RODENTICIDES: WARFARIN, STILL A GOOD MANAGEMENT TOOL

Part 1: First and Second Generation Anticoagulants

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Keywords: anticoagulant, rodenticide, warfarin resistance, antidote, commensal rodents

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Introduction

Rodents have been a menace to man for generations inflicting billions of dollars of crop and commodity damage each year. Rats and mice serve as reservoirs of numerous diseases transmitted to humans, such as plague, leptospirosis, Lyme disease, and rat bite fever (Gratz 2006). Millions of people died during the middle ages because of the spread of plague by rat in Europe.

Rodent control products have been developed over the centuries including traps, glues, and chemical methods (rodenticides). Initial acute, or fast acting products were introduced and contained chemicals having no antidotes, such as arsenic, ANTU (α-naphthylthiourea), sodium monofluroacetate, strychnine, and norbormide. It was not until the late 1950s that rodent control was dramatically changed by the development and marketing of warfarin. The chemical is classified as an anticoagulant, or blood-thinner, and inhibits the production of vitamin K within the rodent, resulting in death over several days.

After warfarin's initial success, other anticoagulants were added to the marketplace, including coumatetralyl, chlorophacinone, pindone, and diphacinone. The compounds came to be known as 'first generation anticoagulants'. These novel rodenticides quickly reduced the use of acute rodenticides which have no antidotes.

Beginning in the early 1980s the more toxic 'second generation' chemicals were introduced into the marketplace, including brodifacoum, bromadiolone, and difethialone. The use of the chemicals soon began to diminish the use of the less toxic first generation group. This took place because of the perceived genetic resistance developed in US rodents. The lower dose baits were seen as the newest rodent management success story.

As early as 1958 there were reports of warfarin resistance in Scotland in the Norway rat (*Rattus norvegicus*) (Boyle, 1960). After prolonged use of warfarin in the US, resistance was documented (based on WHO criteria) and published (Jackson & Kaukeinen, 1972). Consequently, more toxic anticoagulants were synthesized to overcome the genetic resistance reports. The rodent control industry over a period of only a few years, moved from the first to second-generation rodent baits. The marketing strategy was to: 1) implicate first generation rodenticides as ineffective against rats and mice and, 2) argue that the newer baits could kill rodents in a 'single feeding' (2nd generation rodenticides) compared to the 'multiple feeding' required by the 1st generation products. In the professional pest control industry, the goal was to convince the technician that more bait would be required with the less toxic products. It was economically cost effective to use less bait in a rodent control program. It was a good marketing idea, but in reality the story had its flaws.

Warfarin Resistance – fact or fiction

More reports began to surface from Europe with rodent genetic resistance not only to warfarin, but also bromadiolone, coumatetralyl, and difenacoum (Pelz, 2001). The same year Norway rats were observed to be resistant to warfarin, difenacoum, bromadiolone, and coumatetralyl, but not to brodifacoum and difethialone (Lodal, 2001).

A reason behind this resistance development was thought to be possible overuse of anticoagulants, the basic warfarin resistance gene had emerged (Pelz, 2001). Studies in the US indicated that anticoagulant resistance was 'widespread' which led to the promotion of second-generation anticoagulants (Jackson & Ashton, 1992). Others suggested the issue of warfarin resistance was exaggerated and potentially was a minor problem in the US. It was proposed that rodents may exhibit a tolerance to low doses of warfarin because of the ability of the gut flora to produce sufficient vitamin K to counter the effects of the compound (Poché, 1998).

DOI: 10.1564/23jun00

The industry standard for warfarin resistant screening involved the use of a protocol developed by the World Health Organization (WHO, 1982). Wild Norway rats were fed a diet containing 0.005% warfarin for 6 consecutive days in a no-choice design. If the rat consumed an accumulated amount of 12 mg/kg warfarin over the 6-day period and survived, it was classified as resistant.

Dosing rats at 1/5th (0.005%) the EPA recommended concentration of 0.025% warfarin resulted in consistent survival of about 50% of the Norway rats collected from Chicago (Poché 1998). This approach was taken a step farther by re-subjecting surviving rats to the same test 30 days later (Frantz & Padula, 1998). The results were similar with 50% of the Norway rats surviving. If this is a case of genetic resistance, 100% of the rats should have survived, because the animals were deemed resistant in the first test. This process was duplicated multiple times by the authors with the same results: surviving 'warfarin resistant' Norway rats when retested succumbed to the chemical. There was a 30-day latent period so all warfarin and its metabolites had been either excreted or metabolized.

This perceived resistance to warfarin in the US, should be more accurately termed, 'tolerance to low doses of warfarin'. To what extent vitamin K produced by the rats intestinal flora was antidotal enough to counter the effects of the anticoagulant remains to be studied. Studies in humans demonstrated the role of microflora in metabolizing compounds (Manning *et al*, 1988). Flora in the gut of animals has a significant impact on metabolizing compounds (Hill, 1995). This breakdown of first generation rodenticides is part of the reason they pose reduced risk to non-target animals. That issue will be addressed in a subsequent article.

The 'pockets' of resistance as reported from Chicago and Baltimore were studied (Jackson & Ashton, 1992). Norway rats tested in Colorado all succumbed to warfarin at 0.005% after a 6-day exposure. Rats from Chicago subjected to the recommended 0.025% warfarin bait concentration consistently succumbed to the product (100% mortality) (Poché 1998). This is possibly due to geographical differences in Norway rat microflora.

In Europe, Endepols *et al* (2003) studied an area previously thought to be occupied by bromadiolone-resistant rodents. In that instance, the inability to reduce rat numbers had nothing to do with genetic resistance at all. Rather, the insufficient use of properly placed bait stations contributed to inadequate control. Proper baiting techniques ensured a sufficient reduction in rodent numbers. Local authorities had previously concluded that 0.005% bromadiolone bait was not sufficient to control rats and mice, when in fact there was inadequate coverage of bait stations, allowing for constant reinvasion of rodents from neighboring areas. This misinterpretation of field results is not uncommon and has contributed to the misconception that first-generation anticoagulants are ineffective.

Recent work from Uganda in *Rattus spp* has revealed that in a region where no rodenticides have been used, the so called 'resistance gene' is common in the species (Diaz *et al.*, 2010). The issue of warfarin resistance in rodents remains to be completely studied using modern methods.

Single-feeding claims on product labels

In evaluating 20 years of laboratory anticoagulant efficacy data at Genesis Laboratories it was surmised that all anticoagulants have a similar lethality pattern. The time from initial ingestion of bait until death does not differ statistically between first and second generation anticoagulants.

This argument is not made to refute the fact that second generation anticoagulants may kill a rodent in a single feeding, but deals more with the misinterpretation of the statement. The average person reading a rodenticide label with the statement 'kills in a single feeding' will assume the rodent will eat the bait once then, die. Similarly, most pest control professionals believe that second generation baits are faster acting and require less product. In reality, this is not the case. Studies on the biology of Norway rats and house mice (*Mus musculus*) have revealed that they may visit food sources some 80 and 200 times, respectively, during a 24-hour period. With both species, consumption takes place at about 88% of the visits (Meehan, 1984). In the real world, a 'single-dose bait' does not exist.

The single-feed claim test protocol developed by the EPA stipulates a rodenticide exposure for a 24-hr period: bait is presented with a choice of a challenge (placebo) diet, and mortality in the test animals must be ≥90% to permit the use the single-feed claim. Is the message communicating what

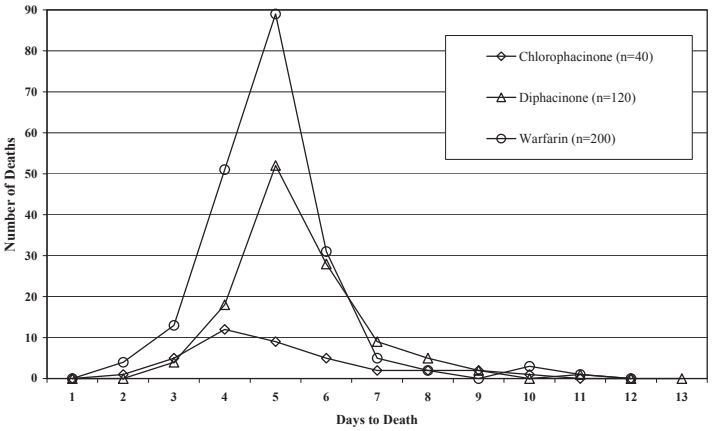

Figure 1. Days to death in house mice – first generation anticoagulants.

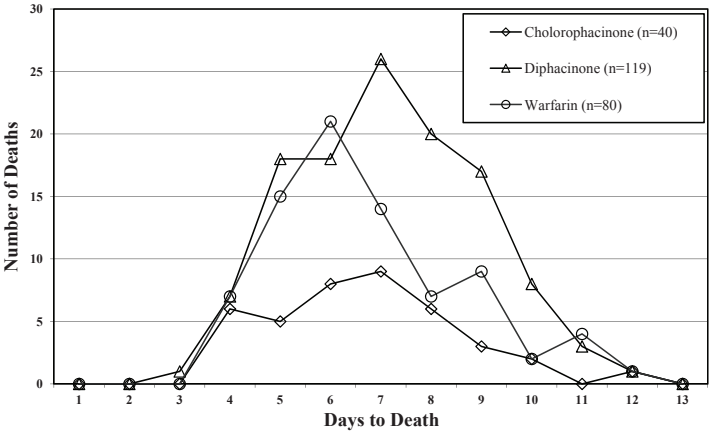

Figure 2. Days to death in laboratory rats – first generation anticoagulants.

takes place in reality? Does this lead to target animal overdosing, resulting in potential secondary hazards? A comprehensive review of the anticoagulants and potential primary and secondary risks was compiled (Erickson & Urban, 2002).

When examining published data on feeding behavior of wild Norway rats, a safe assumption is that a rat eats 20 grams of bait daily for 4 days (Meehan, 1984). The rat then has ingested nearly 50 LD_{50}s of brodifacoum and less than 2 LD_{50}s of warfarin. Warfarin is metabolized and excreted from the rat within 40 hours, while the half-life of brodifacoum exceeds 180 days (Erickson & Urban, 2002). Should a rat consume a sub-lethal dose of brodifacoum in a single feeding subsequent consumption of the bait will result in overdosing and bioaccumulation. Studies with warfarin on various rodent and wildlife species indicate the reduced risk to non-target wildlife when compared to other anticoagulants (Poché & Mach, 2001).

Eason *et al*. (2002) expressed concern over the use of compounds such as brodifacoum stating, "Equally, consideration should be given to banning or restricting the use of second generation anticoagulants where their use is not warranted. Secondary poisoning risk of brodifacoum versus other anticoagulants suggests that switching from brodifacoum to alternative second generation anticoagulants, with similar toxicokinetic profiles would not significantly reduce the risk to non-target species".

Other toxicants, especially bromethalin, are replacing second generation anticoagulants in the US retail market. This compound has not been approved in the European Union because it has no antidote, and is a neurotoxin. The rodent goes into severe convulsions before death. Laboratory and field testing have shown, using standard EPA test methods for Acute Rodenticides, efficacy in house mice ranges from 13–40%, and with rats, less than 40%. The rapid response to the EPA Risk Mitigation measure involving anticoagulants, will result in inferior rodent control products in the US retail market that have the so called 'stop feeding' claim. It is a true statement that because of the extreme discomfort experienced by rodents after eating bromethalin, and subsequent convulsions within minutes, the rodents will stop feeding. Most will survive despite the marketing strategy of 'uses less bait' which helps sway the consumer to buy these products. The rats and mice quickly develop bait shyness which results in poor rodent control and the potential for an increased public health problem.

Time required to eliminate rodents using anticoagulants

Laboratory testing conducted at Genesis Laboratories between 1994 and 2007 utilized the array of testing guidelines developed by the U.S. Environmental Protection Agency (EPA), Office of Pesticide Programs (OPP) guideline series. These studies involved choice tests of anticoagulants to generate efficacy data to support EPA product registrations. Criteria included bait acceptance of 33% (percent test bait consumed divided by the total challenge diet (placebo) and test bait consumption). In addition, a minimum of 90% mortality had to be achieved to consider the data set in the evaluation. Data were compiled to compare time of death from the onset of feeding on baits containing diphacinone, chlorophacinone, warfarin, brodifacoum, difenacoum, bromadiolone, and difethialone.

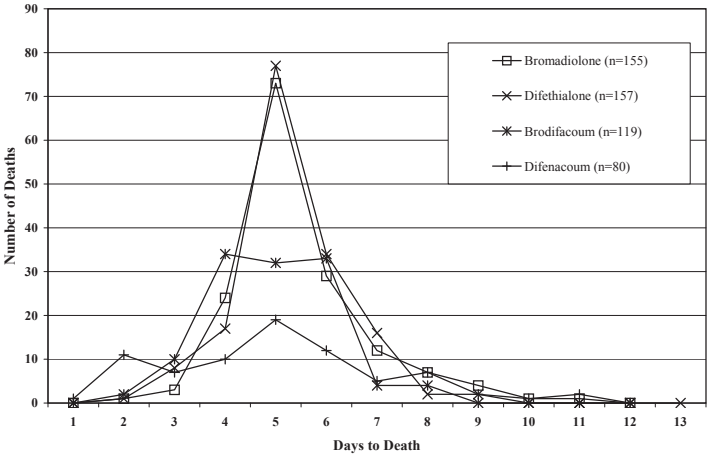

Figure 3. Days to death in house mice – second generation anticoagulants.

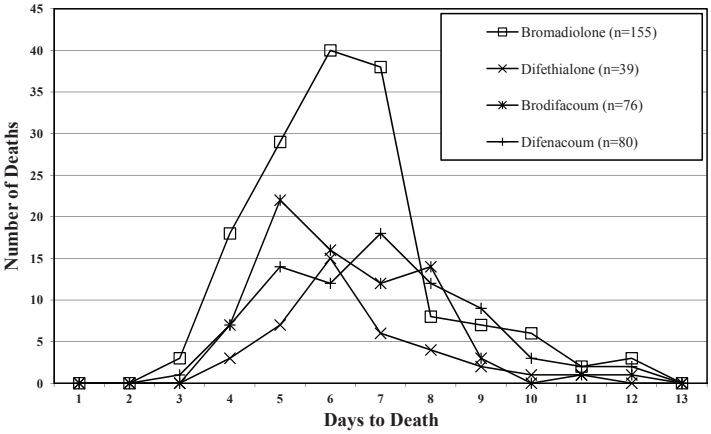

Figure 4. Days to death in laboratory rats – second generation anticoagulants.

The number of rats and mice used varied based on the number of studies conducted. Tests required that a group of 10 males and 10 females be used and that a replication group be presented test material along with a control group. Standard EPA rodent exposure periods for first and second generation anticoagulants were 15 and 3 days respectively. Concentrations of the baits were based on standard active ingredient amounts approved by the EPA and marketed as commercial products: diphacinone 0.005%, chlorophacinone 0.005%, warfarin 0.025%, bromadiolone 0.005%, difenacoum 0.005%, and difethialone 0.0025%.

Data combined from studies with first- and second-generation rodenticides have similar trends in time-to-death for Norway rats and house mice. Figures 1 and 2 present mortality data on house mice and Norway rats in which the first generation compounds were presented to rodents. Figures 3 and 4 display the results from lab tests using second generation rodenticides. With house mice, the mean time until death for brodifacoum and warfarin are similar. In initially compiling these data we had the preconceived notion, as generally thought, that first-generation anticoagulants require more

time to kill rodents. The prevailing thinking within our industry is along the lines that "it takes up to 21 days to kill rodents with warfarin in the field". We suspect part of the reason is that earlier forms of warfarin were not highly purified as they are today. Impurities in warfarin manufactured 30 years ago, were quite distasteful and rendered baits less palatable.

More bait intake results in quicker kill, so the thinking goes, but as these data demonstrate, the acute toxicity of these anticoagulants has no bearing on time-to-death. If one examines the LD_{50} of brodifacoum in rats (0.15 mg/kg) versus warfarin (15 mg/kg), the time until death is almost identical.

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