

Evaluation Manual for the Authorisation of plant protection products and biocides

EU part

Biocides

Chapter 7 Efficacy

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**Board
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Chapter 7 Efficacy

Category: biocides

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PT14 Rodenticides

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GENERAL INTRODUCTION

This chapter describes the data requirements for the assessment of the efficacy of a biocide and the active substance within PT 14, and which evaluation methodologies are applied for the EU framework. This chapter is derived from the TNsG on product evaluation (appendix to chapter 7 on Product Type 14 Efficacy Evaluation of Rodenticidal Biocidal Products) which was recently revised (March 2009).

READER

This chapter deals with the evaluation methodology of efficacy tests for rodenticide biocidal products that is applicable for the EU Biocidal Products Directive 98/8/EC (BPD) [1] for the authorisation of biocidal products (BPD Annex VI).

1. INTRODUCTION

Depending on its field of use a rodenticide may be classified as a biocidal product or plant protection product. This chapter covers the rodenticides in the category of biocides, which are used predominantly for the control of the house mouse (*Mus musculus L.*), brown rat (*Rattus norvegicus Berkenhout*) and the roof rat (*Rattus rattus L.*).

Possible fields of use are:

- In and around residential homes and other places in which people are accommodated;
- In rooms intended for the preparation, processing or storage of food and beverages;
- In empty stores, ships' holds, factories and silos;
- At waste dumps;
- In moist/wet environments such as sewers and watersides.
- On animal husbandry farms (pigs, poultry, cattle, etc.)

1.1. Aim

The aim is to assess efficacy, to ensure that only effective products enter the market.

1.2. Global structure of the assessment

Full assessment of efficacy is conducted on applications for product authorisations. Information on effectiveness and intended uses of the product, together with its active substances, must be sufficient to permit an evaluation of the product, including the nature and benefits that accrue following use of the product in comparison to suitable reference products or damage thresholds, and to define its conditions of use.

Both laboratory and field studies should be performed with the product to evaluate whether the product is effective for the requested use(s) at the specified doses (decision-making scheme, Appendix 1). Data on the palatability of the bait and the mortality from these studies are compared with the specified criteria. The basis is the claim submitted by the applicant.

2. DOSSIER REQUIREMENTS

Data on efficacy are required for every application for authorisation. Data on efficacy are required for every extension of the authorisation, or it should be indicated which data were already submitted in the past. The Competent Authority may always ask for additional information.

The following guidance is designed to be flexible and do not specify rigid protocols to which tests must be conducted. Published or unpublished data from any source will be considered provided the data are valid and relevant to the application. In all cases, the methods have to be described in sufficient detail to make the data reproducible. Ideally, data should be generated using national or international recognised testing methods. However, applicants can also submit data generated using

their own testing strategies where these are conducted and well reported to a sound scientific standard or studies conducted to national or international testing methods. In all cases, the data must allow a specific assessment of palatability and efficacy of the product. Anecdotal evidence will not be acceptable.

Assessment will be made mainly in relation to the claims for the effectiveness of the product made on the product label. This assessment will take into account the pest(s) to be controlled, indoor or outdoor use, the method(s) of application, application rates and use patterns of the product, maximum storage period of the product, together with any other specific claims made for the product.

The pests selected for efficacy testing should be appropriate to the geographic regions in which the product will be used. They should be named on the product label (either common or generic names may be used).

Label claims can be:

FOR USE AGAINST MICE

- this will only require testing against *Mus musculus*³.

FOR USE AGAINST RATS

- this will only require testing against *Rattus norvegicus*, unless there are country specific requirements.

FOR USE AGAINST ROOF RATS

- this will require testing against *R. rattus*.

FOR USE AGAINST RATS AND MICE

- this will require testing against both *R. norvegicus* and *M. musculus*³.

FOR USE AGAINST RATS and/or MICE RESISTANT TO THE FIRST GENERATION ANTICOAGULANTS

- this will require testing against resistant *R. norvegicus* and/or *Mus musculus*³ (for instance warfarin resistance).

A label claim such as 'FOR USE AS A RODENTICIDE' with no further clarification of the target species is not acceptable. This is because it would allow use against rodent species for which the product is not intended (and most likely has not been tested), such as *Sciurus carolinensis* (grey squirrel).

For each target organism that is claimed on the label, a study should be conducted. The brown rat is more sensitive for anti-coagulants than the roof rat; the sensitivity of the roof rat is similar to that of the house mouse. However, their field and laboratory behaviour is totally different in respect of rodenticide applications. Therefore, all claimed target organisms have to be tested.

It should be noted that any efficacy testing conducted in the European Union on rodents should be in accordance with the Protection of Animals used for Experimental and other Scientific Purposes Directive (86/609/EC).

³ In general, data generated using either *M. musculus musculus* or *M. musculus domesticus* would be acceptable.

2.1. Basic activity

Although laboratory testing with wild rodents may be preferable, the difficulty and constraints associated with obtaining and maintaining them for testing purposes is recognised. Therefore for tests conducted within the laboratory, animals sourced from recognised commercially available strains are acceptable. Whilst laboratory strains are acceptable for use in the laboratory, the final stage of testing (semi-natural or field trials) should be conducted using wild rodents.

Where wild animals are used in laboratory or semi-natural studies, these may be live trapped from the wild, reared in either outdoor colonies or under laboratory conditions such that it permits the animals to retain much of their natural physiological and behavioural characteristics. Breeding stock used for rearing wild rodents should not be selected for docile qualities or other characteristics that significantly alter their wild tendencies.

2.2. Laboratory studies

Two laboratory studies should be performed, a mortality test and a choice feeding test. Tests conducted to EPPO or the specimen protocol (Appendix 2) are preferable but other data will be considered on their merits. The study must be representative for the treatment. Depending on the intended aim of the product, the house mouse, roof rat and/or the brown rat should be used as the test animal. Wild strain testing is preferable and is most important for the bait-choice test. However, since this is probably impractical for some applicants, an outbred lab strain (e.g. CD rats) which is likely to exhibit traits of the wild strain may be accepted as surrogate.

2.2.1. The mortality test

The aim of the mortality test is to determine the lethal effect of the product. The test animals are offered bait containing the active substance and the resulting mortality within 10 to 21 days is compared with the specified criterion (§ 4.1). This is normally done in a no choice, single animal cage test, in which the animals are offered only food containing the test substance, but mortality data from a choice test are also acceptable.

The duration of the test (normally 10 to 21 days) should be appropriate to the proposed method of use of the rodenticide. Data must be presented to show the daily intake of laboratory diet prior to the test and product during the treatment period, body weight of test animals (pre and post-test), symptoms of poisoning and days to death.

Rodenticides are divided into groups as follows, based on different mechanisms of action.

- I.** Rodenticides with an acute effect: these can be roughly defined as products that cause death within 24 hours after administration of the lethal dose.
- II.** Rodenticides with a multi-dose effect: these products only cause death after having been ingested several times during a number of days (3-21 days).
- III.** Rodenticides with a single-dose effect and delayed action: these products can be ingested for one or two days but only cause death after a number of days (3-14 days).

The ingestion of acute or multi-dose products leads to a different rodent mortality period of 24 hours and 3-21 days respectively. The duration of the mortality test is therefore different for these groups of products and is also dependent on the rodent involved. Rodenticides with special indications, for instance dust or foam products,

which are taken up orally but are not bait products since they adhere to the rodent fur, require separate laboratory trials, where the conditions are properly simulated.

Where claims such as 'controls warfarin resistant populations' or 'controls rats and mice resistant to first generation anticoagulants' are being made, the test should be conducted on known resistant laboratory or wild-caught strains (the location where wild rodents were obtained should be stated). Resistance of rodent strains can be determined by blood clotting response (BCR) (EPPO Bulletin [4], 1993 ; RRAC Technical Monograph [5], 2003) tests or by feeding studies developed by the World Health Organisation (WHO). As an alternative, 'field' trials of the product against known resistant populations may be conducted (see Section 6.3 of TNsG on Product Evaluation).

2.2.2. *The bait choice feeding trials*

The aim of the bait choice feeding trials is to determine the palatability of the product for the test animal. If conducted on both fresh and aged product it may provide information on the storage stability of the product. This test is preferably done with wild strain animals. In this test design, animals have the choice between a non-poisoned food source (challenge diet) and the bait containing the active substance. Either the amount of bait consumed, in which the active substance is incorporated, or the mortality of the rodents is a measure for the palatability of the bait. Results are compared with the specified criterion (§ 4.1).

Make sure that the challenge diet is a product that the rodent is accustomed to.

Full details of the methods used should be provided and data should be presented to show the daily intake of both untreated diet and product, the palatability ratio (amount of product: amount of challenge diet) or product acceptance (amount of product eaten expressed as a percentage of total [product + challenge diet] consumption) for different sexes of rodent, any signs of poisoning and days to death, with appropriate statistical analysis. When no significant differences exist between the sexes, the data from the two sexes may be combined.

In some cases comparison with normal food intake is inappropriate. For instance when fast-acting rodenticides cause a reduction in feeding activity or when only very small quantities of bait are required to cause effect. Therefore, in these cases the main criterion is not the percentage of consumed bait but the mortality resulting from poison uptake.

2.3. Laboratory studies related to specific product types

2.3.1. *Contact Rodenticides*

In addition to providing an estimation of the oral potency of the product via an acute oral toxicity test, the additional information that should be available in order to demonstrate efficacy will include:

- i)** Estimates of time to death from individually caged rodents exposed to the product for stated periods of time. Reference to EPPO Guidelines (EPPO, 1986) should be made.
- ii)** Evidence from the laboratory that the target rodents will pick up the required dose from the application method is recommended.

2.3.2. *Gassing Agents*

The type of information that should be available in order to demonstrate efficacy will include estimates of the potency of the active substance and product by inhalation.

2.4. Laboratory studies related to specific efficacy claims regarding suitability for use in damp conditions

Where it is claimed that a product is suitable for use in damp conditions, the retention of palatability should be tested in a choice test against the target species, using product that has been stored under damp conditions for at least 5 days.

2.5. Laboratory studies related to specific efficacy claims regarding to storage of the product

When a product is claimed to be effective after a long period of storage, it is necessary to demonstrate that the product will still be effective after the stated storage period. The applicant must either deliver data for palatability of the product at the end of maximum storage or alternatively (in case of a new product) data for a stress test with 'accelerated ageing', i.e. a palatability test with the product which is stored under challenging conditions (for instance: >60% R.H. and > 25 °C) for at least 4 month.

2.6. 'Field' trial / 'semi field' trial

The aim of the field trial is to evaluate, under actual in-use conditions, the palatability of the bait containing active substance and the mortality it causes.

Tests conducted to EPPO or the specimen protocols (Appendix 2 and 3) are preferable but other data will be considered on their merits. Depending of the intended aim of the product, populations of house mice, brown rats or roof rats are used for this trial.

Ideally, sites chosen for field trials should be representative of the range of locations where the rodenticide is to be used (indoor/outdoor), and should be infested with sufficient numbers of the target rodents so that the effectiveness of the product can be clearly demonstrated.

It is advantageous if the rodent infestations on the sites chosen are, as far as possible, discrete and not subject to potential rapid re-invasion. Rodent activity on the site should be determined before and after treatments using at least two standard techniques.

Sketch maps of the sites approximately to an indicated scale showing all the important features including signs of infestation and location of rodenticide application should be provided. Data should be presented to indicate levels of rodent activity both before and after treatment, amounts of bait consumed and all relevant information regarding treatment details.

As an alternative or addition to 'field' trials, evidence of the efficacy of a rodenticide product may be obtained from trials against colonies of wild rodents housed within a semi-natural environment ('semi-field' trial). Such colonies are likely to be family groups, as unrelated animals, particularly males, can be very aggressive towards each other.

2.7 Waivers

In order to prevent unnecessary animal suffering, the no-choice mortality test may be waived when the product has already been approved and listed and only the bait formulation has been changed; however, since the attractivity could be different then, a new bait choice test is obligatory. Reciprocally, when the bait formulation is the same, but the concentration of the

active substance has been decreased, a new mortality test is necessary. In this case, the bait choice test can be waived. If one wishes to submit an application, and if the information to be submitted is based on animal testing, it is mandatory to enquire from the Competent Authority

- whether a pesticide product containing the same active substance has been authorised already;
- and if so, what the name of the registration holder is.

Table.1 Data to be submitted when applying for authorisation of a new rodenticide

New rodenticide	Bait choice feeding trial	Mortality test	'Field' trial
New active substance, for new field of use	+	+	+
Already authorised active substance, for already authorised field of use	+	Data from test with another product based on this already authorised active substance	Data from test with another product based on this already authorised active substance
Already authorised active substance, for new field of use	+	Data from test with another product based on this already authorised active substance	+

+This test should be performed with the product for which authorisation is sought.

In all other cases it is sufficient to submit the results of the tests, which have been performed with an authorised product based on the same active substance. Read across should be allowed to data supplied by other applicants, where access to that data has been granted.

3. METHODOLOGY OF ASSESSMENT

There are many standard test methods currently available that may be appropriate for the assessment of the effectiveness of rodenticides. A list of such test standards is presented in Appendix 4.

In addition to the standard test methods presented in Appendix 4, specimen protocols for a No Choice Test, a Choice Test and a Field Test are presented in Appendices 2 and 3 respectively. These Appendices are intended only to provide further information regarding the types of studies that may be utilised to assess the efficacy of some rodenticides, and some of the factors that should be taken into account.

Any known limitations on efficacy (including resistance) should be considered during the assessment.

- Possible restrictions or recommendations concerning the use of the product in specific environmental or other conditions. State possible factors that can reduce the efficacy, for instance hot, cold or humid environments or the presence of other substances, in addition to the grounds for these. Possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the development of resistant strains and the grounds for these (see also TNsG on product evaluation [2]). State if the product cannot be mixed with, for example, other biocidal products or if the use of the product with other biocidal products is recommended.
- The guidance given on resistance for the corresponding data requirement of the active substance also applies here.

The study results are compared directly with the criteria for efficacy.

4. ASSESSMENT OF AUTHORISATION

4.1. Norms and criteria

A biocidal product may only be authorised if it “possesses a sufficient level of efficacy”.

This is implemented in the following way.

Rodenticides are considered to be efficacious if they satisfy 3 criteria:

- In the mortality test the percentage of dead animals should be normally¹ $\geq 90\%$ within normally 20 days².
- In the bait choice feeding test the percentage of ingested bait containing the product should be normally¹ $\geq 20\%$. When the test results in $\geq 90\%$ mortality, a lower level than 20% of the total food consumption is acceptable.
- In the ‘field’ trial or the ‘semi-field’ trial the percentage of bait consumed after the control operation compared to the amount of bait consumed before the control operation should normally¹ be $\leq 10\%$. When other types of monitoring of the test population are used, such as tracking activity measurement and census by trapping, they should sufficiently show the decrease of the population (this to be decided by the Competent Authority).

4.2. Assessment

If the results of one of two laboratory studies (mortality test and a choice feeding test) do not satisfy the criteria, the product is considered to be insufficiently effective. Authorisation is then considered as not possible as far as efficacy is concerned. In that case a field trial is not necessary. However, if field data are available, that show that the product is effective against wild rodents, the Competent Authority might consider ‘field’ trials overweigh lab trials.

If the results of the laboratory studies do satisfy the criteria, a ‘field’ or ‘semi-field’ trial is then necessary. In the practical use situation, the bait containing active substance competes with other food sources. The degree of reduction or disappearance of the amount of bait is compared with the criterion specified above.

The efficacy of the product after a specified storage time (as claimed on the label) is also taken into account when assessing efficacy of a rodenticide.

For the assessment of resistance is referred to TNsG on product evaluation [2] and the Rodenticide Resistance Action Committee (RRAC) [5].

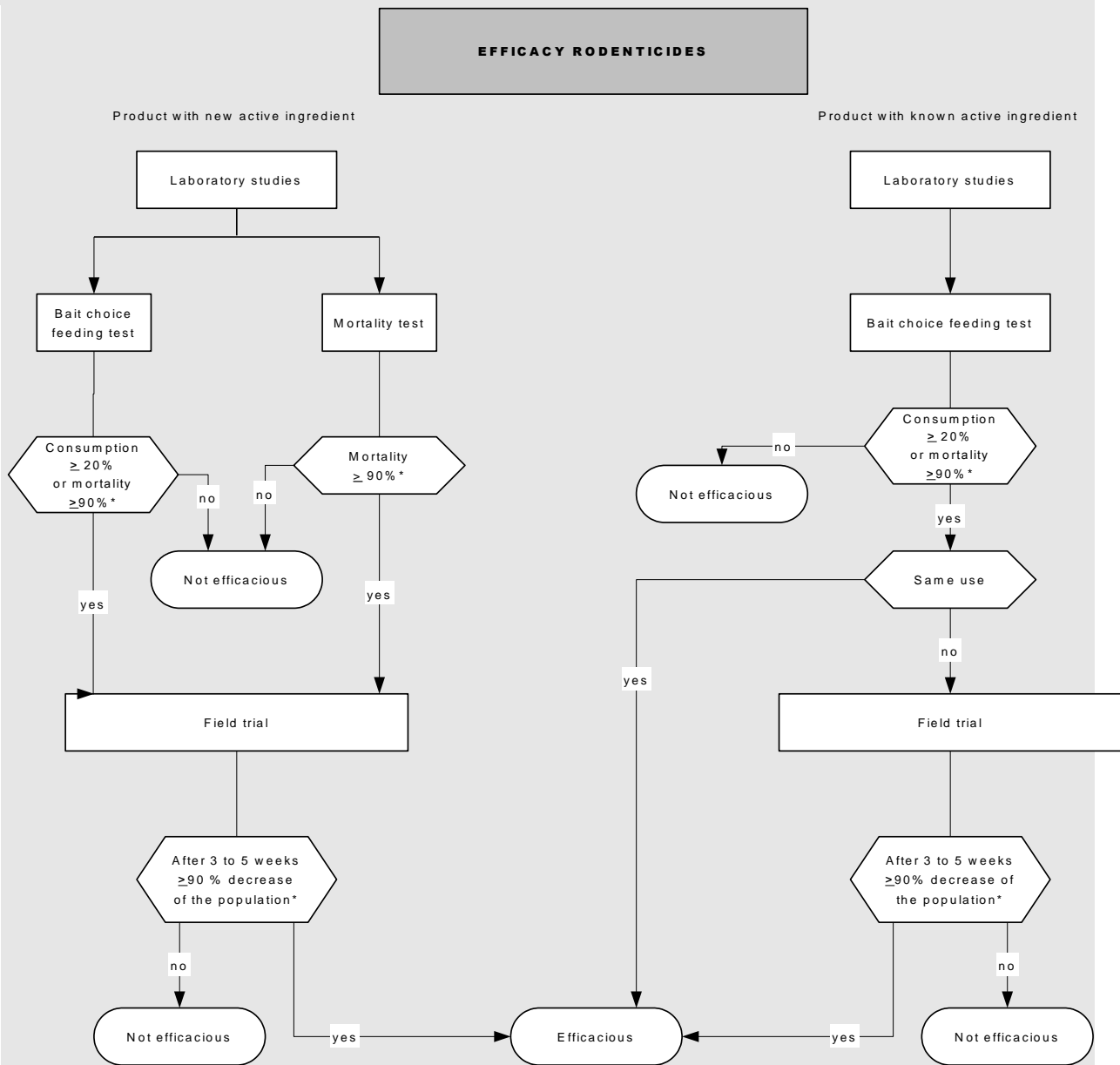
¹ Deviation from this norm is possible but should be explained in the application.

² Except when the claim asks for another time limit.

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Appendix 1 Decision-making scheme assessment Rodenticides



* Deviation from this norm is possible but should be explained in the application.

Appendix 2.1 Laboratory studies for Rodenticides : no choice, mortality test

This guideline describes a protocol of a laboratory study to determine the efficacy of an as yet unauthorised product (rodenticide) against the house mouse, brown rat and roof rat.

To determine the potency of the product against the target species, a no-choice feeding study is conducted against laboratory rodents. The study consists of an acclimatisation period, followed by a pre-test diet take assessment, then a 1- (single dose rodenticide) or, normally^c, a 4-day (multiple dose rodenticide) test period and at least 14 days of post-treatment observation.

A group of 10 (5 males and 5 females) healthy, adult rodents of known strain (STATE) are used in the study. Females should not be pregnant. All animals are weighed (for Norway rats and house mice minimum adult body weights should be 150g and 15 g respectively, at the start of the test) and individually caged. Ambient conditions should conform to those prescribed under current legislation controlling animal experiments. Tap water is freely available throughout the study period.

Pre-test period

The animals are acclimatised to the test conditions for a minimum of 3 days prior to the no-choice feeding period. A feeding dish is placed centrally at the front in each cage and is filled with ground laboratory diet or EPA meal at the desired rate. All other food is removed.

On the third day, a weighed amount of fresh diet is placed in the pot, the quantity to be in excess of the normal daily requirement. After 24 hours, the diet remaining is weighed and the amount eaten by each rat/mouse calculated. Inspection of the figures should confirm that all animals are eating normally from the food pots. Animals not eating normally should be removed from the test.

Test period

During the test period the quantity of biocidal product in each pot should be in excess of the rodent's normal daily requirements. Every 24 hours throughout the test period, any product spillage is collected and any extraneous matter, such as faeces, removed. Unconsumed product is then weighed, and the total amount eaten calculated by subtraction. If the test period is 1 day, the product is then removed and replaced with the normal laboratory diet for the duration of the observation period. If the test period is longer, used product is discarded and replaced with a fresh supply each day in a fresh pot. On the last day, uneaten product is replaced with the normal laboratory diet for the following observation period. Throughout the feeding period the rodents are observed at least twice daily. Daily takes are added up and the amount of active ingredient ingested is calculated.

Observation period

During the observation period the rodents are observed at least once per day and any toxic symptoms and mortality is recorded.

Results

Results should be shown as the percentage mortality during and after the observation period. Any other symptoms should be mentioned.

^c Deviation from this norm is possible but should be explained in the application.

For liquid bait formulations

The test shall be carried out as above with the following exceptions:

- i)** A suitable compounded laboratory diet shall be freely available
- ii)** Tap water shall be withdrawn during exposure to the rodenticide (test period).
- iii)** All procedures relating to the laboratory diet and solid bait shall instead be applied to the tap water and liquid bait, as appropriate.
- iv)** Liquid baits shall be provided in containers with non-drip nozzles or suitable open pots.
- v)** A filled container shall be placed out of reach of the animals in order to check for weight loss due to evaporation.

Appendix 2.2 Laboratory studies for Rodenticides : choice test

This guideline describes a protocol of a laboratory study to determine the efficacy of an as yet unauthorised product (rodenticide) against the house mouse, brown rat and roof rat containing a bait formulation.

A feeding test is conducted to determine the extent to which rodents will eat the product when they are given a free choice between that and their normal food. This type of palatability test is most suited to slow-acting toxicants. The test consists of an acclimatisation period, followed by a pre-test diet take assessment, then a test period of normally^d 4 days and at least 14 days of post-treatment observation.

Pre-test period

For the test, normally⁴ 10 wild or laboratory strain rodents (5 males and 5 females) are required. Laboratory rodents should be healthy, non-pregnant adults of known strain (STATE). Preferably wild adult rodents are used. They should be healthy and obtained from free-living populations (STATE WHERE). On arrival at the laboratory, the wild strains should be treated with an appropriate insecticide to kill ectoparasites and then caged individually. With wild rats especially, it is advisable to place all items (i.e. food pots) required for the test in the cage before each animal is released into it. Wild rodents should be acclimatised to laboratory conditions for at least 3 weeks to ensure that no females are pregnant when the test begins. During this time they should be offered a laboratory animal diet and water should be freely available. To encourage variation in response, animals with body weights throughout the range normally expected for the species should be used as far as possible.

Before the test period begins, it is necessary to ensure that the animals are feeding normally. Following acclimatisation, 2 food pots, placed either side at the front of the cage, are filled with ground laboratory diet or EPA meal. All other food is removed, but water remains freely available. The quantity of food placed in each pot (STATE) should be sufficient to meet each animal's daily needs. All used diet should be discarded and the pot refilled with a fresh supply. This procedure should be repeated for a further 3 days and on the last day the animals should be weighed. Also on the last day, the diet remaining in each pot is weighed and the total amount of food eaten by each rodent calculated (STATE). Any rodent not eating normally by the last day should be discarded.

Test period

The palatability test commences with 2 clean bait containers, one filled with a quantity of the test product (as received from the manufacturer) and the other with a suitable challenge diet (e.g. an EPPO challenge diet [3] or standard laboratory diet). Again, the quantity in each pot should exceed the normal daily requirement for each animal. After 24 hours, the diet remaining in each pot is weighed and the total amount of food eaten by each rodent calculated. All used test and challenge diet is discarded and fresh quantities of each diet are placed in clean pots. In placing the pots back in the cage, the positions of the rodenticide and the challenge diet should be interchanged to avoid place preference. This procedure should be repeated every day during the choice period. After day 4 (3 or 5 is also acceptable) the animals should be returned to the standard laboratory diet.

Observation period

During the observation period the rodents are observed at least once per day and any toxic symptoms and mortality is recorded.

Results

^d Deviation from this norm is possible but should be explained in the application

Results should be shown as the percentage intake of rodenticide and the percentage intake of challenge diet. Also the percentage mortality and any other symptoms should be mentioned.

Liquid bait formulations

The test shall be carried out as above with the following exceptions:

- i) A suitable compounded laboratory diet shall be freely available.
- ii) Tap water shall be used as the control bait.
- iii) All procedures relating to the solid control and test baits shall be applied instead and as appropriate to the liquid control and test baits.
- iv) When the positions of the test and control baits are interchanged the positions of the drinking tubes, if used, should not be interchanged.
- v) Liquid baits shall be provided in containers with non-drip nozzles or suitable open pots.
- vi) A filled container shall be placed out of reach of the animals in order to monitor weight loss due to evaporation.

Appendix 3 Field trial for rodenticides

This guideline describes a protocol and factors to be taken into account when conducting a field trial to determine the efficacy of an as yet unauthorised product (rodenticide) against the house mouse, brown rat or roof rat.

Ideally field trials should:

- i)** be conducted with separate rat and mice populations (as appropriate to label claims).
- ii)** be carried out at sites that are representative of label claims (industrial, commercial, domestic).
- iii)** include sites with ‘known’ anticoagulant resistant populations (if appropriate to label claims).
- iv)** have had no rodenticide treatments over the past 3 months.
- v)** Incorporate lag phases before and after the treatment phase.
- vi)** for testing concentrates, cover a range of bait bases.
- vii)** for product is sold with a specific bait station, include the whole formulation (the bait and its station) in the test.
- viii)** be carried out at 2 or 3 locations

The following suggested method for bait formulations details the extent of the data required, but the methods may be replaced or supplemented by new techniques as appropriate.

Suggested procedure for bait formulations

Trial sites

Each trial site should, as far as possible, comprise a discrete infestation of one target species, with little chance of rapid reinvasion from adjoining areas.

During the entire trial, the baiting sites should be at exactly the same locations, about 2 metres apart for mice, and 5 metres for rats (unless specified differently in the label claim).

At each baiting site, a bait container is placed, the top of which is closed/covered, to protect the bait from weather and avoid spillage. When selecting baiting sites, it is important that the animals can feed without being disturbed.

For mice, 25-50 grams of bait is placed in each feeding tray, while for brown or roof rats 100-200 grams is placed (unless label claims ask for different amounts). In other respects, the test design is identical for both groups. It is important that there is always enough fresh food or bait containing the active substance present.

Before the trial begins, draw a sketch map showing all significant features of the site including signs of infestation.

Data on field efficacy is likely to be more reliable if infestations of Norway rats and House mice are selected on the basis that a stable level of activity is obtained during the pre-treatment assessment. The level of activity can be determined by two of the following (as appropriate to the situation, species etc.):

- i)** Census baiting
- ii)** Tracking techniques
- iii)** Census trapping.

Pre-treatment activity measurement/estimation of numbers

Indices of the target species population should be obtained both before and after the test treatment normally by at least 2 of the following:

Pre-treatment bait census

The position of the census bait points should be indicated on the site sketch plan. Census bait should be laid for at least 4 days to cover the whole infestation in quantities at each bait point which as far as possible exceed the maximum daily take by rodents. The number of census baits should be approximately the same as the planned number of test bait points. Census points should not be located at the same place chosen to lay poison points but should be at different (intermediate) positions. Census bait should be different to the bait base used in the test product.

The number of points where take has occurred and the amount of the take of the census bait, should be recorded daily. An indication of the change in weight of the bait due to moisture loss or uptake should be included.

At the end of the bait census all baits and containers should be removed from the trial site. The total amount of census bait consumed will give an index of population size.

Tracking activity measurement

This is recommended for both rats and mice, and should be measured over at least 3 days, simultaneously with the bait census, using tracking patches/boards laid around the site in numbers similar to the census bait points but as far as possible, not in the same locations. The locations of the patches/boards should be indicated on the plan.

The patches/boards should be inspected for signs of activity and resurfaced daily. A simple scoring system can be devised to assess the number of rodent footprints per patch/board: summing the individual scores gives a daily activity index. When the pre-treatment assessment is complete, the tracking patches/boards may be removed from the site or maintained to provide supplementary information on rodent activity.

Census by trapping

This is recommended for mice only, and should be carried out for a period of at least 3 days using rodenticide-free bait in the traps. Traps should be laid around the site in numbers appropriate to the situation and likely population size.

Animals caught should be marked by fur clipping and subsequently released. The numbers caught should be recorded and used to estimate the size of the population.

The traps should then be removed from the test site during the rodenticide treatment.

Lag period

Once the pre-treatment population measurement has been conducted there should be a lag period, normally 3-14 days (or longer for acute poisons where no pre-baiting is recommended) with no experimental interference (other than tracking) on the site.

Test treatment

The test formulation must be applied in accordance with the label or proposed label, for an appropriate period (normally^e 4 days for acute products and 21 days for multi-dose products). The locations of test bait

^e Deviation from this norm is possible but should be explained in the application

points should, as far as possible, be different from those of the census bait points, traps, and tracking patches/boards.

Where applicable the following items should be recorded:

- i) The locations of the bait points on the plan.
- ii) The amount of bait deposited at each point at each visit and the amount retrieved, including details of the type of container used.
- iii) The number and species of rodents and other animals found dead, and the dates on which they were found.
- iv) The dates of all observations, treatments and censuses.
- v) Any other information deemed relevant. This may include, for example weather conditions, temperature data, site changes instituted by the occupier (including improvements in hygiene and proofing), or supplementary information on rodent tracking activity.

On termination of the treatment all poisoned baits and bait containers should be removed from the trial sites. Similarly rodent bodies should be searched for, removed and disposed of in the appropriate way e.g. burial or burning.

Post-treatment lag period

On completion of the treatment there should be a lag period sufficient to allow poisoned animals to die or survivors to recover from the sub-lethal effects of the rodenticide. This period may be 3-14 days, depending on previous observations of time to death or full recovery. During this period there should be no experimental interference with the site other than tracking.

Post-treatment activity measurement/estimation of numbers

Once the post-treatment lag period is completed, the methods employed to measure pre-treatment activity should be conducted in exactly the same way. Traps, baits and tracking patches should be laid in exactly the same places as in the pre-treatment census.

After each field trial, a comparison of population indices before and after treatment determines how successful the product has been in controlling the target population. The degree of control is expressed as a percentage reduction in the pre-treatment index.

Appendix 4 List of currently available standard test methods for rodenticides

This list may not be exhaustive, and makes no comment on the suitability of particular test methods for efficacy testing.

Standard	Title	Target Organism(s)	Mode of Application
EPA/OPP Protocol Number 1.201	Standard Norway Rat and Roof Rat Anticoagulant Liquid Bait Laboratory Test Method	Norway Rat/Roof Rat	Liquid bait
EPA/OPP Protocol Number 1.202	Standard House Mouse Anticoagulant Liquid Bait Laboratory Test Method	House Mouse	Liquid bait
EPA/OPP Protocol Number 1.203	Standard Norway Rat and Roof Rat Anticoagulant Dry Bait Laboratory Test Method	Norway Rat/Roof Rat	Dry Bait
EPA/OPP Protocol Number 1.204	Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method	House Mouse	Dry Bait
EPA/OPP Protocol Number 1.205	Standard Norway Rat/Roof Rat Anticoagulant Tracking Powder Efficacy Laboratory Test Method	Norway Rat/Roof Rat	Tracking Powder
EPA/OPP Protocol Number 1.212	Standard House Mouse Anticoagulant Tracking Powder Efficacy Laboratory Test Method	House Mouse	Tracking Powder
EPA/OPP Protocol Number 1.213	Standard Norway Rat/Roof Rat Anticoagulant Wax Block and Wax Pellet Laboratory Test Method	Norway Rat/Roof Rat	Wax Block and Wax Pellet
EPA/OPP Protocol Number 1.214	Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method	House Mouse	Wax Block and Wax Pellet
EPA/OPP Protocol Number 1.217	Standard Norway Rat and Rood Rat Anticoagulant Placepack Laboratory Test Method	Norway Rat/Roof Rat	Placepack dry bait
EPA/OPP Protocol Number 1.218	Standard House Mouse Anticoagulant Placepack Penetration Laboratory Test Method	House Mouse	Placepack Penetration
EPA/OPP Protocol Number 1.221	Proposed Norway Rat Anticoagulant Technical and Concentrated Dry Bait Laboratory Test Method	Norway Rat	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number 1.225	Proposed House Mouse Anticoagulant Technical and Concentrated Dry Bait Laboratory Test Method	House Mouse	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number: 1.207	Standard Norway Rat/Roof Rat Acute Liquid Bait Laboratory test method	Norway Rat/Roof Rat	Liquid Bait
EPA/OPP Protocol Number: 1.208	Standard House Mouse Acute Liquid Bait Laboratory Method	House Mouse	Liquid Bait

EPA/OPP Protocol Number: 1.209	Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method	Norway rat/Roof rat	Dry Bait
EPA/OPP Protocol Number: 1.210	Standard House Mouse Acute Dry Bait Laboratory Test Method	House Mouse	Dry Bait
EPA/OPP Protocol Number: 1.211	Standard Norway Rat/Roof Rat Acute Tracking Powder Efficacy Laboratory Test Method	Norway rat/Roof rat	Tracking Powder
EPA/OPP Protocol Number: 1.219	Standard Norway rat/Roof rat Acute Placepack Penetration Laboratory Test Method	Norway rat/Roof rat	Placepack penetration
EPA/OPP Protocol Number: 1.220	Standard House Mouse Acute Placepack Dry Bait Laboratory Test Method	House Mouse	Placepack dry Bait
EPA/OPP Protocol Number: 1.222	Proposed Norway Rat Acute Technical and Concentrated Dry Bait Laboratory Test Method	Norway rat	Technical and concentrated dry bait
EPA/OPP Protocol Number: 1.226	Proposed House Mouse Acute Technical and Concentrated Dry Bait Laboratory Method	House Mouse	Technical and concentrated dry bait
EPA/OPP Protocol Number: 1.227	Proposed House Mouse Acute tracking Powder Efficacy Laboratory Method	House Mouse	Tracking Powder
BBA 9 - 3.1	Richtlinie für die Prüfung von Nagetierbekämpfungsmitteln gegen Hausmause	House mouse	Dry and liquid bait, wax block and pellets, contact rodenticides
BBA 9- 3.2	Richtlinie für die Prüfung von Nagetierbekämpfungsmitteln gegen Wanderratten	Norway Rat	Dry and liquid bait, wax block and pellets, contact rodenticides
EPPO 1982	Guidelines for the Biological Evaluation of Rodenticides No1. Laboratory Tests for Evaluation of the Toxicity and Acceptability of Rodenticides and Rodenticide Preparations	-	-
EPPO 1982	Guidelines For the Biological Evaluation of Rodenticides. Field Tests Against Syanthropic Rodents (Mus musculus, Rattus norvegicus, Rattus rattus)	-	-
EPPO 1986	Guidelines for the Biological Evaluation of Rodenticides. Laboratory and Field Tests for the Evaluation of Rodenticidal Dusts	-	-
ASTM E 565-95	Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions for Commensal Rodents	Norway rat/Roof rat/ House mouse	Dry Bait
ASTM E 593-95	Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions	Norway rat/Roof rat/House mouse	Dry Bait
PP 1/97(2)	Laboratory and field tests for the evaluation of rodenticidal dusts	-	-

PP 1/113(2)	Laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticide preparations	-	-
PP 1/114(2)	Field tests against synanthropic rodents	Mus musculus, Rattus norvegicus, R. rattus	-
PP 1/169(2)	Field rodents	Microtus, Arvicola	-
PP 1/197(1)	Non-target effects of rodenticides	-	-
PP 1/198(1)	Testing rodents for resistance to anticoagulant rodenticides	-	-

6. References

- 1 Biocidal directive (BPD) (98/8/EC)
- 2 Technical Notes for Guidance: TNsG on Product Evaluation; Common principles and practical procedures for the authorisation and registration of products. Available at: <http://ecb.jrc.it/biocides/>
- 3 EPPO guideline PP1/113 for the efficacy of rodenticides, Laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticides preparations. Revised 1998.
- 4 OEPP/EPPO Bulletin, Volume: 23, No: 4, pages: 701-708, year: 1993 Revised methodology for a blood-clotting response test for identification of warfarin-resistant Norway rats (*Rattus norvegicus*)
- 5 Rodenticide Resistance Action Committee, RRAC. A Reappraisal of Blood Clotting Response Tests for Anticoagulant Resistance and a proposal for a standardised BCR Test Methodology Available at: www.rrac.info