	Inventaris Wob-verzoek W15-12								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS 2015159								
1	Aanvraagformulier				x		x	x	
2	Niet-technische samenvatting	x							
3	Projectvoorstel				x			x	
4	Bijlage beschrijving dierproeven 1				x			x	
5	Bijlage beschrijving dierproeven 2				x			x	
6	Bijlage beschrijving dierproeven 3				x			x	
7	Bijlage beschrijving dierproeven 4				x			x	
8	Bijlage beschrijving dierproeven 5				x			x	
9	DEC-advies				x		x	x	
10	Ontvangstbevestiging				x		x	x	
11	Advies CCD		x						x
12	Beschikking en vergunning				x		x	x	
13	Mail beschikking 13-10-2015				x		x	x	
14	Mail terugkoppeling DEC 14-10-2015				x		x	x	

Centrale Commissie Dierproeven

1.

22 SEP. 2015

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.zbo-ccd.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* 90500 / 159

☐ Nee > *U kunt geen aanvraag doen*

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

Naam instelling of organisatie	BioXpert B.V.	
Naam van de portefeuillehouder of diens gemachtigde	[redacted]	
KvK-nummer	54838134	
Straat en huisnummer	Nistelrooise Baan	3
Postbus		
Postcode en plaats	5374RE	Schaijk
IBAN	NL72RABO0183605888	
Tenaamstelling van het rekeningnummer	BioXpert BV	

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	[redacted]	
Afdeling	[redacted]	
Telefoonnummer	[redacted]	
E-mailadres	[redacted]	

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

(Titel) Naam en voorletters		☐ 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 - 7 - 2015
		Einddatum	1 - 7 - 2018
3.2	Wat is de titel van het project?	Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants.	
3.3	Wat is de titel van de niet-technische samenvatting?	Plaatselijke afgifte van testosteron en alendronaat voor een sneller herstel van botbreuken en -defecten en voor het verbeteren van de verankering van botschroeven in bot.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	[redacted]
		Postadres	[redacted]
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 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	[redacted]
Plaats	Schaijk
Datum	3 - 7 - 2015
Handtekening	[redacted]

BioXpert B.V.
Nistelrooise Baan 3
5374 RE Schaijk
The Netherlands
T: +31 (0) 486-463303
F: +31 (0) 486-463498

info@bioxpert.nl
www.bioxpert.nl

Aan: Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Datum: 21 september 2015
Betreft: Aanvraag projectvergunning Dierproeven AVD905002015159

Geachte heer / mevrouw,

Bijgaand de getekende aanvraag Projectvergunning Dierproeven AVD905002015159 met als titel:

Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants..

Deze aanvraag is vandaag met de beveiligde e-mailverbinding ingediend.

Met vriendelijke groet,

[redacted]

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
1.3	Provide the title of the project.	Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants.

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.

Bone tissue is one of the most frequently used tissues for transplantation. Annually more than two million patients are treated worldwide for skeletal complications in the field of orthopedic surgery, plastic surgery, maxillofacial surgery, and neurosurgery. The use of autologous bone grafts is still considered to

be the golden standard, but there are several major disadvantages associated with this technique, for example: low availability of the transplantable tissue, postoperative morbidity and lack of functional shape of the transplant. Moreover, bone graft surgery is considered a painful procedure and usually results in (significant) blood loss. Next to autologous bone grafts, synthetic and natural bone substituting materials are commercially available in granules, blocks or injectable formulations. These materials are based on calcium phosphates (hydroxyapatite) which resemble the bone mineral which only supports bone formation, but doesn't stimulate (induce) bone formation.
Furthermore, each year there are over 6 million bone fractures reported in the U.S. alone. Approximately one third of which require internal fixation devices to help facilitate healing. Despite the use of these devices about 10-15% of the fractures heal very poorly. The development of osteo-inductive medication to improve the healing of such fractures is therefore considered a high medical need. At present, insertion of metal implants in bone is one of the most common of all surgical procedures. It is part of fracture treatment, joint replacement, and dental surgery. The success of these operations is dependent of the fixation of the implants (osseo-integration), which depends on the strength of the bone that holds them. If the bone quality is poor, surgical procedures are modified to provide sufficient mechanical fixation by adding more screws or larger devices, or by protecting the implant from mechanical loading for a considerable postoperative time to allow bone integration. Thus, if the quality of the bone holding the implant could be improved locally, e.g. by coating implants with an osteo-inductive carrier, surgical procedures would become simpler and rehabilitation would become faster. Therefore, the development of bone inducing materials is a very relevant issue in bone (fracture) healing.
Currently, there are very few therapies available to enhance bone growth to improve bone (fracture) healing and/or to improve bone integration of implants. The only product currently available to replace autologous bone grafts is Infuse from Medtronic which is based on the use of BMP-2. Due to the high costs, limited efficacy and, more recently, reported adverse events, this product is not used in The Netherlands. Recently, Shah NJ et al. (PNAS, September 2014, 12847–12852) reported the use of a thin film loaded with growth factor BMP-2 and alendronate to successfully regenerate bone defects in a rat model. Clinical studies have not yet been initiated for this combination. Regarding the use of implants, only one company has developed an implant coated with a bisphosphonate to improve bone integration of implants. This product is currently under clinical evaluation and is pending market approval (AddBIO, Sweden).

Steroidal androgens activate bone forming cells and have shown positive effects on bone mineral density (BMD) and increase bone anabolic biomarkers in clinical trials. However, despite promising results systemic use of steroidal androgens is not approved because of safety concerns, particularly due to potential cardiovascular and blood-clotting issues, prostate stimulation in men and virilizing activity in women (e.g. hirsutism, voice change, and acne). Bisphosphonates are widely used for the treatment and prevention of postmenopausal osteoporosis and have shown to significantly reduce hip and vertebral fractures in clinical trials. Bisphosphonates have relatively low systemic exposure but a high affinity for bone mineral, thus concentrating their effects at localized regions of bone remodeling and inhibiting local osteoclast mediated bone resorption. Bisphosphonates only have a limited capacity to increase osteoblastic activity and may even inhibit bone formation at some sites. This inability to stimulate new bone formation limits the clinical benefit from bisphosphonates in patients with established bone loss where, hence, an anabolic agent would be desirable.

Considering the above, it is Osteo-Pharma´s goal to develop an osteo-inductive carrier that is capable to locally enhance bone growth by simultaneously activating bone forming cells and inhibiting bone resorption cells. This will be achieved by applying locally a combination of a steroidal androgen (testosterone) and a bisphosphonate (alendronate) in that, due to the encapsulation in a biodegradable carrier, will be locally released to aid the bone regeneration process.

The concept of using both a steroidal androgen and a bisphosphonate has been tested in vitro at Oste-Pharma e.g. using the mouse MC3T3 osteoblast cell line. The data obtained indicated stimulation of bone forming cells (osteoblasts) under influence of testosterone and inhibition of bone resorbing cells (osteoclasts) under influence of alendronate. Therefore, these studies indicate that our proposed combination therapy would be able to successfully accelerate bone (fracture) healing as well as to improve implant fixation. To our knowledge, no publications of a combined local treatment of both androgen and bisphosphonates in bone (fractures) have been reported. The next step will be to test the efficacy of this combination therapy in rat and rabbit animal models commonly used in bone fracture repair and for the study of (coated) implants.

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?

The aim of the current project is to provide a local treatment which aids the bone (fracture) healing process, including pathological, traumatic and complicated defects and fractures and to improve osseo-integration of implants. The treatment will comprise of a biodegradable polymer with a combination of testosterone and alendronate for controlled and sustained release in films and microspheres or as a coating on dental and orthopedic screws. Therefore, in vivo experimental studies will be performed in established animal models for fracture repair and implants to evaluate testosterone and alendronate dosing combinations to optimize the amount of new bone formation by means of MicroCT, histology and biomarker analyses.
Our in vitro cell culture data suggest stimulation of bone forming cells (osteoblasts) under influence of testosterone and inhibition of bone resorbing cells (osteoclasts) under influence of alendronate. Therefore, these studies indicate that our proposed combination therapy would be able to successfully accelerate bone (fracture) healing as well as improve implant fixation in in vivo experimental studies.

3.3 Relevance

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

Currently, there are very few therapies available to enhance bone growth to improve fracture healing and/or to improve bone integration of implants. However, these require all very demanding therapy regimens and are cost intensive. Therefore, the medical relevance is to develop a novel therapy that can be used for treatment of bone defects and fractures, in particular to accelerate bone (fracture) healing. This novel therapy is less demanding for the patient, improves the quality of life and reduces healthcare costs.

3.4 Research strategy

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

The project will develop a carrier material loaded with a combination of testosterone and alendronate, to aid the bone (fracture) healing process and to improve osseo-integration of implants. This formulation is tested for its in vitro release characteristics as well as in cell culture systems to study the effect on osteoblast and osteoclast activity. With these in vitro data an optimal concentration of both testosterone and alendronate can be determined to achieve maximum bone regeneration. Therefore, a proof-of-principle study in rats will be performed to investigate if the acquired in vitro data can be translated into an in vivo situation. A sub-periosteal cranial study in rats will be performed to examine changes in bone turn-over by films containing an low, medium or high dose of testosterone and alendronate. A sub-periosteal study is a less invasive method to examine changes in bone biomarkers compared to a critical sized cranial defect. The doses of alendronate and testosterone are based on literature studies and in vitro data. The medium (optimal) concentration will be chosen and a 5 times lower and higher concentration will be chosen. After this proof-of-principle study, the next step is to implant films loaded with the optimal testosterone and alendronate concentrations into critical-sized cranial bone defects. The rat is the smallest animal available in which a flat critical-sized bone defect can be created and has therefore been selected as the model for these experiments. In addition, the effect of a coating containing testosterone and alendronate will be evaluated on dental and orthopedic screws. These screws will be implanted in femoral condyles of New Zealand White rabbits. Finally, a bone substitute (Demineralized Bone Matrix mixed with microspheres) will be used as a carrier for testosterone and alendronate. These implants will be placed in femoral condyles of New Zealand White rabbits as well. Due to the dimensions of the screws and the required size of a bone defect, femoral condyles of rabbits are the smallest animal model before a dog to physically allow implantation of the screws and or to fill a defect.

The first study performed will be to test various concentrations of testosterone and alendronate in the sub-periosteal rat model to determine if the optimal concentrations are used for bone regeneration purposes. (Go/No-Go). Subsequently, the rat sub-periosteal cranial defect model will be tested and rabbit studies will be initiated. For the rabbit implant and bone defect studies, the experiments will start with healthy 12 weeks old animals and if successful (Go/No-Go) will be followed by studies in the

ovariectomized (osteoporosis) model. Prior to all animal studies extensive in vitro cell culture studies have been performed to as guide for dosages to be used in the animal trials. In addition, data available from the literature will incorporated. After conducting these animal studies, bone substitutes, films and coated screws can be used in dental and orthopedic clinical studies.

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.

First, a proof-of-principle study in rats will be performed to investigate if the acquired in vitro data can be translated into an in vivo situation. Therefore, a sub-periosteal cranial study in rats will be performed to examine changes in bone turn-over by films containing an low, medium or high dose of testosterone and alendronate. This will be monitored by detecting biomarkers (PINP and CTx) in blood and by in vivo MicroCT analyses on the bone formation. Before collecting blood from the rats, overnight food intake should be restricted due to interference with biomarker levels. After this proof-of-principle study, the next step is to implant films loaded with the optimal testosterone and alendronate concentrations into critical-sized cranial bone defects. Besides normal healthy female rats, ovariectomized (OVX) rats whose ovaries have been removed will be used. The ovariectomized rat is generally considered a validated model to represent the most important clinical features of estrogen deficiency-induced (or postmenopausal) bone loss in adult females. It is widely used for studies relating to the prevention and treatment of osteoporosis in general as well as studies relating to the healing of osteoporotic fractures as this disease occurs predominantly in females. Therefore, rats (half of them will be ovariectomized) will be examined on their bone regeneration potential in time by means of MicroCT and biomarkers (PINP and CTx) in blood. Also, histological analyses will be performed after the end of the implantation period. In all rat experimental studies the same animals in time will be monitored with MicroCT. Therefore, less animals are needed and also less variation in the results will be expected.
Furthermore, screws will be coated with the optimal amount of testostone and alendronate. These screws will be implanted in femoral condyles of New Zealand White rabbits and will be evaluated in time by means of torgue testing, MicroCT and after the end of the implantation period by histological analyses. Finally, a bone substitute (Demineralized Bone Matrix mixed with microspheres) will be used as a carrier for testosterone and alendronate. These implants will be placed in femoral condyles of New Zealand White rabbits and will be evaluated in time by means of MicroCT and histological analysis on their bone tissue response.

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.

The coherence of the proposed animal experimental studies is to acquire, with an optimal concentration of both testosterone and alendronate, the optimal dose for bone regeneration. First, the efficacy of testosterone and alendronate will be determined in vivo with a sub-periosteal model in rats. These data will be the basis for subsequent studies in the rat and rabbit. With these data, critical-sized cranial defects will be treated with the optimal amount of testosterone and alendronate. Furthermore, coated screws will be implanted in femoral condyles of rabbits. Finally, a bone substitute will be implanted in femoral condyles of rabbits. If successful, these studies would qualify to be used in dental and orthopedic clinical studies.

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	Bone marrow collection with test surgery and MicroCT scanning of Wistar rats
2	Sub-periostal cranial proof-of-principle study in Wistar rats
3	Critical-sized cranial defect in Wistar rats
4	Femoral condyle screw implantation in New Zealand White rabbits
5	Demineralized Bone Matrix (DBM) mixed with testosterone and alendronate loaded microspheres implanted in rabbit femoral defects
6	
7	
8	

9	
10	

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
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: Test surgery and MicroCT scanning of Wistar rats and bone marrow collection

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.

Ten (twelve-week old) female Wistar rats will be used to practice the surgical procedures of the sub-periosteal and cranial defects. Furthermore, blood samples will be collected to set-up the biomarker analyses and in vivo MicroCT will be performed under terminal anesthesia to evaluate the optimal parameters to successfully acquire data within the following animal experiments. Finally, bone marrow will be collected for in vitro cell culture testing.

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

Ten (twelve-week old) female Wistar rats will be used by two technicians next to the principal investigator to practice the surgical procedures of the sub-periosteal and cranial defects. A successful operation means that the sinus (large bloodvessel) directly below the cranium will not be hit during surgery. This is followed directly by in vivo MicroCT scanning under terminal anesthesia to evaluate the optimal parameters to acquire a standardized greyscale (when is it bone or not) for the following animal experiments. This will be performed for all 10 rats. Furthermore, blood samples will be collected to set-up the biomarker analyses. Also, bone marrow will be collected for in vitro cell culture testing of osteoblasts and osteoclasts. Experience with cranial bone defects were gained in the past by the principal investigator. (Link et al., J Biomed Mater Res A. 2013 Nov;101(11):3059-65., J Biomed Mater Res A. 2009 Aug;90(2):372-9. & Biomaterials. 2006 Oct;27(28):4941-7. Both technicians have >5 year experience with working with rat and rabbit models for bone research at MSD/Organon (Gerrits et. al., J Bone Miner Res. 2011 Dec;26(12):2886-98 & Cui et al., Nat Med. 2011 Jun;17(6):684-91). Even though both principle investigator and technicians are skilled in these animal models no studies have been

performed in the last 5 years.

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

No statistical analysis is required to test the surgical procedures. It is estimated that the number of 10 rats would be sufficient to test the surgical procedures adequately and to reach the required level of competency.

B. The animals

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

Taking planned animal experiments in consideration, ten (twelve-week old) female Wistar rats will be used. A rat is the smallest animal available in which a flat critical-sized bone defect can be created. The age of a young adolescent has been chosen as these animals have reached their peak bone mass. Females are chosen because one of the objectives is to perform studies in an osteoporosis model that optimally mimics the human situation. These are generally not performed in male animals as osteoporosis predominantly occurs in women. Such a model will be generated through ovariectomy (OVX). Once 50% loss of Bone Mineral Density (BMD) has been achieved (as determined by MicroCT analysis) the studies will be initiated.

C. Re-use

Will the animals be re-used?

☒ 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.

During the surgical procedures, anesthesia and analgesia will be applied to reduce the discomfort of the rats as much as possible. During MicroCT scanning anesthesia will also be applied.

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

Measures to minimise adverse effects before, during and after surgery will include heating pads to maintain the optimal body temperature of the rats.

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.

Not applicable.

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

☒ 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?

☒ 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.

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

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

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

Surgery will be performed under general inhalation anesthesia. The anesthetics given (type, dosage, frequency) will be in consultation with the IvD.

I. Other aspects compromising the welfare of the animals

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

No long-term adverse effects on welfare are considered, since a continuous procedure under anesthesia within the same day will be performed. The rats will undergo a surgical procedure, meanwhile blood will be collected and consequently MicroCT scanning under terminal anesthesia will be performed.

Explain why these effects may emerge.

No long-term adverse effects on welfare are considered, since a continuous procedure under anesthesia within the same day will be performed. The rats will receive a surgical procedure, meanwhile blood will be collected and consequently MicroCT scanning under terminal anesthesia will be performed.

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

No long-term adverse effects on welfare are considered, since a continuous procedure under anesthesia within the same day will be performed. The rats will receive a surgical procedure, meanwhile blood will be collected and consequently MicroCT scanning under terminal anesthesia will be performed.

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.

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

Indicate the likely incidence.

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').

Non-recovery.

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.

To optimize the use of these animals, additionally bone marrow of both femurs and tibias will be collected for in vitro cell culture testing of osteoblasts and osteoclasts.

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
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: Sub-periosteal cranial proof-of-principle study in Wistar rats

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.

Twelve-week old female Wistar rats will be used to provide anabolic procollagen type I N-terminal propeptide (PINP) and catabolic carboxy-terminal telopeptide of type I collagen (CTX) biomarker data regarding the bone turnover during eight weeks in blood. Both biomarkers will appear in blood if new bone formation has occurred (PINP) or when bone resorption will take place (CTX) In this way, it will give supporting data regarding to bone turnover in time. Since direct bone formation will be measured with MicroCT and histology, biomarkers could serve as an important analyzing tool in future clinical trials, since MicroCT and histology in humans studies will not be possible.
Each rat will receive one film and all experimental groups consists of 3 films each (or no treatment as a neg. control or Parathyroid Hormone (PTH) injections, known to stimulate bone formation, as a pos. control. Blood will be obtained before the start of the surgery and after each two weeks during the implantation period of eight weeks. Specific ELISAs will be used to determine the serum levels of PINP and CTX. Besides these biomarkers, MicroCT data will be gathered before the start of the experiment and after two, four, six and eight weeks implantation period to assess the bone volume and bone mineral density.

	Films containing testosterone (high) and alendronate (n=3)	Films containing testosterone (medium) and alendronate (n=3)	Films containing testosterone (low) and alendronate (n=3)	Only film (n=3)	No treatment (n=3) (Neg. control)	PTH treatment (n=3) (Pos. control)
Wistar rat	PINP, CTX and MicroCT (0, 2, 4, 6 and 8 weeks)					

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

Surgery will be performed under general inhalation anesthesia. To minimize postoperative pain, appropriate analgesics will be administered preoperatively and postoperatively. All anesthetics and analgesics given (type, dosage, frequency) will be decided on in consultation with the IvD. After anesthesia, the rats will be immobilized on their abdomen, and the skull will be washed and disinfected with iodine. To minimize pain, lidocaine will be administered onto the periosteum before incision. A longitudinal incision will be made down to the periosteum from the nasal bone to the occipital protuberance, and soft tissues will be sharp dissected to visualize the cranial periosteum. Subsequently, a midline incision will be made in the periosteum, and the periosteum will be undermined and lifted off in left and right directions on the parietal skull. Consequently, films containing testosterone and alendronate will be placed in the subperiosteal space. The periosteum will be closed using resorbable Vicryl suture material. Subsequently, the skin will be closed using resorbable Vicryl sutures. To minimize postoperative discomfort, treatment with Temgesic will be continued for 2 days postoperatively. Also, the animals will be observed for weight(loss), signs of pain, infection, and proper activity. Blood sample collection of 0.5 ml from the tail vein and MicroCT scanning (under anesthesia) will be performed after the surgery and after 2, 4, 6 and 8 weeks of implantation. Since the release of testosterone and alendronate will be limited to eight weeks, no further blood samples will be collected. Therefore, the rats will be euthanized eight weeks after surgery by an overdose of CO2/O2.

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

In this proof-of-principle study eighteen Wistar rats will be used to evaluate if the proposed combination therapy of testosterone/alendronate can be translated into an in vivo situation. The optimal concentration will be tested next to a lower and higher concentration. These results will give an indication on the in vivo effect and if the biomarkers are measureable in blood. Therefore, an n=3 will be sufficient for this proof-of-principle study. Accordingly, these findings will be taken in consideration for the following large experimental study.

B. The animals

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

Eighteen (twelve-week old) female Wistar rats will be used. This study will be the pilot study for the next large critical-sized defect study. Therefore, the same animal species will be used as for the large critical-sized defect study. A rat is the smallest animal available in which a flat critical-sized bone defect can be created and is considered a well-established model for studying fracture repair. A mouse cranium would be too concave and would be technically challenging to operate (requires microsurgery). The age of a young adolescent has been chosen as these animals have reached their peak bone mass. Females are chosen because it also aimed to perform studies in an osteoporosis model that optimally mimics the human situation. These are generally not performed in male animals as osteoporosis predominantly occurs in women. By OVX such a model will be generated. Once 50% loss of BMD has been achieved (as determined by MicroCT analysis) the studies will be initiated.

C. Re-use

Will the animals be re-used?

☒ 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.

The films containing testosterone/alendronate were tested in in vitro cell culture studies. There, it was shown that these constructs were stimulating osteoblasts, while simultaneously inhibiting osteoclasts. Before we can test these constucts in clinical situations, it is necessary to prove in an animal model that these constucts would be a good alternative for current treatments. Bone turn-over and the behaviour of these films by exposing them into a complex environment can only be studied in animal models. In this study, rats are the lowest animal species in which we can examine the bone turn-over properly. The number of 3 rats per timepoint (18 rats in total) is sufficient for this proof-of-principle study. During the surgical procedures, anesthesia and analgesia is applied to reduce the discomfort of the rats as much as possible.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. Aseptical techniques will be used. Still, if afterwards animals would visually suffer, for instance by >20% weight loss or show pilo-erection, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rats from the study without consultation.

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.

Not applicable.

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

☒ 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?

☒ 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.

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

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

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

Surgery and MicroCT scanning (after surgery, 2, 4 ,6 and 8 weeks) will be performed under general inhalation anesthesia. To minimize postoperative pain, appropriate analgesics will be administered preoperatively and postoperatively. All anesthetics and analgesics given (type, dosage, frequency) will be decided on in consultation with the IvD.

I. Other aspects compromising the welfare of the animals

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

Possibly complications like infections or swelling could occur due to the surgical procedure, resulting in the suffering (anxiety, fear, loss of apetite) of the rats.

Explain why these effects may emerge.

Infections or swelling due to contaminates can't be completely eliminated from the operating theater. Despite all preventive microbiological procedures, even with preoperative antibiotic treatment, few (<5%) of all operations performed results in microbiological infections.

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

The implanted constructs will be manufactured under GLP conditions. After producing the constructs, a final sterilization step will be performed to guarantee sterility. This will also be performed for all other equipment used in the surgical theatre. Therefore, micro-organisms will be reduced to a minimum in the surgical area.

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.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. Still, if afterwards animals would visually suffer, for instance by >20% weight loss or show piloerection, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rats from the study without consultation.

Indicate the likely incidence.

5%

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').

Moderate discomfort due to surgery (exp. groups). Minor discomfort for the control groups.

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.

Samples and their surrounding tissue will be explanted for histological analysis. Therefore, rats will be euthanized.

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
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: Critical-sized cranial defect in Wistar rats

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.

Eighty twelve-week old female Wistar rats will be used to provide MicroCT and histological data of bone formation up to eight weeks. Half of these rats will be ovariectomized being eight-weeks old to mimic an osteoporotic environment. These OVX-rats will be used after 4 weeks of ovariectomy, only when their body mineral density (BMD) is reduced to 50%. Else an additional 2 weeks will be added until the 50% reduction is achieved. Consequently, implants consisting of collagen sponges covered with a film containing testosterone and alendronate will be placed in a critical-sized cranial defects. The collagen sponges will serve as a guiding template for bone regeneration, which were not needed in the proof-of-principle study, since there was no critical-sized defect present. Also, films and sponges will be separately implanted in critical-sized cranial defects next to empty defects as control. The central full thickness bone defect will be drilled with a hollow trephine drill with an outer diameter of 8.0 mm to ensure an standardized defect. Each rat will receive one implant of 8 mm in diameter and all groups consist of ten implants each. Blood will be obtained before the start of the surgery and after each two weeks during the implantation period of eight weeks. Specific ELISAs will be used to determine the serum levels of PINP and CTX. Both biomarkers will appear in blood if new bone formation has occurred (PINP) or when bone resorption will take place (CTX) In this way, it will give supporting data regarding to bone turnover in time, next to the MicroCT and histological data. Besides these biomarkers, MicroCT data will be gathered before the start of the experiment and after two, four, six and eight weeks implantation period. This will be combined with histological data after eight weeks to assess the bone volume and bone mineral density.

	Collagen sponge with testosterone and alendronate film	Only film with testosterone and alendronate	Only collagen sponge	Empty defect
Wistar rats cranial defect	PINP, CTX and MicroCT (2, 4, 6 and 8 weeks) Histology (8 weeks)			
OVX-Wistar rats cranial defect				

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

To obtain ovariectomized rats, a small transverse peritoneal incision of 0.4-0.6 cm will be made with surgical scalpel on the middle part of the abdomen slightly towards right, just near to the second right nipple of the rat. The adipose tissue will be pulled away until the right uterine tube and the ovary surrounded by a variable amount of fat will be identified. The ovary and associated fat will be exteriorized by gentle retraction. The periovarian fat with the ovary will be pulled away from the incision site. After identifying the ovary and uterine horn, a suture will be performed around the area of the distal uterine horns, that will be sectioned thereafter, and the ovaries will be removed. The uterine horn is returned to the peritoneal cavity after the removal of ovaries. The wound will be closed in two layers (muscle and skin) using sterile Vicryl sutures. he procedure will be repeated for the left ovary through a similar kind of incision on the contralateral side. To minimize postoperative discomfort, analgesic treatment will be continued for 2 days postoperatively. Also, the animals will be observed for signs of pain, infection, and proper activity.
The critical-sized cranial defect surgery will be performed under general inhalation anesthesia. To minimize postoperative pain, appropriate analgesics will be administered preoperatively and postoperatively. All anesthetics and analgesics given (type, dosage, frequency) will be decided in consultation with the IvD. After anesthesia, the rats will be immobilized on their abdomen, and the skull will be washed and disinfected with iodine. To minimize pain, lidocaine will be administered onto the periosteum before incision. A longitudinal incision will be made down to the periosteum from the nasal bone to the occipital protuberance, and soft tissues will be sharp dissected to visualize the cranial periosteum. Subsequently, a midline incision will be made in the periosteum, and the periosteum will be undermined and lifted off in left and right directions on the parietal skull. To create a central full thickness bone defect in the parietal cranium, a hollow trephine drill with an outer diameter of 8.0 mm in a dental handpiece will be used. The bone defect will be carefully drilled under continuous cooling with physiologic saline, without damaging of the underlying dura. Then, the created bone segment will be carefully removed, without damaging the underlying sagittal sinus. Following insertion of collagen sponges covered with a film containing testosterone and alendronate, the periosteum will be closed using resorbable Vicryl suture material. Also, films and sponges will be separately implanted in critical-sized cranial defects next to empty defects as control. Subsequently, the skin will be closed using resorbable Vicryl sutures. To minimize postoperative discomfort, treatment with analgesics will be continued for 2 days postoperatively. Blood sample collection of 0.5 ml from the tail vein and MicroCT scanning (under anesthesia) will be performed after the surgery and after 2, 4, 6 and 8 weeks of implantation. Also, the animals will be observed for signs of pain, infection, and proper activity. The rats will be euthanized 8 weeks after surgery by an overdose of CO2/O2.

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

In this experimental study eighty Wistar rats in total will be used to evaluate if the proposed combination therapy of testosterone/alendronate can be translated into an orthotopic critical-sized defect. A Power analysis will be performed to determine the number of animals needed per experimental group to obtain statistically trustworthy data and that it would be implausible that the effect is based on chance. Therefore, an hypothesis will set with continuous variables, which ensures that no unnecessary high numbers of rats will be used. This formula is n=1+2C(s/d)^2 in which the C-value is calculated with the

power (1-β) and the wanted significance level (α). When two groups (experimental versus control) will be compared (with the power of 0.8 and a significance level of 0.05) it will result in a C-value of 7.85. A Bonferroni correction will be applied if more than two groups will be compared with each other. The s- and d- values represent the estimated standard deviation (s) (e.g. 0.7) and the expected difference between the experimental and control groups (e.g. 1). This would lead to an n=8.69 in this example. However, the actual values will be acquired from the previously performed proof-of-principle study of appendix 2. Based on literature an n=10 is a well-accepted number to achieve significant differences.

B. The animals

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

Eighty (twelve-week old) female Wistar rats will be used. This number is an estimate, since the appropriate number of rats will be determined with a power analysis based on the results of the proof-of-principle study of appendix 2.

C. Re-use

Will the animals be re-used?

☒ 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.

The constructs containing testosterone/alendronate films were tested in in vitro cell culture studies. There, it was shown that these constructs were stimulating osteoblasts, while simultaneously inhibiting osteoclasts. Before we can test these constructs in clinical situations, it is necessary to prove in an animal model that these constructs would be a good alternative for current treatments. Bone turn-over and the behavior of these films by exposing them into a complex environment can only be studied in animal models. Therefore, a dose-finding study will be performed (in experiment 2) and the following studies, including the current one, will be performed in phases to reduce the amount of animals as much as possible.
In this study, rats are the lowest animal species in which we can examine the bone turn-over properly. The amount of 10 rats per timepoint (80 rats in total) is sufficient for this experimental study. During the surgical procedures, anesthesia and analgesia is applied to reduce the discomfort of the rats as much as possible.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. A-septical techniques will be used. Still, if afterwards animals would visually suffer, for instance by >20% weight loss or show pilo-erection, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rats from the study without consultation.

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.

Not applicable.

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

☒ 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?

☒ 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.

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

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

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

Surgery and MicroCT scanning (after surgery, 2, 4 ,6 and 8 weeks) will be performed under general inhalation anesthesia. To minimize postoperative pain, appropriate analgesics will be administered preoperatively and postoperatively. All anesthetics and analgesics given (type, dosage, frequency) will be in consultation with the IvD.

I. Other aspects compromising the welfare of the animals

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

Possibly complications like infections or swelling could occur due to the surgical procedure, resulting in the suffering (anxiety, fear, loss of appetite) of the rats.

Explain why these effects may emerge.

Infections or swelling due to contaminates can't be completely eliminated from the operating theatre. Despite, all preventive microbiological procedures, 5% of all operations performed results in microbiological infections.

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

The implanted constructs will be manufactured under GLP conditions. After producing the constructs, a final sterilization step will be performed to guarantee sterility. This will also be performed for all other equipment used in the surgical theatre. Therefore, micro-organisms will be reduced to a minimum in the surgical area.

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.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. Also, the sinus of the brain might be damaged during surgery, resulting in (significant) blood loss. If afterwards animals would visually suffer, for instance by >20% weight loss or show piloerection, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rats from the study without consultation.

Indicate the likely incidence.

5%.

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').

Moderate discomfort.

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.

Samples and their surrounding tissue will be explanted for histological analysis. Therefore, rats will be euthanized.

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
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: 4 Type of animal procedure: Femoral condyle screw implantation in New Zealand White rabbits

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.

Twenty female New Zealand White rabbits (4-5 months) with a weight of approximately 3.5 kg will be used as experimental animals. Each rabbit will receive two screws in a trabecular bone defect as created in the left medial and right medial distal femur. In this way, forty implants will be inserted in twenty rabbits, divided into 2 experimental groups with 2 timepoints (6 and 12 weeks). All screws will receive the same manufacturing treatment, were half of the screws will contain testosterone and alendronate in their coating, while the control screws will have a blank coating. After three, six and nine weeks fluorchromes will be injected to be deposited into the matrix to visualize bone formation in time. Torque testing, MicroCT and histology will be performed after six and twelve weeks of implantation ex vivo to evaluate the screw anchoring and bone tissue response.

	Screws containing testosterone and alendronate	Only screws
New Zealand White Rabbits	Torque testing, MicroCT and Histology (6, and 12 weeks)	

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

Surgery will be performed under general inhalation anesthesia. Anesthesia will be induced by an intravenous injection of Propofol and atropine, and will be maintained by ketamine/medetomidine. The anesthesia will be antagonised after surgery by Antisedan. To reduce the peri-operative infection risk, the rabbits will receive antibiotic prophylaxis (Baytril 2.5% (enrofloxacin), 5–10 mg/kg). During anesthesia, the rabbits will be immobilized on their back and the surgical areas will be shaved and disinfected with povidone-iodine. A longitudinal incision will be made down to the periosteum. Subsequently, a midline incision will be created in the periosteum. The periosteum will be undermined and lifted off the distal femora. With several burrs (with an increasing burr diameter) the defects in the femora will be drilled from the medial direction to obtain undersized cylindrical defects. After insertion of the screws, the periosteum and soft tissue will be closed using resorbable Vicryl sutures. During the implantation period, fluorchromes will be subcutaneously injected in the neck. Directly after surgery and at least two days post-operative, an NSAID (Metacam) will be given. The first injection of oxytetracycline (25 mg/kg) will be administered 3 weeks post-surgery, the mid label with alizarine complexon (30 mg/kg) will be injected 6 weeks post-surgery and the third and last label of calcein green (15 mg/kg) will be injected 9 weeks post-surgery. The rabbits will be euthanized after six and twelve weeks post-surgery by an overdose of sodium pentobartital. Two timepoints are necessary to analyze the effect of bone formation in time and to what extend this would influence the mechanical properties (torque testing) Experience with (trabecular bone defects in) New Zealand White rabbits, including providing the animals antibiotics and final euthanasia, were gained in the past by the principal investigator. (Link et al. Biomaterials. 2008 Feb;29(6):675-82.)

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

In this experimental study twenty New Zealand White rabbits in total will be used to evaluate if the proposed combination therapy of testosterone/alendronate can be translated into an orthotopic defect. A Power analysis will be performed to determine the number of animals needed per experimental group to obtain statistically trustworthy data and that it would be implausible that the effect is based on chance. Therefore, an hypothesis will set with continuous variables, which ensures that no unnecessary high numbers of rabbits will be used. This formula is n=1+2C(s/d)^2 in which the C-value is calculated with the power (1-β) and the wanted significance level (α). When two groups (experimental versus control) will be compared (with the power of 0.8 and a significance level of 0.05) it will result in a C-value of 7.85. The s- and d- values represent the estimated standard deviation (s) (e.g. 0.7) and the expected difference between the experimental and control groups (e.g. 1). This would lead to an n = 8.69. The number of n=10 per group (two implants per rabbit, 40 implants in 20 rabbits) would therefore be chosen, since one rabbit might drop out due to infections or swelling of contaminants. Based on literature an n=10 is a well-accepted number to achieve significant differences.

B. The animals

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

Twenty female New Zealand White rabbits (4-5 months) with a weight of approximately 3.5 kg will be used as experimental animals. New Zealand White rabbits are a well-described model to investigate bone regeneration in a trabecular bone defect. Due to the dimensions of the screws, this is also the smallest animal model before a dog model to physically allow implantation of these screws.

C. Re-use

Will the animals be re-used?

☒ 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.

The concentration of testosterone/alendronate was tested in in vitro cell culture studies. There, it was shown that these constructs were stimulating osteoblasts, while simultaneously inhibiting osteoclasts. Before we can test screws coated with testosterone/alendronate in clinical situations, it is necessary to prove in an animal model that these screws would be a good alternative for current treatments. Bone turn-over and the behavior of these screws by exposing them into a complex environment can only be studied in animal models. Due to the dimensions of the screws, rabbits are the smallest animal species before a dog to physically allow implantation of these screws. The number of 9 rabbits per time point (20 rabbits in total) is statistically sufficient for this experimental study. During the surgical procedures, anesthesia and analgesia is applied to reduce the discomfort of the rabbits as much as possible.

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

If complications such as inflammation or swelling occurs or should the animals suffer visually, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rabbits from the study without consultation.

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.

Not applicable.

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

☐ No

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

Rabbits will be housed individually due to the surgical procedures.

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?

☒ 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.

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

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

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

Surgery will be performed under appropriate anesthetics. To minimize postoperative pain analgesic medication will be given. All anesthetics and analgesics given (type, dosage, frequency) will be decided on in consultation with the IvD.

I. Other aspects compromising the welfare of the animals

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

Possibly complications like infections or swelling could occur due to the surgical procedure, resulting in the suffering (anxiety, fear, loss of appetite) of the rabbits.

Explain why these effects may emerge.

Infections or swelling due to contaminants can't be completely eliminated from the operating theatre. Despite, all preventive microbiological procedures, 5% of all operations performed results in microbiological infections.

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

The implanted constructs will be manufactured under GLP conditions. After producing the constructs, a final sterilization step will be performed to guarantee sterility. This will also be performed for all other equipment used in the surgical theatre. Therefore, micro-organisms will be reduced to a minimum in the surgical area.

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.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. If afterwards animals would visually suffer, for instance by >20% weight loss or would not use both paws normally, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rabbits from the study without consultation.

Indicate the likely incidence.

Not expected.

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').

Moderate discomfort.

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.

Samples and their surrounding tissue will be explanted for torque testing, MicroCT and histological analysis. Therefore, rabbits will be euthanized.

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'.	90500
1.2	Provide the name of the licenced establishment.	BioXpert BV
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: 5 Type of animal procedure: Demineralized Bone Matrix (DBM) mixed with testosterone and alendronate loaded microspheres implanted in rabbit femoral defects

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.

Twenty-five female New Zealand White rabbits (4-5 months) with a weight of approximately 3.5 kg will be used as experimental animals. The samples consist of a commercially available bone filler, demineralized bone matrix (DBM), mixed with testosterone and alendronate loaded microspheres. The microspheres will degrade in time to allow a sustained release of testosterone and alendronate. Each rabbit will receive two samples in a trabecular bone defect as created in the left medial and right medial distal femur. In this way, forty implants (and 10 empty defects) will be placed in twenty-five rabbits. The empty defect will only be analyzed after 12 weeks to evaluate if the created defect will not heal spontaneously. After three, six and nine weeks fluorchromes will be injected to be deposited into the matrix to visualize bone formation in time. Blood will be obtained before the start of the surgery and after each two weeks during implantation period of twelve weeks. Specific ELISA's will be used to determine the serum levels of PINP and CTX. MicroCT and histological analysis will be performed ex vivo after six and twelve weeks of implantation to evaluate the bone tissue response. Ten implants per experimental group will be used, two implants per rabbit.

	DBM + loaded microspheres	DBM + microspheres	Empty defects
New Zealand White Rabbits	MicroCT and histology (6 and 12 weeks)		MicroCT and histology (12 weeks)

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

Surgery will be performed under general inhalation anesthesia. Anesthesia will be induced by an intravenous injection of Propofol and atropine, and will be maintained by ketamine/medetomidine. The anesthesia will be antagonised after surgery by Antisedan. To reduce the peri-operative infection risk, the rabbits will receive antibiotic prophylaxis (Baytril 2.5% (enrofloxacin), 5–10 mg/kg). During anesthesia, the rabbits will be immobilized on their back and the surgical areas will be shaved and disinfected with povidone-iodine. A longitudinal incision will be made down to the periosteum. Subsequently, a midline incision will be created in the periosteum. The periosteum will be undermined and lifted off the distal femora. With several burrs (with an increasing burr diameter) the defects in the femora will be drilled from the medial direction to obtain undersized cylindrical defects. After insertion of the screws, the periosteum and soft tissue will be closed using resorbable Vicryl sutures. During the implantation period, fluorchromes will be subcutaneously injected in the neck. Directly after surgery and at least two days post-operative, an NSAID (Metacam) will be given. The first injection of oxytetracycline (25 mg/kg) will be administered 3 weeks post-surgery, the mid label with alizarine complexon (30 mg/kg) will be injected 6 weeks post-surgery and the third and last label of calcein green (15 mg/kg) will be injected 9 weeks post-surgery. The rabbits will be euthanized after six and twelve weeks post-surgery by an overdose of sodium pentobartital. Experience with trabecular bone defects in New Zealand White rabbits were gained in the past by the principal investigator. (Link et al. Biomaterials. 2008 Feb;29(6):675-82.)

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

In this experimental study twenty-five New Zealand White rabbits in total will be used to evaluate if the proposed DBM mixed with testosterone and alendronate loaded microspheres can be translated into an orthotopic defect. A Power analysis will be performed to determine the number of animals needed per experimental group to obtain statistically trustworthy data. Therefore, an hypothesis will set with continuous variables, which ensures that no unnecessary high numbers of rabbits will be used. This formula is $n=1+2C(s/d)^2$ in which the C-value is calculated with the power ($1-\beta$) and the wanted significance level (α). When two groups (experimental versus control) will be compared (with the power of 0.8 and a significance level of 0.05) it will result in a C-value of 7.85. The s- and d- values represent the estimated standard deviation (s) (e.g. 0.7) and the expected difference between the experimental and control groups (e.g. 1). This would lead to an n = 8.69 in this example. The number of n=10 per group (two implants per rabbit, 50 implants in 25 rabbits) would therefore be chosen, since one rabbit might drop out due to infections or swelling due to contaminants. Based on literature an n=10 is a well-accepted number to achieve significant differences.

B. The animals

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

Twenty-five female New Zealand White rabbits (4-5 months) with a weight of approximately 3.5 kg will be used as experimental animals. New Zealand White rabbits are a well-described model to investigate bone regeneration in a trabecular bone defect. Due to the dimensions of the implants, this is also the smallest animal model before a dog model to physically allow implantation.

C. Re-use

Will the animals be re-used?

☒ 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.

The concentration of testosterone/alendronate was tested in in vitro cell culture studies. There, it was shown that these constructs were stimulating osteoblasts, while simultaneously inhibiting osteoclasts. Before we can test DBM mixed with testosterone and alendronate loaded microspheres in clinical situations, it is necessary to prove in an animal model that these implants would be a good alternative for current treatments. Bone turn-over and the behaviour of these implants by exposing them into a complex environment can only be studied in animal models. Due to the dimensions of the implants, rabbits are the smallest animal species before a dog to physically allow implantation. In this study, rabbits are the lowest animal species in which we can examine the bone turn-over properly. The amount of 9 rabbits per time point (25 rabbits in total) is sufficient for this experimental study. During the surgical procedures, anesthesia and analgesia is applied to reduce the discomfort of the rabbits as much as possible.

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

If complications such as inflammation or swelling occurs or should the animals suffer visually, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rabbits from the study without consultation.

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.

Not applicable.

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

☐ No

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

Rabbits will be housed individually due to the surgical procedures.

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?

☒ 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.

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

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

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

Surgery will be performed under appropriate anesthetics. To minimize postoperative pain analgesic medication will be given. All anesthetics and analgesics given (type, dosage, frequency) will be decided on in consultation with the IvD.

I. Other aspects compromising the welfare of the animals

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

Possibly complications like infections or swelling could occur due to the surgical procedure, resulting in the suffering (anxiety, fear, loss of appetite) of the rabbits.

Explain why these effects may emerge.

Infections or swelling due to contaminates can't be completely eliminated from the operating theatre. Despite, all preventive microbiological procedures, 5% of all operations performed results in microbiological infections.

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

The implanted constructs will be manufactured under GLP conditions. After producing the constructs, a final sterilization step will be performed to guarantee sterility. This will also be performed for all other equipment used in the surgical theatre. Therefore, micro-organisms will be reduced to a minimum in the surgical area.

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.

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

Preoperative antibiotic treatment will be given to significantly reduce, but not eliminate, the 5% risk of bacterial contamination during the surgical procedure. If afterwards animals would visually suffer, for instance by >20% weight loss or the rabbit would not use it both paws, then the animals can be withdrawn from the study. This will always be in consultation with bio-technicians and a veterinarian. If the responsible researchers, the laboratory animal experts and the veterinarian are not reachable, a veterinarian or bio-technician can withdraw the rabbits from the study without consultation.

Indicate the likely incidence.

5%.

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').

Moderate discomfort.

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.

Samples and their surrounding tissue will be explanted for histological analysis. Therefore, rabbits will be euthanized.

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

9.

Format DEC-advies

Maak bij de toepassing van dit format gebruik van de bijbehorende toelichting, waarin elke stap in het beoordelingsproces wordt toegelicht

A. Algemene gegevens over de procedure

1. Aanvraagnummer: AVD905002015159
2. Titel van het project:Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants.
3. Titel van de NTS: Plaatselijke afgifte van testosteron en alendronaat voor het herstel van botbreuken en -defecten en voor het verbeteren van de verankering van implantaten in bot.
4. Type aanvraag:

   X nieuwe aanvraag projectvergunning
5. Contactgegevens DEC:
   - naam DEC: [redacted]
   - telefoonnummer contactpersoon: [redacted]
   - mailadres contactpersoon: [redacted]
6. Adviestraject (data dd-mm-jjjj):

   X ontvangen door DEC: 3 juli 2015

   aanvraag compleet: 9 september 2015

   X in vergadering besproken: 22 juli 2015 en 09 september 2015

   anderszins behandeld

   termijnonderbreking(en) van / tot

   besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen

   X aanpassing aanvraag: 01 september 2015 en 18 september 2015

   advies aan CCD
7. Eventueel horen van aanvrager : n.v.t.
8. Correspondentie met de aanvrager
   - Datum: 25 juli 2015 en 13 september 2015
   - Strekking van de vragen:

Vragen d.d. 25 juli 2015:

- Tekstueel en redactioneel (verduidelijking, aanvulling en onderlinge afstemming van bepaalde tekstpassages en correcte invulling van het formulier, taalgebruik in de NTS).
- Nadere toelichting van het experimental design m.b.t. de evt. daarin opgenomen beslismomenten en nadere toelichting en onderbouwing van het gebruik van het osteoporose model.
- Nadere toelichting en onderbouwing van dierexperiment #1, #2 en #3.
- Onderbouwing van de keuze voor diersoort, leeftijd en sexe.
- Toelichting m.b.t. de te gebruiken anesthetica en analgetica.
- Nadere toelichting en onderbouwing van de toe te passen huisvesting.
- Nadere toespitsing van de toe te passen HEP-criteria.
- Nadere toelichting en onderbouwing van de (maximale) aantallen dieren en van de primaire meetparameters.

Vragen d.d. 13 september 2015:

- Toelichting m.b.t. de te gebruiken anesthetica en analgetica.
- Redactioneel (verduidelijking en aanvulling van tekstpassage in de NTS).

- Datum antwoord: 1 september 2015 en 18 september 2015
- Strekking van het antwoorden: de vragen zijn naar tevredenheid beantwoord, aanvraag na aanpassing volledig en duidelijk.
- De antwoorden hebben geleid tot aanpassing van de aanvraag.

9. Eventuele adviezen door experts (niet lid van de DEC): n.v.t., de DEC zelf beschikt over de relevante expertise.

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet)

2. De aanvraag betreft een nieuwe aanvraag.

3. De DEC is competent om hierover te adviseren.

4. Geen van de DEC-leden is betrokken bij het betreffende project of de aanvrager.

C. Beoordeling (inhoud):

1. Het project is:

X uit wetenschappelijk oogpunt verantwoord

2. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als een substantieel belang. Jaarlijks worden wereldwijd miljoenen patiënten behandeld vanwege uiteenlopende botproblemen. Bovendien is er als gevolg van de gemiddeld hogere leeftijd een toenemend aantal patiënten met botgenezingsproblemen als gevolg van osteoporose. Bij de behandeling van (gecompliceerde) botbreuken en -defecten wordt het gebruik van lichaamseigen botmateriaal nog steeds als de gouden standaard beschouwd. Hieraan zijn echter diverse beperkingen en complicaties verbonden. Zo is er is slechts een beperkte hoeveelheid weefsel beschikbaar, het botweefsel sterft af na transplantatie en is moeilijk vorm te geven. Bovendien betekent het verkrijgen van eigen botweefsel een extra (en pijnlijke) chirurgische ingreep voor de patiënt, met het risico van meer complicaties. Er is verder geen therapie beschikbaar waardoor botdefecten en/of -breuken sneller kunnen genezen. Met dit project wordt beoogd een lokaal toe te passen dragermateriaal te ontwikkelen dat beladen is met een combinatie van testosteron en alendronaat en dat het botgenezingproces en de integratie van implantaten zou moeten bevorderen. Het is de verwachting dat toepassing van deze therapie zal leiden tot tijd- en kostenbesparing in de gezondheidszorg en een verbeterde kwaliteit van leven voor de patiënt.

4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De *in vivo* experimenten worden uitgevoerd in reeds bestaande diermodellen voor dit type studies. Op basis van *in vitro* data zijn reeds aanwijzingen verkregen dat de te testen agentia inderdaad kunnen leiden tot stimulatie van botvormende cellen c.q. inhibitie van botresorberende cellen.

5. Er is in wettelijk opzicht geen sprake van bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren.
6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. De dieren ondergaan maximaal matig ongerief als gevolg van de experimentele procedures en als gevolg van mogelijke complicaties zoals ontstekingen en zwellingen. Dieren die het humane eindpunt bereiken worden uit proef genomen en geëuthanaseerd.
7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen**. Voor dit type onderzoek zijn levende dieren nodig, aangezien de complexe processen die betrokken zijn bij botvorming en -heling maar beperkt buiten het lichaam kunnen worden nagebootst. De te testen agentia zijn vooraf in vitro getest op de gewenste eigenschappen. De te gebruiken diermodellen zijn de kleinste diersoorten die geschikt zijn voor dit type onderzoek.
8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven. De dierstudies zijn gefaseerd opgezet, waarbij tussentijdse beslismomenten zijn opgenomen. Er wordt pas begonnen aan een volgend stadium van de studie als in het voorgaande experiment goede resultaten zijn behaald. Het maximale aantal te gebruiken dieren is realistisch ingeschat. De resultaten uit een voorgaand stadium worden ook gebruikt om het aantal benodigde dieren in de volgende studie te berekenen. Door gebruik te maken van *in vivo* Micro CT is het bovendien mogelijk om de botformatie in de loop van de tijd bij dezelfde dieren te volgen. Dit levert naast betrouwbare informatie tevens een enorme besparing op van het aantal benodigde dieren.
9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Daar waar van toepassing worden experimentele procedures uitgevoerd onder toepassing van adequate anesthesie en analgesie, om zoveel mogelijk ongerief te voorkomen. De dieren worden dagelijks gecontroleerd. Dieren die het humane eindpunt bereiken worden geëuthanaseerd.

   Er is geen sprake van belangwekkende milieueffecten.

10. D

e niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

D. Ethische afweging

Op basis van de overwegingen onder bovenstaand punt C (1 t/m 9) komt de commissie tot de volgende ethische afweging.
Slecht genezende botbreuken en andere slecht genezende botdefecten vormen een belangrijk probleem in de geneeskunde. Dit project beoogt een lokaal toe te passen dragermateriaal te ontwikkelen dat beladen is met een combinatie van testosteron en alendronaat en dat het botgenezingproces en de integratie van implantaten zou moeten bevorderen. Samen met andere nieuwe ontwikkelingen op het gebied van botvervangende materialen zou dat een belangrijke bijdrage kunnen leveren aan de oplossing van dit probleem. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling. Naar het oordeel van de DEC dient het project dan ook een substantieel belang.
Tegenover dit substantiële belang staat het feit dat de dieren in deze experimenten matig ongerief zullen ondervinden. De commissie is er van overtuigd dat bij de dierproeven adequaat invulling gegeven zal worden aan de vereisten op het gebied van de vervanging, vermindering en/of verfijning van dierproeven. Het gebruik van de dieren en het daarbij optredende ongerief is onvermijdelijk, wil men de doelstellingen kunnen realiseren. De doeleinden van het project rechtvaardigen het voorgestelde gebruik van dieren.
De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

1. Advies aan de CCD

X De DEC adviseert de vergunning te verlenen.

2. Het uitgebrachte advies is gebaseerd op consensus.

Centrale Commissie Dierproeven

10.

> Retouradres Postbus 20401 2500 EK Den Haag

BioXpert BV
[redacted]
Nistelrooise Baan 3
5374 RE SCHAIJK

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD905002015159

Bijlagen
2

Datum 24 september 2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 21 september 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD905002015159. 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).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA:	90500
Naam instelling of organisatie:	BioXpert BV
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	54838134
Straat en huisnummer:	Nistelrooise Baan 3
Postcode en plaats:	5374 RE SCHAIJK
IBAN:	NL72RABO0183605888
Tenaamstelling van het rekeningnummer:	BioXpert BV

Gegevens 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 juli 2015
Geplande einddatum:	1 juli 2018
Titel project:	Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants
Titel niet-technische samenvatting:	Plaatselijke afgifte van testosteron en alendronaat voor een sneller herstel van botbreuken en - defecten en voor het verbeteren van de verankering van bostschroeven in bot.
Naam DEC:	[redacted]
Postadres DEC:	[redacted]
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 741,-
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:	Schaijk
Datum:	3 juli 2015

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

BioXpert BV
[redacted]
Nistelrooise Baan 3
5374 RE SCHAIJK

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD905002015159

Bijlagen
2

Datum 24 september 2015
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 24 september 2015
Vervaldatum: 24 oktober 2015
Factuurnummer: 15700159

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD905002015159	€ 741,00

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL28RBOS 056.99.96.066 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 20401, 2500 EK te 's Gravenhage.

Centrale Commissie Dierproeven

12.

> Retouradres Postbus 20401 2500 EK Den Haag

BioXpert BV
[redacted]
Nistelrooise Baan 3
5374 RE SCHAIJK

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD905002015159

Datum 13 OKT. 2015
Betreft Aanvraag projectvergunning Dierproeven

Geachte [redacted]
Op 21 september 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants" met aanvraagnummer AVD905002015159. Wij hebben uw aanvraag beoordeeld.

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. U kunt met uw project "Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants" starten. De vergunning wordt afgegeven van 13 oktober 2015 tot en met 1 juli 2018.
Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie [redacted] gevoegd. Dit advies is opgesteld op 18 september 2015. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Wij nemen dit advies van de commissie over, inclusief de daaraan ten grondslag liggende motivering.
Dit 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).

Met vriendelijke groet,

Centrale Commissie Dierproeven
namens deze:

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	BioXpert BV
Adres:	Nistelrooise Baan 3
Postcode en plaats:	5374 RE SCHAIJK
Deelnemersnummer:	90500

deze projectvergunning voor het tijdvak 13 oktober 2015 tot en met 1 juli 2018, voor het project "Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants" met aanvraagnummer AVD905002015159, volgens advies van Dierexperimentencommissie [redacted]

De functie van de verantwoordelijk onderzoeker is [redacted] Voor de uitvoering van het project is Voorzitter LvD verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 21 september 2015
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 21 september 2015;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 21 september 2015;
c Advies van dierexperimentencommissie d.d. 18 september 2015, ontvangen op 21 september 2015.

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
Test surgery and MicroCT scanning of Wistar rats and bone marrow collection	Ratten (Rattus norvegicus) / Wistar; vrouwelijk; 12-weken oud	10	Terminal / non-recovery	Er worden alleen vrouwelijke ratten gebruikt, omdat het onderzoek gericht is op osteoporose, die komt vaker bij vrouwen dan bij mannen voor.
Sub-periosteal cranial proof-of-principle study in Wistar rats	Ratten (Rattus norvegicus) / Wistar; vrouwelijk; 12-weken oud	18	Matig / moderate	Er worden alleen vrouwelijke ratten gebruikt.
Critical-sized cranial defect in Wistar rats	Ratten (Rattus norvegicus) / Wistar; vrouwelijk; 12-weken oud	80	Matig / moderate	Er worden alleen vrouwelijke ratten gebruikt.
Femoral condyle screw implantation in New Zealand White rabbits	Konijnen (Oryctolagus cuniculus) / New Zealand White; vrouwelijk; 4-5 maanden oud	20	Matig / moderate	Er worden alleen vrouwelijke konijnen gebruikt.
Demineralized Bone Matrix (DBM) mixed with testosterone and alendronate loaded microspheres implanted in rabbit femoral defects	Konijnen (Oryctolagus cuniculus) / New Zealand White; vrouwelijk; 4-5 maanden oud	25	Matig / moderate	Er worden alleen vrouwelijke konijnen gebruikt.

Voorwaarden

Op grond van artikel 10a1 lid 2 Wod zijn aan een projectvergunning voorwaarden te stellen

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

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.

De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.

13.

[redacted]

Van: Info-zbo
Verzonden: dinsdag 13 oktober 2015 14:01
Aan: [redacted]
CC:
Onderwerp: Beschikking AVD905002015159
Bijlagen: Beschikking 159.pdf

Geachte [redacted]

Deze beschikking is ook per post verstuurd.

Met vriendelijke groet,

Centrale Commissie Dierproeven www.centralecommissiedierproeven.nl
Nationaal Comité advies dierproevenbeleid www.ncadierproevenbeleid.nl
.....
Bezuidenhoutseweg 73 | 2594 AC | Den Haag Postbus 20401 | 2500 EK | Den Haag
.....

14.

[redacted]

Van: Info-zbo
Verzonden: woensdag 14 oktober 2015 12:22
Aan: [redacted]
Onderwerp: terugkoppeling besluit op aanvraag AVD905002015159

Geachte DEC leden,

Enige tijd geleden hebben wij een advies van u ontvangen betreffende aanvraag AVD905002015159, 'Local delivery of testosterone and alendronate to aid bone (fracture) healing and to improve osseointegration of implants'.

Wij danken u voor uw advies, en koppelen graag het oordeel van de CCD over deze aanvraag aan u terug. De CCD heeft besloten de vergunning, overeenkomstig uw advies, te verlenen.

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

De aanvrager en verantwoordelijk onderzoeker zijn hierover ingelicht.

We hopen u op deze wijze voldoende geïnformeerd te hebben.

Met vriendelijke groet,

Centrale Commissie Dierproeven **www.centralecommissiedierproeven.nl**
.....
Postbus 20401 | 2500 EK | Den Haag
.....
T: 0900 2800028
E: info@zbo-ccd.nl
Let op: vanaf nu heeft de CCD een nieuw e-mailadres info@zbo-ccd.nl. Heeft u ons oude e-mail adres in uw adressenboek, dan vragen we u om dat aan te passen.