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ABSTRACT: Nearly half a century ago anticoagulant rodenticides changed the nature of rodent control. Warfarin, and succeeding first-generation compounds, provided effective and increasingly safe baits for reducing commensal rodent populations. Environmental deficiencies were overridden by these "miracle" chemicals, but excessive and irresponsible use selected for resistant populations. Second-generation compounds with a single-feeding characteristic have controlled such resistant populations, at least initially. But use extensions to crop and field areas have been held back by registration requirements, costs, and concerns over local effects on predators. New compounds, formulations, and applications in the near future are likely to be quite limited.

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INTRODUCTION

Compound 42, the first anticoagulant rodenticide, was field tested in the U.S. in the late 1940s. It was introduced commercially in the early 1950s as Warfarin (after the Wisconsin Alumni Research Foundation, which had sponsored the development research). Overnight it became the "miracle" rodenticide because of its relative safety (multiple-dose requirement), ease of use (grain baits and "toss" packs), and efficacy (lack of bait shyness).

In the course of time other coumarin and the similar indandione molecules were synthesized. The half-dozen technical products were the basis for a flourishing rodenticide market. These compounds (including chlorophacinine, coumafuryl, diphacinone, isovaleryl indandione, pindone) later were to be designated as first-generation anticoagulants. Less was heard of Compound 1080, zinc phosphide, arsenic, ANTU, strychnine, and thallium, though "old timers" continued to have their favorite (and "secret") formulas using these compounds; and such products did not disappear totally from the OTC market.

Despite the clear demonstration during this period in Baltimore by D.E. Davis (1952) and his associates at the Johns Hopkins University School of Hygiene & Public Health that environmental improvement and sanitation could effectively control outdoor rat populations, rodenticide use continued to be highly favored by those involved—residents and professionals alike. After all, dead rats were visible proof of efficacy, and forced environmental cleanup was not required. Environmental deficiencies could be over-ridden with a chemical "fix."

TOXICITY AND SAFETY

Incredibly small amounts of technical Warfarin are required for effective control. PCOs were accustomed to using 2% zinc phosphide baits, whereas anticoagulants are used at 0.025% or 0.005%. It was difficult for PCOs to fully appreciate the chemical characteristics of this new control tool.

Such formulation use ratios, however, do not appropriately represent relative toxicity. The LD50 statistic is utilized so that chemicals causing acute responses, such as zinc phosphide or 1080, could be compared readily. But when this statistic was determined for the anticoagulants as well, the potential for misuse was high. The acute (single) dose of Warfarin required to kill 50% of a test population of rats was calculated at around 200 mg/kg (Tables 1 and 2). This meant a massive dose was required to kill and that the compound would be regarded of low toxicity.

But wait! That's *not* how anticoagulants are used. Warfarin is fed to rats every day for a week. Then the animal dies from chronic (not acute) causes, such as internal bleeding. The secret is the small daily dose. With recalculation, the LD50 becomes about 2 mg/kg–0.3 mg/kg daily for a week (Table 2); but this then is a 5-day LD50 value (Ashton, Jackson and Peters 1986).

Furthermore, considerable variation exists among species and laboratory strains and between sexes. Generally, fe-

Table 1. Oral LD50 values (mg/kg) for Norway rats.[a]

	"ACUTE"[b]	5-day TEST[c]	
		daily dose	5-day dose
Chlorophacinone	20.5	0.19	0.95
Diphacinone	1.93–43.3	0.21	1.05
Pival	50.0	1.34	6.7
Warfarin	14.5–186	0.33	1.65

[a]Adapted from Ashton, Jackson, and Peters (1986).
[b]Values from literature; domestic rats.
[c]Sprague-Dawley rats (N=40; sexes combined).

Table 2. Acute oral LD50 doses (mg/kg) domestic Norway rat.[a]

Chlorophacinone	20.5
Diphacinone	30
Warfarin	14–323
Brodifacoum	0.22–0.27
Bromadiolone	1.1–1.8
Difenacoum	1.8
Difethialone	0.56
Flocoumafen	0.25–0.56

[a]From ICI (1990).

males are less susceptible than males, wild rats are less susceptible than domestic (laboratory) rats, and house mice are considerably more tolerant than Norway rats (Table 3). In our tests, Pival toxicity for rats deviated considerably from that seen for other products. While anticoagulants generally are effective on rodents (and other mammals) worldwide, care must be exercised in assuming that Norway rat data "fit" all species.

If just numbers are compared, Warfarin thus appears more toxic than Compound 1080 (LD50 = 5 mg/kg). It is the required 5-day sequential feeding period that minimizes hazards with the use of first-generation anticoagulants. All too often in text books and other reference documents the anticoagulant LD50 statistic is not labeled as a 5-day LD5o (or otherwise distinguished from an acute LD50). In fact, the range often is given (e.g., 2-200 mg/kg), which further confuses interpretation. The World Health Organization included Warfarin in a table of technical products classified as "highly hazardous" along with such compounds as sodium cyanide, strychnine, and endrin (WHO 1988). The uninitiated jump to the wrong conclusion relative to hazard. Numerous unnecessary crises have occurred—especially relative to waste bait transport and disposal—that should have been avoided.

This is not to say that first-generation anticoagulants are without use hazards. A predator feeding daily (or at least frequently) on rodents that have tissue residues or stomachs filled with toxic bait can be adversely affected. A single feeding by a predator on the bait itself or on an affected target species will not impact the predator—just as a single feeding will not impact the target species. However, canids do seem more sensitive to diphacinone than other predators, and other species differences have been noted.

Concern over the presence of anticoagulants in the human food chain, especially for pregnant women, prompted the Hawaiian sugar cane industry to discontinue use of these rodenticides in perimeter areas of Hawaiian sugar cane fields. The linkage was with wild pigs feeding on the bait placements and then being hunted and killed for food (Engeman and Pank 1984). Because of this action, zinc phosphide remains the only chemical tool for rodent control in sugar cane.

FORMULATIONS

Initial formulations were grain mixtures (often corn and oats) with a bit of vegetable oil and sugar. In time pellets were created, some using heat processing, others with simple compression. Wax formulations were developed, especially for humid environments. EPA prohibited loose baits in sewer systems due to threat of bait being flushed into waterways. Wax blocks could be restrained and were labeled for such uses.

Always at hand was the conflict between marketing a universal rodent bait or designing distinctive formulations for rats and mice. Smaller formulators tended to follow the unitary approach—but found the competition increasingly stiff. One distinct disadvantage of single products was the higher formulation concentration needed to kill mice, since their LD50 was four times that of rats. Other formulators were able to improve mouse acceptance (and presumably efficacy) with special mixtures of seeds and fine particles.

Packaged baits (whether pellet or loose grain) had the advantage of being an activity measure. Until the package seal was broken, the dry contents remained intact and palatable. A broken package evidenced new rodent activity and was cause for the PCO to increase surveillance.

Modifications then turned to solid bait configurations. The idea of designing baits with multiple gnawing edges to increase feeding caught on, but the difference between the dental apparatus of rats and mice had to be observed. Mice needed smaller ridges and pellet size. Some of these solid baits were designed to be quickly fastened in place to prevent their being dragged away. Attempts at innovation with texture and shape as well as with color (though rats are color blind), flavor and odor will continue.

EPA early on established efficacy criteria that had to be met for product registration. For anticoagulants it was 90% mortality and 33% consumption of the toxic bait in a 15-day choice feeding test.

This 33% consumption threshold has been maintained (except for wax block baits) despite test data indicating that 90% mortality occurs above 25% of the intake being of toxic

Table 3. Daily dose (5-day) oral LD_{50} (mg/kg).[a]

	Domestic[b]			Wild[b]		
	Male	Female	Combined	Male	Female	Combined
Norway Rat						
Chlorophacinone	0.18	0.20	0.19	0.13	0.23	0.16
Diphacinone	0.19	0.23	0.21	0.35	0.35	0.35
Pival	1.21	1.60	1.34	7.60	25.60	12.80
Warfarin	0.29	0.38	0.33	0.39	0.50	0.44
House Mice						
Chlorophacinone	0.38	3.48	1.19			
Diphacinone	0.42	2.83	1.41			
Pival	0.66	3.17	1.19			
Warfarin	0.87	8.00	2.20			

[a]From Ashton, Jackson, and Peters (1986).
[b]N=40; sexes equal.

bait in these laboratory tests (Palmateer and McCann 1976).

When the second-generation anticoagulants came along, some semantic and procedural problems occurred. These compounds killed anticoagulant-resistant rats and mice. (Did rodents proved resistant to *both* coumarin and indandione compounds have to be used in such efficacy tests? Yes.) These compounds acted like acutes, killing after a single feeding—but still some days later. Should the 3-day test for acute toxicants be used? (No.)

Recently EPA has proposed a 24-hour choice test for second-generation compounds that wish to make a single feeding claim (W. Jacobs, personal communication; OPP Designations 1.209 and 1.210, Revisions No. 10 and 9, respectively). We feel a 1-day feeding test should be no-choice, since efficacy is not at issue—only whether a single day's feeding provides a lethal does. Use of a 2-day choice feeding test to establish efficacy for these compounds seems superior to the 3-day (or 5-day) tests with an odd number of days that may result in position-biased test data.

Initially, the concept of "throw" packs was prompted as a fast way of distributing a "safe" rodenticide in rat-infested areas. Gradually the term "place" pack has come into play, giving the idea that even anticoagulants need to be placed in burrows, protected areas, or bait stations.

Bait stations, too, have been modified with some nudging from EPA. Hopefully, we are past trying to characterize bait stations with such absolute designations as "tamper-proof and are looking at probability related "tamper-resistant" criteria relative to material durability, compartment design, accessibility, stationability, and similar characteristics. Baits should be placed in locations *not easily attainable* by children, pets, nontarget wildlife, or domestic animals.

While rodenticides are widely used, their ingestion by nontarget species is very infrequent. For example, data from the National Poison Center Network (1978) indicated that only 0.63% of the more than 125,000 reported human exposures to toxic substances involved rodenticides.

Accidental poisoning of pets, similarly, is of concern. The Animal Poison Control Center (University of Illinois) logged more than 5,000 calls on its 24-hour hotline in 1982 (Buck 1983). About half involved dogs. Only 8% of the total calls concerned anticoagulant rodenticides. Various household products, chemicals, medications, and plants were of far greater concern.

Clearly rodenticides need to be used with care. Appropriate placement is essential. The pest control industry generally has demonstrated its ability to use these rodenticides safely.

RESISTANCE

Anticoagulant resistance (to Warfarin) was first documented in Norway rats from a pig sty near Glasgow, Scotland by Mary Boyle in 1958 (Boyle 1960). Soon thereafter this phenomenon was identified in other areas of the U.K. and then on the continent.

More than a decade later (1971) a North Carolina PCO reported that rats on his farm accounts were getting fat on Warfarin baits. Resistance had arrived in the U.S. (Jackson, Spear and Wright 1971; Jackson and Kaukeinen 1972).

Even so, first-generation anticoagulants dominated the scene for PCOs, government programs, and householders. But inevitably, Warfarin resistance was determined to be cross-resistant, that is, resistance to Warfarin also was resistance to the other first-generation (both coumarin and indandione) compounds.

What Brought on Resistance?

Anticoagulant resistance is inherited; it does not result from repeated exposures to low doses. In control programs with anticoagulants, rats that can eat the baits and survive (i.e., are resistant) have an evolutionary advantage: being parents of the next generation and passing resistant genes to at least some of their offspring.

Warfarin resistance appears monogenic and dominant, being mediated by alterations in several vitamin K metabolizing enzymes that in turn reduce availability of blood clotting factors (MacNicoll 1986,1988). Although monogenic, many alleles may occur. For example, our Chicago (and other U.S.) strains appear less dependent on supplementary dietary vitamin K than the Welsh strain and may be more like the Scottish rats. However, virtually all the U.S. laboratory research has been done with the Welsh allele transferred into laboratory rats; and little attention has been paid to resistant wild Norway rats in the U.S. The relatively recent presence of difenacoum resistance in southern England provides support of the polygenic hypothesis for resistance to second-generation compounds (Greaves and Cullen-Ayres 1988).

Interestingly, anticoagulant resistance in England was largely a rural phenomenon; in the U.S., largely urban. Why?

The basic conditions that facilitate selection for resistance appear to include abundant food, harborage, and persistent (but perhaps inefficient) use of anticoagulant baits. In the U.K., government workers provided pest control services to farmers on a regular basis; in the U.S., it was urban government sanitation workers and some PCOs that did so.

My thesis is that the regular use of Warfarin (and other anticoagulant) baits killed off the susceptible rats (those without the resistant gene). The breeding population by this selection process increased its proportion of resistant rats. Since the environmental conditions usually were not altered, many rats remained isolated from control efforts but were readily available for breeding—and the passing of the resistant gene.

In the 1970s urban rat control became a politically correct activity, and some $15 million in federal funds were allocated annually to states and cities for urban rat control programs. This lasted for more than a decade.

These efforts often facilitated the selection of resistant rats by frequent rodenticide applications and lack of insistence on environmental improvements. Laboratories at Troy (NY) and Bowling Green (OH) were utilized to monitor the resistance problem, and through their joint efforts resistance in Norway rats and/or roof rats was identified in 50 cities (Jackson et al. 1985; Jackson and Ashton 1986). Chicago was perhaps the most famous with more than two-thirds of the populations in target areas being resistant (Ashton and Jackson 1979).

It was evident from PCO reports that house mice also were being selected for resistance in control programs. However, the federal funds supporting the urban *rat* control programs could not be used for surveillance on mice. Consequently, our data are limited to a few sites (Ashton and Jackson 1984). However, our assumption that resistance of house mice to anticoagulants is widespread (as has been demonstrated in Europe) is supported by service records of PCOs.

SECOND-GENERATION ANTICOAGULANTS

Recognition that resistance was widespread as well as the expanding consumer markets provided stimuli for industry's synthesis and development of additional anticoagulant-type compounds which would be effective against resistant populations. These became known as second-generation anticoagulants (Marsh, Howard and Jackson 1980). In addition, they became characterized as single-feeding rodenticides — a single feeding could provide a lethal dose, but the animal would not stop feeding nor would it die until 5+ days later with characteristic internal hemorrhages.

This single-feeding characteristic allowed the introduction of "pulsed baiting" as a control strategy (Dubock 1982). This could be very useful for the PCO but often was not understood. Baits could be placed at intervals (weekly or otherwise) with the knowledge that those animals that fed would die; subsequent bait placements similarly would kill remaining animals. This strategy accounted for dominance and territorial behavior in affected populations as well as population shifts during control programs. Increased operational efficiency was enjoyed, and reduced quantities of toxic baits had to be positioned in the environment, since continuous feeding during the baiting period no longer had to be guaranteed (as with the first-generation compounds).

Veterinarians had to learn that accidental poisoning with the new second-generation compounds was not the same as with Warfarin. The half-life of these new compounds was several months rather than several weeks. While administration of vitamin K was still the indicated therapy and transfusions might be required, continued monitoring—sometimes for weeks—of the animal was necessary (Batten 1985, DuValletal. 1989).

In addition, proposed broadcast field uses posed some problems. While voles in orchards and plantations could be readily controlled with these compounds, avian predators feeding intensively in such treated areas could be adversely affected by the residues in the target rodents (Hegdal and Colvin 1988). The question was whether predator mortality, limited geographically to these specific crop sites, could be tolerated when the larger area predator population was not significantly impacted. So far none of these compounds has been registered for area use, which suggests the attitude of regulatory groups. Any efforts to counter such concerns require population rather than laboratory studies, large teams of skilled personnel, and extensive support—which have not been available in recent years for minor-use materials (Kaukeinen 1982).

One striking benefit from these preliminary studies was documentation that barn owls selectively foraged for voles in distant grassland and marsh areas and did not feed on farmstead rodents, even though the owls themselves roosted and nested in barns and silos. Consequently, rodenticide control of rodents in and around farm buildings would not impact these owls (Hegdal, Colvin and Blaskiewicz 1984). This allowed ICI to retain "in and around farm building" use instructions on their label, whereas other baits were restricted for use "within" farm buildings.

THE FUTURE

In 1990, 70 companies held registrations for 176 first-generation formulations (technical and bait); ten companies had registered 49 second-generation formulations. However, the EPA reregistration process will exact its toll. Warfarin, chlorophacinone, and diphacinone likely will be the only first-generation anticoagulant survivors. General label statements will restrict uses to in and around buildings. Continued use of chlorophacinone for winter vole control in orchards will be requested by LiphaTech. Expansions of other labels are unlikely because of costs unless a realistic risk/benefit climate prevails.

Bitrex as a bait additive to prevent (by its bitter taste) accidental ingestion, already in use by ICI and J.T. Eaton in some of their formulations, may have popular appeal. Encapsulation of the technical material, already tried without much success, probably will not be worth the cost for anticoagulants in the future. Antibiotic additives (to inhibit manufacture of vitamin K by bacteria in the gut) similarly have little support.

Resistance, once established in a population, does not seem to decrease significantly with time, even when anticoagulants no longer are employed. We established that high levels (67%) of first-generation anticoagulant resistance were present in the Chicago target areas in the mid-1970s. A decade later we again tested animals from one of these populations and found about 85% resistant. (City workers and residents had been instructed not to use first-generation anticoagulants, but the ban may not have been wholly effective. Possibly the use of second-generation compounds maintained some selection pressure in the interim.) We don't know if such experience is representative of other resistance sites in the U.S.

Resistance already has been documented in the U.K. for bromadiolone and difenacoum (the latter not being available in the U.S.). Initial reports from Canada suggest bromadiolone resistance there as well (Siddiqi and Blaine 1982), but no reports of resistant populations in the U.S. have surfaced. This may be simply a matter of not having looked carefully enough or conducted appropriate tests. So far, brodifacoum (Talon) has not been tarnished by reports of resistant populations anywhere in North America.

New second-generation anticoagulants may emerge from the EPA labyrinth. Field testing for difethialone has been completed by LiphaTech (Lechevin and Poche, 1988). Flocoumafen is marketed in Europe but is not likely to see registration in the U.S. These are both similar to the existing second-generation compounds. Appropriate alternation of anticoagulants with non-anticoagulants by PCOs and sanitarians will go a long way toward blocking the selection for populations resistant to second-generation compounds.

Environmental uses for rodenticides will have increasing demands for orchards, no-till cropping schemes, and tropical row and tree crops. Uses of anticoagulants in these environments which minimize nontarget species hazard will require careful formulation, placement, and timing. Unfortunately, virtually no corporate resources are being dedicated to field research in support of such new registrations.

Current rodenticides, especially the remaining anticoagulants, will continue to have wide use because of their relative safety, efficacy, and general utility; but there are likely to be fewer allowable applications due to the high costs of data development. Agricultural needs (especially outside the continental U.S.) will increase. U.S. AID-related assistance, however, will have difficulty in participating because compa-

rable rodenticide uses (and labels) do not exist in the U.S., and foreign uses are thereby limited for AID programs. Urban demands everywhere will continue, especially in the absence of coordinated environmental sanitation programs (that is, integrated pest management).

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