for needle-free blood extraction. The aim is to discover whether blood can be released from the injection site using a jet injector.

1. Prick the potential injection sites (ear and abdominal region around nipples) using a lancet.
2. Collect blood using a capillary blood collection tube (Microvette®) and measure blood volume.
3. Measure and record blood glucose concentration for reference by a blood glucose meter. Use the site where there is a suitable release of blood as the location of subsequent jet-injection.
4. Jet inject a mixture of sterile physiological saline and Iodine-based or gold nanoparticle contrast agent. Use a standard "collimated" jet-injection orifice that is known to create an approximately 200 µm diameter cylindrical incision in the skin. The possible jet parameters for jet injection are 50 µL and 150 m/s.
5. Observe if there is any blood released from the injection site and measure the volume and (later) the glucose concentration.
6. Perform a jet injection with the same injectate and jet parameters as last procedure but creating a dispersed jet through a custom orifice.
7. After each injection, measure and record the volume of extracted fluid.

A pig surgery record form will be used to detail and monitor the pigs. (Attached in section G). On completion of study, the animal will be euthanized while under anaesthesia (as per SOP914, attached in section G).

Post mortem, tissue from the belly will be harvested for use in device characterization experiments in the ABI lab.

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) :
Manipulation 1) Subcutaneous delivery of nicotine (25mcg/kg) using needle free injector
Manipulation 2) Subcutaneous delivery of nicotine (25mcg/kg) using needle
Manipulation 3) Jet injection
Manipulation 4) Arterial extraction of blood sample

* D.4b: The extent and duration of the manipulation(s):
Each animal would take approximately 4 hours. Animal will be anaesthetised for the entire procedure. Animals will be euthanized while still under anaesthesia when the study is complete. It is anticipated that part A of the study will take around 2 and a half hour, and part B will take approximately 1 and a half hour.

* 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:
The animals may experience some initial mild discomfort during the administration of the anaesthetic. The animal will be fully anaesthetised for both Part A and Part B of the experiment.

* D.4d: Please explain why this extent of pain or distress is unavoidable:
It is unavoidable to get the pigs under anaesthesia. The distress during the anaesthetic administration will be minimised by handling the pigs in a gentle, calm and quiet manner. A short period of discomfort is tolerated well and allows a smooth transition to surgical anaesthesia.

* D.4e: Describe the pain management plan that has been developed for the alleviation of pain:
The animal will be anaesthetised and euthanised as per SOP914 during the procedure and tissue collection and will be euthanized without regaining consciousness.

Given the use of appropriate anaesthesia, no additional no analgesia is provided for the pigs in these acute terminal studies. The animals will experience brief pain and distress during induction of anaesthesia and remain under surgical depth of anaesthesia until euthanasia on completion of our study. Depth of anaesthesia will be confirmed with palpebral reflexes and experiment will not be initiated until it is ensured that the animals are in a deep anaesthetic state.

* D.4f: Detail the post manipulation care and/or any special housing needs:
N/A

* D.4g: Explain the monitoring procedures and contingencies that will be in place to detect and limit signs of pain or distress:
A welfare monitoring sheet will be used. (Attached in Section G) Vital signs (temperature, heart rate, blood pressure, and end-tidal CO2) will be monitored and recorded throughout the studies. The study will be terminated should any adverse events be encountered.

* 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.
This is an acute, non-recovery study and the animals will be euthanized at study completion. Pigs will be routinely monitored throughout the study and the welfare monitoring sheet will be used to record details. Anaesthesia is known to cause dose dependent respiratory and cardiovascular depression and therefore routine monitoring of the depth or plane of anaesthesia will be carried out. Respiration rate will be maintained via the ventilator and heart rate, blood pressure, ECG and palpebral reflexes will be observed. If the pig cannot be maintained under a satisfactory plane of surgical anaesthesia, it will be euthanized immediately. In addition, if IV access cannot be obtained or maintained, or intubation and ventilation cannot be achieved or maintained, or haemorrhage occurs that cannot be easily controlled, then immediate euthanasia will be performed. These decisions will be taken in close consultation with our experienced animal anaesthetic technician.

* 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?
Yes

* D.9: Describe any other animal welfare or ethical implications of this project:
It could be a concern that the animals will be coming into a new facility and may experience some stress from this. They will only be at the facility for a short time prior to anaesthesia and will be kept together and handled by skilled technicians.

*D.10: Describe why the nominated number of animals is needed: ?☐
The planned experiments are largely technical in nature, being focused on novel medical devices, and pre-clinical validation studies. Biological variability therefore is not formally factored into our primary outcomes, and formal power calculations are therefore not necessary to determine the number of animals needed. This strategy allows us to keep our animal requirement low.
In addition, where possible, multiple experiments (Part A and Part B) will be conducted in each pig, meaning that we are able to be particularly efficient with our overall animal burden for this proposal. This means that with only a relatively small number of animals are needed to achieve several experimental outcomes. Our estimate based on these factors is that 12 animals will be sufficient altogether to meet the current goals, although if the goals are met sooner, then we will use fewer animals.

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: ?☐
Animals and will be delivered on the morning of the study and will be euthanized at the end of the acute study.
When studies are scheduled for consecutive days, it may be possible that two pigs are delivered on the same day. In this case, one pig may be housed overnight in [redacted] Floor pens. When housing of a pig is required, it will be conducted in accordance with the SOP835 which has been approved for the [redacted]

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: ?☐
These refined studies comprise the welfare, anaesthesia and monitoring techniques well established by the experienced staff in the large animal facility. These will be monitored and maintained until euthanasia occurs on completion of the study. Techniques to be introduced or applied will be first extensively tested in a benchtop setting prior to application in experimental animal studies, allowing prior refinement of methods. These approaches allow us to keep experimental failures to a minimum, ensuring high animal use efficiency.

*b. Reduction: ?☐
Where possible, multiple experiments (Part A and Part B) will be conducted in each pig, meaning that we are able to be particularly efficient with our overall animal burden for this proposal. This means that with only a relatively small number of animals are needed to achieve several experimental outcomes. Our estimate based on these factors is that 12 animals will be sufficient altogether to meet the current goals, although if the goals are met sooner, then we will use fewer animals. We will reduce the total number of animals used where possible (e.g. we would test isotonic saline at a similar site on each pig (rather than having a separate control group) to act as a control site for assessing tissue trauma).

*c. Replacement: ?☐

Our research team consists of experts in the field of smoking cessation, development of medical devices, and toxicology. After thorough discussion and literature search through google scholar and pubmed, we were unable to identify any studies that have examined needle-free technology and nicotine use. Given the novelty of this device there are no data to model what will happen to nicotine under these circumstances. It is therefore unavoidable that this device needs to be tested on pigs to provide invaluable information as to the potential safety of such an approach in humans.

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:

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 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:

Taberner, A. Hogan, N. C., & Hunter, I. W. Needle-free jet injection using real-time controlled linear Lorentz-force actuators Medical Engineering & Physics, 2012, 34, 1228-1235 DOI: 10.1016/j.medengphy.2011.12.010

Mitragotri, S. Current status and future prospects of needle-free liquid jet injectors Nature Reviews Drug Discovery, 2006, 5, 543-548 DOI: 10.1038/nrd2076

Le Houezec, J., Jacon, P., & Benowitz, N. L. A clinical pharmacological study of subcutaneous nicotine European Journal of Clinical Pharmacology, 1993, 44, 225-230

Russell, M. A. H., Jarvis, M. J., Jones, G., & Feyerabend, C. Non-smokers show acute tolerance to subcutaneous nicotine Psychopharmacology, 1990, 102, 56-58

Hammann, F., Kummer, O., Guercioni, S., Imanidis, G., & Drewe, J. Time controlled pulsatile transdermal delivery of nicotine: A phase I feasibility trial in male smokers Journal of Controlled Release, 2016, 232, 248-254

Section G: Attachments

Document Name:	Document Version:	In reference to Question No.?	
Pig Surgery Record 001933	1	D2/D4g	
Research Approval 001933	1	AFM Approv	
Details of personnel	1	Section A	
SOP914 Guide	1	D2	
SOP 914 IDAO	1	D2	
Response letter	1		
Revised AFM Approval	1	AFM Approv	

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?
Yes

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: Pig Surgery Record 001933.doc

Needle Free Injection of Nicotine: Pharmacokinetics and tissue effects

AEC approval – 001933

Pig # -	**IDAO #** - SOP914/2
Surgery Date -	Zoletil dosage – 10mg/kg
Breed -	Zoletil dose -
Gender -	Route of administration - IM neck/leg
Weight at surgery -	

Surgery Procedure	Time	Comment
Induction of Anaesthesia		
Intubation		
I.V Fluids start		
Surgery start		
Experiment complete		

Time	Temp.	Iso. %	O2 L	Resp.Rate	Tidal Vol.	CO2	BP	Comment

Comments:

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: Research Approval 001933.pdf

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

[redacted]
Faculty of Medical and Health Sciences,
The University of Auckland,
Grafton, Auckland

Dear Researcher/AEC Secretary,

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

Species: Pig

Sex: Female

Age and or weight: 35 – 50kg

Total number: 12

This is to confirm that the [redacted] can house the following animals:

Number: 12

Duration: Overnight if required

Type of housing: Pens

As detailed in the Ethics application for

Responsible Investigator Name: [redacted]

Department / Institution: ABI

Ethics Project Title (section B2 of application): 001933 Needle Free Injection of Nicotine: Pharmacokinetics and tissue effects - iNDI

[redacted] Manager

Name: [redacted]

Signature: [redacted]

Date: 30/5/17

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: Details of Personnel.pdf

Animal Ethics Application - Details of Personnel **Ref No:** 001933

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 https://www.staff.auckland.ac.nz/en/research-36/research-integrity-ethics-and-biosafety/animal-ethics1.html.
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	Signature	Date	Contact No.
1.	[redacted]	[redacted]	2/06/17	[redacted]
2.			31/5/17	
3.			31/5/17	
4.			31/5/17	
5.			12/6/2017	

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 [redacted] - FM&HS, only these two investigators (*please provide Access card numbers) will be granted 24/7 access to the [redacted] 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 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: SOP 914 April 2017.docx

UNIVERSITY OF AUCKLAND [redacted] STANDARD OPERATING PROCEDURE	Effective From : 31/3/2017~~1/4/2015~~ Review date: 31/3/2020~~3/2017~~ AEC Ref: SOP914

Title: Anaesthesia and Euthanasia of Sheep and Pigs in the [redacted] and MRI.		
	Print name	Signed
[redacted] Authorised	[redacted] (Business Manager)	[redacted]

Table of Contents

1. Purpose

The procedures in this SOP describe the methods to be used by University of Auckland staff for the induction and maintenance of anaesthesia and for the euthanasia of sheep and pigs used for research and teaching purposes under AEC approvals.

2. Definitions

2.1 [redacted]
2.2 MRI – Magnetic Resonance Imaging.
2.3 AWO – Animal Welfare Officer.

3. Responsibility

3.1 The procedures shall only be carried out by University of Auckland animal technicians who have been trained in the handling of large animals (Module 2c), in the use of injectable and inhalation anaesthetics and drugs used for euthanasia, and who have been signed off as competent in their training file.
3.2 All members of staff engaged in this procedure must comply with this SOP and any related SOPs and documentation.

4. Equipment

4.1 Appropriate PPE
4.2 Isoflurane anaesthetic machine and scavenging system
4.3 Ventilator and/or re-breathing bag
4.4 Oxygen cylinder and portable anaesthetic machine for anaesthesia in the MRI
4.5 Anaesthetic monitoring system.
4.6 Operating table.
4.7 Laryngoscope.

5. Procedures

5.1 Pre-anaesthetic checks.

Pre-anaesthetic checks of equipment shall be carried out prior to the induction of anaesthesia in sheep or pigs.

These checks shall include the following:

- Check that there is an adequate supply of oxygen and that it is properly attached to the anaesthetic machine.
- For anaesthetics in the MRI unit, a cylinder of oxygen is required. Check that it contains an adequate amount of oxygen.
- Check that the flowmeters are functioning correctly by opening the cylinder valves and opening the needle valves that control the flow of gas through the flowmeters. The bobbins should rotate when gas is flowing. The gas flow rate is measured from the top of the bobbin.
- Turn off the gas flow using the needle valve and check that the bobbin sinks smoothly back to zero, and is not sticking and giving a false high gas flow rate.
- Check that the emergency oxygen switch on the machine is functioning correctly.
- If an anaesthetic circuit with valves is to be used, open them fully and check that they are operating correctly.
- Check that the Isoflurane vaporizer has been filled, that the bungs are in the correct place and that the control dial moves smoothly over the entire range of possible settings.
- Check that the connection tube is correctly fitted to the proper port with the correct connection fitting.
- Check that when the vaporizer is turned off, no anaesthetic odour can be detected when the oxygen flowmeter is turned on.
- Attach the circuit which will be used to the anaesthetic machine and ventilator, turn on the oxygen supply and check the circuit for leaks by attaching a rebreathing bag and fully closing any valves. Turn the ventilator on and ensure bellows rise fully and fall.
- Open the valves to check they are not sticking.
- Ensure the scavenging tubing is connected to and the system turned on.

After completion of the anaesthesia, the machine shall be shut down as follows:

- Turn the vaporiser and the flow meter to their “OFF” positions.
- Turn the ventilator off.
- Disconnect the oxygen supply.
- Turn off the scavenging unit and disconnect it from the outlet.

Other equipment to be used during the procedure shall also be checked and prepared in advance of administering the anaesthetic.

Monitoring equipment, when used, should be switched on, allowed a period to stabilize if necessary, and its functions checked.

Monitoring alarm limits should be reset from the default settings and then fine-tuned when the individual animal is connected.

6.2 Induction of anaesthesia

6.2.1 Sheep.

- The sheep shall be starved of food overnight prior to the surgery, but water shall be available at all times.
- In order to reduce the amount of stress to an individual sheep in a pen, it should first be transferred into a small catch pen if available. This will reduce the area for avoidance and reduce the need to chase the animal. The smaller the catch pen, the easier it will be to catch the sheep.
- Once the sheep is in the catch pen, the animal handler shall manoeuvre it into a corner, using his/her arms or a portable gate to form a visual barrier.
- The handler shall always approach sheep calmly and slowly initially, in order to minimise stress, but catching or handling should always be done with quick, confident, robust movements. A hood is available if the animal is particularly fractious.
- The sheep can then be backed into a corner of the pen and straddled. The handler must keep a hand under the jaw to control the sheep's movements. The sheep can then walked from the pen into a small wheeled transport box and taken to prep room for anaesthesia.
- Sheep that are in metabolic crates shall be removed from them as safely as possible.
- To induce the anaesthesia, one person shall restrain the animal, while another administers the anaesthetic by intravenous injection in the jugular or cephalic veins.
- The area chosen for the injection site may now be clipped and swabbed with alcohol.
- The anaesthetic can now be administered as per the attached IDAO.

6.2.2. Pigs

- Pigs shall be starved of food overnight prior to the surgery.
- The pig shall be caught and immobilised for ease of injection.
- To catch the pig, ensure the handling area is large enough to perform the task safely, but small enough to restrict the pig's movement, e.g. at the end of a passage way, in a small pen or in a handling crate.

- If a handling crate is not being used, apply gentle pressure with pig board or a leg to the hind-quarters and flank of the pig to keep it still.

- Pigs can also be restrained with ropes or a wire loop around its snout (i.e. around the upper jaw, behind the incisor teeth). However, these methods of restraint cause a greater degree of stress to the pig, and should be avoided whenever possible.

- Once the pig is immobilised, the site for the intramuscular injection is swabbed with alcohol. This site may be either the middle third of the side of the neck, or the rear part of the thigh.

- The injection of anaesthetic is now given as per the attached IDAO.

6.3 Intubation and administration of inhalation anaesthesia in sheep and pigs.

- Following induction, and once the animal has lost its palpebral reflex; it can be lifted onto the operating table in the supine position and strapped down, if necessary. A mechanical lifting device should be used if at all possible.

- Using a speculum or laryngoscope, the anaesthetist shall then identify the animal's larynx, and pass an endotracheal tube of the appropriate size. The cuff of the endotracheal tube shall then be inflated.

- The endotracheal tube shall be immediately connected to the anaesthetic machine and ventilator, and Isoflurane delivered according to the IDAO.

- Any monitoring equipment, if being used, shall also be connected at this time.

6.4 Maintenance and monitoring of anaesthesia.

The depth of anaesthesia shall be monitored at regular intervals, but at a minimum of every five minutes, throughout the surgical procedure.

Anaesthetic depth may be monitored by:

1. Palpebral reflex
2. Respiration rate
3. Heart rate
4. Mucous membrane colour
5. Blood pressure
6. Capnograph/pulse oximeter

The percentage of Isoflurane being delivered may be adjusted if required, as per the IDAO.

Appropriate fluid therapy shall be administered to all anaesthetised animals

6.5 Recovery.

If recovery surgery is being performed, the Isoflurane shall be turned off at the completion of surgery so that the animal is breathing oxygen only.

When the animal is breathing on its own, and the breathing is stable, it may be disconnected from the anaesthetic machine.

When the animal begins to chew, the cuff on the endotracheal tube shall be deflated, the tube gently removed, and the animal placed in sternal recumbency.

Regular monitoring shall continue until the animal is standing, and food and water can be offered to it.

6.6 Euthanasia.

If the surgery is non-recovery, the animal shall be euthanized by the intravenous or intra-cardiac injection of Pentobarbitone, as per the accompanying IDAO, unless an alternative method is used under an AEC approval. Any method of euthanasia shall be completed while the animal is still fully anaesthetised.

The following signs of death shall be confirmed before the animal is removed from the operating table:

1. Cessation of respiration and heartbeat.
2. Lack of a pulse.
3. Fully dilated pupils.
4. Lack of palpebral reflex.

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: 914 IDAO.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.	SOP 914/2
Replaces IDAO no.	SOP 914/1
AEC approval commencement date	1/4/2014 (original approval 8/8/11) [redacted] 1/4/17
AEC/IDAO approval end date	~~1/4/2017~~ 1/4/2020 [redacted]
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:
SOP 914: Anaesthesia, euthanasia and prophylactic treatment of sheep and pigs in the VJU.

ANIMALS	
Species/Breed	Sheep and pigs
Gender	Male and Female
Age	Any
Weight	Any
Method of identification	Ear tags
Number	200 per year
Reproductive status	Not important

For details of Medicines, Administration, Effects and Outcomes and Disposal, please refer to the following Medicines Operating Procedures:

MOP 0027 Isoflurane MOP 0035 Alfaxan MOP 0042 Pentobarbitone MOP 0045 Xylazine MOP 0049 Propofol MOP-0069 Zoletil MOP 0072 Ketoprofen MOP 0073 Oxytetra L[redacted] MOP ~~0074~~ Adrenaline [redacted] 0075	In the following combinations: Anaesthesia: Sheep: Alfaxan or Propofol with or without isoflurane Pigs: Zoletil with isoflurane Adrenaline as required Euthanasia: Sheep and pigs: Pentobarbitone or isoflurane overdose; Xylazine with Pentobarbitone Prophylactic antibiotics: Oxytetra LA

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 details	

STORAGE	
Where will the medicine be stored?	[redacted] 504-B07 and 504-B09

PERSONNEL			
Name	Skill/Qualification	Position	Signature*
[redacted]	Cert Anim Sci & Tech	Team Leader	[redacted]
		~~Technician~~	
	Cert Anim Sci & Tech	Senior Technical Officer	
	LAT	Technician	

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

Authorisation by prescribing veterinarian:

Date: 1/4/2014

Signed: [redacted]

Name: [redacted]

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: Response Letter_27072017.docx

27th July 2017

Dear Sir/Madam

Thank you for reviewing the application for animal ethics approval entitled *"Needle-free Injection of Nicotine: Phamacokinetics and Tissue Effects – INDI*". Please find below our response for each of the questions and comments raised.

1. On page 2 [redacted] is listed as RI, but in Appendix 2 (page 18) [redacted] is cited as RI. Please clarify.

R1. [redacted] is the RI. We have requested this to be corrected on the form (Appendix 2) from the [redacted].

2. Please provide some additional preliminary information that might justify your assumption that it is feasible to deliver pharmacologically-active doses of nicotine across the epidermis. Delivery of an immunogenic dose by this method is well established, but in that case the dose perhaps will be orders of magnitude lower (of the order of 2-5ug per animal) than what you require in your protocol.

R2. Evidence of a pharmacologically-active dose of nicotine delivered across the epidermis has been established by others via needle and syringe (Le Houezec, Jacob et al. 1993). Our team has experience in routinely delivering between 20 µL and 1 mL of fluids across the epidermis using the technique of jet injection (Taberner, Hogan et al. 2012, Hogan, Taberner et al. 2015, Hogan, Anahtar et al. 2017). In the needle-free jet injection technique the jet of nicotine itself acts like a needle to penetrate the skin. We have demonstrated that it is easy to deliver 50 µL (which could contain a 2 mg dose) up 10 mm deep into post-mortem porcine tissue. (Ruddy, Dixon et al. 2017)

3. Please better clarify what each of the 5 individuals will be doing. What are the roles of [redacted]? There should not be people on the application that will not be doing the day to day animal work, (except the RI that may be overseeing/coordinating the project). That person will still take responsibility for all work done.

R3. [redacted] is the RI and will be overseeing the project and be responsible for all the work done. [redacted] will be coordinating the project including drafting of study procedures, liaising with [redacted], and day-to-day management of the project. [redacted] will be responsible for carrying out the experiment and performing the procedures on animals.

4. Please outline the level of experience that Jiali has with animal experimentation.

R4. [redacted] has training and experience in performing jet injection into post-mortem pig tissue.

5. Please explain what measures are proposed to ensure that [redacted] gains some prior knowledge of animal biology and manipulation prior to the commencement of this study.

R5. [redacted] and [redacted] will both accompany the ABI gut group (led by [redacted]) during their next scheduled pig experiment, to observe their procedures and manipulation throughout the experiment. Once the experiment is concluded and the animal has been sacrificed, [redacted] and [redacted] (who has done this before) will excise skin and tissue from the belly of the animal for use in our benchtop injections, under the supervision of [redacted] and [redacted] (our PhD student

who has harvested pig belly, post-mortem, several times.) [redacted] will learn the procedures of tissue handling and preservation, and use some of this tissue in her experiments in the lab, prior to this proposed animal study.

6. Need an age range, as opposed to an age of 12 weeks.

R6. We have revised and given an age range of 9-12 weeks.

7. Please consider providing the left over tissue from the pigs to other researchers for secondary work once you have finished experiments.

R7. The left over tissue from pigs will be harvested for used in jet injection studies; other tissues will be made available to other researchers for secondary work upon completion of the experiments.

REFERENCES

Hogan, N. C., M. N. Anahtar, A. J. Taberner and I. W. Hunter (2017). "Delivery of immunoreactive antigen using a controllable needle-free jet injector." Journal of Controlled Release **258**: 73-80.

Hogan, N. C., A. J. Taberner, L. A. Jones and I. W. Hunter (2015). "Needle-free delivery of macromolecules through the skin using controllable jet injectors." Expert Opin Drug Deliv **12**(10): 1637-1648.

Le Houezec, J., P. Jacob and N. L. Benowitz (1993). "A clinical pharmacological study of subcutaneous nicotine." European Journal of Clinical Pharmacology **44**(3): 225-230.

Ruddy, B. P., A. W. Dixon, R. M. J. Williams and A. J. Taberner (2017). "Optimization of portable electronically-controlled needle-free jet injection systems." IEEE/ASME Transactions on Mechatronics.

Taberner, A., N. C. Hogan and I. W. Hunter (2012). "Needle-free jet injection using real-time controlled linear Lorentz-force actuators." Medical Engineering & Physics **34**(9): 1228-1235.

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: AFM Approval 001933.pdf

THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES

[redacted]
Faculty of Medical and Health Sciences,
The University of Auckland,
Grafton, Auckland

Dear Researcher/AEC Secretary,

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

Species: Pig

Sex: Female

Age and or weight: 35 – 50kg

Total number: 12

This is to confirm that the [redacted] can house the following animals:

Number: 12

Duration: Overnight if required

Type of housing: Pens

As detailed in the Ethics application for

Responsible Investigator Name: [redacted]

Department / Institution: ABI

Ethics Project Title (section B2 of application): 001933 Needle Free Injection of Nicotine: Pharmokinetics and Tissue Effects - iNDI

[redacted] Manager

Name: [redacted]

Signature: [redacted]

Date: 28/7/17