DRAFT GUIDANCE DOCUMENT ON ASSAYS FOR TESTING THE EFFICACY OF BAITS AGAINST COACKROACHES

Introduction

1. This document provides guidance on conducting tests to determine and assess the efficacy of test substances and/or the effectiveness of test products used as baits for the control of cockroach species in indoor environments. It is based upon a German guideline (Federal Environment Agency 2001) and incorporates information from the American Product Performance Guidelines 810-3500 (US EPA 1998).

2. The following species are considered candidates for testing as they are species of potential public health importance in the European region:
   - German cockroach, Blattella germanica (Linné, 1767)
   - Oriental cockroach, Blatta orientalis (Linné, 1758)
   - American cockroach, Periplaneta americana (Linné, 1758)
   - Brown-banded cockroach, Supella longipalpa (Fabricius, 1798)

Of these, the German and the Oriental cockroach are the most prevalent species throughout Europe, thus constituting the test organisms most often used here, while in North America the American cockroach replaces the latter one in relative frequency of occurrence and, therefore more often serves as test organisms there. Information on the distribution of these pests and their public health importance is abundantly available (Rust et al. 1995, Cochran 1999, Pai et al. 2004, Gore & Schal 2007, Rust 2008).

Test product and application

3. A bait product usually contains one or more insecticidal active ingredients (a.i.) combined with a cockroach attractant. Active ingredients comprise toxicants with knock-down and lethal effects on all mobile stages and insect growth regulators active against nymphal stadia or instars. The present guidance document applies to all blatticides that are effective as baits. This includes formulations for use in bait stations as well as those applied in the open (e.g. in cracks and crevices). The guidance document does not deal, however, with the effectiveness of bait stations.

4. A cockroach bait should be attractive and effective even when alternative food sources are abundantly available (Nalyanya G et al 2001). In addition, it should maintain its insecticidal activity, consistency and attractiveness for the claimed period, even at damp locations such as kitchens, bathrooms and conduit pipes.

Test arenas

5. A test arena is made of easy to clean materials (glass, plastic) and its design caters for the needs of cockroaches as far as size and hiding space is concerned. Examples of suitable test arenas, which are essentially Ebeling boxes, are depicted in Fig. 1, 2 and 3 (Ebeling 1966, Appel 1992, Le Patourel 1998,
Durier & Rivault 2000a, Appel 2003). A dual-chamber system using circular boxes is shown in Figure 4. Test arenas consisting of one single large test arena (1 m²), quadratic or circular are equally appropriate (Durier & Rivault 2000a, Durier & Rivault 2002, Wang & Bennent 2006). The latter one is quite often used, particularly in laboratories in North America.

6. To avoid contamination of arenas by insecticide residues, arena grounds are completely covered by filter paper sheets, fit accurate to size and taped tightly to the walls, thus preventing cockroaches from slipping under.

7. Test arenas consist of two chambers, a ‘feeding’ or ‘activity’ chamber’ and a ‘harbourage chamber’ connected by a translucent Plexiglas™-tube (500mm long, diameter 50mm). Chambers should be rectangular (245 x 395 x 300mm; 1.12m², Annex: Fig 1-3) or cylindrical (1,01m³, Fig. 4). An impassable (chemical or electrical) barrier should be applied to the upper inner surface to prevent cockroach escape. In cylindrical boxes, a fluoropolymer (Fluon™) is applied, while the inner top of rectangular boxes can be coated by caterpillar glue or petrolatum. The latter ones are less suitable if early instars are tested. Surface areas are reduced (by a 0.1 m band of Fluon) to 0.86 m² and 0.78 m², respectively. In addition, arenas are covered by nylon screens of appropriate mesh size (e.g. mosquitos-mesh netting). The feeding chamber contains one 50 ml water vial, placed into centrally between the two baits (Fig 1). The food placement in a Petri-dish is placed into the corner (Fig 1 and 2). The harbourage chamber contains two ‘shelter (or harbourage) units’ at opposite corners or walls. Each consists of three 4 mm plywood panels (100 x 100 mm) separated by 10 mm spacers (surface area: 30 x 30 mm) when small test organisms are tested (B. germanica and S. longipalpa). Spacers should be 15 mm in height for tests with larger organisms, e.g. B. orientalis and Periplaneta spp.

8. If instead of the one described above, a single-chambered system is used, its area should be 1 m². Other details, i.e. sizes, placement and the distance to the wall et cetera of water, baits and/or alternative food should equal those described for the two chambered system (Durier & Rivault 2000a, Durier & Rivault 2002, Wang & Bennent 2006).

9. Large circular arenas (ground area 1m², Annex: Fig. 2) have been in use by some companies, especially for screening bait stations. Details should be searched for in the literature.

**Bait placement**

10. An aliquot of bait is deployed per box. It is placed into a position that is symmetrical to the food container relative to the opening. If scattered baits or gel baits are tested, the bait is placed onto a small Petri dish cover (40 mm diameter, 7 mm height).

11. Weight of the test product is taken prior to and at the end of the test. Three additional bait specimens are placed into an open bowl outside the test arena and kept there during the entire test period; their weight is taken in the same fashion. Comparison of the two will allow the determination of weight changes resulting from exogenous factors, e.g. moisture loss in gels.

12. For testing photosensitive gel baits, the translucent tubes are replaced by opaque ones. These are positioned in such a way that both of their entrance points are flush with the walls. Optimal diameter of the tubes varies with the species deployed: 9-10mm (Blattella spec, Supella spec.), 16-18 mm (B. orientalis), and 18-22mm (Periplaneta spp) or equivalent.

13. Fresh bait is replenished in all boxes if necessary. This is usually the case, when approximately 75% of the bait has been consumed in one test arena. However, certainly this may not be done when testing a product, for which a ‘single dose’ claim is under investigation.
Test organisms

14. Due to the specificity of baits, only effects against species that have been tested should be claimed (single species claims). In case a ‘general’ claim is to be made, such as ‘for use against cockroaches’, usually the two species most prevalent to the region are tested.

15. Standard susceptible strains should be used, reared in a laboratory at standard conditions and fed on insecticide free diets (e.g. Harlan Teklad™ rodent diet or other suitable diets, for example dog or cat food) (Schrader et al 2007). For the special purpose of reflecting field conditions, it might be an objective to include the testing of field-caught strains from urban centres where resistance is known to be a problem.

16. Test organisms should be in good health. However, there are some microbial pathogens that have been implicated as agents of chronic infections in laboratory roach colonies, such as bacteria (Serratia spec), protozoa (Gregarina spec.), fungi (Herpomyces spec, Metarhizium spec), nematodes (Blatticola blattae), or acanthocephales (Moniliformis moniliformis). If existing, their possible influence should be investigated and controlled for (Lopes & Alves 2005, Libersat & Moore 2000).

17. In Europe, species most often tested are B. germanica and B. orientalis, and on occasion S. longipalpa, while P americana is sometimes used as a model species for large cockroaches. In North America, however, B. germanica and P americana constitute the species most often tested with the demand for testing B. orientalis being low. For a product to be marketed with a ‘general’ label claim against cockroaches, efficacy against the two species that are most prevalent in the region should usually be tested.

18. For testing adulticides against B. germanica, 3-14 day old adult cockroaches of both sexes (only non-gravid females) are used, while the age range might be expanded in other species, for example B. orientalis, P americana and/or others to include adults aged up to 21 days. They are released to each arena by random allocation of a representative sample drawn from the rearing facility.

19. For testing larvicides, developmental stages (early instars: L1/L2; medium stages: L3/4; late stages: L5/6) are installed.

20. Size of test populations deployed varies with species and life stages tested. For arenas as proposed here, Table 1 displays recommended population sizes. Information on recommended test duration, effects investigated et cetera is displayed in Table 2.

21. Carbon dioxide anaesthesia can be used to facilitate roach transfer from breeding containers to test arenas. However, this may be done only once, as it affects insects’ behaviour (Branscome et al 2005). Other procedures to facilitate transfer include the use of ‘chill tables’.

22. Test organisms are allowed to habituate in the arenas for three days. Acclimatisation is considered successful, when – at day light conditions – most test organisms reside in the hiding towers. Before beginning the test, dead and moribund organisms are removed from the arenas and replaced by fresh ones. It is equally acceptable to account for test organisms having died or being moribund during the habituation period by reducing relevant numbers accordingly when doing the statistical calculations.

Test design – Test conditions

23. Tests can be designed as no-choice test (no alternative food available) or as choice test (alternative food available). No-choice testing is employed to determine if a bait matrix is palatable to cockroaches and to detect repellency to the bait matrix and/or level of active ingredient in the formulation. Other purposes of no-choice testing include the screening for the amount of active ingredient required to kill cockroaches consuming the bait and detecting behavioral avoidance of a bait type or bait station,
especially in cockroaches collected from urban areas. Choice testing, in contrast, will give additional insights that are important, but not necessarily evident from no-choice tests; this relates particularly to an evaluation of the attractiveness of test baits in comparison to alternative food sources.

24. Room temperature should be maintained fairly constant. It might depend upon the climatic region with the range potentially spanning from a low 19° C to a high 35 or 40° C, where the latter can be used when products are tested for which efficacy in hot climates is claimed. It is recommended that testing be carried out at temperatures that are designated as ‘room’ temperatures in the region where the product be sold. This is around 22 ± 3° C in temperate regions, for example Central Europe, but may certainly differ in other regions. Relative humidity is maintained at 55 ± 10%, if no particular requirements apply (such as the testing of baits designed for damp locations, at which occasion a company might wish to carry out tests at a higher humidity). Conditions are recorded throughout the test. Arenas can be 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.

25. Water should be supplied ad libitum.

26. A minimum of three replicate groups are dosed with each test concentration. The numbers of test individuals per replicate group and dose level (treatment group) as well as the number of replicates in the entire study need to be established prior to conducting the tests. As the improvement in power wears off substantially as the number of replicates increases beyond five, it is usually sufficient to conduct three, four or five replicate tests at each dose level. The precise number will depend on the size of the variances between and within the replicates. A power analysis should be carried prior to conducting tests in order to determine adequate sample sizes and number of replicates required. Examples are displayed in Table 1.

27. Food used in the choice and/or control tests should be rodent laboratory pellets or dog chow/biscuits and oat flakes. Food must be free of any insecticide. When investigating specific issues, food could be used that is akin to the test bait formulation.

28. Egg cases deposited during the test and/or gravid females are transferred to another container and kept there at least for seven days (Blattella, Supella) or ten weeks (Blatta, Periplaneta) for products for which relevant claims are made. Oothecae and gravid females are collected and kept separately during day one and two, and combined for all subsequent days. This allows investigating any ovicidal effects of the bait and/or its potential effects on eclosion.

Use of reference products

29. At least two negative (not treated) controls should be included. Since negative controls are employed 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, others prefer using the regular feed as negative control and this is as well acceptable.

30. A reference product (‘positive control’) should be included in each trial. This should preferably be a product registered for the intended use and with the same mode of action according to the IRAC scheme (IRAC 2010).

Effects

31. Cockroaches in supine position and not responding by movements of antennae or extremities upon prodding are classified as dead. Insects in supine position and those exhibiting uncoordinated or sluggish movements are classified as moribund. Moribund test organisms are counted as dead, if they die within the test duration (21 days). See Annex for a sample reporting form.
32. Mortality is counted at day 1, 2 and at 24 h intervals up to 21 days and is recorded separately for gender, developmental stage and location where dead bodies were found. In addition, all unusual behaviour is recorded. Dead cockroaches are removed from the arenas unless a reason exists not to do so, for example if secondary transfer of toxicity to the colony mates is under investigation (Flynn 1966, Buczkowski 2001, Buzkowski 2008).

33. If control mortality exceeds five percent in any specific developmental stage or gender, mortalities are corrected by control mortality using Abbott’s formula (Abbott 1925):

\[
\text{Efficacy} = \frac{(\text{test mortality rate}) - (\text{control mortality rate}) \times 100}{100 - (\text{control mortality rate})}
\]

where control refers to ‘negative control’

34. Time-mortality responses can be analysed by adequate statistical analyses (e.g. probit analyses) to yield lethal time values, LT_{50} and LT_{95} (Finney 1971). However, non-parametric analysis will allow the characterization of data without assuming an underlying normal distribution. Recently, therefore, several groups have switched to performing non-parametric tests, such as failure analysis, e.g. Weibull analysis or event analysis, e.g. Kaplan-Meier survival analysis. A significance level of \( p = 0.05 \) is usually considered sufficient. Further advice can be obtained from an OECD Guidance Document (OECD 2006). While 95% performance standards are usually considered in Europe (European Commission 2011), US-EPA is proposing use of a 90% performance standard.

35. Bait consumption is calculated as follows:

\[
\text{BC in mg} = \frac{[(\text{MTSB in mg}) - (\text{MTSE in mg})] \times (\text{MCSE in mg})}{(\text{MCSB in mg})}
\]

Where BC = bait consumption; MTSB = mass of test substance at baseline; MTSE = mass of test substance at test end; MCSB = mass of control substance at baseline; MCSE = control substance mass at test end.

**Efficacy evaluation**

36. Efficacy is usually considered sufficient, if 95% control (corrected according to Abbott) in a no-choice-test and 95% in a laboratory choice-test is achieved, respectively.

**Validity of the test**

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

- Average mortality in (negative) controls must not exceed 10% at the end of the test.
- Mortality rate in one (negative) control must not exceed 15% at the end of the test.

**Test report**

38. The test report should contain the following information:

**Test Substance:** chemical identification data (CAS number), physical nature and physicochemical properties
**Test Conditions:** Test design: number and description of test arenas, number of test insects per replicate, number of replicates, temperature and humidity recordings.

**Test Organisms:** Scientific name, strain, stage, age, health status, source, method of rearing and handling including specification of feed used, day of preparation.

**Disease Symptoms in Test Organisms:** abdominal swelling, changes in body colour, odour, mobility and/or behaviour.

**Data Management:** Results should be summarised in tabular form, showing for each treatment and control group, the number of cockroaches used, mortality observed at each observation time and number of cockroaches 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.

**References**


Appel AG. Laboratory and field performance of an indoxacarb bait against German cockroaches (Dictyoptera: Blattellidae). *J Econ Entomol* 2003;96:863-70


Buszkowski G, Scherer CW, Bennett GW. Horizontal transfer of bait in the German cockroach: indoxacarb causes and tertiary mortality. *J Econ Entomol* 2008;101:894-901


Le Patourel GN. Effect of arena size on behaviour and mortality of the oriental cockroach Blatta orientalis in arenas with a cypermethrin deposit adjacent to harbourage access points. Med Vet Entomol 1998;12:67-73


Lopes RB & Alves SB. Effects of Gregarina parasitism on the susceptibility of Blattella germanica to some control agents J Invertebr Pathol 2005; 88(3):261-4


Pai HH, Chen WC, Peng CF. Cockroaches as potential vectors of nosocomial infections. Infect Control Hosp Epidemiol 2004;25:979-84


Rust, MK. 2008. Chapter 2: Cockroaches in Public Health Significance of Urban Pests (pp.53-84). Editors X. Bonnefoy, H. Kampen, and K. Sweeney, World Health Organization Regional Office for Europe, Copenhagen, Denmark:


ANNEX

Table 1
Recommended Size of Test Populations per Arena (dual chamber box) for different species

<table>
<thead>
<tr>
<th>Species</th>
<th>Adults*</th>
<th>Adults and Larvae**</th>
<th>L1-L3***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
<td>♂</td>
</tr>
<tr>
<td>Blattella spec, Supella spec.</td>
<td>25</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Blatta</td>
<td>25</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Periplaneta spp.</td>
<td>25</td>
<td>25</td>
<td>30</td>
</tr>
</tbody>
</table>

* Tests with adults only (first two columns)
** Test with adults and larvae, and tests with larval stages only

Table 2
Recommended duration of tests, developmental stages and variables investigated when testing efficacy of cockroach bait formulations

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Life Stages</th>
<th>Test Duration (weeks)</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance used as adulticides</td>
<td>Adults, L_{5+6}</td>
<td>3</td>
<td>Knock down, recovery, mortality,</td>
</tr>
<tr>
<td>Juvenoids</td>
<td>L_{1+2}, or gravid females</td>
<td>8</td>
<td>Duration of development; deformities, sterility, mortality</td>
</tr>
<tr>
<td>Chitin synthesis inhibitors</td>
<td>L_{3+4}</td>
<td>6-8</td>
<td>Deformities, behavioural anomalies mortality</td>
</tr>
</tbody>
</table>
Figure 1. Arenas for testing cockroach baits: dual chamber box with rectangular units, side view

Figure 2. Arenas for testing cockroach baits: harbourage unit of dual chamber box, top view
Figure 3 Arenas for testing cockroach baits: feeding unit of dual chamber box, top view.
Fig. 4. Arenas for testing cockroach baits: schematic representation of circular arenas, top view
Abbreviations

a.i. active ingredient
BC bait consumption
d day/s
FP food placement area
h hour/s
HU hiding unit
IGR Insect Growth Regulator
Imp moribund test organisms
LT$_{50}$, LT$_{95}$ lethal times: the time until which 50% and 95% of test organisms are dead, respectively
MCSB baseline mass of control substance
MCSE mass of control substance at the end of the test
MTSB baseline mass of test substance
MTSE mass of test substance at the end of the test

Glossary

Active ingredient The insecticide component of a formulated product
Health Status Populations will be checked for clinical symptoms of diseases and/or parasites like Gregarina spec., Serratia spec., Moniliformis spec., Metarhizium spec., etc.
Moribund approaching death
Mortality the state of a test organism in which all vital functions have ceased
Ootheca Egg case, the structure in which female cockroaches place their eggs and encase them with a hard covering.
Example of reporting form for the testing of bait substance/product
(for use in dual chamber boxes)

Test Design: …………………………. (Choice/No-Choice Test)

Test Item: …………………………………….. Date / Time: ………………………

Roach Species: ………………………….. Start of Habituation Date/Time: …………..

Number of Test Organisms: ………m; …….. fem

<table>
<thead>
<tr>
<th>Test Arena No.</th>
<th>Name/Date /Time</th>
<th>Control Time</th>
<th>Harbourage Chamber</th>
<th>Feeding Chamber</th>
<th>∑ m + dead</th>
<th>Oothecas removed</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Outside HU*</td>
<td>Inside HU</td>
<td>Outside FP*</td>
<td>Inside FP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>mb</td>
<td>vital</td>
<td>mb</td>
<td>vital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H 0 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H 3 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Legend: HU = Hiding Unit; FP Food Placement Area; mb = number moribund test organisms; vital: number of organisms that are not moribund

Placement of Test Organisms into Arenas

1 h
3 h
6 h
24 h
48 h
72 h
96 h
7 d
8 d
9 d
10 d
11 d
14 d
15 d
16 d
17 d
18 d
21 d
End