Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (http://www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate Research Centre
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 4 — Type of animal procedure: Pharmacokinetics (PK) of CoV antivirals in NHP

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

Before the evaluation of the efficacy of an antiviral compound in an NHP model for CoV infection, the pharmacokinetics of the compound different dosages of the compound need to be tested in order to determine the effective compound concentration in plasma, after administration (the 'bioavailability'). If the results of such a PK study are positive, then studies are planned to test the efficacy of the compound in macaques for use as a therapeutic drug (Appendix 4).
In general, the study set-up is as follows: a group of animals will receive the compound in different doses. Typically, one group of animals will be given the compound. This group will provide baseline data of the main PK parameter, like clearance of compound from the body, volume of distribution, and half-life of the compound. Then, experimental groups will receive the compound in different dosages. After administration, the animals will be bled at regular time points to determine the concentration of the compound in the blood.
The primary outcome parameters for the PK are:
1. the maximum concentration of the active compound in plasma, and
2. the total amount of the active compound in plasma in time

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

A typical PK study consists of groups of animals that are given the antiviral compound at different dosages and/or different routes of administration. One group will first receive the compound by intravenous bolus to derive the main PK parameters (clearance, volume of distribution, half-life). The other groups will be given the compound at different dosages. The route of compound administration in the latter groups will depend

on the antiviral compound, and will be done via intravenous administration, or orally. The used dosages will be calculated on basis of PK studies in other animal models. Typically, blood samples will be collected at regular time points until 24 h post-administration and will then be performed daily until the end of the study (maximally 7 days post-administration).
The PK study may be designed as a study in which the animals are first given a low dose of compound. Then, after a wash-out period during which the compound is cleared from the body, the animals can be administered a second, higher dose of the compound, and blood will be collected at regular time points. Plasma from blood samples will then be analyzed for PK parameters and, if applicable, for anti-compound immune responses.
As a follow-up to the single-dose PK study, animals may also receive antiviral therapeutics by repeated administration to determine if this prolonged bioavailability leads to either unwanted accumulation of the substance in the blood, or to an equally unwanted decrease because repeated-dosing influences the body metabolism. A prolonged bioavailability is essential when anti-CoV therapeutics are used as a prophylaxis, i.e. they should protect against CoV infection for a certain period. Essentially, blood sampling will be like a single-dose study, with multiple samplings the first day after administration, followed by daily blood collection until the next drug administration. The maximum number of procedures as outlined in the table below is based on the current state of the art, future experiments, however, may require higher maximum numbers. The details of each future study, regarding the NHP species used, route of infection, dose used, etc., will be submitted for approval to the Animal Welfare Body (Instantie voor Dierenwelzijn; IvD).
Table. Maximum number of repeats per procedure.

Procedure	Maximum	Duration
Sedation	40	15-120 min
IV bolus	15	30 min
Gavage (compound)	15	30 min
Tube feeding	15	30 min
Blood sample	45	30 min

Procedure	Maximum	Duration
Sedation	35	15-120 min
IV bolus	4	30 min
Gavage	12	30 min
Tube feeding	15	30 min
Blood sample	45	60 min

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

A group size of 3 animals is the standard group size for PK studies. From previous PK studies in macaques, it was concluded that experimental groups of three animals are sufficient to obtain a pharmacokinetic profile in blood of the compound. This group size is sufficiently large to allow insight in the variance between the individual animals and allows us to identify possible outliers.
Block randomization will be used for the group composition. The animals will be randomly allocated to treatment groups according to a randomized block design based on the sex and weight of the animals.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
4	Rhesus or cynomolgus macaque	Purpose bred	adult	48	M/F	Not applicable	Not applicable

Provide justifications for these choices

Species	The experiments will be performed in rhesus macaques (*Macaca mulatta*) or cynomolgus macaques (*M. fascicularis*). All animals are purpose bred at our institute, or incidentally they will be obtained from a certified supplier. Both mature male and female animals can be used. In contrast to other animal species that are used in virus research, i.e. rodents, NHP have the advantage that their body surface area/mass ratio, drug metabolism, pharmacokinetics, and anatomical structure are highly comparable to that of humans (1-5). Consequently, drugs are metabolized in a similar way in NHP as in humans, and also exert their mode of action similarly. This renders NHP an important preclinical animal model to investigate the pharmacokinetics of potential anti-CoV compounds for human use.
Origin	All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier
Life stages	Adult animals will be used
Number	A group size of 3 animals is the standard group size for PK studies. From previous PK studies in macaques, it was concluded that experimental groups of three animals are sufficient to obtain a pharmacokinetic profile in blood of the compounds. This group size is sufficiently large to allow insight in the variance between the individual animals and allows us to identify possible outliers. In a PK study 2 groups of 3 animals will receive 3 different dosages of the drug. Per compound we will test 6 different dosages (5 dosages + 1 standard control dose) given in two groups of 3 animals = 6 animals per compound. Then, for each compound, a repeat-dose administration is planned with a group of 3 animals. These animals will be selected from the animals already used in the single-dose PK study. Thus, for each compound we plan to use 6 animals. We plan to test 8 compounds over a 5-year period: N = 48
Gender	Males or females can be used
Genetic alterations	Not applicable
Strain	Not applicable

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

In case an animal suffers pain, oral or parenteral analgesia may be administered after consultation with the veterinarian.

Describe which other adverse effects on the animals' welfare may be expected?

1. Stress because of recovery from sedation
2. Reduced food intake due to repeated daily sedations for blood collection and compound administration
3. Discomfort due to administration of compound via gavage

Explain why these effects may emerge.

1. Animals will be repeatedly sedated for blood sampling and compound administration. Nausea can sometimes be observed during recovery from the sedation.
2. Animals will be sedated daily during the PK study for blood collection. Therefore, they may lose bodyweight during that period.
3. Insertion of the tube may cause local irritation

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1. Recovery of the animals is monitored, and the veterinarian will intervene if animals do not recover fast enough.
2. Animals will receive tube feeding via gavage (applied during sedation) or an adapted calorie rich diet. Insertion of the feeding tube will be done by experience caretakers. In case irritation occurs, this will be mild and no extra measures need to be taken

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☒ No > Continue with question F

☐ Yes > Describe the criteria that will be used to identify the humane endpoints.

Indicate the likely incidence.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

The cumulative discomfort is estimated as moderate. This is mainly caused by the daily sedations after compound administration, or potential side-effects caused by the compound.

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	All compounds have been tested extensively for efficacy and toxicity in *in vitro* assays. If possible, *in silico* modeling of the pharmacokinetics will be applied (6). This computer-simulation may result in a drastic decrease of the number of animals necessary, or even make *in vivo* studies superfluous. It is not yet known which antivirals will be investigated, and if *in silico* modeling is achievable for all compounds, but before *in vivo* PK studies will be performed, all other options, including *in silico* PK, will be investigated and the results will be presented to the AWB. In cases that *in silico* PK modeling is not feasible, *in vivo* toxicity studies will be performed in rodents. Then, only when no side effects or signs of toxicity are found in the rodent model, and if no other suitable animal model for anti-coronavirus compound testing is available, the compounds will be evaluated in nonhuman primates.
Reduction	In this animal procedure, the pharmacokinetic profile of anti-CoV compounds is determined in NHP, before their use in antiviral compound efficacy testing in the same animal species. A group size of 3 animals is the standard group size for PK studies. From previous PK studies, it was concluded that experimental groups of three animals are sufficient to obtain a pharmacokinetic profile of the compounds in blood of macaques. This group size allows us insight in the variance between the individual animals, and at the same time allows us to identify possible outliers. The number of animals needed will be kept to a minimum by testing of different dosages of a compound, successively administered to the same animal.

Refinement	Animals will be socially housed with a socially compatible cage mate. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food as per guidelines for macaques (7). A nutritious and calorie-rich diet is administered (or tube feeding) when the animals are daily sedated for blood sampling or compound administration. This reduces the possible negative effects on bodyweight of frequent sedation. Compound administration and blood sampling take place under sedation, and at the same time the animals will be weighed and examined. The animals are trained to collaborate as much as possible on invasive actions such as giving anaesthesia or compound administration. In consultation with our collaborators, the number of blood samplings, and the collected volumes of blood will be reduced to a minimum. The animals are trained to collaborate as much as possible voluntarily on invasive actions such as giving anaesthesia or compound administration. In consultation with our collaborators, the number of blood samplings, and the collected volumes of blood will be reduced to a minimum.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☐ No > Continue with question I.

☒ Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that will be used in these experiments, have possibly been used in previous procedures. Animals that have pre-existing antibodies against recently emerged coronaviruses are not suitable. In view of the long life of the animals of this species re-use of animals will take place within the limitations described in art 1e of the Wet op de Dierproeven.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☒ No > Provide information on the destination of the animals.

☐ Yes > Explain why it is necessary to kill the animals during or after the procedures.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☐ Yes > Will a method of killing be used for which specific requirements apply?

☐ No > Describe the method of killing.

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

References

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