



## SUMMARY

This investigation is part of a research program funded and steered by the Rodenticide Resistance Action Committee (RRAC) of CropLife International to develop and proof methods, which are required to assess the degree of resistance and to draw conclusions for the effective control of populations containing resistant individuals. The aim was to determine whether the level of reduced susceptibility, characterised by the INR-based blood clotting (BCR) resistance-test constitutes “practical resistance”. Earlier BCR resistance tests indicated a high degree of resistance to bromadiolone among Norway rats (*Rattus norvegicus*) on farms in the Muensterland area, Westphalia, which were subjects of three experiments in this study. Rats were trapped and subjected to BCR-tests using different multiples of the ED<sub>50</sub> for the susceptible baseline strain in order to assess the incidence of resistance and the resistance factor for bromadiolone. Subsequently, standardised treatments with 0.005% bromadiolone grain bait were conducted. Control success was determined by pre- and post-treatment census.

The incidence of resistance to bromadiolone was assessed at 30% with resistance factors at 10 and 15, for females and males respectively in experiment 1. In experiments 2 and 3, incidence of resistance approached 100% with resistance factors at 10. Control success after the subsequent treatments was 72% in experiment 1, and zero and 20% in the other two experiments.

With resistance factors of the Westphalia bromadiolone-resistant Norway rats at or higher than 7 and 10 for females and males respectively, and with a high incidence of resistance, no control was possible with common strength bromadiolone bait.

The INR-based BCR resistance test can provide useful data to predict the practical effect of reduced susceptibility or resistance to an anticoagulant. The test method should be recommended in resistance management for assessing the degree of resistance.

## INTRODUCTION

Practical resistance to warfarin, coumatetralyl and bromadiolone was first proven in Muensterland area, Westphalia during feeding trials in the early 1980s (Schnorbach, Bayer). Resistance spots of advanced level appear on farms there with control problems, when rats are cross-resistant to coumatetralyl and bromadiolone. Warfarin-resistance was shown to be the basic resistance for this advanced resistance to develop (Pelz et al. 1995), and was characterised by the Y139C variant of the VKORC1 gene (Rost et al. 2004). This variant was also confirmed in rats from the farms introduced here. Resistance factors to bromadiolone range on the highest ever confirmed level at 15 in homozygous advanced resistant rats from Westphalia (Endepols et al. 2007), appearing with an incidence at 40% to 70% on these farms. Lower bromadiolone resistance factors at 3 to 6 had no significant effect on the outcome of a control operation using common strength bromadiolone bait in Wales (Buckle et al. 2007). It was our aim to investigate whether the high level of bromadiolone resistance found in these resistance spots constitutes “practical resistance”. This investigation is part of a research program funded and steered by the Rodenticide Resistance Action Committee (RRAC) of CropLife International to develop and proof methods, which are required to assess the degree of resistance and to draw conclusions for the effective control of populations containing resistant individuals.

## METHODS: Field Trials

The field trials were carried out according to RRACs’ standard protocol in four phases:

- Implementation of the trial;
- Pre-baiting census 5 days; following 4 days break;
- Baiting period: 42 days (trial 1); 35 days (trials 2+3); following 4 days break;
- Post-baiting census (5 days).

Census method: Consumption of rolled oats during 24 hours; + Foot print index on tracking plates for confirmation.

Baiting method: Distribution of baiting-points according to structural analysis of the estate using the Baytool computer program (Endepols et al. 2003).

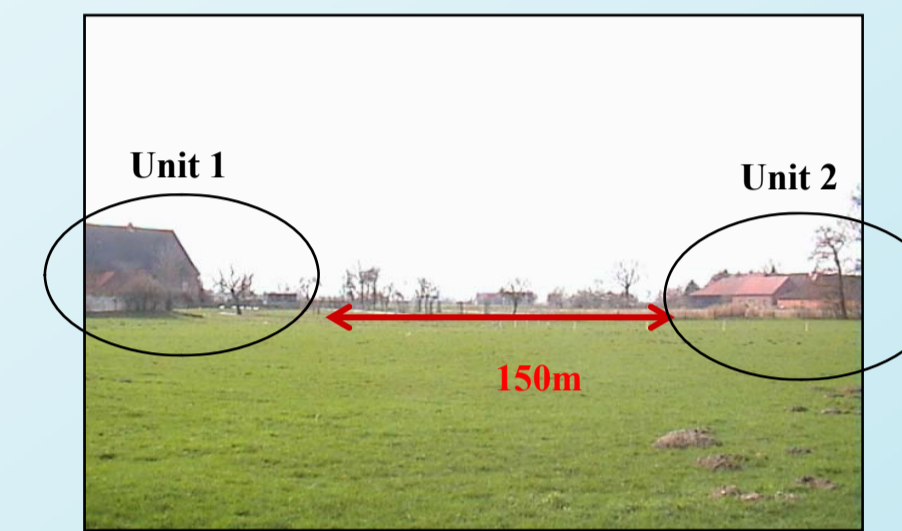
After the field trial, a control measure with difethialone paste bait was conducted for complete eradication.

## METHODS: Locations

Trials No. 1 and No. 2 were conducted on farm I, and trial No. 3 was conducted on farm II.

### Farm I

The trial site is a traditional farm with fattening pigs (two units: 500 animals + 400 animals). The trial site consists of two farm-units separated by a path with a bordering hedge. Distance between the two units is 150 meters, and migration of rats is frequently seen. Both units were considered as one infestation.



### Farm II

A traditional farm on which cattle (150 animals) and fattening pigs (400 animals) are kept.



## RESULTS: Trial No. 1

Degree of Resistance (RF) test dose = 15x / 10x ED <sub>50</sub>	Pre-baiting Census (1 day):	2,663g
INR ≤ 5.0: 2/12 (17%)	% Survivors: (Census)	29%
INR ≤ 10.0: 6/12 (50%)	Bait consumed:	9.95kg

- 17% of the tested rats were classified non-responders with an INR ≤ 5.0 after treatment with ED<sub>50</sub>-multiples of 15 and 10, respectively.
- Assuming these multiples fit well with the RF as determined earlier in this population, the incidence of resistance is supposed at 34%.
- BCR test result fits well with control success in field trial.

## RESULTS: Trial No. 2

Incidence of Resistance (IR) test dose = 2.5x ED <sub>50</sub>	Degree of Resistance (RF) test dose = 15x / 10x ED <sub>50</sub>	Pre-baiting Census (1 day):	2,140g
5/5 (100%)	INR ≤ 5.0: 1/11 (9%)	% Survivors: (Census)	107%
		Bait consumed:	43.4kg

- The IR was assessed at 100%.
- As only 9% of rats were tested non-responders with 10x and 15x ED<sub>50</sub>, a lower RF is supposed.
- Treatment success confirmed previously determined IR, and confirming the RF to be lower than the multiples chosen for BCR-test.

## RESULTS: Trial No. 3

Incidence of Resistance (IR) test dose = 2.5x ED <sub>50</sub>	Degree of Resistance (RF) test dose = 10x / 7x ED <sub>50</sub>	Pre-baiting: Census (1 day)	1,512g
3/3 (100%)	INR ≤ 5.0: 5/12 (42%) INR ≤ 10: 7/12 (58%)	% Survivors: (Census)	80%
		Bait consumed:	25.5kg

- The IR was estimated high (at 100%, with low n).
- In BCR resistance tests with multiples of 7 and 10 of the ED<sub>50</sub>, respectively, 42% of the tested rats were classified non-responders.
- Assuming these multiples fit well with the RF, the IR was 84%.
- The BCR test results fit well with control success in field trial (80% survivors).

## METHODS: BCR resistance-tests

A blood clotting response (BCR) resistance-test protocol was employed to determine the incidence of resistance as well as resistance factors, which relies on the WHO sensitivity standard, developed in conjunction with baselines for most anticoagulants (RRAC 2003, Prescott et al. 2007). Animals were treated with certain multiples of the ED<sub>50</sub> of the CD-strain of susceptible Norway rat. Low multiples were chosen to determine the incidence of resistance, and higher multiples were administered to assess the resistance factor. Animals showing an INR (International Normalised Ratio, related to blood clotting activity of WHO standard) below 5.0 were classified non-responders.

Number of tested rats: Trial 1: 12 rats  
Trial 2: 16 rats  
Trial 3: 15 rats

Dosages for incidence (IR) of bromadiolone resistance:

Trials No. 2 + 3 only: 2.5 x ED<sub>50</sub> – 1.17mg/kg (males) and 1.65mg/kg (females).

Dosages for investigation of the degree (Resistance Factor RF) of bromadiolone resistance:

Trial No. 1 + 2: 15 x ED<sub>50</sub> (males) = 7.05mg/kg  
10 x ED<sub>50</sub> (females) = 6.61mg/kg

Trial No. 3: 10 x ED<sub>50</sub> (males) = 4.70mg/kg  
7 x ED<sub>50</sub> (females) = 4.62mg/kg

## CONCLUSIONS

The standardised INR-based BCR resistance test allows a reliable assessment of incidence of resistance and of resistance factors in wild rat populations.

Resistance factors may vary slightly within one area due to factors like variability, seasonality, feed etc.

In contrast to rats with resistance factors at 3 – 6, rats of a strain with resistance factors at or higher than 10 for bromadiolone can not be controlled with common strength bait.

## REFERENCES

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