# RECENT NORWAY RATS STUDIES USING WARFARIN

**RICHARD M. POCHÉ**, Genesis Laboratories, Inc., 10122 N.E. Frontage Road, Wellington, Colorado 80549.

ABSTRACT: Warfarin resistance in the Norway rat (*Rattus norvegicus*) has been studied over the past 30 years. To determine the status of this resistance phenomenon wild Norway rats were collected from Colorado and Chicago, Illinois. As reported previously, warfarin resistance in the Chicago area exceeds 50%, while rats from Colorado remain very susceptible to warfarin. The theory that true genetic resistance may not exist was examined, implying that geographic variation in intestinal flora contribute to the rapid degradation of warfarin after ingestion, along with production of sufficient Vitamin K in the bacteria to reverse the effect of warfarin. Antibiotics in combination were tested with warfarin and demonstrated that efficacy in the laboratory can be increased by using the combination in a bait form.

KEY WORDS: warfarin resistance, Norway rat, potentiation, antibiotics, efficacy, bacteria

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## INTRODUCTION

The anticoagulant rodenticide warfarin was developed during the 1940s and quickly took hold as an effective means of rodent control and replaced many of the more toxic and acute compounds. By 1958, Boyle (1969) reported Norway rats (*Rattus norvegicus*) developed resistance to warfarin on farms in Scotland. Reports surfaced within the next 20 years from various parts of the globe indicating that the compound was losing its effectiveness. Such genetic resistance was reported from the United States by Jackson and Kaukeinen (1972).

As a result of these increasing reports of Norway rats' resistance, more potent second generation anti-coagulants were developed to overcome the perceived resistance problems. These newer rodenticide products contained such compounds as brodifacoum, bromadiolone, difenacoum, flocoumafen, and difethialone.

During 1995 wild Norway rats were collected from Chicago and Colorado. These were subjected to the routine World Health Organization (WHO) screening test for warfarin resistance (WHO 1982). The purpose of this study was to determine if Norway rats in the Chicago area, previously documented as warfarin resistant, remained resistant to warfarin after the extensive use of second generation anticoagulants. Although cross resistance has been reported from various parts of the globe, an objective was to determine if resistance remained in Chicago. It was during that time that the search for other mechanisms involved in warfarin resistance in rats began. Why was there a perceived failure of warfarin as a rodenticide? Although Chicago and Colorado, or Iowa and Colorado, are not geographically separated, the consideration of what other mechanism might be involved in the reduced toxicity of warfarin based on geography remained in question.

Consideration to drug potentiation, or the interaction of warfarin with other compounds was examined from the literature. Previous studies have demonstrated the potentiation of non-steroid anti-inflammatory drugs on warfarin (Sirdhara and Krishnamurthy 1992). As early as 1974 Lewis et al. (1974) found that phenylbutazone potentiated anticoagulant effects.

## METHODS

Wild Norway rats were trapped using Tomahawk live traps. Rat infested areas of Chicago suburbs and feedlots in eastern Colorado were the source of wild rats used in this study. The areas selected in Chicago had not been recently baited by the Bureau of Rodent Control. Likewise, trap sites utilized in Colorado did not have recent rodent control efforts. Traps were placed in and around garages, along building walls, and vacant lots and were baited with a grain bait. Traps were monitored each morning. In Chicago, trapped animals were brought to the nearby regional office of the Bureau of Rodent Control warehouse, dusted with an EPA approved flea powder and placed in cages individually until transport back to the Genesis Laboratories facility in Colorado. Rats were trapped between April 1995 and October 1997. Rats were acclimated from two to six weeks before testing. The temperature within the test room was maintained at approximately 20 to 25°C and a photoperiod of 12 hours light:12 dark was maintained for the duration of the test. The average humidity was maintained between 25 to 55%.

### Liquid Provision of Tetracycline

The WHO testing protocol for warfarin resistance in Norway rats was used for screening rats in 1995. Subsequently, since resistance was approximately 50%, additional rats in 1996 and 1997 were trapped for further study. Treatment and control groups were established. Control groups received 50 ppm warfarin-treated cracked corn for six consecutive days in a no-choice test. Separate treatment groups of rats were presented with 5 and 20 mg tetracycline per ml of water *ad libitum* for three days before presentation of the 50 ppm warfarin bait. A separate group was given one 750 mg/kg dose of tetracycline hydrochloride via oral gavage three days before presentation of the warfarin bait. After obtaining interesting results in 1996, grain bait were formulated incorporating antibiotics.

### Preparation of Antibiotic Baits

The 0.005% warfarin bait was prepared using a Hobart Mixer. Cracked corn was used as the carrier.

An appropriate solvent was used to facilitate proper mixing of the warfarin in the mix. Baits were prepared containing of 0.005% warfarin and either tetracycline or metronidazole at various concentrations.

There were three treatment groups of Norway rats: warfarin control, warfarin with tetracycline, and warfarin with metronidazole. A treatment group consisted of eight Norway rats (four males and four females). Three replications of each level were subjected to the experimental design.

The treated baits were offered on six consecutive days. Each cup contained 40 grams and consumption was weighed every 24 hours. Consumption was recorded and additional feed was added so each cup contained 40 grams. At the end of the six-day test period, the test material was removed and Manna Pro Lab Cubes were provided *ad libitum* during the ten-day post-test observation period.

Observations were recorded twice daily during the exposure and post-treatment periods. Signs of warfarin intoxication and mortality were recorded, as well as bait consumption.

## RESULTS AND DISCUSSION

Representative results of the Norway rat warfarin screening for animals collected from Colorado and Chicago are presented in Tables 1 through 4. During 1995, 187 (144 males and 43 females) rats were trapped from Chicago and screened for warfarin resistance. Of these, 55% survived the 50 ppm warfarin diet presented for six consecutive days.

Fifty-three Norway rats collected from Colorado were examined for warfarin resistance. All rats died after the six-day exposure. These data support previous claims that the Chicago Norway rat population was genetically resistant to warfarin while rats from Colorado were non-resistant.

Norway rats given tetracycline-treated water for three days before presentation of 50 ppm warfarin diet were more susceptible to the bait (Table 5). Mortality in the control groups, those rats fed 50 ppm warfarin without the antibiotic, averaged 54.3%; while the treated groups receiving 5 and 20 mg per ml, respectively, attained 90% mortality.

The group of rats gavaged once with 750 mg/ml tetracycline then exposed to a 50 ppm warfarin diet did not respond in a similar manner. Mortality was only 60%, indicating that repeated doses were more efficacious in depleting the intestinal flora.

During 1997, additional research was conducted on tetracycline and metronidazole treated (Borchert 1997). A summary table of that study is presented in Table 6. Test groups treated with warfarin control, warfarin plus tetracycline, and warfarin with metronidazole additive, resulted in mortality of 74.1, 92.6, and 92.6%, respectively. There was no significant difference in consumption for the three formulations tested.

The results of adding certain antibiotics to warfarin indicate that efficacy can be enhanced when controlling Norway rats. Test groups exposed to 50 ppm warfarin including an antibiotic achieved high mortality. In the past, the nominal concentration of most warfarin baits sold in the U.S. was 250 ppm, more than adequate a concentration to attain effective Norway rat control.

Drug interactions with warfarin have been reported previously and the number of compounds that potentiate the effects are numerous (Wells et al. 1994). The exact mechanisms involved, however, remain nebulous.

The addition of tetracycline and metronidazole, as well as other antibiotics to warfarin, increases the toxicity over warfarin alone and is attributed the bacteria in the intestinal tract. Although previous studies indicated that earlier forms of antibiotics did not appreciably affect the efficacy of warfarin (Derse 1963), newer and stronger antibiotics are more efficacious in eliminating gut bacteria. It is felt that the mechanism in this case involves the action of the antibiotic on the intestinal flora of the Norway rat. Geographic variation within species and number of bacteria results in differing impact on the reaction to chemicals entering the gastrointestinal tract of the rat, and possibly the mouse. Warfarin is a relatively unstable compound and degrades into less toxic metabolites. The half-life in the gastro-intestinal tract is a mere 42 hours (Ford 1993). Dupont (1996) reported the effective half-life of approximately 40 hours. About 92% of the parent compound is excreted in the urine with a small percentage being the parent material.

Once reaching the gut of the rat, warfarin bait begins to degrade, based on the types of bacteria present. The degradation rate in areas such as Chicago influences the amount of parent compound available for absorption into the rodent system.

Warfarin elimination is almost entirely by metabolism by hepatic microsomal enzymes to inactive hydroxyiated metabolites by reductases to reduce metabolites (warfarin alcohols). The warfarin alcohols have minimal anticoagulant activity (DuPont 1996). The metabolites are principally excreted into the urine and to a lesser extent into the bile. The metabolites of warfarin have identified and include dehydrowarfarin, two diastereoisomer alcohols: 4'-, 6-, 7-, 8-, and 10-hydroxywarfarin.

Bacteria capable of producing more vitamin K, the antidote for warfarin, also contributes to the reduced toxicity of warfarin in certain populations of Norway rats. It is suggested that a combination of factors involving the degradation of warfarin by bacteria and the production of vitamin K by the intestinal flora contribute to reduced toxicity of the rodenticide. This geographic variation in the ability of Norway rats to tolerate warfarin, is probably attributed to regional differences in intestinal flora species. The issue of Norway rat genetic resistance to warfarin in the U.S. requires careful re-examination again, especially in light of the regulatory restrictions being placed on more toxic anticoagulants.

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Table 1. Results of warfarin resistance screening test for Norway rats collected in Colorado during 1995.

| Animal No. | Sex | Body Wt. (g) | Warfarin Consumption (g) | Consumption/kg Body Weight (g) | Mortality |
|---|---|---|---|---|---|
| CO-101 | F | 277 | 73.5 | 265.3 | Yes |
| CO-102 | F | 251 | 77.5 | 308.8 | Yes |
| CO-103 | F | 187 | 110.7 | 592.0 | Yes |
| CO-104 | F | 204 | 115.7 | 567.2 | Yes |
| CO-106 | F | 190 | 86.0 | 452.6 | Yes |
| CO-107 | F | 180 | 67.3 | 373.9 | Yes |
| CO-108 | F | 234 | 78.8 | 336.8 | Yes |
| CO-109 | F | 204 | 73.5 | 360.3 | Yes |
| CO-110 | F | 277 | 110.7 | 399.6 | Yes |
| CO-111 | F | 207 | 91.0 | 439.6 | Yes |
| CO-112 | F | 180 | 84.7 | 470.6 | Yes |
| CO-113 | F | 283 | 105.2 | 371.7 | Yes |
| CO-114 | F | 208 | 109.2 | 524.0 | Yes |
| CO-115 | F | 201 | 84.4 | 419.9 | Yes |
| CO-116 | F | 198 | 93.5 | 472.2 | Yes |
| CO-117 | F | 200 | 95.6 | 478.0 | Yes |
| CO-118 | F | 189 | 76.2 | 403.2 | Yes |
| CO-119 | F | 223 | 81.2 | 364.1 | Yes |
| CO-120 | F | 196 | 80.9 | 412.8 | Yes |
| CO-121 | F | 266 | 135.0 | 507.5 | Yes |
| CO-122 | F | 268 | 120.2 | 448.5 | Yes |
| CO-124 | F | 192 | 84.9 | 442.2 | Yes |
| CO-126 | F | 236 | 86.4 | 366.1 | Yes |
| CO-127 | F | 245 | 92.4 | 377.1 | Yes |
| CO-128 | F | 217 | 113.7 | 524.0 | Yes |
| CO-129 | F | 291 | 90.5 | 311.0 | Yes |
| CO-130 | F | 264 | 116.0 | 439.4 | Yes |
| CO-131 | F | 294 | 70.9 | 241.2 | Yes |
| CO-132 | F | 297 | 74.0 | 249.2 | Yes |

Table 2. Results of warfarin resistance screening test for Norway rats collected in Colorado during 1995.

| Animal No. | Sex | Body Wt. (g) | Warfarin Consumption (g) | Consumption/kg Body Weight (g) | Mortality |
|---|---|---|---|---|---|
| CO-1 | M | 358 | 75.5 | 210.9 | Yes |
| CO-2 | M | 326 | 76.5 | 234.7 | Yes |
| CO-4 | M | 346 | 102.2 | 294.8 | Yes |
| CO-5 | M | 278 | 80.8 | 290.6 | Yes |
| CO-6 | M | 316 | 80.3 | 254.1 | Yes |
| CO-7 | M | 394 | 90.7 | 230.2 | Yes |
| CO-8 | M | 331 | 81.6 | 246.5 | Yes |
| CO-9 | M | 223 | 95.6 | 428.7 | Yes |
| CO-10 | M | 355 | 113.9 | 320.8 | Yes |
| CO-11 | M | 323 | 93.6 | 289.8 | Yes |
| CO-12 | M | 333 | 122.8 | 368.8 | Yes |
| CO-13 | M | 310 | 12.7 | 41.0 | Yes |
| CO-14 | M | 277 | 37.3 | 134.7 | Yes |
| CO-15 | M | 325 | 85.0 | 261.5 | Yes |
| CO-16 | M | 272 | 88.0 | 323.5 | Yes |
| CO-17 | M | 357 | 82.4 | 230.8 | Yes |
| CO-18 | M | 450 | 115.7 | 257.1 | Yes |
| CO-19 | M | 325 | 104.1 | 320.3 | Yes |
| CO-20 | M | 280 | 95.6 | 341.4 | Yes |
| CO-21 | M | 322 | 84.0 | 260.9 | Yes |
| CO-22 | M | 262 | 55.8 | 213.0 | Yes |
| CO-23 | M | 373 | 131.1 | 351.5 | Yes |
| CO-24 | M | 313 | 82.9 | 264.9 | Yes |
| CO-25 | M | 171 | 72.0 | 421.1 | Yes |

Table 3. Results of warfarin resistance screening test for Norway rats collected in Chicago during 1995.

| Animal No. | Sex | Body Wt. (g) | Consumption/Kg Body Wt. (g) | Mortality |
|---|---|---|---|---|
| IL-33 | M | 389 | 164.3 | Yes |
| IL-34 | M | 369 | 313.8 | Yes |
| IL-35 | M | 319 | 149.5 | Yes |
| IL-36 | M | 400 | 42.0 | No |
| IL-37 | M | 434 | 206.0 | No |
| IL-38 | M | 393 | 230.5 | No |
| IL-39 | M | 413 | 138.0 | No |
| IL-40 | M | 358 | 169.3 | No |
| IL-41 | M | 309 | 250.8 | Yes |
| IL-42 | M | 399 | 105.8 | Yes |
| IL-43 | M | 388 | 229.4 | No |
| IL-45 | M | 367 | 220.7 | Yes |
| IL-46 | M | 467 | 177.9 | Yes |
| IL-47 | M | 378 | 222.5 | Yes |
| IL-48 | M | 276 | 285.5 | Yes |
| IL-49 | M | 449 | 143.2 | Yes |
| IL-50 | M | 472 | 152.5 | Yes |
| IL-51 | M | 255 | 239.6 | No |
| IL-52 | M | 356 | 294.4 | No |
| IL-53 | M | 227 | 291.2 | No |
| IL-55 | M | 392 | 79.8 | Yes |
| IL-56 | M | 478 | 193.7 | No |
| IL-57 | M | 357 | 148.5 | No |
| IL-59 | M | 348 | 136.2 | Yes |
| IL-60 | M | 463 | 259.8 | Yes |
| IL-61 | M | 393 | 169.7 | Yes |
| IL-62 | M | 361 | 265.9 | No |
| IL-63 | M | 415 | 121.7 | No |
| IL-64 | M | 297 | 88.2 | Yes |
| IL-65 | M | 425 | 152.7 | Yes |

Table 4. Results of warfarin resistance screening test for Norway rats collected in Chicago during 1995.

| Animal No. | Sex | Body Wt. (g) | Consumption/Kg Body Wt. (g) | Mortality |
|---|---|---|---|---|
| IL-131 | F | 294 | 325.5 | No |
| IL-132 | F | 349 | 50.1 | Yes |
| IL-133 | F | 178 | 286.5 | Yes |
| IL-135 | F | 228 | 307.0 | Yes |
| IL-137 | F | 282 | 74.8 | Yes |
| IL-138 | F | 263 | 238.0 | Yes |
| IL-139 | F | 206 | 243.7 | Yes |
| IL-140 | F | 317 | 263.4 | Yes |
| IL-141 | F | 418 | 177.0 | Yes |
| IL-142 | F | 250 | 457.6 | Yes |
| IL-143 | F | 300 | 371.7 | Yes |
| IL-144 | F | 305 | 251.1 | No |
| IL-145 | F | 292 | 440.4 | No |
| IL-146 | F | 292 | 337.7 | No |
| IL-147 | F | 368 | 270.4 | No |
| IL-148 | F | 350 | 242.3 | Yes |
| IL-150 | F | 286 | 147.2 | Yes |
| IL-151 | F | 322 | 329.8 | No |
| IL-152 | F | 335 | 253.4 | No |
| IL-153 | F | 347 | 276.4 | No |
| IL-154 | F | 195 | 275.9 | Yes |
| IL-155 | F | 339 | 283.8 | Yes |
| IL-156 | F | 384 | 194.0 | No |
| IL-157 | F | 367 | 4.1 | No |
| IL-158 | F | 329 | 65.3 | No |
| IL-159 | F | 324 | 370.1 | Yes |
| IL-160 | F | 318 | 294.3 | Yes |
| IL-161 | F | 281 | 324.2 | No |
| IL-162 | F | 349 | 222.6 | No |
| IL-163 | F | 227 | 241.4 | Yes |

Table 5. Summary of tetracycline studies completed during 1995-96.

| Amount of Tetracycline in water mg/ml | Group ID | Number of Rats | Mortality |
|---|---|---|---|
| 0 | T-2 | 10 | 50% |
| 0 | T-4 | 10 | 80% |
| 0 | T-6 | 12 | 33% |
| 5[1] | T-1 | 10 | 90% |
| 20 | T-3 | 10 | 90% |
| 750[2] | T-5 | 10 | 60% |

[1] Three-day exposure to tetracycline in water.
[2] Single oral gavage.

Table 6. Results of laboratory tests on Norway rats fed 0.005% warfarin diet as control, and diet treated with tetracycline and metronidazole (Borchert 1997). The three groups had three replications of eight rats each.

| Treatment Type and Replication Number | Average Bait Consumption (g) | Average Warfarin Consumed (mg/kg) | Average Days Alive | Percent Mortality |
|---|---|---|---|---|
| Warfarin (1) | 84.4 ± 22.2 | 14.9 ± 5.25 | 7.1 ± 3.8 | 88.9 |
| Warfarin (2) | 77.9 ± 21.9 | 14.7 ± 4.7 | 9.8 ± 5.3 | 66.7 |
| Warfarin (3) | 80.1 ± 25.3 | 14.5 ± 4.3 | 10.2 ± 4.9 | 66.7 |
| Tetracycline (1) | 85.4 ± 25.1 | 21.7 ± 4.47 | 6.4 ± 1.5 | 100.0 |
| Tetracycline (2) | 87.2 ± 31.0 | 15.9 ± 6.5 | 8.0 ± 3.6 | 88.9 |
| Tetracycline (3) | 75.6 ± 17.1 | 14.9 ± 6.8 | 8.1 ± 3.6 | 88.9 |
| Metronidazole (1) | 91.6 ± 28.8 | 15.6 ± 3.7 | 7.3 ± 3.7 | 88.9 |
| Metronidazole (2) | 71.6 ± 19.8 | 17.4 ± 5.0 | 8.4 ± 3.3 | 88.9 |
| Metronidazole (3) | 80.7 ± 15.7 | 14.3 ± 4.5 | 6.4 ± 1.2 | 100.0 |

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