G. NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Skeletal Mechano-Pathobiology
Key Words	Bones, Joints, Mechanobiology, Osteoarthritis, Osteoporosis, Pathophysiology
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Disorders of the skeletal system may result from hereditary or acquired pathologic processes. Impairments may result from degenerative processes as well as traumatic events. Two of the most prevalent skeletal conditions are osteoporosis and osteoarthritis, both of which will increase even further as a consequence of increasing longevity and lifestyles. Those conditions result in fracture, chronic pain, impaired quality of life, higher levels of morbidity and mortality and provide a challenge in terms of management and health economic costs. There is therefore a continuing need to advance our basic knowledge on the remodelling, repair and regeneration of the skeletal system in order to translate understanding of mechanisms to new clinical strategies in prevention and management of skeletal disease.

Our project uses animal models of these disorders, genetically modified rodents and a range of specific protocols to further understand the mechanisms and essential factors regulating skeletal tissues remodelling and repair from development to ageing with the ultimate goal of providing directions for drug developments to alleviate osteoarthritis and osteoporosis and the impacts of their consequences such as pain and fragility fractures. Our work under a previous Licence has indeed led to successful transition into proof-of-concept studies for the treatment of osteoporosis, with successful avoidance of ovariectomy- and neurectomy-induced bone loss with the compound Naquinate. Also in the alleviation of gait abnormalities that develop in line with advancing osteoarthritis in the STR/Ort strain of mouse in response to Sulforadex^{tm} administration in vivo.

There is a still continuing need to translate understanding of mechanisms to new clinical strategies to improve prevention, diagnosis, control and treatment of skeletal disease. For example, despite considerable advances, the mechanisms controlling the response of cells to specific biological and defined mechanical stimuli have not been fully elucidated. This is true not only for bone tissue, which has been the initial focus of mechano-biological research, but also for other non-calcified musculoskeletal tissues where the lack of knowledge of these specific mechanisms is even greater.

Our aim is to build upon current knowledge and extend understanding to variables such as genetics, mechanical environment, hormonal changes and ageing. The fact that musculoskeletal disorders lead to significant human suffering, this work is focussed on furthering our understanding of the mechanisms controlling normal physiological function of joints and bone in the pathogenesis of osteoarthritis and osteoporosis. They also align with a One Medicine approach and seek to similarly inform drug development in the treatment/prevention of canine osteoarthritis.

In each of the protocols we aim to emulate some aspect of the human pathbiology in order to understand and interfere with the pathophysiological processes. In both osteoarthritis and osteoporosis, there is a multi-cell involvement with complex systems that cannot currently be addressed in tissue culture or non-animal model

systems alone. In osteoarthritis, the main surgical model that we propose to use is akin to sports injury in people which go on to develop osteoarthritis, therefore these models are important paradigm for interventional studies to ameliorate the outcome.

Our program of work will principally investigate:

1. The identification of the molecular mechanisms and novel regulators involved in skeletal tissues repair and functions.
2. The response of specific skeletal tissues to defined mechanical and biological stimulation and how this response is compromised with age and disease.
3. The interactions between biological factors and mechanical loading for the maintenance of skeletal tissues during ageing.
4. The influence of age, disease, mechanical and biological factors on the repair processes of skeletal tissues and structures.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The aim of our project is to gain better understanding of the factors and their mechanisms of action, regulating bone and joint physiology for diseases in which these tissues are affected. Overall, our research aims to improve the quality of life and mobility of people with bone and joint pathologies. Ultimately, we hope to help develop directions for treatment options for those pathologies and for the associated pain.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use a maximum of 9,000 rats and mice, mainly mice over a period of five years. The rodent species to be used are appropriate because their fundamental skeletal biology is very similar to humans in many regards and there is the advantage that genetic models, probes and antibodies are available. The most appropriate models of osteoporosis and joint disruption are in rodents. Sample sizes to be used are based on previous work and a calculation to estimate the minimum number of rodents required for establishing significant differences between groups.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

All procedures to be undertaken are performed in rodents and do not exceed “moderate” in severity. We have developed over the years appropriate animal models of skeletal diseases and protocols that aimed at investigating the remodelling responses of skeletal tissues to their mechanical and biological environment. All these experiments are performed by appropriately trained experimenters and are essential for the success of this project. Animals will be sacrificed by Schedule 1 at the end of experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The processes involved in achieving adaptive changes in bone architecture and mass and those involved in the degeneration of joint that are likely promoted by mechanically-derived loads are incompletely understood and can only realistically be replicated using live animal models. *In vitro* organ culture systems appear capable of at least partly replicating the events whereby these mechanical stimuli are applied and may therefore be useful in examining the immediate and short term responses to such application, but they are completely incapable of replicating the longer term osteogenic response in bone to create functionally appropriate changes in architecture and mass. These *in vitro* approaches also fail to produce the range of structural abnormalities in joint architecture that can be seen, sometime after, in response to abnormal loading in the intact joint. Monolayer cell culture can sometimes be used to replicate selected aspects of both of these types of responses but they fall short of providing integrated, organ-level, physiologically intact environment in which such responses are normally coordinated.

These *in vitro* and cell culture based alternatives have been, and will be, used by us as replacements wherever possible to examine some selected aspects of the responses we aim to more fully decipher. We have fully acknowledged their strengths, reviewed their use for others, but appreciate their limitations (see also 3Rs, above).

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We always aim to reduce the numbers of animals we use. Power analyses are applied in order to identify the minimum number of animals that we need to use in order to answer the specific question being posed. For instance, we have established that our tibial bone loading studies require group sizes of no more than eight to secure statistical significance. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce the numbers of animals required still further; the possibility of exploiting such controls is another area in which future reduction in numbers may be achieved. This may not always be possible, however, but efforts will be made in all initial investigations to secure the validity of internal control samples.

The principles of our experimental design have been already established in our on-going programme of study and so there is little need in performing studies to modify our bone loading programme. This is not necessarily the case for joint loading but advances are being made all the time and it is our hope that during this particular programme of study that we will have identified an optimised osteogenic loading protocol; an important step, as it will mean that we more fully understand the mechanical drivers of osteoarthritis – a vital advance in our understanding.

If loaded animals are also simultaneously treated with compounds that may modify bone remodelling then animals will be previously randomised and blinded (see also The 3Rs, above).

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

We have chosen to focus particularly on rodents. This decision has been made as it will provide us with the potential to explore the role of specific genes in the response we identify, through the use of mutant and transgenic mouse models. Indeed, the choice to develop the tibial bone (de Souza et al., 2005) and joint loading model (Poulet et al., 2011) in the mouse was made with this purpose firmly in mind. These models are being replicated by other groups and represent the fore-front of this *in vivo* approach to address questions in bone and joint mechanobiology.

Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. The alternative strategies which others have used to attain similar end-points frequently involve surgery and our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia (see also The 3Rs, above).