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Malcolm R. Hadler
Sorex Limited

Alan P. Buckle
Research & Development Manager, ICI Public Health

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MALCOLM R. HADLER, Managing Director, Sorex Limited, Widnes, Cheshire, WA8 8TJ, UK

ALAN P. BUCKLE, Research & Development Manager, ICI Public Health, Fernhurst, Haslcmere, Surrey, GU27 3JE, UK

ABSTRACT: The anticoagulant rodenticides were discovered in the 1940s and their advantages of efficacy and safety quickly resulted in their use dominating the practice of rodent control in temperate countries. However, the development of resistance to the early compounds within a decade stimulated research culminating in the invention of anew class of anticoagulant, the second generation compounds, active against resistant strains but also overall far more potent than those previously available. A novel baiting strategy, pulsed baiting, was developed to make full use of this valuable characteristic. Pulsed baiting has enabled the use of second generation anticoagulants in situations where early products were of limited value, particularly in tropical agriculture. The future of this highly-successful group of compounds is reviewed in relation to resistance and the difficulty and cost of developing further rodenticides.

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INTRODUCTION

Few modern pesticide groups have such long a history of successful use as the anticoagulant rodenticides. Their origin lies in the discovery that dicoumarin was the active agent responsible for a haemorrhagic condition found in the USA in cattle that had been fed spoiled sweet clover hay (Link 1944).

Early interest in anticoagulants was concentrated upon their therapeutic use for the treatment of thrombosis. Workers at the Wisconsin Alumni Research Foundation were responsible for the isolation of dicoumarin and for the synthesis of a number of analogues in an attempt to increase the potency of the parent compound. The 42nd member of this series, subsequently called warfarin, proved to be the most active.

The rodenticidal potential of these compounds seems to have been recognised independently in the USA and in the UK. According to Mills (1955), Link noted that warfarin "might make a good exterminating agent" following the observation that laboratory rodents died of haemorrhage. In the UK, workers researching dicoumarin had made a similar observation. When contacted by a pest controller who was unable to obtain rodenticides due to post-war shortages, they handed over a sample for trial. The first controlled trials being carried out in London in 1946-47.

The unpublished reports of these trials and O'Connor (1948) indicate that the significant advantages of anticoagulants over previously available rodenticides were recognised remarkably quickly. In an eighteen-month period, the foundations were laid upon which all subsequent developments took place.

Dicoumarin was introduced as a commercial rodenticidc in the UK in 1949 and warfarin was registered for sale in the USA in 1950. Because of its greater potency, warfarin superseded dicoumarin and very rapidly became the dominant rodenticide, a position it was to retain on a world scale for many years.

THE MODE OF ACTION OF ANTICOAGULANTS

The anticoagulants represented a significant advance because they overcame nearly all of the negative aspects of their predecessors, the acute poisons. In order to understand why this should be it is necessary to understand the mode of action of anticoagulants.

When the carcass of an anticoagulated rodent is analysed, by far the largest residue of anticoagulant is found in the liver, the site of action. Within the liver, Vitamin K is utilised to drive the production of blood clotting factors II, VII, IX & X by the gamma-carboxylation of glutamic acid residues of precursor proteins. Concomitant with this reaction, the active form of vitamin K, a hydroquinone (KHj), is converted to an inactive 2,3-epoxide. Some of this is excreted but the majority is reactivated by an epoxide reductase enzyme. The reactivated vitamin recycles and represents the major source of vitamin K in the clotting factor synthesis cycle. Small losses are replaced from extraneous sources, gut bacteria and from food (Fig. 1).

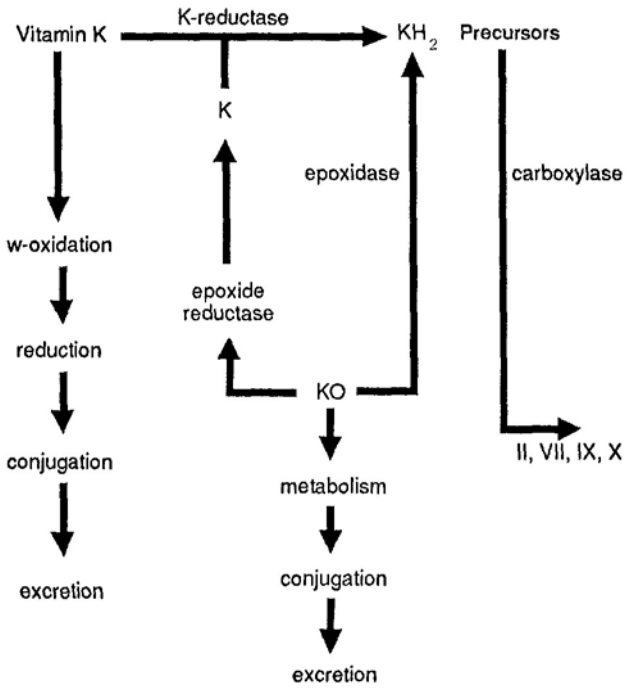

Figure 1. The relationship between the metabolism and the physiological action of Vitamin K_1. (After Park 1982).

All available anticoagulants seem to share the same site of action; they block the epoxide reductase enzyme (Silverman 1980). The recycling of activated vitamin K is therefore stopped and there is insufficient incoming vitamin K to maintain the carboxylation reaction. The production of clotting factors is critically reduced and eventually, when the supply of factors already present has degraded, the clotting mechanism fails and haemorrhaging begins.

Because the mechanism is common to them all, there is no significant difference in the time to death between anticoagulants once the enzyme is blocked. What can differ is the time taken to achieve this. Also, once more because of their common mode of action, the anticoagulants do not differ in the sites at which the lethal haemorrhages occur.

The treatment of poisoning in the case of an accident is also common to all compounds. The synthesis of clotting factors can be restarted by the addition of appropriate amounts of Vitamin K4. Frequent administration is necessary until the anticoagulant has cleared. Different anticoagulants require different treatment periods dependent upon their half-lives at the site of action, varying from some hours for warfarin to several weeks for the more potent products (Anon. 1988, MacKintoshetal. 1988).

THE ADVANTAGES OF ANTICOAGULANTS

With an understanding of the mode of action of anticoagulants, it is worth reviewing the advantages that were so quickly recognised by the early workers. These were, and still are, the reasons why the anticoagulant group of rodenticides came to dominate the practice of rodent control.

Prior to the introduction of anticoagulants, the only compounds available were fast-acting single-dose toxicants, such as thallium sulphate, red squill, arsenic, zinc phosphide, and the first purpose-built rodenticide, ANTU.

Each suffered one common fault, they produced symptoms very rapidly. Rodent behaviour is such that an individual encountering a new food for the first time will normally test feed and may not take a substantial quantity for many hours, or even days (Buckle et al. 1987). If the bait causes distressing symptoms during the test feeding period, the rodent is intelligent enough to recognise cause and effect and becomes 'poison' or 'bait shy.'

The only way to reduce the impact of bait shyness is to prebait for several days with unpoisoned bait base and then to substitute toxic bait when rodents have accepted the new food. Effective prebaiting requires considerable persistence, expertise and effort.

The potency of the acute rodenticides varied considerably, with their LD_{50}'s ranging between 5 and 100 mg/kg. In order to ensure a lethal intake in a single feed, the concentrations of active ingredient in bails had to be high, normally between 0.5 and 5%. The early compounds had little if any selective toxicity and the high concentrations utilised meant that baits represented a considerable hazard to non-target species. This problem was further exacerbated by the fact that few had an effective antidote and their speed of effect made treatment impractical even if one had been available. These considerations have lead to the imposition of rigorous restrictions on the use of acute rodenticides in many countries. The only one now widely used is zinc phosphide.

In the case of anticoagulants, the delay between the ingestion of a potentially lethal dose and the onset of symptoms is such that the rodent does not recognise their relationship. In addition, the effect itself is relatively painless in most cases. Bait shyness does not occur, prebaiting is unnecessary, and compared to most other toxicants, anticoagulants may be considered relatively humane (Rowsell et al. 1979).

Furthermore, a practical treatment was available in the event of accidental ingestion and, because of their potency, even the earliest could be used at concentrations more appropriately measured in parts per million (ppm), and therefore palatability was inherently far less of a problem than it had been with the fast-acting products.

THE DEVELOPMENT OF ANTICOAGULANTS

Commercial anticoagulant rodenticides are either indanediones or hydroxycoumarins, with the exception of one thiocoumarin. Following the introduction of warfarin, a number of compounds were developed during the nineteen fifties and these are now collectively known as the "first generation anticoagulants." Principal amongst these were the hydroxycoumarins, coumachlor (1951), coumafuryl (1953) and coumatetralyl (1956). Amongst the indane-diones were diphacinone (1952) and chlorophacinone (1961). Pindone was first developed as an insecticide and its rodenticidal properties were not utilised until 1953. A common feature of the first generation compounds is that they are chronic toxicants: they are more potent if administered in small daily doses than if given as a single dose. For example the acute oral LD_{50} in rats of warfarin is variously quoted at between 10-20 mg/kg (Lund 1982) and 323 mg/kg (Hagan and Radom-ski 1953). The sub-acute oral LD_{50} by contrast is generally accepted to be about 5 daily doses of 1 mg/kg for warfarin and for most of the first generation products. The reason for this apparent anomaly appears to relate to their half-lives. Yacobi and Wingard (1972) determined the biological half-life of warfarin in Sprague-Dawley rats as being between 5 and 28 hours. Warfarin is relatively easily detoxified by the action of mixed-function oxidase enzymes and converted to lower toxicity and easily excreted hydroxylated metabolites (Barker et al. 1969). In order to maintain an effective concentration at the active site it is necessary constantly to renew anticoagulant removed by the action of these enzymes.

The compounds discussed above came to dominate the practice of rodent control world-wide in the period 1950-1965. They were used to such effect that it is perhaps not surprising that resistance genes should be selected.

RESISTANCE TO FIRST GENERATION ANTICOAGULANTS

Warfarin resistance was discovered first in *Rattus norvegicus* in the UK (Boyle 1960), then in Denmark, the Netherlands, the United States, Germany and France. Resistance was also detected in *Mus domesticus* (Rowe and Redfern 1965) and in *Rattus rattus* (Greaves et al. 1976). Concern increased when it was found that cross-resistance existed to all first generation anticoagulants (Rowe and Redfern 1965, Greaves and Ayres 1969, Hadler and Shadbolt 1975).

The occurrence of resistance in all of the major commensal species to all of the anticoagulants then available rekindled research in an area which had been dormant as a consequence of the commercial dominance of these products. Greaves and Ayres (1969) showed that, in a Welsh strain of resistant

Table 1. No-choice feeding of anticoagulant to groups of house mice for up to 21 days.

Anticoagulant	Concentration	Mortality	% Mortality
Brodifacoum	20	12/12	100
Difenacoum	50	14/15	93
Coumatetralyl	50	3/13	23
Chlorophacinone	250	6/13	46
Diphacinone	125	0/9	0

Table 2. 10-day no-choice feeding tests of anticoagulants against homozygons Welsh resistant Norway rats.

	Mortality at Concentration (ppm)						
Anticoagulant	250	200	100	50	20	10	5
Warfarin	0/5	0/5	—	—	—	—	—
Diphacinone	0/5	—	—	—	—	—	—
Chlorophacinone	1/5	—	—	—	—	—	—
Difenacoum	5/5	—	5/5	10/0	10/10	7/10	—
Brodifacoum	—	—	—	—	5/5	5/5	5/5

R. norvegicus, the resistance gene is single, dominant and autosomal. Hermodson et al. (1969) showed that rats of this strain, homozygous for resistance, have a dietary requirement for vitamin K of twenty times normal. This suggested a change of an acceptor site on the reductase enzyme such that affinity to Vitamin K was reduced.

Unpublished work with the 2-chloro analogue of vitamin K_1 (Lowenthal and MacFarlane 1967), indicated that this known anticoagulant was not resisted, in fact it was more active against resistant individuals. This indicated the possibility of producing analogues which would block the resistant enzyme and that the acceptor site corresponding to the 3-phytyl side chain of vitamin K_1 had not been modified.

Consideration of the structures of a number of failed anti-malarial agents which had some anticoagulant activity suggested that the stable substituted 3-biphenyl side chains of these vitamin K-like l:4-naphthoquinones could be mimicking the 3-phytyl side-chain of vitamin K.

SECOND GENERATION ANTICOAGULANTS

Substitution of stereochemically similar side-chains onto the 4-hydroxycoumarin moiety produced a series of compounds which was both effective against resistant strains and considerably more potent than any previously used anti-coagulant (Hadler and Shadbolt 1975).

Two compounds were selected from the series for development, difenacoum and brodifacoum. These were the first commercially available 'second generation anticoagulants.' The term was coined to describe compounds effective against strains resistant to previously available products. Difenacoum was first registered in the UK in 1975, followed by brodifacoum in 1978. Tables 1 and 2 illustrate the comparative potencies of these two compounds compared with that of first generation products in resistant house mice and Norway rats.

Meanwhile, in 1967, workers in France had synthesized a series of warfarin alcohol analogues with similar side-chain substitutions, although activity against resistant strains was not apparently recognised until one of these, bromadiolone, was introduced in France in 1978 (Grand 1976).

Since the introduction of difenacoum, brodifacoum and bromadiolone, two further compounds, flocoumafen and difethiolone, have been added to the list of commercially available second generation anticoagulants. Apart from their ability to control rodents resistant to previously available anticoagulants, the second generation products are characterised by their higher overall potency (Table 3). The acute oral toxicity of second generation products can be of the order of 100 times greater than that of their predecessors.

A PRACTICAL PATTERN OF USE FOR THE SECOND GENERATION ANTICOAGULANTS-PULSED BAITING

The first generation products were described as chronic rodenticides because several consecutive and substantial daily feeds of poisoned bait were required to deliver a lethal dose. This characteristic makes it necessary, when using them, to maintain large quantities of bait at the site of treatment, often for some weeks, and to visit the site frequently to replace eaten bait to ensure the continuous availability of rodenticide. This technique is known as surplus, or saturation, baiting (Dubock 1984).

As well as activity against resistant rodents, the second generation anticoagulants possess another highly advantageous characteristic. They are so potent that a lethal dose can be delivered when an animal consumes bait on a single occasion and as only a fraction of its daily food requirement. For example, an adult *R. norvegicus* will consume about 20-30 grammes of food daily and an adult *M. musculus* typically 2-5 grammes. Thus, 0.005% brodifacoum bait will deliver an LD_{50} dose to a rat in only 1.3 grammes of bait and to a mouse in only 0.2 grammes.

These observations, based on laboratory experiments (e.g. Redfern et al. 1976), led to speculation that brodifacoum could be potentially effective as a 'single application' rodenticide (Rennison and Dubock 1978). Trials were therefore conducted with brodifacoum baits against Norway rats on UK farms to test the efficacy of single bait applications of 1,4 and 7 days duration. Contrary to expectations, complete control was not achieved with any of these regimens. They resulted in 41, 51 and 68% mortality respectively. It was concluded that, to achieve satisfactory levels of control, bait must be available for longer than a seven-day period because, clearly, a proportion of rats do not feed sufficiently from bait points in the first week to acquire a lethal dose. It was also concluded that those rats that took bait during the first week, and succumbed, were likely to have fed on several occasions thereby consuming more bait than necessary to cause death.

These considerations gave rise to the concept of 'pulsed baiting' (Dubock 1984) in which limited quantities of bait are applied at approximately weekly intervals. Those animals that feed during the early stages of the treatment consume the available bait completely, finding none left when they return to the bait points subsequently. They die before another application, or 'pulse,' of bait is laid for those animals that are more reluctant to begin feeding on the poison. Two or more

Table 3. Acute oral LD_{50} of active ingredients and baits of several anticoagulants against Norway rats and house mice. (After Dubock 1979).

Anticoagulant	LD_{50} (mg/kg) to albino Norway rats (ppm)	Normal bait LD_{50}	
		Concentration	(g of bait/250g rat)
Brodifacoum	0.26	50	1.3
Bromadiolone	1.126	50	5.6
Difenacoum	1.8	50	9.0
Coumatetralyl	16.5	375	11.0
Diphacinone	3.0	50	15
Pindone	50.0	250	50
Chlorophacinone	20.5	50	102.5
Warfarin	186.0	250	186.0

Anticoagulant	LD_{50} (mg/kg) to albino house mouse (ppm)	Normal bait LD_{50}	
		Concentration	g of bait/ 25g mouse
Brodifacoum	0.40	50	0.2
Difenacoum	0.80	50	0.4
Bromadiolone	1.75	50	0.9
Warfarin	374.00	250	37.0
Diphacinone	141.00	50	70.5

additional pulses may be required to achieve complete control of rat and mouse infestations. The mechanism of this process was displayed during field trials of one of the more recently introduced highly-potent second generation anticoagulants, flocoumafen (Buckle 1985).

The comparative performance of three compounds, difenacoum, bromadiolone and brodifacoum, in pulsed baiting programmes was compared by Greaves et al. (1988). These authors found that treatment efficacy was directly related to the toxicity of the baits used. Thus, fewer bailing rounds and less bait was required to achieve complete control of resistant *R. norvegicus* infestations with brodifacoum baits than with baits containing either difenacoum or bromadiolone.

The use of pulsed baiting with compounds such as brodifacoum and flocoumafen offers valuable advantages to the rodent control practitioner. Firstly, relatively small quantities of bait are required and less labour is needed to apply it during baiting programmes, resulting in lower treatment costs. Also, for periods during treatments, no bait is exposed because it has all been consumed by the target rodents (Buckle 1985), thus reducing the primary hazards of the treatment. There is also a reduction in the quantity of bait eaten by rodents resulting in reduced levels of residues in targets and potentially lower secondary non-target hazard (Dubock 1984).

This system is now in use in rodent control world-wide and is integral to the labeled use patterns of the second generation compounds. It is particularly applicable in agriculture. Smallholders cultivating tropical field crops were always unwilling to employ the saturation baiting technique necessary to ensure the full efficacy of the first generation anticoagulants and consequently these compounds were never widely used in tropical agriculture. Similarly, the technique of prebaiting was never widely accepted in the use of the acute compounds, resulting in their frequent failure (e.g. West et al. 1972). Thus, until the introduction of the second generation compounds, tropical smallholders never had available to them a rodenticide bait and pattern of use appropriate to their needs. Field trials have now been conducted world-wide to develop safe and efficient patterns of use for these products in a very wide range of agricultural crops (e.g. Kaukeinen and Rampaud 1986, Hoque and Olvida 1988).

SAFETY IN USE

One of the major potential benefits of the anticoagulant rodenticides over their acute predecessors was that of improved safety. The features which lead to this are, in particular, the presence of an antidote, Vitamin K_1, and the slow mode of action which provides adequate time in cases of accidental poisoning for diagnosis and treatment (Anon. 1988).

However, in spite of these safety features, as with all pesticides, the use of anticoagulants carries with it a degree of risk, particularly because this class of compounds contains potent mammalian toxophores. An exacerbating factor is the frequently commensal nature of the rodent pest problem, which means that rodenticides are often applied peridomestically, where accidental exposures to man and his companion and domestic animal are likely. Additionally, 'rat poisons' are attractive to those who would mis-use them as vertebrate killing agents outside labeled use patterns and for unlawful purposes unrelated to rodent control.

In spite of all these factors, the anticoagulant rodenticides have a remarkable history of safe and efficient use over

a period exceeding 40 years. Few published records exists which place this history into context but, perhaps, the most fully documented evidence on accidental exposure to harmful substances is published in the USA. Litovitz et al. (1989) record that, of more than one million human exposures, only 3.8% were associated with pesticides; a category which includes rodenticides as only a minor component. Exposures to analgesics, cleaning agents and cosmetics by far exceed in number exposures to pesticides.

Although the number of exposures to rodenticides is relatively small, the demographic profile of these incidents is of great interest to those attempting to develop even safer products. The data presented by Litovitz et al. (1989) is typical in showing that the vast majority of exposures are via the oral route, involve children under the age of six and occur, presumably, when they accidentally encounter rodenticide bait placements. A major advance in ensuring a favourable outcome in such cases is the use of a human taste deterrent in rodenticidal preparations (Johnson 1988). This substance, 'Bitrex,' is not perceived by rodents but its inclusion in bails at an appropriate concentration renders them highly objectionable to humans (Kaukeinen and Buckle, these Proceedings). This will serve to reduce the quantity of bait ingested during accidental exposures, although the numbers of recorded exposures may actually increase when baits contain 'Bitrex' because their unpleasant taste will more likely cause 'consumers' to report the episode.

Companion animals are also occasionally exposed to rodenticides because of the often close association between the places where they are housed and the sites of rodent infestation. Care in bait placement, the use of bait stations of suitable construction and baits with low intrinsic palatability to companion animals will serve to reduce the frequency and severity of these exposures to very low levels but it is impossible to eliminate this risk altogether.

ENVIRONMENTAL IMPACT

It is unlikely that anticoagulant rodenticides will have any major adverse environmental impact (Kaukeinen 1982). Their mode of use generally involves the localised placement, often within bait containers, of small quantities of bait with very low toxicant loadings. The contamination of either aquatic or soil systems with these compounds remains therefore a remote possibility envisaged only in the event of serious mis-use.

However, the most problematic aspect of the potential of anticoagulants to have an impact is the hazard they may pose to animals that prey upon poisoned rodents or scavenge their dead bodies. All the second-generation compounds arc equally persistent, and even some of the earlier compounds are retained in animal tissues at sub-lethal levels for relatively long periods (Parmar et al. 1987, Huckle et al. 1989). It is known, therefore, that where target rodents are the dietary mainstay of a carnivorous non-target species, individuals of the latter species may be at risk (e.g., Hegdal and Colvin 1988). To date, however, there is no indication that the occasional mortalities caused by anticoagulants have had any affect on non-target populations, as such losses are rapidly made good by breeding (Kaukeinen 1982, North 1985).

One of the most thoroughly investigated interactions is that between Barn owls and anticoagulant rodenticides in the UK. In spite of the prolonged and intensive use of compounds such as coumatetralyl, difenacoum, bromadiolone and brodifacoum, there is no evidence that this has been to the detriment of Barn Owl populations (Percival, 1990). However, there is scope for studies to monitor the population levels of non-target animals, particularly predatory birds, and for the development of potent anticoagulants that are more rapidly eliminated from animal tissues than those currently available.

THE FUTURE

Five highly potent second generation anticoagulants have been commercialised and three of them, brodifacoum, flocoumafen and difethiolone, each to a greater or lesser extent, can be said to possess the capability of killing rodents after a single feed. These three compounds have acute oral LD_{50}S to *R. norvegicus* in the range 0.2-0.4 mg/kg. It is the experience of one of us (MRH), having worked with numerous experimental analogues, that none with significantly higher toxicity has ever been found. It appears that, after 45 years of development of anticoagulants with progressively greater potency, the upper limit has at last been reached.

Although the chronic action of the anticoagulant rodenticides is an advantage in preventing the development of bait shyness, it also has the disadvantage that animals which have consumed a lethal dose of poison may continue to feed on bait for several subsequent days. This is both wasteful and increases secondary hazard. It would be beneficial to have a potent anticoagulant which caused death after, say, 2-3 days rather than the 5-6 days that is characteristic of the presently available compounds. However, the onset of symptoms of anticoagulation and death occurs only after the reservoir of various blood clotting factors have been fully depleted. This mechanism is fundamental to the mode of action of all compounds and it seems, at the moment, unlikely that an anticoagulant with a faster action can be produced.

Several of the second-generation anticoagulants have been used extensively world-wide for 15 years or more. This constitutes a substantial selection pressure favouring the emergence of resistant strains. So far, however, resistance to these compounds is very localised. In southern-central England populations of *R. norvegicus* resistant to difenacoum are found in a locality coincident with a long- established area of warfarin resistance (Greaves et al. 1982). Resistance to both difenacoum and bromadiolone is present in Norway rats in Denmark (Lund 1984) and the Danish Pest Infestation laboratory has also identified populations of House mice resistant to the same compounds (Anon. 1990). In both foci it is possible, in laboratory tests, to demonstrate that rodents are less sensitive to the more potent compound brodifacoum (e.g. Gill and MacNicoll 1991), although this altered level of tolerance falls short of resistance in practical terms.

There is much speculation on what the future holds in terms of resistance to the second generation anticoagulants. Some workers feel that the levels of resistance described above presage the development of ever more resistant populations of rodents. There is an alternative view, however, that resistance may have reached an upper limit in the levels presently observed in southern England (Greaves and Cullen-Ayres 1988). This is because possession of resistance genes carries with it the cost of impaired ability to synthesize Vitamin K. Some resistant rodents require twenty times more dietary Vitamin K than their susceptible counterparts and

further development of resistance may thus be physiologically self-limiting. Whatever the future holds for resistance in temperate countries where anticoagulant use is intensive, it seems unlikely that resistance to the second generation compounds will become a serious practical problem in the tropics. This is implied by the fact that resistance to the first generation compounds, widespread in North America and in many European countries, is of very limited importance elsewhere.

Given that anticoagulants are designed to be mammalian toxicants, their record of safety in use is impressive. Significant selective toxicity has been achieved in favour of many non-target animals (Dubock and Kaukeinen 1978, Bowler et al. 1984, Lechevin and Poche 1988) but there would still seem to be a benefit in developing a product with even greater specificity. However, the increasingly rigourous regulatory requirements currently being introduced in the USA, Europe and elsewhere have caused companies to direct resources towards the defense of existing compounds rather than to the development of new ones. Furthermore, development costs have soared. For example, the cost of introducing flocoumafen during the 1980s was more than ten times greater, in real terms, than that of developing difenacoum a decade earlier. These costs, and the existence already of five highly potent compounds, would make it necessary to consider carefully the commercial justification for the development of a new, more specific anticoagulant, even if such a compound was technically feasible.

Anticoagulant rodenticides have a long history of effective and safe use world-wide. It seems likely that those currently available will continue to dominate the practice of rodent control for many years to come.

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