		Inventaris Wob-verzoek W17-07								
			wordt verstrekt				**weigeringsgronden**			
	nr.	**document NTS 2016782**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	1	Aanvraagformulier				x		x	x	
	2	NTS	x							
	3	Projectvoorstel			x					
	4	Bijlage animal procedure 1			x					
	5	Bijlage animal procedure 2			x					
	6	Bijlage animal procedure 3			x					
	7	Ontvangstbevestiging				x		x	x	
	8	Mail verzoek om aanvulling				x		x	x	
	9	Mail verzoek om aanvulling DEC				x		x	x	
	10	DEC advies				x		x	x	
	11	Advies CCD		x						x
	12	Beschikking en vergunning				x		x	x	

Centrale Commissie Dierproeven

20 DEC. 2016

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☒ Ja > *Vul uw deelnemernummer in* 10700 / 782

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Maastricht university	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	50169181	
Straat en huisnummer	Minderbroedersberg	4-6
Postbus	616	
Postcode en plaats	6200MD Maastricht	
IBAN	NL04 INGB 0679 5101 68	
Tenaamstelling van het rekeningnummer	Universiteit Maastricht	

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]	☒ Dhr. ☐ Mw.
Functie		
Afdeling		
Telefoonnummer		
E-mailadres		

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	[redacted]	☒ Dhr. ☐ Mw.
Functie		
Afdeling		
Telefoonnummer		
E-mailadres		

1.6	*(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.	(Titel) Naam en voorletters	[redacted] ☒ Dhr. ☐ Mw.
		Functie	[redacted]
		Afdeling	[redacted]
		Telefoonnummer	[redacted]
		E-mailadres	[redacted]
1.7	Is er voor deze projectaanvraag een gemachtigde?	☐ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*	
		☒ Nee	

2 Over uw aanvraag

2.1	Wat voor aanvraag doet u?	☒ Nieuwe aanvraag > Ga verder met vraag 3
		☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.2
		☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn
		Vul uw vergunde projectnummer in en ga verder met vraag 2.3
2.2	Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?	☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier
		☐ Nee > Ga verder met vraag 3
2.3	Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?	☐ Nee > Ga verder met vraag 3
		☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 2 - 2017
		Einddatum	1 - 2 - 2022
3.2	Wat is de titel van het project?	Development of new pharmacological strategies to treat atrial fibrillation.	
3.3	Wat is de titel van de niet-technische samenvatting?	Ontwikkeling van nieuwe medicijnen voor de behandeling van boezemfibrileren	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-UM
		Postadres	Postbus 616, 6200MD Maastricht
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1441 Lege

☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	
Plaats	Maastricht
Datum	14 - 12 - 2016
Handtekening	

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht university
1.3	Provide the title of the project.	Development of new pharmacological strategies to treat atrial fibrillation.

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☒ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Atrial fibrillation (AF) is an arrhythmia characterized by fast (up to 600 beats per minute) and

irregular activations in the atria. In both AF patients and large animal models of AF, the arrhythmia is progressive in the sense that the duration of AF episodes gradually increases. The progressive, self-perpetuating nature of AF can be explained by the fact that AF causes changes in the atria that increase the stability of the arrhythmia. These changes include a shortening of the action potential duration and effective refractory period (electrical remodeling) and alterations in tissue structure (structural remodeling). The latter process includes altered connexin expression,[1] myocyte hypertrophy[2] and atrial fibrosis[3]. Whereas electrical remodeling takes place rapidly, within 1-2 days,[4, 5] structural remodeling progresses gradually over a time course of months[6, 7]. The stability of AF (measured as the duration of AF episodes or as the amenability to pharmacological cardioversion) increases over a comparable slow time course of months.[8, 9]
Using high-density epicardial contact mapping of AF, our group has shown that progression of structural remodeling and AF stability go hand in hand with an gradual increase in complexity of the AF conduction pattern, both in experimental and clinical studies.[9-15] With the duration of the arrhythmia, the number of simultaneous fibrillation waves and the occurrence of conduction block increase, leading to a more dissociated pattern of fibrillatory conduction.[9, 11]

Our aim is to test the efficacy and safety of a number of novel drugs targeted at ion channels. The larger context of this work is a European network, the 'TrainNet' ITN, in which a number of electrophysiologists, pharmaceutical companies and computer modellers collaborate. A major advantage of this approach is that compounds are currently being tested in cultured cells and computer models. This will allow us to identify the most promising compounds without requiring animal experiments and thus greatly reduce the number of animal experiments. However, the lead compounds will still have to be tested in an animal model with hearts that resemble the human heart in size, electrophysiological properties and AF propagation pattern.

The ion channels targets that have been identified for this project are:
- the small conductance calcium-dependent potassium channel, I_{SK}
- the acetylcholine-activated potassium current $I_{K,ACh}$
- the late sodium current $I_{Na,late}$
- the inward rectifier current I_{K1}
- combined sodium current (I_{Na}) and potassium current (I_K) blockade

In the current project, these novel antiarrhythmic strategies will be assessed in the same animal model under equal conditions. This will allow their relative merits to be evaluated and compared directly.

The small conductance calcium-dependent potassium channel, I_{SK}
Small conductance Ca^{2+}-activated SK channels, underlying the I_{SK} current, are unique among ion channels due to their ability to sense changes in intracellular Ca^{2+} concentration and couple this to cellular electrical activity. Traditionally, these channels have been thought to be almost ubiquitously expressed with the exception of the heart. However, recently published reports indicate that SK channels are present in cardiac tissue and can have very important functions both under normal conditions and in pathophysiological settings.[39] Importantly, pharmacological blockage of I_{SK} has an effect on the human atrial AP in isolated human muscle bundles.[40] In rodent models of acutely induced AF, SK channel blockade can protect against AF.[41] This altogether makes SK channels a very promising new target for treating AF.
As inhibition of SK channels can be obtained by different means (pore blocker versus allosteric modulator) an extensive pharmacological program is being executed within our network, and the lead compound from this approach will be tested in a goat model of AF.

In isolated perfused dog atria, I_{SK} blockade prolonged atrial repolarization, and thereby had a pro-arrhythmic effect.[42] However, in anesthetized dogs with electrical remodeling (one week of rapid atrial pacing), an I_{SK} blocker has a strong antiarrhythmic effect.[43] The effect in conscious animals and in models with structural remodeling has not been investigated. The effect of I_{SK} blockade on the fibrillation pattern in electrically remodelled atria has also not been investigated.

The Acetylcholine-activated potassium current $I_{K,ACh}$

The acetylcholine-activated K^+ ($I_{K,ACh}$) current, conducted through $I_{K,ACh}$ channels, is primarily expressed in the atria and the sinus node. $I_{K,ACh}$ is activated following vagal activation, through G_i receptors, which in the nodal tissue contributes to slowing of pacemaking and in the atria shortens the action potential. In myocytes from AF patients and animal models of AF, a constitutively activated current component is found upregulated[44]which has been suggested to enhance the arrhythmicity of the atria.[45] The fact that AF can be triggered by vagal activation further supports the notion that $I_{K,ACh}$ plays a pivotal role in the initiation and perpetuation of the arrhythmia.[46] Pharmacological inhibition of $I_{K,ACh}$ in pre-clinical models has been suggested to constitute an atrial-selective target for safe and effective treatment of AF.[47]

In a dog model of AF (electrical remodeling caused by 8 weeks of rapid atrial pacing with a controlled ventricular rate), two investigative $I_{K,ACh}$ blockers had a high efficacy for terminating AF.[48, 49] The effects in conscious animals and in models with structural remodeling have not been investigated. The effect of $I_{K,ACh}$ blocker on fibrillation patterns are not known.

The inward rectifier current I_{K1}

In both AF patients and in large animal models of AF, the stability of AF increases with time, meaning that the duration of AF episodes gradually increases with time, and that restoration of sinus rhythm becomes more difficult to attain. One of the earliest changes in the atria during AF is a dramatic reduction in the duration of the action potential. Concomitantly, the atrial refractory period decreases, leading to an increase in the activation frequency during AF. This process of so-called 'electrical remodeling' is thought to be responsible for the early phase of AF stabilization.[4, 50] Experimental studies have demonstrated that electrical remodeling is caused by changes in expression of various ion channels. The most important of these are the L-type calcium current (I_{CaL}, reduced by 70%), the transient outward potassium current (Ito, reduced by 70%) and the inward rectifier potassium current (I_{K1}, increased by 100%). In principle, all these changes can contribute to the decrease in action potential duration (APD). However, computer simulations show that the reduction in I_{CaL} and I_{to} only play a modest role, whereas the increase in I_{K1} has a major contribution to APD shortening.[51] Therefore, it is to be expected that blockade of this current will cause a much larger prolongation of the APD and be a provide a more effective method to terminate AF. We will test this hypothesis by testing the antiarrhythmic properties of an I_{K1} blocker.[52] The (poorly selective) I_{K1} blocker chloroquine terminated AF in perfused sheep hearts in which AF was induced with a high dose of acetylcholine[53] or by acute stretch[54]. The effects in conscious animals, and models with electrical and structural remodeling have not been investigated.

The late sodium current $I_{Na,late}$

The sodium current (I_{Na}), which mediates the upstroke of the action potential in the working myocardium, normally inactivates quickly and completely. However, under pathological conditions, I_{Na} may not inactivate completely, producing a depolarizing current during the plateau and repolarization that prolongs the action potential and increases the likelihood of arrhythmogenic afterdepolarizations.[55] This persistent sodium current, $I_{Na,late}$ is also increased in AF patients.[56, 57] Ranolazine, an older anti-anginal drug that also suppresses $I_{Na,late}$ has been effective against AF in

some animal models and perfused preparations.[55] However, ranolazine also blocks many other ion channels, complicating its use as an anti-arrhythmic drug.[58] Nevertheless, novel agents that block $I_{Na,late}$ more selectively remain a highly attractive strategy for the treatment of AF.[59] Therefore, we will test the efficacy of a selective blocker of the persistent sodium current in goat models of AF. The atrial effects of the selective $I_{Na,late}$ blocker GS-458967 have been investigated in perfused preparations.[60-62] The same blocker terminated AF in pig models of autonomically-triggered AF (combined administration of acetylcholine and epinephrine) under anesthesia[63] Eleclazine, another $I_{Na,late}$ blocker terminated AF in the same model[64] and in a pig model of ischemia-induced AF[65]. The effects of selective $I_{Na,late}$ blockers in conscious animals and models with electrical and structural remodeling, and the impact on fibrillation pattern in these models, have not been investigated.

Combined I_{Na} and I_K blockade

Of the conventional antiarrhythmic drugs, both I_{Na} blockers (e.g. flecainide) and I_K blockers (e.g. dofetilide) have been proven effective in early stages of AF.[8, 9, 66] The effects of both classes of drugs have been extensively characterized in animal models, including the goat model of AF. However, the efficacy of these drugs declines as structural remodeling progresses during later stages of the disease. Intriguingly, a recent publication using computer model of atrial tissue shows that a combination of I_{Na} and I_K blockers has a synergistic effect in terminating AF.[67] Although this is a promising concept, it has yet to evaluated in a relevant animal model of AF. As such, combined I_{Na}/I_K blockade can be considered as a state-of-the-art standard of the efficacy that can be attained with current antiarrhythmic drugs. As such, it will be included in the current project as a comparison with the novel drug targets detailed above.

Regional differences in ion channel expression

The expression of ion channels and the shape of the action potential show regional variability within the atria.[71,72] Most importantly, the expression level of several ion channels, and their relative change during electrical remodeling, is known to differ between the left and right atrium.[29, 30] These differences in the electrophysiology and electrical remodeling lead to a gradient in activation frequency between the left and right atria, and therefore leads to differences in their contribution to the maintenance of AF.[29-35] Previous studies have suggested the importance of the inter-atrial connections to AF maintenance,[36, 37] and their altered behavior in AF patients[38]. Furthermore, a recent study (un published own observations) revealed that those goats that were resistant to antiarrhythmic drugs had large electrophysiological differences between the right and left atrium. We believe that these gradients correspond to the relative contributions to the maintenance of AF. To obtain insight into the mechanism of cardioversion of novel antiarrhythmic drugs, we will determine that intrinsic contribution of left and right atrium by ablation of the inter-atrial connections (primarily Bachmann's bundle, the main right to left connection and the coronary sinus musculature) during sacrifice experiments.

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3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

Our aim is to test the efficacy and safety of a number of novel drugs targeted at ion channels. The larger context of this work is a European network, the 'AFib TrainNet' ITN, in which a number of electrophysiologists, pharmaceutical companies and computer modellers collaborate.
As detailed under paragraph 3.1, the ion channels targets that have been identified for this project are:

1. the small conductance calcium-dependent potassium channel, I_{SK}
2. the acetylcholine-activated potassium current $I_{K,ACh}$
3. the late sodium current $I_{Na,late}$
4. the inward rectifier current I_{K1}
5. combined sodium current (I_{Na}) and potassium current (I_K) blockade

In the current project, these novel antiarrhythmic strategies will be assessed in the same species under equal conditions. This will allow their characteristics and relative merits to be evaluated and compared directly in a number different models within the goat that reflect different populations of AF patients, as detailed above.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Atrial fibrillation (AF) is the most frequent cardiac arrhythmia seen clinically. In a patient suffering from AF, the regular electrical impulses generated in the heart by the sinus node are overruled by disorganized electrical impulses, making the atria "quiver" or fibrillate. As a direct result, patients suffer from palpitations, fatigue, reduced exercise tolerance and a diminished overall quality of life. This electrical disturbance also results in an uncoordinated contraction of the atrial muscle, whereby the atrial chambers do not empty properly. The result is increased risk for blood clots and thereby stroke and heart attack – this risk is increased up to five-fold as compared to a non-sufferer. Furthermore, the chaotic electrical activity of the atria is associated with additional comorbidities; i.e., it often impacts the larger chambers of the heart, the ventricles, resulting in ventricular arrhythmia. AF is thus generally associated with increased morbidity and mortality, and thereby results in reduced work capacity; both in terms of sick leave and early retirement.
AF occurs in between 1 and 2% of the general population. Currently, more than 6 million Europeans suffer from this arrhythmia, and its prevalence is expected to increase by more than two-fold during the next 40 years due to increased life expectancy of the general population. Thus, AF is said to assume epidemic proportions. The risk of the disease rises with age: about 5% of 65-year-olds and 10% of 75-year-olds are expected to suffer from AF. Due to the acute risk of blood clot formation, but also because initially short periods of AF (paroxysmal) become more persistent and

subsequently chronic,[4, 5] AF should be diagnosed and treated as early as possible. The unmet demand for effective and safe antiarrhythmics poses not only a serious public health problem, but also an economical one, as atrial fibrillation is associated with profound healthcare costs, annual costs related to management of AF estimated to reach €13.5 billion in the European Union.[68, 69] According to the European Society of Cardiology (ESC), at least 1% of the healthcare budget of European countries is currently spent on AF management.
Despite improvements in healthcare, the prognosis related to AF has not improved and, if anything, mortality and hospitalizations related to AF are increasing. Current options for pharmacological therapy are limited by both low efficacy and side effects, including life- threatening ventricular arrhythmias and severe extra-cardiac toxicities. The "Guidelines for the management of Atrial Fibrillation", as published by the ESC, call for an urgent need for development of safe antiarrhythmic drugs.[70] In particular, there is an unmet need for antiarrhythmic drugs that are still efficacious in structurally remodeled atria.
This research project will explore new therapeutic targets for the treatment of AF in man. We will in-depth evaluate the effect at different stages of AF and in combination heart failure. In case of positive results this study will allow the next step towards evaluation in humans.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

Justification of models of atrial fibrillation
As atrial electrophysiological properties differ substantially between different substrates for AF, the investigational compounds directed against these targets will be tested in goats with 3 weeks of AF, paroxysmal AF, in heart failure with AF and in goats with 4 months of AF. These animal models reflect distinctly different patient populations, i.e. patients with persistent and longstanding persistent 'lone AF' (AF without underlying structural heart disease) and AF with underlying structural heart disease.
Numerous previous studies have used dogs with rapid atrial pacing (RAP) for 6-8 weeks to study AF-induced remodeling. However, because dogs with RAP develop heart failure, the ventricular rate in most of these studies was controlled by creating AV block and pacing the ventricles at a slow rate. In this model, electrical remodeling develops fully, but the degree of structural remodeling is very limited, because atrial structural remodeling strongly depends on a high ventricular rate.[16-18] Results from this model therefore reflect a state of complete electrical remodeling, without significant structural remodeling. Goats with RAP, without ventricular rate control, do not develop heart failure, even after 6 months,[9] and are therefore more comparable to AF patients, in which progression towards heart failure during chronic AF is also a rare occurrence.
AF in goats will be maintained by RAP, without ventricular rate control, as previously described.[5, 6] We have demonstrated that 3 weeks of RAP in the goat model leads to persistent AF (i.e. non-self-terminating AF) with complete electrical remodeling, whereas 4-6 months of RAP is characterized by both complete electrical remodeling and significant structural remodeling.[9, 12] At this later stage, conventional anti-arrhythmic drugs are no longer effective, as a result of AF-induced structural remodeling.[8, 9]
In this project, CHF will be induced by rapid ventricular pacing (RVP). In patients, congestive heart failure (CHF) is a major risk factor for AF. About 50% of patients with severe CHF develop AF, often leading to a exacerbation of heart failure.[19] Atrial remodeling induced by CHF is distinctly different from that induced by AF. Although changes in atrial cellular electrophysiology do take

place, the atrial action potential does not shorten,[20, 21] and therefore electrical remodeling is not considered to be an important factor in the CHF-induced increase in AF stability. In contrast, widespread and dramatic atrial structural remodeling has been observed in CHF models.[20] The nature of atrial structural remodeling differ significantly between models of AF and CHF.[20] Whereas long-term AF causes endomysial fibrosis,[12] CHF causes large areas of replacement fibrosis.[20] The latter type of fibrosis typically occurs as a result of myocyte death, which does indeed in models of heart failure,[22] but probably plays a very minor role in AF-induced remodelling.[23, 24]
The difference between these variegated substrates for AF[25] is underscored by their differential sensitivity to antiarrhythmic drugs such as dofetilide[26] and rotigaptide[27], 'upstream' therapy such as pirfenidone and even electrical cardioversion.[28] Successful anti-arrhythmic drug treatment should be effective both in AF patients at early (electrical remodeling) and later (electrical and structural remodeling) stages of the disease, and in patients with AF caused by underlying structural heart disease, such as CHF.
To determine the range of efficacy mode of action of our novel anti-arrhythmic drugs, we will therefore test these compounds in the different stages of AF as patients may present themselves in the clinical ward. After a first evaluation (phase1) of relevant dosing, we will start (phase 2) with the least complex manifestation of persistent AF. This will be resembled by a group of goats with an AF-duration of 3 weeks. At this stage of continuous AF maintenance AF has become non-selfterminating. The AF stability in this group is entirely dependent the degree of electrophysiological changes. Therefore it is expected that this group of animals will have a high susceptibility to drug induced AF termination. Next, in case of positive findings, in phase 3 we will further investigate the compound in models of different type of fibrosis, endomydial fibrosis (around bundles) due to prolonged existence of AF or replacement fibrosis due to heart failure. Both conditions will promote AF stability yet with distinctly different AF patterns. Finally we will determine the electrophysiological profile of the compound in the first days of paroxysmal (self terminating) AF.

Strategy
This research project is part of a European network focused on the development of new pharmacological strategies for the treatment of AF. Within this network we have a collaboration between cellular electrophysiologists, computer modelers and toxicologists. New compounds will be first tested in in vitro settings. Compounds will only be evaluated in this study if they pass toxicology tests and exhibit promising results on cellular electrophysiology. These lead compounds will be evaluated in a phased study design consisting of 3 phases.

In **phase 1**, appendix 1, we will determine the pharmacokinetic properties of the potential compounds to assess a relevant dosing regime in the goat and goat specific safety. For targets 1 to 4 (see section 3.2) one compound will be identified for further evaluation.

In **phase 2,** appendix 2, we will determine the effect of the compound(s) when AF has become non-self-terminating. Here we will explore the drug potency by performing AF termination experiments in the awake goat. Moreover, we will address basal electrophysiological properties to unravel the compounds mechanism of action at the organ level.
Based on the current potency of antiarrhythmic drugs it is expected that AF will not terminate in all goats. The resistance of AF to the drugs might be caused by cross talk between the left and right atrium. To address this potential mechanism we will measure the electrical activity at a multitude of locations on the atria. In case of left to right gradients a second group of experiments will be

included. Here we will explore the significance of the Bachmann's bundle in interatrial cross talk by performing bachamnn's bndle ablations.

In **phase 3**, appendix 3, we will further address the effects of the compounds in 3 different models of AF (paroxysmal AF, CHF combined with AF and 4 months of AF). These models reflect the different underlying structural and electrophysiological properties in different patients populations seen in clinic.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

All studies will be performed in chronically instrumented goats allowing for repetitive electrophysiological measurements in the same experimental animal. The effects of investigational compounds on AF stability, conduction patterns and refractory period will be investigated. Also, the electrophysiological mechanism of cardioversion will be studied in these groups of goats by extensive epicardial mapping. These data and MRI data of the atrial tissue will be fed into the 3D computer models for AF, which will be used for further testing and development of antiarrhythmic drugs, potentially reducing the number of animal experiments required in future drug development.

Phase 1 (appendix 1) pharmacokinetics
The pharmacokinetic properties of the lead compounds will be evaluated in this protocol. The pharmacokinetics will be investigated in healthy goats without arrhythmia. During administration of the compound, body surface ECG recordings are performed to assess global cardiac effects. Throughout infusion and washout repetitive blood samples are taken to determine the plasma concentration. Based on these data we will establish a pharmacokinetic model that will be tested in a final experiment. During this experiment we will also perform electrophysiological measurements to screen for an antiarrhythmic potential.
These investigations will deliver the following outcome;
1. Clinical relevant dosing regime,
2. Demonstration of a potential atrial antiarrhythmic effect,
3. General cardiac effect on the ECG,
4. Safety within the goat.
Go/no go criteria to move on to phase 2 are;
1. Proof of an antiarrhythmic potential in normal atria,
2. Safety in the goat.

Phase 2 (appendix 2) effect on non-selfterminating AF in a substrate of electrical remodeling only.
Here the first evaluation of the antiarrhythmic effects of the compound(s) during AF will be assessed. We will use the goat model of short term atrial fibrillation. Goats will be implanted with electrodes around the heart for the induction and acquisition of atrial signals. AF will be induced by means of continuous monitoring of the rhythm. Consequently, the heart rhythm is paced into AF once sinus rhythm is detected. Continuous monitoring and automated pacing into AF is maintained until AF becomes non-selfterminating. Once AF has reached this point of stability, AF termination experiments will be performed. About 1 week later a final open chest experiment will be performed for detailed quantification of electrophysiological properties. High coverage and density electrical mapping, measurements of electrical activity at >500 electrodes, will give insight into the electrical

dynamics (e.g. number of waves, activation frequency, etc) of both atria. In case right to left differences are observed, we will investigate the significance of the inter-atrial interaction through the Bachmann's bundle in a second group of experiments. Here we will investigate the effect of Bachmann's bundle ablation on AF dynamics and the potency of the compound(s).
These investigations will deliver the following outcome;
- Efficacy of the new compounds to terminate persistent AF with electrical remodeling only,
- Insight into the mechanism of AF termination by these compounds,
- Identification of the relevance of interatrial cross talk for AF maintenance in the presence of the antiarrhythmic compound.

Go/no go criteria to move on to phase 3 is:
- Proof of the ability to terminate AF

phase 3 (appendix 3) effect on non-selfterminating AF in a substrates of electrical and structural remodeling.

Once the compound(s) has proven the effective to terminate AF we will further address its effects in 3 other models of AF. The compound(s) will be investigated in **1) Paroxysmal AF, 2) short term AF in combination with heart failure** and **3) non-selfterminating AF with electrical remodeling and structural remodelling**. For all models electrodes will be implanted around the heart, ventricle and atrium. The location and duration of pacing is varied dependent on the different models.

1) In the model of **paroxysmal AF** multiple awake experiments will be conducted to describe the effects in a changing electrical substrate. The goats will be implanted with electrodes on the atria and ventricle for sensing and pacing. The electrodes will exteriorized through a cable between the shoulder blades. This will allow computer programmed AF induction as above described.
During paroxysmal AF fast changes in ion current expression occurs. The effect of an antiarrhythmic drugs may vary strongly dependent on the time point of administration. Therefore we will investigate the compound(s) at 3 early time points of AF induced remodelling. In the first time point, sinus rhythm, the heart has normal electrical properties. The second time point will be after about 2 days of AF inductions. Here the episodes (paroxysms) are still relatively short (minutes) and the ion currents have undergone partial remodeling. The final time point will be at about 10-14 days of AF where remodeling of the ion currents is complete and AF episode durations are in the magnitude of hours. Remodeling in this stage of AF is still fully reversible. Therefore multiple compounds can be investigated in the same animal.
In phase 2 we evaluated the effect of new compound on 5 newly defined targets. Here we will add another target in which we investigate the combination of antiarrhythmic drugs. Note that basic properties are already described in literature and therefore do not need to go trough phase 1 and 2. If for a combination a synergistic effect can be described we will do a study with this combination in phase 2 with non-selfterminating goats.

2) In a model with **heart failure and short term AF**
The goats will be implanted with electrodes on the atria and ventricle for sensing and pacing. The electrodes will be exteriorized through a cable between the shoulder blades. This will allow computer programmed AF induction as above described. In combination with AF heart failure will be induced. Rapid pacing of the ventricles will induce heart failure. In this model of structural remodeling, characterized by replacement fibrosis.
We will assess the potency of the compound(s) to terminate AF. Furthermore, we will explore electrophysiological effects in detail in a final experiment. Similar experiments to phase 2 are

performed in these goats.

3) **Non selfterminating AF with structural remodelling**

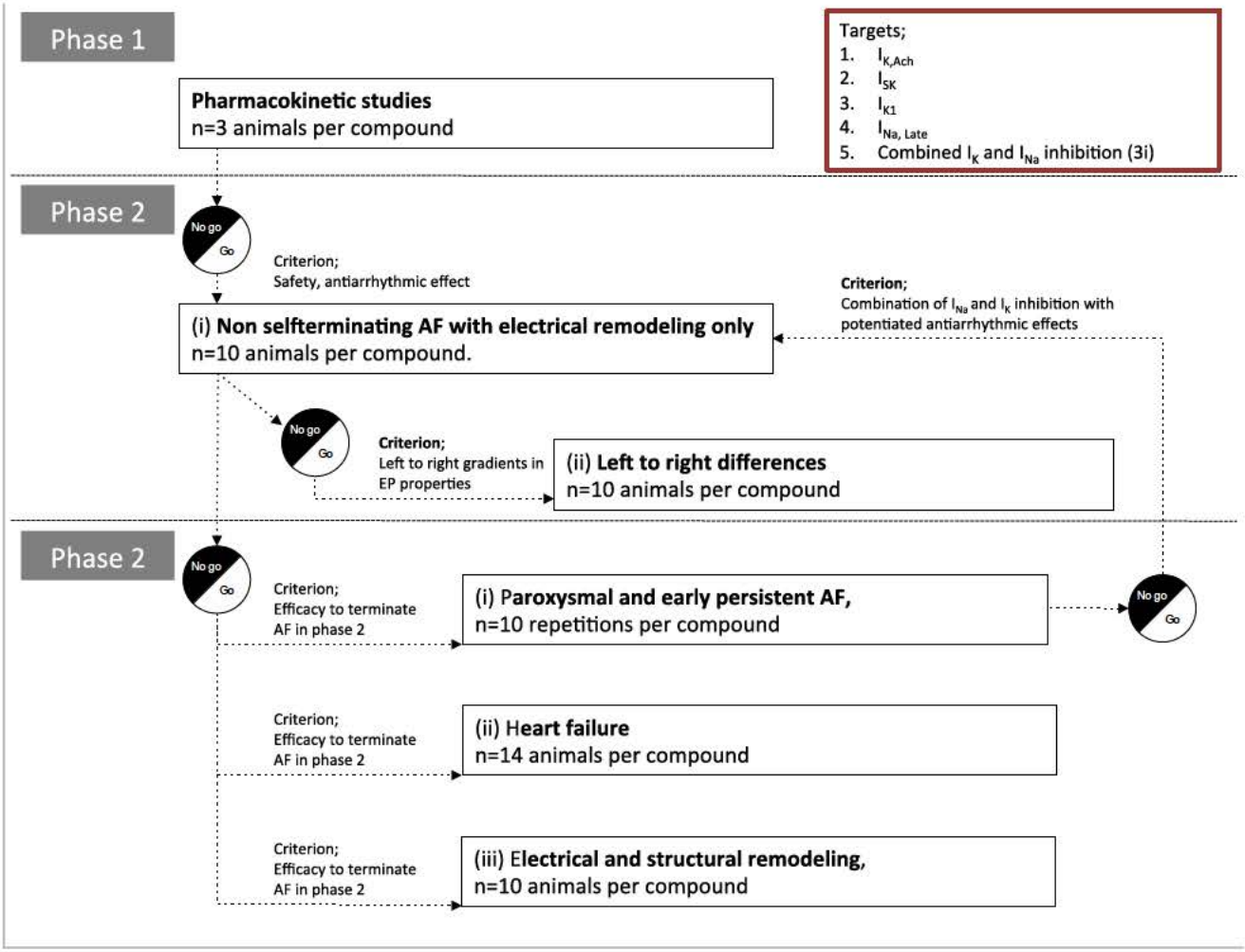
In the experiments with non-selfterminating AF with structural remodeling, we will monitor AF stability by evaluating the amenability to pharmacological termination. The goats will be implanted with electrodes on the atria and ventricle for sensing and pacing. The electrodes will be exteriorized through a cable between the shoulder blades. This will allow computer programmed AF induction as above described.

To monitor the amenability to pharmacological termination we will perform multiple AF termination experiments to identify the point where AF became resistant to antiarrhythmic drugs. To take structural changes into account as a possible cause of this resistance we will perform MRI scans of the atria. Scans to quantify the degree of structural remodeling will be performed at two time points, before AF induction and when AF has become resistant to the antiarrhythmic compound. In a finale experiment (comparable to phase 2) detailed information on the electrophysiological effect will be obtained.

These investigations will deliver the following outcome;

1. Identification if a compound has an antiarrhythmic potential at different stages of AF induced remodeling and in the presence of heart failure
2. Evaluation of the possible synergistic effect of a combination of drugs,
3. Acknowledgement if a compound can both terminate and prevent AF

A schematic overview of the project design is given below.



3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

Atrial fibrillation is an arrhythmia with a progressive nature. It starts with short-lasting episodes and progresses into a persistent state. Different aspects of AF-induced remodeling have been described to contribute to a changing substrate for this arrhythmia. However, other morbidities such as heart failure also develop a substrate for AF stabilization. The efficacy of currently available drugs are limited and dependent on the underlying substrate. An important consideration for the treatment of these patients with conventional drugs is the propensity to induce lethal ventricular arrhythmia. This project aims to establish new pharmacological treatment therapies for patients with atrial fibrillation. More specifically we aim to develop more potent and safe strategies. We have identified 5 atrial (anatomy) and/or AF specific (arrhythmia) targets to treat AF. Targets that are not exploited in current treatment options. We will investigate compounds that affect these targets in different models of AF that reflect the different patient conditions as seen in the cardiology ward. We have chosen for a phased design to explore the compounds for safety and efficacy. In phase 1 we will establish the pharmacokinetic action of the compound. The milestone in phase 1 is to prove an antiarrhythmic potential. In phase 2 we will study in detail the electrophysiological effect on AF itself. Milestones here are termination of non-selfterminating AF and a well-defined characterization of its antiarrhythmic mechanism. In phase 3 we will extend our understanding on the antiarrhythmic effect in different more complex atrial substrates. This might contribute to a future strategy that might be applicable in all patients. Otherwise our research

strategy can identify the appropriate target for a specific substrate.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Pharmacokinetic studies
2	Non-selfterminating AF with only electrical remodelling
3	Effects in paroxysmal AF, heart failure and permanent AF
4	
5	
6	
7	
8	
9	
10	

Centrale Commissie Dierproeven

4

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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht university
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Pharmacokinetic studies

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.

This study is designed to explore new therapeutic targets for the treatment of atrial fibrillation. We aim to specify the effect of potential atrial specific targets. In this appendix we will investigate the pharmacokinetic properties, the safety and antiarrhythmic potential of the newly developed compound in the goat. These compounds are developed by our academic and industrial partners within the research network 'AFib trainet'. The compounds they identified as (most) potent on atrial cellular electrophysiological properties with no or limited effect on cells originating from the ventricle, are potential candidates. Further, all compounds are screened for biological safety before they are tested in the goat. Thus compounds choosen have proven antiarrhythmic potential with an profile that has a high biological safety.

The targets that we will asses are:

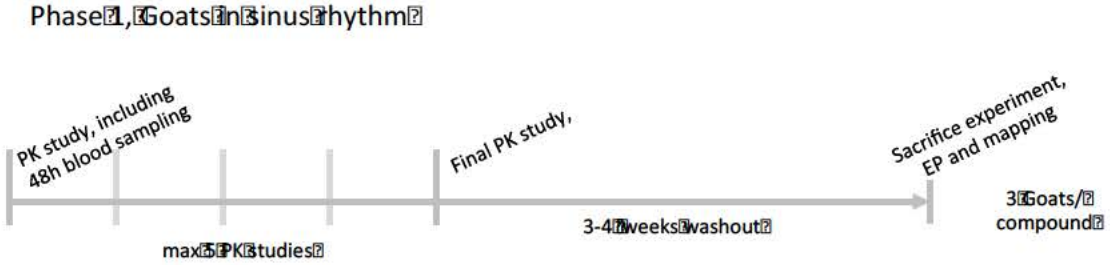
1. $I_{K,Ach}$
2. I_{sk}
3. I_{K1}
4. $I_{Na,Late}$

The primary outcomes of these investigations are:

1. Clinical relevant dosing regime in goats,
2. Demonstration of a potential atrial antiarrhythmic effect,
3. General cardiac effect on the ECG,

4. Safety within the goat.

Based on these studies we will decide wether to continue with the investigation of the compound. The continuation of a compound to the second phase (appendix 2) will only be performed if the



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

Pharmacokinetic study
Pharmacokinetic studies (PK) are performed to determine the optimal drug infusion regimes to reach relevant predicted plasma levels, based on *in vitro* evaluations. Healthy untreated goats will be infused with the compound of interest. For the infusion of the drug we will place a intravenous line in one of the superficial veins. In consultation of a pharmacologist, dosing and infusion rates will be determined. During infusion, goats will be monitored with continuous ECG recordings and visual inspection of the physical signs. During the infusion and washout multiple blood samples will be taken to obtain plasma levels. Blood samples will be taken up to 48 hours after infusiuon. In the particular case that the (expected) half life of a compound is >24hours, we will draw samples (max15) up to 5 days after the infusion. In 1 goat, up to a maximum of 5 PK studies will be perofrmed. PK studies will be executed with at least 1 week intervals. About 5 ml blood samples will be sufficient for the determination of plasma concentrations. Thus the maximal amount of blood withdrawel will be about 75ml in 2-5 days. After the last PK study, we will wait 3 to 4 week for the washout.

Sacrifice experiment
After accomplishment of the pharmacokinetics, the goats will undergo a final experiment. In this terminal experiment the effects on the surface of a healthy heart will be measured. For this terminal experiment the goat will be anesthetized. The chest will be opened to expose the heart for direct measurements of the electrophysiologic parameters. Additionally catheters will be introduced to monitor the blood pressures. Once all electrophysiological measurements will be performed, the heart will be excised for further *in vitro* analysis.

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

This phase of the project can be considered as a pilot phase. We will limit the number of animals per compound to 3. Previous studies in goats suggested that 3 animals are sufficient to model the pharmacological dynamics of different compounds. Only if the PK experiments prove to be safe and give an indication of an antiarrhythmic potential, the second phase will be initiated.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Species

We will make use of the goat model of AF, which is essential for the later phases of this project. These experiments will provide essential information about dosing in the goat. Since the experiments in phase 2 will be executed in goats, we also chose to determine the pharmacokinetics in the goat in the first phase. We chose the goat model of AF because it is well characterized and closely resembles human electrophysiology elementary characteristics such as cycle length, refractory period and number of waves.[9-13] Heart size is crucial for AF stabilization. Smaller hearts (e.g. rodents) can not harbour chronic AF. The heart weight/size of a goat truly resembles humans heart. In a currently running project on goats, the heart weight was 323±65g, in accordance with a recent publication (2012) by the "American journal of forensic medical pathology", where Molina et al. found that the average heart weight for adult humans is 331±57g.

Sex

Female goats will be used for this study. We chose the female gender because of:

1) Goat studies in literature, for example reference 4-9 and 12-16, are based on female goats;
2) In contrast to male goats, the tranquil nature of female goat behaviour allows measurement in awake state;
3) The availability of male goats is limited. Most male goats are slaughtered for food consumption at young age.

Origin

The goats will be purchased from a local goat breeder.

Number of animals.

Pharmacokinetic studies will be performed in 3 animals per compound. Currently, the potential compounds are under investigation in *in vitro* studies. It might be that for each target more than one compound will be identified to have a relevant antiarrhythmic potential. We will chose the compound with the highest potential to be evaluated in the goat. However, if (although unforeseen) side effects will occur or lack of an electrophysiological effect will be shown in sacrifice experiments, we would desire to test a second best compound, according to the *in vitro* screening. Therefore, we will test a maximum of 8 compounds.

We will investigate 1 compound per target. We would like to investigate 4 targets with essentially 4 compounds (one per target). However, as mentioned above, it might occur that the compound of our first choice will not have the desired of effect or may be inappropriate in the goat. In that case we would like to be able to test our second best candidate. That results in 4 targets X 2 compounds leading to 8 conditions. Thus, 8 conditions X 3 animals make a **total number of 24 animals.**

The nature of these experiments and analysis of blood samples are simple. We believe that adjustment of the numbers is not needed on forehand.

Life stage

Size and age are important determinants for AF stabilization. Therefore, we will use adult goats with a limited range of age. Approximately 1-5 years.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

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.

D. 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

These experiments are the first step towards to test the effect of the componds on AF. In preparation of testing the therapeutic benefit of the selected compounds on AF, we will first determine pharmacokinetic properties in heathy goats. The goat is choosen with future experiments, described in appendix 2 and 3, in mind because it is a representative model of human AF. In AF a complex interaction between physiological systems in the body (inflammation, hemodynamics, neurohumoral etc.) are involved. At later phases (appendix 2 and 3) of this project, the different stages of AF-induced remodelling are crucial and therefore we need to obtain specific pharmacokinetic knowledge. This project can only be investigated in an *in vivo* model with intact physiology. The *in vitro* therapeutic targets are identified by our collaborators within this research. All targets within this study have been extensively studied in *in vitro.*

Both large and small animal models are considered. Models in rat and mice are suitable to investigate processes on a cellular level. However, due to their small heart size, any non-self-terminating AF cannot be induced. It is, instead, of great importance in this project to investigate the efficacy of the compounds in stable non-selfterminating AF. For larger animal models, dogs, pigs, sheeps and goats can be considered. The drawback of dog and pig models of AF is the fast ventricular rate leading to heart failure. Therefore these models are in principle a combination of AF and heart failure. Alternatively, goats and sheep can be chosen. We have several arguments to prefer the goat model. Firstly, most AF research in awake animals is obtained in goats. Secondly, goat hearts tend to be a bit bigger resembling more the human heart size. Finally, we have a large expertise in goats and our research facilities and equipment has been developed to perform measurements in the goat.

Reduction

Reduction will be achieved by choosing the minimal number of animals to base our pharmacokinetic models on. Multiple PK studies will be done in the same animal, thereby reducing total number of on animals needed. Moreover, these experiments are a crucial go/no go time point in this project.

Refinement

The interventions in the goats will lead to limited discomfort. During the time window of frequent blood sampling an intravenous line will be placed. This will lead to the reduction of vein puncture required during blood sampling. Goats will always be group-housed in order to keep them in normal housing conditions. During the terminal experiment the animals will receive adequate general anaesthesia.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Upon arrival, the goats will be housed in the animal facilities to get familiar their new surrounding and care takers. Goats are domesticated animals and get quickly habituated to their new

surrounding and contact to humans. The goats will be housed with congeners and group housing. General anaesthesia (including analgesia) will be used during the terminal experiments.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Target 1-3 are compounds which are newly developed. We have a direct collaboration with the developers of these compounds. Because of this short linik we will be the first to test the compound in a large animnal model.
All targets we will investigate in this project are also reconized by others as possible new therapeutic strategies. Individual currents and inhibition of them were investigated in a range of different electrophysiological models. Most of the published data is derived from isolated cells or wedge preperations. Only occasionally the measurements were performed in intact animal models. These models have in common that AF was always acutely induced, e.g, by stretch or autonomic stimulation. These models do not create a substrate of persistent AF (clinnical setting) but need continuous drug administration for AF maintenance. Furthermore, data described in the whole animal models reflect local cellular properties but lack information on conduction properties during AF itself.
To obtain the latest state of knowledge we performed a literature review based on Pubmed.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

X No

Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

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

X No > Continue with question H.

☐ 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

During the pharmacokinetic studies, repetitive blood sampling is needed. This will lead to limited discomfort and, to our opinion, does not need additional pain relieve.
For the terminal experiment general anaesthesia (including analgesia)will be used. Ecg, respiration gasses, blood pressure and physical appearance will monitor the depth of anaesthesia.

I. Other aspects compromising the welfare of the animals

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

Adverse events due to drug administration.

Explain why these effects may emerge.

Despite toxicity screening, these compounds might lead to unexpected adverse events. These side effects might be species specific and therefore not detected in the toxicity screening phase of the drug development.

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

Firstly, all drugs underwent toxicity screening before tested in our model. Secondly, the pharmacokinetic study will be tested in a low dosage.
The goats will be frequently monitored (ecg, breathing, physical appearance) to minimize severity of possible adverse.

J. 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 K.

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

- Loss of normal body condition as scored with body condition inqueries and or bodyweight loss of 25% compared to the body weight before the start of experiments. These end points apply over the whole period the goats are in protocol.
- Adverse drug events caused by the administered compound, such as neurological or gastrointestinal tract anormalities.

Indicate the likely incidence.

Body weight (5-7.5%)
It is unlikely that goats will loose weight due to the pharmacokinetic studies. Possibles other reasons for body weight reduction are a lack of habituation or undelying dissease gained in earlier stages of life. In DEC project a similar model was used and here 2 out of 32 goats were taken out of experiment because of a reduced body weight.

Adverse events (<1%)
We expect the likelihood of reaching humane end point to be very small. Experience with other antiarrhythmic compound has so far never led to these end points.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

The goats will experience discomfort because of 2 components in this protocol.
1 Pharmacokinetic studies → infusion line placement for blood sampling and compound infusion
2 Sacrifice experiment → induction of anaesthesia
We believe that pharmacokinetic studies causes the highest degree of discomfort.

For these pharmacokinetic studies the expected level of discomfort is **mild**.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The animals will be sacrificed during the final anesthetized experiment. This is essential to obtain relevant electrophysiologic measurements for the go/no go decision.

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

Centrale Commissie Dierproeven

5

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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht university
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Non-selfterminating AF with only electrical remodelling

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

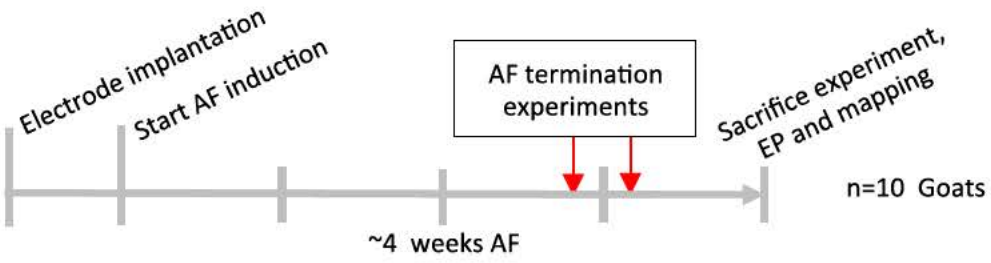
Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

This study is designed to explore new therapeutic targets for the treatment of atrial fibrillation. We aim to specify the effect of potential atrial specific targets. These targets are either trans-membrane ion currents uniquely expressed in the atrium or an optimized combination of sodium and potassium currents. In this appendix we will investigate the antiarrhythmic properties of the compounds in a model of persistent AF. Persistent AF is the stage of AF progression to a level of stability that AF does terminate spontaneously.

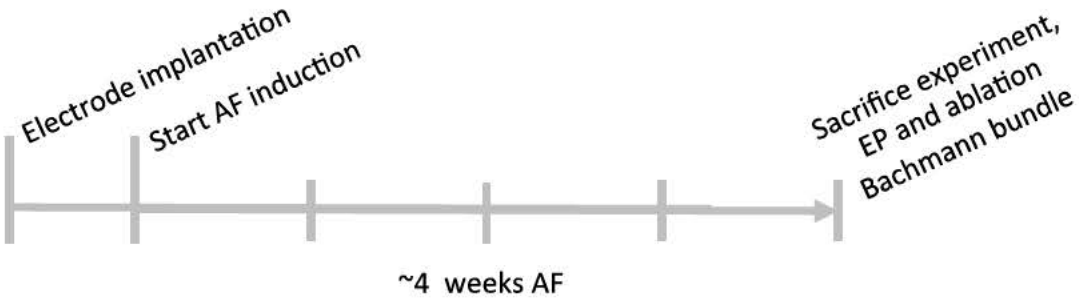
Phase 2i

Goats will undergo an implantation procedure by which electrodes are placed on the heart for the measurement of electrophysiological properties. A 2-3 weeks of recovery will be taken into account before the actual experiment starts. First the goats will be stimulated (paced) to induce AF. For the induction of AF the goat will be connected to an automated computer protocol. This system is able to detect normal sinus rhythm. The goat will be paced into AF once sinus rhythm is detected. The AF episodes will first last for short periods (seconds to minutes) but will progress into non-selfterminating (persistent) AF in about 2-4 weeks. This remodelling process is termed AF begets AF and accompanied by changes in electrophysiology, autonomic balance and mild structural adaptations. When AF has become non-selfterminating up to 2 AF termination (cardioversion) experiments will be performed in the awake goat. These two experiments will be performed in two consecutive weeks. During these AF termination experiments iv-lines, for compound administration and blood sampling, will be placed and atrial and ventricular signals continuously recorded. The

i


Electrode implantation
Start AF induction
AF termination experiments
Sacrifice experiment, EP and mapping
n=10 Goats
~4 weeks AF

ii



The groups and targets we will assess are dependent on the outcome 2i. Only those compounds expressing large interatrial differences in effect on the cycle length and conduction patterns of the arrhythmia will be investigated phase 2ii. In theory this might be group 1-7 from phase 2i.

The primary outcomes for phase **2ii** are;

1. We will identify differences in regional effect of the antiarrhythmic compounds,
2. We will quantify the degree of interatrial cross talk during persistent AF,
3. We will determine the relevance of interatrial cross-talk for AF maintenance in the presence of the antiarrhythmic compound.

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

Electrode implantation
The implantation will be performed under general anaesthesia. A single left sided thoracotomy will be performed to expose the left atrium. A plaque containing multiple electrodes for sensing and pacing will be placed on the heart. Before closure of the thoracic cavity signal quality will be tested. This test is performed to assure signal quality and prevention of both ventricular and phrenic nerve stimulation during AF induction. With the electrodes in the correct position, the chest will be closed and the electrode cable will be tunnelled to the back of the goat where the cable is exteriorized. A 2-3 weeks of recovery will be taken into account before the actual experiment starts.

AF maintenance
After a recovery period, the goats will be individually housed in specially designed cages for AF induction and monitoring. This restricted housing only allows one animal per cage. However, the cages are designed in such a manner that animals can see, hear and smell each other. This type of housing will be maintained until AF becomes persistent(approximately 2-4 weeks).

AF termination experiment
Cardiac signals, both atrial and ventricular, will be recorded troughout the experiment. Signals can be obtained by connecting the goat to a cardiac amplifier. In this experiment i.v. lines are placed for the infusion of the compound and blood sampling. First, measurements of baseline conditions are measured during infusion of vehicle only. After these measurements the recording is continued during infusion and part of washout of the drug. The experiment may take up to 3 hours.

Sacrifice experiment
The goats will undergo a final experiment after washout (>5 half-lives) of the last AF termination experiment. In this terminal experiment the effects on electrophysiological properties will be measured. For this terminal experiment the goats will be anesthetized. The chest will be opened to expose the heart for direct measurements electrophysiologic parameters. Additionally catheters will be introduced to monitor blood pressures. Once all measurements are performed, the heart will be excised for in vitro analysis.

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

Study design
We have considered using the baseline conditions of the individual goats to determine the effect size of the compounds. However, the measurements of the electrophysiological properties are time-consuming. This might introduce a time affect on the measurements due to unknown factors or factors that are difficult to control. Therefore we believe it is desirable to include a time matched control group. Nevertheless for each individual animal repeated measures are conducted. This will allow the use of a linear mixed model. The advantage of this approach is that subjects with missing data point(s) will not be dropped from the analysis, leding to a reduction in the number of animals.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Species
We will make use of the goat for or electrphysiological studies. Models of different stages of AF in the goat are well characterized and closely resembles human electrophysiology elementary characteristics such as cycle length, refractory period and number of waves.[9-13] Heart size is crucial

for AF stabilization. Smaller hearts (e.g. rodents) can not harbour chronic AF. The heart weight/size truly resembles the size of humans. In a currently running project the heart weights was 323±65g in contrast a recent publication (2012) in the American journal of forensic medical pathology Molina et al. found an average heart weight of 331±57g.

Sexe
Female goats will be used for this study. We choose the female gender because of;

1) Goat studies in literature, for example reference 4-9 and 12-16, are based on female goats;
2) In contrast to male goats, the tranquil nature of female goat behaviour allows measurement in awake goat;
3) Availability of male goats is limited. Most male goats are slaughtered for consumption at young age.

Origin
The goats will be purchased from a local goat breeder.

Number of animals.
Phase 2i Historic data on drug studies has shown that 8 goats are sufficient to demonstrate relevant differences after drug administration[8,9]. Elementary electrophsysiological properties like refractory period, fibrillation frequency and conduction velocity of fibrillation waves determine the number of animals. Based on previous experiments we expect a dropout of about 15%. Dropout might occur due to electrode failure, infection and technicalities during the extensive terminal experiment. We need **10 animals/group** after the correction for dropout.
The total number of animals will depend on decisions made based on our go/no go criterions.
The maximal number animals is **70goats**

Phase 2ii. The same number of animals per group apply to phase 2ii. We do not know on forhand if right to left differences are stable or specifically altered after the administration of the compounds. If we assume that all drugs have comparable effects in the left and right atrium two groups of goats (1 compound (group 1-5) and 1 control groups (7)) in phases 2ii will suffice. If different regional effects will be observered for the different compounds we might need to do these experiments for all compounds.
The maximal number animals is **60goats**

Life stage
Size and age are important determinants for AF stabilization. Therefore, we will use adult goats with a limited range of age. Approximately 1-5 years.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

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.

D. 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
The complex interaction between physiological systems in the body (inflammation, hemodynamics, neurohumoral etc.) are involved in AF induced remodelling. At later phases of this project the different stages of AF induced remodelling are crucial and therefore need to apply to this group as well. Therefore, this project can only be investigated in an *in vivo* model with intact physiology. The *in vitro* therapeutic targets are identified by our collaborators within this research. All targets within this study have been extensively studied in *in vitro.*
Both large and small animal models are considered. Models in rat and mice are suitable to investigate processes on a cellular level. However, due to their heart size no non-selfterminating AF can be induced. It is of great importance in this project to investigate the efficacy of the compounds in stable non-selfterminating AF. For larger animal models the dogs, pig sheep and goats can be considered. The drawback of dog and pig models of AF is the fast ventricular rate leading to heart failure. Therefore these models are in principle a combination of AF and heart failure. Alternatively, goats and sheep, who do not develop heart failure, can be chosen. We have several reasons to prefer the goat model. Firstly, most AF research in awake animals is obtained in goats. Secondly, goat hearts tend to be a bit bigger resembling more the human heart size. Finally, we have a large expertise in goats and our research facilities and equipment has been developed to perform measurements in the goat

Reduction
This project application constitutes a phased design. Moreover, we have choosen for a repeated meausres protocol where for large pasrt the gopat can serve as its own control. In combination with a linear mixed model we will achieve high power with limited number of animals.

Refinement
The animal model has been developed in our laboratory. Over the past two decades we have adjusted a number of aspects concerning the procedures. For instance, by refining the electrode design we are able to prevent infections at the porte d'entrée. Redesign of cages allows the goats the have some freedom of movement when they are housed in their monitoring cages.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Upon arrival the goats will be housed in the animal facilities to get familiar their new surrounding and care takers. Goats are domesticated animals and get quickly habituated to their new surrounding and contact to humans. After surgery the goats need to be housed individually because group housing has a high risk of electrode damage. To limit this the effect of this restriction goats will always be housed with congeners. They will be able to see and each other.

General anaesthesia (including analgesia) will be used during implantation and the terminal experiments. Peri and postoperative analgesia will be applied accordingh to the 2015 GVSOLAS guidelines for pain management of laboratory animals.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Target 1-3 are compounds which are newly developed. We have a direct collaboration with the developers of these compounds. Because of this short linik we will be the first to test the compound in a large animnal model.
All targets we will investigate in this project are also reconized by others as possible new therapeutic strategies. Individual currents and inhibition of them were investigated in a range of different electrophysiological models. Most of the published data is derived from isolated cells or wedge preperations. Only occasionally the measurements were performed in intact animal models. These models have in common that AF was always acutely induced, e.g, by stretch or autonomic stimulation. These models do not create a substrate of persistent AF (clinnical setting) but need continuous drug administration for AF maintenance. Furthermore, data described in the whole animal models reflect local cellular properties but lack information on conduction properties during AF itself.
To obtain the latest state of knowledge we performed a literature review based on Pubmed.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

After surgery the goat needs to be housed individually to prevent failure of the electrodes. After recovery from surgery the goat will be housed in a smaller cage. T goat will be housed in narrow cage in which the goat can only walk backward and forward. The actual duration for AF to become persistent is highly variable but the far majority of the goats will have persistent AF within 2 weeks. In rare occasions this might be delayed to 4 weeks. This is necessary to prevent turning because the goat is connected to a computer system that controls and monitors the rhythm of the goat. Turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable. This type of housing will started at AF induction until sacrifice.

G. Location where the animals procedures are performed

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

X No > Continue with question H.

☐ 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

All surgical procedures will be executed under adequate general anaesthesia. The depth of anaesthesia will be monitored by ECG, respiratory gasses and physical appearance. For open chest sacrifice of goats with AF, additional blood pressure monitoring will be used since "normal" heart rate regulation absent. Due to AF an irregular and fast heart rate is present.
Peri and postoperative analgesia will be applied accordingh to the 2015 GVSOLAS guidelines for pain management of laboratory animals.

Postoperative analgesia will be given after electrode implantation.
All anaesthetic and analgetic drugs will be chosen in consultation with the designated veterinarian.

I. Other aspects compromising the welfare of the animals

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

The main risk of the lone AF model in the goat are;

- infection at the porte d'entrée
- electrode failure
- confined housing
- Reduced food intake

Explain why these effects may emerge.

Infection: To allow computer control of the atrial rhythm, the connector of the electrode needs to be exteriorized. This leaves a small opening in the skin. If the cable with wires is poorly fixated, the cable could move in and out the skin leading to local infection.

Electrode failure: Multiple electrodes are needed for stimulation and sensing. Some sites on the heart may not have signal quality adequate for analysis or stimulation. Furthermore, bending and tension can break the wires. In addition, there is a risk that the goat can bite or otherwise dislocate electrode wires.

Confined housing: During the early stage of AF induction (until AF is persistent), the goat will be housed in narrow cage in which the goat can only walk backward and forward. This is necessary to prevent turning because the goat is connected to a computer system that controls and monitors the rhythm of the goat. Turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable.

Reduced food intake: Although food intake was not affected by in previous projects, we now observed weight loss in some goats in a current project. It is still unclear yet why this occured. It might be due to the change of location (breeder to the animal facilties), change in diet, the AF model itself or other (yet) unknown causes.

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

Infection: The design of the cable has been adopted in such a way that wound healing encapsulates the cable. The encapsulation further fixates the cable. Additionally, the goat will receive antibiotics five days post operatively.

Electrode failure: Firstly, the goat will wear harness of elastic fabric. The location of the cable and connector is covered with a resilient fabric. During computer controlled stimulation the goat needs to be housed in a cage with limited freedom of movement but may return to a normal cage once AF has become persistent. Secondly, we will implant an array of electrodes. This allows us to change sensing and stimulation sites to optimize signal quality.

Confined housing: The stables are desgned in a mode the goats can see, hear and smell congeners. We chose this approach to come close to normal group housing.

Reduced food intake: Food will be daily monitored and body weight will be at least weekly monitored. We will also further investigate possible the possible factors listed above as possible comfounder. To minimize this adverse effect we also adopted weight loss criterium as a human end point.

J. 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 K.

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

- Uncontrollable infections. Infections are identified by an increased body temperature above 40^0
- Pain despite adequate analgesic medication. Pain will be recognized by; piloerection; grinding of the teeth; apathy.
- reduction of body condition compared to normal in combination with bodyweight loss (max 25%)

Indicate the likely incidence.

In a previous project in adult goats, 3 out of 28 goats were taken out of protocol due to a reduction on body weight of >20%. Therefore we expect an incidence of about 10%
Note: for the animal number calculation, we have stated a total dropout rate of15% because we expect additional missing data due to technical limitations.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

The goats will experience discomfort because of 4 components in this protocol.
1 Electrode implantation → recovery from surgery.
2 AF induction → confined housing
3 Pharmacological experiments → infusion line placement for drug infusion and blood sampling
4 Sacrifice experiment → induction of anaesthesia
We believe that electrode implantation causes the highest degree of discomfort because of the recovery from open chest implantation.

Summarized, for these studies the expected level of discomfort is **moderate**.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The animals will be sacrificed during the final anesthetized experiment. This is essential to obtain relevant electrophysiologic measurements.

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

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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10700
1.2	Provide the name of the licenced establishment.	Maastricht university
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 Type of animal procedure: Effects in paroxysmal AF, permanent AF and heart failure.

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.

This study is designed to explore new therapeutic targets for the treatment of atrial fibrillation. We aim to specify the effect of potential atrial specific targets. These targets are either trans-membrane ion currents uniquely expressed in the atrium or an optimized combination of sodium and potassium currents. In this appendix we will investigate the antiarrhythmic properties of the compounds in 3 models of AF.

I. Paroxysmal and early persistent AF, the earliest stages of AF induced adaptions. Here AF is still unstable and spontaneously cardioversion occurs after minutes.
II. AF in the presence heart failure. Heart failure will result in significant levels of fibrosis in the atria. The level of fibrosis and the presence of heart failure are strong predictors of AF. In heart failure large strands of replacement fibrosis are seen but conduction patterns are still relatively organized.
III. Permanent AF. In this model we let AF continue to exist up to 4-6 months. This will result in moderate fibrosis and structural changes. In this model diffuse fibrosis is present in combination with high complexity of conduction patterns.

Groups and targets we will investigate are listed below. Targets 1-4 will depent on a positive outcome to restore sinus rhythm in persistent AF (2i).;

1. $I_{K\ Ach}$,
2. I_{sk},
3. I_{K1},

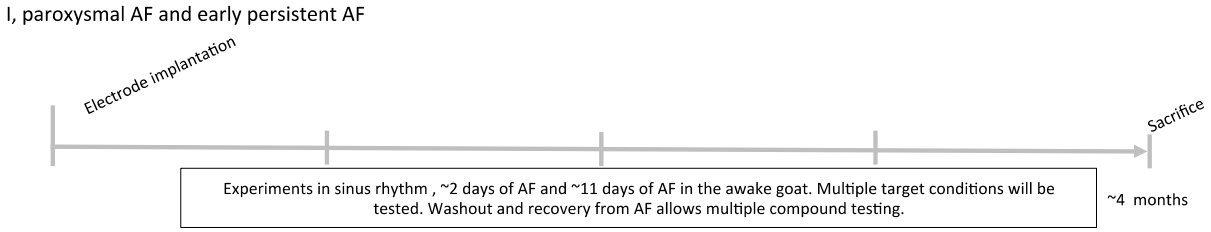
4. $I_{Na\ Late}$
5. Potassium channel inhibition in combination with a sodium channel inhibitor,
6. Positive control, a drug commonly used in clinical practice[#],
7. Time matched control (commonly negative control) [*].

* time matched controles are included into the study to correct for possible time effects at sacrifice experiments. Because measurements will take hours a possible change may occur in electrophysiological parameters due to a change in avariety of conditions (e.g. temperature, inflammation due to surgery, anesthesia, blood loss, etc.

[#]A positive control is included to demonstrate possible superiority of the new compound(s) compared to clinical available antiarrhythmic drugs.

I. Paroxysmal AF

Goats will undergo the same implantation procedure as appendix 2. Electrodes will be placed on the heart for the measurement of electrophysiological properties in the awake goat. A 2-3 weeks of recovery will be taken into account before the actual experiment starts. In this model compound will be tested in normal sinus rhythm and early stages of AF. In this early stage, AF episodes are short lasting (paroxysmal) and terminate spontaneously. The adaptations as a result of AF induced remodelling are still reversible in this early stage of AF. Therefore, we can perform repeated experiments at 3 different stages of AF induced remodelling. Experiments will be performed in sinus rhythm when AF is absent, after 2 days of AF and after about 11 days of AF. During the experiments the goat will be infused with saline or antiarrhythmic drug and basic electrophysiological measurements will be performed. Between experiments sufficient time for drug washout and restoration of baseline electrical properties are allowed. This protocol will also be used to evaluate the combination of sodium and potassium current inhibitors. The evaluation of the combined drugs will be executed independent of the outcome of protocol 2i.



The primary outcomes for phase **3i** are;

1. Identification if a compound has an antiarrhythmic potential at different stages of AF induced remodelling,
2. Evaluation of the possible synergistic effect of a combination of drugs,
3. Acknowledgement if a compound can both terminate and prevent AF

II. Heart failure in combination with AF

Goats will undergo an implantation procedure by which electrodes are placed on the heart for measurement of electrophysiological properties. A 2-3 weeks of recovery will be taken into account before the actual experiment starts. After recovery, the ventricles will be stimulated to induce heart failure. Simultaneously, AF will be induced with an automated computer protocol. This system is able to detect normal sinus rhythm. The goat will be paced into AF once sinus rhythm is detected. The AF episodes will first last for short periods (seconds to minutes), but they will progress into stable (persistent) AF in about 2-4 weeks. Heart failure and AF will be induced for a maximum of 4 weeks. When AF has become persistent up to 2 AF termination (cardioversion) experiments will be performed. During these experiments the compound will be administered and

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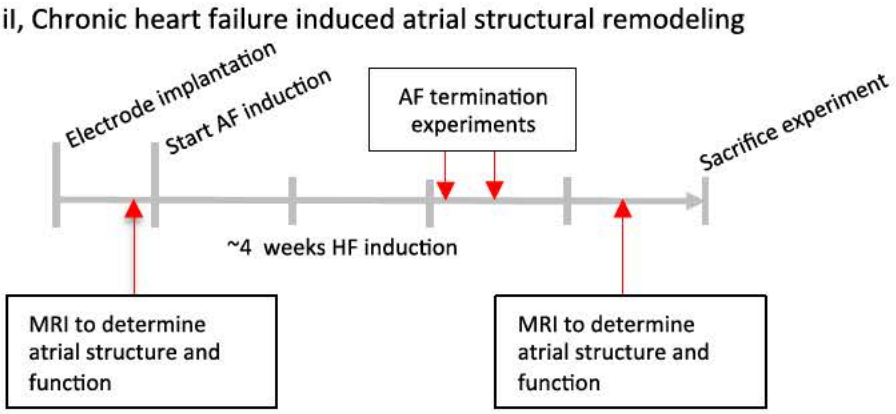

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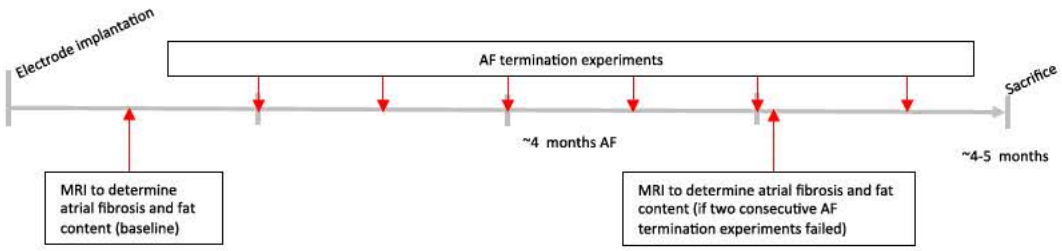
with an interval of 2 weeks (max 10). No more cardioversion experiments will be performed if two consecutive experiments fail to terminate AF. The transition from persistent to drug refractory AF is accompanied with structural remodelling. To assess the amount of structural remodeling needed for a compound to fail, we will perform MRI scans in combination with recordings on the body surface. Two scans are needed to quantify the degree of structural remodelling for each goat. At the end of the protocol a terminal experiment will be executed to measure the electrophysiological properties.
The total duration of the experiment is limited to 5.5 months (recovery from surgery and AF maintenance). This protocol will only be conducted if the compound resulted in termination of AF in protocol 2i.

iii, long term persistent AF



1. Effect of the targets on the electrophysiology in atria with diffuse fibrosis,
2. The relation of structural remodelling and the efficacy of the compounds.

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

Electrode implantation (3i, ii & iii)
The implantation will be performed under general anaesthesia. A single left sided thoracotomy will be performed to expose the left atrium. A plaque containing multiple electrodes for sensing and pacing will be placed on the heart. Before closure of the thoracic cavity signal quality will be tested. This test is performed to assure signal quality and prevention of both ventricular and phrenic nerve stimulation during AF induction. With the electrodes in the correct position, the chest will be closed and the electrode cable will be tunnelled to the back of the goat where the cable is exteriorized. A 2-3 weeks of recovery will be taken into account before the actual experiment starts.

AF induction and maintenance (3i, ii & iii)
After a recovery period, the goats will be housed in specially designed cages for AF induction and monitoring. This restricted housing only allows one animal per cage. However, the cages are designed in such a manner that animals can see, hear and smell each other. The type of protocol determines the duration of this type of housing. AF induction is maintained for 2 days or 11 days for protocol 3i. For protocol 3ii and 3iii AF will be maintained until AF becomes persistent (approximately 2-4 weeks).

Electrophysiological experiments (3i)
Cardiac signals, both atrial and ventricular, will be recorded throughout the experiment. Signals can be obtained by connecting the goat to a cardiac amplifier. In this experiment, i.v. lines will be placed for the infusion of the compound and for blood sampling. First, measurements of baseline conditions are measured during infusion of vehicle only. After these measurements the recording is continued during infusion and part of washout of the drug. We will perform the electrophysiologic measurements in sinus rhythm, after ~ 2 and ~11 days of AF. The experiment may take up to 3 hours.
The goats will have limited awareness of most measurements. Occasionally we will assess certain properties at higher heart rates which the goat might experience. We believe that the combination of I.V. placement and measurements will cause mild discomfort. To prevent excessive accumulation of discomfort we will limit our experiments to 18, per animal.

Electrophysiological and AF termination experiments (3ii & 3iii)
Cardiac signals, both atrial and ventricular, will be recorded throughout the experiment. Signals can be obtained by connecting the goat to a cardiac amplifier. In this experiment an i.v. line is placed for the infusion of the vehicle and the compound. First, measurements of baseline conditions are taken during infusion of vehicle only. After these measurements, the recording is continued during infusion and part of washout of the drug. The experiment may take up to 3 hours.

MRI Scans (3ii & 3iii)
For the MRI scan the goats will be brought under general anesthesia. Before the scan, a series of electrodes will be placed on the body surface. This will allow the reconstruction of the electrical activity on the atrial activity while the structural properties arte obtained through the scan. Two scans will be performed per animal.

Sacrifice experiment (3i, ii & iii)
In a terminal experiment the effects on electrophysiological properties will be measured. For this terminal experiment the goats will be anesthetized. The chest will be opened to expose the heart for direct measurements electrophysiologic parameters. Additional catheters will be introduced to monitor blood pressures. Once all measurements are performed, the heart will be excised for *in vitro* analysis.

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

Study design
For each individual animal repeated measures are conduction. This will allow the use of a linear mixed model. The advantage of this approach is that subjects with missing data points will not be dropped from the analysis, achieving a higher statistical power.

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

Species
We will make use of the goat for or electrphysiological studies. Models of different stages of AF in the goat are well characterized and closely resembles human electrophysiology elementary characteristics such as cycle length, refractory period and number of waves.[9-13] Heart size is crucial for AF stabilization. Smaller hearts (e.g. rodents) can not harbour chronic AF. The heart weight/size truly resembles the size of humans. In a currently running project the heart weights was 323±65g in contrast a recent publication (2012) in the American journal of forensic medical pathology Molina et al. found an average heart weight of 331±57g.

Sex
Female goats will be used for this study. We choose the female gender because of:

1) Goat studies in literature, for example reference 4-9 and 12-16, are based on female goats;
2) In contrast to male goats, the tranquil nature of female goat behaviour allows measurement in awake goat;
3) Availability of male goats is limited. Most male goats are slaughtered for consumption at young age.

Origin
The goats will be purchased from a local goat breeder.

Number of animals.
3i. Paroxysmal AF
Historic data on drug studies has shown that 8 goat are sufficient to demonstrate relevant differences after drug administration[8,9]. Elementary electrophysiological properties such as refractory period, fibrillation frequency and conduction velocity of fibrillation waves determine the number of animals. Based on previous experiments we expect a dropout of about 15%. Dropout might occur due to electrode failure, infection and technicalities during the extensive terminal experiment. We need **10 animals/compound** after the correction for dropout.
Calculation of the number of animals

- For each compound we will perform measurements at 3 time points of AF-induced remodelling. This cycle is reversible and therefore it can be repeated several times. One cycle of experiments will take about 4 weeks. This will allow **4 compound/condition testing cycles in one goat**.
- We have **5 targets and 1 control** ($I_{K,Ach}$, I_{sk}, I_{K1}, $I_{Na,Late}$ and a positive control drug) studied in previous phases of this project. Note that no time matched control is needed because the goat can serve as its own control at each condition.
- For the evaluation of the combination of sodium and potassium current inhibition we do not know yet which combination of compounds and doses will need to be evaluated. This will be dependent on the predictions based on our computer models. If we assume that we will use 1 sodium channel blocker and 2 different potassium channel blockers that will be identified as potential synergistic combinations and each drug will tested in 2 doses will have **8 conditions to test**.

Considering the above-described variables we have a total of 14 compounds/conditions. Four of the compounds/conditions can be assessed in 1 goat. Thus, if we divide 14 by the compounds/conditions that can be assessed in one goat we get $3^{1/2}$. Rounding this up we will need 4 groups of 8 animals to be able to test all conditions. Therefore we need **a total of 32 animals.**

3ii. Heart failure in combination with AF
Historic data on drug studies has shown that 8 goat are sufficient to demonstrate relevant differences after drug administration[8,9]. Elementary electrophysiological properties like refractory period, fibrillation frequency and conduction velocity of fibrillation waves determine the number of animals. We will accounting for a dropout of 40%. This drop out is expected to be higher than e.g. 3i. Here we have both risks of drop out AF induction and heart failure. Drop out of animals may occur due to reaching the hman end points, technical failures such as signal quality and physiological instability during anesthesia. After correction for drop out we need **14 animals/group**. We will investigate a maximum of 5 targets and use 2 control groups.
This will bring the **total number of animals to 98.**

3iii. Permanent AF
Historic data on drug studies has shown that 8 goat are sufficient to demonstrate relevant differences after drug administration[8,9]. Elementary electrophysiological properties like refractory period, fibrillation frequency and conduction velocity of fibrillation waves determine the number of animals. Based on previous experiments we expect a dropout of about 15%. Dropout might occur due to electrode failure, infection and technicalities during the extensive terminal experiment. After the correction for dropout we need **10 animals/group**. We will investigate a maximum of 5 targets and use 2 control groups.. This will bring the **total number of animals to 70.**

Life stage
Size and age are important determinants for AF stabilization. Therefore, we will use adult goats with a limited range of age. Approximately 1-5 years.

C. Re-use

Will the animals be re-used?

X No, continue with question D.

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

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.

D. 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
The complex interaction between physiological systems in the body (inflammation, hemodynamic, neurohumoral etc.) are involved in AF induced remodelling. At later phases of this project the different stages of AF induced remodelling are crucial and therefore need to apply to this group as well. Therefore, this project can only be investigated in an *in vivo* model with intact physiology. Our collaborators within this research project explored the *in vitro* therapeutic targets. All targets within this study have been extensively studied in *in vitro.*
Both large and small animal models are considered. Models in rat and mice are suitable to investigate processes on a cellular level. However, due to their heart size no non-selfterminating

AF can be induced. It is of great importance in this project to investigate the efficacy of the compounds in stable non-selfterminating AF. For larger animal models the dogs, pig sheep and goats can be considered. The drawback of dog and pig models of AF is the fast ventricular rate leading to heart failure. Therefore these models are in principle a combination of AF and heart failure. Alternatively, goats and sheep can be chosen. We have several reasons to prefer the goat model. Firstly, most AF research in awake animals is obtained in goats. Secondly, goat hearts tend to be a bit bigger resembling more the human heart size. Finally, we have a large expertise in goats and our research facilities and equipment has been developed to perform measurements in the goat.

Reduction
This project application constitutes a phased design. Moreover, we have chosen for a repeated measures protocol where for large part the goat can serve as its own control. In combination with a linear mixed model we will achieve high power with limited number of animals.

Refinement
The animal model has been developed in our laboratory. Over the past two decades we have adjusted a number of aspects concerning the procedures. For instance, by refining the electrode design we are able to prevent infections at the *porte d'entrée*. Re-design of cages allows the goats the have some freedom of movement when they are housed in their monitoring cages. The technical approach of protocol 3i, 3ii and 3iii are the same except for the site of stimulation or duration of stimulation. Therefore, the above-mentioned refinements apply to all protocols.
For protocol 3ii we will take additional measures to prevent rapid deterioration of ventricular function by close monitoring the ventricular function with echocardiography.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Upon arrival the goats will be housed in the animal facilities to get familiar their new surrounding and care takers. Goats are domesticated animals and get quickly habituated to their new surrounding and contact to humans. The goats will be housed with congeners and group housing when possible.
General anaesthesia (including analgesia) will be used during the terminal experiments.

Repetition and duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Target 1-3 are compounds which are newly developed. We have a direct collaboration with the developers of these compounds. Because of this short linik we will be the first to test the compound in a large animnal model.
All targets we will investigate in this project are also reconized by others as possible new therapeutic strategies. Individual currents and inhibition of them were investigated in a range of different electrophysiological models. Most of the published data is derived from isolated cells or wedge preperations. Only occasionally the measurements were performed in intact animal models. These models have in common that AF was always acutely induced, e.g, by stretch or autonomic stimulation. These models do not create a substrate of persistent AF (clinnical setting) but need continuous drug administration for AF maintenance. Furthermore, data described in the whole animal models reflect local cellular properties but lack information on conduction properties during AF itself.
To obtain the latest state of knowledge we performed a literature review based on Pubmed.

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

No

X Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

3i. Paroxysmal AF
Goats will need to be individually housed to prevent failure of the implanted electrodes electrodes. Majority of the time the goat will be housed in a normal cage.
However for the time points of 2 days of AF and 11 days of AF the goat needs to be housed for the limited time periods, in a designated cage for AF maintenace. In this narrow cage the goat is connected with cable to a computer system for rhythm management. The3 goat can only walk back and forward. This is necessary to prevent turning, turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable.

3ii. Heart failure and AF
After surgery the goat needs to be housed individually to prevent failure of the electrodes. After recovery from surgery the goat will be housed in a smaller cage. In this narrow cage the goat is connected with cable to a computer system for rhythm management. The goat can only walk back and forward. This is necessary to prevent turning, turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable. This type of housing will be continued until the goat will sacrificed.

3ii. Permanent AF
After surgery the goat needs to be housed individually to prevent failure of the electrodes. After recovery from surgery the goat will be housed in a smaller cage. In this narrow cage the goat is connected with cable to a computer system for rhythm management. The goat can only walk back and forward. This is necessary to prevent turning, turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable. This type of housing will be continued until AF has become persistent. Once AF has become persistent no continuous monitoring is needed and the goat will be housed in a normal sized cage. However we will house the goat in the narrow cage after each AF termination experiment to assure that AF is maintained when the drug is washed out.

G. Location where the animals procedures are performed

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

X No > Continue with question H.

☐ 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.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

All surgical procedures will be executed under adequate general anaesthesia. Peri and postoperative analgesia will be applied accordingh to the 2015 GVSOLAS guidelines for pain management of laboratory animals.

The depth of anaesthesia will be monitored by ECG, respiratory gasses and physical appearance. For open chest sacrifice of goats with AF, additional blood pressure monitoring will be used since "normal" heart rate regulation absent due to AF an irregular and fast heart rate is present.

All anaesthetic and analgetic drugs will be chosen in consultation with the designated veterinarian.

I. Other aspects compromising the welfare of the animals

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

Infection (3i, ii & iii)
The main risks of the lone AF model in the goat are infection at the porte d'entrée and electrode failure.

Electrode failure and confined housing (3i, ii & iii)

Decompensating heart failure (3ii)
Goats in the heart failure group might also experience progressive deterioration of ventricular function leading to acute decompensation.

Reduced food intake (3i, ii & iii)

Explain why these effects may emerge.

Infection (3i, ii & iii)
To allow computer control of the atrial rhythm, the connector of the electrode needs to be exteriorized. This leaves a small opening in the skin. If the cable with wires is poorly fixated, the cable could move in and out the skin leading to local infection.

Electrode failure (3i, ii & iii)
Multiple electrodes are needed for stimulation and sensing. Some sites on the heart may not have signal quality adequate for analysis or stimulation. Furthermore, bending and tension can break the wires. In addition, there is a risk that the goat can bite or otherwise dislocate electrode wires.

Confined housing (3i, ii & iii)
During the early stage of AF induction (until AF is persistent), the goat will be housed in narrow

cage in which the goat can only walk backward and forward. This is necessary to prevent turning because the goat is connected to a computer system that controls and monitors the rhythm of the goat. Turning could potentially lead to damage to the electrodes or connection cables. The goat might also get entangled with the cable.

Decompensating heart failure (3ii)
The sensitivity to CHF can have rather large variation. Therefore, despite intensive monitoring irreversible heart failure may occur.

Reduced food intake
Although food intake was not affected by in previous projects, we now observed weight loss in some goats in a current project. It is still unclear why this occured. It might be due to the change of location (breeder to the animal facilties), change in diet, the AF model itself or other (yet) unknown causes.

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

Infection (3i, ii & iii)
The design of the cable has been adopted in such a way that wound healing encapsulates the cable. The encapsulation further fixates the cable. Additionally, the goat will receive antibiotics five days post operatively.

Electrode failure (3i, ii & iii)
Firstly, the goat will wear harness of elastic fabric. The location of the cable and connector is covered with a resilient fabric. Secondly, during computer controlled stimulation the goat needs to be housed in a cage with limited freedom of movement but may return to a normal cage once AF has become persistent. Thirdly, we will implant an array of electrodes. This allows us to change sensing and stimulation sites to optimize signal quality.

Confined housing (3i, ii & iii)
The stables are desgned in a mode the goats can see, hear and smell congeners. We chose this approach to come close to normal group housing.

Decompensating heart failure (3ii)
Frequent echodiography will be performed. In case of a rapid drop of cardiac function the pacemaker frequency will be reduced and diuretics will be given.

Reduced food intake
Food will be daily monitored and body weight will be at least weekly monitored. We will also further investigate possible the possible factors listed above as possible comfounder. To minimize this adverse effect we also adopted weight loss criterium as a human end point.

J. 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 K.

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

- Uncontrollable infections. Infections are identified by an increased body temperature above

40^0

- Pain despite adequate analgesic medication. Pain will be recognized by; piloerection; grinding of the teeth; apathy.
- reduction of body condition compared to normal in combination with bodyweight loss (max 25%)
- **3ii specific.** Acute decompensation due to heart failure. Heart failure may be identified by ascites, impaired breathing (tachypnea, cyanosis) due to pulmonary edema, edema in the periphery* (anasacra) and strongly reduced cardiac function based on echo parameters. Edema on the chest wall will occur after surgical implantation of the electrodes. This transient edema in reaction to surgery will not be considered as a humane end point.

Indicate the likely incidence.

In a previous project in adult goats, 3 out of 28 goats were taken out of protocol due to a reduction on body weight of >20%. Therefore we expect an incidence of about 10%. Note: for the animal number calculation, we have stated a total dropout rate of 15% because we expect additional missing data due to technical limitations.
For group 3ii we expect a higher degree of dropout due to acute decompensation during CHF induction. Therefore we have account for a total drop out of 40%.

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

3i The goats will experience discomfort because of 4 components in this protocol.
1 Electrode implantation → recovery from surgery.
2 AF induction → confined housing
3 Pharmacological experiments → infusion line placement for drug infusion and blood sampling
4 Sacrifice experiment → induction of anaesthesia
We believe that electrode implantation causes the highest degree of discomfort because of the recovery from open chest implantation.
For these experiments the expected level of discomfort is **moderate**.

3ii The goats will experience discomfort because of 5 components in this protocol.
1 Electrode implantation → recovery from surgery.
2 AF induction → confined housing
3 Heart failure → animals might go into acute decompensation leading to dyspnea and severly reduced cardiac performance
4 Pharmacological experiments → infusion line placement for drug infusion and blood sampling
5 Sacrifice experiment → induction of anaesthesia
We believe that heart failure causes the highest degree of discomfort .
For these experiments the expected level of discomfort is **severe**.

3iii The goats will experience discomfort because of 4 components in this protocol.
1 Electrode implantation → recovery from surgery.
2 AF induction → confined housing
3 Pharmacological experiments → infusion line placement for drug infusion and blood sampling
4 Sacrifice experiment → induction of anaesthesia
We believe that electrode implantation causes the highest degree of discomfort because of the recovery from open chest implantation.
For these experiments the expected level of discomfort is **moderate**.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

☐ No

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

The animals will be sacrificed during the final anesthetized experiment. This is essential to obtain relevant electrophysiologic measurements.

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

Centrale Commissie Dierproeven

7

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Onze referentie
Aanvraagnummer
AVD107002016782

Bijlagen
2

Datum 15 december 2016
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [redacted]

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 15 december 2016. Het gaat om uw project "Development of new pharmacological strategies to treat atrial fibrillation.". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD107002016782. Gebruik dit nummer wanneer u contact met de CCD opneemt.

Wacht met de uitvoering van uw project
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

Factuur
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016782

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016782

Gegevens aanvrager

Uw gegevens	
Deelnemersnummer NVWA:	10700
Naam instelling of organisatie:	Universiteit Maastricht
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	50169181
Straat en huisnummer:	Minderbroedersberg 4-6
Postbus:	616
Postcode en plaats:	6200 MD MAASTRICHT
IBAN:	NL04 INGB 0679 5101 68
Tenaamstelling van het rekeningnummer:	Universiteit Maastricht

Gegevens verantwoordelijke onderzoeker	
Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Datum:
15 december 2016
Aanvraagnummer:
AVD107002016782

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Gegevens verantwoordelijke uitvoering proces

Naam:	[redacted]
Functie:	[redacted]
Afdeling:	[redacted]
Telefoonnummer:	[redacted]
E-mailadres:	[redacted]

Over uw aanvraag

Wat voor aanvraag doet u?	[x] Nieuwe aanvraag [ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn [ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 februari 2017
Geplande einddatum:	1 februari 2022
Titel project:	Development of new pharmacological strategies to treat atrial fibrillation.
Titel niet-technische samenvatting:	Ontwikkeling van nieuwe medicijnen voor de behandeling van boezemfibrileren
Naam DEC:	DEC-UM
Postadres DEC:	Postbus 616, 6200MD Maastricht
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1.441,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:	[x] Projectvoorstel [x] Beschrijving Dierproeven [x] Niet-technische samenvatting
Overige bijlagen:	[x] DEC-advies

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Maastricht
Datum:	14 december 2016

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Universiteit Maastricht
[redacted]
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Centrale Commissie Dierproeven
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0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD107002016782

Bijlagen
2

Datum 15 december 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 15 december 2016
Vervaldatum: 14 januari 2017
Factuurnummer: 16700782

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD107002016782	€ 1.441,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

8

[redacted]

Van: Info-zbo
Verzonden: woensdag 1 februari 2017 10:55
Aan: [redacted]
Onderwerp: RE: vraag bij de behandeling van AVD107002016782

Geachte [redacted]

De juiste versie van de brief is bij het dossier gevoegd. Uw antwoord is voldoende om het dossier compleet te maken, De CCD heeft besloten uw aanvraag te vergunnen zoals aangevraagd. U ontvangt nog deze week de beschikking en vergunning,

Vriendelijke groet, [redacted]

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.....
T: 0900 2800028
E: info@zbo-ccd.nl (let op: nieuw emailadres!)

Van: [redacted]
Verzonden: woensdag 1 februari 2017 9:27
Aan: Info-zbo
Onderwerp: Re: vraag bij de behandeling van AVD107002016782

Beste,

Ik kom er net achter dat ik gister avond de verkeerde versie van de antwoordbrief heb toe gestuurd. Hopelijk ben ik nog op tijd voor de verweking hiervan. Bij deze wil ik de juiste versie indienen.

Groeten,
[redacted]

From: Info-zbo <info@zbo-ccd.nl>
Date: Tuesday, 31 January 2017 at 14:55
To: [redacted]
Cc: [redacted]
Subject: RE: vraag bij de behandeling van AVD107002016782

Geachte [redacted]

Op 12 januari heeft de CCD u onderstaande vraag voorgelegd. Door storingen in de mailbox kan het zijn dat wij uw antwoord niet ontvangen hebben. Zou u dit nogmaals in kunnen sturen of wanneer u nog niet heeft geantwoord dit willen doen?

Met vriendelijke groet, [redacted]

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Van: Info-zbo
Verzonden: donderdag 12 januari 2017 14:20
Aan: [redacted]
CC: [redacted]
Onderwerp: vraag bij de behandeling van AVD107002016782

Geachte [redacted]

U heeft bij de CCD een aanvraag tot projectvergunning ingediend. Het betreft uw project "Development of new pharmacological strategies to treat atrial fibrillation´ met aanvraagnummer AVD107002016782. Uw aanvraag is behandeld in de afgelopen CCD vergadering en de CCD wil u vragen de aanvraag aan te vullen voordat een besluit genomen kan worden. U beschrijft in uw aanvraag het uitvoeren van *in vitro* experimenten voordat u onderzoek in proefdieren gaat uitvoeren. De beschreven *in vitro* studies hebben betrekking op de functionaliteit van de te selecteren componenten. Zijn er ook *in vitro* studies uitgevoerd die betrekking hebben op de effectiviteit van een component en zou dit de opzet van de dierstudies zoals beschreven in bijlage 3.4.4.1 verder kunnen verfijnen door kinetisch moduleren/ PK-PD (in vitro, in silico, andere diermodellen)?

Kunt u dit toelichten? De behandeltijd van uw aanvraag is opgeschort todat uw aanvullingen ontvangen zijn, met vriendelijke groet, [redacted]

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9

[redacted]

Van: Dec Secretariaat [redacted]
Verzonden: dinsdag 10 januari 2017 8:43
Aan: 'Info-zbo'
Onderwerp: RE: vraag om aanvullend advies bij AVD107002016782

Categorieën: Dossier: [redacted]

Geachte commissie,

Hierbij de reactie van de DEC-UM:

Het betreft een thoracotomie bij geiten ten behoeve van plaatsing van 1 electrode-plaatje op een atrium met postoperatief (indien noodzakelijk pijnstilling en) huisvesting gedurende 2-4 weken in een hok, waarin de dieren niet kunnen omdraaien, maar wel contact kunnen onderhouden met soortgenoten in dezelfde ruimte. De betreffende operatie wordt uitgevoerd onder algehele anesthesie en is daarmee te classificeren als matig. Voor de uiteindelijke inschatting van het totale ongerief moet natuurlijk tevens de postoperatieve huisvesting gedurende 2-4 weken in een hok, waarin de dieren niet kunnen omdraaien worden meegewogen. In de EU richtlijn wordt aan huisvesting in een metabole kooi met matige beperking van de bewegingsvrijheid gedurende een langere periode (tot en met 5 dagen) als ongeriefsclassificatie matig toegekend. In de EU richtlijn wordt tevens aangegeven belang te hechten aan de diersoort bij de definitieve indeling van de procedure naar ernst. In de landbouwhuisdiersector was het niet ongebruikelijk herkauwers en paarden aangebonden te huisvesten gedurende meerdere (winter)maanden, waarbij de bewegingsvrijheid beperkt was ondermeer ten aanzien van de mogelijkheid zich te keren. Deze klassieke huisvestingsmethode is bezwaarlijk als matig ongerief te classificeren indachtig de persoonlijke verzorging de dieren ondermeer middels borstelen door de veehouder. De DEC-UM heeft goede nota genomen van de opmerking onder verfijning in het betreffende PV dat "Redesign of cages allows the goats to have some freedom of movement when they are housed in their monitoring cages" oftewel er is meer bewegingsvrijheid in vergelijking tot aangebonden herkauwers ter verfijning, maar nog immer de onmogelijkheid te keren. Naar de mening van de DEC-UM is het totale ongerief bij deze geiten zeer wel passend bij matig mede indachtig het feit dat de operatie ook niet als zeer zwaar kan worden beschouwd. De classificatie ernstig ongerief lijkt echter een overmatige classificatie daar de procedure, zoals die wordt toegepast bij deze geiten, niet vergelijkbaar lijkt met het ongerief bij procedures als 'immobilisatiestress om hartstilstand bij ratten te induceren' of een test 'met gedwongen zwemsessies of oefeningen met uitputting als eindpunt'.

De DEC-UM hoopt hiermee uw verzoek volledig te hebben beantwoord.

Met vriendelijke groet,

[redacted]
Ambtelijk secretaris Dierexperimentencommissie
[redacted]**DEC-UM**

[redacted]
www.maastrichtuniversity.nl/dec

Postbus 616, box 48, 6200 MD Maastricht
[redacted]

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: donderdag 5 januari 2017 11:55
To: Dec Secretariaat [redacted]
Subject: vraag om aanvullend advies bij AVD107002016782

Geachte leden van DEC-UM,

U heeft aan de CCD advies uitgebracht over aanvraag AVD107002016782 getiteld: "Development of new pharmacological strategies to treat atrial fibrillation.". Bij de behandeling van deze aanvraag zou de CCD u om aanvullend advies willen vragen over de beschreven ongerief classificatie. De dieren ondergaan een invasieve open thorax chirurgie en worden anders dan in bijlage III van de Richtlijn gehuisvest. De afwijkende huisvesting beperkt de dieren in hun bewegingsvrijheid, ze kunnen beperkt alleen voor- en achterwaarts bewegen en deze vorm van huisvesten kan minimaal 2-4 weken duren. In combinatie met de invasieve chirurgie kan de CCD zich voorstellen dat het cumulatief ongerief voor de dieren die dit betreft (bijlage 3.4.4.2 en gedeeltelijk uit bijlage 3..4.4.3) eerder als ernstig dan als matig geclassificeerd moet worden. Uit onderdel K. in de bijlage dierproeven :" We believe that electrode implantation causes the highest degree of discomfort because of the recovery from open chest implantation"
Lijkt er geen rekening gehouden met dit cumulatieve effect maar is de ongerief classificatie afgeleid van de procedure die op zichzelf het hoogste ongerief veroorzaakt.

Graag hoort de CCD hier uw aanvullende advies over,

Met vriendelijke groet, [redacted]

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DEC-advies PV 2016-013/[redacted]

Preambule;
De DEC-UM verzoekt U eventuele aanvullende vragen rechtstreeks aan de aanvrager te stellen met een afschrift aan de DEC-UM.

A. Algemene gegevens over de procedure

1. **Aanvraagnummer:** 10700

2. **Titel van het project:** *Development of new pharmacological strategies to treat atrial fibrillation.*

3. **Titel van de NTS:** *Ontwikkeling van nieuwe medicijnen voor de behandeling van boezemfibrileren.*

4. **Type aanvraag:**
   X **nieuwe** aanvraag projectvergunning

5. **Contactgegevens DEC:**
   - naam DEC; *DEC-UM*
   - telefoonnummer contactpersoon; [redacted]
   - e-mailadres contactpersoon; [redacted]

6. **Adviestraject:** (data dd-mm-jjjj):
   X ontvangen door DEC-UM 03-11-2016
   ☐ aanvraag compleet
   X in vergadering besproken 11-11-2016
   ☐ anderszins behandeld
   ☐ termijnonderbreking(en) van / tot
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☐ aanpassing aanvraag
   ☐ advies aan CCD

7. **Afstemming IvD:**
   - De aanvrager heeft het projectvoorstel afgestemd met de IvD *dd. 03-11-2016*.

8. **Eventueel horen van aanvrager**: ***N.V.T.***

9. **Correspondentie met de aanvrager:**
   - Datum 16-11-2016
   - Gestelde vragen en antwoorden:

3.1 Achtergrond
Algemene opmerking:
1. **Vraag:** U geeft aan cardiale fibrose te willen monitoren middels MRI. Hiertoe dienen de dieren tweemaal te worden blootgesteld aan anesthesie. Heeft U monitoring van fibrose middels bloedonderzoek overwogen als alternatief hiervoor?

Antwoord:
Vorming van fibrose in het atrium is een lokaal proces. Een aantal lokale stimuli zoals lokale ischemie, rek en inflammatie factoren uitgescheiden door adipocyten zijn de aanleiding tot de vorming van fibrose.

Vanwege van dit zeer lokale karakter zijn deze markers niet meetbaar in het bloed of anders niet specifiek voor het atriale remodellingsproces. Bovendien zal de MRI-scan de anatomische locatie identificeren. De samenhang van zowel fibrose als golfpatronen zal bijdragen in het begrip van het mechanisme van een antiaritmicum.
Geen aanpassing van het protocol uitgevoerd.

3.4 Onderzoeksstrategie
3.4.2
Algemene opmerking:
1. De DEC-UM zou het verschil tussen fase 2 en 3 iets meer gedefinieerd willen zien. Er wordt gesproken over "these models". De DEC-UM adviseert deze te benoemen.

Reactie:
Onder punt 3.4.1 en 3.4.2 is nu explicieter aangegeven dat het in essentie om 4 modellen van AF gaan. Een zelf terminerend AF model (paroxysmal), een niet-zelf terminerend model zonder fibrose, en 2 modellen van niet-zelf terminerend AF met 2 verschillende types van fibrose
De tekst is aangepast en in grijs gearceerd.

3.4.4
Appendix 1
Vragen:
A. Experimentele aanpak en primaire uitkomstparameters.
1. **Vraag:** Waar baseert u de keuzes van de stoffen op?

Antwoord:
In de tekst wordt nu aangegeven dat alleen stoffen worden getest die in *in vitro* studies een werkelijk elektrofysiologisch effect hebben aangetoond. Daarnaast wordt de stof op toxiciteit gescreend. Die stoffen met een profiel van een goede toepasbaarheid worden gekozen.
De tekst is aangepast en in grijs gearceerd.

B. De Dieren.
2. **Vraag:** U beschrijft onder aantallen het gebruik van 8 compounds, in de volgende zin schrijft U 4 targets, 2 compounds. Dat begrijpt de DEC-UM niet, test U 8 stoffen of 2 stoffen in 4 targets, kunt U dit verduidelijken?

Antwoord:
We zullen altijd maar 1 target beïnvloeden met 1 enkele stof. Dus als alle stoffen effectief zijn en geen gevaar voor de dieren opleveren hebben we 4X3=12 dieren nodig. Effect en veiligheid is hier gekozen als go/no go moment. Mocht het blijken dat het de compound van onze eerste keuze geen positieve keuze oplevert, willen we een tweede kandidaat compound kunnen testen. Uiteraard moet in *in vitro* studies deze compound een relevant elektrofysiologisch effect hebben. Mocht dit in het ergste geval in alle 4 de targets voorkomen dan moeten we alle experimenten opnieuw doen. Dus 2X12=24 dieren
In het protocol is nu de tekst uitgebreid om deze overweging helderder over te brengen.

3. **Vraag:** U spreekt van een limited range of age. U kiest er echter voor dieren te gebruiken in de leeftijd van 1-5 jaar? Is dat niet tegenstrijdig?

Antwoord:
Geiten kunnen ongeveer 8 tot 12 jaar oud worden. In dat opzicht is 1-5 jaar een "limited range". We zijn het met de commissie eens dat dit wel een zeer breed bereik is. We hebben echter dit bereik op advies van de IVD verbreed naar 1-5 jaar. De argumentatie hierbij is dat de geiten niet direct voor onderzoeksdoeleinden worden gefokt en daardoor beschikbaarheid niet altijd gegarandeerd kan worden. Vanuit wetenschappelijk oogpunt is het vooral van belang dat de dieren nog van jonge leeftijd zijn (<5 jaar) en dat alle groepen in hetzelfde leeftijdsbereik bevinden. Bijvoorbeeld allemaal van 1-3jaar of anders van 3-5 jaar. Op basis van praktische overwegingen gaat de voorkeur uit naar 1-3 jaar.
Geen aanpassing van het protocol uitgevoerd.

4. **Vraag:** Is er iets bekend over uitval? Gaarne toelichten.

Antwoord:
Er wordt geen rekening gehouden met uitval. De analyses (plasma concentratie) en handelingen (plaatsing van infuuslijnen) zijn makkelijk uitvoerbaar. Daarnaast laat het afgenomen bloedvolume toe om meerdere plasma analyses uit te voeren. Omdat de geit meerdere grote oppervlakkige venen heeft zal het plaatsen van een infuus ook geen probleem opleveren. Daarom denken we geen rekening dienen te houden met uitval.
Geen aanpassing van het protocol uitgevoerd.

J. Humane eindpunten.

5. **Vraag:** Gaarne periode van gewichtsverlies aangeven. Zou disfunctie van het cardiovasculair systeem hier ook niet misstaan, zoals ontstaan van oedeem? Moet hier uitgebreidere infectie ook niet worden vermeld evenals bij andere appendices?

Antwoord:
De periode is nu aangegeven.
In deze appendix wordt geen AF opgewekt. De stoffen worden enkel aan gezonde dieren gegeven om de farmacokinetiek te kunnen berekenen. Dientengevolge worden voor deze experimenten geen elektrodes geïmplanteerd en wordt de cardiale functie niet door ritmestoornissen beïnvloed. Er is daarom geen reden om cardiovasculair gerelateerde complicaties, zoals oedeem, te verwachten. Dit geld ook voor de elektrodes, deze worden niet geïmplanteerd en kunnen daardoor niet tot een ontsteking leiden.
Om te onderstrepen dat in de groep dieren onder appendix 1 enkel gezonde (niet geopereerde) dieren gebruikt worden is in sectie A van appendix 1 en in paragraaf 3.4.2 bij de beschrijving van stage 1, expliciet vermeld dat de dieren geen ritmestoornissen hebben.
De tekst is aangepast en in grijs gearceerd.

6. **Vraag:** In de humane eindpunten noemt u geen effecten van het opwekken van AF, is dat noodzakelijk en kunt u de (bij)effecten eventueel weergeven als humaan eindpunt of is dat zinloos?

Antwoord:
Zoals onder punt aangegeven wordt in deze dieren geen AF opgewekt.

Appendix 2

Vragen:

A. Experimentele aanpak en primaire uitkomstparameters.

1. **Vraag:** I.c.m. 2: U wilt "time matched controls" gebruiken, Uw leeftijd range varieert echter van 1-5 jaar. Kunt U, indien U bang bent voor effecten in de tijd mogelijk gerelateerd aan de fysiologische veroudering, een smallere leeftijd kiezen of is dat niet noodzakelijk?

Antwoord:
De keuze voor een time-matched control is niet gemaakt op basis van fysiologische veroudering maar op basis van elektrofysiologische veranderingen die tijdens of ten gevolde het experiment kunnen optreden. In het "sacrifice experiment" kunnen twee potentiele verstorende factoren onze metingen beïnvloeden.

1. De AF terminatie experimenten kunnen theoretisch een residueel effect hebben op het electrische gedrag. Dit is niet waarschijnlijk omdat uit eerdere studies is gebleken dat herhaaldelijk initiëren en termineren van AF geen effect heeft op basale elektrofysiologische parameters in een normaal hart of een hart met kort durend AF.
2. Een belangrijker punt is de duur van het "sacrifice experiment" zelf (daarom time matched). Gedurende uren zullen metingen op het oppervlak van het hart uitgevoerd worden. We weten uit eerdere experimenten dat over een 1 uur het AF patroon stabiel is. Over langere periodes, zoals de totale duur van het experiment, hebben we echter geen informatie.
   Het experiment is zo gekozen dat de eerste metingen in een hart met AF en een fysiologisch zout infuus wordt uitgevoerd.

Daarna zullen identiek dezelfde metingen worden herhaald in de afwezigheid van de compound. Naar verwachting zal het gehele meetprotocol >5 uur duren. In deze periode kunnen veranderingen in externe factoren (temperatuur, anesthesie, compositie van het bloed, inflammatie etc.) plaats vinden die mogelijk de metingen verstoren. Helaas kan een cross-over protocol niet opgezet worden i.v.m. de washout tijd van de compounds welke het protocol langer zal maken. Om niet ten onrechte veranderingen aan de compound toe wijzen willen we een time-matched controle includeren.

In de uiteindelijke studie zal in een kleinere leeftijdsbereik gehanteerd worden. Waarschijnlijk 1-3 jaar. Op basis van de mogelijkheden bij de start van het project zal hierin een definitieve keuze gemaakt worden.

In appendix 2 en 3 staan de functie van de positieve en negatieve controle nu expliciet benoemd.

J. Humane eindpunten.

2. **Vraag:** Zou disfunctie van het cardiovasculair systeem hier ook niet misstaan, zoals ontstaan van oedeem?

Antwoord:

Tijdens AF hebben de geiten een snelle en chaotische activiteit in de atria. Ondanks deze snelle activiteit is in de geit de frequentie in de kamers beperkt. Dit is een groot voordeel van het geiten model van AF t.o.v. andere grote dier modellen omdat de geit geen contractiele dysfunctie ontwikkeld. In meerdere projecten hebben we na maanden van AF geen verandering in hartminuut volume, linker kamerfunctie of oedeem vorming geconstateerd. Dit punt wordt benoemd onder het kopje "replacement" van deze appendix en onder punt 3.4.1.

Ter verheldering is nu het uitblijven van heart failure expliciet benoemd.

3. **Vraag:** Hoe ziet u hier AF zelf? Kan dat een reden zijn voor een humaan eindpunt?

Antwoord:

Het welzijn bij patiënten met AF wordt door met name drie aspecten beïnvloed.

1. Afname van inspanningstolerantie. Patiënten met een gecomprimeerde pompfunctie kunnen tijdens een AF aanval een sterke afname van inspanningstolerantie ervaren. Echter bij patiënten met een normale pompfunctie is het acuut wegvallen van de atriale bijdrage aan de pompfunctie van beperkte waarde. Dit wordt ook geïllustreerd door het relatief vaak voorkomen van asymptomatisch AF. Zoals beschreven het antwoord op vraag 2 van appendix 2 leidt AF ook op lange termijn niet tot een verstoorde pomp functie. De geiten hebben daarom dus geen hinder van een verminderde cardiovasculaire functie.
2. Palpitaties of het ervaren van de onregelmatige hartslag. Een zeer groot aantal episodes van AF wordt niet door patiënten ervaren. Dit neemt niet weg dat voor een bepaalde groep patiënten het hebben van een onregelmatige het leven ernstig beïnvloed. Echter bij het opwekken van AF, met een lage stroomsterkte van de stimulator, is nooit enige reactie van de geit te zien. Verder neemt de fysieke activiteit van de geit ook niet af.
3. Complicaties van trombo-embolische events. Het grootste gevaar van AF is de vorming van stolsels in de atria. Indien deze losschieten kan dit tot een beroerte leiden. In de vele jaren van AF onderzoek in ons lab, hebben wij in de geit geen events geïdentificeerd die bijvoorbeeld leidde tot neurologisch consequenties voor de geit.

Op basis van deze overwegingen denken we dat AF zelf geen (meetbaar) effect heeft op het welzijn van de geit. Daarom verwachten wij geen extra humane eindpunten te moeten opnemen.

Geen aanpassing van het protocol uitgevoerd.

Appendix 3

Vragen:

B. De Dieren.

1. **Vraag:** U geeft aan dat een groepsgrootte van 8 dieren voldoende ("sufficient") is. Kan het niet met minder?

Antwoord:
Voor sommige parameters zoals bijvoorbeeld een refractaire periode zou een kleiner aantal dieren misschien mogelijk zijn. Maar om het elektrofysiologische mechanisme dat bijdraagt aan het termineren van AF zijn een combinatie van verschillende factoren van belang. Een greep aan parameters zijn bijvoorbeeld de activatie frequentie, snelheid van geleiding, het aantal golven, ectope activiteit etc. De samenhang van deze parameters bepaald in welke theoretische concepten het effect van de compound past. Uit ervaring van eerdere projecten blijkt dat ongeveer 8 dieren afdoende zijn.
Geen aanpassing van het protocol uitgevoerd.

2. **Vraag:** Ook hier de vraag waar baseert U de aantallen targets op, waarom komt U tot deze aantallen? Waarvoor gebruikt U twee controle groepen?

Antwoord:
Deze vraag is voor ons niet geheel helder. Hopelijk beantwoorden we hieronder de vragen van de DEC.
Het aantal targets dat wij willen bestuderen wordt door een aantal aspecten bepaald. Zoals in de achtergrond van de PV staat beschreven zijn we van mening dat target 1-5 wetenschappelijk en klinisch interessant zijn. Deze targets zijn atriaal en of AF specifiek. Voor al deze targets zijn stoffen in ontwikkeling binnen ons netwerk. De beschikbaarheid van compounds, met een bewezen antiaritmische potentie in vitro, bepalen welke targets bestudeerd kunnen worden. In theorie is het mogelijk dat alle targets bestudeerd worden.
De time-matched controle wordt alleen gebruikt in de modellen "short term AF in combination with heart failure" en in "non-selfterminating AF with electrical remodeling and structural remodelling". We denken dat voor een correcte opzet van de studie de time-matched controle nodig is. In antwoord op vraag 1 van appendix 2 zijn we hier uitgebreider op in gegaan. We wensen de positieve controle groep mee te nemen omdat we de mogelijk betere werking van de nieuwe drugs willen aantonen.
In appendix 2 en 3 staan de functie van de positieve en negatieve controle nu expliciet benoemd.

H. Pijn en pijnbestrijding.

3. **Vraag:** Hoe ziet u hier AF zelf? Kan dat een reden zijn voor een humaan eindpunt?

Antwoord:
Zie antwoord op vraag 3 appendix 2.
Geen aanpassing van het protocol uitgevoerd.

I. Overige aantasting van het welzijn en maatregelen.

4. **Vraag:** Hierin wordt gevraagd wat er gedaan wordt om het te voorkomen, hier staat meer beschreven wat er technisch gedaan wordt m.n. bij electrode failure.

Antwoord:
Het is voor ons niet geheel duidelijk wat met deze opmerking bedoeld wordt. In de appendix wordt gevraagd welke aspecten een aantasting kunnen zijn. In het document staan 5 punten opgenoemd. Infectie, uitval een geit ten gevolge een kapotte elektrode, aangepaste huisvesting, decompensatie tgv hartfalen en verminderde eetlust. Vervolgens behandelen we waarom we denken dat deze complicaties voorkomen. Tot slot worden alle vijf de punten behandeld hoe we hiermee omgaan om het risico tot een minimum te beperken. In principe zijn dit inderdaad veelal technische aanpassing maar allen dragen bij aan het beperken van het ongerief dus in onze ogen relevant om te benoemen.

Infectie → procedure aangepast om deze te voorkomen
Uitval tgv beschadigde elektrode → design en hok aangepast
Huisvesting → zo ontworpen dat de huisvesting zo veel mogelijk groepshuisvesting benaderd.

Decompenserend hartfalen → monitoring voor vroege detectie en behandeling met diuretica en verandering van de pace frequentie.
Voedselinname → exacte reden is nog onbekend. Hier gaan we systematisch verder naar kijken.
Geen aanpassing van het protocol uitgevoerd.

J. Humane eindpunten.
5. **Vraag:** Zou hier oedeem als klinisch verschijnsel niet misstaan?
Antwoord:
Oedeem vorming is inderdaad voor het hartfaalmodel een relevante complicatie. Dit is nu opgenomen in de tekst. Echter oedeem kan ook optreden ter hoogte van de operatie wand na implantatie. Hiervoor is dan ook een uitzondering gemaakt
Aangepast en in grijs gearceerd.

NTS
Opmerking: 3.3. en 3.5 lijken niet in overeenstemming qua aantallen.
Punt 3.3 is gecorrigeerd en in grijs gearceerd.

- Datum antwoord 23-11-2016
- Verstrekte antwoorden: Zie hierboven.
- De antwoorden hebben wel geleid tot aanpassing van de aanvraag.

10. **Eventuele adviezen door experts:** (niet lid van de DEC-UM) ***N.V.T.***

B. Beoordeling (adviesvraag en behandeling)

1. Is het project vergunningplichtig (dierproeven in de zin der wet)? Indien van toepassing, licht toe waarom het project niet vergunningplichtig is en of daar discussie over geweest is. ***JA***

2. De aanvraag betreft een **nieuwe** aanvraag.

3. Is de DEC competent om hierover te adviseren? ***Ja***

4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom. ***N.V.T.***

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft.

   Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. Het is helder welke handelingen en ongerief individuele dieren zullen ondergaan.
   De DEC-UM vertrouwt erop dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. Gezien bovenstaande is de DEC-UM van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.

2. Geef aan of er aspecten in deze aanvraag zijn die niet in overeenstemming zijn met wet- en regelgeving anders dan de Wod? Denk hierbij aan bijvoorbeeld de Flora en fauna wet en Wet dieren. Indien van toepassing, leg uit om welke aspecten het gaat en waarom hier sprake van is.

N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

3. Beoordeel of de in de projectaanvraag aangekruiste doelcategorie(ën) aansluit(en) bij de hoofddoelstelling. Nevendoelstellingen van beperkt belang hoeven niet te worden aangekruist in het projectvoorstel.

   Het projectvoorstel heeft inderdaad kenmerken van zowel fundamenteel als translationeel onderzoek.

   Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een reële relatie is tussen beide doelstellingen.

   Zie antwoord op vraag C5.

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden.

   De belangrijkste belanghebbenden in dit fundamenteel en toegepast wetenschappelijke project dat gericht is op de ontwikkeling van nieuwe geneesmiddelen ter behandeling van atrium fibrilleren, zijn de proefdieren, de onderzoekers, de doelgroep/patiënten en hun naasten, en ook de medische wetenschap en de samenleving als geheel.
   Waarden die voor de proefdieren in het geding zijn: De integriteit van de dieren zal worden aangetast door de experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven en de opoffering aan het eind daarvan. Gedurende de proeven zullen de dieren gering, matig of ernstig ongerief ondervinden.
   Waarden die voor de onderzoekers bevorderd worden: De onderzoekers zullen medisch-wetenschappelijke kennis verkrijgen en delen met de wetenschappelijke gemeenschap. De onderzoekers vergaren kennis over de werking en het therapeutische effect van bepaalde farmaca op atrium fibrilleren. Uiteindelijk kan meer kennis daarover leiden tot verbeterde therapeutische mogelijkheden in de cardiologie.
   Waarden die voor patiënten bevorderd worden: Meer kennis over nieuwe farmaceutische strategieën in de behandeling van atrium fibrilleren levert, behalve een beter begrip van deze aandoening, ook bouwstenen voor een verbeterde therapie.
   Dat kan leiden tot vermindering van ziektelast en verbetering van levensverwachting. Hierdoor kan uiteindelijk de kwaliteit van leven verbeterd worden van deze patiënten en hun naasten.
   Groei van medische kennis op een gebied waar daaraan behoefte is, wordt eveneens bevorderd door het onderhavige onderzoek. Atrium fibrilleren komt nu al veel voor en zal gezien de stijgende levensverwachting steeds meer mensen treffen. Daarom heeft dit onderzoek ook belang voor de samenleving als geheel.

6. Geef aan of er sprake kan zijn van substantiële milieueffecten. Zo ja, benoem deze, leg uit waarom daar sprake van kan zijn en of geef aan of deze effecten afgedekt worden door specifieke wetgeving.

   N.v.t., daar dit buiten de taakstelling van de DEC valt overeenkomstig artikel 18a.2.b van de Wod.

   Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw antwoord toe.

   Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat gezien de wetenschappelijke output, de verworven interne- en externe financiering alsmede de aandacht voor de drie V's.

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw antwoord toe.

   De DEC-UM is er van overtuigd dat het projectvoorstel aansluit bij recente wetenschappelijke inzichten en geen hiaten bevat die de bruikbaarheid van de resultaten in de weg zullen staan. De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder gekozen en sluiten aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kunnen naar de mening van de DEC-UM leiden tot het behalen van de doelstelling in het kader van het project.

   Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en de aanvrager voldoet aan de in de Wod voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw antwoord toe. ***N.V.T.***

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van de richtlijn. Indien niet aan deze minimale eisen kan worden voldaan omdat het om wetenschappelijke redenen noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht toe waarom wel/niet.

    De DEC-UM heeft zich ervan verzekerd dat zulks het geval is.

11. Beoordeel of het ongerief als gevolg van de dierproeven realistisch is ingeschat en geclassificeerd, waarbij uitgegaan wordt van de kans op angst, pijn, stress en/of ziekte bij individuele dieren.

    De DEC-UM vertrouwt erop dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen.

12. Geef aan op welke wijze de integriteit van de dieren wordt aangetast.

    De integriteit van de dieren zal worden aangetast door: De experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven. De dieren worden aan het eind van de proef opgeofferd.

13. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw antwoord toe.

Naar de mening van de DEC-UM zijn de humane eindpunten zorgvuldig beschreven en is de inschatting van het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken eveneens zorgvuldig beschreven in de projectaanvraag.

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn? Onderbouw uw antwoord.

    De DEC-UM is van mening dat de doelstellingen van de proef niet behaald kunnen worden, anders dan met de aangevraagde dieren, daar geschikte vervangingsalternatieven ontbreken, zoals beschreven in onderhavig projectvoorstel.

15. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Onderbouw uw antwoord.

    Naar de mening van de DEC-UM is het aantal te gebruiken dieren realistisch ingeschat en wel zodanig dat niet meer dan nodig, maar ook niet minder dan nodig dieren worden gebruikt voor het behalen van een betrouwbaar wetenschappelijke resultaat zulks mede gebaseerd op statistische analyse middels een poweranalyse.

16. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd? Licht uw antwoord toe.

    De DEC-UM heeft zich ervan verzekerd dat de aanvrager al het mogelijke zal doen om het eventuele ongerief voor de proefdieren te identificeren, te verminderen en waar mogelijk te voorkomen. Hierbij heeft de DEC-UM onder andere de pijnbestrijding en huisvesting in haar beoordeling betrokken.

17. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Onderbouw uw antwoord.

    Voor zover de DEC-UM kan beoordelen zijn de kennis en kunde van de onderzoeksgroep adequaat en mede gezien het daartoe strekkende antwoord van de aanvrager in de projectaanvraag heeft de DEC-UM reden aan te nemen dat onnodige duplicatie achterwege blijft.

    Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd? Geef ook aan welke maatregelen verder zijn getroffen om bij fok of aankoop van dieren het aantal in voorraad gedood te beperken.

    In onderhavige projectaanvraag worden wel dieren van een eenvormig geslacht gebruikt. Uit de projectaanvraag blijkt dat de onderzoeker zich bewust is van het nadeel van het gebruiken van dieren van een eenvormig geslacht zulks in relatie tot de vermindering van proefdieren in voorraad gedood en de onderzoeker heeft in de projectaanvraag naar de mening van de DEC-UM dit voldoende onderbouwd. Alhoewel de DEC-UM vermindering van proefdieren in voorraad gedood toejuicht is zij overigens van mening dat dit aspect met name met de centrale dienst proefdieren en de aanvrager kortgesloten dient te worden daar de DEC niet betrokken is bij de fok

en aankoop van proefdieren.

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van de richtlijn. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht dit toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd.

    Naar de mening van de DEC-UM is dit genoegzaam beschreven in de projectaanvraag door de aanvrager.

20. Indien dieren worden gedood, is adoptie of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.

    Adoptie is ten aanzien van onderhavige aanvraag niet opportuun daar het hier niet handelt om niet-humane primaten, honden, katten of landbouwhuisdieren.

    NTS

21. Is de niet-technische samenvatting een evenwichtige weergave van het project en begrijpelijk geformuleerd?

    Naar de mening van de DEC-UM is zulks het geval.

D. Ethische afweging

1.
Rechtvaardigt het verkrijgen van kennis over de werking en het eventuele therapeutisch effect van bepaalde farmaca op atrium fibrilleren, de opoffering en het geringe, matige, dan wel ernstige ongerief dat de dieren wordt aangedaan in het voorliggende project "*Development of new pharmacological strategies to treat atrial fibrillation*?".

2.
Waarden die voor de proefdieren in het geding zijn: *matig/ernstig nadeel.*
Waarden die voor onderzoekers bevorderd worden: *substantieel voordeel.*
Waarden die voor de doelgroep bevorderd worden: *matig voordeel.*
Algemeen: *relevante groei van medische kennis.*

De DEC-UM is van mening dat de belangen van de samenleving in het algemeen en de patiënten en hun naasten in het bijzonder binnen het project "*Development of new pharmacological strategies to treat atrial fibrillation"* zwaarder wegen dan de belangen/waarden van de proefdieren.
Voor de betrokken proefdieren leiden deze proeven, na mild, matig of ernstig ongerief, tot de dood. Zij worden door de experimenten in hun welzijn geschaad. De integriteit van de dieren wordt geschaad door: de experimentele handelingen en het leven met de gevolgen daarvan gedurende de proeven en het opofferen aan het eind van de proeven. Indien de doelstellingen bereikt worden, zal dit project echter leiden tot inzicht in de werking en het therapeutische effect van bepaalde farmaca op atrium fibrilleren. Kennis van mogelijk nieuwe farmaceutische strategieën bij de behandeling van atrium fibrilleren kan het therapeutische arsenaal in de cardiologie vergroten. Hierdoor kan uiteindelijk de levensverwachting, ziektelast en kwaliteit van leven verbeterd worden van patiënten en hun naasten.
Atrium fibrilleren komt nu al veel voor. Het risico op atrium fibrilleren stijgt met de leeftijd. Atrium fibrilleren zal gezien de stijgende levensverwachting steeds meer mensen treffen. Daarom heeft dit onderzoek ook belang voor de samenleving als geheel. Vandaar dat de DEC-UM het onderhavige onderzoek van substantieel belang acht.

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD107002016782
Bijlagen
1

Datum 2 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted]

Op 15 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Development of new pharmacological strategies to treat atrial fibrillation." met aanvraagnummer AVD107002016782. Wij hebben uw aanvraag beoordeeld.

Op 1 februari 2017 heeft u uw aanvraag aangevuld. U heeft op ons verzoek het in vitro proces om de componenten te selecteren meer toegelicht. Uw antwoord is toegevoegd aan het dossier en meegewogen in de besluitvorming.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project "Development of new pharmacological strategies to treat atrial fibrillation." starten. De vergunning wordt afgegeven van 3 februari 2017 tot en met 1 februari 2022.

Overige wettelijke bepalingen blijven van kracht.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, vanwege de ongerief classificatie Ernstig, zoals bedoeld in artikel 10a1, lid 1d en lid 3, in de wet. Meer informatie over de eisen bij een beoordeling achteraf vindt u in de bijlage.

Datum:
2 februari 2017
Aanvraagnummer:
AVD107002016782

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-UM gevoegd. Dit advies is opgesteld op 15 december 2016. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 10 januari 2017 heeft de DEC gereageerd op onze vragen. Op ons verzoek is er aanvullend advies gegeven over de chirurgie en de afwijkende huisvesting in relatie tot de ongeriefclassificatie.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
2 februari 2017
Aanvraagnummer:
AVD107002016782

Centrale Commissie Dierproeven
namens deze:

[redacted]

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Universiteit Maastricht
Adres:	Postbus 616
Postcode en plaats:	6200 MD MAASTRICHT
Deelnemersnummer:	10700

deze projectvergunning voor het tijdvak 3 februari 2017 tot en met 1 februari 2022, voor het project "Development of new pharmacological strategies to treat atrial fibrillation." met aanvraagnummer AVD107002016782, volgens advies van Dierexperimentencommissie DEC-UM. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is Onderzoeker. Voor de uitvoering van het project is Hoofd electrofysiologie onderzoekslijn verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 15 december 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 15 december 2016;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 15 december 2016;
c Advies van dierexperimentencommissie d.d. 15 december 2016, ontvangen op 15 december 2016.
d De aanvullingen op uw aanvraag, ontvangen op 1 februari 2017

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1 Pharmacokinetic studies				
	Geiten (Capra aegagrus hircus) /	24	100% Licht	
3.4.4.2 Non-selfterminating AF with only electrical remodelling				
	Geiten (Capra aegagrus hircus) /	130	100% Matig	
3.4.4.3 Effects in paroxysmal AF, permanent AF and heart failure.				
	Geiten (Capra aegagrus hircus) /	200	50% Ernstig 50% Matig	

Aanvraagnummer:
AVD107002016782

Voorwaarden
Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen
In dit project worden dierproeven toegepast die vallen in de categorie ernstig volgens artikel 10b van de wet en wordt daarom voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk februari 2023 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Aanvraagnummer:
AVD107002016782

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.

Beoordeling achteraf
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.