Proceedings of the Tenth International Conference on Urban Pests
Rubén Bueno-Marí, Tomas Montalvo, and Wm. H Robinson (editors) 2022
CDM Creador de Motius S.L., Mare de Deu de Montserrat 53-59,
08930 Sant Adrià de Besòs, Barcelona, Spain

AN INNOVATIVE CHOLECALCIFEROL RODENTICIDE BAIT

SHARON HUGHES
BASF plc, St Michael's Industrial Estate, Widnes, Cheshire, England, WA8 8TJ

Abstract Alternatives to anticoagulants have presented limitations in their use and/or efficacy. BASF have developed a non-anticoagulant rodenticide bait containing cholecalciferol (vitamin D_3). A beneficial feature of cholecalciferol is that it is effective against all anticoagulant resistant strains of rats and mice and the development of resistance to cholecalciferol is unlikely. Laboratory studies with BASF's cholecalciferol bait against anticoagulant resistant strains Y139F and L120Q resulted in 100% mortality within 4 days. This effectiveness has been further confirmed with field trials against Y139F and L120Q rats. In the past cholecalciferol rodenticide baits have been less palatable (rat palatability ratios of below 1.0). The BASF cholecalciferol bait has been formulated to be palatable (rat palatability ratio above 4.0). Cholecalciferol is regarded as a subacute rodenticide and has a quicker mode of action compared to anticoagulants. The intrinsic properties of both cholecalciferol and the palatable BASF bait matrix result in a quicker time to individual rodent death, mean of 2 - 4 days in laboratory studies. Field trials have shown that 100% control is possible with only 7 days of baiting. Efficacy was demonstrated against *Rattus norvegicus, Rattus rattus, Rattus exulans and Mus musculus* including anticoagulant resistant strains.

Key words Non-anticoagulant, anticoagulant resistance, *Rattus norvegicus*, *Rattus rattus*, *Mus domesticus*,

INTRODUCTION

Worldwide, anticoagulant rodenticides baits are the fundamental tool used for rodent pest management (Buckle and Eason, 2015). While there is no practical resistance to baits containing the more potent anticoagulants flocoumafen, difethialone and bromadiolone, resistance to other less potent anticoagulants exists and is spreading. In many countries, there is no viable alternative to anticoagulant baits that is effective against both rats and mice. However, BASF have developed a non-anticoagulant rodenticide bait containing only cholecalciferol (vitamin D_3) as the active substance. In mammals, cholecalciferol toxicity causes hypercalcemia and the calcification of soft tissues such as heart, kidney, liver, stomach (inducing a stop-feeding effect) and lungs and death. The resulting "stop-feeding" effect, means that cholecalciferol rodenticide baits must be both potent and palatable to ensure a lethal dose of bait is eaten before this effect occurs.

Due to the quicker mode of action compared to anticoagulants, cholecalciferol baits have the potential to cause bait shyness and hence reduced levels of control, especially if the bait is not sufficiently palatable/acceptable to the target pests. To negate any potential bait shyness pre-baiting is used as in general pre-baited rodents accept treated baits more readily (Lund 1988). However, the presented studies show that the BASF cholecalciferol bait is highly palatable and efficacious in both rats and mice without the need for pre-baiting. No anticoagulant is required to be added to boost efficacy. Cholecalciferol is effective against all anticoagulant susceptible and anticoagulant resistant rats and mice. The mode of action of cholecalciferol is such that the risk of the development of resistance is very low as it is an essential component for life and growth of mammals including rodents. If resistance to cholecalciferol (vitamin D3) were to develop in rodents, individuals would be at a huge competitive disadvantage as any decreased sensitivity to vitamin D_3 would likely have extreme effects on physiological processes and development. A palatable and efficacious cholecalciferol bait would be an essential tool in anticoagulant resistance management.

MATERIALS AND METHODS

The method for the choice feeding studies and field trial studies either complied with or was a modification of that prescribed in the Biocidal Products Directive Technical Notes for Guidance on Product Evaluation, Product Type 14.

All choice feeding studies had laboratory diet as the control diet. The control diet (ground laboratory diet) was expanded rat and mouse diet, provided by Harlan Laboratories UK Ltd, England.

Animals used were; *Rattus norvegicus*, *Rattus rattus*, *Rattus exulans* and *Mus domesticus.* The target rodents were either wild (as in the field trials), wild-derived (Welsh, Hampshire and Berkshire rats and bromadiolone resistant mice from BASF) or laboratory, anticoagulant susceptible strains (supplied by Harlan Laboratories UK Ltd, England).

Data Analysis. Statistical analysis (ANCOVA) was carried out to establish if there was a significant difference between the palatability of the BASF cholecalciferol bait and each of the competitor cholecalciferol baits to compare the total individual amounts of bait eaten with the total individual food consumption. ($p = 0.05$).

RESULTS AND DISCUSSION

Choice feeding studies (4 days) were undertaken using male anticoagulant susceptible *R. norvegicus* against either the BASF cholecalciferol bait (n=50) or Storm® (flocoumafen 0.005% wax block bait) bait (n=50). The mean times to death were 2.8 days for the BASF cholecalciferol bait and 5.7 days for Storm. The BASF cholecalciferol bait and Storm bait effected 100% mortality by day 3 and day 10, respectively.

The results of laboratory choice feeding studies on the BASF cholecalciferol bait against both anticoagulant susceptible and anticoagulant resistant *R. norvegicus* and *M. domesticus* are summarised in Table 1 and Table 2, respectively. The rats were singly caged and mice held as a colony in a pen *i.e.* simulated use studies. Results show that the BASF cholecalciferol bait was effective against all strains of rats and mice tested, including the resistant L120Q strains. In rats and mice there was no practical difference in days to death between each of the strains tested.

Table 1. Efficacy of BASF Cholecalciferol bait following choice feeding studies on single caged anticoagulant susceptible and anticoagulant resistant *R. norvegicus.*

Rat strain	**Anticoagulant resistance status**	Number of rats used (♂+ ♀)	**Mortality %**	**Mean time to death, days**
Wistar	susceptible	10 ♂+ 10 ♀	100	2.4
Y129S (Welsh)	Resistant to first generation anticoagulant rodenticides (FGARs)	10 ♂+ 10 ♀	95	2.7
L120Q (Hampshire)	Resistant to FGARs. Tolerant to difenacoum & bromadiolone	10 ♂+ 10 ♀	100	3.4
L120Q (Berkshire)	Resistant to FGARs. Resistant to difenacoum bromadiolone bromadiolone	10 ♂+ 10 ♀	100	3.2

Table 2. Efficacy of BASF Cholecalciferol bait following choice feeding studies against anticoagulant susceptible and anticoagulant resistant *Mus musculus* in simulated use studies.

Species	**Number of trials**	**Mean days baiting for control (range)**	**Trials at 7 days baiting (or less)**	**Mean Control achieved, %**
M. musculus	4	11.3 (7 - 21)	2	94
R. norvegicus	15	10.5 (6 - 23)	10	93
R. rattus	5	6.4 (5 - 10)	4	99

Choice feeding (palatability) studies against individually caged male and female *R. norvegicus*, Figure 1, were undertaken on the BASF cholecalciferol bait and three competitor cholecalciferol baits. The results are reported as the mean of the individual palatability ratios and the standard error of the mean.

Individual palatability ratio = Total amount of test bait eaten, g
Total amount of control diet eaten, g

Statistical analysis of covariance (ANCOVA) shown that the BASF cholecalciferol bait is significantly more palatable than each of the competitor cholecalciferol rodenticide baits. This enhanced palatability is essential in negating the effect of bait shyness.

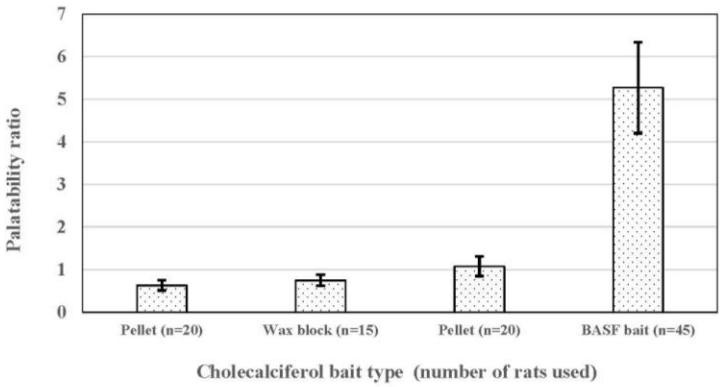

Figure 1. Palatability ratio and for the BASF cholecalciferol bait and competitor cholecalciferol baits.

To support global use of this bait one of the main rodent pest species is *Rattus exulans*, laboratory choice feeding studies were undertaken on the BASF cholecalciferol bait against wild-caught *R. exulans*. The bait effected 100% mortality within 7 days.

The definitive proof that a rodenticide bait is efficacious against target rodent pest species is successful field trials against those species. Field trials were conducted in the UK, France, Germany and the USA in both urban and rural settings. Census diet and tracking score were used as indexes to measure the rodent infestation pre and post bait treatment. As a result of the high palatability of the BASF cholecalciferol bait and the speed of action of cholecalciferol, control was possible in many trials with only 7 days of baiting. The results of a typical rat rural field trial are shown in Figure 2, indicating 100% control with 7 days of baiting.

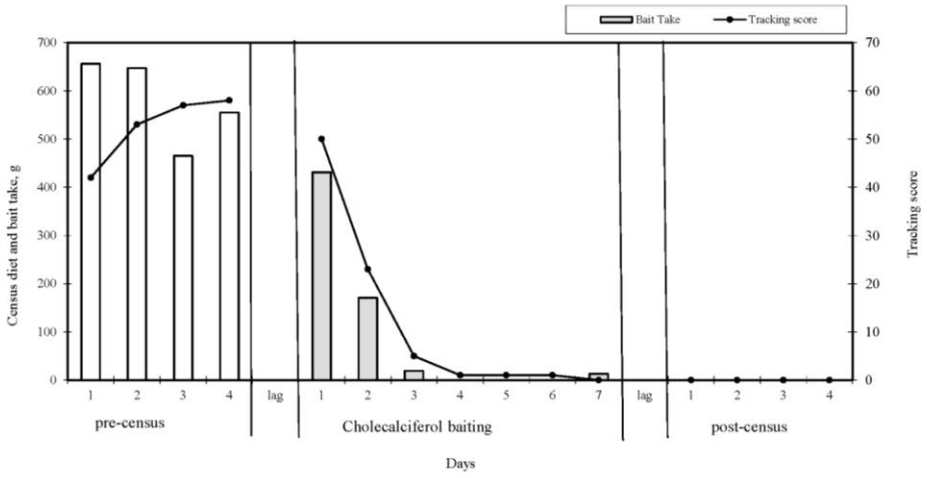

Figure 2. Field trial on BASF Cholecalciferol bait against *R. norvegicus* in a rural environment.

Extensive trials were undertaken on the target pest species and a summary of the results are shown in Table 3. For *M. musculus* of the 4 trials undertaken 2 required 7 days or less of baiting to effect control with the mean baiting duration 11.3 days. For *R. norvegicus*, of the 15 trials undertaken 10 required 7 days or less of baiting to effect control with the

mean baiting duration 10.5 days. Similarly, for *R. rattus* of the 5 trials undertaken, 4 required 7 days or less of baiting to effect control with the mean baiting duration 6.4 days. The mean level of control for *M. musculus*, *R. norvegicus* and *R. rattus* were 94, 93 and 99%, respectively.

Table 3. Summary of field trials undertaken on BASF Cholecalciferol bait against *M. musculus*, *R. norvegicus* and *R. rattus.*

Species	Number of trials	Mean days baiting for control (range)	Trials at 7 days baiting (or less)	Mean Control achieved, %
M. musculus	4	11.3 (7 - 21)	2	94
R. norvegicus	15	10.5 (6 - 23)	10	93
R. rattus	5	6.4 (5 - 10)	4	99

Two field trials were also undertaken against anticoagulant resistant *R. norvegicus*. The first site was in France, where the rats were identified, by DNA sequencing, as Y139F - resistant to FGARs and bromadiolone. To effect 100% control against these anticoagulant resistant rats required 12 days of baiting with the BASF cholecalciferol bait. The second site was in undertaken in South England where the rats were identified, by DNA sequencing, as L120Q - resistant to FGARs, bromadiolone and difenacoum. The population of approximately 2,000 rats was also considered to be extremely neophobic. After 18 days of baiting with the BASF cholecalciferol bait at this highly challenging site, 83 % control was achieved. Unfortunately, after 18 days the baiting was terminated due to harvesting and subsequent significant re-invasion affecting population assessment.

CONCLUSIONS

The BASF cholecalciferol bait is palatable and efficacious against both anticoagulant susceptible and resistant strains of rats and mice. Field trials have shown that pre-baiting is not required. This bait will be an invaluable resistant management tool for the effective control of rodent infestations.

REFERENCES CITED

Buckle, A.P. and Eason, C.T. 2015. Control methods: Chemical. Rodent Pests and Their Control: 2nd Edition 2015, p123-154

Lund M. 1988. Rodent behaviour in relation to baiting techniques. EPPO Bulletin. Volume 18 (2): 193