4. APPENDIX C1: Ethics Clearance Certificate and Modifications and Extensions Documents

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/66/C

APPLICANT: Ms Z Hayiyana

SCHOOL: Therapeutic Sciences
DEPARTMENT: Pharmacy
LOCATION: Medical School

PROJECT TITLE: In vivo assessment of topical ocular drug delivery systems in rabbits

Number and Species

51 New Zealand white rabbits

Approval was given for to the use of animals for the project described above at an AESC meeting held on 20141125. This approval remains valid until 20161124.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

Application form error (p 10) - not all rabbits to be euthanased at stated time point
Include pilot study to determine which protocol is optimal for cataract development
- daily injection of sodium selenite, or intra-vitreal injection of diquat dibromide

Signed: _____ Date: 3rd DECEMBER 2014
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 28th NOVEMBER 2014
(Registered Veterinarian)

cc: Supervisor: Professor V Pillay
Director: CAS

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AESC 2015 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Zikhona Hayiyana

b. Department: Pharmacy and Pharmacology

c. Experiment to be modified / extended

	AESC NO		
Original AESC number	2014	66	C
Other M&Es : This serves to request to be allowed to re-group the allocated animals and to re-arrange the sampling points			

d. Project Title: *In vivo* assessment of topical ocular drug delivery systems in rabbits

		No.	Species
e.	Number and species of animals originally approved:	51	*rabbits*
f.	Number of additional animals previously allocated on M&Es:	0	0
g.	Total number of animals allocated to the experiment to date:	51	51
h.	Number of animals used to date:	0	0

i. Specific modification / extension requested

1. Re-grouping of animals

Following the recommendations from the AESC to include a pilot study to test which of the two drugs will effectively induce cataracts I would like to rearrange grouping of the rabbits allocated to me.

51 rabbits were allocated for this project. TI would like to arrange them in the following groups:
6 rabbits: Pilot study: To test drug delivery routes (Topical vs. Intravitreal)
6 rabbits: Pilot study: Cataract development (3 for each group)
36 rabbits: Experimental study (2 groups of 18 rabbits instead of 3 groups of 15: Thus for each sampling time points, n=6 will be used instead of 5)
3 rabbits: Pilot study for drug dosing

2. Dosing change

Originally a dose of 30nmol/g was indicated for administration, this dose is the highest dose. I would like to use a lower dose (0.01%-/„ Abdul-Hussein B.A. and Alzubaidy A.A., 2014) in the pilot study using the 3 rabbits

3. Alteration of time points

For the pilot study assessing intravitreal route of administration, I would now like to use a single termination point after a week instead of the two time points in the original application.

3. Addition of co-worker: Dr. Aubrey Makgotloe (Ophthalmologist)

j. Motivation for modification / extension:

1. Following recommendation from the ethics committee to do a pilot study to test which agent is more effective in developing cataract, 6 animals will be allocated to this (3 for each group).

I would like to remove one of the study groups from the original protocol as literature (Abdul-Hussein B.A. and Alzubaidy A.A., 2014 and Agarwa A. *et al.*, 2013) recommends that the prophylactic and treatment interventions be combined and not separated. As a result instead of using 3 groups with 15 rabbits, we will now use 2 groups with 18 rabbits each.

2. Six rabbits will be used for the originally approved pilot study to test the routes of drug delivery. The remaining 3 rabbits of the 51 allocated will be used to test the higher approved dose of sodium selenite (30nmol/g) if the lower dose is ineffective.
The increase in sample size for each sampling time point, n=6 instead of 5 is for improved for statistical analysis, to achieve precision and accuracy.

3. Dr. Makgotloe will provide expert assistance with the study.

Date: 02.07.2015 Signature:

RECOMMENDATIONS: Approved
i. Reallocation of rabbits to groups as detailed above.
ii. Use of the low dose in the pilot study and thereafter if it produces the desired outcomes.
iii. Inclusion of Dr Makgotloe as a co-worker.

Date: 6th July 2015 Signature:
Chairman, AESC

AESC 2015 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Zikhona Hayiyana

b. Department: Pharmacy and Pharmacology

c. Experiment to be modified / extended

	AESC NO		
Original AESC number	2014	66	C
Other M&Es : This serves to request to be allowed to increase the dose of cataract inducing agents and extend the pilot study			

d. Project Title: *In vivo* assessment of topical ocular drug delivery systems in rabbits

		No.	Species
e.	Number and species of animals originally approved:	51	*rabbits*
f.	Number of additional animals previously allocated on M&Es:	0	0
g.	Total number of animals allocated to the experiment to date:	51	51
h.	Number of animals used to date:	12	12

i. Specific modification / extension requested

1. Extension of the cataract development pilot study from 1 week to 3 weeks/ until cataract develops and increasing the dose of the cataract inducing agents (0.01%w/, to 0.05%w/,)

a. Instead of testing the higher doses on the 3 rabbits as previously requested, a new request is being made to increase the dose on the 6 existing rabbits as it would be a waste to terminate them and begin the same pilot on a new group.

2. Addition of co-worker: Dr. Saadiah Goolam (Ophthalmologist)

j. Motivation for modification / extension:

1. Following the administration of the lower doses of cataract inducing agents in the 6 rabbits allocated for cataract development pilot study, it has been found that after a week, there are no visible signs of cataract on the lens when examined using a slit lamp microscope and

ophthalmoscope, therefore, this warrants for further observation and a possible increase in dose of the cataract inducing agents to ensure that a reproducible cataract development method is obtained.

3. Dr. Goolam will provide expert assistance with the study.

Date:20.07.2015 Signature:

RECOMMENDATIONS: Approved

i. Increase in dose from 0.01%w/v to 0.05%w/v.
ii. Increase in observation period from 1 week to 3 weeks. The rabbits should be examined at least weekly for the development of cataracts.
iii. Inclusion of Dr. Saadiah Goolam as a co-worker.

Date: 20 July 2015 Signature:
Chairman, AESC

AESC 2015 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Zikhona Hayiyana

b. Department: Pharmacy and Pharmacology

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2014	66	C
Other M&Es : A request is being made to use the 3 rabbits to continue the cataract development pilot			

d. Project Title: *In vivo* assessment of topical ocular drug delivery systems in rabbits

		No.	Species
e.	Number and species of animals originally approved:	51	*rabbits*
f.	Number of additional animals previously allocated on M&Es:	0	0
g.	Total number of animals allocated to the experiment to date:	51	51
h.	Number of animals used to date:	12	12

i. Specific modification / extension requested

(1) Use of the three rabbits originally intended for the testing of a higher dose

- A request is being made to use these rabbits at an age range of 4-5 weeks old. This is because literature suggests that younger rabbits are more prone to developing cataract as evidenced by their common use in similar studies (Peighambarzadeh S.Z. and Tavana M., 2014).

(2) Modification of cataract inducing agent administration procedures

- Rabbit one will receive a subcutaneous injection on the **neck** of 0.01%$^{w}/_{v}$ sodium selenite
- Rabbit two will receive an intravitreal injection of 0.01%$^{w}/_{v}$ sodium selenite by inserting a needle 4mm behind the limbus in sclera (measured using a sterile caliper)
- Rabbit three will receive an intravitreal injection of 120nmol/g diquat dibromide by inserting a needle 4mm behind the limbus in sclera (measured using a sterile caliper).
- The rabbits will be anaesthetized by ketamine before each injection, this eliminates pain and discomfort. However should any pain and discomfort present, opioids, NSAIDs or local anaesthetics may be used. Buprenorphine and carprofen are common choices (Kohn D.F. *et al.*, 2007). Local anaesthetics such as ketamine may also be used (Kohn D.F. *et al.*, 1997)
- The rabbits will be monitored and assessed for cataract development for 2 weeks or until cataract develops which may be earlier in younger rabbits.

j. Motivation for modification / extension:

- The results obtained with the cataract development pilot showed detectable lens opacification (mostly on the periphery) two weeks after administration of cataract inducing agents. By the end of three weeks, the lens opacification had progressed to involve the whole lens. The repetition of these administrations will also confirm the reproducibility of the cataract producing procedures before proceeding to a large scale study, i.e. The main study. Figure 1 below shows a clear lens (a) and a lens opacification (b).

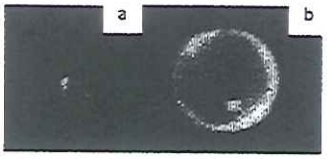

Figure 1: Images showing a normal clear lens (a) and a cataractous lens (b)

- Younger rabbits are more prone to developing cataract (Peighambarzadeh S.Z. and Tavana M., 2014). This means that using the younger rabbits may generate the cataract earlier.
- Some studies have used these optimized cataract inducing procedures which yielded positive results (Abdul-Hussein B.A. and Alzubaidy A.A., 2014)

References

1. Abdul-Hussein B.A. and Alzubaidy A.A., Role of Topically-Applied Zinc Sulfate in Prevention of Sodium Selenite-Induced Cataract in Rabbits, International Journal of Advanced Research 2(3): 1014-1022 (2014).

2. Peighambarzadeh S.Z. and Tavana M., 2014, Attenuation of experimental cataract by vitamin C in rabbits, WALIA 30(1): 204-207 (2014).

3. Kohn D.F., Martin T.E., Foley P.I., Timothy H Morris T.H., Swindle M.M., Vogler G.A. and Wixson S.K., Guidelines for the assessment and management of pain in rodents and rabbits, Journal of the American Association for Laboratory Animal Science 46(2): 97-108 (2007).

4. Kohn D.F., Wixson S.K., White W.J. and Benson G.J., Anesthesia and analgesia in laboratory animals, Academic Press, American College of Laboratory Animal Medicine Series (1997).

Date: 26.08.2015 Signature:

RECOMMENDATIONS: Approved
i. Pilot study using rabbits in age range of 4-5 weeks old.
ii. Interventions as described above.
iii. Liaise with the CAS regarding the use of analgesics during interventions.

Date: 27th August 2015 Signature:
Chairman, AESC