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GUIDANCE DOCUMENT ON THE TESTING OF EFFICACY OF BAITS, FOR INDOOR USE, AGAINST GARDEN ANTS

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Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2013

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FOREWORD

This *Guidance Document on the Testing of Efficacy of Baits, for Indoor Use, against Garden Ants* has been developed under the auspices of the Task Force on Biocides (TFB).

A first draft document was developed by Germany and discussed at a TFB meeting in 2011. It was further reviewed, revised and approved by the TFB in 2012. The draft Guidance Document was approved by the 25th Meeting of the Working Group of National Co-ordinators of the Test Guidelines Programme (WNT) in April 2013. The Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 10th July, 2013.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

INTRODUCTION

1. This document is intended to provide guidance when testing and/or evaluating effectiveness of biocide products (public health insecticides) used as baits for the control of *Lasius* ant colonies nesting in, or in close proximity to, buildings. This document does not deal with efficacy consideration for products used as pesticides, for example products aimed at outdoor ant elimination.

2. Guidance is provided in the design and evaluation of bait tests applied in buildings and courtyards, on balconies and terraces as well as those used in perimeter applications (within a maximum of 60 cm outside the wall of buildings), but the latter ones only if related to infestations indoors. The guidance document takes into account guidelines published previously (Commission des Essais Biologiques 1997, Umweltbundesamt 2001, US-EPA 2004).

3. Target populations include colonies of the common black or garden ant *Lasius niger* (Linné 1758), the brown wood ant *Lasius brunneus* (Latreille 1798), *Lasius emarginatus* (Olivier 1791), and *Lasius neglectus* (Van Loon, Boomsma and Andrásfalvy 1990). The *Lasius* genus is widespread in Europe and also occurs in Asia, Africa and North America; *L. niger* is the species the most often found in buildings in Central European countries (Deutsche Ameisenschutzwarte 2011, Seifert 2007). There are many reasons in support of a preference for *L. niger* over other species, such as the ease of finding and capturing young queens in autumn as they build a nest in an artificial environment without much effort, the manageability and easy handling of the resulting colonies, including the possibility for their monitoring and observation. All these factors favour the use of *L niger* over a potential use of other *Lasius* species, that – owing to their more reclusive living – are not easily traceable and often have special needs that make their maintenance more demanding.

4. The assessment of baits efficacy is usually made in relation to label claims made for the product. This guidance is aimed at testing baits that claim to demonstrate efficacy in the 'control of ant colonies', *i.e.* their complete elimination or 'colony extermination', not those claiming to 'kill' ants, as a relevant claim refers to the elimination of individual ants only. Furthermore, the guidance does not apply to the control of colonies of the mentioned species nesting outdoors.

Test products and its application

5. The present guidance applies to all products against *Lasius* ants that are effective as baits. These comprise gel, granular and liquid baits.

6. An ant control bait usually consists of a mixture of four components: (i) an insecticide, which should be slow-acting or delayed in action to ensure transfer by the worker ants to and throughout the entire colony; (ii) an attractant, usually foods or a pheromone increasing bait acceptability and attractiveness; (iii) a palatable matrix responsible for the physical structure of the bait (*i.e.* its particle size); and (iv) other materials added for specific purposes, such as emulsifiers, preservatives and/or antimicrobial agents.

7. In ants, individuals store food in their crops to feed it later to other colony members by mouth-to-mouth exchange of the regurgitated liquid or food. This phenomenon, referred to as trophallaxis, is an important component of larval care in the majority of the ant families of *Myrmicinae*, *Dolichoderinae* and *Formicinae* (Wheeler 1918, Cassill and Tschinkel 1996, Dussutour and Simpson 2008). Likewise, other colony members (worker ants, queens) are catered for; this is an interesting habit for a colony eradication

strategy to be effective. In addition, it implies that ant toxicants need to be efficient over a wide dose range as trophallaxis may dilute a toxicant to sub-lethal doses (Rust et al 2004, Soeprono and Rust 2004), jeopardizing success of a treatment. Another point for consideration is that foragers affected by the toxicant may not feed their nest mates, and instead move the colony. This is the reason why the application of residual high-toxicity spray insecticides only eliminates the small number of ants that forage on the surface; it is not suitable to eradicate an entire infestation, as not all colony members can be reached by this way.

8. In practice, application of baits in bait stations is considered the most suitable delivery method. These should be placed on locations used by ants or in areas where they are foraging. Trap utilisation must be without risk for the user, *i.e.* delivered in child-resistant containers. The use of bait stations is usually required for outdoor applications, and open application should be restricted to specific indoor operations, where baiting in trap devices is not feasible, *e.g.* for crack and crevices treatments.

9. Assessments of effectiveness are made in relation to the products' label claims. It will take into account the species to be controlled, the method(s) of application, application rates and use patterns of the product.

Test organisms – *Lasius niger*

10. As *Lasius niger* can be easily maintained under laboratory conditions, this species is recommended as test organism in simulated use tests. *Lasius niger* can be maintained in a gipsum nest system that allows observations to be carried out during the entire development of the colony (Figures 4 and 5 in the Annex) (Iglisch 2000, Umweltbundesamt 2001). In general, *Lasius brunneus* and *Lasius emarginatus* can be maintained in the same fashion. As the latter species have similar social systems, food preferences and feeding habits are similar to those of the black garden ant, results gained in simulated use tests with *Lasius niger* populations are suitable to cover these species. In any case, the origin of the test organisms should be documented as well as how the ant colonies were maintained.

11. While the establishment of colonies is not an issue here, it is to note that this is best done by capturing a queen at times of nuptial flights. More information can be found on the Internet as to when, where and how to do so in the best way. In general the queen is placed on a sponge in a small gypsum nest in a translucent container (often referred to as a "formicarium"). Water and food are provided only after the first workers appear. Upon growth, the colony is generally transferred to a glass tank containing earth.

PRE-TESTS

Laboratory Acceptance and Individual Toxicity Test

12. The test is carried out with three replicate a negative control group that consists of workers fed a sugar solution. Ants that have been starved groups (*i.e.* 10-15 worker ants per replicate) and for about three days are given a sugar solution presented in a small bowl (*e.g.* lid of a film can). Once foraging has begun, the bowl is changed for another one containing the bait formulation. When around 10-15 worker ants are feeding from the bait, the bowl is transferred (with the ants) to a box; the behaviour of the ants is observed and the time span determined until the crop is filled ("social stomach"). The occurrence of toxic effects as evidenced by changes in behavior, compared with the untreated controls, is then followed up (in intervals of 15 or 30 min) until death occurs. While the first information is a useful measure of the baits attractiveness, the latter one is an indication of the latency period.

Trophallaxis Test

13. Since baits entered into a colony undergo significant dilution in the social food flow, it is of great importance to formulate a bait in a fashion that the toxic dose delivered will be in the middle between too low a dose insufficient to kill the entire colony and an overdose killing worker ants too quickly, that is before the entire colony will be reached via trophallaxis. To this end, a trophallaxis test can be carried out. In such a test, a colony fragment of about 150 workers (a second one will serve as negative control) is transferred to a box that contains a test tube as a 'nest' (Fig. 1 and 2 in the Annex). Water is provided in the lower part of the test tube, closed off by a cotton wad. During a habituation phase, dead worker ants are replaced. Ants are starved three days prior to the test to ensure empty crops. To start the test, five worker ants are allowed to feed on the bait before they are released again to the box. In addition, a sugar solution is placed into the box, thus serving as an alternative feeding source. The number of dead ants and those with symptoms is recorded daily, up to the mortality endpoint – which can take 14 days or more. In this test, which is quite easy to carry out, active substances suitable for bait formulations usually achieve mortalities exceeding 90% within 14 days.

SIMULATED USE TESTS

Test Arenas

14. For simulated use testing, arenas with enclosures are casted that can host a nest of a *Lasius niger* colony (Fig. 5 in the Annex). The arena needs to be designed to cater for the minimum requirements of *L. niger* ants as far as size and space is concerned. The container with the nest described here contains seven sections and the actual nest is accommodated in section 5 (Figure 5), which consists of a chambered plaster board covered by a glass plate and, in addition, a plastic plate (elements 6 and 7, figure 5). The latter one blacks out the nest chamber, but its removal allows for observation of the colony members' condition and/or activity. Arenas that are designed in a similar fashion are equally acceptable, as long as they cater adequately for the needs of *L. niger* ant colonies. This should be documented.

15. A constant and high humidity needs to be maintained in the nesting chamber to prevent ants from migrating to other areas. Therefore, pads of highly absorbent material, *e.g.* cotton and sponge mat (elements 2 and 4, Figure 5) are placed between gypsum plates and moistened regularly to humidify the nest sections.

16. The test design should be configured in a way so that ants can forage in a fashion similar to their natural behaviour. To this end, the test arena setup comprises two separate arenas (boxes) connected by two tubes enabling ants to move between them. Dimensions of the various elements of the boxes are displayed in Figure 3. One box contains a nest chamber, while the other contains food, sugar water and the test bait (Figures 6 and 7). Caterpillar glue is applied around the top (30 mm) to prevent from escaping; this can also be achieved by applying talcum of liquid teflon.

Test Populations

17. Populations of *Lasius niger* are maintained at standard conditions (Iglisch 2000). Colonies should have a well-defined population of larvae, pupae and worker ants, *i.e.* $\geq$ 300 larvae at all developmental stages, about 80 pupae, $\geq$ 500 worker ants and a queen.

18. Colonies with egg masses are particularly suitable since this not only allows for monitoring development of the colony, but of the queen's reproductive activities as well. However, egg masses are not always available in colonies of this size, and can be absent during the winter months. Hence, presence of egg masses is not a requirement.

Test Design and Conditions

19. Room temperature should be maintained constant at a temperature designated as room temperature in the region where the product is sold. This is around 22 ± 3° C in temperate regions, for example Central Europe; it may differ in other regions. Relative humidity is maintained at 45 ± 15%. Conditions are recorded throughout the test. Arenas are kept at normal periods of light and dark with seasonally adjustment to the length of the photoperiod; likewise a standardized photoperiod can be chosen with a minimum of 8 hours darkness each day.

20. Water is supplied *ad libitum* in vials with cotton inserted into the top to provide ants with easy access. Water is replenished daily and the vials and cotton changed entirely once a week.

21. Tests can be designed as no-choice test (no alternative food available) or as choice test (alternative food available). No-choice testing is used to determine if a bait matrix is palatable for ants and to detect repellency and/ or behavioral avoidance of the bait. Another important objective of the no-choice testing is the screening for the amount of active ingredient required to kill ants consuming the bait. If laboratory pre-tests were promising (see paragraphs 12 and 13), no-choice tests might be waived. Choice testing will allow additional observations compared to the no-choice tests: an evaluation of the attractiveness of test baits in comparison to alternative food sources. Food preferences vary considerably among ant species, hence good bait acceptability in choice tests is a requirement when bait products are to be assessed.

22. Test organisms are allowed to habituate in the test arena for a minimum of seven days. During this period, ants are fed flies (10 dead flies twice per week; deep frozen) and sugar or honey solutions. Flies can be replaced with other insects, for example crickets or cockroach nymphs. Food not eaten is discarded. Habituation is considered successful when worker ants visit the water and food area regularly. This is evidenced by left-over, remains of pupae and faeces accumulated around the feeding area.

23. Baits are offered for a period of nine weeks (no-choice-tests) and twelve weeks (choice tests), respectively. If the queen is still alive at the end of this period, the test bait is removed and the colony is observed during additional five weeks enabling the assessment of whether a colony was able to survive and might be re-established or not. This may be possible if the surviving queen is able to produce vital eggs and if surviving worker ants are still able to tend to the queen, the eggs, and the larvae.

24. A negative control is included. Since negative controls are used to confirm that the test is not observing an unrelated effect, some groups favor provision of negative controls that should equal the bait as close as possible except for not containing the active ingredient. However, if this is not available, the regular food as negative control is as well acceptable. Relevant information is to be documented.

25. To verify the sensitivity of ants and precision of the test method, a toxic standard (if available) should be used or a reference product/positive control should be included in each trial; the latter should be a product registered for the intended use and with the same mode of action (IRAC 2011).

Assessment criteria

26. The following observations are made:

Observations within the nest

- Numbers of worker ants, pupae, larvae and eggs are estimated once per week. More frequent observations are recommended (twice weekly) if changes have occurred (see below). Counts in control populations should be done simultaneously.

Changes in the population's social system within the nest to be recorded

- Death of the queen or cessation of egg production
- Number of morbid and dead ant stages (worker ants, larvae, pupae)
- Changes in ants behaviour
- Signs of neglected brood care, *e.g.* stagnation of larval development
- Reduced or no development of pupae
- Signs for neglected nest care, *e.g.* size changes of waste piles
- Appearance of coloured guts, baits containing a dye

Observations outside the nest

Changes in ants' health and behaviour are recorded daily

- Number of visits to the test bait by worker ants, decreasing?
- Number of ants that exhibit uncoordinated, disoriented and/or extremely slow locomotion
- Number of dead workers and corpses of dead workers are removed
- Number of worker corpses at the glue barrier of the test boxes, increase?
- Size of waste piles around the nest, any or no increase over time?

27. A successful bait product is able to achieve complete control of the ant population. This may be assumed if the social system in the colony is destroyed, specifically if the queen is irreversibly affected (resulting ultimately in death or sterility) and/or when all worker ants have died. Hence, evidence is considered sufficient, if:

- the queen is dead; and/or
- all worker ants are dead.

For criteria used within the authorisation process of biocide products (BP) in member states of the European Communities, refer to paragraph 40 of this document.

Validity of the Test

28. For a test to be valid, the following conditions apply:

- The average mortality in the controls must not exceed 10% at the end of the test.
- The mortality rate in one control must not exceed 15% at the end of the test.

FIELD TESTS

29. Testing products' efficacy at field conditions is complex and requires long-term monitoring. This is particularly so because ant food preferences may not only vary among species but seasonal variation in these preferences may exist in colonies and only long term field tests may demonstrate how appropriate baits are in eliminating entire colonies (hence, demonstration of product efficacy against all target species is an essential requirement for the authorization of bait products).

30. In Central European regions, the lifecycles of *Lasius niger, Lasius brunneus* and *Lasiu marginatus are* as follows: female worker ants emerge between mid February till May. The normal swarming times (nests in buildings usually at the earlier date) for the different species are:

- *L. brunneus:* from mid-May to late June
- *L. emarginatus:* from mid-June to late August
- *L. niger:* mid-July to early August

In *L. niger*, winged reproductive ants develop from May to August and mating occurs. The females hibernate to start a new colony as a queen in the following year. Between May and February of the following year, worker ants are observed only rarely within infested buildings in Central Europe (a time span that may certainly differ in other regions). Hence, field tests should preferably be carried out when worker ants start to appear in buildings.

31. Field tests against *Lasius brunneus, Lasius emarginatus*, and *Lasius niger* are carried out in infested residential buildings. Apartment buildings and detached single family dwellings are suitable locations as their infestations can be assessed quite easily. Information on test sites is recorded and should include the size of buildings, furnishings, usage modalities, hygienic conditions, alternative food source available, nest location, time course of the infestation, and other nest locations in close proximity, if any.

32. As an alternative, if appropriate field sites are not sufficiently available, garden colonies of *L niger* can be used instead and colonies prepared using flower pots or those below larger stones. No information is currently available about whether this approach gives reproducible results in line with the existing evaluation strategies. Hence this would await validation.

33. Sites should be selected from two or three different regions, where the product will be sold.

34. Pre-treatment baiting should be carried out in early spring when worker ants first start appearing. A saturated sugar water solution that has been left standing for three days at room temperature is used for pre-treatment baiting. Five days are considered sufficient to attract workers in mild infestations.

35. The best locations for placing baits are chosen after pre-treatment baiting. Bait is delivered at the same time each day, in order to habituate the ants to forage for food at these sites. All rooms, floors and stairways within the infested building are supplied with baits, as is the immediate outdoor vicinity, as long as outside temperature does not fall below 5 °C. As *Lasius brunneus*, *Lasius emarginatus*, and *Lasius niger* do not engage in extensive trail building to food sources, worker ants trapped in the sugar water indicate the presence of an infestation. Bowls of bait placed in the perimeter are covered with glazed ceramic flowerpot bowls, to prevent use by non-target insects and/or wetting by rainfall.

36. Site specific differences in infestation intensity can be detected either by comprehensive 24 hour infestation assessment or by a pre-treatment baiting test. If more than 100 workers are found at or in a bowl and trails to it, the bowl is replaced by a box containing the test bait. An additional box (or two, if necessary) is placed at the start of the trail, both, inside and around the building. If less than 100 workers

are found at a bowl or the trail to it, the bowl is replaced by only one box of test substance. Only locations with bowls visited by ants serve as test locations. Infestations are graded and recorded prior to, during the treatment as well as after the treatment.

37. Different monitoring tactics are being considered, dependent on the type of active ingredient. It might be useful to start monitoring shortly after bait application, as ant activity during this time can provide useful information. Then, assessment can be done one, two, four, eight, and 12 weeks after bait placement. In severe infestations, assessments are carried out every three to six months, *i.e.* in June and August if test baiting started in February.

38. Field test duration covers the period until possible recurrence of worker ants in buildings. This occurs in Central Europe after the overwintering period in the following spring; hence, the test period covers 12 months. Other suggestions include baiting until no bait is consumed anymore.

Effectiveness Criteria

39. Complete control is achieved if no worker ants are found when baited with sugar-water-solution at the end of the field test period. In countries of Central Europe this is in the following spring. If, however, worker ants still visit the sugar bowls, the test product does not successfully control populations of *Lasius brunneus*, *Lasius emarginatus*, and *Lasius niger*. If worker ants are still observed at the sugar water at the end of the five day test, the population in the infested building may have been considerably reduced; however the queen may not have been killed which will entail a rebuilding of the colony.

EVALUATING BAIT SUCCESS

40. In the European countries, biocidal products may be authorized if they exhibit a 'sufficient level of efficacy'. As far as ant bait products are concerned, the Technical Notes for Guidance (EC TNsG 2011) state that this level is met when the reduction of the ant population exceeds 90% within a few weeks in a simulated use test. This translates into nine weeks (no-choice test) and twelve weeks (choice- test), respectively. In field tests, a minimum of 90% population reduction should be achieved after two to four weeks, according to the TNsG (2011). Due to the long duration of the field test this is obviously not feasible in the present case, and a longer test period needs to be considered to demonstrate complete eradication of an ant colony.

Test Report

41. The test report should contain the following information:

Test Substance: Chemical identification data (CAS number), physical nature and physicochemical properties, Product: date of production, date of expire, batch number.

Test Species: Scientific name, brood stages, age, health status, source, method of rearing and handling including specification of feed used, day of preparation.

Test Arena: Size and design of test arenas, materials, drawings and photography.

Test Conditions: Test design: number of test arenas, number of replicates; temperature and humidity recordings.

Data Management: Results should be summarised in tabular form, showing for each treatment and control group, mortality counts observed at each observation time and number of ants with adverse behaviour. Data management procedures and statistical analysis are recorded.

Deviations: Any deviation is recorded and mentioned in the report. Deviations are possible, but should be justified.

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Glossary

Active Ingredient	the insecticide component of a formulated product
Formicarium	a vivarium containing an ant colony.
Mortality	the state of a test organism in which all vital functions have ceased
Colony	a group of ants constructing a nest and rearing offspring in a cooperative fashion
Trophallaxis	The transfer of alimentary liquids and/or food among colony members

ANNEX

VISUAL REPRESENTATION OF EXPERIMENTAL SET UP

Figure 1. Ant Box (200 x 200 mm)

Figure 2. Container (Test Tube, length: 150 mm, Ø 15 mm) for ant nest as used in a Trophallaxis Test (G. Heller 2012)]

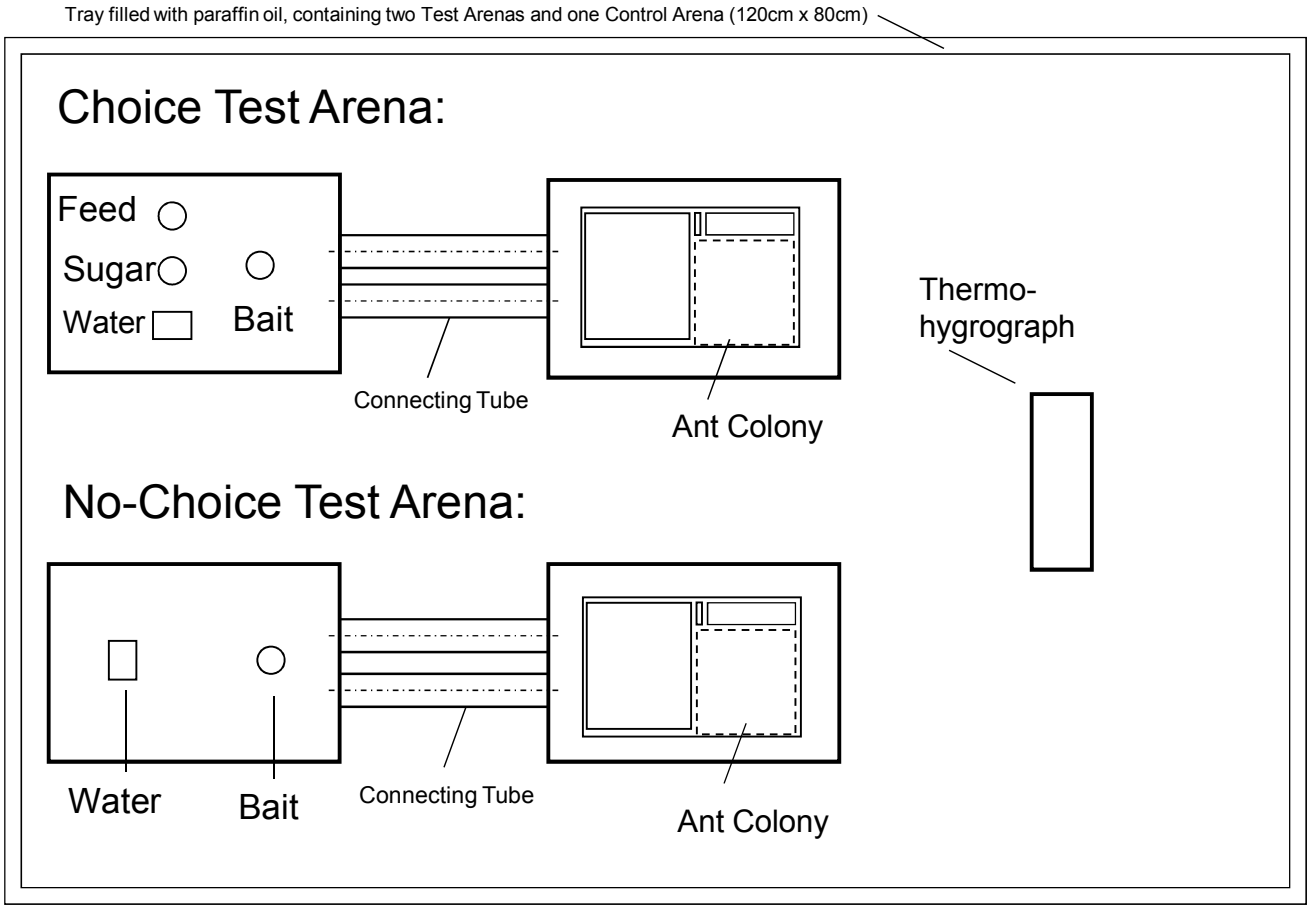

Figure 3. Tray for Experimental Set up of Simulated Use Test, top view (Umweltbundesamt 2001)

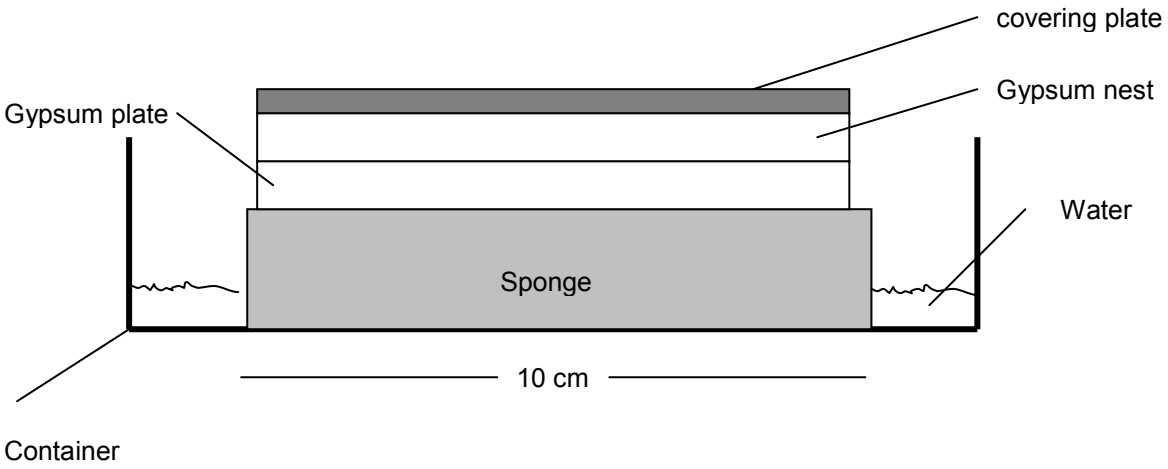

Figure 4. Experimental Set up Gypsum nest, side view (Umweltbundesamt 2001)

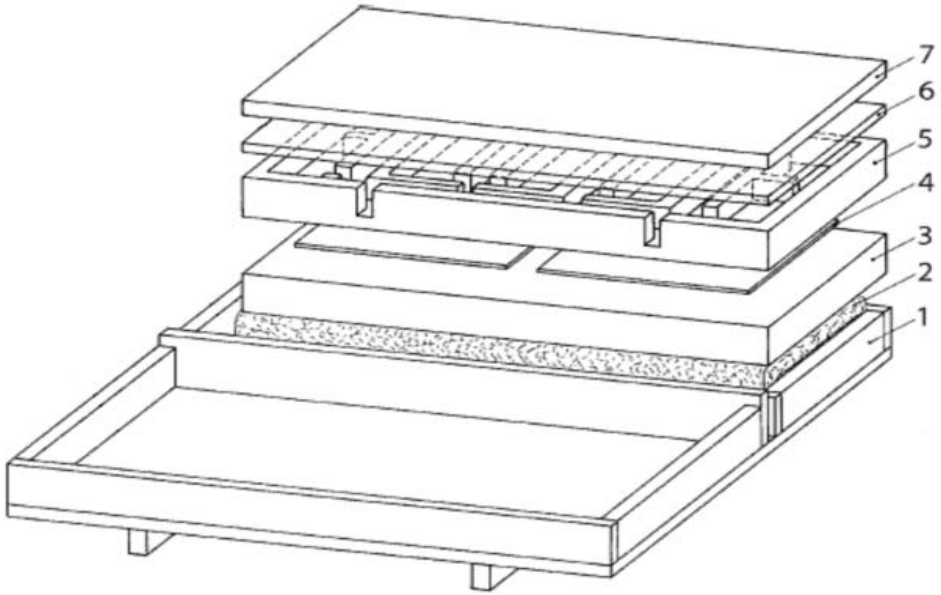

Figure 5. Experimental Set Up (1= plastic tray, 2 = absorbent cotton, 3= gypsum board, 4 = absorbent sponge mat, 5= tray for nest of ant colony, 6= translucent cover plastic, 7= plastic cover) (Umweltbundesamt 2001)

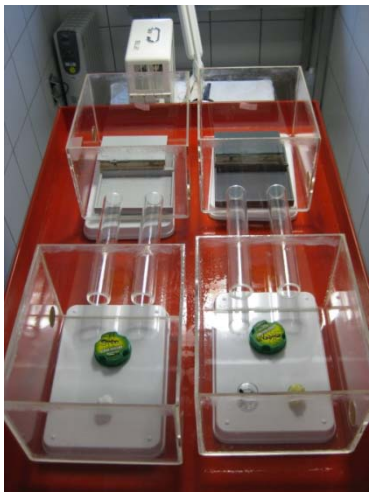

Figure 6. Experimental set-up, foreground: box containing food and test bait; and background: box containing nest of ant colony.

Figure 7. Experimental set-up, foreground: box containing nest of ant colony; and background: box containing food and test bait.

(Umweltbundesamt 2001)