

1. Why control grey squirrels?

The grey squirrel (*Sciurus carolinensis*) is a 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 (<http://www.mammal.org.uk/wp-content/uploads/2018/06/MAMMALS-Technical-Summary-FINALNE-Verision-FM2.pdf>) at present circa 2.7 million grey squirrels are estimated to live in the UK, against circa 287,000 native red squirrels (*Sciurus vulgaris*).

i) The impact of grey squirrels 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 and 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 loss of revenue in the forestry industry of approximately £15 million.

ii) The impact of grey squirrels on red squirrels

The greys are one of the main factors contributing to the extinction of reds in many areas of the UK. This is due to the spread of a disease called squirrelpox virus (SQPV) and by direct competition for resources such as food.

iii) Impact on songbirds

Some studies suggested that the grey squirrels are among the main predators of songbirds in some environments and may be a large factor in the decline in some of our species (e.g. *A Review of the Impact of Mammalian Predators on Farm Songbird Population Dynamics Prepared for SongBird Survival July 2006 by R.Brown, School of Biological and Chemical Sciences, Birkbeck, University of London.*)

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 decades.

Why do we need fertility control when there are culling methods available?

Culling such using shooting or trapping can 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 for grey squirrels represent a possible option of population control when used either as a standalone method or as an additional tool to culling.

3. 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 wildlife management tool for around 20 years. The NWMC includes the Non-Native Species Secretariat and expertise in all different types of wildlife management in addition to being the UK centre for humaneness of trap

testing. The NWMC also boasts the most extensive captive wildlife research centre in the UK and one of the best in Europe.

Two drugs will be tested in the next few years for their contraceptive effectiveness on grey squirrels: a contraceptive vaccine and a fertility inhibitor known as *ContraPest*. The contraceptive 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 European partners, including the NWMC. The NWRC provides Federal leadership and expertise to resolve wildlife conflicts that threaten the Nation's agricultural and natural resources, human health and safety and property.

ContraPest (<https://senestech.com/contrapest/faq/>) is registered in the US as a fertility inhibitor for rats but this contraceptive has never been tested on grey squirrels. The NWRC is collaborating with the company producing this drug to initiate studies on grey squirrels.

4. 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 signals the production of sex hormones (e.g. estrogen, progesterone, and testosterone). By binding to GnRH, the antibodies reduce GnRH's ability to stimulate the release of these sex hormones. All sexual activity is decreased, and animals remain in a non-reproductive state as long as a sufficient concentration of antibodies is present.

ContraPest contains two active ingredients that act synergistically to suppress follicle development and sperm production. The active ingredient 4-vinylcyclohexene diepoxide (VCD) causes the premature ovarian failure, and the second active ingredient, triptolide, specifically stops growing follicles in the ovary and sperm production in the testes.

5. 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 has proven effective for use with other wildlife, including California ground squirrels, Eastern fox squirrels, prairie dogs, Norway rats, feral cats, goats, cattle, horses, badgers, kangaroos, wild boar, white-tailed deer, bison and elk.

Studies on this injectable product carried out by NWMC have included the use on feral goats with 85-92% infertility achieved for 4 years after a single dose, and captive wild boar in which one injection caused 92% of females to remain infertile for at least 6 to 8 years .

The oral GnRH vaccine has been trialled in a pilot study on laboratory rats, which showed that 60% of 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 (between 2 and 10 fold). To determine whether this is the case, trials are currently being conducted on laboratory rats using the encapsulated form of the fertility agent.

No studies have been conducted on ContraPest in grey squirrels but this contraceptive, available as a liquid formulation, has successfully reduced reproduction in rats and mice.

6. Does it work on both males and females?

Yes to both - the vaccine targets the parent hormone that makes both female and male sexual hormones, the vaccine can render both males and females infertile.

ContraPest has been successfully tested in male and female rodents.

7. Are there any health or welfare effects?

No side effects were observed in the first studies on the oral contraceptive vaccine nor for ContraPest. 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.

8. How is the vaccine delivered to the animals?

The vaccine will be encapsulated (rather like many of today's common tablets) but using a natural capsule of a pollen or spore grain (known as a SPEC). These SPECs 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.

ContraPest is delivered in a liquid formulation.

9. 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 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 uptake by target and non-target species will be monitored in captivity (for target species) and in the field using camera traps.

10. What are the risks to the environment or to other animals?

The risks to the environment and to non-target species 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 minimized (see above).

The fertility of squirrels' predators is unlikely to be affected using a contraceptive that is a vaccine (protein) and not a reproductive hormone. This means the vaccine will be broken down in the tissues and the stomach of the host animal 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 mean that it is very unlikely that any active product 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.

For ContraPest, the active ingredients metabolize to inactive within 15 minutes, minimizing risk to predators animals. ContraPest does not alter rat behavior or make them more vulnerable to predation, therefore reducing the chances of secondary exposure.

11. Are there any risks to humans?

The bait delivery will be in species specific hoppers 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 bait is likely to be registered as a biocide and regulations relating to its use will need to be followed (as is the case for other biocides such as rodenticides). This may include adding human taste repellents (such as Bitrex, which is common practice for rodenticides) to the final formulation. Other requirements are likely to include risk assessments appropriate to the site, with product information and labels detailing safe use.

For ContraPest, it is anticipated that the same precautions recommended for the field applications of this contraceptive will be adopted.

12. 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) and the duration of the action of the contraceptive. The project is also testing how different densities of hoppers will affect grey squirrel bait uptake from the hoppers, to maximise the proportion of squirrels exposed to the contraceptive. These data will be used to assess how we can target enough animals for fertility control to reduce the number of animals in an area.

13. 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 6-8 years.

14. 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.

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

The research is focussed on the development and delivery of an effective contraceptive for grey squirrels. Based on data collected in the field by this project, the model will assess the effort and time required to eradicate local population of squirrels using culling, fertility control or a combination of these methods.

16. Hasn't this been tried before in squirrels unsuccessfully?

Forest Research undertook a study (with collaboration from NWMC) in 2013* looking at a different oral contraceptive Diazacon (which affects serum cholesterol levels). The study was partially successful with a proportion of the squirrels being affected by the drug. However fertility could not be measured as although the treated animals did not breed the control (untreated) squirrels also did not produce any litter.

In the present study the fertility agent works through a completely different biological systems (targeting the GnRH or the ovary and testes). The NWRC 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 indicators such as sperm count will be monitored. For the vaccine trials, captivity studies will also allow us to measure the levels of antibodies as a measure of effectiveness of the vaccine. Anti-GnRH antibodies are routinely measured in GnRH-based vaccine studies as indicators of infertility.

* 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.

17. How many doses will be needed ?

In the initial trials with an oral GnRH-based vaccine, 6 doses delivered within a month were sufficient to make 60% of the rats infertile. The NWRC will be testing different doses and concentrations of the vaccine and of ContraPest. 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.

18. 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.

The possibility of registering ContraPest for other species will be discussed with the company producing this drug.

19. How long to have a contraceptive that can be used in the field?

The aims of the 5 year study are to test the effectiveness, potential side effects and delivery of a contraceptive for controlling grey squirrels. It is anticipated that, once captive studies have been completed, in Year 4 and 5 the NWRC will work with the relevant UK authorities towards assembling 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 a new contraceptive will 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 3 to 5 years. HSE added that it is unlikely that the process could be completed within 3 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.

20. Can we use "old" warfarin dispensers to deliver contraceptives in the future?

The NWRC 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 and recommended to deliver contraceptives to grey squirrels.