

### **1. Why control grey squirrels?**

The grey squirrel (*Sciurus carolinensis*) is an invasive non-native species, which was introduced to the UK from North America in 1876. Populations of grey squirrels grew rapidly since multiple introductions. According to the Mammal Society, at present circa 2.7 million grey squirrels are estimated to live in the UK, against circa 287,000 native red squirrels (*Sciurus vulgaris*):

<https://www.mammal.org.uk/science-research/mammal-review-2018-technical-summary/>

#### **a) Impact on the UK treescape**

Grey squirrels damage our forests by stripping bark from trees' main trunks (at the base and up in the canopy) and branches. Bark stripping affects tree growth and timber value and may cause broadleaved trees to die, with consequent costs of restocking. Planted or naturally regenerated trees aged between 10 to 40 years are most vulnerable to bark stripping. Bark stripping increases the susceptibility of trees to various pathogens, such as fungi, that can be transmitted by squirrels. This impact is resulting in devastation of the broadleaved woodlands of the United Kingdom and causing many millions of pounds of lost revenue to the forestry industry.

#### **b) Impact on red squirrels**

Grey squirrels are one the main reason for the disappearance of red squirrels in most of the UK. This is due to the spread of a disease called squirrel pox virus and by direct competition for resources such as food.

#### **c) Impact on songbirds**

Some studies suggested that the grey squirrels are among the main predators of songbirds and may be a large factor in the decline of some of our species <sup>1</sup>.

### **2. How are the grey squirrels currently controlled?**

Grey squirrel management is currently carried out by various methods of culling such as trapping and shooting. The use of warfarin for grey squirrel control has not been available since July 2016. Despite extensive culling, the number of grey squirrels has grown in the last few decades.



*Fig. 1. Bark stripping by grey squirrels.*

### **3. Why do we need fertility control when there are culling methods available?**

Shooting and trapping can be expensive. For instance, in 2015 Red Squirrel Northern England culled 21,000 grey squirrels in the North of England at an estimated cost of £60 per squirrel. Culling alone has so far failed to bring the problem under control because the reproductive rate of grey squirrels far exceeds the numbers culled. Oral contraceptives represent a possible option of grey squirrel control when used either as a standalone method or as an additional tool to culling.

### **4. Who is doing this research?**

The National Wildlife Management Centre, which is part of the Animal and Plant Health Agency (an executive agency of Defra). The NWMC provides evidence, impartial advice and services, primarily to Defra, to resolve human-wildlife conflicts and to support policy and decision making, drawing on expertise in wildlife management, invasive non-native species, wildlife disease, animal ecology, population modelling, and animal welfare. The NWMC has been studying fertility control in different species, as a wildlife management tool, for around 20 years. The NWMC includes the Non-Native Species Secretariat and expertise in all different types of wildlife management.

An oral vaccine will be tested in the next few years for its contraceptive effectiveness on grey

squirrels. This vaccine has been developed by the National Wildlife Research Center (NWRC), which is part of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), in collaboration with NWMC and other European partners. The NWRC provides Federal leadership and expertise to resolve wildlife conflicts that threaten agricultural and natural resources, human health and safety and property.

### 5. How do contraceptives work?

The contraceptive vaccine stimulates the production of antibodies that bind to the Gonadotrophin Releasing Hormone (GnRH), a hormone in an animal's body that is responsible for the production of sex hormones (e.g. oestrogen, progesterone, and testosterone). By binding to GnRH, the antibodies reduce the GnRH's ability to stimulate the release of these sex hormones. Animals remain in a non-reproductive state as long as a sufficient concentration of antibodies is present.

### 6. Has this been shown to be effective in other animals?

A similar contraceptive vaccine (GonaCon), developed by the NWRC (see above) is currently registered as an injectable single-dose contraceptive for white-tailed deer, horses and donkeys. In addition to these species, GonaCon was effective on other wildlife species, including California ground squirrels, Eastern fox squirrels, prairie dogs, Norway rats, feral cats, goats, cattle, horses, badgers, kangaroos, wild boar, bison and elk. The NWMC carried out several studies on this contraceptive, and showed that a single dose of GonaCon caused infertility in 85-92% of feral goats for 4 years, and in 92% of female wild boar for at least 6 to 8 years. Pilot studies of a novel oral contraceptive vaccine, conducted by the NWMC and collaborators, also showed that 60% laboratory rats were rendered infertile when the agent was delivered directly in the animals' mouth. It is hoped that encapsulating (see below) this vaccine may increase this efficacy and duration of contraceptive effect. To determine whether this is the case, trials are currently being conducted on laboratory rats using a new formulation of the oral contraceptive vaccine. This formulation involves the use of natural capsule of a pollen or spore grain (known as a SpECs). The SpECs containing the vaccine will then be added to

a bait. When the animals eat the bait, the 'sticky' SpECs attach to the intestine and release the fertility agent into the blood stream. This encapsulation technique has been used already to deliver many different types of chemicals including the common painkiller Ibuprofen.

### 7. Does it work on both males and females?

Yes – as the vaccine targets the parent hormone that makes both female and male sexual hormones, it can render both sexes infertile.

### 8. How is the vaccine delivered to the animals?

The vaccine will be added to a bait and the bait delivered in a squirrel-specific hopper (see Q21). The palatability of bait to grey squirrels will be tested in captivity and field trials.



Fig. 2 Hopper used to deliver baits to grey squirrels.

### 9. Are there any health or welfare effects?

No side effects were observed in the first studies on the oral contraceptive vaccine. In some species, the injectable vaccine caused an injection site reaction similar to that produced by other injectable vaccines, whilst in other species no side effects have been observed.

### 10. Will non-target species be affected?

The vaccine is mammal-specific, so birds are not at risk. In order to reduce the possibility of non-target mammals being exposed, a species-specific delivery system (grey squirrel feeding hopper) will be tested. To mitigate the risk of spillage of bait containing contraceptives, the project will formulate the bait as a paste, such as hazelnut paste, that animals must consume inside the hopper. Bait palatability and bait uptake by target and non-target species will be monitored in captivity (for target species) and in the field using camera traps.

### **11. What are the risks to the environment or to other animals?**

The risks to the environment and to non-target species, including predators and scavengers, will be evaluated as part of the data required for the registration package. The hoppers and the bait formulation will ensure that bait uptake by non-target species is minimised (see above).

The fertility of squirrel predators is unlikely to be affected using a contraceptive that is a vaccine (protein) and not a reproductive hormone. The vaccine will be broken down in the tissues and the stomach of the squirrels and hence it is likely that the amount of active product (if any) passed onto predators would be very small and unlikely to have any biological effect. The breaking down of the protein vaccine by the stomach and tissues will also mean that it is very unlikely that any active components will be passed into the environment via excretory products such as faeces or urine. Full detailed data on secondary hazards will be collected when registration is considered for this vaccine.

### **12. Are there any risks to humans?**

The bait will be delivered in grey squirrel- specific hoppers, clearly labelled with hazard signs and placed on trees so that the risk to non-users would be negligible. In addition, the quantities of the appropriate active ingredient for squirrels are very unlikely to have an effect on humans (unlike rodenticides which are known to be highly toxic to humans) either from direct ingestion or eating of squirrels. The final formulation may include adding human taste repellents (such as Bitrex, which is common practice for rodenticides). Other requirements in the registration dossier will include assessing risks for users, with product information and label detailing safe use.

### **13. Can we target enough animals to reduce the population?**

The project is developing a model aimed at evaluating the impact of fertility control on the population of grey squirrels. In parallel, the project will assess the proportion of the population of squirrels that consume the bait containing the contraceptive at different times in the year (e.g. summer or winter). The project is also testing how different densities of hoppers affect grey squirrel bait uptake, to maximise the proportion of

squirrels exposed to the contraceptive. These data will be used to assess how we can target enough grey squirrels for fertility control to reduce the number of animals in an area.

### **14. By when do you hope to start using the contraceptive in the wild?**

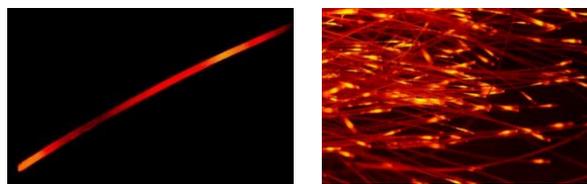
If all trials and formal registration of the vaccine are successful, it is possible that vaccine could be brought to market within six to eight years. Pilot trials will be conducted in the wild as part of the current five- year project.

### **15. Will oral contraception be publicly supported?**

A recently published Forest Research survey on social acceptability of methods used to manage grey squirrels in the UK found that contraception is considered the most acceptable control method available, with 61.5% responders indicating that it is either acceptable or highly acceptable.

### **16. By how much are you hoping to reduce the population, and in which geographical areas?**

Based on data collected in the field by this project, a mathematical model will assess the effort and time required to eradicate local populations of squirrels using culling, fertility control or a combination of these methods. The current project has employed the bait marker Rhodamine B (which is incorporated in hair and can be seen under UV light as indicator of bait uptake) to assess the proportion of a grey squirrel population that could be targeted by oral contraceptives.



*Fig. 3. Rhodamine B (RB), detectable as fluorescent bands in whiskers (left) and hair (right) of animals that ingested RB-treated baits.*

### **17. Hasn't this been tried before in squirrels unsuccessfully?**

Forest Research undertook a study (with collaboration from NWMC) in 2013<sup>2</sup> looking at a different oral contraceptive, Diazacon, which

affects cholesterol levels. In this study fertility could not be measured: although the treated squirrels did not breed the control (untreated) animals also did not produce any litter.

In the present study the fertility agent works through a completely different biological systems (targeting the GnRH). The NWMC set up a breeding colony of grey squirrels to test the effects of fertility control agents in captivity. Besides litter production, other indicators such as hormone concentration and reproductive physiology parameters will be monitored. For the vaccine trials, captivity studies will also measure the levels of antibodies, routinely measured in GnRH-based vaccine studies as indicators of infertility.

#### **18. How many doses will be needed?**

In the initial trials with an oral GnRH-based vaccine, six doses delivered within a month were sufficient to make 60% of the rats infertile. The NWMC will be testing different doses and concentrations of the vaccine.

Once the number of doses and the frequency of doses to make animals infertile are optimised, the data collected in the field on patterns of bait consumption by squirrels will be used to design field applications that maximise grey squirrels' uptake of baits containing contraceptives.

#### **19. Could these contraceptives be used on other species?**

The GnRH hormone is very similar across all mammal species and indeed the injectable vaccine has successfully reduced fertility in many wildlife species. It is expected that an oral GnRH-based contraceptive will be effective on a wide range of mammals, although the dose and the frequency of application might vary between species. The possibility of applying the same vaccine to control other wildlife would scale up field applications, thus increasing the potential commercial return of this drug for investors.

#### **20. How long to have a contraceptive that can be used in the field?**

The aims of the five-year study are to develop an oral contraceptive for grey squirrels and to assess how this contraceptive could be delivered in field applications. It is anticipated that, once captive

studies have been completed, in years four and five, the NWMC will work with the relevant UK authorities towards starting to assemble a registration dossier that will eventually make the contraceptive available for field use.

In 2015, the UK's Health and Safety Executive (HSE) stated that any new contraceptive would be registered as a biocide and estimated that the whole process, from starting to compile a dossier to final biocidal product authorisation, will require approximately three to five years. HSE added that it is unlikely that the process could be completed within three years if new data must be generated as part of the compilation of the dossier. HSE also estimated that the cost of generating and compiling an active substance dossier was likely to be £2-4 Million. By liaising with the regulatory authorities and by starting to collect data for the registration dossier, it is anticipated that the five-year project will reduce the time and cost of the registration process.

#### **21. Can we use 'old' warfarin dispensers to deliver contraceptives in the future?**

The NWMC is testing a modified version of the bait hopper originally designed by Forest Research to deliver warfarin. Once it is proven that grey squirrels can feed from these hoppers but other species (including red squirrels) cannot access the bait, we will know whether the 'old' dispensers can be modified to deliver contraceptives to grey squirrels.

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<sup>1</sup>Brown R. 2006. *A Review of the Impact of Mammalian Predators on Farm Songbird Population Dynamics Prepared for Song Bird Survival*. School of Biological and Chemical Sciences, Birkbeck, University of London.

<sup>2</sup> Mayle, B., Ferryman, M., Peace, A., Yoder, C., Miller, L., Cowan, D. 2013. The use of DiazaCon™ to limit fertility by reducing serum cholesterol in female grey squirrels, *Sciurus carolinensis*. *Pest Management Science* 69, 414-424.