WARFARIN RESISTANCE REVISITED

STEPHEN C. FRANTZ, Wadsworth Center, New York State Department of Health, Empire State Plaza, P. O. Box 509, Albany, New York 12201-0509.

CONSTANCE PADULA MADIGAN, Center for Environmental Health, New York State Department of Health, 2 University Place, Western Avenue, Albany, New York 12207.

ABSTRACT: Roughly 50 years ago, the Wisconsin Alumni Research Foundation developed warfarin, the first anticoagulant rodenticide. This product was something close to that desired elusive "magic bullet" of pest management. Warfarin effectively killed rats and mice, required multiple feedings, and had a good margin of safety for non-target species. The widespread adoption of anticoagulants somewhat changed the conduct of rodent control with a shift in interventions toward toxicants and away from education and physical measures. The discovery of warfarin resistance in the United States in *Rattus norvegicus* in 1971, and later in *Mus musculus* and *Rattus rattus*, heralded in another shift in rodent pest mitigation. This shift was the development of more toxic anticoagulant products capable of killing with one or a few feedings and with concomitantly greater risks to non-target species. Development of the more toxic products both anticoagulant and non-anticoagulant continues today, although there is an increasing trend favoring comprehensive approaches (i.e., integrated pest management [IPM]) which: emphasize educating clients and reducing causative conditions; diminishing the role of toxicants; and, when necessary, using products of the least practical toxicity. In this paper, the concept of counteracting anticoagulant resistance is blended with the sometimes necessary use of anticoagulant rodenticides as part of IPM. Nationwide data from the former New York State Department of Health Rodent Control Evaluation Laboratory (in cooperation with the Centers for Disease Control's former Urban Rat Control Program) are examined regarding warfarin resistance in *Rattus norvegicus*. In samples from two dozen project cities, population resistance levels ranged from 1.6% to 76.2% using the standard World Health Organization (WHO) testing criteria. However, most survivors (i.e., resistant rats) of the initial test succumbed upon one or more re-exposure(s) to warfarin using the same WHO testing protocol. The results are surprising and have implications on interpreting the phenomenon of anticoagulant rodenticide resistance and on the pragmatic designing of rodent management programs.

KEYWORDS: rodenticides, anticoagulant resistance, warfarin, Norway rat, baiting strategies, IPM

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INTRODUCTION

A new class of rodenticides became available in the 1940s with the introduction of warfarin by the Wisconsin Alumni Research Foundation, Madison, Wisconsin. The advantage of warfarin (and closely related hydroxycoumarin compounds) was that it was effective in killing rats and mice with a relatively low dose when consumed regularly over a period of several days. Further, a large amount of warfarin bait consumed at one time would not effectively kill; thus, this new rodenticide had a built-in safety factor regarding non-target species such as cats, dogs, and children. Proper baiting procedures should prevent access to baits by non-target species, and certainly should prevent the repeated ingestion necessary for intoxication. In essence, warfarin was a product that was close to that elusive "magic bullet" of pest management. An unfortunate outcome of this discovery was that rodent control became largely an issue of chemical intervention with less emphasis placed on public health education, housekeeping, storage practices, sanitation, and exclusion (proofing and stoppage). Not surprisingly, anticoagulants have been the most preferred rodenticides since World War II.

The identification of warfarin resistance in the United States (known in Europe since 1958) in *Rattus norvegicus* in North Carolina in 1971, and later in *Mus musculus* and *Rattus rattus* (Jackson et al. 1985), heralded in another shift in rodent pest mitigation. This shift was the industry's increased interest in the development of more toxic anticoagulant products (e.g., brodifacoum, bromadiolone) capable of killing with one or a few feedings. Unfortunately, the more potent anticoagulants also have greater risks to non-target species. Development of the more toxic products, both anticoagulant (e.g., difethialone) and non-anticoagulant (e.g., bromethalin), continues today. While not remarkable in thoroughness nor consistency, there is an increasing trend in some sectors of the pest management industry favoring comprehensive approaches (i.e., integrated pest management [IPM]) which: emphasize educating clients and reducing causative conditions; diminishing the role of toxicants; and, when necessary, using chemical products of the least practical toxicity (Frantz and Davis 1991). Of course, concomitant with changes in the industry are necessary changes in the public's perception of what to expect in an IPM program.

In this presentation, the authors reexamine the definition of "anticoagulant resistance" in Norway rats (*Rattus norvegicus*) and how rodent control programs might counteract anticoagulant resistance. In fact, warfarin products themselves may be more useful than was thought during the heyday of "super rat" preachments. Nationwide warfarin resistance data are examined from the New York State Department of

Health's former Rodent Control Evaluation Laboratory (a technical adjunct to the Centers for Disease Control and Prevention's former Urban Rat Control Program).

MATERIALS AND METHODS

Animals

All animals used in this study were warfarin-resistant Norway rats (*Rattus norvegicus*) belonging to one of three different source groups. An animal was "resistant" if it had survived the standard warfarin resistance screening test; that is, it survived six days on .005% warfarin bait, nine days on placebo (post-test), and had consumed a total warfarin dosage of at least 12 mg/kg (Brooks and Bowerman 1973, 1974).

Two source groups were comprised of wild-trapped rats from project cities that had been sampled during the nationwide anticoagulant rodenticide resistance surveillance program, a service conducted in conjunction with the federally funded CDC Urban Rat Control Program (Frantz and Padula 1980). One group, mixed source, contained rats from 23 project cities of the United States and Puerto Rico where resistance levels in the sampled rat populations ranged from 1.6% to 25.0%. The second group of wild rats came from only the Chicago, Illinois project where resistance levels in two different sampled populations were 59.7% and 76.2%.

The third group of rats were all F_1 offspring of various combinations of wild-trapped Chicago rats, some of which had been identified as resistant (through the screening test) and others which were of unknown susceptibility (untested) to warfarin. Of 106 of the F_1 Chicago offspring, 76.4% proved to be warfarin resistant upon initial warfarin screening. This offspring group provided age-related data for comparison with the wild-trapped groups which were of unknown age when they arrived at the laboratory.

Test Procedures

All wild-trapped rats were singly caged in mesh-floored cages within about 24 hours of their arrival at the laboratory and held for a minimum of three weeks before being screened for anticoagulant rodenticide resistance (as described in Frantz and Padula 1980). All F_1 Chicago offspring were weaned and singly caged at an age of approximately four weeks; they were then held until about 150 days of age before being screened for resistance.

During the period before anticoagulant resistance screening, all animals received a diet of laboratory food pellets (Wayne Lab Blox, Allied Mills, Inc., Libertyville, Illinois) which contained "added" vitamin K. The overall vitamin K activity of Lab Blox is unknown, but its use may add to the homogeneity of the test animals by minimizing variations in vitamin K status, particularly of wild-trapped animals. Both food and water were provided *ad libitum*. At pre-selection, one week before initiation of pre-test, animals received lab meal (Purina 5001 Lab Chow, Ralston Purina Co., St. Louis, Missouri), containing no added vitamin K. Later, this meal was used as the pre-test diet and then as the base for the warfarin bait in each test or retest.

Pre-selection and selection criteria were that animals were in a healthy condition, not pregnant, without obvious wounds or other pathologies, and weighed at least 150 grams (Frantz and Padula 1980). These criteria are essentially the same as given in the standard WHO procedure (1970) and in Jackson et al. (1975), and were used before each screening test or re-test procedure.

The pre-test, test and post-test procedures were essentially the same as described in Frantz and Padula (1980) and are presented as an algorithm in Figure 1; resistance criteria also remained unchanged from the authors' previous work.

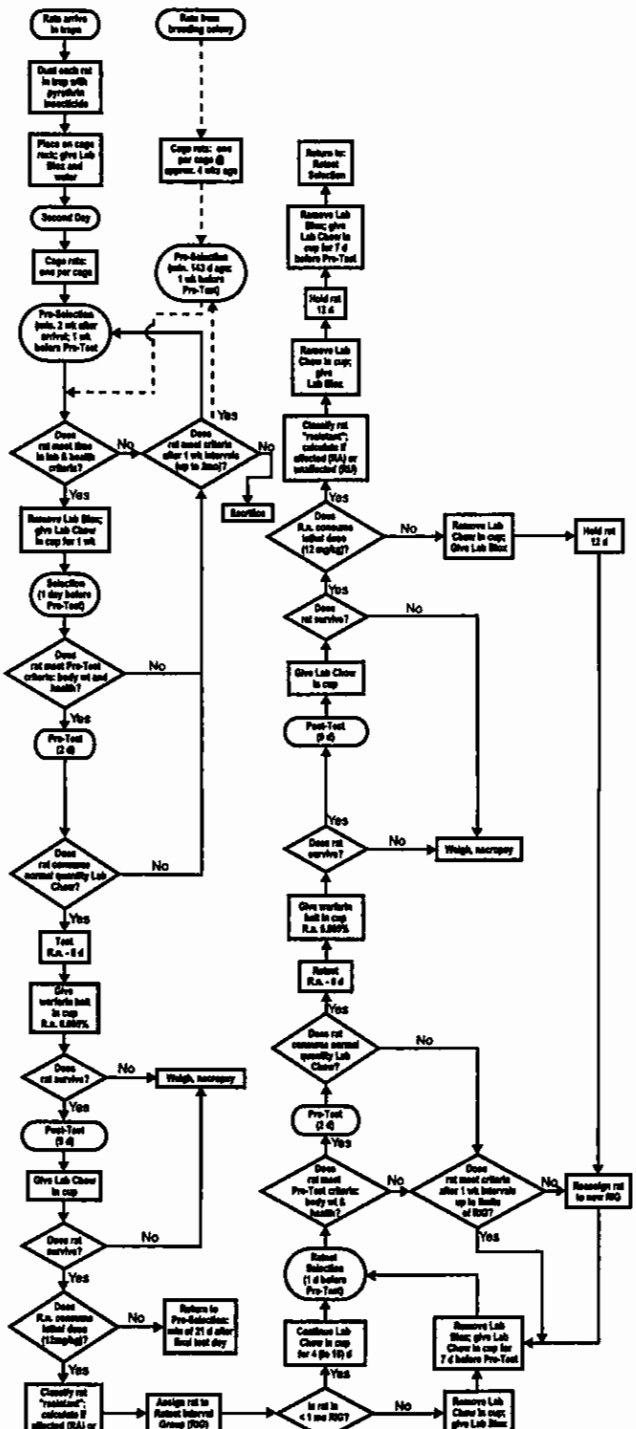

Figure 1. Basic laboratory procedure for anticoagulant resistance screening and retesting of Norway rats.

As resistant animals were identified by the standard screening procedure, they were assigned to one of three retest interval groups (RIG)—or recovery interval groups—depending on the interval between the last day an animal received warfarin bait in the screening test and the first day it was to receive its second laboratory exposure to warfarin in the first retest ($retest_1$) procedure. The three retest interval groups were defined as follows:

Retest Interval Group (RIG)	Days Since Last Received Warfarin Bait Limits	Range Used
<1 month	15-27	15-27
1-2 months	28-59	28-50
>6 months	180-730	196-633

Once an animal was in the time range of its assigned RIG, it was again tested (i.e., re-tested) by the same procedure as in the standard warfarin resistance screening (see Figure 1). Note that procedural differences occur just prior to Retest Selection due to the necessary timing requirements of the RIGs. That is, in the <1 month group, the authors wanted to retest at 15 days whenever possible; but there was not sufficient time for a nine day post-test, seven days on Lab Chow before $pre\text{-}test_2$, and a two day $pre\text{-}test_2$—a total of 18 days. Therefore, the three steps were merged; in essence, the $post\text{-}test_1$ remained nine days, and $pre\text{-}test_2$ remained two days, but the time between these steps was reduced to four days. If, for some reason, an animal did not meet basic test criteria (body weight, health, etc.) (see Frantz and Padula 1980) at that time, it was held for another week or up to 16 days. After 16 days, the animal was reassigned to a RIG with a longer interval between screening and retest. Rats assigned to the other two RIGs which did not meet criteria were treated similarly.

Many animals surviving the $retest_1$ were placed back on a Lab Blox diet, held 12 days, returned to Lab Chow for nine days (seven days + two day pre-test), and then retested repeatedly (e.g., $retest_2$, $retest_3$, $retest_4$, etc.) until they died (to be reported elsewhere). For all retests after the first, the interval between warfarin exposures was fixed at 30 days. Note that some animals surviving the first retest ($retest_1$) were removed from this study for use in other tests requiring resistant rats.

RESULTS AND DISCUSSION

In the <1 month category, 52 rats from mixed sources (excluding Chicago) were retested with 59.6% (31/52) mortality; 18.0% (11/61) mortality resulted when this test was repeated with Chicago-trapped rats (see Table 1). In the second category of 1 to 2 months (see Table 1), 61.2% (30/49) of the mixed-source rats died, whereas 14.7% (10/68) of the Chicago rats died. Repeating this test (1 to 2 month RIG) with 17 of the F_1 Chicago offspring resulted in a mortality of 5.9% (1/17). In the third RIG category of >6 months (see Table 1), 47 mixed-source rats were retested with 83.0% (39/47) mortality; only six Chicago-trapped rats were retested and one died (16.7%).

While test results beyond $retest_1$ will be discussed elsewhere, it is worth noting that few mixed-source rats survived $retest_3$. That is, most animals of mixed-source origin (excluding Chicago) tested from each of the three RIG categories succumbed upon their fourth exposure to warfarin bait in no-choice tests. Chicago-trapped rats in the <1 month, 1 to 2 month, and >6 month groups commonly survived $retest_8$, $retest_{10}$, and $retest_3$, respectively. Thus, some Chicago rats survived 11 lethal doses of warfarin rodenticide, the last 10 of which were consumed at 30 day intervals.

From these data, it appears that mortality for most rats is not significantly affected by the recovery time interval (RIG) for at least the categories of <1 month and 1 to 2 months. The high mortality among mixed-source rats in the >6 month category may be age related. For Chicago rats in this latter category, not enough data are available for analysis. Source (geographic origin), however, is clearly important. Upon first retest, Chicago rats have a significantly greater probability of survival than those animals from mixed sources.

Thus, the most significant finding of these data is that "resistant" (as by standard WHO screening measures) Norway rats from many geographic locations are likely to die upon re-exposure to warfarin, the very product which is used to identify or define their resistance. That is, in a baiting program with warfarin it appears that it should be possible to continue to effectively use warfarin bait *if* a time period of at least two or more weeks without warfarin exposure is allowed between baiting cycles. In fact, the two-week hiatus would be a good time to complete more sustaining, non-toxic interventions such as public health education, housekeeping, storage practices, sanitation, and exclusion (proofing and stoppage). Even in the Chicago area, or other areas that might be identified with similar anticoagulant resistance characteristics, rats will not be "resistant" to such non-toxic interventions that are a significant part of a properly conducted IPM program.

While it should be somewhat easier for rats to consume a normally lethal dose of warfarin in the field situation because of the higher warfarin concentration (.025% in most commercial baits vs .005% in no-choice laboratory tests), bait acceptance might be negatively affected by the higher warfarin concentration and by the availability of other food materials (Jackson et al. 1975). Thus, the need for interventions to limit food resources (e.g., sanitation) is underscored. The uninterrupted use of warfarin baits over long periods of time should be discouraged because such practices would select for resistance (behavioral or other).

A second issue of importance raised by these data is how to define the "resistance" of rats being utilized in efficacy tests of rodenticidal products designed to kill warfarin resistant rats. If a product is tested against "resistant" rats from many geographic areas, the efficacy results become unclear when more than half of such rats might have succumbed to warfarin as shown with the mixed-source test group. Repeated baiting cycles using warfarin (with 30-day intervals of no warfarin) might well

Table 1. Results of resistant[a] wild Norway rats' (*Rattus norvegicus*) second exposure (no-choice feeding test) to .005% warfarin bait.

Source of Rats	Time Interval to Retest$_1$ (months[b])	Rats Retested (Number)	Mortality at Retest$_1$ (Percent)
Mixed Wild-Trapped[c]	<1	52	59.6
	1-2	49	61.2
	>6	47	83.0
Chicago Wild-Trapped	<1	61	18.0
	1-2	68	14.7
	>6	6	16.7
Chicago Lab-Bred[d]	1-2	17	5.9

[a]As determined by the standard warfarin screening test (Brooks and Bowerman 1973 and 1974)
[b]Number of months since exposed to warfarin bait
[c]Excluding Chicago Wild-Trapped rats
[d]F_1 offspring of Chicago Wild-Trapped rats

effectively reduce most rat populations without the adverse consequence of increased risk for non-target species intoxication.

CONCLUSIONS

These data raise interesting questions regarding the significance of the warfarin resistance "problem" and how to effectively conduct efficacy tests for products designed to counteract warfarin resistance. Although many details remain to be clarified, these studies support the need to emphasize a non-chemical strategy for rodent control efforts. Environmental sanitation and rat proofing would go far to eliminate food and harborage resources and thus curb breeding activity—affecting all animals in the population as demonstrated decades ago by Davis (1950), Holloway (1947), Orgain and Schein (1953), and others. Elimination of the food alternatives would also increase bait acceptance whenever the chemical strategy is necessary. Under environmentally improved conditions, it should be possible to kill resistant animals in most localities with the standard anticoagulants (including warfarin) and adjusted baiting schedules, rather than switching to rodenticide baits which have a higher risk to humans, pets, livestock, and/or wildlife.

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