Understanding resistance

The future of anticoagulant resistance in Europe was the subject of an insightful and instructive paper at PestEx in April by Dr Alan Buckle of the University of Reading. *Pest* technical advisory board member, Richard Strand, of the Pest Information Consultancy reports.

The term 'super-rat' was coined as long ago as the 1960s, to describe then warfarin resistant rodents. Images of blue jump-suited, red caped flying rats were conjured up by the popular press! Mice didn't really make the news despite the fact that, when I first started my pest control career in the mid 1970s, my colleagues were almost in despair about how to control mice, rather than just feeding them.

The advent of second-generation anticoagulants (SGARs) in the early 1980s seemed to be the answer although, there was always the niggling concern that '… if it's happened once, it can happen again'.

So here we are 40 years on, better able to analyse and understand the biochemical mechanisms that lead to resistance and in a political environment where the very use of second-generation anticoagulants is under question.

At PestEx, Dr Alan Buckle explained some of the complex work that has been done on the mechanisms of rodenticide resistance

At this spring's PestEx Dr Alan Buckle of the University of Reading tackled some complex work done on the mechanisms of resistance and translated it into layman's terms. It proved an interesting and instructive session.

We all may have our own idea as to what resistance is and to ensure that we were all 'singing from the same hymn sheet'. Dr Buckle opened the session with a definition of resistance:

> "Anticoagulant resistance is a major loss of efficacy in practical conditions where the anticoagulant has been applied correctly, the loss in efficacy being due to the presence of a strain of rodent with a heritable and commensurately reduced sensitivity to the anticoagulant."
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> *Croplife International's – Rodenticide Resistance Action Committee*

He explained that resistance is not simple. At a molecular level there are a number of changes, properly called 'mutations', that can bring about anticoagulant resistance. These arise from substitutions of amino acids at specific points along the chains of amino acids that form molecules, such as the enzyme vitamin K1 epoxide reductase (or VKORC1). This enzyme is found in the endoplasmic reticulum of liver cells and is the one affected by anticoagulant rodenticides.

Such substitutions alter the shape of the 'chain-like' enzyme molecule preventing anticoagulants from binding to the enzyme, thereby negating the anticoagulants' deleterious effect on the blood clotting mechanism and so causing resistance.

VKORC1 has been mapped and it has been noted that all of the key mutations occur between amino acid molecule numbers 100 and 140 along the chain.

Because mutations involve the substitution of different amino acids at different points, the resistance may exhibit itself in different ways. When a resistance type is identified it is named by the normal amino acid, the location on the protein chain and the substituted amino acid. For example, the resistance that occurs amongst rats throughout much of Berkshire, Hampshire and Wiltshire, is labelled 'leucine120glutamine' (abbreviated to L120Q).

Dr Buckle described five different types of resistance that have been identified across Europe, one or two prevalent in some countries, others in other countries. All five types are present in the UK!

As previously alluded to, the different types of mutation lead to resistance exhibiting itself in different ways, against different rodenticides. All five types render rats resistant to all of the first-generation anticoagulant rodenticides (FGARs) so that's warfarin, chlorophacinone and coumatetralyl.

The good news is that rats exhibiting any of the five mutations remain susceptible to some of the SGARs, notably brodifacoum, difethialone and flocoumafen. Indeed, Dr Buckle pointed out that: "Whilst there was no difethialone data for some mutations, by extrapolation we expect it to be effective against them all."

For the widely-used products based on difenacoum and bromadiolone the picture is mixed. Rodents carrying the Y128Q and Y139S mutations remain largely susceptible to these actives, L120Q rodents are resistant

whilst those rodents with the Y139C and Y139F may, or may not, be resistant.

Geographical spread

So what countries have what type of resistance?

- The most widespread is tyrosine139cystine (Y139C) mutation which is found in the UK, France, Germany, Belgium and Holland. As well as resistance to the FGARs, this may lead to resistance to bromadiolone and difenacoum.
- Tyrosine139phenyalanine (Y139F) is the next most widely spread being found in the UK, France and Belgium. It has a similar impact as Y139C.
- Leucine128glutamine (L128Q) and leucine120glutamine (L120Q) are found widely in the UK and also in France, whilst tyrosine139serine (Y139S) is limited to the UK, specifically Wales. L120Q is the world's most extreme form of resistance. Present across much of central southern England, it confers resistance to all FGARs and has a severe effect on the efficacy of baits containing bromadiolone and difenacoum.
- With the exception of the UK where resistance is widespread (although with localisation of the various types of resistance) in other countries, most notably Germany, resistance tends to be restricted to certain areas.

Interesting times

As ever, we are condemned to live in interesting times. The extent and complexity of the resistance problem starts to become clear at a time when the very future of anticoagulants is in the balance!

Dr Buckle drew attention to a statement by

the European Chemicals Agency (ECHA) that: "All anticoagulants are toxic to reproduction." Because of this, the ECHA has proposed a 'Specific Concentration Limit' of 30 ppm (parts per million) of active in baits – ALL anticoagulant baits; not just SGARs. To put this in perspective, the concentration of actives in FGARs ranges from 250 ppm to 500 ppm. Dr Buckle observed that at 30 ppm efficacy would be negligible, effectively removing these products from the market.

The SGARs currently available have a concentration of, or about, 50 ppm (difethialone products have a concentration of 25 ppm and are therefore inside the proposed limit). The impact would not be quite as drastic as on first-generation products, but the lower the concentration of active in the bait, the more adverse the possible impact on the development of resistance!

Any bait with a concentration above the 30 ppm limit would not be available to amateurs. The prospect of anticoagulants being restricted to professional users only may seem like music to the ears of pest controllers. The party spoiler, as Alan Buckle pointed out, is the label statement about the products being 'toxic to reproduction'. If clients don't bar the products from their premises altogether, it is likely to be open season for the 'No win, no fee' lawyers every time someone contracts a health problem, no matter what the cause.

Note: both these points were also discussed at the Global Summit of Pest Management Services see page 24 in this issue.

If this concern was not enough, Dr Buckle drew attention to a number of both illogical and knee-jerk reactions by European national governments in response to the resistance data.

The German government has already made SGARs 'professional use only' – when 78% of mice are already resistant to them. The Dutch government has restricted the FGAR chlorophacinone to 'professional use only' – mice exhibit Y139C resistance. Scandinavian governments are restricting the more potent SGARs to 'indoor use only', just at the time that the UK government is easing this constraint.

In conclusion, Dr Buckle observed that if you do not use anticoagulant rodenticides, you cannot induce resistance to them. He therefore proposed the following strategy:

- Use all possible measures to prevent rodent infestation;
- Use non-anticoagulant alternatives wherever possible to control infestations, for example trapping, gassing and non-anticoagulant rodenticides;
- Where FGARs are effective, continue to use them;
- Where FGARs are not effective and/or there is evidence that there is resistance to them, select a suitable SGAR.