MONASH University

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(Revised December 2014)

APPLICATION FORM FOR THE USE OF ANIMALS FOR SCIENTIFIC PURPOSES IN RESEARCH AND TEACHING

MARP-2

ANIMAL ETHICS COMMITTEE

AEC NUMBER	

Project Type	☒ Research ☐ Undergraduate Teaching ☐ Training in Procedural Techniques

Project Title	Evaluating in vivo effects of glucagon-like peptide-1 (GLP1) nanoparticles in obese rats.
Animal Use Categories (Refer to List of Categories attached)	1.1, 1.2, 2.1, 4.2

Standard Operating Procedures	x **No ☐Yes: Title/AEC Number:**

SOPs indicated are to be read in conjunction with the application. Detail any variations from the SOP.

Proposed Start Date April 2016 actual start determined at time of AEC approval	**Proposed Finish Date March 2018** Actual finish determined at time of AEC approval

	Title & Full Name	Qualifications & Position	Department / Institution

DECLARATION BY CHAIRPERSON OF AEC

I certify that the procedures/ personnel/ location in this project has been considered and approved by the Animal Ethics Committee **for the period** ..07../..04.../2016. **to** ...31../..12../2019..

Chairperson's signature	AEC	Date
	MARP-2	07/04/2016

Dr Rick Lang
Print Name

Conditions of Approval:

1. Monash University Investigators must not deviate from approved application without a written AEC approval and must adhere to all requirements of the AEC;
2. Any variation proposed to the project, and the reasons for that change, must be submitted to the AEC for approval and must not be implemented until written approval is granted;
3. All matters pertaining to the conduct of the approved project are to be reported to the AEC, which maintains oversight in accordance with Monash University licence conditions;
4. A record of details of any animals used in the project must be retained;
5. Project must only be conducted in approved premises;
6. The AEC must be notified any incidents and adverse events that may impact on the welfare of the animals and any changes to approved investigators;
7. Annual Progress Report & Victorian government Animal Use Return must be provided to the AEC at the end of each calendar year and a Completed Report within 6 months of end of approval.
8. Condition of Approval: Report back on Part I and considered at an AEC meeting before can proceed with Part II

MONASH UNIVERSITY ANIMAL ETHICS APPLICATION FORM December 2014

This form is based on requirements from the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition 2013 (the Code) and relevant Victorian legislation. In particular, applications must conform to the special requirements of **Justification**, **Replacement**, **Reduction** and **Refinement**

- Before proceeding access the Regulations, Guidelines and Codes which are available at http://intranet.monash.edu.au/researchadmin/animal/regguide/index.html

- It is the applicant's responsibility to ensure that the application submitted to the AEC is complete, with all questions answered in sufficient detail as requested, and in lay language. Applications which do not comply with the guide lines risk being held up or deferred, and may be refused consideration by the AEC.

- Ensure you are using the **current version** of the application form by downloading it from the web page each time.

- The Chief Investigator is responsible for ensuring that all regulated approvals and clearances have been obtained prior to undertaking any research. Researchers who wish to conduct animal research in States and Territories other than Victoria must meet the particular State/Territory licensing requirements. Researchers are advised to contact the Monash Animal Ethics Office immediately as there may be a requirement for additional state licensing to be undertaken by Monash University.

- An understanding of the Code and relevant legislation is the responsibility of the Investigators.

- This form cannot be used for applications to the AMREP AEC, who have their own application form.

- Ensure all pages are numbered.

- The Declaration by the Chairperson of the AEC must be signed to indicate approval of the application. Animal Work CANNOT BEGIN without this signature.

- If you have any problems or queries, or would like to comment on the form contact the Monash Animal Ethics Office on ☎9905 5121 or ☎9905 9907
  E-mail animal.ethics@monash.edu

N. B. The Animal Ethics Office will provide advice on draft applications

Privacy Information: The information on this form is collected for the primary purpose of assessing your animal ethics application. Other purposes of collection include attending to administrative matters, corresponding with you and statistical analyses. If you chose not to complete all the questions on this form it may not be possible for Monash University to assess your application. Personal information may also be disclosed to the relevant state Government or National Health & Medical Research Council. You have a right to access personal information that Monash University holds about you, subject to any exceptions in relevant legislation. If you wish to seek access to your personal information or inquire about handling of your personal information, contact the University Privacy Officer on 9905 9589.

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Chief Investigator	Dr Chooi Yeng Lee	PhD; Lecturer	School of Pharmacy, Monash University Malaysia.
Person to act in Chief Investigator's absence	Prof Iekhsan Othman	PhD; Professor	School of Medicine and Health Sciences, Monash University Malaysia.
Investigator responsible for animal care	Dr Chooi Yeng Lee	PhD; Lecturer	School of Pharmacy, Monash University Malaysia.

ANIMAL USE: include all animals used eg embryos, neonates, pregnant animals and any expected animal by-catch in trapping protocols.

Species **Include descriptive or family term i.e. mouse, frog**	**Strain** **Indicate with {*} if genetically modified**	**Sex**	**Age**	**Total**	**Source** (Insert the numerical value for the source of animal(s) from the list below)
Rat	Sprague Dawley	Male	6 weeks	88	1

Source of Animals	1) Monash Animal Research Platform (MARP) 2) Purpose-bred by Monash investigator (*PROVIDE Monash Breeding Colony Approval Number below*) 3) Obtained from another Australian institution or Commercial Supplier (*PROVIDE the name and address of the supplier below*) *4)* Imported from Overseas Institution or Commercial Supplier (*PROVIDE the name and address of the supplier below)* 5) Privately owned 6) Pound/Refuge (*PROVIDE the name and address of the pound/refuge below*) 7) Animals in Natural Habitat or captured from Natural Habitat (*PROVIDE details in Special features/permits section below)* *8)* Saleyard *9)* Reuse or Sourced from another approved project (*PROVIDE ethics approval number, the techniques animals have been subjected to, the time between projects etc below)* **DETAILS:** MARP. Rats will be bred in the animal breeding centre at the Monash University Malaysia campus.
Housing Location	x Monash Animal Holding Facilities (SPECIFY below) [2] ☐ Research or Teaching laboratory (SPECIFY building & room no. below) ☐ Off campus Fieldwork (identify the location by street address or Melway ref) ☐ Interstate (SPECIFY below) ☐ Overseas (SPECIFY below) ☐ other (SPECIFY below) **DETAILS:** Monash University Malaysia, Building 3, Level 8, Room 8302. The room temperature will be maintained at 23°C with 12 hours day/night cycle.
Special features/permits/ etc	☐ Yes x No **DETAILS:** -
If transporting animals - describe transportation vehicle [3]	☐ Monash Animal Research Platform Transportation ☐ Other (SPECIFY details below) **DETAILS:** Not applicable
Acclimatisation period	x Yes ☐ No **DETAILS:** 7 days

Type of Housing - e.g. size of caging, individual or group housing, Enrichment	x standard animal facility husbandry conditions for these animals apply. ☐ animals housed greater than 6 months (SPECIFY housing in detail below) ☐ housed individually (JUSTIFY; and SPECIFY housing and LENGTH of individual confinement in detail below) ☐ other (SPECIFY housing in detail below) **DETAILS:** Photo of the rat cage was attached. The cage body is polycarbonate, and wire lids is stainless steel, and the dimensions are 430 x 270 x 150mm. We keep two rats per cage. Bedding is made of natural kenaf. The bedding will be changed 2 to 3 times every week, depending on the bedding condition.
Location of the animals during the experiment	☐ Monash Animal Holding Facilities (SPECIFY below) [2] x Research or Teaching laboratory (SPECIFY building & room no. below) ☐ Off campus Fieldwork (identify the location by street address or Melway ref) ☐ Interstate (SPECIFY below) ☐ Overseas (SPECIFY below) ☐ other (SPECIFY below) **DETAILS:** Monash University Malaysia, Building 3, Level 6, room 3-6-07A.
Are animals held in laboratories?	x No ☐ Yes (SPECIFY duration of laboratory holding) **DETAILS:** If Yes, are records of temperature & humidity maintained for the duration of the holding in the laboratory? ☐ Yes ☐ No

1. Before ordering animals consult with your animal facility manager to ensure your animals can be accommodated.
2. Declaration of Animal Facility Manager must be signed.
3. Monash University researchers require animal ethics approval before animals are transported to Monash facilities from overseas / interstate / local for specific projects.

Source of Funding/Granting Body NB For successful grants the Ethics Approval must cover all animal research in the grant, list all investigators performing scientific procedures involving animals and cover the entire period of the grant	☐ Research group/Departmental/School funds. ☐ Applying for grant funding (SPECIFY granting body below) x Funded by grant (SPECIFY granting body, ID number & Title, below) ☐ Commercially funded (SPECIFY details below.) **DETAILS:** Granting body: Ministry of Education (MOE), FRGS grant. ID number: 2110366-120-00 Title: Suppressing appetite and body weight gain through delivery of glucagon-like peptide-1 (GLP1) to the brain using novel GLP1-loaded pH-sensitive inorganic nanoparticles.

Questions 1 - 6 - ANSWER ALL QUESTIONS IN LAY LANGUAGE

ACRONYMS AND GLOSSARY
Where scientific terms are unavoidable, an explanation of these terms must be given. This glossary is to be used for scientific terminology where lay terms are not available but not as a substitute for lay language. **Arrange words in alphabetical order**.

Word	Lay explanation
L-cells	A type of cells found in the small intestine.
Metabolism	A process that breaks down the original or parent compound to product that will be excreted from the body.
Nanoparticles	Spherical particles that is nano size in scale.
Peptide	A molecule consists of two or more amino acids linked in chain via amide bond.

JUSTIFICATION

Question 1 AIMS, BENEFITS AND SIGNIFICANCE OF THE PROJECT

(1a) Background
Provide brief background information about the project.

Glucagon-like peptide-1 (GLP1) is a peptide secreted from the small intestinal L cells. It is released after feeding and stimulates insulin secretion. Given its physiological function, GLP1-based anti-diabetic drugs have been used clinically to control both the glucose level and body weight. However, GLP1 is not stable in our body and is rapidly broken down into inactive molecules, and this has restricted its therapeutic use. In this study, we formulate nanoparticles that would serve as a carrier for GLP1 and protect GLP1 from rapid metabolism, thus increase the presence of GLP1 in the circulation. Exogenous GLP1 which will be used as a standard and test compound, has the same amino acid sequence as the endogenous GLP1, and the peptide is purchased commercially.

(1b) Aims
Describe the aims of the experiments.

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1) To assess the ability of GLP1 nanoparticles to prolong the residence time of GLP1 in the circulation.

2) To assess the effectiveness of GLP1 nanoparticles in preventing long-term body weight gain and diet-induced elevation in glucose level.

(1c) Potential Benefit
What is the potential benefit to humans or animals as per the Code Section 1.6 which states that "Projects must only be undertaken:

cross one or more of the following:

- x to obtain and establish significant information relevant to the understanding of **humans**;
- ❑ to obtain and establish significant information relevant to the understanding of **animals**
- x to maintain and improve **human** health and welfare;
- ❑ to maintain and improve **animal** health and welfare
- ❑ to improve animal management or production;
- ❑ to obtain & establish significant information relevant to the understanding, maintenance or improvement of the natural environment
- x to achieve educational objectives in science as specified in the relevant curriculum or competency requirements"

(1d) Significance
Referring to 1(c), describe the scientific, environmental and / or educational significance of the project and its relation to previous studies.

GLP1 nanoparticles which have sustained release property will increase the presence of GLP1 in the systemic circulation. This reduces the administrative dose, thus potential side effects of GLP1, and may be used clinically for long term control of body weight gain and glucose level.

REPLACEMENT

(1e) Replacement

Explain why animals are needed for the project, including:

- a list of any potential alternatives to animal use;
- whether any of these alternatives would be used, and if not;
- why alternatives are unsuitable.

There is no alternative to animal use. Animals provide a physiological condition comparable to human body, which is necessary in pre-clinical testing of a new drug formulation.

REDUCTION & REFINEMENT

Question 2 DESCRIPTION OF DESIGN AND PROCEDURES incorporating Key Principles of Reduction & Refinement

(2a) SCIENTIFIC DESIGN

- Give a clear **description of the scientific design** of the experiments. This should give an overall view of the design including choice of independent & dependent variables, methods of measurement and analysis.
- Justify animal use: why this species/ strain/ sex/ age?
- If this is a disease/acute injury model, include a description of how the model works, effects of progression of the disease process, identify humane end-points (cross reference to 3c) and previous experience with the model.

The experiments consist of two stages, which will be carried out in this order: 1) Pharmacokinetics study, 2) In-vivo effects of the GLP1 nanoparticles. In both studies, GLP1 and GLP1 nanoparticles will be suspended in phosphate buffered saline.

Pharmacokinetics study will be carried out on a predetermined dose of GLP1, and nanoparticles that carry equal dose of GLP1. Test compound will be injected into the tail vein and blood samples will be collected from the jugular vein. The duration of each testing will be carried out to a maximum of 3 hours. We are not sure about the clearance rate of GLP1 in this preparation but as shown in Bioconjugate Chem 16: 377-382 (2005), a 2 hour sampling period appeared to be suitable. We may prolong the procedure to 3 hours. Rats will be killed without recovery from anaesthesia.

To study in-vivo effects of the formulation, rats will be fed either a control or a high fat-diet (HFD) for a period of 4-6 weeks. The main purpose of HFD feeding is to induce obesity in the animals. Previous data suggested that an approximately 6 weeks on a high fat-diet will establish an obese model (this was in line with the write-up in our previously approved ethics MARP-2013-064). The data is not published.

Besides the diet, animals will be administered with a fixed dose of GLP1 or GLP1 nanoparticles. The concentration of GLP1 we proposed to use will not give us information about the LD50 because the concentration is not within the toxic level. We may however, know the ED50 of the GLP1. . GLP1 will reduce food intake only when the blood glucose level is higher than the normal level. Therefore, GLP1 may not reduce body weight of the control animals. But the body weight of animals will be monitored, whereby if 10% weight loss is observed, the animal will be humanly killed. Throughout the 4-6 weeks study period, the body weight of each rat, and food intake per cage will be recorded twice a week, while glucose level will be monitored every week. Glucose level will be elevated but we are not sure about the minimum level that is

required. We may find out from this study. The frequency of glucose measurement was clarified. Based on past experiment, we need not monitor the glucose levels regularly or regulate it (for a 6 weeks HFD treatment). We define obesity as animals having body weight that is 20% higher than the control group.The current formulation is not suitable for oral administration. IV (in contrast to intramuscular and subcutaneous) will allow direct delivery of drug to the systemic circulation, thus we know exactly how much is delivered in the blood circulation. Animals will be administered GLP1 or GLP1 nanoparticles by IV up to a total of 3 times throughout the study period ie. once in weeks 3, 4 and 5.

At the end of the treatment, animals will be anaesthetised and blood will be collected from the vena cava. Different tissues will be harvested for a series of bioassays. Rats will be killed without recovery from anaesthesia.

Rats are normally used in pharmacokinetics study because the sampling procedures are easier to be carried out in rats than in smaller animals such as mice. The pharmacokinetics profile will be relevant in determining the frequency of dosing in chronic study, and this explains the use of the same species in the subsequent study. Six weeks old is a suitable age to commence the study.

The above study is not a disease model. It is a model of metabolic disorder.

(2b) WHAT HAPPENS TO THE ANIMALS?

- Give a clear **step-by-step** description of **all procedures to be carried out on each group of animals** (including controls) from the time they are obtained until the time the project is completed.
- Use of a flow chart / time line is recommended.
- Indicate the animal use category for each procedure.
- Include full details where relevant of handling and restraint, manipulations, age of animal, number and volume of blood samples taken.
- If performing surgery full details must be provided including aseptic technique, incision site, procedure & suturing.
- The AEC expects that all possible procedures will be refined to lessen the impact of the procedures that may compromise the animals' well-being or cause pain or distress

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Sprague Dawley (SD) rats will be housed two per cage, and in a temperature- and humidity-controlled room with a 12 h light-dark cycle. Animals will be allowed free access to control diet and water for 7 days before the start of treatment.

In the pharmacokinetics study, rats will be divided into three groups (n = 8 per group, 24 rats in total), and will be administered with either empty nanoparticles (NPs) dispersed in phosphate buffered saline (PBS), or nanoparticles (NPs) that contain 1 μg/kg of GLP1, or 1 μg/kg of GLP1. The NPs or GLP1 will be administered into the tail vein, and blood samples collected from the jugular vein at 0 min, every 10 min interval up to 60 min, 90 min and 120 min (and may include 150 min and 180 min).

The effective dose (ED) 50 of GLP1 in this preparation will be determined. Prior to administration of the test substance, the rat will be anaesthetised by intraperitoneal injection of ketamine/xylazil (at a ratio of 10:1). An incision will be made to expose the trachea and nearby region. The jugular vein will be cannulated using polyethylene tubing (PE 50). (animal use category 1.2). The pharmacokinetics of GLP1 in rats, where GLP1 will be administered intravenously, has been reported by other researchers, and the dose of GLP1 used was

1 µg/kg. Therefore, we will test our formulation using the same GLP1 dose. The materials that we will be using to formulate the nanoparticles, ie. the carrier of GLP1, have been tested in our laboratory and they did not show any toxic effect in animals (AEC approval number MARP/2012/087).

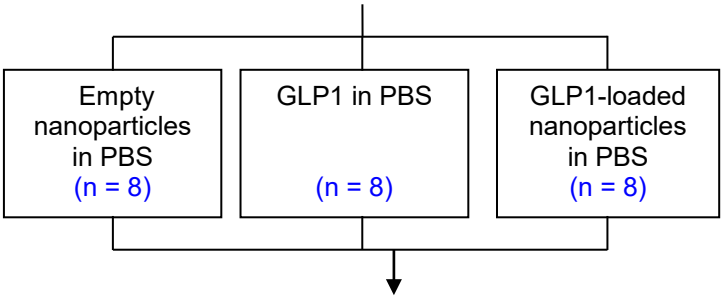
In the subsequent chronic study, pre-treatment, 12 h fasting glucose levels (from 2000 hours of Day 0 to 0800 hours of Day 1) will be measured from the tail vein of all rats using a glucometer. (animal use category 2.1). Blood will be obtained via a tail nick from the middle segment of the tail. The area will be disinfected with an alcohol wipe before a small amount of blood is delivered to a glucose strip attached to the glucometer. A drop of blood is sufficient for each measurement. A nearby area will be disinfected for blood taking if a second measurement is required. Otherwise, the procedure will be done only once. Glucose level will be measured once a week. (animal use category 2.1).

The SD rats will be divided into diet-induced obesity (DIO) and control groups (n = 32 per group, 64 rats in total), and fed with diet containing 45% kcal% fat and 10% kcal% fat, respectively. (animal use category 2.1). Obesity is confirmed when the body weight of DIO rats are 20% higher than control rats. This can be determined because the body weight of each rat as well as food intake per cage will be recorded twice a week. The control and DIO group will now be subdivided into four groups (n = 8 per group, 8 groups altogether), and will be administered via the tail vein the following test substance: empty NPs or GLP1 NPs dispersed in PBS (GLP1 NPs contain 1 µg/kg of GLP1), 1 µg/kg GLP1, or PBS only. (animal use category 4.2). Test substance will be administered once in the 3rd, 4th and 5th weeks of the study, meaning a total of 3 times throughout the study. . The general health of the animals during the 6 weeks period will be monitored using the general health rodent monitoring sheet (Appendix A). At the end of the treatment period, rats will be anaesthetised by ketamine/xylazil injection. An incision will be made at the abdomen and 5 mL of blood will be withdrawn from the vena cava. Animals will be killed without recovery from anaesthesia (animal use category 1.2). The liver, kidneys and the brain will be harvested for further analyses.

Below are flowcharts and timelines of each of the study.

a) Pharmacokinetics study. Timeline: April 2016 – July 2016

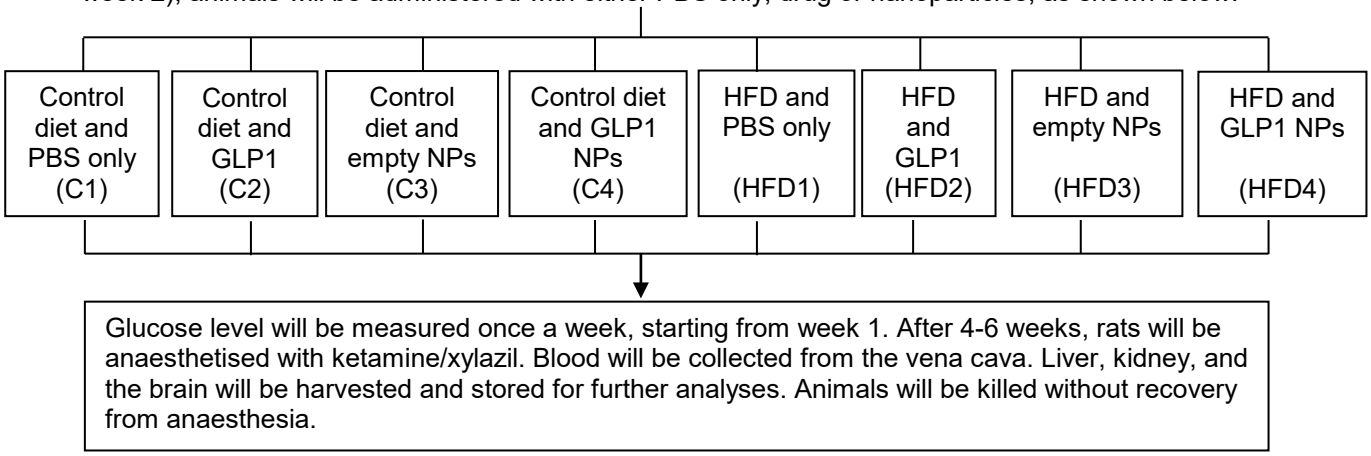
After a 7 days of acclimatisation period, male Sprague Dawley rats (n = 24) will be divided into 3 groups, and each group will be administered via the tail vein one of the following drug or formulation. Injection will be made after animals have been anaesthetised with ketamine/xylazil (at a ratio of 10:1).



Blood will be collected from the jugular vein at 0 min, ie. before administration of drug or formulation, and at specific time points for up to a maximum of 3 h. The amount of GLP1 in the blood will be measured by using the ELISA method. Animals will not be subjected to other treatment. Animals will be humanly killed as they will not recover from anaesthesia.

b) In vivo effects of GLP1 nanoparticles. Timeline: August 2016 – March 2018 (The study described below will be carried out during this period).

After a 7 days of acclimatisation period, male Sprague Dawley rats (n = 64) will be divided into 8 groups. Blood glucose level will be measured for each rat before animals are treated with different diet. Each group will receive either a control diet or a high fat-diet (HFD) throughout the study period of 4 to 6 weeks. When the body weight of the HFD groups is significantly higher than the control groups (approximately at the end of week 2), animals will be administered with either PBS only, drug or nanoparticles, as shown below.



(2c) Refinement

Give details of any treatments or substances administered to the animals as part of the experiment. e.g. drugs, chemicals, hormones, biological or radioactive agents administered to the animal as outlined above. Cross reference with Q2b. Do not include details of anaesthetics or analgesics here (see Q4a &4b)

Compound / Agent / Vehicle	High fat diet	Phosphate buffered saline (PBS)	Empty nanoparticles in PBS	GLP1 in PBS	GLP1 nanoparticles suspended in PBS
Route of administration	Ad libitum	Intravenous injection	Intravenous injection	Intravenous injection	Intravenous injection
Dosage / Dose rate	45% kcal% fat (diet composition was attached)	-	Equal amount of nanoparticles	1 µg/kg	1 µg/kg GLP1
Volume	Not applicable	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Frequency of administration	4-6 weeks	In-vivo: Once in weeks 3, 4 and 5..	Once in pharmacokinetics study. In-vivo: Once in weeks 3, 4 and 5.	Once in pharmacokinetics study. In-vivo: Once in weeks 3, 4 and 5.	Once in pharmacokinetics study. In-vivo: Once in weeks 3, 4 and 5.
Purpose	To induce obesity	As control	As control	To control body weight gain in rats	To control body weight gain in rats
Used previously in this laboratory?	Yes	Yes	Yes	No	No
Possible adverse effects of administration or withdrawal of	No	No	No	Not likely as none has been reported in other study that uses the same dose.	Not likely as none has been reported in other study that uses the same dose.

drugs?					

(2d) Reduction
The AEC expects experiments to be designed to MINIMISE THE NUMBERS of animals used.
Give a breakdown in tabular form of numbers of animals in each experimental group.

a) The pharmacokinetics study:

Treatment groups	Empty nanoparticles in PBS	GLP1 in PBS	GLP1 nanoparticles in PBS
Number of rats	8	8	8

b) Study to determine in-vivo effects of GLP1 nanoparticles:

Treatment groups	Control rats				HFD-treated rats			
	C1	C2	C3	C4	HFD1	HFD2	HFD3	HFD4
Number of rats	8	8	8	8	8	8	8	8

Justify the number of animals per group on the basis of statistical analysis, power calculations, advice from a Biometrician or prior experience. NB All animals used for training personnel or students must be justified and listed here unless covered by separate ethics approval.

Generally, 6 rats per treatment group are the minimum number required in order for the statistical analysis to be considered significant. Eight animals will be used in this study as steps to ensure that we achieve at least n = 6 because data from in vivo study usually varies, hence some data may be excluded.

In the past, data from samples subjected to various analyses, such as ELISA and gene expression, showed high variation. Reasons are not clear but it is actually common that each treatment group has 10 subjects or n = 10 instead.

Is there an opportunity for the sharing of tissues or animals with other researchers?

No.

Question 3 MONITORING
Where observation / monitoring sheets are used, attach proforma
http://www.monash.edu.au/researchoffice/animal/approval/app-forms/monitor-rec.html

(3a) Pain or Distress

Anticipate and describe any possible impact on the animals caused by each procedure/treatment/model in the experimental protocol that may compromise their well-being or cause pain/distress.
Include previous observations where possible.

In the pharmacokinetics study, blood sampling from the jugular vein will be done in animals under anaesthesia.

The general health of rats in the in-vivo study will be monitored according to the monitoring sheet in Appendix A. The feeding of high fat diet will gradually increase the body weight gain, but the condition may be reversed following treatment with GLP1 or GLP1 nanoparticles. Rats may feel distressed during intravenous

administration of the test compounds but this occurs only briefly each time. Injection of ketamine/xylazil intraperitoneally to anaesthetise the animals prior to animal dissection may also induce pain or distress.

(3b) Monitoring

i) **HOW** will the wellbeing of the animals be monitored i.e. what signs will be monitored and how frequently? N.B. relates to conscious, non-anaesthetised animals.	Signs to be monitored are as listed in Appendix A. Monitoring will be done daily for 4-6 weeks.
ii) **WHO** will be monitoring the animals under experimentation? NB The responsibility for monitoring animals under experimentation lies with the Chief Investigator.	The Chief Investigator
iii) **WHO** will be responsible for emergencies and how will it be ensured that nominees can be contacted?	All investigators.
iv) Given that Animal Facility staff attend the Animal Facility daily, **WHAT** is the longest period the animals under experimentation will be left unchecked by the **investigators** (including weekends & Public Holidays)?	One day
v) Are Animal Facility staff to be asked to take any **special** responsibilities in addition to routine husbandry? If yes, staff must be named on the application in sections 6a & 6c.	☐ **YES** X **NO**
vi) What is the maximum time individual animals are to be held under **experiment**?	6 weeks
vii) What is the maximum time individual animals are to be held **in the animal facility?**	7 weeks, inclusive of the acclimatisation period.

(3c) Experimental Endpoints/Euthanasia criteria

At what point would you intervene to kill or treat the animal to alleviate its pain or suffering?
Specify **in detail** what clinical signs, change in behaviour, loss in body weight, tumour size, changes to normal feeding and drinking behaviour etc you would use in making this decision.

Rats that show a single score of 2 or a cumulative score of 3 in any of these parameters, ie. drinking, eating, breathing, faeces, urine or vocalisation (Appendix A).

Question 4 SPECIFIC PROCEDURES

(4a) Anaesthesia
N.B. copy the table for each anaesthetic agent or mixture used or different animal group.

Anaesthesia? X Yes ☐ No **Local and /or General** If YES,	
Agent	Ketamine and Xylazil
Route	Intra-peritoneal injection
Dose/Volume	100 mg/kg Ketamine and 10 mg/kg Xylazil (volume: 0.5 mL each)
Duration	Terminal
Procedure & species for which anaesthesia is be used.	Anaesthetic will be injected using a 25 gauge syringe needle to the animal"s lower right quadrant of the abdomen.

Monitoring depth of anaesthesia.	Checking response from pinching of the paw.
Monitoring recovery from anaesthesia.	There will be no recovery from anaesthesia.
If animals are undergoing multiple anaesthesia, SPECIFY.	Not applicable.

(4b) Analgesia*

*It is the expectation of the AEC that analgesia will be routinely used after every painful procedure. Non-use must be well justified.
N.B. copy the table for each different animal group or analgesic agent used.

Analgesia ☐ **Yes** ☐ No - Justify X Not applicable.	
If YES,	
Agent	
Route	
Dose/Volume/Frequency	
Routinely Used? If NO, specify when.	☐ **Yes** ☐ No

(4c) Euthanasia

It is the expectation of the AEC that animals will be humanely killed in a quiet, clean environment away from other animals.

Are animals humanely killed at the end of the experiments? X **Yes** ☐ **No**	
If **NO**, what is their fate? ☐ Death as an endpoint ie. where investigator will not intervene to kill the animal humanely before death occurs (justify in Question 3c, animal use category 4.15). BAW approval required. ☐ Re-use (complete Question 4d) ☐ Release of trapped wildlife ☐ Other	
Indicate Euthanasia method for each species (including agent, route of administration, dose and volume)	DETAILS: Exsanguination under anaesthesia.
Who will be killing the animals?	The investigator
Disposal	X Standard animal facility disposal procedures of this animal species ☐ Other (SPECIFY)

(4d) Re-use /Recapture

☐ Re-use

Have/will any animals been used in other experiments? If yes, give approval number and brief details of the prior and/or subsequent experimentation and justify their use in this project. ☐ Recapture

Are animals likely to be recaptured? If yes, detail effects of repeated capture.

Animals will not be re-used or recaptured.

(4e) Neuromuscular Block

Neuromuscular Block? ☐ **Yes** X No If YES,	
Agent	
Route	
Dose	

Monitoring	

(4f) Other Safety Hazards

Ensure all details of the use of these materials are included in the table in 2c.

Ionising radiation?	☐ **Yes** X **No**	Agent:
Carcinogen/teratogen/cytotoxic drugs?	☐ **Yes** X **No**	Agent:
Pathogenic organisms?	☐ **Yes** X **No**	Agent:
Health Risks to staff?	☐ **Yes** X **No**	How affected?
Health Risks to other animals?	☐ **Yes** X **No**	How affected?

If YES, to any of the above prepare a Risk Assessment and complete a Risk Control Worksheet indicating how the health and welfare of animals and research and animal facility staff will be safeguarded. The AEC may request a copy of these documents when considering your application. Available from the OHS&E web page **http://www.adm.monash.edu.au/ohse/documents/**

Have all clearances / approvals been applied for/ given by the relevant committees and Safety Officers? If NO, give an explanation.	☐ **Yes** ☐ **No** ☐ **Pending** X **NA**

(4g) Human Ethics

Does the project involve the use of the following?

(A) Human stem cell or stem cell lines?	☐ **Yes** X **No**
(B) Human organs, tissues, cells, cell lines or fluids?	☐ **Yes** X **No**
(C) Study of human/animal behavioural interactions?	☐ **Yes** X **No**
Has Human Research Ethics Committee (HREC) approval been applied for/given by the HREC Monash University Standing Committee on Ethics in Research involving Humans?	☐ **Yes** X **No** **Approval Number**
If yes to (A) (B) or (C) then the Monash University Human Ethics Office (9905 5490) should be consulted in order to determine whether an application is required.	

Question 5 GENETIC MODIFICATION

Does this project involve genetically modified (GM) animals?	☐ **Yes** X **No** **If "YES" answer the questions below**
Briefly define the Genetic Modification/Mutation	**Details:**
Indicate how the GM animals are used	☐ Creating genetically modified animals ☐ Experimenting on existing genetically modified animals ☐ Cross Breeding existing genetically modified animals
Gene Technology Regulator Category	☐ OGTR Exempt Dealing (e.g. Animals with genetically modified somatic cells introduced) ☐ OGTR Notifiable Low Risk Dealing Physical Containment 1 (PC1) (e.g. Genetically modified mice and rats) ☐ OGTR Notifiable Low Risk Dealing Physical Containment 2 (PC2) (e.g. Genetically modified mice and rats with conferred advantage) ☐ Randomly induced mutation that not regulated by OGTR (e.g. Chemical mutagenesis, Ethyl Nitrosourea –ENU) ☐ Naturally occurring mutation (e.g. Severe Combined Immune Deficiency (SCID), Nude Mice)
Are there any known or unknown but anticipated animal welfare	☐ **Yes** ☐ **No** **If "YES", outline the issues and how they will be dealt with.**

issues (eg mortality, appearance, behaviour, responsiveness, nutritional requirements etc)?	
Institutional Biosafety Committee (IBC) approval of dealing (if applicable)	☐ **Yes** ☐ **No** ☐ **Pending**

Question 6 PERSONNEL DECLARATION

Any person proposing to carry out any scientific procedures on animals under this application must be named in this application, and sign the following section. Failure to do so is an offence under the Victorian Prevention of Cruelty to Animals Act 1986.

(6a) LIST ALL PERSONNEL INVOLVED IN ANIMAL USE IN THE PROJECT.

Copy the box for each person involved.

Full Name including title	Dr Chooi Yeng Lee
Staff ID	035831
and / or **Student ID**	-
Email	chooi.yeng.lee@monash.edu
Qualifications	PhD, MSc, BSc.
Position (i.e. lecturer, PhD student)	Lecturer
Department / Institution	School of Pharmacy, Monash University Malaysia
Scientific Licence*	Not applicable
Role/Responsibility for the project	Chief Investigator
Attended Monash Animal Ethics information session?	☐ Yes X No

List the procedures this person will be doing in this project and their level of experience with this species

Technique/ procedure	Species	Level of Experience: Approx. number of times you have performed the procedure in this species			Specify Trainer's name (if none or limited)
		None (<5)	Limited (eg 5-20)	High (eg >20)	
Animal monitoring	Rat and mice			X	-
Clearance study	Rat and mice			X	-
IV injection	Rat and mice			X	-
Animal dissection, blood and tissues collection	Rat and mice			X	-

Full Name including title	Professor Iekhsan Othman
Staff ID	023841
and / or **Student ID**	
Email	Iekhsan.othman@monash.edu
Qualifications	PhD, DIC, BSc(Hons)
Position (i.e. lecturer, PhD student)	Professor
Department / Institution	Jeffrey Cheah school of Medicine and Health Sciences, Monash University Malaysia
Scientific Licence*	Not applicable

Role/Responsibility for the project	Co-investigator
Attended Monash Animal Ethics information session?	☐ Yes x No

List the procedures this person will be doing in this project and their level of experience with this species

Technique/ procedure	Species	Level of Experience: Approx. number of times you have performed the procedure in this species			Specify Trainer's name (if none or limited)
		None (<5)	Limited (eg 5-20)	High (eg >20)	
Animal handling				X	
Animal monitoring				X	
Animal dissection and tissues coillection				X	

For a listing of Monash Scientific Licences, see the web
http://intranet.monash.edu.au/researchadmin/animal/approval/scientificlicences.html

(6b) DECLARATION BY CHIEF INVESTIGATOR

- I hereby declare that I have the appropriate qualifications and experience to perform the procedures described in this Application or to ensure that they are done correctly.
- I have read the provisions of Part III of the Prevention of Cruelty to Animals Act 1986, including Amendments, (the Act) and the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition 2013 (the Code)
- I accept responsibility for the conduct of the experimental procedures detailed above, in accordance with the requirements of the Act and the Code and any other conditions laid down by Monash University.
- I understand that I am required to promptly supply annual and completed reports as a condition of approval.
- I further declare that the procedures described in this Application do not constitute unnecessary repetition of work previously carried out by other research workers or myself (the Code)
- I declare that all persons engaged in this project have been adequately instructed in, and are competent to perform, procedures they are to carry out. If they are not already skilled in the procedures, I will be responsible for ensuring that they obtain the necessary training, so that each procedure on an animal will be carried out in the most appropriate manner.
- I declare that all persons engaged in this project have copies / or access to copies of this Application form and any relevant standard operating procedures.
- I acknowledge and declare that I am responsible for ensuring that all regulated approvals, permits and clearances have been obtained prior to undertaking any research described in this Application.
- I consent for my name and this Application document to be provided to Monash University AEO and kept on file and in a confidential database. I understand that this information may be used in reports made to Monash University or any government department with legal authority to access this information.
- I consent for my name and this Application document to be provided to, and kept on file with this AEC.

....3 /4 / 2016.....
Signature: Chief Investigator Date

.....CHOOI YENG LEE.....
Print Name

(6c) DECLARATION OF ALL PERSONNEL INVOLVED IN ANIMAL USE

- I consent for my name and this Application document to be provided to Monash University AEO and kept on file and in a confidential database. I understand that this information may be used in reports made to Monash University or any government department with legal authority to access this information.
- I consent for my name and this Application document to be provided to, and kept on file with this AEC.

....24 / 3 / 2016.....
Signature: Chief Investigator Date

.....CHOOI YENG LEE.....
Print Name

(6c) DECLARATION OF ALL PERSONNEL INVOLVED IN ANIMAL USE

- I hereby declare that I have the appropriate qualifications and experience or will be trained by the person specified to perform the procedures as described in this Application.
- I have read the above application.
- I have read the provisions of Part III of the Prevention of Cruelty to Animals Act 1986 (including Amendments).
- I have read the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition (2013)
- I agree to submit to the authority of the Chief Investigator, and Scientific Licence Nominee listed in the section below for the purposes of collaboration on this project.
- I consent for my name and this Application document to be provided to Monash University AEO and kept on file and in a confidential database. I understand that this information may be used in reports made to Monash University or any government department with legal authority to access this information.
- I consent for my name and this Application document to be provided to and kept on file with this AEC.

Name of associated investigator/s	Signature	Date
Prof Iekhsan Othman		24/3/16

(6d) DECLARATION BY ANIMAL FACILITY MANAGER

This declaration must be signed by the Manager of the Animal Facility where animals will be housed.

- I have discussed this project with the applicant and have indicated that the required animals can be maintained in the animal facility subject to space availability.

.....
Signature: Animal Facility Manager

Mah Yong Cheng
.....
Print Name

24/3/16
.....
Date

ANIMAL MONITORING SHEET

AEC Project No._____ Investigator _____
Animal ID No._____ Species /Strain/ _____
Animal Details (sex, age etc) _____

- Each animal is examined and observed for abnormalities at each time point (weekly or daily as appropriate)
- Observations are recorded in the table
- Normal clinical signs are recorded as "N"
- Abnormalities are recorded as "A" and severity is scored in brackets eg Breathing: A (3)
- Comments concerning abnormalities are recorded in the comments section of the table
- Additional observations tailored to the monitoring requirements for each animal experiment are to be added at "Other"

CLINICAL OBSERVATION (N or A)	**DATE**						
UNDISTURBED							
Activity							
Breathing							
Eating							
Drinking							
ON HANDLING							
Alert							
Body temperature (^{o}C)							
Drinking							
Eyes							
Faeces							
Nose							
Breathing							
Urine							
Vocalisation							
OTHER (specify)							
COMMENTS							
INITIALS:							

_____ _____

Signature of Chief Investigator **Date**

CLINICAL SIGNS SEVERITY SCORE

SIGNS	0	1	2	3
Activity	normal	isolated, abnormal posture	huddled/inactive OR overactive	moribund OR fitting
Alertness/Sleeping	normal	dull or depressed	little response to handling	unconscious
Body condition*	normal	thin	loss of body fat, failure to grow	loss of muscle mass
Body weight*	normal weight and growth rate	reduced growth rate	acute weight loss 10% OR chronic weight loss 15%	acute weight loss >10% OR chronic weight loss >15% OR failure to grow
Breathing	normal	rapid, shallow	rapid, abdominal breathing	laboured, irregular, skin blue
Coat	normal	coat rough	unkempt; wounds, hair thinning	bleeding or infected wounds, or severe hairloss or self mutilation
Dehydration	none	skin less elastic	skin tenting	skin tenting & eyes sunken
Drinking	normal	increased OR decreased intake over 24 hrs	increased OR decreased intake over 48 hours	constantly drinking OR not drinking over 24 hours
Eating	normal	increased OR decreased intake over 24 hours	increased OR decreased intake over 48 hours	obese OR inappetence over 48 hours
Eyes	normal	wetness or dullness	discharge	eyelids matted
Faeces	normal	Faeces moist	loose, soiled perineum OR abnormally dry +/- mucus	running out on handling OR no faeces for 48 hrs OR frank blood on faeces
Movement/ gait	normal	slight incoordination OR abnormal gait	incoordinated OR walking on tiptoe OR reluctance to move	staggering OR limb dragging OR paralysis
Nose	normal	wetness	discharge	coagulated
Urine	normal		abnormal color/volume	no urine 24 hrs OR incontinent, soiled perineum
Vocalisation	normal	squeaks when palpated	struggles and squeaks loudly when handled/palpated	abnormal vocalisation
Other				

* these criteria may not apply in some situations (eg tumor growth, obesity/metabolic studies)

SPECIAL HUSBANDRY REQUIREMENTS**

None required.

EUTHANASIA/HUMANE EXPERIMENTAL ENDPOINT CRITERIA**

CLINICAL SIGN	ACTION
Animals showed any of the above clinical signs that reaches a severity score of 2.	Humanely kill.
Animals showed a cumulative score of 3 from either drinking, eating, breathing, faeces, urine or vocalisation.	Humanely kill.

** as approved by the AEC, relevant to each specific situation

SCIENTIFIC MEASURES (ie data or tissues to be collected as part of the experimental use)

(eg animals that are killed should be weighed and have their bodies placed in labelled bags and refrigerated)

In this study, blood samples will be collected from the vena cava. Liver, kidney, the intestine, pancreas and adrenal glands are harvested and kept in either RNAlater solution or flash frozen with liquid nitrogen for further analyses. Visceral and reproductive adipose tissues are also removed and weighed. Animal carcasses will be weighed and their bodies placed in labelled bags and refrigerated.

Reference: Morton, D.B. (1997) A scheme for the recognition and assessment of adverse effects in animals. In: Developments in animal and veterinary sciences, 27. *Animal Alternatives, Welfare and Ethics*. pp 235-240. Eds van Zutphen, L.F.M. and Balls, M. Elsevier Science B.V.

MONASH UNIVERSITY ANIMAL USE CATEGORIES

The Monash University Animal Use Categories are to be filled in on the "Application for Approval of a Research or Teaching Project Involving the Use of Animals". Please identify which of the following categories best describe your experiments for each of the species proposed. In some cases experiments may fall into two or more categories.

1.1 No experimentation on living animals (i.e. animals are killed painlessly for biochemical analysis, or in vitro, cell tissue or organ studies).

1.2 Experiments under anaesthesia, without recovery (i.e. animals are fully anaesthetised for the duration of the experiment, and are killed at its conclusion without recovery from anaesthesia).

1.3 Observation of wildlife in their natural habitat.

1.4 Observation of laboratory animal behaviour.

2.1 No anaesthesia, minor procedures used (eg. injection, blood sampling, minor clinical observations, minor dietary manipulations).

2.2 Polyclonal Antibody raising

2.3 Monoclonal antibody raising with in vitro production

3.1 Experiments or surgery under anaesthesia, with subsequent recovery of the animal, and minor post-operative sequelae (eg. following biopsies or cannulations).

3.2 Experiments or surgery under anaesthesia, with subsequent recovery of the animal, and significant post-operative sequelae.

3.3 Experiments involving the transplantation of cells, tissues or organs.

4.1 Studies on the biology of pain or of the responses to physical stresses (eg. heat, cold, burning, ionising radiation).

4.2 Studies on un-anaesthetised animals of the toxic actions of drugs or other chemical agents or of infectious agents.

4.3 Experimental procedures on un-anaesthetised animals requiring immobilisation or extended period of restraint other than normal caging.

4.4 Studies involving experimental induction of abnormal foetal growth.

4.5 Experiments on individual animals that last for more than 3 months.

4.6 Experiments involving the restriction of food or water intake, or other major dietary intervention.

4.7 Studies on animals prone to serious chronic disease (eg studies on mutant strains of animals such as stroke-prone rats, transgenic animals with experimentally induced disease).

4.8 Laboratory studies designed to produce substantial and overt changes in behaviour by physical or chemical means.

4.9 Laboratory studies designed to create or simulate a disease state.

4.10 Laboratory studies involving genetically modified animals - (OGTR NLRD and exempt dealings).

4.11 Monoclonal antibody raising with in vivo ascites production

4.12 Laboratory studies involving Non-Human Primates

4.13 Laboratory studies involving genetically modified animals where OGTR categories do not apply eg Chemical and Radiation induced mutations, cloning and mitochondrial transfer.

4.14 Experiments involving the cloning of an animal.

4.15 Experiments involving 'death as an end point' (no humane euthanasia criteria specified).

5.1 Experiments involving collaboration with commercial interests.

5.2 Experiments involving collaboration with overseas laboratories.

5.3 Field Work / Off Campus Work

6.1 Other experimentation not covered above.