Validation report –

Results of an International Ring test

According to the Draft Guideline

Predatory mite reproduction test in soil (*Hypoaspis (Geolaelaps) aculeifer*)

Compiled by

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Content:

0. Executive Summary

A new Test Guideline has been developed, which is designed to be used for assessing the effects of chemical substances in soil on the reproductive output of the soil mite species *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae). *H. aculeifer* represents an additional trophic level to the species for which guidelines are already available. The main endpoint is the reproduction of the mites without discrimination and quantification of the different stages of the reproductive cycle. Based on already standardised OECD and ISO test guidelines as well as ideas from literature, the ad-hoc working group HASTE (*Hypoaspis aculeifer* Soil Test) prepared a draft guideline using OECD format in 2005. This group has about 30 members from different countries, representing authorities, industry (including contract laboratories) and universities.

Using the draft guideline as a starting point, HASTE developed a study protocol which was afterwards used in an international ring test. Twelve laboratories performed in total 48 tests using two test chemicals (dimethoate and boric acid) and two test designs (NOEC, ECx). Only five tests were not valid. Seven tests (plus further work) were intended to clarify methodological details, in particular to determine the optimal test duration. Originally, a test duration of 16 days was proposed but finally 14 days were determined to be more suitable and the results are at least as sensitive. The change of the test duration was the only relevant change of the test method caused by the ring test experiences.

The results determined in the ring test proved the suitability of the proposed test method. The LC50 values for both test chemicals differed by less than a factor of 2.5 from the mean and no statistically significant differences were found between laboratories.The EC50 values from the dimethoate tests differed by less than a factor of two from the mean and no statistically significant differences were found between laboratories. In the case of boric acid the range of EC50 values was broader (they differed by less than a factor of five from the mean), but still there were no statistically significant differences between laboratories. Smaller treatment effects could be detected in the tests with higher numbers of replicates.

After the discussion of the results of the ring test in a HASTE workshop (January 2007), the group decided to recommend to include the final draft version of this test guideline into the OECD test guideline program. This recommendation is based on the evidence summarised in this report and in particular its Annexes.

1. Introduction

1.1 Background

The proposed test guideline is intended to be used for the environmental risk assessment of chemical substances, in particular plant protection products (PPPs). The detailed requirements for this test are laid down in the Terrestrial Guidance Document (EC 2002). However, the test is also suitable for other chemical substances. Its applicability is supported by the fact that ecotoxicological studies using the same and very closely related mite species as proposed here are known in the literature for many years (e.g. Schlosser & Riepert 1992). Finally, proposals for standardised tests with *Hypoaspis aculeifer* have already been made (Schlosser & Riepert 1991/92; Krogh & Axelsson 1998; Bakker et al. 2003).

The test is designed to estimate the effects of a test chemical on the reproductive output of the predatory soil mite *Hypoaspis (Geolaelaps) aculeifer*. The mites are exposed under controlled conditions to the chemical which has been mixed into the test substrate (usually OECD artificial soil). *H. aculeifer* is considered to be a relevant representative of soil fauna and predatory mites in particular for the following reasons:

- It is worldwide distributed (Karg 1993).

- The ecology of this species is well-known (e.g. Ruf 1991; 1995; 1996; Heckmann et al. 2007)

- Ecotoxicological background information is also available (e.g. Bakker et al. 2003; Heckmann et al. 2005)

- In the laboratory, it is easy to culture and to handle.

Therefore, this species was proposed as part of a battery of standard soil tests (Løkke et al. 2002), since it represents an additional tropic level to the species for which guidelines are already available.

The proposed draft guideline has been developed by the HASTE (*Hypoaspis aculeifer* Soil TEst) group. The group was founded at a meeting at the BBA in Braunschweig (Germany) on January 31, 2003. Since then, the group met once or twice per year in order to discuss various versions of the draft guideline. A first draft, already written in OECD format, was developed in late 2004. The practical work of the ring test started in late 2005, using the sixth version as its basis. It was supported by the German Environmental Agency (UBA, Dessau; S. Schmitz) and the Dutch National Institute for Public Health and Environmental Protection (RIVM, Bilthoven; E. Smit).

During a final meeting of the HASTE group in Bilthoven (The Netherlands) the results of the ring test were discussed and a new version (already the ninth one) of the guideline ready to be submitted to OECD was agreed-on. Already in late 2005, The Netherlands agreed to act as a lead country for this new guideline within OECD. Afterwards, encouraging comments from various member countries were received. This document will be used for another commenting round. It consists of four parts:

- A summary report, presenting the main methodological experiences from the ring test as well as the most important results of the this exercise

- Some information about the HASTE group in Annex I;

- The newest (ninth) version of the draft guideline, providing all the details how to perform the test, as Annex II

- In a third Annex, a detailed listing of the individual test results including their statistical evaluation is provided.

1.2 Information on the ring test

In theory, in ecotoxicology a new test is developed after a specific requirement has been identified by authorities. In reality, test ideas were often developed as part of a study with other aims (e.g. basic ecological investigations on stress reactions). Using such information as a starting point, the idea has to be transformed into a standardised test method, usually in one laboratory followed by an inter-laboratory comparison study with the aim to produce a draft test guideline. The final step of this process would be the performance of an international ring test in order to validate the method. All details are published by the responsible standardisation organisation (here: OECD (2005a)).

The HASTE group had 32 members from seven countries and with very different background, i.e. governmental institutions, universities, chemical industry and contract laboratories. Its Organizing Committee / Advisory council consist of nine persons, representing the main institutional groups as well as countries.

In total, 12 laboratories participated in the ring test. Most of them (eight) were contract laboratories while two belonged to the chemical industry. One public institution as well as one university did also participate. About two third (seven) of the ring test participants are located in Germany. The regional background of the remaining institutions is as follows: United Kingdom (two), Denmark (one), The Netherlands (one) and Switzerland (one).

In the following, names and addresses of the HASTE ring test participants are listed.

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1.3 Work performed in the HASTE Ring test

The work performed in the ring test followed closely the draft guideline which in fact differed only slightly from the newest version provided in Annex II. Few differences will be discussed more in detail in Chapter 2.2. In the following list the main features of the practical work are summarized:

Name:	Predatory mite reproduction test in soil
Guideline:	Proposal of an expert group in accordance with the collembolan test (ISO 11267), the enchytraeid test (OECD 220) and experiences from the literature (Krogh & Axelsen 1998; Bakker et al. 2003)
Test species:	***Hypoaspis (Geolaelaps) aculeifer***
Test design:	Start with adult mated females, 14 days old, exposure for 16 days
Experimental design:	ECx and NOEC approach (all tests were performed twice)
Substrate:	Artificial soil (OECD 1984) with 5% peat
Exposure:	Test substance mixed into the soil (20 g DW)
Parameter:	Number of surviving females after 16 days; number of offspring after 16 days, population growth rate (optional)
Performance:	20 ± 2°C; light : dark cycle 16:8
Food:	Cheese mites or other suitable food
Extraction method:	Berlese, handsorting
Reference substance:	Dimethoate, boric acid The test substance dimethoate was provided by BASF AG.
Statistical analysis:	See Chapter 3: Statistical support was provided by Syngenta AG: Jealott's Hill International Research Centre, Ecological Sciences Bracknell, RG42 6 EY, United Kingdom.

In total, 48 test runs were performed in the ring test. While seven of them can be classified as "non-standard methodological tests", performed in order to clarify details of the test method, 41 tests were conducted according to the Draft Guideline. Out of 22 tests with dimethoate only two were invalid. The respective numbers for the tests with boric acid are 19 (total number), including three invalid tests. It should be noted that most of the individual laboratories provided up to four tests (two designs time two test substances), but in some cases only one or two data sets were delivered.

2. Methodological Overview

This chapter is divided into two parts. Firstly, the experiences made with the standard tests, i.e. those performed according to the draft test guideline are described. In the second part, the aims and results of the additional tests "non-standard" tests are discussed.

2.1 Compilation of experiences made with the standard tests

This compilation is organised like the draft guideline; i.e. it starts with those aspects of the test performance which may influence the test results and ends with a brief overall assessment of the quality of the data provided. Finally, some recommendations how to proceed are given. In order to facilitate reading, the information (which has been collected from the ring test participants in standard forms) is provided either in tables or figures.

The source of the mites used in the test is given in Table 1. Actually, two third of them derive from animals collected in The Netherlands and were cultured for many years at Mitox (Amsterdam). This situation raised the question whether the use of mites from other sites (or strains?) may influence the test results. For this reason, tests with mites from different sites were performed at the University of Bremen (see Chapter 2.2). While this situation may raise questions it has also to be stated that the fact to have a reliable source (including species determination) clearly improves and facilitates the test performance.

Tab. 1: Origin of test organisms:

Source:	Dimethoate	Boric acid	Sum (%)
Mitox (NL)	12	11	66
Koppert (NL)	2	2	10
Katz (D)	4	2	14
Biol. Crop Prot. (UK)	2	2	10
Sum	22	19	100

Relatively few responses were received from the participants regarding the culturing conditions, which can be interpreted as a hint that no problems occurred. Actually, based on the experience with this species, this outcome was expected. In the different laboratories light regimes varied, but no conspicuities regarding this point were observed. Nearly all participants used the mite *Tyrophagus putrescentiae* as food source, while only in one laboratory juvenile collembolans (*Folsomia candida*) were provided as food. No problems were reported in all cases.

According to the Draft Guideline, the mites should be 28 – 35 days old at the beginning of the test. In the tests with dimethoate, the age ranged from 21 – 35 days (on average 29 days (Fig. 1)), while they were slightly older in the tests with boric acid: 27 – 35 days (on average 30 days (Fig. 2)). In the non-valid tests, the age of the mites was in the required range (dimethoate: 28 + 34 days; boric acid: 28, 33 + 34 days). Thus, the validity of a test run is not correlated with the age of the mites. One participant proposed to increase the age of the mites by about a week, i.e. 30 – 40 days at the beginning of the test. However, this proposal was rejected because most participants made good experiences with the proposed range of 28 – 35 days.

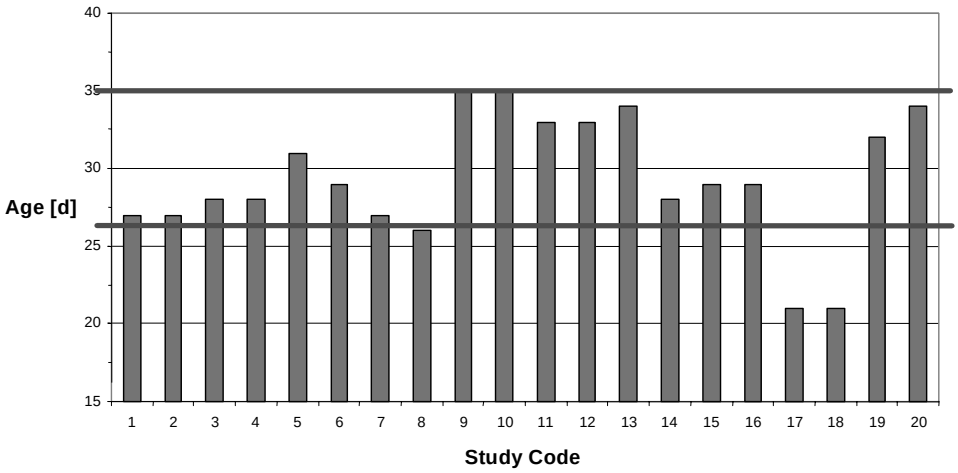


Fig. 1: Age of the mites at the beginning of the tests with dimethoate (in red: range required by the draft guideline)

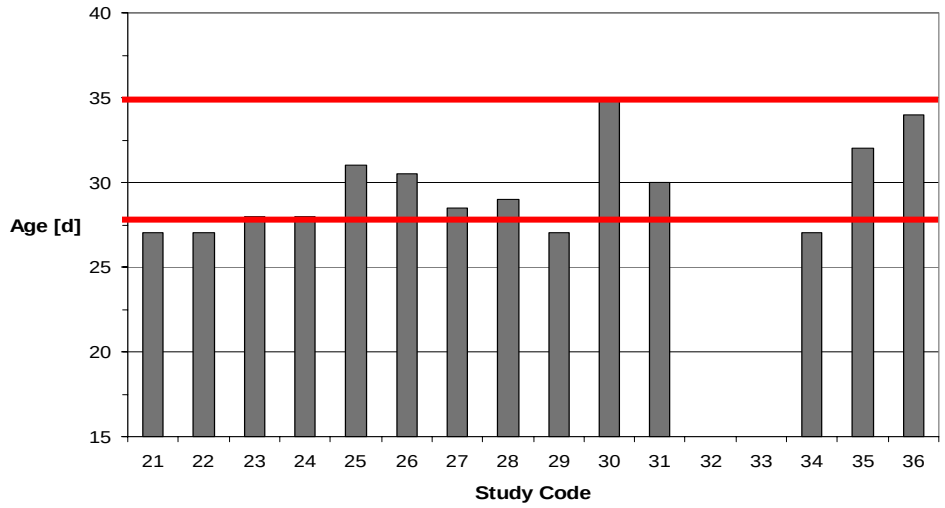


Fig. 2: Age of the mites at the beginning of the tests with boric acid (in red: range required by the draft guideline)

The preparation of the test substrate OECD artificial soil did also not cause problems. It should be highlighted that this is the first standard test in which the peat content of the artificial soil is decreased from 10 to 5%. Most of the participants used glass vessels for the tests, but few were made of plastics. Their size differed considerably, but no correlation with the occurrence of invalid tests or other problems were reported. Out of 36 valid tests, in two cases the amount of soil per test vessel was not reported and in three cases more soil (24 – 30 g) than required was added. In all invalid tests the amount of soil was correctly 20g.

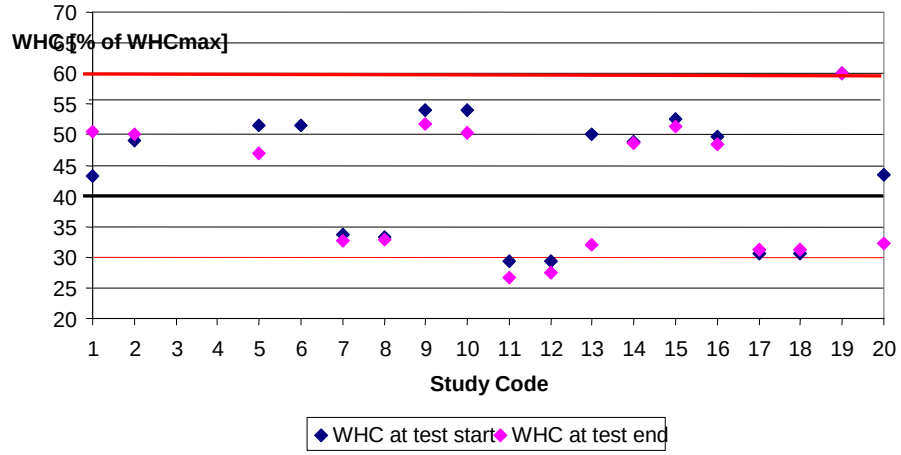


Fig. 3: Mean soil moisture (% WHCmax) at the end of the tests with dimethoate (in red: range required by the draft guideline)

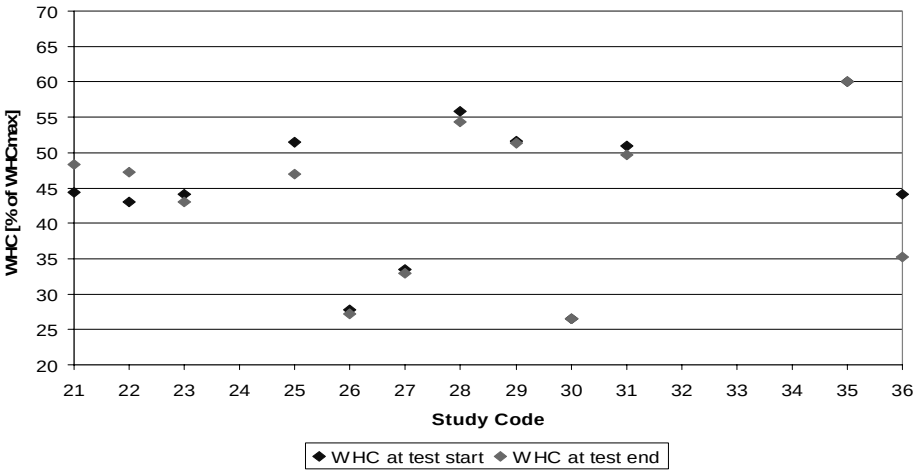


Fig. 4: Mean soil moisture (% WHCmax) at the end of the tests with boric acid (in red: range required by the draft guideline)

The soil moisture at the end of the test (% WHC) was too low in 12 out of a total number of 36 valid tests (required: 40 – 60 % WHC) (Figs. 3 + 4). In the two and three non-valid tests with dimethoate and boric acid it was partly too low (28 % and 26 or 27%), partly not reported. Therefore, dryness has probably affected negatively mite reproduction, but it is surely not the sole reason, because there were more tests with relatively dry soil at the end of the test but with acceptable numbers of juveniles. This situation cannot be clarified in detail because it is not known for how long the soil was drier than required.

The pH values in soil should have been in the range of 6.0 ± 0.5. As can be seen from Figures 5 and 6, the pH value was never too low. In one test (slightly in three) out of 36 valid tests it was too high. However, the pH value in non-valid tests (dimethoate: 6.0 – 6.3; boric acid: 6.1 – 6.3) was within the required range, meaning that pH did not affect the validity of the tests.

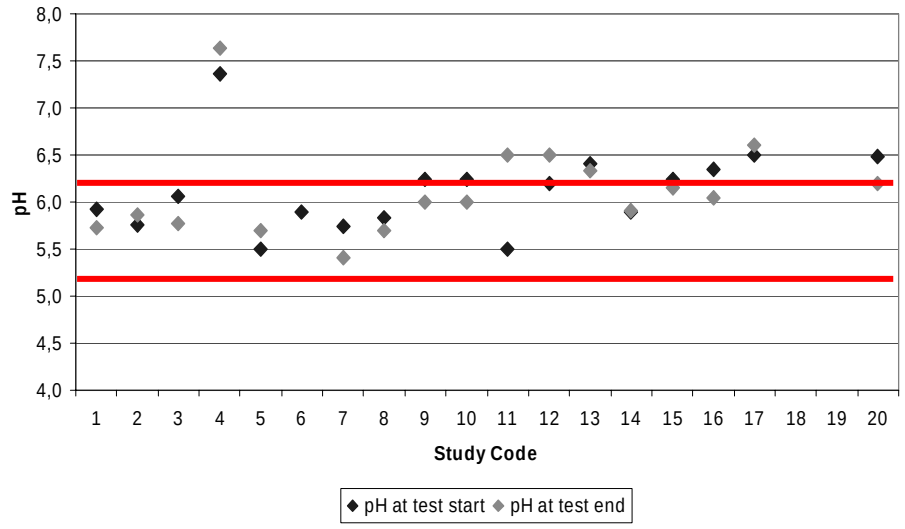


Fig. 5: Mean soil pH at the start and the end of the tests with dimethoate (in red: range required by the draft guideline)

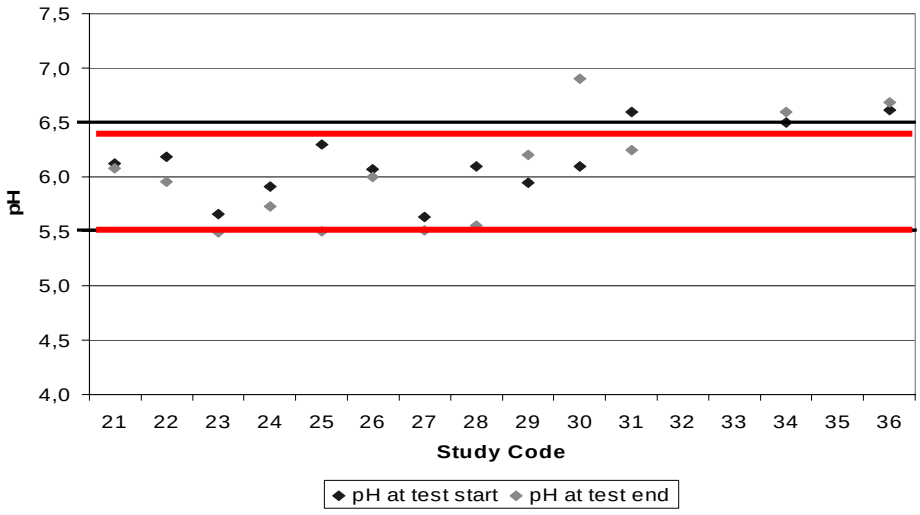


Fig. 6: Mean soil pH at the start and the end of the tests with boric acid (in red: range required by the draft guideline)

According to the OECD Draft guideline, the test organisms should be introduced into the test vessels within 60 minutes. With two exceptions this step was performed in all tests in less than two hours. In the non-valid tests, the introduction of the mites occurred in less than one hour. Since there was no hint at all that this modification of the draft guideline did affect validity, it was proposed to change the text of the draft accordingly.

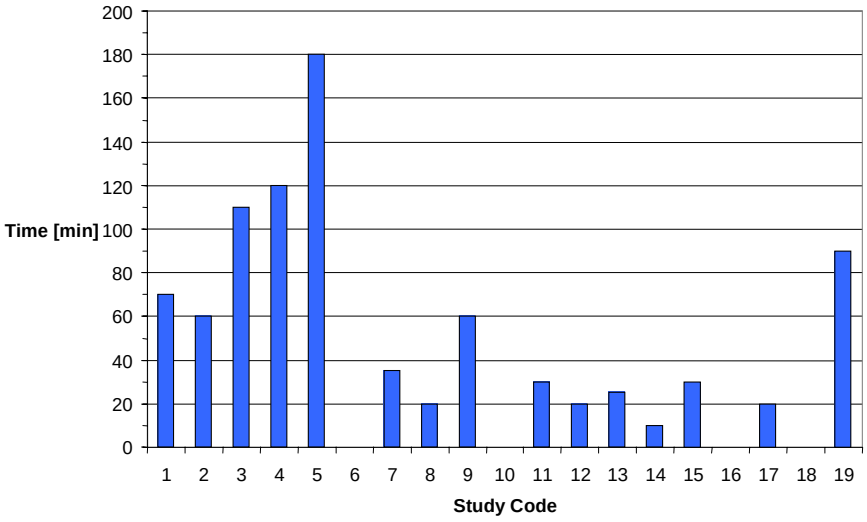


Fig. 7: Introduction of test organisms in the tests with dimethoate

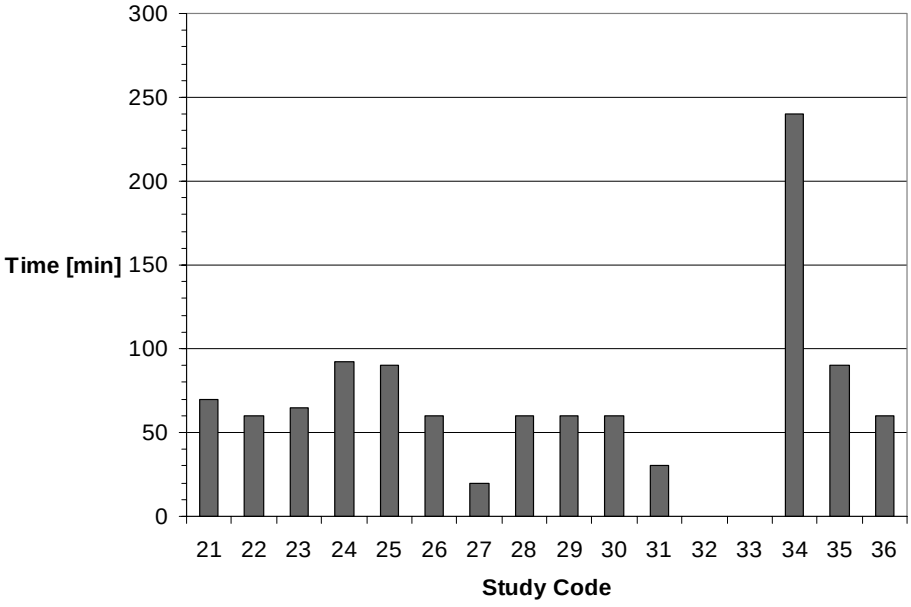


Fig. 8: Introduction of test organisms in the tests with boric acid

Concerning the number of replicates it differed in 7 test runs with dimethoate from the draft guideline requirements, while changes were made in the test with boric acid. The test duration was not reported or longer than expected (16 days) in 14 out of 41 tests. Mainly caused by practical reasons (e.g. weekends) the test was running two days longer, in one case even for 21 days. Concerning other test properties like temperature, humidity, light regime and feeding, responses are not available from all participants. However, with one exception (no light; feeding only twice during the test) no conspicuities with test validity were observed.

According to the draft guideline the extraction efficiency should be higher than 90 %. Out of 36 valid tests only for 24 tests the efficiency was given (Figs. 9 + 10). In five cases the efficiency was lower than 90%. The efficiency in the non-valid tests is not known in two and between 88 – 95 % in three tests, meaning that there is probably no relationship with the validity status of the tests.

Fig. 9: Extraction efficiency (% of introduced mites) at the end of the tests with dimethoate (in red: range required by the draft guideline)

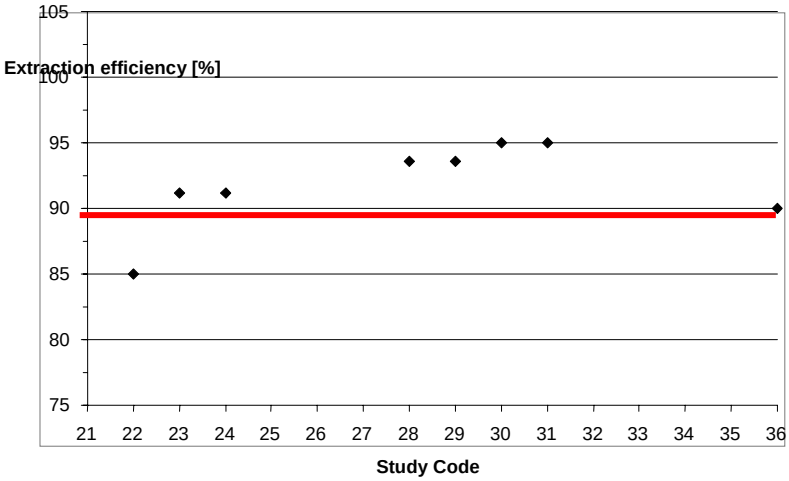


Fig. 10: Extraction efficiency (% of introduced mites) at the end of the tests with boric acid (in red: range required by the draft guideline)

In the following, the deviations from the OECD draft guideline are quantified, separately for the tests with dimethoate and those with boric acid (almost all participants performed firstly the tests with dimethoate before moving on to the test with boric acid).. As a general tendency, the amount of valid tests increased with increasing experience with this test system.

Summary of valid dimethoate tests (20):

No deviations: 11 tests (= 55%)

Minor deviations: 6 tests (= 30%)

The minor deviations were for example a brief overstepping of pH or temperature values, mainly caused by established laboratory routine or simple mistakes.

Major deviations: 3 tests (= 15%)

For instance, no abiotic measurements were made, replicates got lost or feeding was only done twice. Again, these deviations were mainly caused by mistakes.

Summary of valid boric acid tests (16): Summary of invalid tests (5):

No deviations: 3 tests

Minor deviations: 0 test

Major deviations: 2 tests

(e.g. no abiotic measurements, no lid on the test vessel)

In general, no single factor could be identified why a test is invalid (i.e. number of juveniles too low). However, dryness of the test substrate as well as individual mistakes are probably the most important ones. The test performance and test result quality was evaluated on the basis of 22 criteria (i.e. all issues discussed in the Chapter so far but in more detail). In the following, the outcome of this evaluation presented is for the two test substances separately. The numbers indicate how many participants reported a specific criterion in a sufficient way (all participants = 100%).

No deviations: 11 tests (= 69%)

Minor deviations: 1 test (= 6%)

For example, accidentally 20 instead of 10 animals were put into one replicate.

Major deviations: 2 tests (= 12.5%)

The major deviations were actually the same like in the tests with dimethoate.

Not reported: 2 tests (= 12.5%)

Dimethoate:

Maximum conformity with OECD draft:	100%	(preparation of artificial soil)
Minimum conformity with OECD draft:	45%	(moisture content at test end)
Maximum issue not specified:	30 %	(egg laying period)

Usually, important issues are reported by 80 – 100% of all participants.

Boric acid:

Maximum conformity with OECD draft:	81%	(culture temperature, test vessels)
Minimum conformity with OECD draft:	37%	(temperature during extraction)
Maximum issue not specified:	50 %	(egg laying period)

Usually, important issues are reported by 50 – 81%.

2.2 Results from the non-standardized tests

In these tests the effects of test duration, prey type and *Hypoaspis* strain on the test outcome were tested. The issues of prey type and *Hypoaspis* strain were important to clarify since the previous version of the guideline leave these points open up to now. The test duration was questioned for practical reasons and because of the problem of the presence of F1-adults.

2.2.1 Test duration (14 versus 16 days)

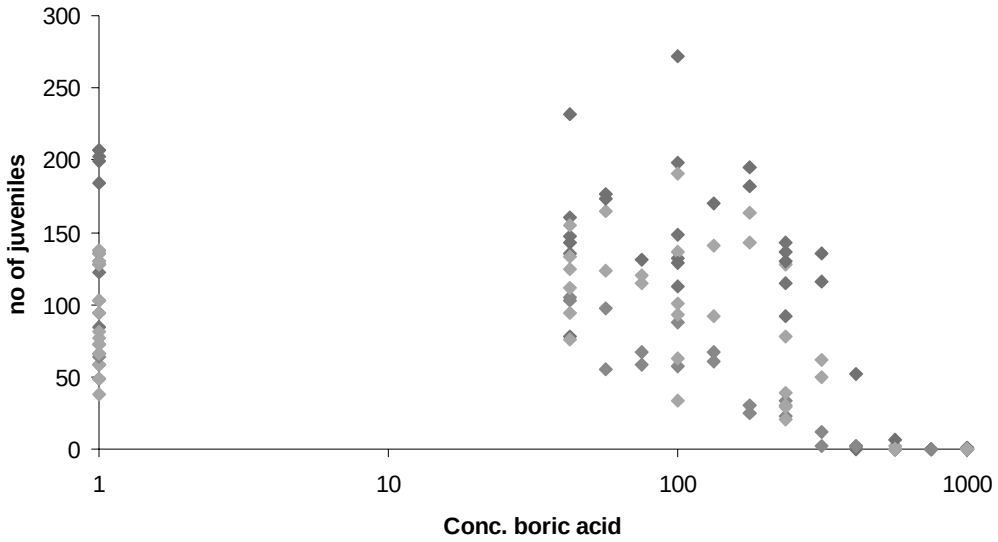


Fig. 11: Number of Juveniles at test end at different concentrations of boric acid at a test duration of 14 days (red / orange / yellow diamonds) compared to a test duration of 16 days (greenish diamonds). Tests were performed at Bremen University laboratory.

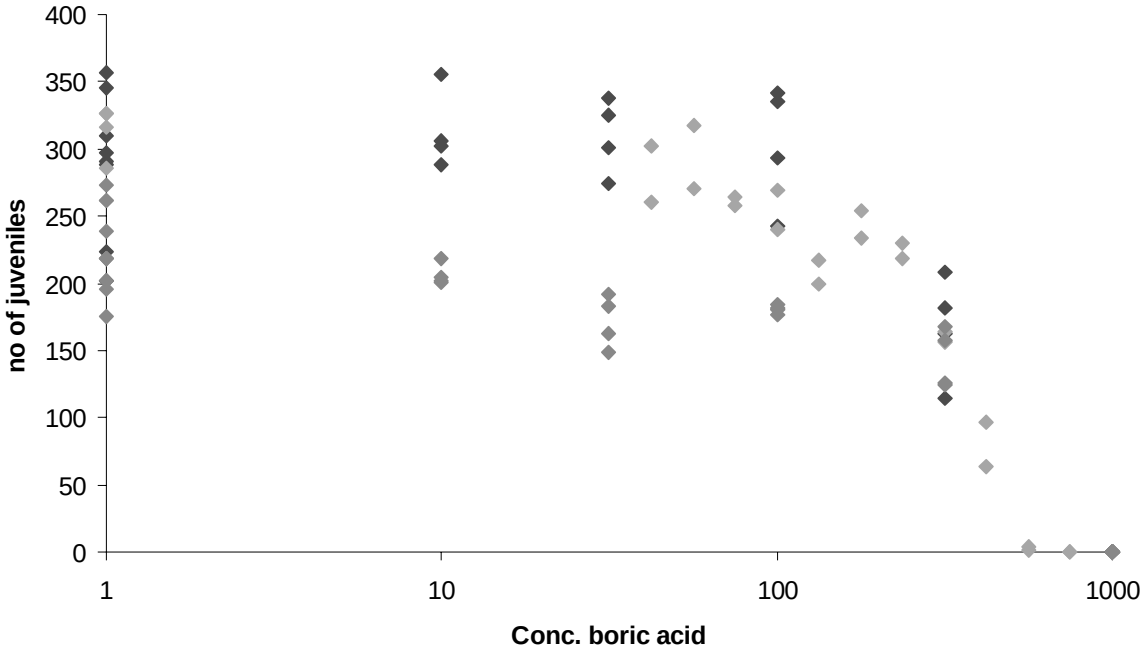


Fig. 12: Number of Juveniles at test end at different concentrations of boric acid at a test duration of 14 days (orange diamonds) compared to a test duration of 16 days (greenish diamonds). Tests were performed at the ECT laboratory

The shorter test duration of 14 days would facilitate the test handling in laboratory schedules because with 14 days no work on weekends has to be planned. But the more important concern was that after 16 days of test F1 adults could already be present in the test containers. Since these adults cannot be distinguished from the parent generation, such results would probably lead to wrong interpretation.

Therefore, tests were performed in the University of Bremen and ECT Oekotoxikologie GmbH. According to these results the number of mites is lower in the test containers after a test duration of 14 days tests compared to the number found after a test duration of 16 days (Figs. 11 + 12). In terms of sensitivity, no difference was found in the tests with the two durations. In addition, the variation is slightly lower in the 14 days tests, meaning that NOEC values could be lower than in the tests with a duration of 16 days (Tab. 2). At least the effect values (EC50, NOECs) are on a similar level.

In conclusion the 14 days tests could be less variable, slightly more sensitive, provide results two days earlier and make no weekend work necessary. In addition the 14 days tests are on the safe side in respect to the occurrence of F1 adults, meaning that a misinterpretation of the mortality rate would be avoided. So there are many arguments to favour 14 days as recommended test duration instead of 16 days.

Tab. 2: LC50, EC50 and NOEC values for tests with 14 days and 16 days duration. Tests were performed at the ECT lab. Data from Diploma Thesis of C. Scholer

Duration	**Dimethoate**		
	LC50	**$NOEC_{Repro}$**	**EC50**
14 days	4.7	**< 1.0**	5.0
16 days	4.4	3.2	5.6
Duration	**Boric acid**		
	LC50	**$NOEC_{Repro}$**	**EC50**
14 days	**755.4**	**10**	370.9
16 days	> 1000	100	347.3

2.2.2 Prey organisms

Two prey species (*Tyrophagus putrescentia* and *Caloglyphus michaeli*) were compared using criteria like handling and test performance in studies performed at the University of Bremen. The first mite species is frequently used as prey since it has a good nutrition value, but it is very small and swift. The other mite species is thought to be of lower nutritional value (Heckmann et al., 2006), but it is large and hardly leaves the substrate it is exposed on.

The results show that there was hardly any effect on test performance and sensitivity when using the two different mite species with different nutritional quality (Fig. 13). Therefore, prey type seems to be not an important issue for this test. The predatory mite test showed its robustness in this respect. But results could be different for other chemicals and prey species from different orders because such prey species could differ in terms of the exposure pathway and their sensitivity toward the tested chemical.

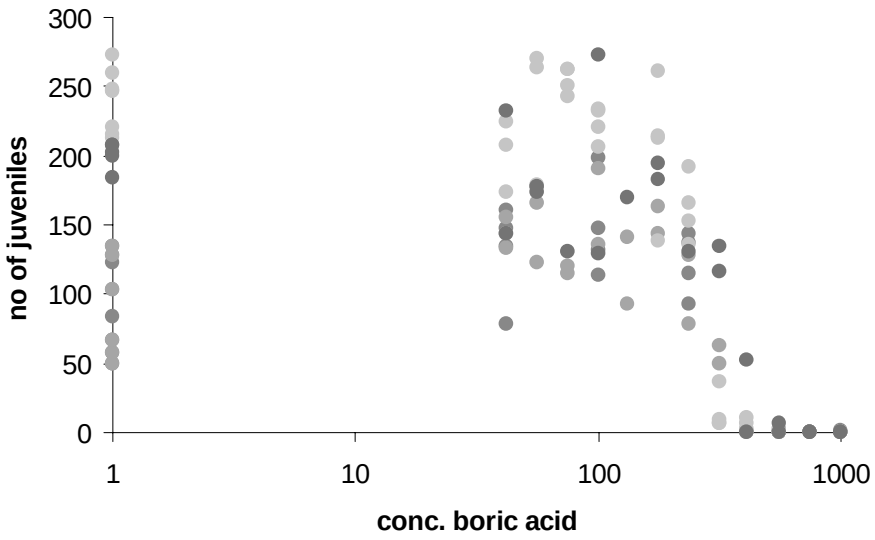


Fig. 13: Number of Juveniles at test end at different concentrations of boric acid at with *Caloglyphus michaeli* as prey (orange dots) compared to *Tyrophagus putrescentiae* as prey (green dots). Tests were performed at the Bremen University lab.

2.2.3 *Hypoaspis* strain

The comparison of the two *Hypoaspis* strains from Bremen and those from Mitox, Amsterdam showed that they differed considerably in their life history traits (Fig. 14 + 15). The Amsterdam strain was somewhat more fertile and had a slightly quicker juvenile development.

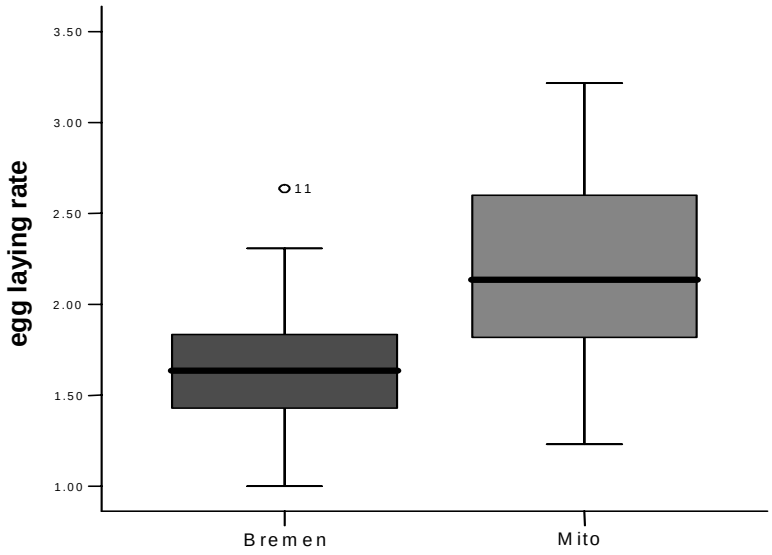


Fig. 14: Average number of eggs per day (first 18 days) of *Hypoaspis aculeifer* mites from the Bremen strain (red column) compared to the Amsterdam, Mitox strain (blue column).

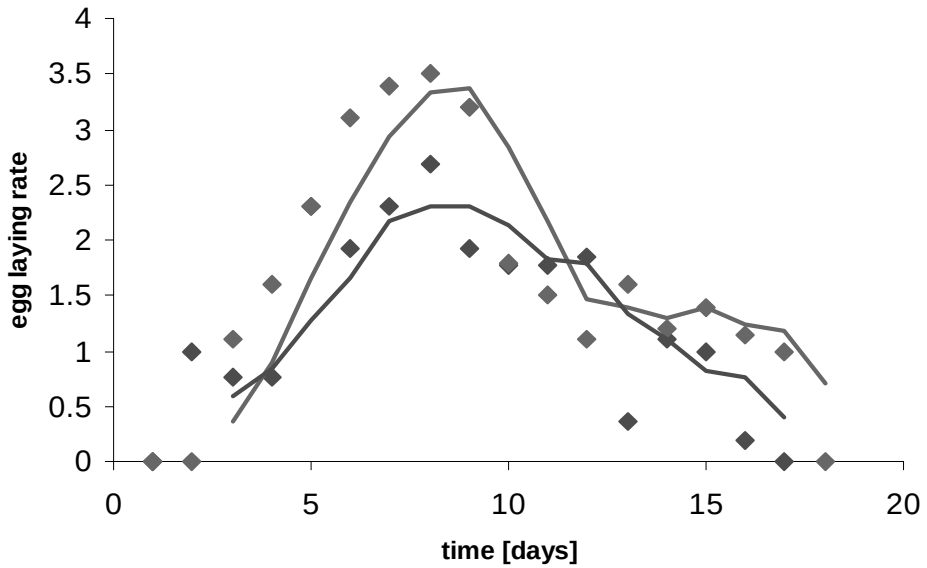


Fig. 15: Number of eggs per day of *Hypoaspis aculeifer* mites from the Bremen strain (red) compared to the Amsterdam, Mitox strain (blue) during the early adult stage of the mite.

In conclusion there is no obvious difference in sensitivity towards chemicals between the two strains (data not shown but included in overall results). Therefore it is not necessary to define a specific strain to fulfil the aims of the guideline. But there are differences in life history traits between strains and those could have consequences for defining validity criteria, like the minimum number of offspring and variability.

3. Summary of Results

The purpose of this chapter is to report the statistical analyses performed on the data available from a ring test of the new OECD draft guideline (OECD 2005b). The test guideline is designed to be used for assessing the effects of chemicals in soil on the reproductive output of the soil mite species *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae). Two test items were used in the ring test, namely dimethoate and boric acid.

3.1 Experimental design

The new draft guideline (OECD 2005b) recommends two different experimental designs depending on whether a NOEC or ECx (based on reproduction) is to be determined. Each of the twelve laboratories involved in the ring test carried out one or more tests using a NOEC design, an ECx design, or a combined NOEC/ECx design. These designs are discussed for each test item below.

Dimethoate: The NOEC design comprised five or six test concentrations of dimethoate and a control, with four replicates per test concentration and eight replicates per control. The test concentrations used in the NOEC design ranged from 0.85 to 11.2 mg a.s./kg soil (dw). The ECx design comprised between eight and twelve test concentrations of dimethoate and a control, with two replicates per test concentration and four or six replicates per control. The test concentrations used for the ECx design ranged from 0.18 to 100 mg a.s./kg soil (dw). The combined NOEC/ECx design used by laboratory 10 comprised ten test concentrations of dimethoate and a control, with two or four replicates per test concentration and eight

replicates for the control. The test concentrations used by laboratory 11 for the NOEC/ECx design ranged from 1 to 13.3 mg a.s./kg soil (dw). For details see Table 1a (Annex III).

Boric acid: The NOEC design comprised five or six test concentrations of boric acid and a control, with four replicates per test concentration and six or eight replicates per control. The test concentrations used in the NOEC design ranged from 10 to 1000 mg a.s./kg soil (dw). The ECx design comprised between seven and twelve test concentrations of boric acid and a control, with two or three replicates per test concentration and four or six replicates per control. The test concentrations used for the ECx design ranged from 1.8 to 1800 mg a.s./kg soil (dw). The combined NOEC/ECx design used by laboratory nine comprised eleven test concentrations of boric acid and a control, with four replicates per test concentration and eight replicates for the control. The test concentrations used by laboratory 10 for the combined NOEC/ECx design ranged from 42.2 to 1000 mg a.s./kg soil (dw). For further details see Table 1b (Annex III).

At test initiation, ten mated adult female soil mites (*H. aculeifer*) were introduced into each test vessel. At test termination (after 16 days exposure), the number of surviving females and the number of juveniles per test vessel were determined. Mortality was determined as the number of missing females. The mortality and reproduction data are summarised in Tables 2.1a to 2.20a in Annex IIIa (dimethoate) and Tables 2.1b to 2.16b in Annex IIIb (boric acid), and shown in full in Tables 1.1a to 1.20a in Annex IIIa (dimethoate) and Tables 1.1b to 1.16b in Annex IIIb (boric acid). It should be noted that mortality and reproduction data are missing in (i) dimethoate test 12 (lab. 6, NOEC design): replicates 5, 6, 7 and 8 of the control and replicates 3 and 4 of the 7.5 and 10 mg a.s./kg soil (dw) treatments, (ii) dimethoate test 13 (lab. 6, ECx design): replicates 5 and 6 of the control, (iii) dimethoate test 18 (lab. 10, NOEC/ECx design): replicate 4 of 1.8 mg a.s/kg soil (dw) treatment and replicate 2 of 5.6 mg a.s./kg soil (dw) treatment, (iv) boric acid test 13 (lab. 9, ECx design): replicate 3 of the control, replicate 1 of the 75 mg a.s./kg soil (dw) treatment, replicate 2 of the 133 mg a.s./kg soil (dw) treatment and replicate 2 of the 237.1 mg a.s./kg soil (dw) treatment.

3.2 Summary of Control Data

The information of the controls as summarised in Tables 3a (dimethoate) and 3b (boric acid) may be used to establish the validity criteria, i.e. the minimum level of survival and number of offspring that should be met in the controls for a test to be considered valid.

Table 3a: Dimethoate: Summary of control data

Test	Lab.	Design	Mean % Mortality	Min. juveniles per replicate	Max. juveniles per replicate	% CV
1	1	NOEC	3.75	318	385	7
2		ECx	6.67	311	344	4
3	2	NOEC	2.50	308	355	5
4		ECx	3.33	365	437	7
5	3	NOEC	2.50	188	259	10
6		ECx	3.33	265	326	8
7	4	NOEC	5.00	179	294	17
8		ECx	3.33	102	207	23
9[a]	5	NOEC	5.00	186	321	20
10[b]			5.00	209	262	8
11[a]		ECx	5.00	186	321	24
12	6	NOEC	7.50	165	214	11
13		ECx	12.50	80	151	25
14	7	NOEC	3.75	300	387	8
15		ECx	5.00	216	304	11
16	8	NOEC	7.50	144	199	12
17		ECx	6.67	172	204	7
18	10	NOEC/ECx	2.50	237	337	12
19[c]	11	ECx	2.50	48	119	38
20	12	NOEC	1.25	214	296	12

[a] these controls were shared between the two tests

[b] this test was not part of the ring test (14 days exposure rather than 16)

Table 3b: Boric acid: Summary of control data

Test	Lab.	Design	Mean % Mortality	Min. juveniles per replicate	Max. juveniles per replicate	% CV
1	1	NOEC	1.25	268	363	12
2		ECx	1.67	301	388	10
3	2	NOEC	2.50	381	471	7
4		ECx	0.00	358	398	4
5	3	NOEC	1.67	198	275	14
6	4	NOEC	1.25	103	153	16
7		ECx	3.33	126	154	8
8	5	NOEC	22.50	202	357	19
9[b]		NOEC	8.75	175	273	15
10		ECx	10.00	219	326	15
11	6	ECx	6.67	123	182	14
12	8	ECx	5.00	168	251	16
13	9	ECx	8.00	184	207	5
14		NOEC/ECx	2.50	213	272	9
15c	11	ECx	2.50	48	119	38
16	12	NOEC	2.50	176	257	11

[b] this test was not part of the ring test (14 days exposure rather than 16).

[c] this control was shared with dimethoate test 19

From Table 3a and 3b it can be seen that: (i) the average female control mortality at the end of the test ranged from 0 to 22.5%, (ii) the number of juveniles produced per control replicate at the end of the test ranged from 48 to 471, and (iii) the coefficient of variation based on the number of juveniles produced per control replicate at the end of the test ranged from 4 to 38%.

3.3 Method of Analysis and Results

3.3.1 Mortality Data

3.3.1.1 Estimation of Median Lethal Concentration (LC_{50})

The median Lethal Concentration (LC_{50}) is defined as the concentration which results in 50% mortality of the test population. The LC_{50} values from the individual tests were obtained by fitting a linear probit model to the mortality per replicate, with logarithm (base 10) of nominal concentration as the explanatory variable (Collett 1999). Since mortality was observed in one or more replicates of the control groups of all twenty dimethoate tests (see Table 1a) and fifteen of the sixteen boric acid tests (see Table 1b), the probit model included an additional parameter to account for natural (or background) mortality. The probit model was

fitted using the method of maximum likelihood, in which the binomial distribution of the data was taken into account. If possible, confidence limits for the LC_{50} estimate were determined using Fieller's method. Goodness of fit of the model was assessed using the likelihood ratio residual deviance. The probit model was fitted using the PROC PROBIT procedure in SAS version 9.1 (SAS 2002-2003). The LC_{50} values and their associated lower and upper 95% confidence limits are shown in Tables 4a (dimethoate) and 4b (boric acid), below.

Table 4a: Dimethoate: LC_{50} values with 95% lower and upper confidence limits

Test	Lab.	Design	LC_{50}	Lower	Upper
1	1	NOEC	3.66	3.33	4.03
2		ECx	4.23	3.48	4.84
3	2	NOEC	4.04	.	.
4		ECx	4.53	3.04	5.12
5	3	NOEC	3.86	.	.
6		ECx	2.47	2.08	2.93
7	4	NOEC	4.62	3.95	5.07
8		ECx	5.44	.	.
9	5	NOEC	3.67	3.25	4.09
10*			4.80	3.86	5.64
11		ECx	4.07	3.30	4.77
12	6	NOEC	7.13	6.47	7.93
13		ECx	3.74	2.89	4.51
14	7	NOEC	3.53	2.72	4.12
15		ECx	5.59	.	.
16	8	NOEC	5.80	5.03	6.42
17		ECx	6.40	5.56	7.27
18	10	NOEC/ECx	25.54	15.26	95.62
19	11	ECx	4.10	2.94	5.07
20	12	NOEC	2.64	2.31	2.95

* this test was not part of the ring test (14 days exposure rather than 16).

Table 4b: Boric acid: LC_{50} values with 95% lower and upper confidence limits

Test	Lab.	Design	LC_{50}	Lower	Upper
1	1	NOEC	19416.42	.	.
2		ECx	20906.28	.	.
3	2	NOEC	587.49	.	.
4		ECx	749.18	.	.
5	3	NOEC	991.57	.	.
6	4	NOEC	8267.48	.	.
7		ECx	567.51	470.84	665.35
8	5	NOEC	1567.43	.	.
9*			751.48	495.12	1347.41
10		ECx	668.43	523.41	791.06
11	6	ECx	748.65	657.10	820.12
12	8	ECx	164221.87	.	.
13	9	ECx	396.66	282.72	517.16
14		NOEC/ECx	762.72	.	.
15	11	ECx	6926684.28	.	.
16	12	NOEC	293.34	251.71	326.60

* this test was not part of the ring test (14 days exposure rather than 16).

LC_{50} values have been obtained for all twenty dimethoate tests and all sixteen boric acid tests. Confidence limits around the LC_{50} values have been obtained for sixteen of the twenty dimethoate tests and for six of the sixteen boric acid tests. The LC_{50} value for dimethoate test 18 (namely 25.54 mg a.s./kg soil (dw)) and for boric acid tests 1, 2, 6, 8, 12 and 15 (namely 19,416.42, 20,906.28, 8,267.48, 1,567.43, 164221.87 and 6,926,684.28 mg a.s./kg soil (dw) respectively) were obtained from the probit model by extrapolation and, in each case, are higher than the highest concentration tested. The mean dimethoate LC_{50} value is 4.44 mg a.s./kg soil (dw), with minimum and maximum values of 2.47 and 7.13 mg a.s./kg soil (dw) respectively (excluding dimethoate test 18). The mean boric acid LC_{50} value is 651.71 mg a.s./kg soil (dw), with minimum and maximum values of 293.34 and 991.57 mg a.s./kg soil (dw) respectively (excluding boric acid tests 1, 2, 6, 8, 12 and 15). The individual LC_{50} values, together with the mean, minimum, and maximum values are shown graphically in Figure 16a (dimethoate) and Figure 16b (boric acid).

Figure 16a: Dimethoate LC_{50} values obtained from nineteen predatory mite reproduction tests in soil. [Note: The LC_{50} value for dimethoate test eighteen has been excluded as it was obtained by extrapolation and is higher than the highest concentration tested.]

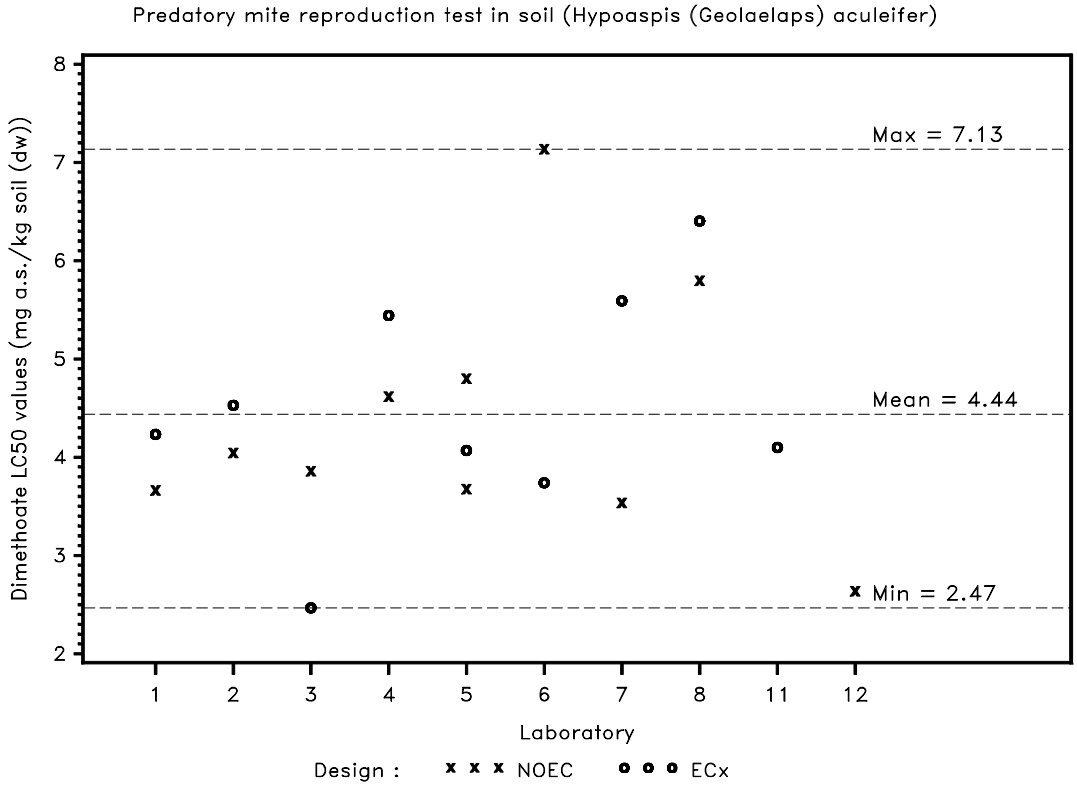
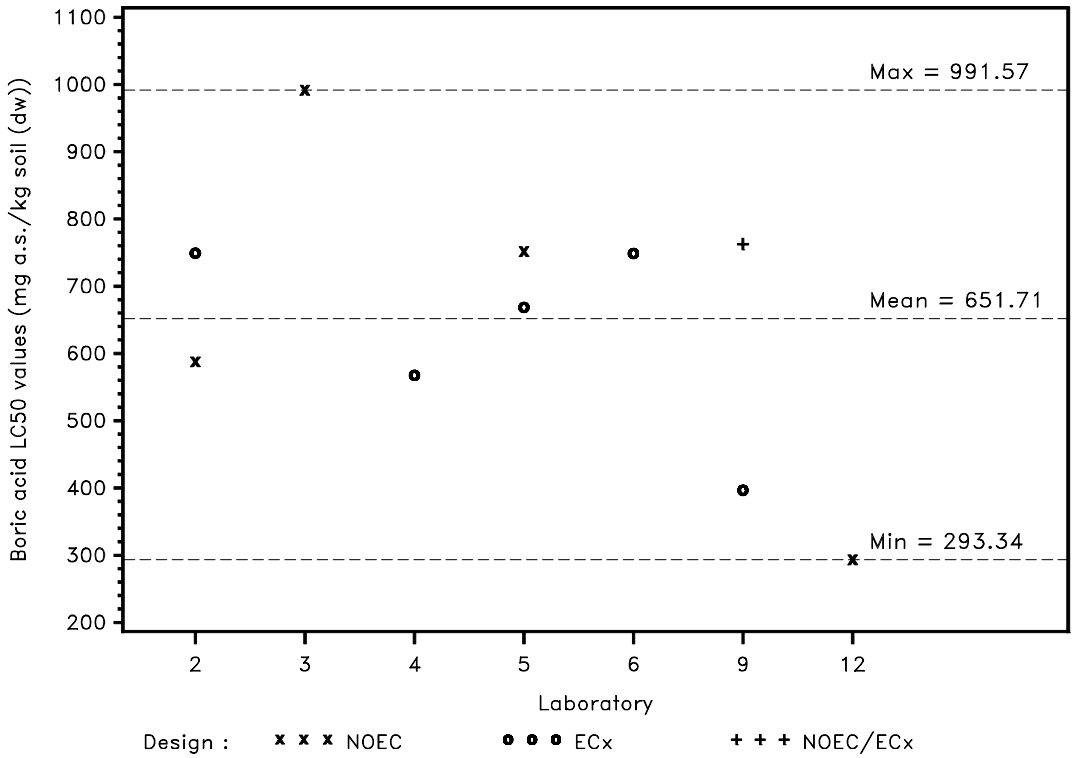


Figure 16b: Boric acid LC_{50} values obtained from ten predatory mite reproduction tests in soil. [Note: The LC_{50} values for boric acid tests one, two, six, eight, twelve and fifteen have been excluded as, in each case, they were obtained by extrapolation and are higher than the highest concentration tested]



Figures 3.1.2a to 3.20.2a in Annex IIIa (dimethoate) and Figures 3.1.2b to 3.16.1b in Annex IIIb (boric acid) display the observed mortality data and fitted probit models. Control data has been included on the figures in Annex IIIa (dimethoate) at 0.1 mg a.s./kg soil (dw) and on the figures in Annex IIIb (boric acid) at 1 mg a.s./kg soil (dw) (rather than 0 mg a.s./kg soil (dw)) as the x-axis has been displayed on the log (base 10) scale (and log of zero does not exist). The values of 0.1 and 1 mg a.s./kg soil (dw) for dimethoate and boric acid tests respectively were chosen as they are lower than any of the concentrations used in any of the tests. The model parameters are shown in Table 3.1a in Annex IIIa (dimethoate) and Table 3.1b in Annex IIIb (boric acid). From Tables 3.1a and 3.1b it can be seen that the estimated natural mortality from the fitted probit models ranged from 1.19 to 15.31%.

Results of the goodness of fit test are shown in Table 3.2a in Annex IIIa (dimethoate) and Table 3.2b in Annex IIIb (boric acid). From Table 3.2a it can be seen that there is evidence that for eight of the twenty dimethoate tests (namely tests 5, 7, 10, 11, 14, 16, 17 and 19) the probit model displays a significant lack of fit. A similar observation can be made for six of the sixteen boric acid tests (namely tests 1, 6, 8, 10, 13 and 14; see Table 3.2b) For these tests, the confidence limits around the LC_{50} values have been inflated using a heterogeneity factor (based on the likelihood ratio residual deviance).

3.3. 1.2 Estimation of No Observed Effect Concentration (NOEC)

The No Observed Effect Concentration (NOEC) is defined as the highest test concentration that does not produce a statistically significant adverse effect on mortality when compared to the control. The NOEC values from the individual tests were obtained by comparing the mean mortality at each test concentration with the control using a two-tailed Fisher's exact test with a stepdown Bonferroni adjustment (the

Bonferroni-Holm adjustment) with a 5% type one error rate (Sokal & Rohlf 1995). Statistical analyses to determine the NOEC values were carried out using the PROC MULTTEST procedure in SAS version 9.1 (SAS 2002-2003).

For each test the minimum detectable treatment effect (MDTE) was computed. The MDTE is the smallest level of mortality (after adjusting for any control mortality) on a test item treatment which would be declared statistically significant. The MDTEs are unique to their respective tests, since they are functions of the number of individuals on the control and test item treatments, the number of comparisons made against the control and the response rate on the control treatment. They were computed as follows: The number of individuals allocated to the control and test item treatment and the number that died in the control were fixed at the corresponding values in the test. Separate contingency tables were created by varying the number of dead individuals on the test item treatment from zero to the total number exposed. P-values for each table were determined (using a two-tailed Fisher's exact test). The cut-off p-value depends upon the overall number of comparisons made in the test and is computed as 0.05 divided by the number of test item treatments. Contingency tables with p-values less than the cut-off are significant. Abbott's correction (Abbott 1925) was used to adjust the percentage mortality on the test item treatments from the significant tables. The MDTE is the smallest positive adjusted value from the significant tables. The dimethoate NOEC values (Table 5a) range from 0.75 to 7.50 mg a.s./kg soil (dw) and the boric acid NOEC values (Table 5b) range from <42.4 to 1000 mg a.s./kg soil (dw) .

Table 5a: Dimethoate: NOEC values

Test	Lab.	Design	NOEC
1	1	NOEC	1.80
2		ECx	3.20
3	2	NOEC	3.20
4		ECx	3.20
5	3	NOEC	1.37
6		ECx	1.80
7	4	NOEC	4.00
8		ECx	3.20
9	5	NOEC	1.78
10			3.16 (not part of the ring test: 14, not 16 days of exposure)
11		ECx	3.16
12	6	NOEC	5.60
13		ECx	1.80
14	7	NOEC	3.00
15		ECx	5.35
16	8	NOEC	4.10
17		ECx	4.62
18	10	NOEC/ECx	7.50
19	11	ECx	0.75
20	12	NOEC	1.00

Table 5b: Boric acid: NOEC values

Test	Lab.	Design	NOEC
1	1	NOEC	316.0
2		ECx	562.0
3	2	NOEC	524.9
4		ECx	565.3
5	3	NOEC	500.0
6	4	NOEC	60.0
7		ECx	320.0
8	5	NOEC	1000.0
9*			316.0 (not part of the ring test: 14, not 16 days of exposure)
10		ECx	562.3
11	6	ECx	563.0
12	8	ECx	1000.0
13	9	ECx	177.8
14		NOEC/ECx	<42.2
15	11	ECx	749.9
16	12	NOEC	200.0

* this test was not part of the ring test (14 days exposure rather than 16).

The minimum detectable treatment effect values are shown in Table 3.6a in Annex IIIa (dimethoate) and Table 3.6b in Annex IIIb (boric acid). These values, together with the minimum and maximum values are shown graphically in Figure 17a (dimethoate) and Figure 17b (boric acid). The level of control mortality in boric acid test 8 was such that it was also possible to detect a decrease in mortality relative to control if the unadjusted observed mean mortality on a test item treatment was <2.5%. In all of the situations where laboratories tried both NOEC and ECx designs the NOEC designs were able to detect smaller treatment effects than the ECx designs could.

Figures 3.1.1a to 3.20.1a in Annex IIIa (dimethoate) and Figures 3.1.1b to 3.16.1b in Annex IIIb (boric acid) display the observed mortality data. The results of Fisher's exact test are shown in Table 3.4a in Annex IIIa (dimethoate) and Table 3.4b in Annex IIIb (boric acid).

Figure 17a: Dimethoate Minimum detectable treatment effect (after adjusting for control mortality), expressed as a percentage of exposed individuals, obtained from twenty predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps) aculeifer*)

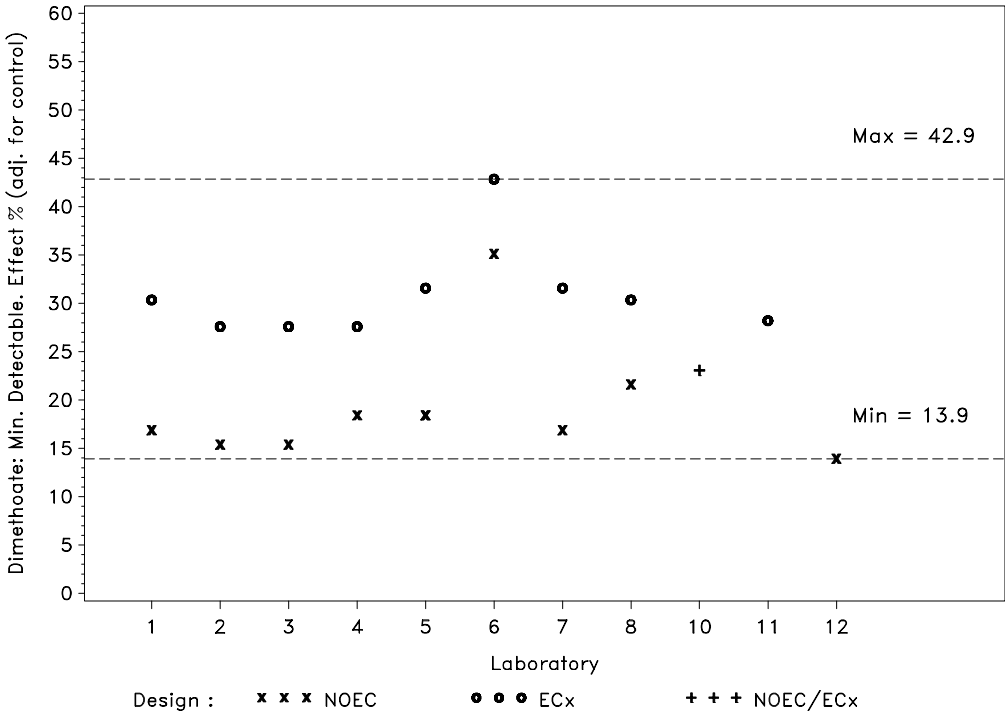
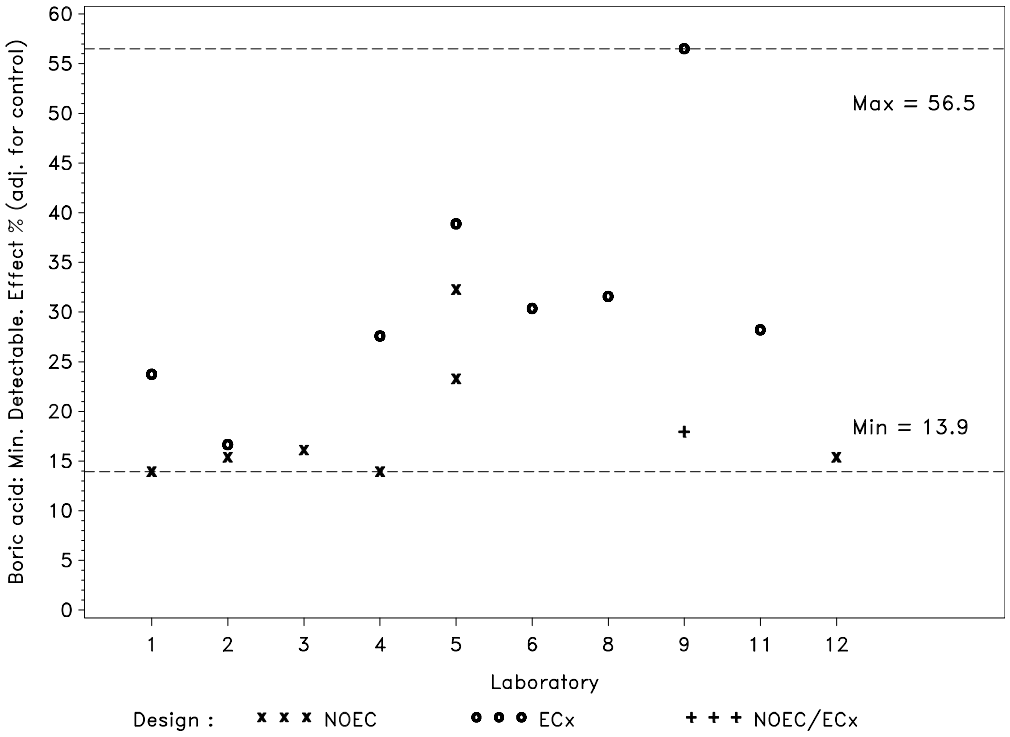


Figure 17b: Boric acid Minimum detectable treatment effect (after adjusting for control mortality), expressed as a percentage of exposed individuals, obtained from sixteen predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps) aculeifer*)



3.3.2 Reproduction Data

3.3.2.1 Estimation of Median Effective Concentration (EC_{50})

The median Effect Concentration (EC_{50}) is defined as the concentration which results in a 50% reduction in reproduction relative to the control. The EC_{50} values from the individual tests were obtained by fitting a logistic model to the number of juveniles per replicate, with nominal concentration as the explanatory variable (Van Ewijk & Hoekstra 1992).

The general form of the logistic model is:

$$y = \frac{\alpha}{1+\left(x/EC_{50}\right)^{\beta}},$$

where y = number of juveniles per replicate, α, β and EC_{50} are model parameters ($\alpha > 0, \beta > 0, EC_{50} > 0$), and x = nominal concentration.

The EC_{50} is included in the model specification as a parameter, and has been estimated directly, along with its approximate confidence limits. The background response, alpha (□), is also included in the model specification as a parameter. Goodness of fit of the model was assessed by partitioning the residual error into lack of fit error and pure error (Draper & Smith 1998). The logistic model was fitted using the NEWTON iterative method of parameter estimation within the PROC NLIN procedure in SAS version 9.1

(2002-2003). Any derivatives of the model that were needed to fit the model were automatically derived by the SAS procedure.

EC_{50} values and their associated confidence limits have been obtained for all twenty dimethoate tests and all sixteen boric acid tests. The mean dimethoate EC_{50} value is 4.93 mg a.s./kg soil (dw), with minimum and maximum values of 2.67 and 8.27 mg a.s./kg soil (dw) respectively. The mean boric acid EC_{50} value is 296.08 mg a.s./kg soil (dw), with minimum and maximum values of 70.83 and 402.35 mg a.s./kg soil (dw) respectively. The individual EC_{50} values, together with the mean, minimum, and maximum values are shown graphically in Figures 18a (dimethoate) and 18b (boric acid).

The EC_{50} values and their associated lower and upper 95% confidence limits are shown in Tables 6a (dimethoate) and 6b (boric acid).

Table 6a: Dimethoate: EC_{50} values with 95% lower and upper confidence limits

Test	Lab.	Design	EC_{50}	Lower	Upper
1	1	NOEC	5.09	4.61	5.56
2		ECx	5.13	4.42	5.83
3	2	NOEC	5.62	-2.08	13.32
4		ECx	5.55	-2.58	13.68
5	3	NOEC	5.50	-10.68	21.69
6		ECx	3.11	2.35	3.87
7	4	NOEC	3.98	3.27	4.69
8		ECx	4.11	2.33	5.88
9	5	NOEC	3.97	3.42	4.52
10*			6.07	5.48	6.66
11		ECx	3.91	3.35	4.48
12	6	NOEC	8.27	7.58	8.97
13		ECx	5.08	3.98	6.18
14	7	NOEC	3.97	3.73	4.21
15		ECx	6.28	5.78	6.77
16	8	NOEC	5.97	5.26	6.68
17		ECx	5.38	4.85	5.91
18	10	NOEC/ECx	3.63	3.37	3.90
19	11	ECx	5.34	4.06	6.63
20	12	NOEC	2.67	2.35	2.99

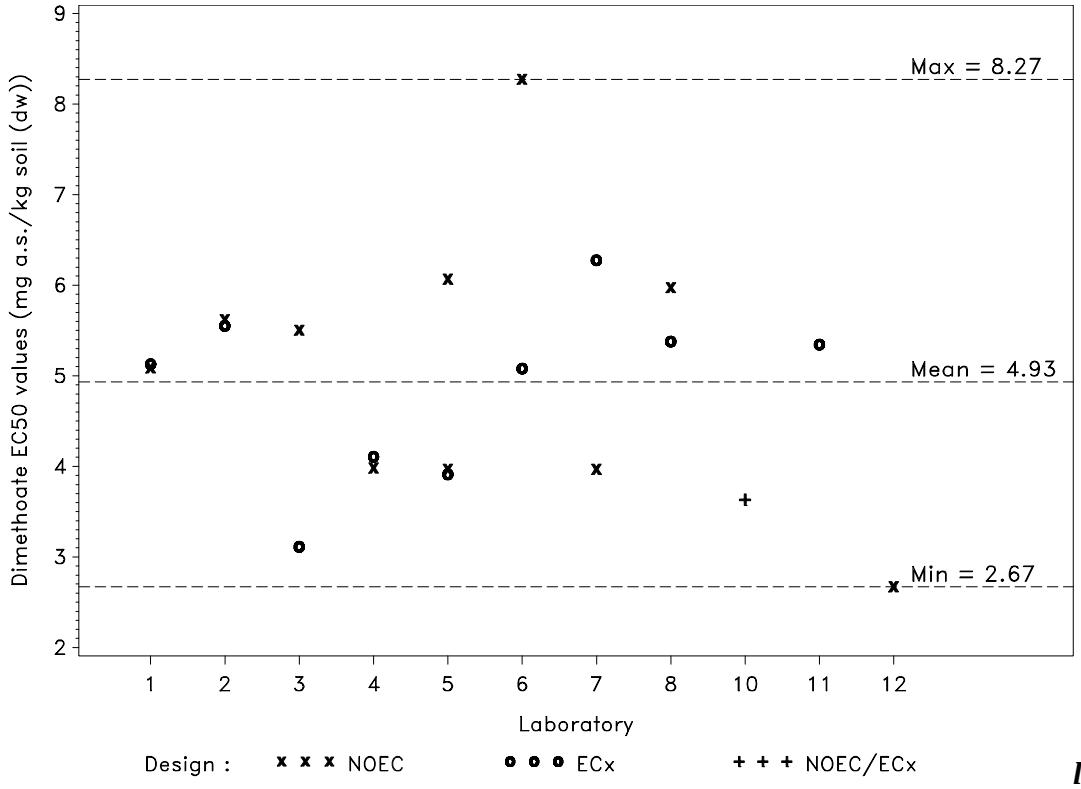
* this test was not part of the ring test (14 days exposure rather than 16).

Table 6b: Boric acid: EC_{50} values with 95% lower and upper confidence limits

Test	Lab.	Design	EC_{50}	Lower	Upper
1	1	NOEC	383.0	268.6	497.4
2		ECx	402.4	313.0	491.7
3	2	NOEC	356.4	333.1	379.8
4		ECx	331.2	313.9	348.4
5	3	NOEC	259.8	-2324.1	2843.7
6	4	NOEC	250.0	-23550.7	24050.7
7		ECx	70.8	36.9	104.8
8	5	NOEC	320.0	-1687.2	2327.1
9*			390.1	245.0	535.2
10		ECx	332.4	291.7	373.2
11	6	ECx	363.6	330.9	396.3
12	8	ECx	270.2	162.0	378.3
13	9	ECx	337.3	291.0	383.5
14		NOEC/ECx	256.1	242.4	269.7
15	11	ECx	233.2	-614.9	1081.2
16	12	NOEC	180.8	133.7	227.9

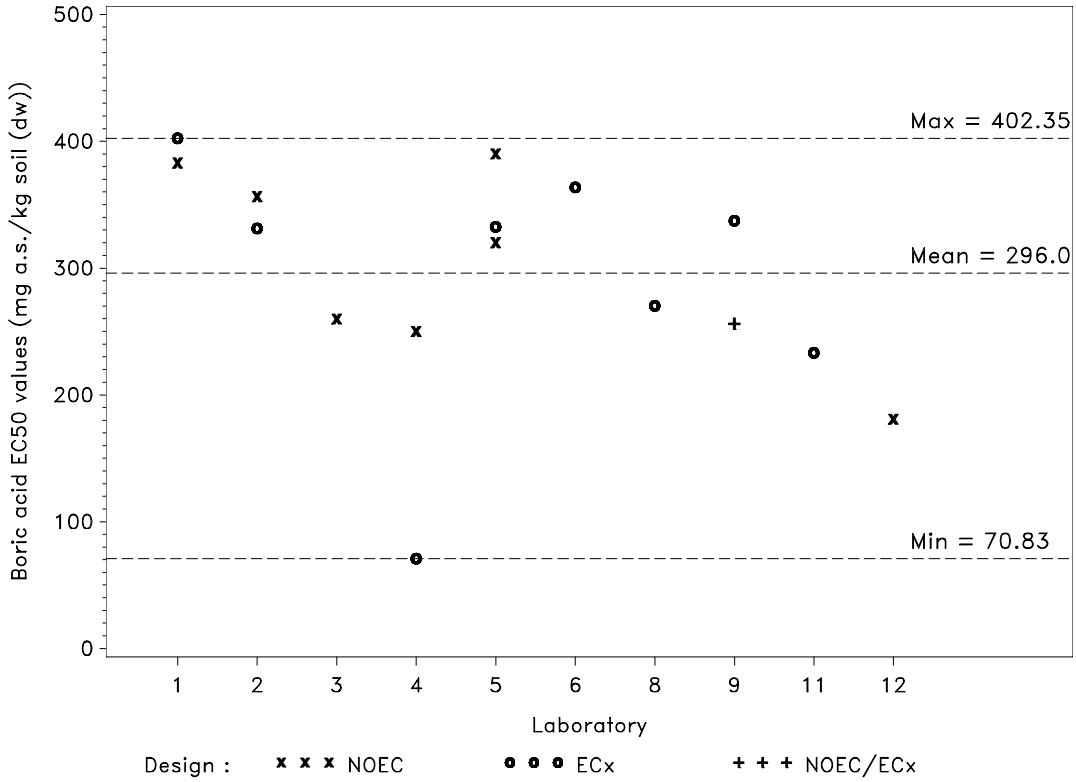
* this test was not part of the ring test (14 days exposure rather than 16).

**Figure 18a: Dimethoate EC_{50} values obtained from twenty predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps).*



acu ***leifer*)**

Figure 18b: **Boric acid EC_{50} values obtained from sixteen predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps) aculeifer*).**



Figures 4.1.2a to 4.20.2a in Annex IIIa (dimethoate) and Figures 4.1.2b to 4.16.2b in Annex IIIb (boric acid) display the observed reproduction data and fitted logistic models. Control data has been included on the figures in Annex IIIa (dimethoate) at 0.1 mg a.s./kg soil (dw) and on the figures in Annex IIIb (boric acid) at 1 mg a.s./kg soil (dw) (rather than 0 mg a.s./kg soil (dw)) as the x-axis has been displayed on the log (base 10) scale (and log of zero does not exist). The values of 0.1 and 1 mg a.s./kg soil (dw) for dimethoate and boric acid tests respectively were chosen as they are lower than any of the concentrations used in any of the tests. The model parameters are shown in Table 4.1a in Annex IIIa (dimethoate) and Table 4.1b in Annex IIIb (boric acid). From Table 4.1a and 4.1b it can be seen that the estimated background response from the logistic model ranges from 76.0 to 413.2.

Results of the goodness of fit test are shown in Table 4.2a in Annex IIIa (dimethoate) and Table 4.2b in Annex III4b (boric acid). From Table 4.2a and 4.2b it can be seen that for two of the twenty dimethoate tests (namely tests 10 and 18) and for one of the sixteen boric acid tests (namely test 9) the logistic model displays a significant lack of fit. For completeness, a hormesis model was also fitted (Van Ewijk and Hoekstra 1992). Hormesis was found to be significant in dimethoate tests 4, 6, 8, 16, 17, 18, 19 and 20, and boric acid tests 4 and 10. However the EC_{50} values obtained from the hormesis model for these tests (not reported here) differ to those from the logistic model by less than 0.9 and 15 mg a.s./kg soil (dw) (for the dimethoate and boric acid data respectively).

3.3.2.2 Estimation of No Observed Effect Concentration (NOEC)

The No Observed Effect Concentration (NOEC) is defined as the highest test concentration that does not produce a statistically significant adverse effect on reproduction when compared to the control.

The NOEC values from the individual tests were obtained by performing an analysis of variance (ANOVA) and then by comparing the mean number of juveniles at each test concentration with the control using a two-tailed Dunnett's test (Hsu 1996) with a 5% type one error rate. In an attempt to satisfy the assumption of homogeneity of variance, test concentrations with low or zero variability were excluded from the analysis (see Table 5a (dimethoate) and Table 5b (boric acid) for details). Following analysis, the assumption of homogeneity of variance was checked using Levene's test (only possible for tests with three or more replicates in one or more test concentrations) and the assumption of normality was checked using Shapiro-Wilk's test.

For each test the minimum detectable difference (M.D.D.J) was computed and expressed as a percentage of the control response. The M.D.D.J is the smallest difference between the mean number of juveniles on a test item treatment and the mean number on control which would be declared statistically significant (given the observed variation and the actual replication). It is computed as follows:

$$M.D.D.J = D_{crit} \times \sqrt{MSE \times \left(\frac{1}{n_c} + \frac{1}{n_t} \right)}, \qquad M.D.D.J \text{ as \% of Control} = \frac{M.D.D.J}{\text{Control mean}}$$

where :

D_{crit} = Critical value for Dunnett's test (2 - sided, 5% level, accounting for unequal replication)

MSE = Pooled estimate of residual (error) variation

n_c and n_t = Replication on the control and test item treatments respectively

Statistical analyses to determine the NOEC values were carried out using the PROC GLM procedure in SAS version 9.1 (SAS 2002-2003).

For completeness, NOEC values from the individual tests were also obtained (for tests using a NOEC or NOEC/ECx design only) using a two-tailed Mann-Whitney test (Hollander & Wolfe 1999) with a stepdown Bonferroni adjustment (also known as the Bonferroni-Holm adjustment) with a 5% type one error rate. The NOEC values obtained using this non-parametric approach (not reported here) were either the same or higher than those obtained using the parametric approach. Thus the NOEC values from the parametric approach can be considered worse case values. The NOEC values are shown in Tables 7a (dimethoate) and 7b (boric acid). From these Tables it can be seen that the dimethoate NOEC values ranged from 1 to 7.49 mg a.s./kg soil (dw) and that the boric acid NOEC values ranged from 10 to 316.2 mg a.s./kg soil (dw).

Figures 4.1.1a to 4.20.1a in Annex IIIa (dimethoate) and Figures 4.1.1b and 4.16.1b in Annex IIIb (boric acid) display the observed reproduction data. The results of Dunnett's test are presented in Table 4.4a in Annex IIIa (dimethoate) and Table 4.4b in Annex IIIb (boric acid).

Table 7a: Dimethoate: NOEC values

Test	Lab.	Design	NOEC
1	1	NOEC	3.20
2		ECx	3.20
3	2	NOEC	3.20
4		ECx	3.20
5	3	NOEC	3.50
6		ECx	1.80
7	4	NOEC	2.00
8		ECx	3.20
9	5	NOEC	1.78
10*			1.00
11		ECx	3.16
12	6	NOEC	5.60
13		ECx	5.60
14	7	NOEC	3.00
15		ECx	5.35
16	8	NOEC	5.12
17		ECx	3.55
18	10	NOEC/ECx	2.40
19	11	ECx	7.49
20	12	NOEC	1.00

* this test was not part of the ring test (14 days exposure rather than 16).

Table 7b: Boric acid: NOEC values

Test	Lab.	Design	NOEC
1	1	NOEC	316.0
2		ECx	316.0
3	2	NOEC	162.0
4		ECx	180.7
5	3	NOEC	250.0
6	4	NOEC	240.0
7		ECx	32.0
8	5	NOEC	100.0
9*			10.0
10		ECx	100.0
11	6	NOEC	316.0
12	8	ECx	178.0
13	9	ECx	316.2
14		NOEC/ECx	177.8
15	11	ECx	177.8
16	12	NOEC	100.0

* this test was not part of the ring test (14 days exposure rather than 16).

The results of Levene's test for homogeneity of variance and Shapiro-Wilk's test for normality are shown in Table 4.5a in Annex IIIa (dimethoate) and Table 4.5b in Annex IIIb (boric acid). From Tables 4.5a and 4.5b it can be seen that there is no evidence that the assumption of normality is invalid for any of the twenty dimethoate tests and for fifteen of the sixteen boric acid tests. There was some evidence to suggest that the assumption of normality is invalid for boric acid test 13, although the p-value was only just significant ($p=0.043$). Since ANOVA is robust to small departures from normality this result was not considered a cause for concern. There is evidence that the assumption of homogeneity of variance is invalid for four of the dimethoate tests (namely tests 1, 3, 7 and 9) and for three of the boric acid tests (namely tests 4, 5 and 14). Therefore responses for these seven tests were log (base 10) transformed and re-analysed. The transformation resulted in a non-significant Levene's test for dimethoate tests 1 and 7 only. However for these two tests, NOEC values for the untransformed and transformed responses were identical.

The minimum detectable difference values are shown in Table 4.7a in Annex IIIa (dimethoate) and Table 4.7b in Annex IIIb (boric acid). The individual M.D.D.J values, together with the minimum and maximum values are shown graphically in Figure 19a (dimethoate) and Figure 19b (boric acid). Since the data were untransformed the M.D.D.J values also represent the minimum detectable increase in juveniles. In all of the situations where laboratories tried both NOEC and ECx designs the NOEC designs were able to detect smaller differences than the ECx designs could.

Figure 19a: **Dimethoate Minimum Detectable Difference in Juvenile reproduction, expressed as a percentage of the control response, obtained from twenty predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps) aculeifer*).**

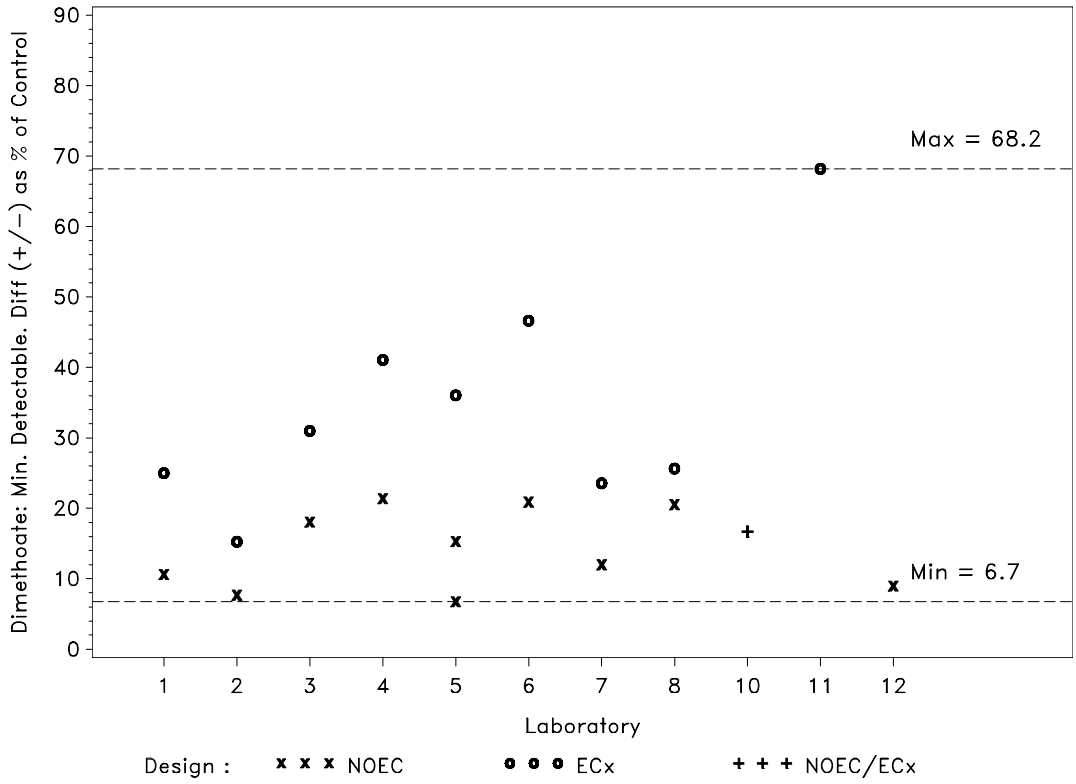
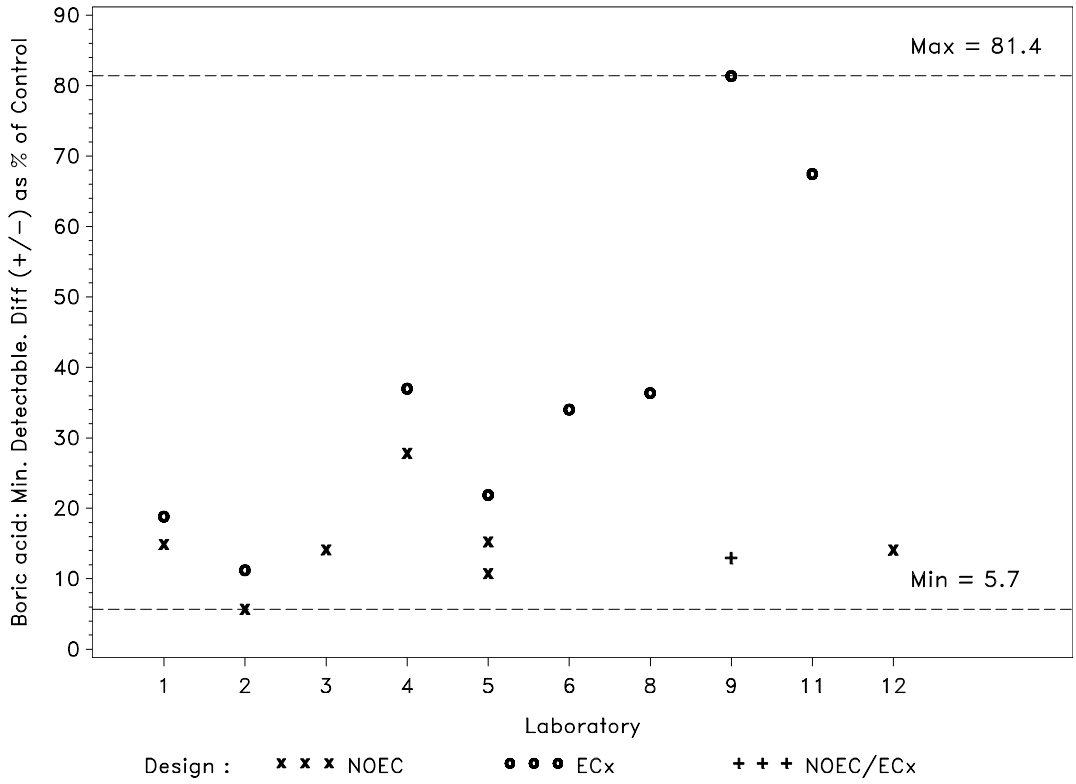


Figure 19b: Boric acid Minimum Detectable Difference in Juvenile reproduction, expressed as a percentage of the control response, obtained from sixteen predatory mite reproduction tests in soil (*Hypoaspis (Geolailaps) aculeifer*).



3.3.2.3 Power of the Mann-Whitney Test

For dimethoate and boric acid tests using the ECx design it is not possible to detect a difference (of any size) between a test concentration and control as significant using the two-tailed Mann-Whitney test (with a stepdown Bonferonni adjustment). This can be explained as follows. For dimethoate and boric acid tests using the ECx design we wish to compare between seven and twelve test concentrations with a control, with at most three replicates per test concentration and six replicates per control. Let us consider one such comparison. In order to apply the Mann-Whitney test, the nine responses (three from the test concentration and six from the control) are ranked from highest to lowest. The number of possible ways of arranging these ranks over the test concentration and control is ${}^{9}C_{3} = {}^{9}C_{6} = 84$. In a two-tailed Mann-Whitney test the unadjusted p-value for the most extreme arrangement (where all three responses from the test concentration are higher or lower than the six responses from the control) is 2/84 = 0.024. This is the smallest unadjusted p-value possible for dimethoate and boric tests using the ECx design. Since the stepdown Bonferroni adjustment will always multiply the smallest p-value by the number of treatment comparisons to get the adjusted p-value, the smallest adjusted p-value possible for dimethoate and boric acid tests using the ECx design is 7 x 0.024 = 0.167, i.e. non-significant. Hence we see that for dimethoate and boric acid tests using the ECx design, the two-tailed Mann-Whitney test (with a stepdown Bonferroni adjustment) cannot detect a difference (of any size) as significant.

3.4 Discussion

The LC_{50} values obtained from the nineteen dimethoate tests (excluding test 18 - see Section 4.1.1) show a good level of agreement. All the individual dimethoate test results differ by less than a factor of two from

the mean in each case. Furthermore, there was no overall statistically significant difference in dimethoate LC_{50} values between laboratories.

The LC_{50} values obtained from the ten boric acid tests (excluding tests 1, 2, 6, 7, 12 and 15 - see Section 4.1.1) show a reasonable level of agreement. All the individual boric acid test results differ by less than a factor of two and a half from the mean in each case. Furthermore, there was no overall statistically significant difference in boric acid LC_{50} values between laboratories.

The EC_{50} values obtained from the twenty dimethoate tests show a good level of agreement. All the individual dimethoate test results differ by less than a factor of two from the mean in each case. Furthermore, there was no overall statistically significant difference in dimethoate EC_{50} values between laboratories.

The EC_{50} values obtained from the sixteen boric acid tests do not show as good a level of agreement as those obtained from the twenty dimethoate tests. All the individual boric acid test results differ by less than a factor of five from the mean in each case. There was no overall statistically significant difference in EC_{50} values between laboratories.

In general, smaller treatment effects could be detected in the tests with higher numbers of replicates.

All twenty dimethoate tests and sixteen boric acid tests, using a NOEC design, an ECx design, or a combined NOEC/ECx design, were able to provide LC_{50} and NOEC estimates based on mortality, and EC_{50} and NOEC estimates based on reproduction. Hence, when testing dimethoate or boric acid, one experimental design can be used to determine all these estimates, provided the doses are chosen appropriately.

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