

## Anticoagulant resistance in the Norway rat and Guidelines for the management of resistant rat infestations in the UK

### 1. Background, history and development of resistance

#### The introduction of the anticoagulant rodenticides and their use

The development of the anticoagulant rodenticides in the early 1950s revolutionised rodent control. Up to this point the limitations of the acute rodenticides had made it virtually impossible to achieve a 100% clearance of a rodent population and repeated applications of acute rodenticides to rapidly recovering and “shy” rodent populations were required.

The “chronic” anticoagulant rodenticides revolutionised rodent control and for the first time complete control of target rodent populations was possible and practical.



The main reason why the anticoagulants work so much better than the alternative rodenticides is that they are slow acting. Minimum time to death is two to three days, with average times to death being seven to eight days. In some cases death may not occur for 14 days. This enables the rodents to keep feeding on the anticoagulant rodenticide baits until a lethal dose has been consumed. If other aspects of the rodenticide treatment have been conducted correctly, then 100% mortality can be achieved.

The essential elements of a chronic rodenticide treatment are to ensure not only that the baits are placed correctly, as would be a requirement for any rodenticide treatment, but that the rodents in an infestation are able to feed on the anticoagulant baits on a daily basis, possibly for several weeks, even though the daily consumption of bait may be very low. This is termed “surplus” or “saturation” baiting. It is essential that the baits are visited and replaced frequently, daily if necessary.

The first of the anticoagulants to appear on the market in the early 1950s was warfarin. Within a few years additional anticoagulants were available in the UK, including diphacinone, coumatetralyl and chlorophacinone. These are now collectively known as the “first-generation anticoagulants”. They all have broadly similar levels of toxicity to the commensal rodents, although there is some variation between them in their toxicity to other species.

#### Development of anticoagulant resistance

The initial widespread success of these early anticoagulants was not maintained. Resistance not only to warfarin but to all the first-generation compounds was detected in the UK in some Norway rat and house mouse populations by the late 1950s and early 1960s.

The first documented case of anticoagulant resistance was identified in 1958 during a routine field trial of the indane-dione anticoagulant, diphacinone, on a pig farm in central Scotland. Following an initial knockdown, the survivors were not controlled after a 30-day exposure to diphacinone and a subsequent baiting of 18 days using warfarin. Laboratory feeding tests carried out in Scotland and at the MAFF laboratories in Tolworth gave a survival rate of almost 90%.

The infestation was eliminated by April 1959 but by September 1961 the farm was again reported to be heavily infested with rats. During this period tests were carried out at many sites where resistance was suspected or reported but in almost all cases the reason for lack of control was found to be poor technique. By 1962 resistance had been confirmed by field trial and laboratory tests at 7 sites within an area of 200 square kilometres.

Following the warfarin treatments, survivors were eradicated using the acute rodenticides, zinc phosphide; arsenious oxide; ANTU; or by trapping. Although subsequent checks after six to nine months identified a low level of rat activity this was thought to be as a result of animals surviving the treatment rather than re-infestation. The acute rodenticides are no longer available and, while it is unlikely that this level of commitment would be practicable today, it did demonstrate the benefit of good control in the management of resistant rats.

In 1960, resistance was identified in the Anglo/Welsh border area based around Welshpool and Shrewsbury. In an attempt to curb the spread of the outbreak, a cordon sanitaire using acute poisons was set up. This was relatively unsuccessful and was abandoned in 1966. Detailed monitoring of this Welsh resistance showed that it spread radially at about 4.8 km per year.

Subsequently, resistance was identified in the Kent/Sussex border areas of southern England and then in Hampshire.

Although Norway rat resistance to the first-generation anticoagulants was found first in the United Kingdom it was subsequently identified in many other industrial countries. Whilst resistance may be present in some areas this does not mean that it is present in all areas.

The development of resistance encouraged the search for more potent anticoagulants that could be used to control the resistant populations. In the early 1970s, difenacoum was marketed and effectiveness against resistant populations of both Norway rats and house mice was claimed. At about the same time, bromadiolone was developed with similar claims.

## **Resistance to the second-generation anticoagulants**

Difenacoum and bromadiolone were called “second-generation anticoagulants”. Both however required application using the saturation or surplus baiting techniques discussed earlier.

Within a few years of the arrival of difenacoum and bromadiolone on the market, populations of Norway rats resistant to either difenacoum or bromadiolone, or both, were being identified, particularly in central southern England. Subsequently, individual sites with resistance to either difenacoum and/or bromadiolone were identified during the 1980s and 1990s in a number of areas including East Anglia, Yorkshire and Lincolnshire.

The development of the two most recent second-generation anticoagulants, brodifacoum and flocoumafen, has brought with it the opportunity to use an alternative baiting strategy. The new strategy has been termed “pulsed” baiting. In practice this simply means that the higher toxicity of these compounds allows some flexibility with the bait application regime. Visits to replace baits may be made less frequently, perhaps at weekly intervals, and the quantity of bait placed can be reduced.

The increased toxicity of the later anticoagulants may result in faster control. However the minimum time to death and average time to death of individual rats remains the same as for the first-generation anticoagulants. The time taken to control the typical Norway rat infestation with these compounds is about 14-28 days.

The increased toxicity of both brodifacoum and flocoumafen brought with it a perceived increased risk to non target species. For this reason the use of these two compounds is restricted in the UK to Indoor Use only.

## ANTICOAGULANT RODENTICIDES

### **First Generation Anticoagulants**

Warfarin (including sodium warfarin)  
Chlorophacinone  
Coumatetralyl

### **Second Generation Anticoagulants**

Difenacoum  
Bromadiolone  
Brodifacoum     *Pulsed Baiting – Indoors*  
Flocoumafen     *Pulsed Baiting - Indoors*

## 2. Definitions of resistance

The following general definition of anticoagulant resistance was proposed in 1994 by Dr John Greaves and is now widely used.

“Anticoagulant resistance is a major loss of efficacy in practical conditions where the anticoagulant has been applied correctly, the loss of efficacy being due to the presence of a strain of rodent with a heritable and commensurably reduced sensitivity to the anticoagulant”.

Some other terms are also used in relation to the resistance phenomenon.

**Resistance factors** – the factor by which the dose of rodenticide required for a susceptible rodent population must be multiplied to achieve the same effect in a resistant rodent population.

**Technical resistance** – this term is used in cases where resistance tests identify resistance but where resistance factors are low and the resistance has no observable practical effect.

**Practical resistance** – this term is used in cases where resistance tests identify resistance and resistance factors are sufficiently high so that an acceptable level of control is unlikely to be achieved.

## 3. General resistance strategy. Integrated Pest Management.

The control of rat populations should never rely upon the use of chemical control measures alone. It is essential that an integrated pest management programme (IPM) is implemented. When dealing with resistant rat populations it is more important than ever that such procedures are followed.

In addition to the use of chemical control measures, an IPM programme against a resistant population of rats will utilise the following:

- Trapping
- Environmental and habitat management (restriction of access to food, water and harbourage)
- Proofing, exclusion and restriction of movement.

Further reference to the use of these procedures may be found in a variety of publications including “Anticoagulant Resistance Management Strategy for Pest Management Professionals, Central and Local Government and Other Competent Users of Rodenticides” from the Rodenticide Resistance Action Committee (RRAC) and available on their website (<http://www.rrac.info>). Please note, however, that not all compounds mentioned in RRAC advice are now available in the European Union.

## 4. DNA testing for anticoagulant resistance

### General

Until recently, deciding whether a rat was resistant to anticoagulants or not depended on catching it alive and using one of several expensive and time-consuming laboratory tests. Needless to say these tests were not much used as they were largely impractical for routine resistance monitoring. But recently researchers in Germany led by Dr Hans-Joachim Pelz made a critical break-through. They identified which part of the genetic code of rats and mice carried the DNA sequence, or gene, which alters when rodents become resistant to anticoagulants. The gene they discovered affects the enzyme vitamin K epoxide reductase, a crucial enzyme in the vitamin K cycle and the one blocked by all anticoagulant rodenticides. The gene was given the name VKORC1 and the sequence of chemicals (nucleotides) used in its construction was decoded. Knowing the gene's DNA sequence, it became possible for the first time to look for changes, or mutations, which resulted in anticoagulant resistance in rodents.



Pelz, and his many co-workers, went on to study the amino-acid sequence of the VKORC1 gene from Norway rat and house mouse resistance areas in Germany, France, Denmark and the UK. In a bench-mark paper published in 2005 in the journal *Genetics* they showed there were many different mutations of the gene. A fascinating pattern began to emerge. Anticoagulant resistance in Norway rats had evolved many times over the years, with different mutations in different places. But occasionally the same mutation was found in rats from different countries indicating either that the same mutation emerged several times or that the rat populations developed from the same original stock.

Often, much-heralded scientific advances offer little practical benefit to pest control technicians. But few have not found a troublesome rodent infestation and wondered whether anticoagulant resistance was the cause. In the past, wondering was usually as far as it got as resistance testing was so expensive and took far too long. Scientists in the UK now have the capacity to conduct routine DNA anticoagulant resistance assays. These include Dr Colin Prescott (University of Reading) and Dr Dougie Clarke (University of Huddersfield). To be able to say if the rodent is resistant or not, these tests require only the tip of the tail, which can be sent in the post. A sensible sample size would be 10 – 20 tails. So the common question “have I got resistance?” can now be answered.

This new DNA technology holds great promise. Not only will it enable more effective rodent control in problem areas, but it will allow us to make more effective use of existing anticoagulants.

### Collection of tissue sample for DNA extraction

Before collecting samples for DNA analysis it is advisable to contact the laboratory where the samples will be sent to obtain instructions about rat trapping and sample collection. The following paragraphs provide outline guidance.

The tissue sample to be collected will be the 2cm tip of the rat tail. The samples should be collected from Norway rats that have been dead for less than 24 hours, and should be immediately transferred into clean small plastic or glass collection tubes that contain a small volume of alcohol (surgical spirit). The sample tubes should then be stored in a freezer (at -21°C) as quickly as possible, and no longer than 4 hours after collection.

Care should be taken to clean instruments that are used to take the tail sample before and after each collection, to ensure that there is no cross-contamination of DNA between samples. This could involve thorough cleaning with a tissue drenched with surgical spirit.

Surgical spirit is available from all chemists, and contains ethanol to which a small amount of methanol has been added to render it unfit to drink. It is used to sterilize surfaces and to cleanse skin abrasions and sores.

## Interpretation of DNA sequencing results

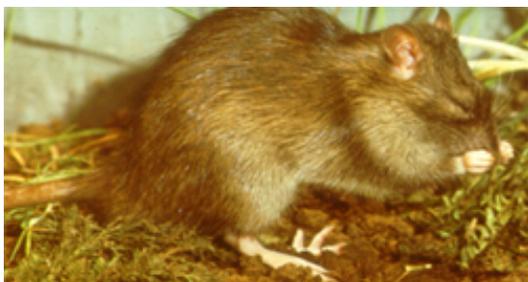
The information obtained from the DNA analysis of rodents requires careful interpretation. The following paragraphs provide information that will support better understanding of analytical results and allowed reasoned decisions to be made about actions to be taken at sites where resistance is discovered.

### Homozygous and heterozygous

The genetic code of all advanced animals is made up of two sets of genes, one obtained from the mother and one from the father. If the two genes carried by an individual for a particular characteristic, such as susceptibility to anticoagulants, are the same, the individual is said to be "homozygous". If the genes are different, the individual is said to be heterozygous. If the genes are different then the actual characteristic possessed by the individual will depend on which of the two genes is dominant.

### Incidence of resistance

The actual degree of resistance shown by rodent populations will depend on the type and frequency of the resistance gene in the population. If DNA tests show that a population has a high proportion of animals carrying the resistance gene (i.e. a high incidence), and a high proportion of those are homozygous resistant animals, then we might expect that the resisted compound(s) will be largely ineffective against that population. Conversely, if the resistance gene is very rare in the population, and those animals that carry it are mainly heterozygous, the resisted compound(s) would be largely effective. However, when resisted compounds are used against populations where a resistance gene is present, even at a low incidence, the animals carrying the resistance gene have a selective advantage. Those animals will be more likely to survive while the susceptible animals will die. Consequently, the incidence of resistance in the population will increase until, eventually, control problems may occur.



### “Scottish resistance” - Leu128Gln (or L128Q)

This mutation is the one found at the site of the first occurrence of anticoagulant resistance in Scotland. It has subsequently been found in rats from the North-west of England and from Yorkshire. It was also present in a sample of rats taken for DNA resistance testing in France.

The gene is known to confer strong practical resistance to warfarin and diphacinone. Coumatetralyl may still retain some effectiveness against rats that carry it although efficacy is somewhat impaired. All second-generation anticoagulants are fully effective against this strain.

### “Welsh” resistance - Tyr139Ser (or Y139S)

Resistance was found in a large focus on the Anglo-Welsh border centred on the town of Welshpool soon after the original discovery in Scotland. Welsh resistant rats are now known to carry the Tyr139Ser mutation. To date this mutation has only ever been found in the original focus, although the extent of its spread is now unknown.

Welsh resistant rats have very high resistance factors to the first-generation anticoagulants and these compounds are virtually ineffective against them. Before the second-generation compounds were introduced, coumatetralyl had some limited effect against Welsh resistance but is not now recommended for use at this focus. The second-generation compounds are fully effective against Welsh resistant rats.

### “Gloucestershire” resistance - Tyr139Cys (or Y139C)

The resistance in rats which has been present for decades in Denmark and North-west Germany is caused by this resistance mutation. Recently, DNA testing in the UK has discovered rats from the counties of Gloucestershire, Yorkshire, Lincolnshire and Norfolk that also carry this mutation. The resistance in Gloucestershire is long-standing, although not much researched, and therefore this type of resistance in the

UK has been called “Gloucestershire” resistance. Tyr139Cys mutation has probably been in the UK undetected for many years.

Few trials have been conducted in the UK against this strain and most of what we know about it comes from trials in Denmark and Germany. This mutation confers strong practical resistance against the first-generation anticoagulants. However, field trials of coumatetralyl conducted in Germany against populations containing some Tyr139Cys rats resulted in a degree of success, but not full population control. This strain shows some resistance to the second-generation anticoagulants, particularly where populations contain a high percentage of resistant animals and homozygous animals are frequent. The efficacy of bromadiolone particularly is poor against animals carrying this mutation and, although difenacoum is generally more effective, complete infestation elimination may be difficult even with that compound. If populations are indoors they should be treated with either brodifacoum or flocoumafen. Recent field trials in Germany showed that the former compound is fully effective against these rats. [See Section 5 for further advice.]

### **Hampshire/Berkshire resistance - Leu120Gln (or L120Q)**

A third major resistance focus discovered in the UK was in Hampshire, with some farms over the border in south Berkshire also having this resistance. It was known in early research papers as “difenacoum-resistance” but the strain shows resistance to other compounds as well. Resistant rats from this focus carry the mutation Leu120Gln. The term “Hampshire resistance” is now used when referring to this strain in order to make it clear that rats there are not only resistant to difenacoum.

This resistance focus has been the cause of considerable discussion for some time because research work provided inconsistent results. In particular, the significant degree of resistance seen in early field trials was not supported by laboratory studies, which showed quite low resistance factors among Hampshire rats, for both difenacoum and bromadiolone. It was later postulated that a reluctance to take poisoned baits, which was sometimes very pronounced in the area, exacerbated the resistance problem and this has since been confirmed. Practical experience in the Hampshire focus has shown that bromadiolone may be more effective than difenacoum in this area.

Some time after the initial discovery of “Hampshire resistance”, rat infestations were identified in north-west Berkshire that were even more resistant to difenacoum and bromadiolone. In the laboratory, some of these rats were found to survive a seven-day feed on low-strength brodifacoum bait (5ppm or 0.0005% - a tenth of field strength), and these individuals were termed “brodifacoum resistant”. However, as full strength brodifacoum baits would be fully effective against these animals, this low level of resistance is “technical resistance” and not “practical resistance”. Subsequent field trials against a rat infestation in an area north-west of the town of Newbury, showed for the first time that there was full “practical resistance” to the second-generation anticoagulant bromadiolone, with a high likelihood that difenacoum was also fully resisted. The type of resistance in this area became known as “Berkshire resistance”.

A difficulty in interpreting the results of resistance tests of rats from this area is that both Hampshire and Berkshire resistant rats carry the same mutation (Leu120Gln). The genetics of this situation remains in question, although it is postulated that Berkshire resistance is conferred by the presence of the Leu120Gln mutation as well as some second, and as yet unknown, mutation or group of mutations.

What can we say with confidence about the use of rodenticides in this area? Firstly, resistance is such that the first-generation anticoagulants are broadly ineffective. Secondly, that bromadiolone is more likely to be effective than difenacoum in some parts of this resistance focus. However, there is growing practical experience that, in some parts of the focus it is very likely that both bromadiolone and difenacoum are largely ineffective. A recent, carefully-monitored practical treatment has shown that brodifacoum is fully effective against Leu120Gln rats. Previous treatment records at the site, showing the complete failure of difenacoum and bromadiolone baits, suggest that the resistance there was of the Berkshire type. [See Section 5 for further advice.]

### **Kent resistance - Tyr139Phe (or Y139F)**

Anticoagulant resistance was found in Kent in 1968 and surveys conducted between then and 1972 found resistant rats across a substantial part of west Kent and eastern Sussex, from the Thames estuary to the south coast. Little was heard subsequently from this focus until a recent report of the failure of rodent control

on animal-rearing facilities in the centre of the resistance area identified earlier. DNA analysis of rats taken from the site revealed the mutation Tyr139Phe for the first time in the UK. This is one of the most common resistance mutations found in France and it also occurs in Belgium. Limited published information is available on the level of resistance to anticoagulants conferred by this mutation, and none is available from the UK, but published reports from the continent suggest that bromadiolone is less effective than difenacoum against this strain. Indeed, the former was the active substance which was found to have failed in the recent (2009) reports from Kent. [See Section 5 for further advice.]

### Other mutations

Several other mutations have been discovered in UK rats, in particular Arg33Pro, Ala26Thr, Tyr30Asn and Phe66Cys. We do not know enough about these mutations to say anything about the practical effectiveness of anticoagulants against rats that carry them.

## 5. Further advice about dealing with anticoagulant resistant populations

If you have submitted rat tails for DNA analysis and the result is obtained that rats you are treating carry a resistance gene, what should you do next?

Useful guidelines about the most effective anticoagulants in different parts of the UK are provided in the preceding sections of this document and these are summarised in Table 1. You may also wish to contact the UK Rodenticide Resistance Action Group directly. This can be done either through the website (<http://www.pesticides.gov.uk/rags.asp?id=702>) or through the British Pest Control Association and National Pest Technicians Association. Good advice about the treatment of resistant rat infestations is also provided in the document mentioned previously from the Rodenticide Resistance Action Committee and available at the RRAC website (<http://www.rrac.info>). However, some of the rodenticides proposed as alternatives to anticoagulants, such as zinc phosphide and calciferol, are no longer available for use in the EU.



Generally, the use of products containing bromadiolone and difenacoum may be effective in controlling anticoagulant resistant rats across much of the UK. However, the resistance mutations Tyr139Cys (Gloucestershire resistance), Leu120Gln (Berkshire resistance), and possibly Tyr139Phe (Kent resistance), confer levels of resistance to either bromadiolone or difenacoum, or both, such that treatments using them may be ineffective. If practical applications of both difenacoum and bromadiolone have been found to be unsuccessful against rats carrying these mutations, then no further applications of these compounds should be carried out. Further applications of ineffective anticoagulants will exacerbate resistance at the site and constitute an unnecessary and unacceptable risk to non-target animals. If populations are

indoors they should be treated with either brodifacoum or flocoumafen. A recent, carefully-monitored practical treatment outdoors, but in and around buildings, using an “emergency extension of an existing approval” has shown that brodifacoum is fully effective against Leu120Gln rats. Previous treatment records at the site, showing the complete failure of difenacoum and bromadiolone baits, suggest that the resistance there was of the Berkshire type. Treatments using brodifacoum against rats carrying the Tyr139Cys mutation were similarly effective in Germany. If resistant populations are outdoors, then alternative control measures such as trapping, gassing and habitat modification to reduce the rodent carrying capacity of the site should be attempted. If these measures are either unsuccessful or impractical, consideration should be given to an application to the Health and Safety Executive for the limited, emergency use of either brodifacoum or flocoumafen around the infested buildings.

Further advice and information on anticoagulant resistance can also be obtained from your rodenticide distributor or manufacturer, who may know more about anticoagulant resistance in your particular area. If you are in a known resistance area, one of these sources will be able to give general advice about tackling resistant rats and more specific advice about what anticoagulant baits will work best.

Table 1. The different anticoagulant active substances and their effectiveness against the resistance mutations found in rats in the UK. A cross means that the active substance should not be used against that strain and a tick means that it may be used with a reasonable expectation of a successful outcome. Some treatments may be effective using bromadiolone and/or difenacoum against resistant rats carrying the Gloucestershire, Hampshire and Kent genes, although complete eradication may not be achieved. [Please note that under current regulatory rules in the UK, brodifacoum and flocoumafen may only be used against indoor infestations of rats and mice.]

Active Substance	Resistance mutation					
	Scotland (Leu128Gln)	Wales (Tyr139Ser)	Gloucestershire (Tyr139Cys)	Hampshire (Leu120Gln)	Berkshire (Leu120Gln)	Kent (Tyr139Phe)
warfarin	X	X	X	X	X	X
chlorophacinone	X	X	X	X	X	X
coumatetralyl	X	X	X	X	X	X
difenacoum	✓	✓	?	?	X	?
bromadiolone	✓	✓	?	?	X	X
brodifacoum	✓	✓	✓	✓	✓	✓
flocoumafen	✓	✓	✓	✓	✓	✓

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