RECENT DEVELOPMENTS IN ANTICOAGULANT RODENTICIDE RESISTANCE STUDIES: SURVEILLANCE AND APPLICATION IN THE UNITED STATES

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ABSTRACT: Since anticoagulant rodenticide resistance was first discovered in the United States in 1971, it has become apparent that the phenomenon is widespread. In cooperation with the Center for Disease Control, a nationwide surveillance program was initiated in 1977 to obtain statistically valid samples of rats from federally funded projects of the Urban Rat Control Program. A summary is given of the basic sampling, testing, and analysis components of this study. Problems encountered in all aspects of the first three years of the program are discussed along with results from the 40 completed samples. The 16 cities with significant Anticoagulant Resistance Problem Areas are distinguished from those in which resistance has merely been observed. Levels of resistance in various rat populations are discussed and recommendations are made in support of integrated pest management programs. Recent findings from retesting resistant rats, half of which die, are presented with regard to application of the surveillance program.

INTRODUCTION

Since resistance to anticoagulant rodenticides in the United States was first discovered in Norway rats (Rattus norvegicus) in rural North Carolina in 1971 (Jackson et al. 1971), it has become clear that the phenomenon is widespread in both urban and rural rodents and in all three major domestic rodent species (Brooks and Rowe 1979). Anticoagulant resistance in Norway rats and house mice (Mus musculus) is apparently due to an autosomal gene with dominant effect while in roof rats (Rattus rattus), a multifactorial basis has been indicated (Greaves and Ayres 1976, Greaves, et al. 1976, Wallace and MacSwiney 1976).

In resistant animals, an enzymatic alteration occurs which reduces the normal antagonist effect of anticoagulants on vitamin K, thus preventing the usual hemorrhage and death. However, resistant animals are abnormally sensitive to vitamin K deficiency. Compared with susceptible Norway rats, heterozygous resistant animals have been reported to require 2-3 times as much vitamin K and homozygotes may need nearly 20 times as much just to survive when on a vitamin K deficient diet (Greaves et al. 1977).

Numerous papers concerning anticoagulant resistance in urban areas are available but most of the data indicate only that resistance was present or absent without clear statistical limits regarding the populations under scrutiny. The lack of statistically based sampling procedures has somewhat limited the generalizations that could be made from previous reports; however, a wealth of conceptual and programmatic information has been generated and much of this has been adapted for our current surveillance program.

An initial nationwide program was established in 1972 for surveillance of anticoagulant rodenticide resistance in urban rat populations (Brooks and Bowerman 1974, WHO 1970); the program was revised in 1977 to improve the sampling procedures and the validity of test results (Frantz 1977 and 1979). In essence, this is a monitoring program for the 63 federally funded projects of the Urban Rat Control Program in the U.S. and Puerto Rico. For sampling purposes, there are more than 63 sampling units because large projects have physically distinct areas, each of which should be sampled.

The main program objectives are to identify and maintain baseline data regarding the level and distribution of anticoagulant resistance in urban rat populations, and to assist the Center for Disease Control (CDC), U.S. Public Health Service, Atlanta, in making recommendations for more effective rodent pest management when significant resistance problems are encountered. Until June 1979, the Environmental Studies Center at Bowling Green State University (Bowling Green, OH) was also involved in this program; their data are included in this report.

METHODS AND MATERIALS

Selection of Participants

Projects to be sampled are selected quarterly by the CDC in consultation with the Rodent Control Evaluation Laboratory (RCEL). Highest priority is given to projects which have recently been initiated, have not impacted on existing rat problems, or have a history of resistance problems (Envir. Health Serv. Div., Envir. Studies Cent., and Rodent Control Eval. Lab., 1978). Participants are to be notified by their Regional Office at least 3 months prior to the initiation of the sampling procedure in order that all preparations can be made to complete the study in accordance with the collecting protocol.

Collection of Sample

Prior to actually trapping rats, a detailed map of the area to be sampled (sampling unit) must be submitted to RCEL for verification of its conformity to sampling standards (readability, number and location of blocks to be sampled, etc.), and to be divided into subunits to enhance distribution of the trapping effort. Sixty-four rats are requested from each participating project; the sample should

be collected within a 3-month period (Frantz 1977 and 1979). Considering the life-span of wild rats and the fact that one field-worker should be able to tend 30-50 traps per day, the 3-month criterion is both biologically and pragmatically reasonable.

Rats are shipped in their traps, packed into corrugated paper cartons, via air freight. This method is simple and economical and casualties are infrequent when packing specifications (Frantz 1977) are carefully observed. Most shipping problems have involved time delays due to strikes, bad weather and inadequate attention by the air freight carriers.

Acclimatization to Lab

Rats arriving at RCEL are acclimatized to laboratory/cage conditions for a minimum of 3 weeks during which time food and water are provided ad libitum (Fig. 1). For two weeks rats receive a basal diet of laboratory pellets (Wayne Lab Blox*, Allied Mills, Inc., Libertyville, IL) which contains added vitamin K. The overall vitamin K activity of Lab Blox is unknown, but its use here may enhance the homogeneity of the sample by minimizing variations in vitamin K status. This is a real concern (Drummond and Wilson 1968) since many of the rats arrive from areas with ongoing anticoagulant poisoning campaigns.

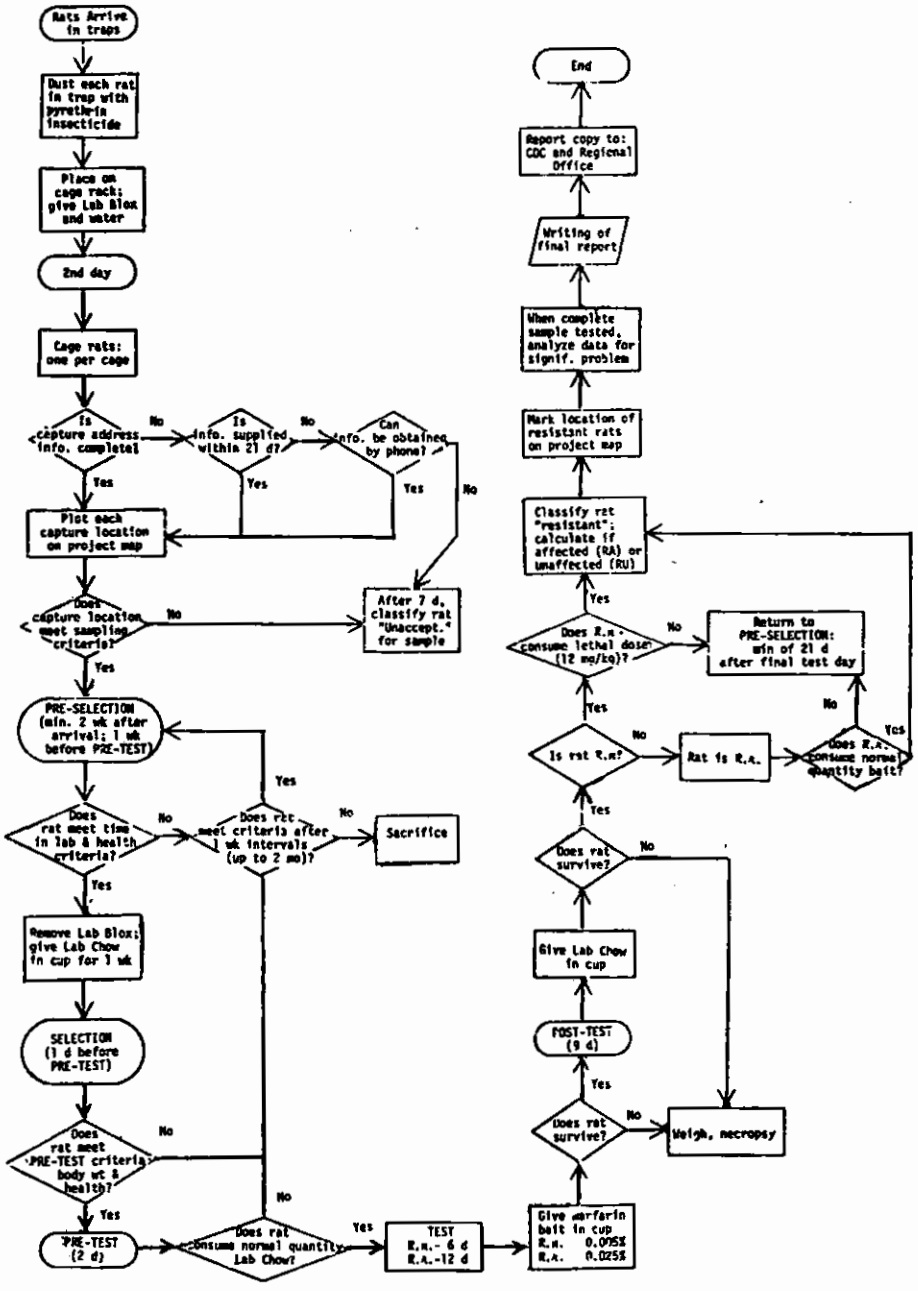

Fig. 1. Basic lab procedure for anticoagulant rodenticide resistance study.

*The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service, the U.S. Dept. of Health and Human Services, or the New York State Dept. of Health.

For the third week, animals receive lab meal (Purina 5001 Lab Chow*, Ralston Purina Co., St. Louis, MO), containing no added vitamin K. Lab meal is later used in food cups as the pre-test food and then as a base for the anticoagulant test bait. Throughout our studies, rats are housed individually in suspended cages with wire mesh bottoms which allow feces to fall through; this minimizes coprophagy which can supply vitamin K to the rat (Mameesh and Johnson 1959, Greaves and Ayres 1973).

Selection of Sample Rats

Each incoming rat is assigned, in numerical order, an accession (ID) number which is marked on a project's map. If an animal meets all sampling criteria (Frantz 1979), it is classified "acceptable" and so indicated on the map. If an acceptable rat dies before testing, the project map is examined to see if a previously classified "unacceptable" rat from the same basic location can be reclassified to "acceptable". If more than one animal could be accepted, priority is given to that animal with the lowest number indicating its longer period of residence in the lab. For this reason, we test all unacceptable rats with complete address information. When necessary, we request additional animals from a project in order to improve the distribution of the sample.

Project Reminders

In order to encourage projects to submit a sufficient number of rats within the proper time period, we have developed three types of reminder documents (copies are available upon request). An Acknowledgement Report is sent to a project to acknowledge each shipment of rats, review any problems with the shipment and review the current status of a sample. Whenever a designated project fails to submit rats for any two-week period, we notify the CDC with a Two-Week Notification so that they may investigate. A Sample Update Memo is sent to a project whenever it completes half of the sample; this usually includes a project map showing locations of acceptable rats and indicates areas which need to be trapped in order to give a properly distributed sample.

Pre-Test Procedure

The pre-test, test and post-test procedures and criteria are similar to the standard WHO procedure (WHO 1970) with modifications as given in Jackson et al. (1975) and Frantz (1979). For the pre-test, lab meal is offered for 2 days in food cups with consumption measured daily; $\geq$1.0 g spillage is accounted for in these calculations. Rats not consuming normal quantities during pre-test are removed from a test and used at a later date (Fig. 1).

Test Procedure

The warfarin we use in our bait formulation is provided as a purified concentrate in cornstarch from Raltech Scientific Institute (formerly the Wisconsin Alumni Research Foundation [WARF]), Madison, WI; this material is diluted to the appropriate concentration (by weight) with lab meal. Norway rats are offered water and 0.005% warfarin bait for 6 days, ad libitum, no choice (roof rats are offered 0.025% bait for 12 days). Daily, bait consumption is measured and signs of anticoagulant intoxication (sluggishness and/or bleeding) and mortality are recorded. All dead animals are weighed and then necropsied to confirm anticoagulant effects (Fig. 1).

Post-Test Procedure

For 9 days following the last feeding on warfarin bait, surviving rats are given only lab meal in cups; daily consumption measurements and observations continue as in the test period. Rats surviving the 9 days are weighed, returned to the diet of lab pellets, and set aside for future work. The warfarin dosage consumed is computed using initial body weight; final weight is used as an indicator of illness or refusal of food during the study.

Resistance Criteria

The chance that a normal Norway rat will survive the 6-day test (Drummond and Wilson 1968), the 9-day post-test, and a total warfarin dosage of 12 mg/kg is quite small (Brooks and Bowerman 1973 and 1974); animals meeting or exceeding these limits are classified resistant. Though this definition has been used for years at RCEL and at the Center for Environmental Studies, it is stricter than in other studies including those of the U.S. Environmental Protection Agency (Palmateer, pers. comm.). A roof rat is classified resistant if it survives the test and post-test periods with normal bait consumption (Jackson et al. 1975).

In addition to resistant Norway rats are those which survive the test and post-test periods, but have not consumed a warfarin dosage of $\geq$ 12 mg/kg. These animals, called poor feeders, are held for at least 21 days from the last day of toxicant feeding and then returned to pre-selection (see Fig. 1), whereas many investigators would classify such animals as resistant.

Types of Resistance

Resistant Norway rats can be divided into two groups: resistant affected (RA) and resistant unaffected (RU). Resistant affected rats are those in which their food intake dropped for 2 or more days, during test days 3-6 and/or post-test days 1-9, to 75% or less of the average intake for test days 1 and 2. Resistant unaffected rats do not exhibit the depression of food intake as described

for RA animals. Other investigators have found distinctions among resistant Norway rats (Drummond and Wilson 1968, Lund 1966) and some criteria for divisions have been proposed (Jackson et al. 1975); however, the categories described here tend to be more conservative than others.

Statistical Analysis Procedures

Results of the testing procedure are used to determine if there is statistical evidence at the 0.10 level that the observed proportion of resistance is significantly greater than 0.05 (Frantz 1979). Up to 5.0% resistance is accepted as the normal percentage of resistance for urban rat populations in the U.S. and Puerto Rico (Brooks and Bowerman 1975, Envir. Health Serv. Div. et al. 1978) and no further analysis is indicated. Although the critical percentage for statistical significance varies somewhat with the sample size, an observed rate of resistance of 10% or more in a sample of 64 rats "flags" the sampling unit as an Anticoagulant Resistance Problem Area (ARPA).

Secondly, a Chi-square test is used to determine whether or not the observed level of resistance is the same in all subunits of the sampled area. In a gross sense, this identifies whether the resistance problem is localized (confined to several nearby blocks) or generalized (involving many blocks; encompassing much or all of the sampling unit).

Lastly, if significant differences are found between the resistant levels in the subunits, a modified Chi-square test can be used to determine which subunit(s) of the study area differ. This modification is based upon partitioning of the subunits into groups and is planned ahead (Fleiss 1973). The decision on which subunits should be combined will be suggested by the observed proportions in each of the subunits. Greater observed differences are required to achieve significant results and, in this sense, this is a relatively conservative test (Therriault, pers. comm.). The details of this and other statistical procedures used in the surveillance program will appear in a separate publication.

Results of the statistical analyses allow us to more precisely focus appropriate pest management strategies on that portion of the ARPA with the greatest problem. In conjunction with this, we indicate on the project map which rats proved to be resistant. This map accompanies the summary analysis and final report which is submitted to CDC. The CDC notifies each project of the study results by memorandum through the appropriate Regional Office of the U.S. Department of Health and Human Resources.

RESULTS AND DISCUSSION

Sample Submission Time

In order to overcome some initial confusion and communication problems, extra time was allowed for the first group of projects selected to submit samples. This leniency resulted in some excessive times as shown in Table 1 (see: Initial Start). As we gained more experience, projects were vigorously encouraged to submit complete samples within the 90 days mandated in the collecting protocol.

The average times for projects completed in 1977, 1978 and 1979 were 181.3, 163.9, and 125.2 days respectively (refer to Tables 1 and 2). Our use of the detailed Acknowledgement Report, Two-Week Notification and Sample Update Memo has helped to shorten the submission times.

Of the 56 samples designated to be collected since April 1977 (through those completed testing as of 31 March 1980), 16 (28.6%) were discontinued. This occured mainly because too few rats were submitted within a reasonable time. For example, if only 25 rats were submitted by the end of the eleventh week, it would be unlikely that the sample could be completed after another week, and the projects' sampling work should be discontinued.

Five samples have been discontinued then restarted after a short time interval which accounts for some of the excessive "total project involvement" times reported in Table 1. Note that this figure includes any lag period from when a project was officially designated to begin sampling until the first acceptable rat was received; it also includes the time during which rats were being submitted, both "initial" and "restart". For example, of the 490 days listed for Hartford, CT, 120 days ("restart") were required for submission of the sample we actually studied; 337 days ("initial") were required for their first attempt at sampling; and, by subtraction, a lag period of 33 days preceded their submission of the first acceptable rat.

We are concerned with total project involvement times because the sampling work should fit conveniently into a projects' overall workload. A project notified 90 days prior to the expected start date should be able to complete the sample within 90 days; hence, the "total project involvement time" (as reported in Table 1) would be 90 days as described in the policies and procedures manual (Envir. Health Serv. Div. et al. 1978). Often, neither of these time intervals is observed and virtually all submission times have exceeded 90 days. The bulk of the delays is related to: project personnel misinterpreting the collecting protocol; lack of communication between supervisors and field staff; and inefficient trapping methods. At any rate, the "total involvement" times of Table 1 do reflect a need for closer interagency cooperation.

Laboratory Study Time

If all rats of a sample were received and tested as a group under the best of circumstances, a minimum of 38 days would be required for Norway rats and 44 days for roof rats (Fig. 1). RCEL's average time for when "last rat received until last rat tested" is 61.5 days (Table 1). Some extra

Table 1. Time involvement summary for anticoagulant rodenticide resistance surveillance program[1] (for studies finished: April 1977-March 1980).

Federal Region	State:Project	Sample Submission Time (no. days)			Laboratory Time (no. days)	
		Initial start actual[2]	Restart actual[2]	Total proj. involvement time[3]	Last rat rec'd. until last rat tested	Last rat tested until analysis sent to CDC
I	CT: Hartford	(337)	120	490	49	139
	MA: Boston	(99)	123	288	67	116
II	NJ: Camden	175	--	221	66	5
	Jersey City	122	--	124	40	13
	NY: NYC-Bushwick	189	--	229	66	24
	NYC-Lower East Side	214	--	247	51	4
	Rochester	143	--	161	56	5
	PR: Mayaguez	127	--	211	80	53
	Ponce	--	--	Discontinued	--	--
	San Juan	315	--	391	58	165
III	DC: Washington	225	--	285	40	15
	MD: Baltimore	(266)	168	475	72	23
	PA: Chester	323	--	390	60	228
	Clairton	125	--	158	46	30
	Harrisburg	(86)	161	286	42	33
	Philadelphia	133	--	157	79	6
	VA: Chesapeake	180	--	325	50	9
	Portsmouth	95	--	147	97	242
IV	AL: Mobile	(313)	204	569	69	2
	FL: Ft. Lauderdale	76	--	87	18	21
	Miami	160	--	200	52	3
	Pensacola	86	--	126	54	23
	Pensacola (Roofs)[1]	--	--	Discontinued	--	--
	Tampa (Roofs)	102	--	320	49	1
	GA: DeKalb County	140	--	194	37	171
	KY: Louisville[4]	381	--	421	NA	222
	TN: Memphis	121	--	121	67	206
V	IL: Chicago-Austin	26	--	73	122	0
	Chicago-Englewood	23	--	30	71	21
	Chicago-Austin[4]	148	--	166	NA	NA
	Chicago-Englewood[4]	127	--	166	NA	NA
	IN: Gary[4]	--	--	Discontinued	--	--
	Indianapolis[4]	--	--	Discontinued	--	--
	MI: Benton Harbor	133	--	170	65	47
	Detroit[4]	201	--	208	NA	41
	Saginaw	--	--	Discontinued	--	--
	OH: Cincinnati[4]	240	--	278	NA	36
	Cleveland[4]	183	--	231	NA	111
	Columbus[4]	28	--	41	NA	145
VI	AR: Pine Bluff[4]	--	--	Discontinued	--	--
	Pine Bluff (Roofs)[4]	--	--	Discontinued	--	--
	LA: New Orleans[4]	--	--	Discontinued	--	--
	New Orleans (Roofs)[4]	--	--	Discontinued	--	--
	TX: Houston (6 TA's)	127	--	139	66	68
	Houston (CT 503)	--	--	Discontinued	--	--
	Houston[4]	--	--	Discontinued	--	--
	Houston (Roofs)-6 TA's	--	--	Discontinued	--	--
	Houston (Roofs)[4]	--	--	Discontinued	--	--
VII	MO: Kansas City	--	--	Discontinued	--	--
	St. Louis	63	--	69	49	5
	St. Louis[4]	125	--	165	NA	295
	NE: Omaha	105	--	178	107	5
IX	CA: Los Angeles[4]	108	--	158	NA	188
	Ontario (Roofs)[4]	--	--	Discontinued	--	--
	San Francisco[4]	--	--	Discontinued	--	--
	West Oakland[4]	238	--	347	NA	257

1 = Samples are of Norway rats, R. *norvegicus*, except when noted Roofs, R. *rattus*, after the project name
2 = Number days from first acceptable rat to date last acceptable rat received in lab
3 = Number days from designated initial start to date last acceptable rat received in lab (excluding time before restart)
4 = Analysis by Environmental Studies Center, Bowling Green State University, Bowling Green, OH
NA = Data not available

time is required, of course, for administrative matters, but the main delays can be attributed to: rats that must be kept several weeks to meet test weight criteria; and poor feeder Norway rats that have completed all test procedures and must be held another 21 days before returning to pre-selection (see Fig. 1).

The statistical analyses of test results should be completed in a perfunctory fashion and submitted to CDC so that countermeasures might be initiated. Nearly all samples (tested at RCEL) in Table 1 with analysis times exceeding 53 days are those in which test results were available before all analysis procedures were developed. Excluding this group, RCEL's average time for "last rat tested until analysis sent to CDC" is 18 days and well within the 30-day mandate (Envir. Health Serv. Div. et al. 1978). The average "turn-around time" for RCEL's "in-lab" portion of the study (from when last rat received until analysis sent to CDC; excluding the initial group as above) is 79.4 days.

Rats Submitted for Sample

The percentage of rats that are "acceptable" out of all rats submitted may indirectly reflect how well project personnel have interpreted and adhered to the collecting protocol. For the 40

Table 2. Sample results for anticoagulant rodenticide resistance surveillance program[1] (for studies finished: April 1977-March 1980).

Federal Region	State:Project	Number rats		Percent rats		Significant problem[2]	Year completed
		Rec'd. alive	Acceptable	Acceptable	Resistant		
I	CT: Hartford	73	66	90.4	15.2	Yes	1978
	MA: Boston	80	56	70.0	8.9	Yes	1978
II	NJ: Camden	55	53	96.4	0.0	No	1979
	Jersey City	101	77	76.2	2.6	No	1979
	NY: NYC-Bushwick	81	53	74.6	0.0	No	1978
	NYC-Lower East Side	107	70	65.4	2.8	No	1979
	Rochester	72	61	84.7	1.6	No	1979
	PR: Mayaguez	91	65	71.4	15.4	Yes	1979
	Ponce	39	28	--	--	--	Discontinued
	San Juan	67	61	91.0	18.0	Yes	1978
III	DC: Washington	102	70	68.6	5.7	No	1978
	MD: Baltimore	75	55	73.3	9.1	Yes	1978
	PA: Chester	82	59	72.0	10.2	Yes	1978
	Clairton	90	65	72.2	20.0	Yes	1978
	Harrisburg	83	66	79.5	4.5	No	1979
	Philadelphia	112	61	54.5	0.0	No	1979
	VA: Chesapeake	81	70	86.4	7.1	No	1978
	Portsmouth	71	56	78.9	25.0	Yes	1978
IV	AL: Mobile	87	77	88.5	2.6	No	1978
	FL: Ft. Lauderdale	67	64	95.5	0.0	No	1978
	Miami	78	75	96.2	1.3	No	1977
	Pensacola	84	68	81.0	0.0	No	1979
	Pensacola (Roofs)	22	22	--	--	--	Discontinued
	Tampa (Roofs)	99	64	64.6	1.6	No	1978
	GA: DeKalb County	79	64	81.0	10.9	Yes	1978
	KY: Louisville[3]	93	64	68.8	10.9	Yes	1978
	TN: Memphis	60	56	93.3	10.7	Yes	1978
V	IL: Chicago-Austin	74	63	85.1	76.2	Yes	1979
	Chicago-Englewood	62	62	100.0	59.7	Yes	1979
	Chicago-Austin[3]	93	62	66.7	71.0	Yes	1979
	Chicago-Englewood[3]	89	69	77.5	43.5	Yes	1978
	IN: Gary[3]	66	40	--	--	--	Discontinued
	Indianapolis[3]	11	8	--	--	--	Discontinued
	MI: Benton Harbor	79	59	74.7	8.5	Yes	1979
	Detroit[3]	125	71	56.8	1.0	No	1977
	Saginaw	21	10	--	--	--	Discontinued
	OH: Cincinnatti[3]	91	52	57.1	0.0	No	1979
	Cleveland[3]	120	60	50.0	10.0	Yes	1977
	Columbus[3]	124	71	57.2	12.7	Yes	1978
VI	AR: Pine Bluff[3]	63	25	--	--	--	Discontinued
	Pine Bluff (Roofs)[3]	11	0	--	--	--	Discontinued
	LA: New Orleans[3]	41	18	43.9	--	--	Discontinued
	New Orleans (Roofs)[3]	38	26	68.4	--	--	Discontinued
	TX: Houston (6 TA's)	102	64	62.7	14.1	Yes	1979
	Houston (CT 503)	44	30	--	--	--	Discontinued
	Houston[3]	54	34	--	--	--	Discontinued
	Houston (Roofs)-6 TA's	12	12	--	--	--	Discontinued
	Houston (Roofs)[3]	20	13	--	--	--	Discontinued
VII	MO: Kansas City	3	2	--	--	--	Discontinued
	St. Louis	83	63	75.9	6.3	No	1979
	St. Louis[3]	80	61	76.2	0.0	No	1978
	NE: Omaha	81	56	69.1	7.1	No	1979
IX	CA: Los Angeles[3]	81	63	77.8	1.3	No	1978
	Ontario (Roofs)[3]	55	16	--	--	--	Discontinued
	San Francisco[3]	35	10	--	--	--	Discontinued
	West Oakland [3]	99	52	52.5	5.8	No	1978

[1] = Samples are of Norway rats, *R. norvegicus*, except when noted Roofs, *R. rattus*, after the project name
[2] = Statistical evidence at 0.10 level that proportion of resistance in population significantly exceeds 0.05 (Frantz, 1979)
[3] = Analysis by Environmental Studies Center, Bowling Green State University, Bowling Green, OH
[4] = Statistical borderline; consider as significant problem

samples completed, an average of 73.1% of the rats received alive were acceptable for the samples (Table 2). Major reasons for not accepting rats are: too many rats trapped in a block; rats trapped in adjacent blocks; poor distribution of trapping effort; and incorrect/incomplete capture address. Many of these difficulties would be reduced if more careful attention were given to the sampling guidelines; we openly encourage projects to contact us directly should any problems arise with their sampling work.

Resistance Identified

Anticoagulant rodenticide resistance was found in at least one animal in 33 of the 40 samples (Table 2). However, the fact that a project does or does not have resistant individuals is of little consequence. Our interest is in those samples with statistical evidence that the percentage of resistance in the population significantly ($p \leq 0.10$) exceeds 5.0% (Frantz 1979). Such evidence was found in 48.7% (19/39) of the acceptable Norway rat samples from 16 cities in 12 states and Puerto Rico. Only one sample of roof rats has been completed thus far (Tampa, FL) and the level of resistance was insignificant. In all cases, the resistance was generalized throughout much of the sampling unit studied; no significant localized resistance has been identified. Figure 2 shows the geographical distribution of the latest study results, a distinction is made between those cities with an Anticoagulant Resistance Problem Area (ARPA) and those without.

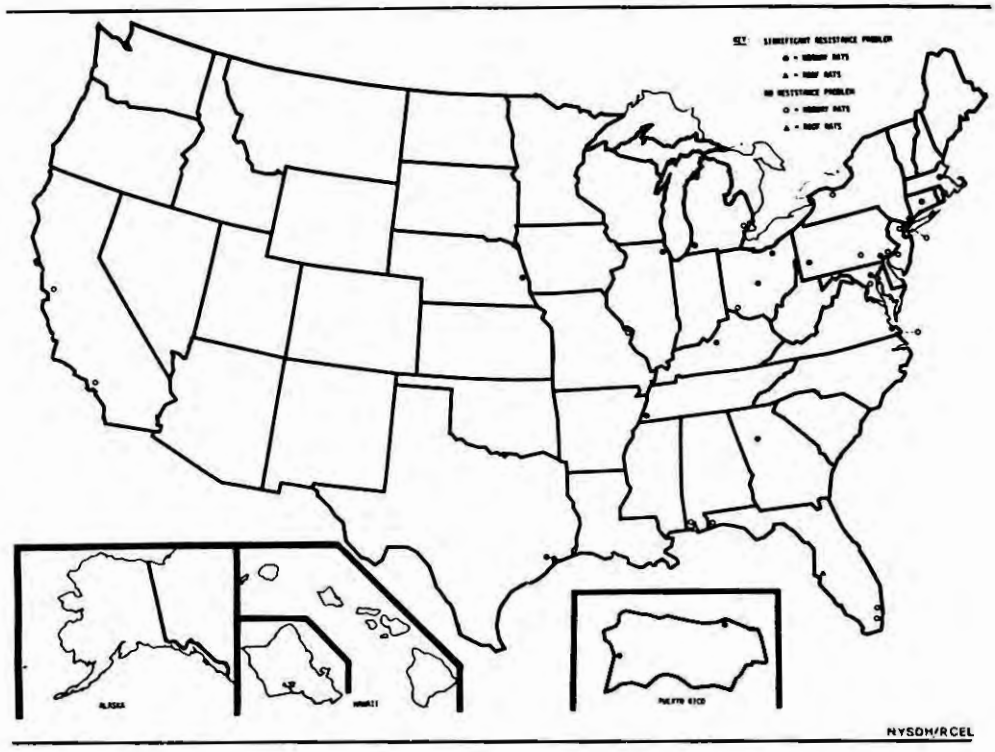

Fig. 2. Geographic distribution of anticoagulant rodenticide resistance identified in sampled projects of the federally funded Urban Rat Control Program: the United States and Puerto Rico (April 1977-March 1980).

Only Chicago has submitted two samples from the same sampling units in order for a comparison to be made over time (Table 2). The 1978 samples from Austin and Englewood showed 71.0% and 43.5% resistance respectively; these levels increased in the 1979 samples to 76.2% and 59.7%. These resistance levels are the highest reported for the entire surveillance program; and, of the resistant rats, the proportion of resistant unaffected animals (77.1% for Austin and 64.9% for Englewood in 1979) exceeds that for all other samples (usually we find only one or two RU animals per sample). In addition, the 1979 Englewood sample showed a significant ($\chi^2 = 3.43$: $.05<P<.10$) increase in the proportion of resistance in the population.

The Chicago results are of particular concern because countermeasures emphasizing the application of acute rodenticides were initiated at least a year ago. Considering our latest data, it appears that the chemical strategies employed in Chicago deserve reconsideration and new impetus is necessary for intensively and promptly implementing other management practices such as community participation, improved sanitation, and rat stoppage.

Retest Phenomenon

Knowing that some projects will continue to use anticoagulant rodenticides even when resistance has been identified in their particular rat populations, we have begun to explore the consequences of a resistant Norway rat's reexposure to warfarin bait. Details of this work will be published at a later date; but, in essence, resistant rats are retested in the same manner as the standard no-choice test after a predetermined period of time has elapsed since their previous exposure to warfarin. Currently, the bulk of our data is for animals retested 30-59 days after their initial test. In a group of 53 resistant (affected and unaffected) Norway rats, 27 (50.9%) died when re-exposed to warfarin bait at the same concentration to which they were originally classified as resistant. Twenty-two (41.5%) of the group were once again resistant and four (7.5%) were poor feeders. The "resistant again" animals were retested at 30-day intervals until all died by the end of the fourth retest.

Thus, resistant Norway rats may eventually succumb even to warfarin bait when a recovery period is allowed between exposures. This is a different situation from that reported by Lund (1972) and Jackson, et al. (1975) in which rats survived and reproduced for several months when on a continuous diet of only warfarin bait. A particularly disturbing aspect of the retest findings is that we must now question the significance of claims that a particular product "will kill resistant rats". As suggested by Drummond and Wilson (1968), and earlier by Lund (1966), previous exposure to warfarin may make some rats more susceptible. With this in mind, it may be worthwhile for the U.S. Environmental Protection Agency to re-examine its efficacy criteria for claims that a rodenticide will kill resistant rats.

We are continuing to study the retest phenomenon to determine its significance in terms of management practices. The data do confirm the concept of resistance to anticoagulants because rats that can survive one typically lethal dose (not to mention additional doses) of warfarin cannot be considered "normal". In the field where 0.025% warfarin baits are used, some of the animals identified in the lab as resistant would be killed; however, these animals will be more difficult to kill (than susceptible rats), especially if they have alternative food sources to dilute the bait and if the alternative foods are rich in vitamin K.

Management Recommendations

Support for non-chemical strategies of rodent pest management are well-founded (Davis 1972, Frantz and Comings 1976, Jackson and Marsh 1978) and some new perspectives based on our resistance studies are presented below. First, the RA/RU distinction is probably not simply one of genotype, though it may have some genetic basis as discussed by others (Drummond and Wilson 1968, Greaves and Ayres 1976, Jackson et al. 1975, Lund 1966). In fact, genotypic variation is known to influence expression of the resistant gene and this can be further complicated by differences in basic physiology (e.g. metabolic and absorption rates) and/or behavior (e.g. sequencing and rate of feeding). Behavior differences may be particularly important in RA/RU distinctions since an individual's classification may change with subsequent exposures to warfarin baits (Frantz, unpublished data).

Resistant unaffected animals would generally be at a competitive advantage immediately after an anticoagulant poisoning cycle because they would be better able to escape negative environmental factors such as predation and inclement weather (Jackson et al. 1975). Also, since these animals remain mobile after intoxication they could maintain an adequate diet and, perhaps, could select available vitamin K rich food sources. Thus, the RA/RU distinction is probably functional with regard to poisoning work.

Secondly, from the genetic standpoint, it appears that heterozygous resistant rats (at least with R. norvegicus) are favored in a resistant population (Greaves et al. 1977). That is, the homozygous susceptible rats succumb to anticoagulant poisons and the homozygous resistant animals are likely to succumb because of their high vitamin K requirement. Hence, if poisoning with anticoagulants is continued in an area where resistance is found, the level of resistance in the rat population will not necessarily increase (Greaves et al. 1977). However, if a habitat was rich in vitamin K resources (e.g. garbage and animal food) it seems reasonable to suspect that more homozygous resistant animals would be able to survive and the proportion of resistance in the population could increase.

Obviously, if we limit the human-supplied vitamin K and harborage resources through sanitation and rat-stoppage, there will be a concomitant overall decrease in the rat population. Such management strategies would be non-selective from the standpoint of genotype or the RA/RU distinction and the probability of reinfestation would also be lowered. The supplementary use of acute rodenticides (or perhaps the use of the new "acute" anticoagulant materials) would enhance this work, but should not supplant non-chemical strategies.

CONCLUSIONS

The Rodent Control Evaluation Laboratory has the task of identifying and maintaining baseline data on the level and distribution of anticoagulant rodenticide resistance in rat populations of cities participating in the federally funded Urban Rat Control Program. The methods for conducting this work in a statistically sound manner are laborious and difficult, particularly from the standpoint of inter-agency coordination and communication. However, our most recently developed monitoring techniques are improving the quality of the samples of rats submitted, and therefore, validity of the data is also improving.

The criteria for resistance in this program are somewhat conservative, but, even so, significant resistance levels have been identified in nearly half of the 39 samples of Norway rats tested. In Chicago, resistance exceeds 75% in at least one rat population and the bulk of these animals tends to be resistant unaffected, a difficult group to kill with anticoagulants. Some of our recent studies show that some resistant animals can survive sequential intoxications with a typically lethal dose of warfarin though about half of the animals succumb to the first re-exposure.

It seems reasonable that alternative food resources readily available to rats might dilute anti-coagulant rodenticide bait intakes and, if rich in vitamin K, might further decrease the anticoagulant effect. Adoption of a properly integrated pest management approach (Anon. 1979) would probably go far towards preventing many of the control difficulties associated with resistant rat populations.

If an urban rat population is to be kept within tolerable limits, it should be monitored periodically in order to make decisions regarding what pest management strategies should be used, when, and for how long. In the surveillance program, 5% resistance in the population is the tolerable limit. For countermeasures, we consider the non-chemical strategies of public health education, sanitation, good housekeeping and rat stoppage to be of particular value because they address the causative conditions which support the rat infestations. We must not lose sight of the fact that rat control is largely a people problem; focusing all attention on the rats per se will usually lead to only temporary relief.

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