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Evaluation of: "Draft Guidance Document on Assays for Testing the Efficacy of Baits against Cockroaches

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I have gone through the document and I have a number of comments, which I have given below. The numbers refers to the numbering of paragraphs in the original document.

Main document

Pt. 4. It is argued that the product should maintain its insecticidal activity in the claimed period in damp locations, but only laboratory tests are described in the guideline. The humid conditions are an important point and should be considered to be part of the guideline.

Pt. 7. In pt.5 you say: "*Test arenas consisting of one single large test arena (1m$_2$), 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.*"
The last sentence is irrelevant, and not in accordance with my experience. You have a very detailed description of the "two champers system" although you say in pt. 5 that "the one chamber system" is just as good – see citation. I agree with the statement that for many purposes the one chamber system is quite good, but then it should be described in this point, pt.7, as well.
From the given measures I cannot understand how you end up with 1.12m^2 as it refers to the champers in the text, while 1.12m^2 is actually the

total surface area of 2 champers + the Plexiglas tube (inner or outer surface?), I can not see the relevance.
Why this detailed description of the two chamber system, but not of the one chamber system, which is less complicated and just as good?

The reduction of the surface area by fluon is only of limited interest, when we know the size of the cages and only works with baits (not surface treatments).
The references to figure number are confusing. Apparently fig 1 in the text is actually fig 3 and fig 2 should maybe refer to the talk about harborage in the text.

Pt 8. Considering the problems with the area of the champers above in pt 7, I would appreciate a comment on the 1m^2 . Is this the ground area or is the walls also part of the area. It is a very big arena, in my experience a ground area of approximately 0,4 m^2 is sufficient.

Pt 9. This is a wrong reference to figure 2. Do you consider these circular arenas for OK or why is it mentioned?

Pt 10. Implying the use of the two chamber systems – there is no opening in a one chamber system. It is very important to emphasize that in the design there must be a swopping condition. In each replica there must two parts: one with the baits in position one, and then the whole thing repeated, with the two baits swopped. The bias from draft, light/shadow etc in a laboratory can be quite big.

Pt. 11 and 13. In some designs humidity in the arena is higher than in the room as such, as the ventilation in the laboratory often is very high and is reduced significantly in the arenas due to the nylon-netting. At the same time the water supply in the arena adds to humidity. I therefore always prefer to position my “weight-control” in a Petri dish inside the arena next to the test bait, covered by a suitable mech.
In pt 13 when new baits are introduced, weight-controls must also be changed. I’m not at all sure why this is suggested, what information do you get from a setup like this?

Pt. 12. Once again only the two chamber system is described, not the alternative, which in this case is a lot simpler. A one chamber system with a lit on makes the test simple. What is the reason for different tube-diameters compared to Pt7’s 50mm?

Pt. 15. Efficacy evaluation based solely on “Standard” laboratory strains should never be accepted. Laboratory strains are very good for comparison between products or active ingredients, but in my opinion such test can never

be the sole background for a positive efficacy evaluation. If trials are done solely with susceptible lab strains then adequate field trials or semi field-trials (with an adequate field-strain) must be presented to give a fair profile of efficacy. I think it may be over-kill to say you always need a resistance profile on the strain, but it definitely makes conclusions more transparent.

Pt. 19. There is no correlation between what is written here and what is said about juvenoids in table 2. where L1+2 and gravid females is mentioned, while in this text no females are mentioned, but groups of all nymphs are, which they are not in the table. Gravid females is only relevant when we are working with *B. germanica*.

Pt. 26. It has to be emphasized that replicates has to be true replicates. In my experience the most widespread error are tests done in say three arenas, with all arenas standing in the same room and all the test animals drawn from the same batch. In such cases all the data can be pooled together as there is only one "replication" in reality and there is no reason to do any statistical testing. Concerning replications please see my comment on pt. 34.
I cannot see how table 1 says any thing about power tests. But I can see a problem with the different numbers of males, females and nymphs. How do you use this in a power test, considering the well known differences in palatability, "search enthusiasm" etc between sexes and nymphs (you have even introduced different numbers of the stages and sexes in each cohort)? I think you have to explain this, to make sure that nobody misunderstands your intensions.

Pt. 27 I do agree on the rodent pellets, dog biscuits and oat-flakes, but I'm not fund of dog chow. Often the protein content is very high and it is well known to affect the cockroaches in a number of ways. A protein/carbohydrate ratio of 25/75 or less should be sought.

Pt. 29. The controls have to be the same strain and batch as the test insects.

Pt. 31. What is the conclusion if the moribund test organisms do not die within the test duration?

Pt. 34. Probit analysis is not applicable to serial-mortality data as observations on the same group of cockroaches over time are correlated. If probit analysis should be used for time data it is necessary to have separate trial-unit for each of the test periods and with sufficient replications. This is time-consuming and expensive, but also at very precise method. If correlated data are chosen then Weibull analysis has to be used (in SAS; LIFETEST).

Pt. 37. It is not very ambitious to have a 10% control-mortality. If 10% of cockroaches die within 21 days, provided they are not very old to begin with, it indicates that the colony is not very healthy. I would accept 10%, but would be concerned, that something in the setup or the rearing was not running as it should. It is evident that you must be even more concerned if one has to accept a 15% mortality in one of the replications. (This goes for laboratory reared strains. If tests are done with field-collected cockroaches, high dead rates in the controls must be expected).

Pt. 38. Test conditions. It has been a rather bad habit to refer to all sorts of national guidelines, by different cotes. It is important that a copy of the guideline in English is part of the report. When evaluations are done it is not unusually to have to search back several decades to find reports showing aspects of a problem. It is then very complicated if you have to find some obscure guideline to find the methods used.

There must be a description of the test product, not only the test substance.

Test organisms. If the cockroaches are starved before the test, the duration of starvation has to be given.

Data management: All raw data should be given in an appendix.

Appendix.

There is a confusing lack of correlation between the figure numbers in the text and in the appendix. Fig 4. States that it is a top view, but to me it looks like a side and top view. The indicated placement of baits and water supply is contradictive to the text.

Table 1. In my opinion, the use of the word “Larvae” is not appropriate when we are working with hemimetabolic insects – it should be nymphs. What is *** supposed to show. Why do you suggest different number of specimen’s in the trials?

It is important to stress that this is only the laboratory part of an evaluation, as for a full dossier on cockroach baits, field test should also be presented. The suggested report form is not in correlation with table 1: there is no room for the nymphs, not sufficient space for remarks, and very important no space for the signature of the technician doing the assessments. I personally prefer one sheet per control-time.

For some reason the whole guideline apparently are advocating for a two chamber system, but I cannot see the reason for this choice, so please explain

why you think this should be used, compared to the much simpler one chamber test . I fully appreciate that it is a good setup for many behavioral studies, but I do not se the point here.

In my opinion it must be clear that choice test is extremely important and that it has to be made always. Some reduced palatability can easily be suppressed if there is no choice.

In my opinion the guideline still need some further consideration? Hopefully I have covered the most important points. My comments above are just my attempt to suggest improvements based on my experience both as researcher and evaluator for the Danish EPA. If you find it necessary to discuss some of my points, please fell free to contact me on karl-martinv.jensen@agrsci.dk

Kind regards

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