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# Transitional Guidance on the Biocidal Products Regulation

## Transitional Guidance on Efficacy Assessment for Product Type 14 Rodenticides

**December 2016**

**TRANSITIONAL GUIDANCE**

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# PREFACE

This Transitional Guidance is to be applied to applications for active substance approval and product authorisation submitted under the Biocidal Product Regulation (EU) No 528/2012 (the BPR). This document describes the BPR obligations and how to fulfil them.

A "Transitional Guidance" is a document that has been initiated under the "old" Biocidal Products Directive 98/8/EC and because it has been finalised before the relevant new BPR guidance document has been fully developed, it is being made available as a Transitional Guidance document until such time as the relevant new document is ready for publication.

This Transitional Guidance document has been discussed and supported by the Efficacy Working Group of the Biocidal Products Committee (BPC). The document has undergone a "transitional" consultation with the Biocidal Competent Authorities and Accredited Stakeholder Organisations and additionally had a Public Consultation by the Commission on an earlier version - comments from the Public Consultation were addressed and incorporated into the earlier draft.

The document will be included into the Volume II Efficacy, Assessment and Evaluation (Parts B+C) of the new BPR guidance structure when it is published early in 2017; there will be no further consultation on this document before that time.

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# Tables



**NOTE to the reader:**
This Transitional Draft Guidance will be reformatted when it is incorporated into the New Guidance Structure. When this is completed, the finalised version will be uploaded onto the website of ECHA. No consultation will be made to do this.

# General introduction

This document provides guidance on the methodology for the evaluation of the efficacy of rodenticide biocidal products according to the common principles laid down in Annex VI of the BPR in order to demonstrate that the condition for granting an authorisation in Article 19(1)(b)(1) of the BPR is fulfilled (i.e. the rodenticide is sufficiently effective).

# 1. Introduction

Depending on its intended purpose, a rodenticide may be regulated as a biocidal product or as a plant protection product[1]. This document covers the rodenticides under the BPR, which are used predominantly for the control of the house mouse (*Mus musculus.*), brown rat (*Rattus norvegicus*) and the roof rat (*Rattus rattus*). Also other target species such as water voles (*Arvicola amphibius*), bank vole (*Myodes glareolus*), common voles (*Microtus arvalis*), field or wood mice (*Apodemus* spp.) and the grey squirrel (*Sciurus carolinensis*) are considered.

The four standard fields of use are given below with examples of possible fields of use:

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

Since the majority of rodenticides are bait products, most of this guidance deals with the evaluation of the efficacy of baits. In the text it is indicated where it specifically concerns bait products or concerns other types of rodenticides.

## 1.1 Aim

The aim of this document is to provide guidance on how to assess the efficacy of rodenticides, in order to ensure that only sufficiently effective products are authorised

[1] Biocidal product (PT14): Rodenticides used for the control of mice, rats or other rodents (by means other than repulsion or attraction) outside plant growing areas, for example in farms, cities, industrial premises etc, and inside plant growing areas not to protect plant or plant products.

Plant protection product: Rodenticides applied in plant growing areas (agricultural field, greenhouse, forest) to protect plants or plant products temporarily stored in the plant growing areas in the open without using storage facilities.

Where a product is used in both situations (as PPP and BP), it will need dual authorisation for the relevant use in accordance with the last subparagraph of Article 2(2) of the BPR. See also http://ec.europa.eu/food/plant/protection/evaluation/borderline_en.htm

and therefore placed on the market for use. Animal welfare considerations are also taken into account.

## 1.2 Global structure of the assessment

Full assessment of efficacy is conducted on applications for product authorisations.

Information on effectiveness and intended use(s) of the product, together with its active substance(s), must be sufficient to permit an evaluation of the product and to define its conditions of use.

Efficacy studies (see section 2 below for the type of testing required) should be performed with the product to evaluate whether the product is effective for the intended use(s) at the specified doses. Efficacy tests should be performed with the product (in its final formulation) for which the authorisation is sought, and the composition of the test-product should be provided in the efficacy reports (especially for field tests and palatability tests). Any efficacy data from scientific literature are considered only as supportive data and should not replace efficacy data obtained from efficacy tests, which should be performed according to recognised standards. Data on the mortality and, in case of bait products palatability of the bait, resulting from these studies are compared with the specified criteria. The basis for the evaluation is the uses specified in the application (i.e. draft SPC) submitted by the applicant.

# 2. Dossier Requirements

Data on efficacy are required for every application for authorisation. The following information on effectiveness is required for each biocidal product in accordance with Annex III of the BPR:

1. Function (e.g. rodenticide) and mode of control (e.g. killing);
2. Representative organism(s) to be controlled and products, organisms or objects to be protected;
3. Effects on representative target organisms;
4. Intended concentration at which the active substance will be used and application rate;
5. Mode of action (including time delay);
6. The intended uses for the product;
7. Efficacy data to support these intended uses, including any available standard protocols, laboratory tests or field trials used including performance standards where appropriate and relevant;
8. Any known limitations on efficacy:
    - 8.1. Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies;
    - 8.2. Observations on undesirable or unintended side effects for example, on beneficial and other non-target organisms.

**Efficacy testing**

It should be noted that any efficacy testing conducted in the European Union on rodents should be in accordance with the principles set under Directive 2010/63/EU[2] on the

[2] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

protection of animals used for scientific purposes. However, field trials with rodenticide products to control wild rodent infestations under actual use conditions that are carried out to demonstrate the results of already obtained data on palatability, mortality and humaneness are not considered animal procedures for the purposes of Directive 2010/63/EU.

For all types of rodenticides, efficacy has to be demonstrated in a laboratory trial and a field trial or alternatively in a semi-field trial and a field trial for each target organism submitted in the application, unless specified otherwise in this guidance. For roof rats it is also acceptable to demonstrate efficacy:

- in two or more well-conducted semi-field trials (for description see section 2.6 below), since in some regions infestations of roof rats are quite rare; or
- Two (or more) well-conducted field trial(s) in regions with infestations of roof rats.

In general it applies that tests should be of high quality to be considered for evaluation. For animal welfare reasons, in laboratory tests, the number of animals per test should be restricted to a minimum.

Positive results in field trials may outweigh negative results[3] in laboratory studies, but only under the following conditions:

- there is at least one other laboratory study (or semi-field trial) with positive results for each study with negative results and;
- there is at least one field trial of high quality with positive results.

Positive results in laboratory studies cannot outweigh negative results in field and semi-field trials.

In case of testing only in semi-field or field trials (roof rats):

- at least two well-conducted semi-field tests or one field trial should have positive results, respectively.

The following guidance is designed to be flexible and does not specify rigid protocols to which tests must be conducted. Published or unpublished data from any source will be considered provided the data are scientifically 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 internationally recognised testing methods and in accordance with the principles set under Directive 2010/63/EU on the protection of animals used for scientific purposes. However, applicants can also submit data generated using their own testing strategies where these are conducted and well reported to a sound scientific standard. In all cases, the data must allow a specific assessment of efficacy and, in case of bait products, palatability of the product. Anecdotal evidence will not be acceptable.

Assessment will be made in relation to the effectiveness of the product for the intended uses in the draft SPC submitted with the application. This assessment will take into account the animals that are considered to be harmful and are to be controlled (target species), indoor or outdoor use, the method(s) of application, application rates, use patterns of the product, maximum storage period (shelf life) of the product, together with any other specific terms and conditions concerning the use of the product.

The target species selected for efficacy testing should be appropriate to the geographic regions in which the product will be used. They should be named in the draft SPC for the

[3] Negative results are those showing insufficient efficacy against the evaluation criteria (see section 4.1 of this Guidance).

product (either common or generic names may be used). Please note that in some countries specific rodent species are protected and no control action against them is permitted.

**Intended uses**

Examples of intended uses given in the draft SPC associated with the target organisms are :

- for use against house mice:
  - this will require testing against *Mus musculus*.
- for use against rats
  - this will require testing against *Rattus norvegicus* and *Rattus rattus*.
- for use against brown rats
  - this will require testing against *Rattus norvegicus*.
- for use against rats and house mice
  - this will require testing against *Rattus norvegicus*, *Rattus rattus* and *Mus musculus*.
- for use against rats in sewers
  - this will require testing against *Rattus norvegicus* with specifically treated bait (see section 2.4 below)
- for use against voles
  - this will require testing against at least two vole species which differ in size and behaviour, for example, water voles (*Arvicola amphibius*), bank vole (Myodes glareolus) and common voles *Microtus arvalis*.
- for use against a field mice (wood mice) species
  - this will require testing against the specified target species, for example the long-tailed field mouse/wood mouse (*Apodemus sylvaticus*) or yellow-necked field mouse (Apodemus flavicollis).
- for use against [name of target species]
  - this will require testing against the given target species. an example could be the grey squirrel (*Sciurus carolinensis*).

General intended uses given in the draft SPC, such as 'for use as a rodenticide' or ‘for use against mice’, with no further clarification of the target species are not acceptable. This is because it would allow use against rodent species for which the product is not tested and/or not intended. Concerning the target species, intended uses have to be species-specific (both for products authorised for professional and non-professional users).

Testing has to be species-specific, and for each target organism that is given in the draft SPC, a study should be conducted. This is because the biology, behaviour and susceptibility of target species, even within taxonomic groups such as rats, voles or mice, may differ considerably. For example, the brown rat (*R. norvegicus*) is more sensitive for anticoagulants than the roof rat (*R. rattus*), whereas it has been observed that the roof rat is more neophobic and will be less likely to accept baits than the brown rat. Mice are taxonomically very unspecific and may be applied to a broad range of species (e.g. *Mus musculus*, or various *Apodemus* species) with different biology, behaviour and susceptibility against the active substances. Vole species differ considerably in their size and habitat. Therefore, all target organisms given in the draft SPC have to be tested. If the authorisation of a rodenticide with a less specific intended use, such as ‘for use against voles’ or ‘for use against mice’ is applied for, the product has to be tested at least against all representative species of the respective taxonomic group. For voles there are products authorised under the plant protection products (PPP) legislation, but under some circumstances, there can be a need for biocidal product

approvals (e.g. in case of invasions near buildings and disease spreading).

Resistance claims are allowed for products based on actives with a mode of action other than anticoagulants. For products based on anticoagulants there is differing opinions of permitting claims by Member States[4] and therefore, until further discussions and decisions are made, such intended resistance claims must be considered on a case by case basis in discussion with the Member States. An intended use such as 'for use against rats and/or mice resistant to the first generation anticoagulants', is generally not possible, because test animals which are resistant to first generation anticoagulants are difficult to define and their degree of susceptibility may vary. Moreover, when a case of resistance is recognised in a field situation, it is generally advisable to use non-chemical methods like mechanical or electronic traps, rodenticide with non-anticoagulant mode of action, or the most potent anticoagulant rodenticides, and the use instructions in the draft SPC should generally contain a paragraph about resistance management. Therefore, a general intended use concerning resistance on an anticoagulant product may not be regarded as informative, since resistance generally refers to the active substance rather than a specific product.

## 2.1 Test animals

Although laboratory testing should preferably be performed on second generation wild animals housed in groups, 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.

In accordance with Directive 2010/63/EU, Articles 7 and 9 and Section A, 3.2. of Annex III, , semi-field trials should preferably be conducted using wild rodents or their offspring. Although not preferred, it is possible to use strains that resemble wild strains in semi-field trials as an alternative. These strains should be outbred strains (e.g. Long Evans or Lister Hooded rats) which retain the behavioural characteristics of wild rodents, which includes neophobia, anxiety, and fully capable sensory organs (no impairment of seeing, hearing, smelling or taste). When laboratory strains that resemble wild strains are used, a short description of the behavioural characteristics as well as reasoning for the choice of the respective strain as test animals should be provided. Generally, the diet which rodents (laboratory and wild strain) receive prior to the tests can be crucial for their behaviour towards bait products. It is therefore important that, as far as possible, the study reports should also include information on the dietary history of the test animals. It is recommended that test animals should receive a rather broad diet during breeding. Where wild animals are used in laboratory or semi-field 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.

OECD Guidance Document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2002) must be considered. Unnecessary suffering must be avoided (e.g. excessive weight loss/severe dehydration, persistent convulsions, cannibalism/self-mutilation, etc.) and animals should be checked regularly. Moribund animals should be euthanized in line with the requirements to apply humane end-points by using clinical signs to determine impending death.

[4] This issue is under review and discussion and the guidance will be updated if the situation regarding resistance claims for anticoagulants changes.

Field trials should be conducted on wild rodent infestations and are not considered animal experiments provided the respective tests on efficacy, palatability and humaneness have been confirmed under controlled laboratory studies.

The purpose of Article 62 of the BPR is to minimise the number of tests on animals and not duplicate any studies on vertebrates that might be required by the BPR. While the objective is clear for laboratory tests and semi-field trials, for which animals are used on purpose, for field trials the situation can be seen from a different perspective. Where a field trial is carried out under real life conditions and the rodents subject to such field trial would have been to be killed/controlled in any case by using other authorised products, then it is considered that such field trial does not involve any duplication of testing. Therefore, field trials for PT 14 would be exempted from Article 62 of the BPR.

Concerning laboratory tests and semi-field trials, the objectives of Article 62 (of BPR) would be achieved by data waiving where there were already tests with a fully comparable bait containing an active substance with similar or lower toxicity (see Table 1 in section 2.7 below). In such cases read-across could be accepted provided that, where relevant, a LoA (Letter of Access) is presented by the applicant.

## 2.2 Laboratory studies for bait products

For testing the efficacy of bait products, two types of laboratory studies are available, mortality tests (i.e. no-choice feeding tests) and choice feeding tests. Since mortality tests give very little information in addition to data from the bait choice feeding testing and in order to reduce the number of animal experiments, mortality tests (i.e. no-choice feeding tests) are not recommended and are not required. However, many applicants may have no-choice studies on their products as they have been conducted in the past. These can still be submitted as part of the data package but no new studies should be conducted.

Tests conducted to EPPO or the specimen protocol (Appendix 1 of this Guidance) 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, brown rat or other species 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 outbreed lab strain (e.g. CD rats) which is likely to exhibit traits of the wild strain is accepted as surrogate.

Rodenticides with special indications, for instance 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 (see section 2.3 below).

### 2.2.1 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 efficacy after a long period of storage of the product (see section 2.5 below). This test is preferably done with wild strain animals. In this test design, animals have the choice between a non-toxic 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 an indication that the bait is sufficiently palatable for a lethal dose to be ingested. Results are compared with the specified criterion (see section 4.1 below).

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. Clinical observations should be conducted to determine mode of action, degree of suffering, duration of toxicosis prior to unconsciousness, etc. These data are optional but provide useful information, especially on new active substances.

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, the main criterion is not the percentage of consumed bait but the mortality resulting from poison uptake.

### 2.2.2 Bait choice feeding trials with voles

The test protocol for choice test against voles in the laboratory should be principally the same as for rats and house mice.

## 2.3 Laboratory studies related to contact rodenticides and gassing agents

### 2.3.1 Contact rodenticides

The information that should be available in order to demonstrate efficacy will include:

i) Estimates of time to death from individually or group 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

Rodenticidal gassing agents are typically used in gas-tight buildings, ships, airplanes, containers and storage locations or for burrow fumigation. 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 when applied as described in the use instructions in the draft SPC for the product.

There are no internationally recognised standardised test protocols for testing efficacy of rodenticidal gassing agents. In general, the dossier requirements are the same as with bait products. No-choice tests are not necessary. The dossier should include simulated use-tests as well as field tests. Simulated use tests should be conducted in gastight containers. The size of the container, duration of exposure as well as the concentration of the fumigant in the container should reflect a real-usage situation.

It has to be noted that the use of gassing agents in sealed rooms, buildings, ships, airplanes or containers (generally denoted here as "rooms") is different from use in burrows (generally denoted here as "rodent burrows"). Hence, it has to be declared for which use an authorisation is applied for. For each type of use a field study must be conducted.

Generally, during each experiment the concentration of gas has to be monitored. The test reports should contain a detailed description of gas concentration, position of measurement points as well as the analytical method. The absence or presence of sorptive materials has to be documented.

Field tests for burrow fumigants should follow the protocol for rodent baits. It has to be demonstrated that rodent populations in infected objects can be eliminated. The study has to include a description of the burrow (location in the infested object, position of

entrance holes), for example, Ross, (1986), and Méthode CEB n°254 (2013) listed in Appendix 3 of this Guidance. The methods for a population census before and after application as well as the mortality criteria are the same as for bait products (see Appendix 2 of this Guidance).

Field tests for rooms should include an estimation of the population size, but it is recognised that a feeding census is often not possible (e. g. in containers). In these cases, cages with the respective target organisms (mice, rats) should be introduced to the field object. Their placement should reflect the expected distribution of rodents in the object. It is important that some cages should be placed at spots which would represent "worst case scenarios", i.e. places with air draft (since a room or container may not be perfectly airtight) or in hideouts. The test report should contain a detailed description of placement of the cages, as well as number, age and sex of the test rodents. Exposure time should be according to the use instructions in the draft SPC . After exposure, the number of dead rodents within the sealed room/compartment and/or inside the cages must be determined. Field tests with no scientifically comprehensible data on population reduction or mortality will not be accepted. In cases where a sufficient number of caged rodents have been introduced to field objects for efficacy testing, simulated use tests can be waived. The mortality criteria are the same as for baits.

Considering the risks linked to the presence of rodents in an airplane, an efficacy of 100% is necessarily required. Indeed rats and mice (these latter being able to hide in places of low volume and completely inaccessible in airplanes) can cause damage, besides the problems of public health, which affect the safety of the airplane and the passengers. Besides possible damage linked to the urine on the electronics, these rodents possess incisors with continuous growth which oblige them to eat away permanently at any type of materials (threads, girdles, steering cables, printed circuits.). There is therefore no tolerance threshold, because a single rodent can cause irreversible damage. In order to make sure that the dose administered according to recommendations and within the framework of fumigation under actual conditions, achieves the required mortality concentrations, the following requirements have to be carried out:

- during fumigation, the measurements of the "CT" (measured effective concentration x time of fumigation) must be systematically taken. The aircraft to be fumigated may not be completely airtight and gas leaks may occur, therefore measures need to be taken for the required 100% efficiency;
- for every trial, the data for the calculation of the "CT" are to be collected from the start of fumigation with statements of concentration (two minimum test points according to the type of airplanes) made at regular intervals (frequency of five minutes) for the duration of fumigation as claimed by the applicant. It is suggested that these data should be collected for two operations of fumigation;
- to make sure that there is good distribution of the gas at lethal concentrations in the entire airplane, rats in individual cages (five rats per test point) must be placed next to all the concentration test points. This will allow estimation of the relation between the measurements, the "CT" and the mortality of the rodents;
- a statement of temperature and humidity should be made.

In case a gassing agent is used in combination with a specific device or is part of a device (e.g., traps), results from laboratory choice tests as well as (semi-) field tests should to be submitted. A no-choice test is not necessary; (semi-) field tests should have the same protocol as field tests for baits. A population census like in bait tests before and after application is needed. The mortality criteria are also the same as for baits.

## 2.4 Laboratory studies related to specific efficacy claims regarding suitability of bait products for use in damp conditions

Where it is claimed that a product is suitable for use in sewers or under damp conditions, the retention of palatability (such as the effect of the heat and humidity on palatability) should be tested in a choice test[5] against all claimed target species, using product that has been specifically pre-treated to simulate such conditions. Please note that sewers are generally only infested by the brown rat.

For this purpose, the bait product must be exposed to a warm and humid surrounding for at least five days. Bait which is pre-treated in such conditions, may be tested either with experimental animals or, preferably, in a semi-natural test system (pen test). The total number of animals should be 10 to 20.

Below a preferred test protocol is described. Other test protocols will be considered on their merits and are acceptable provided they are scientifically justified.

The bait portions/blocks must be weighed before treatment and then exposed to preferably 30°C to 35 °C and 80 to 99% RH for five days. Stable conditions can best be achieved in a climate chamber. The bait should be placed in a water-permeable clay bowl, which itself is placed in a water-tight clay dish. The clay dish contains water, which permeates through the wall of the clay bowl with the bait, so that the surface of the clay bowl is permanently wet to simulate the moist surface of sewer walls. Each pre-treated bait portion/block is applied to the test animals for one day. The bait portions/blocks are then removed and replaced with new pre-treated bait. Since bait exposure to warm and humid conditions is for five days, the baits must be pre-treated stepwise, so that for each testing day, bait with exactly the same pre-treatment time will be applied. The test chamber or test cage is not acclimatised, i.e. the test animals do not experience specifically warm or humid conditions. The bait is replaced daily with freshly pre-treated bait and is offered in a wet clay bowl to maintain surface moisture, so that the bait remains wet and does not dry out during the 24 h exposure to the test animals. Specific acclimatisation of test chambers/cages to high temperatures and humidity is therefore unnecessary and not advisable, as the test animals will most likely originate from laboratory colonies which are kept under normal conditions (i.e. moderate humidity and temperature). High temperatures and humidity may cause them to react with behavioural disturbances.

To determine the bait consumption, bait is removed from the test chambers/cages each day and weighed back. After this, the bait should be dried, preferably by placement in a drying oven at 30 to 36 °C (note: since most bait blocks contain a significant portion of paraffin, the temperature for drying must not be too high). Bait portions/blocks are then weighed until no further weight decrease can be measured (i.e. the bait lost all water and is dry).

To calculate the bait uptake, it must be taken into account that the initial weight of the bait is fresh weight, whereas the final weight after bait application to the rats and subsequent drying is the dry weight. Thus, the difference between both is not exactly the amount of bait consumed by the rats, since fresh baits may contain moisture (which adds to the fresh weight at the beginning of the experiment, but is removed after drying for the final weight determination). Hence, the water content of bait must be determined by placing five untreated bait portions for each product in a drying oven until no further weight decrease is determined. The difference between the fresh and dry weight is then taken into account for the determination of the amount of bait uptake (Equation [1]):

[5] Field tests may be accepted in case of a controlled situation without re-entry of rats, but laboratory studies are preferred.

$$[1] \quad b = f - \frac{d}{(1-w)}$$

Where:

*b* is the amount of bait taken up

*f* is the fresh weight of the bait prior to heat and humidity exposure

*d* is the dry weight after bait application, consumption and drying

*w* is the proportion of water content of the bait (determined through drying of untreated bait).

The relative portion of bait taken up by the test animals in relation to overall food consumption can be then calculated as (Equation [2]):

$$[2] \quad c = \frac{\Sigma b}{\Sigma b + \Sigma a} \times 100$$

Where:

*c* is the percentage of consumed bait during the test

*b* the amount of bait taken up (corrected after Equation [1])

*a* is the amount of challenge diet taken up.

## 2.5 Studies related to specific efficacy claims regarding to the shelf life of bait products

When a bait product is claimed to be effective after a long period of storage, it is necessary to demonstrate that the product will still be effective and palatable after the stated storage period (i.e. shelf life). Analytical studies on active substance content are therefore not sufficient to support shelf life claims of bait products.

Based on expert opinion, most bait products have been found to be effective and palatable for 24 months (with preservatives) . Efficacy testing should therefore only be provided for:

- bait products with preservatives that claim a shelf life of longer than 24 months;
- bait products without preservatives that claim a shelf life of longer than 12 months;
- bait products for which the degradation of the active content is >10% and assessment of the degradation on the efficacy is needed to substantiate the shelf life claim

For bait products with a shorter shelf life claim than stated above, no efficacy tests on aged bait (i.e. product at the end of maximum storage) have to be provided. For these products it is sufficient to provide tests on fresh bait (i.e. newly produced product).

For bait products with a longer shelf life claim, the applicant must deliver data on the palatability of the product at the end of maximum storage for all target organisms claimed. The palatability of the aged product preferably is tested in bait choice feeding trials, but can be tested in field trials, provided these tests are scientifically valid (see section 2.6 below). Accelerated ageing studies, i.e. palatability studies in which the product tested is stored under challenging conditions, are not acceptable as these cannot simulate longer storage periods.

## 2.6 Field trial and semi field trial

The following text describes the field and semi-field testing of bait products, but is also largely valid for other rodenticide products.

### 2.6.1 Field trials

The aim of the field trial is to demonstrate the results on the effectiveness (palatability, mortality and humaneness) obtained during laboratory studies of the rodenticide product containing active substance under actual use conditions for the purposes of marketing authorisation. Field trials should only be performed once efficacy, palatability and humaneness have been confirmed in laboratory (semi-field) studies under Directive 2010/63/EU.

Tests conducted to EPPO or the specimen protocols (Appendices 2 and 3) are preferable but other data will be considered on their merits. Depending on the intended use(s) of the product, populations of the respective target organisms (house mice, brown rats, roof rats or others) 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. The amount of bait applied at each bait point and the distance range between bait points should correspond to those given in the draft SPC. Replenishment of the bait should follow intervals given in the draft SPC . Bait exposure should normally be for 4 days for acute products and 30-40 days for multi-dose products after the first bait uptake or less when full control is achieved. 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.

### 2.6.2 Semi-field trials

As an alternative or addition to 'field' trials, evidence of the efficacy of a rodenticide product may be obtained with semi-field trials (otherwise referred to as pen trials). A semi-field trial simulates field conditions under controlled laboratory conditions. Bait acceptance and bait uptake in the field is strongly influenced by the social behaviour of the target species. Both rat species (*R. norvegicus* and *R. rattus*) as well as house mice (*M. musculus*) are social animals, and food exploration is largely social in these species. Hence, the most important field condition to be simulated is the presence of conspecifics, i.e. the semi-field trial has to be conducted with groups of rodents. Group size should be at least 10 animals in tests with both rat species and at least 10 animals in tests with house mice. Sex ratio should be approximately 1:1 although single sex groups may be used with robust justification, e.g. to avoid unacceptable levels of aggression. Groups should consist of related animals to avoid intraspecific aggression. The test animals should either be directly caught in the field, or be bred from wild catches, as only wild-strain rodents show the typical behaviour of the target species which could be expected in the field. A test with laboratory strain rodents cannot be regarded as a proper simulation of field conditions.

The test arena should provide shelter for the animals, as well as sufficient space for the animals to roam. The minimum space requirement would be ≥ 0.5 m² per rat and 0.25 m² per mouse. If possible, cage enrichment such as branches, ladders, tunnels and wooden nest boxes with nest material may be provided and details on this should be given in the test report. Cage enrichment should be designed in a way that daily inspection for dead rodents and spilled bait material and feed causes only minimum disturbance.

The rodents have to be familiarised for at least three days with the test arena prior to bait exposure. The semi-field trial is always a choice test, and a suitable challenge diet must be provided together with the bait. The amount of bait applied should correspond to the amount given in the draft SPC. Bait exposure should normally be for 4 days for acute products and 30-40 days for multi-dose products. Bait exposure must be followed by a 14 day post baiting observation period.

### 2.6.3 Field tests with voles

For efficacy testing of products against voles, the test protocols for house mice and rats are only suitable when the infestation is inside a building. Efficacy testing outside of buildings should be conducted with a specific protocol. In contrast to rats and house mice, voles excavate and inhabit galleries (tunnels beneath the surface) for food exploration and nesting.

For each field test with voles, one test plot and one control plot should be investigated. Principally, the test protocol is the same for oral baits and gassing tablets/pellets. The pre-treatment and post-treatment censuses are conducted by counting occupied galleries. For this, at least ten galleries should be opened on each plot (treatment and control). After 24 h, the number of refilled galleries is then counted. The number of refilled single openings is set into relation to the number of openings as an indicator for vole activity. Depending on the vole species, an alternative census method could be the closing of burrow openings. Reopening of burrows is then counted as a sign for activity. During the treatment, vole activity should be controlled after 5 and 10 days with the same method.

Application of the rodenticidal product should follow the use instructions in the draft SPC. Normally, one bait portion has to be placed in each gallery. Replenishment of the bait should follow intervals given in the use instructions in the draft SPC. Bait exposure should be for 14 days. The efficacy is then calculated as (Equation [3]):

$$E = 100 * \left(1 - \frac{t2 * c1}{t1 * c2}\right)$$

Where:

*E* is the efficacy,

*t* are treated plots

*c* are control plots,

*t1* and *c1* are the ratios of refilled galleries/open galleries before treatment

*t2* and *c2* are the ratios of refilled galleries/open galleries after treatment.

Treatment and trials with oral bait should be undertaken in spring or autumn, as in the winter not much activity is to be expected, and in summer other food sources than the bait are too abundant.

## 2.7 Waivers

Waiving of laboratory trials or semi-field trials will reduce animal testing. For bait products, because the composition of the bait determines the palatability and hence efficacy of the product, even small changes in ingredients may affect the attractiveness. This may differ between target organisms and is difficult to predict in advance.

### 2.7.1 Semi-field trials

Laboratory testing of bait products (bait choice test or semi-field trial) should always be requested for new active substances, or if a product was altered regarding the active substance concentration and/or bait formulation. One exception would be if there were already test data with a fully comparable bait, i.e. containing a different active substance

but otherwise the same or similar formulation with the same mode of action and similar or lower toxicity; (see Table 1 below for a ranking of toxicity of existing active substances), in such cases read-across could be accepted; however if the two formulations contained the same active substance, then the concentration of the active substance would need to be the same.

### 2.7.2 Field trials

Field trials are always required when the composition of a product is changed. Exceptions could possibly include changes of minor importance in ingredients that are likely not to have an effect on palatability or efficacy, such as change in colour of a product. In case of waiving, the applicant needs to provide a robust justification why no testing was performed.

Read-across between species is generally unacceptable unless the applicant can demonstrate that there is no significant difference in the susceptibility and behavior of the species.

**Table 1: Toxicity ranking of known active substances used in anticoagulant rodenticides based on LD 50 (acute) data of brown rats and house mice compiled from CA-Reports, ranking from high (1) to lower toxicity (3)**

| Rank of toxicity | Active substance |
|---|---|
| 1 | Flocoumafen, brodifacoum, difethialone |
| 2 | Bromadiolone, difenacoum |
| 3 | Chlorophacinone, warfarin, coumatetralyl |

## 2.8 Biocidal Product Families (BPF)

A BPF of rodenticide baits may contain several bait products with different formulations, for example, various grain, block, paste and gel products. Each bait formulation should be allocated to a different meta-SPC[6]. Each bait formulation within the BPF has to be tested, because it cannot be predicted which form is the least palatable. It would also be difficult to select one product that could be regarded as a 'worst case scenario' for testing all the formulations. Within a given meta-SPC, an individual product should only be tested to consider the minimum level of efficacy within the concentration ranges of the active substance in that meta-SPC.

# 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 3 of this Guidance.

In addition to the standard test methods presented in Appendix 3, specimen protocols for a Choice Test and a Field Test are presented in Appendices 1 and 2 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

[6] See Q&A pair number 6 in Annex IV of the Note for guidance "Implementing the new concept of biocidal product families" (CA-Nov14-Doc.5.8 – Final.rev2). [https://circabc.europa.eu/w/browse/c309ae58-bdd7-421d-a678-8d8ac361d4e0]

factors that should be taken into account.

Any known limitations on efficacy (including resistance) should be considered during the assessment. Possible restrictions, risk mitigation measures, or recommendations concerning the use of the product in specific environmental or other conditions can be considered. 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 should be stated. Possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the selection and spread of resistant strains and the grounds for these (see TNsG on Product Evaluation and a report on risk mitigation measures for anticoagulant rodenticides as biocidal products [7] . 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 (see section 4.1 below).

# 4. Assessment of authorisation

## 4.1 Norms and criteria

In accordance with Article 19(1)(b)(1) of the BPR, a biocidal product may only be authorised if it is sufficiently effective. This is implemented in the following way.

In general rodenticide products are normally considered to be sufficiently effective if the following results can be achieved:

- required results in laboratory test and semi-field test:
  - ≥90% mortality within a relevant time frame
- required results in field test:
  - Monitoring of the test population should show a ≥90% decrease of the population

Rodenticide bait products are considered to be sufficiently effective if the following results can be achieved:

- required results in the bait choice feeding test, semi-field test and sewer test (if claimed):
  - ≥90% mortality. The percentage of ingested bait containing the product should be normally ≥20%, but it may be lower because a mortality of ≥90% the product would still be effective. In case of a bait ingestion <20%, justification should be provided.
- required results in field test:
  - feeding on census bait after treatment should be reduced by at least 90% from the levels of feeding on census baits before treatment. When other types of quantitative 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 (≥90%).

The efficacy of the product after a specified storage time (e.g. shelf life as claimed in the use instructions in the draft SPC) is also taken into account when assessing efficacy of a rodenticide bait.

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[7] "Risk mitigation measures for anticoagulant rodenticides as biocidal products" [https://circabc.europa.eu/sd/a/343a61cd-b8d4-40af-9e5c-4f763aea3240/CA-Nov14-Doc.5.1%20-%20draft_final_report_RMM.docx6].

Deviations from the norms are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case-by-case basis, consulting the other CAs where appropriate and decide whether it is acceptable or not.

In order to promote the development of new types of products (less toxic, more humane), a mortality <90% could be acceptable when the product is used as an accompanying method, (i.e. used with another product to demonstrate efficacy). but not as a stand alone product. However, mortality of these new type of products should not be <50%. The use of a product as an accompanying method should be reflected in the use instructions in the draft SPC.

For the assessment of resistance, reference is made to TNsG on Product Evaluation . Information on resistance testing techniques is also available from the Rodenticide Resistance Action Committee (RRAC)] and Prescott *et al*. (2007).

# 5. References

Prescott, C. V., Buckle, A. P., Hussain, I. and Endepols, S. (2007) A standardised BCR resistance test for all anticoagulant rodenticides. International Journal of Pest Management, 53 (4). pp. 265-272. ISSN 0967-0874.

Rodenticide Resistance Action Committee, RRAC. A Reappraisal of Blood Clotting Response Tests for Anticoagulant Resistance and a proposal for a standardised BCR Test Methodology [www.rrac.info].

Ross, 1986. Comparison of fumigant gases used for rabbit control in Great Britain. Proceedings of the Twelfth Vertebrate Pest Conference (1986). [http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1053&context=vpc12].

# Appendix 1. Laboratory studies for rodenticides : bait choice test

This appendix 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. This protocol can be applied to other target organisms (e.g. voles).

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[8] 3-5 days and at least 14 days of post-treatment observation.

**Pre-test period**

For the test, normally 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) in accordance with Directive 2010/63/EU, Articles 7 and 9 and Section A, 3.2 of Annex III . On arrival at the laboratory, the wild strains should be treated with an appropriate insecticide to kill ectoparasites and then be housed in small groups (no more than five per cage) of the same sex and treatment group if no aggressive behaviour is expected, preferably in solid floor cages with appropriate environmental enrichment. Animals may be housed individually only if scientifically justified. 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, two food pots, placed either side at the front of the cage, are filled with cereals, such as wheat, broken wheat, or a wheat-based mixture or 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. Food uptake should be determined, therefore all unused food (i.e. food left in the pot) and scattered food must be collected and taken into account by weighing to determine how much of the food has not been eaten. All unused diet (i.e. food left in the pot and scattered food) should be discarded and the pot refilled with a fresh supply, to ensure it is palatable. This procedure should be repeated for a further 3 days and on the last day (of this pre-treatment period) the animals should be weighed. Also on the last day, the diet remaining in each pot and scattered food, 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 and the other with a suitable challenge diet (e.g. an EPPO challenge

[8] Deviation from this norm is possible but should be explained in the application.

diet[9] 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 signs of toxicity and mortality are recorded. Humane end-points should be applied in line with Directive 2010/63/EU to all animals showing clinical signs that can determine impending death.

Guidance Document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2002) must be considered.

**Results**

Results should be shown as the percentage intake of rodenticide and the percentage intake of challenge diet (see section 2.2.1 for further details). Also the percentage mortality and any other symptoms should be mentioned.

**Liquid bait formulations**

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

- a suitable compounded laboratory diet shall be freely available;
- tap water must be used as the control bait;
- all procedures relating to the solid control and test baits must be applied instead and as appropriate to the liquid control and test baits;
- when the positions of the test and control baits are interchanged the positions of the drinking tubes, if used, should not be interchanged;
- liquid baits must be provided in containers with non-drip nozzles or suitable open pots;
- a filled container must be placed out of reach of the animals in order to monitor weight loss due to evaporation.

[9] 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.

# Appendix 2. Field trial for rodenticide baits

This appendix describes a protocol and factors to be taken into account when conducting a field trial to determine the efficacy of an as yet unauthorised rodenticide bait product against the house mouse, brown rat or roof rat. This protocol can be applied to other target organisms (e.g. voles).

Ideally field trials should:

- be conducted with separate rat and mice populations (as appropriate to the intended uses in the draft SPC);
- be carried out at sites that are representative of the intended uses in the draft SPC (for example industrial, commercial, domestic);
- include sites with 'known' anticoagulant resistant populations (if appropriate to the intended uses in the draft SPC);
- have had no rodenticide treatments over the past 6 weeks;
- Incorporate lag phases before and after the treatment phase;
- for testing concentrates, cover a range of bait bases;
- for product that is sold with a specific bait station, include the whole device (the bait and its station) in the test;
- be carried out at 2 or 3 locations (i.e. a trial site sufficiently far away from the next, dependent on the roaming pattern of the test organism; e.g. Sites >30 m apart for Norway rats (Buckle and Smith 2015).

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, taking into account distances as specified in the intended use, local structure and rodent activity as established prior to the trial. See also the Good Practice Document released by Cefic (http://www.cefic.org/Documents/Industry%20sectors/EBPF/Guideline-on-Best-Practice-in-the-Use-of-Rodenticides-in-the-EU.pdf), and the field trial protocol released by the RRAC (www.rrac.info/releases/technical-monographs/).

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.

The amount of bait applied in each feeding point should correspond to the amount given in the use instructions in the draft SPC. In general, for mice, the amount of bait applied in each feeding point is less than for brown or roof rats. 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 brown 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.):

- census baiting;
- tracking techniques;
- census by live trapping;
- electronic methods of census.

***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 quantitative methods. Other methods, such as electronic remote detection systems, can be used as additional information for example, in combination with bait census.

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 live traps. Live 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 live 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 draft SPC for an appropriate period (normally[10] 4 days for acute products and 30-40 days for multi-dose products). The locations of test bait 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:

- the locations of the bait points on the plan;
- the amount of bait deposited at each point at each visit and the amount retrieved, including details of the type of container used;
- the number and species of rodents and other animals found dead, and the dates on which they were found;
- the dates of all observations, treatments and censuses;
- 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 for example, 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.

[10] Deviation from this norm is possible but should be explained in the application.

# Appendix 3. 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.

**Table 2: List of standards**

| 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 | Brown 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 | Brown 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 | Brown 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 | Brown 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 | Brown 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 | Brown 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 | Brown Rat/Roof Rat | Liquid bait |
| EPA/OPP Protocol Number: 1.208 | Standard House Mouse Acute Liquid Bait Laboratory Method | House Mouse | Liquid bait |

| Standard | Title | Target Organism(s) | Mode of Application |
|---|---|---|---|
| EPA/OPP Protocol Number: 1.209 | Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method | Brown 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 | Brown Rat/Roof Rat | Tracking Powder |
| EPA/OPP Protocol Number: 1.219 | Standard Norway rat/Roof rat Acute Placepack Penetration Laboratory Test Method | Brown 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 Prufüng 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 | Brown 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 Synanthropic 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 | Brown rat/Roof rat/ House mouse | Dry Bait |

| Standard | Title | Target Organism(s) | Mode of Application |
|---|---|---|---|
| ASTM E 593-95 | Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions | Brown rat/Roof rat/ House mouse | Dry Bait |
| EPPO Standards/97(2) | Laboratory and field tests for the evaluation of rodenticidal dusts | - | - |
| EPPO Standards /113(2) | Laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticide preparations | - | - |
| EPPO Standards /114(2) | Field tests against synanthropic rodents | Brown rat/Roof rat/ House mouse | - |
| EPPO Standards /169(2) | Efficacy trials with rodenticide baits under practical conditions against Voles (*Arvicola terestris and Microtus spp*.) in their subterraean galleries" | Voles (Microtus, Arvicola) | - |
| EPPO Standards /197(1) | Non-target effects of rodenticides | - | - |
| EPPO Standards /198(1) | Testing rodents for resistance to anticoagulant rodenticides | - | - |
| RRAC rat field trial protocol 2013 | Field Trial to Evaluate the Efficacy of Rodenticide Baits for the Control of Rats (*Rattus norvegicus*) | Brown Rat/Roof Rat | Dry Bait |
| OECD | OECD Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Human Endpoints for Experimental Animals Used in Safety Evaluation (2002) http://www.oecd-ilibrary.org/environment/guidance-document-on-the-recognition-assessment-and-use-of-clinical-signs-as-human-endpoints-for-experimental-animals-used-in-safety-evaluation_9789264078376-en | - | - |
| EPPO Standards PP1 2004 | 2nd edition, volume 5, EPPO, Paris (2004), 48-56. | Voles | - |
| BBA (1963) | Richtlinie 9-2, Richtlinien für die Prüfung von Nagetierbekämpfungsmitteln gegen Schermaus (in German) | Voles | - |
| BBA (1980) | Richtlinien für die amtliche Prüfung von Pflanzenbehandlungsmitteln 18-3.3, Richtlinie für die Prüfung von Rodentiziden gegen Schermaus im Forst (in German) | Voles | - |
| Méthode CEB n°254 (2013) | Méthode d'essai d'efficacité pratique de générateurs de gaz fumigants pour lutter contre la taupe (*Talpa europaea*) et le campagnol terrestre (*Arvicola terrestris*) dans leurs galeries souterraines au champ. | Voles, moles | Gassing agent |

| Standard | Title | Target Organism(s) | Mode of Application |
|---|---|---|---|
| Méthode CEB n°257 (2014) | Méthode d'essai d'efficacité pratique d'appâts rodenticides pour lutter contre les campagnols (A*rvicola terrestris, Microtus spp.*) dans leurs galeries souterraines au champ | Voles, moles | Bait |

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