Deltamethrin + broflanilide

Small scale collaborative trial for the determination of deltamethrin and broflanilide content

in Long-Lasting Insecticide-treated Nets (ITN/LN), coated onto polyester

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by

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1. Background

This small-scale collaborative trial aims to provide an initial insight into the performance of a new analytical method that is intended for determining deltamethrin and broflanilide content in long-lasting insecticide-treated nets (LN/ITN), coated onto polyester.

This method is set up to determine both active ingredients simultaneously and in long-lasting insecticide-treated nets that contain the two molecules cited above. Even if an analytical CIPAC method exists for broflanilide and deltamethrin technical materials, given the particular type of formulation the long-lasting insecticide-treated nets are, simply conduct an extension of the method is estimated not appropriate. The decision to develop one single combined method capable of measuring both molecules at the same time explains why the existing CIPAC method for the determination of deltamethrin in long-lasting coated nets has not been used.

The chromatography used is a reverse phase liquid chromatography with ultraviolet detection (HPLC-DAD).

2. List of participants

Five laboratories agreed to participate in the deltamethrin + broflanilide small scale collaborative trial, three provided results on time. A number has been assigned randomly to each of them. Two laboratories did not provide results, one of them due to an analytical issue: they do not have the recommended HPLC column. They used another one but observed an overlapping of 2 peaks.

All five participants are listed below, in alphabetical order. The author thanks all these participants for their contribution to this trial.

Table 1 List of participants to the trial

Brenda Checa	**Ministerio de Desarrollo Agropecuario**	PANAMA
Nam Thu Le	**Vestergaard Sàrl**	VIET NAM
Marie Baes	**Walloon Agricultural Research Centre (CRA-W)**	BELGIUM
Jia Jian Loo	**TÜV SÜD PSB Pte Ltd**	SINGAPORE
Woramon Suriyachan	**Department of Medical Sciences (DMSc)**	THAILAND

3. Active ingredients, general information

DELTAMETHRIN

333

ISO common names

Deltamethrin (BSI, E-ISO), deltamethrin ((f) F-ISO)

Synonyms

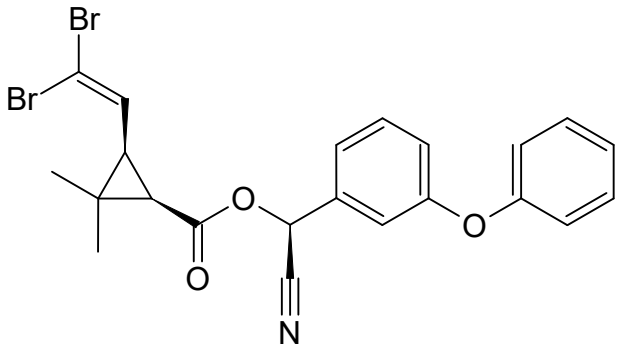
decamethrin (rejected common name)

Chemical names[1]

IUPAC: (*S*)-alpha-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CA: (*S*)-cyano(3-phenoxyphenyl)methyl (1*R*,3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural formula



Empirical formula

$C_{22}H_{19}Br_2NO_3$

Relative molecular mass

505.2

CAS Registry number

52918-63-5

CIPAC number

333

EEC number

258-256-6

[1] Source : The Pesticide Properties Database (PPDB) - https://sitem.herts.ac.uk/aeru/ppdb/

BROFLANILIDE
994

ISO common name

Broflanilide (ISO 1750 approved)

Synonyms

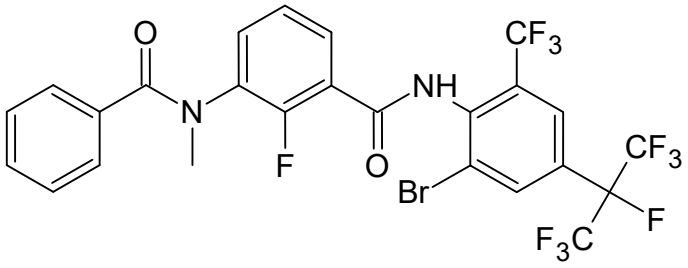
TENEBENAL™, MCI-8007, BAS 450 I, MLP-8607, Reg. No. 5672774, LS5672774, LSP5672774, MAI-7316

Chemical names[2]

IUPAC: N-(2-bromo-4-(1,1,1,2,3,3,3-heptafluoropropan-2-yl)-6-(trifluoromethyl)phenyl)-2-fluoro-3-(N-methylbenzamido)benzamide

CA: 3-(benzoylmethylamino)-N-(2-bromo-4-(1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl)-6-(trifluoromethyl)phenyl)-2-fluorobenzamide

Structural formula



Empirical formula

$C_{25}H_{14}BrF_{11}N_2O_2$

Relative molecular mass

663.29

CAS Registry number

1207727-04-5

CIPAC number

994

[2] Source : The Pesticide Properties Database (PPDB) - https://sitem.herts.ac.uk/aeru/ppdb/

4. Samples and reagents provided

As mentioned above, technical materials (TC) were not tested in this study, but the samples were only long-lasting insecticide-treated nets (LN/ITN).

Five samples of long-lasting insecticide-treated nets (LN/ITN) were sent by Vestergaard Sàrl to the participants. Each sample consists of 5 pieces of 25 cm x 25 cm that were cut from the same net, as explained in Section 5.1. The 5 samples come from different batches of long-lasting insecticide-treated net, coated onto polyester, containing a declared content of

deltamethrin 2.1 g/kg
+ broflanilide 3.8 g/kg.

Table 2 List of samples provided

Sample code	Batch n°	Quantity per participant
LN/ITN 1 / xx (= lab number)	KT-11.25	5 pieces of 25 cm x 25 cm
LN/ITN 2 / xx (= lab number)	KT-12.25	5 pieces of 25 cm x 25 cm
LN/ITN 3 / xx (= lab number)	KT-13.25	5 pieces of 25 cm x 25 cm
LN/ITN 4 / xx (= lab number)	KT-14.25	5 pieces of 25 cm x 25 cm
LN/ITN 5 / xx (= lab number)	KT-15.25	5 pieces of 25 cm x 25 cm

The participants also received analytical standards and internal standard:

Table 3 Certified analytical standards provided

Nature	Batch n°	Purity	Quantity per participant
Deltamethrin	BCCF1130	98.6%	250 mg/bottle
Broflanilide	A150640A503-1YS	99.83%	200 mg/bottle

Table 4 Internal standard provided

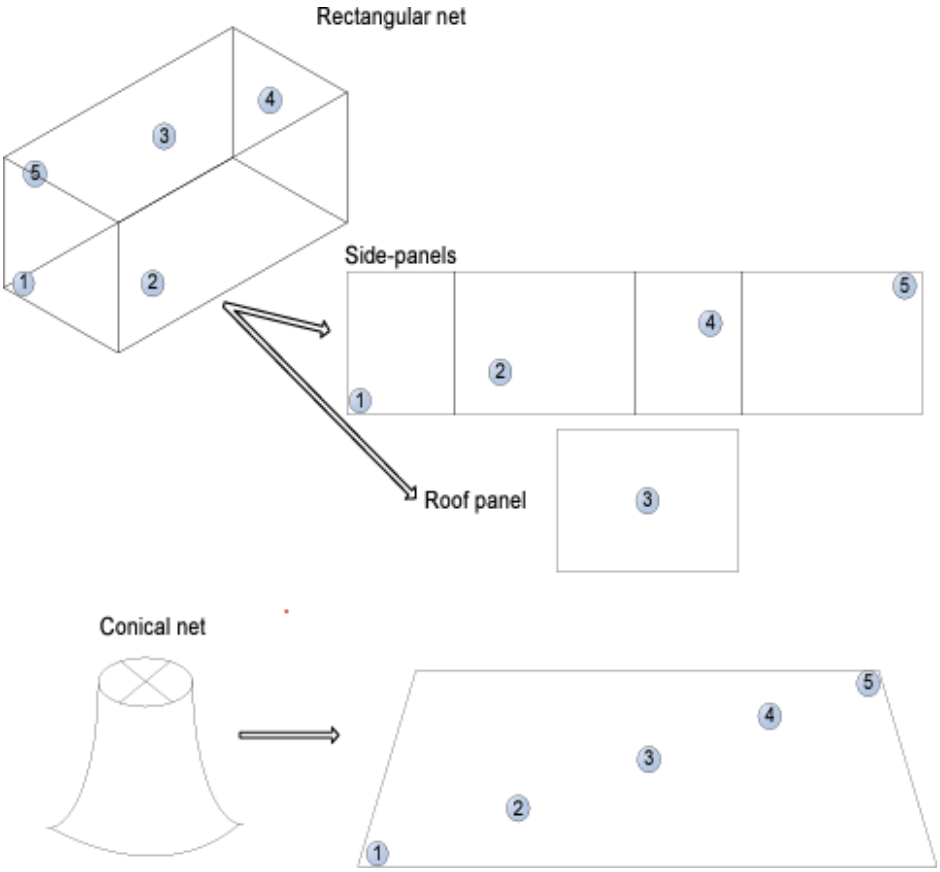
Nature	Batch n°	Purity	Quantity per participant
Dibutyl phthalate	MKCT3246	99.6%	600 mg

5. Preparation of LN/ITN samples

5.1 Preparation of LN/ITN samples by Vestergaard Sàrl

The samples of long-lasting insecticide-treated nets were supplied and prepared by Vestergaard Sàrl (Hanoi, Viet Nam), according to the Manual on the development and use of FAO and WHO specifications for chemical pesticides – second edition[3], as illustrated hereafter:

Figure 1 General method for sampling rectangular and conical nets



[3] FAO and WHO. 2022. Manual on the development and use of FAO and WHO specifications for chemical pesticides – Second edition. Rome and Geneva. https://doi.org/10.4060/cb8401en.

Each sample of long-lasting insecticide-treated net consists of 5 pieces of 25 cm x 25 cm that were cut with scissor from the same net, on a convenient diagonal across the width, in order to obtain a representative laboratory sample. These 5 pieces were pooled together, put into an aluminum foil and identified with a code “LN/ITN x (= sample number) / yy (= lab number)”.

This procedure was performed 5 times, once for each participating laboratory. The samples (LN/ITN 1, LN/ITN 2, LN/ITN 3, LN/ITN 4 and LN/ITN 5) were sent to each participating laboratory. Some of the samples were accidentally found to have a marker dot. The participants were asked not to include these dots in sample weighing.

Figure 2 Picture of a sample with a marker dot.

5.2 Sampling procedure, to be done by each participating laboratory

Cut the 5 net pieces into small pieces (max. 5 x 5 mm) and mix it carefully to get a homogeneous sample, representative sample of the entire net.

6. Analytical method

6.1 Performance of the method

The analytical method provided had to be applied in full on 2 different days, named ”DAY 1“ and ”DAY 2“.

Each sample was analyzed in duplicate, on two different days. Calibration working solutions and internal standard solution had to be freshly prepared on both days.

Details of the extraction process, of the chromatographic analysis and of calculation of the active ingredients content are also reported in the document CIPAC 5411/m.

6.2 Scope

This method is intended for determining deltamethrin and broflanilide content in long-lasting insecticidal net (LN/ITN), coated onto polyester.

6.3 Outline of method

The sample is extracted with acetonitrile using dibutyl phthalate as internal standard. Deltamethrin and broflanilide contents are determined by reverse phase liquid chromatography with ultraviolet detection (HPLC-DAD).

6.4 Sampling

The sampling procedure is detailed in Chapters 5.1 and 5.2.

6.5 Identity test

Deltamethrin **HPLC.** Use the HPLC method below. The retention time of deltamethrin in the sample solution should not deviate by more than 1% from that of the calibration solution.

Broflanilide **HPLC.** Use the HPLC method below. The retention time of broflanilide in the sample solution should not deviate by more than 1% from that of the calibration solution.

6.6 Deltamethrin and broflanilide content

6.6.1 Reagents

Deltamethrin (DM), certified analytical standard of known purity

Broflanilide (BFA), certified analytical standard of known purity

Dibutyl phthalate (CAS RN 84-74-2), internal standard *(ISTD)* of known purity

Acetonitrile, analytical reagent and HPLC grade

Methanol, HPLC grade

Water, HPLC grade

Mobile phase, methanol/water 72:28, v/v

6.6.2 Equipment

Semi–micro-analytical balance: capable of ± 0.1 mg readability

Volumetric flasks of 100 ml and of suitable volume (to prepare the internal standard stock solution)

Volumetric pipettes of 1 ml, 2 ml, 4 ml, 5 ml, 6 ml and 8 ml or electronic pipette able to dispense these volumes

Conical flasks of 50 ml (or of suitable volume)

100 ml cap glass bottles or flasks

Ultrasonic bath

Solvent filtration unit with 0.45 µm PTFE filters

HPLC column, stainless steel, 250 mm x 4.6 mm (i.d.), packed with BDS Hypersil™ C18 (5 µm), or equivalent material with same selectivity.

High performance liquid chromatograph (HPLC), equipped with a constant flow pump, an auto-sampler capable of delivering 10 µl, a column oven and an UV detector capable of measuring at 236 nm.

Integration software

6.6.3 Preparation of solutions

a. *Internal standard stock solution*

Prepare a stock of 1.0 mg/ml internal standard solution. For example, weigh, accurately to the nearest 0.1 mg, about 100 mg of dibutyl phthalate into a 100 ml volumetric flask. Add acetonitrile and place the flask in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20 °C ± 1 °C with acetonitrile (solution $ISTD_{stock}$). Mix thoroughly.

Ensure that a sufficient quantity of this solution is prepared for all the samples and calibration solutions to be analysed.

[concentration of about 1.0 mg dibutyl phthalate / ml].

b. Deltamethrin and broflanilide calibration stock solutions

Weigh in duplicate, accurately to the nearest 0.1 mg, about **25 mg** of deltamethrin (s_{DM} mg) and about **45 mg** of broflanilide (s_{BFA} mg) analytical standards into two separate 100 ml volumetric flasks, each flask containing both analytical standards. Add acetonitrile and place the flasks in an ultrasonic bath until complete dissolution. Allow the solution to cool to room temperature and fill to the mark at 20 °C ± 1 °C with acetonitrile (solutions C_{DM+BFA} and C^*_{DM+BFA}). Mix thoroughly.

[concentrations of about 0.25 mg deltamethrin / ml, and of 0.45 mg broflanilide / ml].

c. *Deltamethrin and broflanilide calibration working solutions*

Prepare the following calibration solutions into conical flasks at room temperature, using the calibration stock solution C_{DM+BFA} as described in the below table (= calibration solutions C_1, C_2, C_3, C_4 and C_5).

Add the internal standard and deltamethrin + broflanilide solutions at 20 °C ± 1 °C and using a volumetric pipette.

Table 5 Preparation of the calibration working solutions C_1 to C_5 from C_{DM+BFA} stock solution

Code	$ISTD_{stock}$	C_{DM+BFA}	Deltamethrin (µg/ml), approx.	Broflanilide (µg/ml), approx.	Acetonitrile	Final volume
C_1	1 ml	2 ml	25	45	Up to volume	20 ml
C_2	1 ml	4 ml	50	90	Up to volume	20 ml
C_3	1 ml	5 ml	62.5	112.5	Up to volume	20 ml
C_4	1 ml	6 ml	75	135	Up to volume	20 ml
C_5	1 ml	8 ml	100	180	Up to volume	20 ml

[concentration of about 0.05 mg dibutyl phthalate / ml].

Use C^*_{DM+BFA} to check the accuracy of the weighing of C_{DM+BFA}: for that, prepare a C^*_3 using the calibration stock solution C^*_{DM+BFA} as described in the below table (= calibration solution C^*_3).

Add the internal standard and deltamethrin + broflanilide solutions at 20 °C ± 1 °C and using a volumetric pipette.

Table 6 Preparation of the calibration working solution C^*_3 from C^*_{DM+BFA} stock solution

Code	$ISTD_{stock}$	C^*_{DM+BFA}	Deltamethrin (µg/ml), approx.	Broflanilide (µg/ml), approx.	Acetonitrile	Final volume
C^*_3	1 ml	5 ml	62.5	112.5	Up to volume	20 ml

[concentration of about 1.0 mg dibutyl phthalate / ml].

Store the stock and working calibration solutions out of direct sunlight and in a refrigerated (<10 °C) zone.

d. *<u>Preparation of samples solutions for LN/ITN</u>*

Cut samples according to Chapters 5.1 and 5.2.

Weigh in duplicate, accurately to the nearest 0.1 mg, about 500 mg of LN sample cut in small pieces into a 50 ml extraction glass bottle with a cap. Add precisely at 20 °C ± 1 °C and by volumetric pipette, 1 ml of internal standard stock solution and 19 ml of acetonitrile. Tighten the bottle cap, gently shake the bottle to ensure that all the net sample is immersed in the extraction solvent. Put the sample bottle into an ultrasonic

bath and sonicate for 15 min. Note that the net sample is not dissolved. Allow the solution to cool to room temperature and mix thoroughly. Filter an aliquot of the solution through a PTFE filter with maximum 0.45 µm pore size, before filling an injection vial (sample solutions S_1 and S_2).

Blank solution should be prepared following the previously described conditions, but without adding any LN sample (= Solution "blank ISTD"). For example, dilute 1 ml of internal standard stock solution into 19 ml of acetonitrile before filling an injection vial.

6.6.4 Operating chromatographic conditions (typical)

Column	stainless steel, 250 mm x 4.6 mm (i.d.), packed with BDS Hypersil™ C18 (5 µm), or equivalent material with same selectivity	
Column temperature	30 °C	
Flow rate	isocratic, 1.25 ml/min	
Injection volume	10 µl	
Detector wavelength	236 nm	
Run time	about 50 min. Run time may be increased for column clean-up to avoid interferences of co-formulants	
Retention times:	broflanilide	about 10 min
	dibutyl phthalate	about 11 min
	deltamethrin	about 41 min

6.6.5 System equilibration

Pump sufficient mobile phase through the column to equilibrate the system.

Inject 10 µl portions of the 2 calibrations working solutions C_3 and C^*_3 before analysis and repeat the injections until retention times does not deviate by more than 1.0% from the mean for three successive injections, for both active ingredients. Ensure that the relative response factors ($f_{i\,DM}$ *vs* $f^*_{i\,DM}$ and $f_{i\,BFA}$ *vs* $f^*_{i\,BFA}$) does not deviate by more than 2.0%, for both active ingredients. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula:

$$f_{i\ DM\ or\ BFA} = \frac{I_r \times s_{DM\ or\ BFA} \times P_{DM\ or\ BFA} \times V_{DM+BFA\ transferred}}{H_{s\ DM\ or\ BFA} \times V_{stock\ DM+BFA} \times V_{working\ cal\ DM+BFA}}$$

where:

$f_{i\,DM\,or\,BFA}$ = individual response factor, for deltamethrin or broflanilide

$H_{s\,DM\,or\,BFA}$ = peak area of deltamethrin or broflanilide in the calibration solution (C_3 or C^*_3)

I_r = peak area of internal standard in the calibration solution (C_3 or C^*_3)

$s_{DM\ or\ BFA}$ = mass of deltamethrin or broflanilide reference standard in the calibration stock solution C_{DM+BFA} and C^*_{DM+BFA} (mg)

$P_{DM\ or\ BFA}$ = purity of deltamethrin or broflanilide reference standard used to prepare the calibration stock solution C_{DM+BFA} and C^*_{DM+BFA} (g/kg)

$V_{DM+BFA\ transferred}$ = volume of the calibration stock solution (C_{DM+BFA} or C^*_{DM+BFA}) transferred to prepare the working calibration solution (C_3 or C^*_3), (ml, typically 5 ml)

$V_{stock\ DM+BFA}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+BFA} or C^*_{DM+BFA}) (ml, typically 100 ml)

$V_{working\ cal\ DM+BFA}$ = total volume of the calibration working solution (C_3 or C^*_3), (ml, typically 20 ml)

If the peak retention times differ significantly from the values given, then adjust the flow rate accordingly.

6.6.6 Determination

After equilibration of the chromatographic system, inject the solvent and blank solutions first to ensure that there is no interference at the retention time of deltamethrin, broflanilide or dibutyl phthalate. Then, inject the sample extracts in duplicate. Bracket each 2 to 4 sample extracts with a calibration solution (C_1 to C_5) as follows: calibration solution C_1, calibration solution C_1, sample solution S_1, sample solution S_1, sample solution S_2, sample solution S_2, calibration solution C_2, calibration solution C_2, and so on for further samples (C_1, C_1, S_1, S_1, S_2, S_2, C_2, C_2, S_3, …)

Measure the relevant peak areas.

Calculate the content of deltamethrin and broflanilide in the sample solutions by comparing the ratio of peak area of deltamethrin or broflanilide to the peak area of dibutyl phthalate in the sample solutions with that of the standard solutions, on basis of a calibration curve established with standard solutions (C_1 to C_5) bracketing the sample solutions.

6.6.7 Data handling

Set up the calibration curves for deltamethrin and broflanilide by plotting the ratio of peaks areas (peak area of deltamethrin or broflanilide *vs* peak area of dibutyl phthalate) versus the deltamethrin or broflanilide concentration in µg/ml. Calculate the equation of the linear regression obtained.

$$y - axis = \frac{H_{w\ DM\ or\ BFA}}{I_q}$$

$$x - axis = \frac{s_{DM\ or\ BFA} \times P_{DM\ or\ BFA} \times V_{DM+BFA\ transferred}}{V_{stock\ DM+BFA} \times V_{working\ cal\ DM+BFA}}$$

where:

$H_{w\ DM\ or\ BFA}$ = peak area of deltamethrin or broflanilide in the sample solution

I_q = peak area of internal standard in the sample solution

$s_{DM\ or\ BFA}$ = mass of deltamethrin or broflanilide reference standard in the calibration stock solution $C_{DM + BFA}$ (mg)

$P_{DM\ or\ BFA}$ = purity of deltamethrin or broflanilide reference standard used to prepare the calibration stock solution $C_{DM + BFA}$ (g/kg)

$V_{DM+BFA\ transferred}$ = volume of the calibration stock solution (C_{DM+BFA}) transferred to prepare the working calibration solutions (C_1 to C_5), in ml (typically 2, 4, 5, 6 and 8 ml, respectively)

$V_{stock\ DM+BFA}$ = volume of the volumetric flask used to prepare the calibration stock solution (C_{DM+BFA}) (ml, typically 100 ml)

$V_{working\ cal}$ = total volume of the calibration working solution (C_1 to C_5) $_{DM+BFA}$ in ml (typically 20 ml)

Express the amount of deltamethrin and broflanilide in the samples in g of deltamethrin and in g of broflanilide per kg of sample, taking into account of dilution factor and sample weight.

Content of deltamethrin or broflanilide in the samples:

$$= \frac{C_{DM\ or\ BFA} \times D}{W}\ g/kg$$

where:

$C_{DM\ or\ BFA}$ = concentration of deltamethrin or broflanilide in the sample solution (μg/ml), found using the equation of the calibration curve

D = dilution factor of the sample solution (typically 20 ml)

W = weight of the sample (mg)

7. Remarks from the participants

7.1 Comments received

Comments received from participants are listed below. Responses (R./) are provided where appropriate.

1. The run time was extended to 56 minutes because the retention time of deltamethrin is 50.2 minutes and to ensure that the column is properly cleaned.

   R./ The retention times observed for this laboratory are longer than those of the analytical method. This laboratory used a different column than the one mentioned in the method. However, this laboratory obtained good results, and their column can be proposed as an alternative.

2. The run time was 60 minutes.

   R./ The run time of the analytical method is about 50 minutes but can be increased for clean-up of the column. The change is in line with the method.

3. The preparation of the calibration solution (stock and working solution), ISTD solution, and samples solution of LN were adjusted to the mark at more than 20°C, which is necessary to deviate from the protocol because of the effect of the weather in summer. However, all solutions were prepared simultaneously under the same conditions.

   R./ Solutions can be thermostatized to avoid this problem. In this case, all solutions were prepared simultaneously, which balances the volume variation for all the solutions.

4. Standard solutions and sample solutions are prepared in 25 ± 3 °C:

   Day 1: 15 Apr 2025 at 24.1 °C.
   Day 2: 16 Apr 2025 ar 25.2 °C.

   All standards and samples are prepared simultaneously and at the same temperature.

   R./ Same comment as before.

5. Nets were cut around max. 10x 10 mm square. It is more feasible and produces less fine polyester than max. 5x5 mm square.

   R./ It is a change to the method, but it can be considered minor since the size of the cut piece is not significantly (compared to the entire net pieces) different from the one specified in the method. This laboratory obtained good results, the method can be changed for the sampling as follows: “Then, cut all the quarters in small pieces

of max 5 mm x 5 mm" becomes "Then, cut all the quarters in small pieces of max 10 mm x 10 mm"

6. In the outline of the method (page 3), state that DM and BFA contents are determined by the normal phase chromatography. But this method uses the C18 column, and high polarity of the mobile phase, which is a characteristic of the reverse-phase chromatography.

   R./ It is indeed an error in the protocol that needs to be corrected. All participants performed the trial by reverse phase HPLC.

7. There should be clarified if the method demonstrates that the inactive isomer of deltamethrin (e.g. *R*-alpha isomer) could be separated.

   R./The analytical method has been validated for (among others) the non-interference of the peak of deltamethrin *R*-alpha isomer [*αR*,1*R*,3*R*-isomer] *vs* peaks of deltamethrin, broflanilide or dibutylphthalate. Since this isomer is a non-relevant impurity of deltamethin, it is usually not mentioned in the analytical method. However, it can be added as a note.

In conclusion, we do not notice anything critical that could affect the analytical method.

7.2 Chromatographic conditions used by the participants

Table 7 **Summary of the instrument and column used by the participants and deviation to the proposed chromatographic conditions.**

Instrument	HPLC column type	Particule size, lenght, internal diameter	Deviations to the proposed operating chromatographic conditions
Agilent 1260 Infinity LC system	Waters XSelect CSH C18	5 µm x 250 mm x 4.6 mm (i.d.)	56 minutes run time
Waters, Arc HPLC	Phenomenex Luna BDS Hypersil™ C18	5 µm x 250 mm x 4.6 mm (i.d.)	60 minutes run time
Thermo Scientific, Vanquish Core	BDS Hypersil™ C18	5 µm x 250 mm x 4.6 mm (i.d.)	-

8. Evaluation and discussion

8.1 Screening for valid data

The statistical evaluation was completed according to the document "CIPAC Guidelines for Collaborative Study Procedures for Assessment of Performance of Analytical Methods", which conforms to the DIN ISO 5725 rules.

The data was examined for outliers and stragglers using Grubbs' test, that shows the between-laboratories variance. The tests were carried out at the alpha level of 1% for outliers and 5% for stragglers.

8.2 Determination of active ingredients content

Among the five laboratories that agreed to take part in the deltamehrin + broflanilide small scale collaborative trial, three provided results on time (see Chapter 2 for more details).

Results of these three laboratories were taken into consideration for statistical treatment.

8.3 Deltamethrin content in long-lasting (coated onto polyester) insecticide-treated net (LN/ITN)

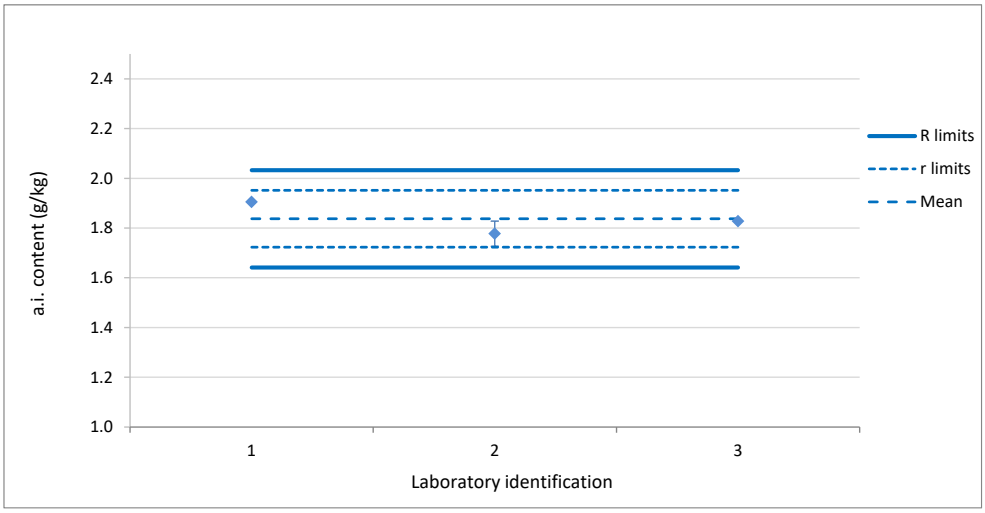
Tables 8 shows all results obtained and a summary of the statistical evaluation is given in table 9.

Table 8 Determination of deltamethrin content in LN/ITN formulations, in g/kg

	LN/ITN 1			LN/ITN 3			LN/ITN 2		
	DAY-1	DAY-2	DAY-1	DAY-2	Mean	Mean	DAY-1	DAY-2	Mean
Lab 1	1.91	1.90	1.94	1.97	**1.95**	**1.91**	1.89	1.91	**1.90**
Lab 2	1.83	1.73	1.76	1.67	**1.71**	**1.78**	1.79	1.70	**1.74**
Lab 3	1.83	1.83	1.73	1.73	**1.73**	**1.83**	1.73	1.76	**1.75**

	LN/ITN 4			LN/ITN 5		
	DAY-1	DAY-2	Mean	DAY-1	DAY-2	Mean
Lab 1	1.98	1.99	**1.99**	1.93	2.03	**1.98**
Lab 2	1.84	1.77	**1.81**	1.81	1.74	**1.78**
Lab 3	1.79	1.85	**1.82**	1.78	1.81	**1.80**

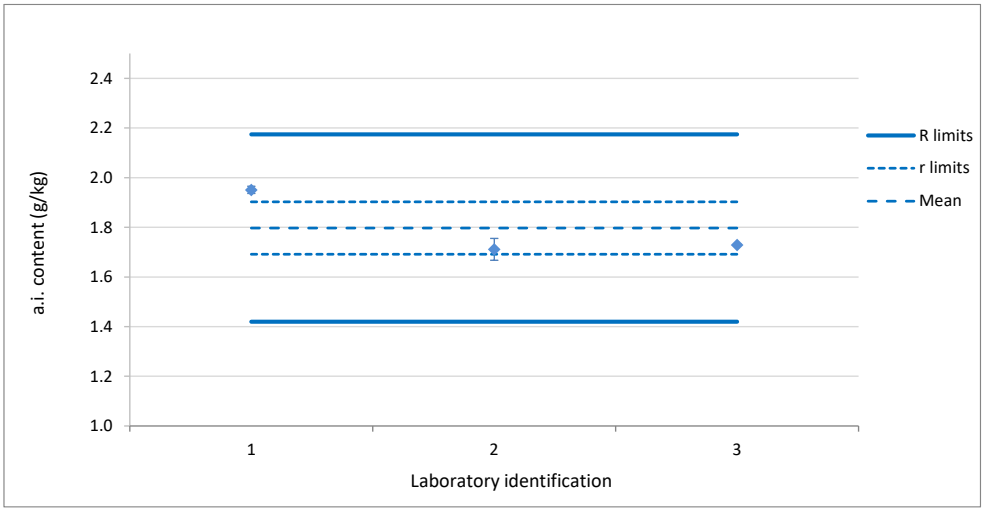
Figure 4 Determination of deltamethrin content in LN/ITN 1, batch n° KT-11.25



	DM LN/ITN 1
$\bar{x}$	1.84 g/kg
L	3 labs
s_r	0.04 g/kg
s_L	0.06 g/kg
s_R	0.07 g/kg
r	0.11 g/kg
R	0.20 g/kg
RSD_r	2.25 %
RSD_R	3.84 %
$RSD_{R(Horw)}$	5.16 %
HorRat	**0.74**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

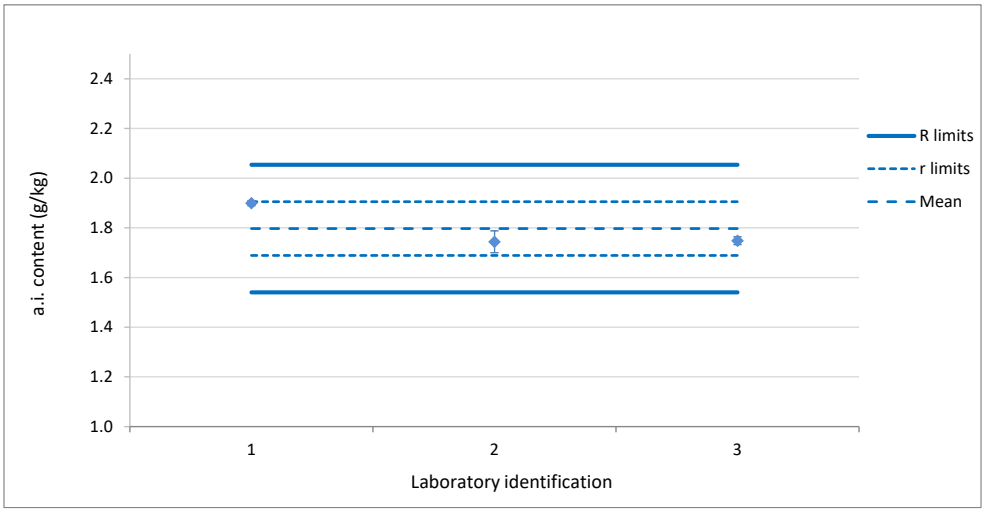
Figure 5 Determination of deltamethrin content in LN/ITN 2, batch n° KT-12.25



	DM LN/ITN 2
$\bar{x}$	1.80 g/kg
L	3 labs
s_r	0.04 g/kg
s_L	0.13 g/kg
s_R	0.14 g/kg
r	0.11 g/kg
R	0.38 g/kg
RSD_r	2.12 %
RSD_R	7.57 %
$RSD_{R(Horw)}$	5.18 %
HorRat	**1.46**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

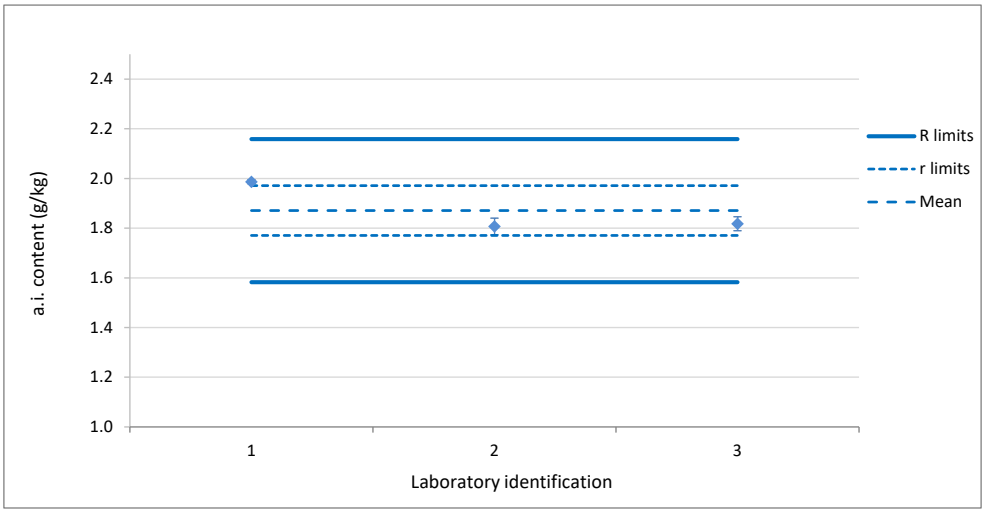
Figure 6 Determination of deltamethrin content in LN/ITN 3, batch n° KT-13.25



	DM LN/ITN 3
$\bar{x}$	1.80 g/kg
L	3 labs
s_r	0.04 g/kg
s_L	0.08 g/kg
s_R	0.09 g/kg
r	0.11 g/kg
R	0.26 g/kg
RSD_r	2.17 %
RSD_R	5.15 %
$RSD_{R(Horw)}$	5.18 %
HorRat	**0.99**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

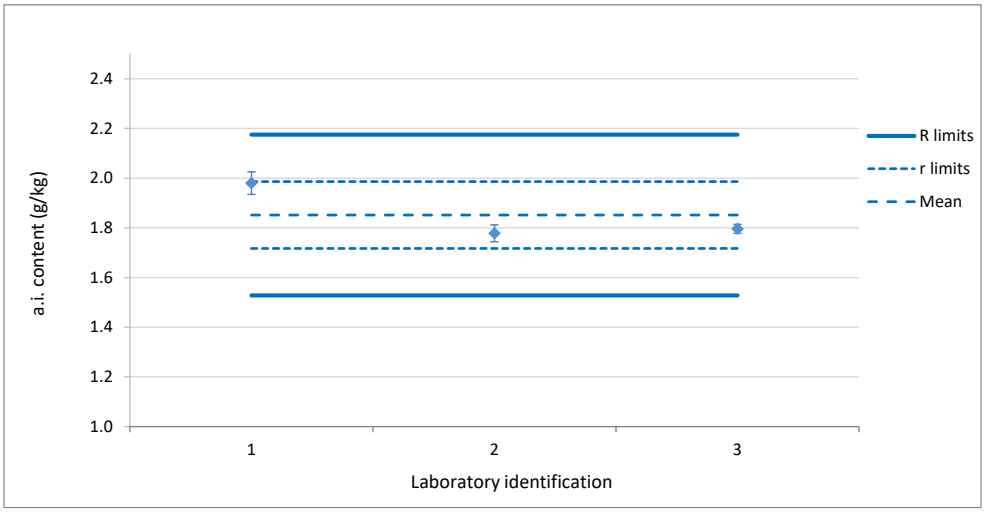
Figure 7 Determination of deltamethrin content in LN/ITN 4, batch n° KT-14.25



	DM LN/ITN 4
$\bar{x}$	1.87 g/kg
L	3 labs
s_r	0.04 g/kg
s_L	0.10 g/kg
s_R	0.10 g/kg
r	0.10 g/kg
R	0.29 g/kg
RSD_r	1.93 %
RSD_R	5.56 %
$RSD_{R(Horw)}$	5.15 %
HorRat	**1.08**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

Figure 8 Determination of deltamethrin content in LN/ITN 5, batch n° KT-15.25



	DM LN/ITN 5
$\bar{x}$	1.85 g/kg
L	3 labs
s_r	0.05 g/kg
s_L	0.11 g/kg
s_R	0.12 g/kg
r	0.13 g/kg
R	0.32 g/kg
RSD_r	2.62 %
RSD_R	6.31 %
$RSD_{R(Horw)}$	5.16 %
HorRat	**1.22**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

Table 9 Summary of the statistical evaluation deltamethrin content in LN/ITN formulations – with all data (no Grubb's outliers have been identified)

	Deltamethrin content				
	LN/ITN 1	**LN/ITN 2**	**LN/ITN 3**	**LN/ITN 4**	**LN/ITN 5**
$\bar{x}$	1.84	1.80	1.80	1.87	1.85
L	3	3	3	3	3
s_r	0.04	0.04	0.04	0.04	0.05
s_L	0.06	0.13	0.08	0.10	0.11
s_R	0.07	0.14	0.09	0.10	0.12
r	0.11	0.11	0.11	0.10	0.13
R	0.20	0.38	0.26	0.29	0.32
RSD_r	2.25	2.12	2.17	1.93	2.62
RSD_R	3.84	7.57	5.15	5.56	6.31
$RSD_{R(Horw)}$	5.16	5.18	5.18	5.15	5.16
HorRat	**0.74**	**1.46**	**0.99**	**1.08**	**1.22**

0.3 ≤ HorRat ≤ 1 : acceptable

0.3 > HorRat or 1 < HorRat ≤ 2 : acceptable in case of a reasonable explanation

HorRat > 2 : not acceptable

8.4 Broflanilide content in long-lasting (coated onto polyester) insecticide-treated net (LN/ITN)

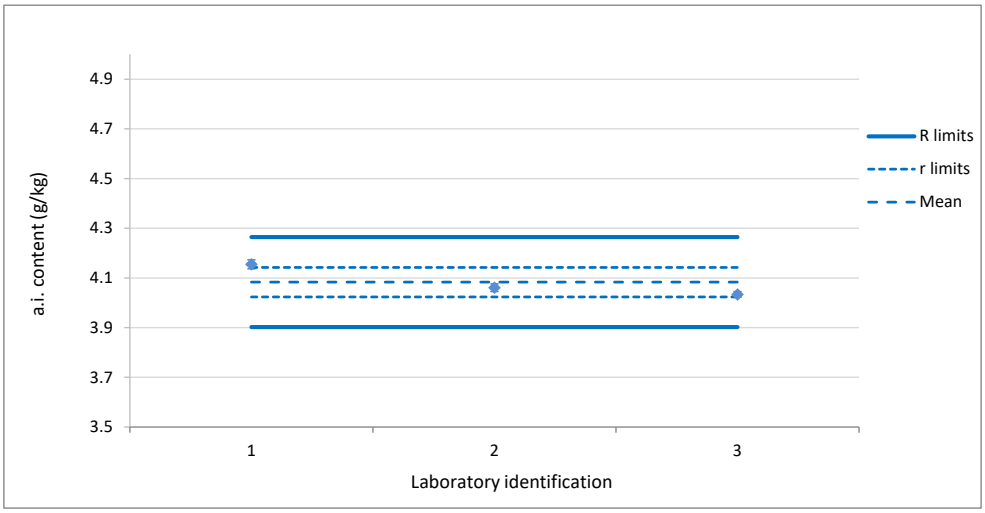
Table 10 shows all results obtained and a summary of the statistical evaluation is given in table 11.

Table 10 Determination of broflanilide content in LN/ITN formulations, in g/kg

	LN/ITN 1			LN/ITN 2			LN/ITN 3		
	DAY-1	DAY-2	Mean	DAY-1	DAY-2	Mean	DAY-1	DAY-2	Mean
Lab 1	4.17	4.14	**4.15**	4.35	4.36	**4.36**	4.25	4.25	**4.25**
Lab 2	4.08	4.05	**4.06**	4.03	4.01	**4.02**	4.09	4.07	**4.08**
Lab 3	4.04	4.02	**4.03**	4.01	3.99	**4.00**	3.99	4.06	**4.03**

	LN/ITN 4			LN/ITN 5		
	DAY-1	DAY-2	Mean	DAY-1	DAY-2	Mean
Lab 1	4.33	4.30	**4.32**	4.29	4.42	**4.35**
Lab 2	4.09	4.09	**4.09**	4.07	4.07	**4.07**
Lab 3	4.06	4.11	**4.08**	4.02	4.09	**4.05**

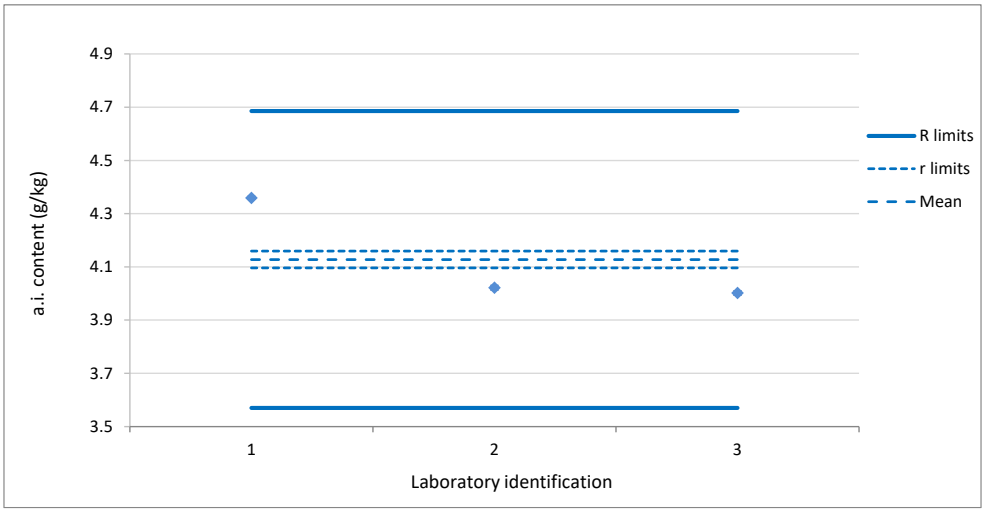
Figure 9 Determination of broflanilide content in LN/ITN 1, batch n° KT-11.25



	BFA LN/ITN 1
$\bar{x}$	4.08 g/kg
L	3 labs
s_r	0.02 g/kg
s_L	0.06 g/kg
s_R	0.07 g/kg
r	0.06 g/kg
R	0.18 g/kg
RSD_r	0.53 %
RSD_R	1.60 %
$RSD_{R(Horw)}$	4.58 %
HorRat	**0.35**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

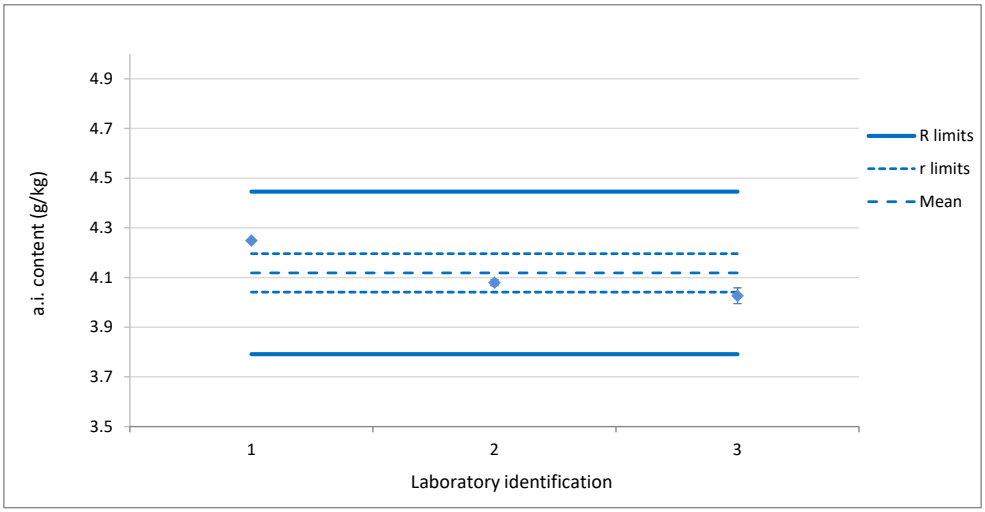
Figure 10 Determination of broflanilide content in LN/ITN 2, batch n° KT-12.25



	BFA LN/ITN 2
$\bar{x}$	4.13 g/kg
L	3 labs
s_r	0.01 g/kg
s_L	0.20 g/kg
s_R	0.20 g/kg
r	0.03 g/kg
R	0.56 g/kg
RSD_r	0.27 %
RSD_R	4.87 %
$RSD_{R(Horw)}$	4.57 %
HorRat	**1.07**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

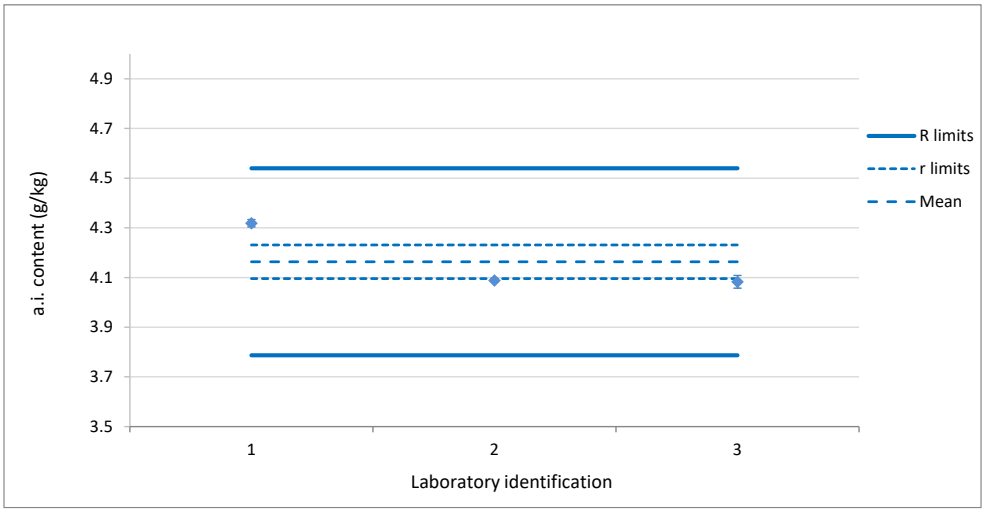
Figure 11 Determination of broflanilide content in LN/ITN 3, batch n° KT-13.25



	BFA LN/ITN 3
$\bar{x}$	4.12 g/kg
L	3 labs
s_r	0.03 g/kg
s_L	0.11 g/kg
s_R	0.12 g/kg
r	0.08 g/kg
R	0.33 g/kg
RSD_r	0.68 %
RSD_R	2.87 %
$RSD_{R(Horw)}$	4.57 %
HorRat	**0.63**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

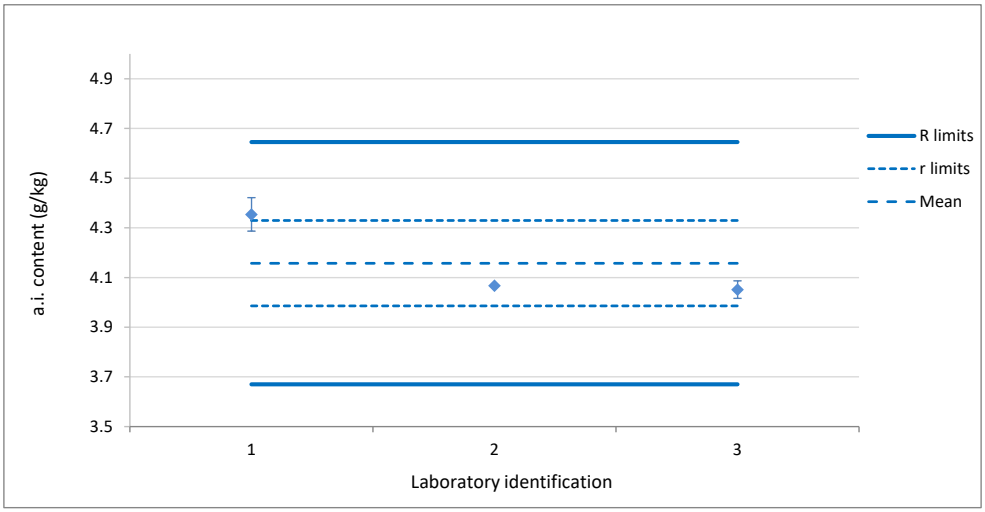
Figure 12 Determination of broflanilide content in LN/ITN 4, batch n° KT-14.25



	BFA LN/ITN 4
$\bar{x}$	4.16 g/kg
L	3 labs
s_r	0.02 g/kg
s_L	0.13 g/kg
s_R	0.14 g/kg
r	0.07 g/kg
R	0.38 g/kg
RSD_r	0.59 %
RSD_R	3.26 %
$RSD_{R(Horw)}$	4.56 %
HorRat	**0.71**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

Figure 13 Determination of broflanilide content in LN/ITN 5, batch n° KT-15.25



	BFA LN/ITN 5
$\bar{x}$	4.16 g/kg
L	3 labs
s_r	0.06 g/kg
s_L	0.16 g/kg
s_R	0.18 g/kg
r	0.17 g/kg
R	0.49 g/kg
RSD_r	1.49 %
RSD_R	4.23 %
$RSD_{R(Horw)}$	4.56 %
HorRat	**0.93**

The vertical bars represent the difference between Day 1 and Day 2 and the dots indicate the mean of Day 1 and Day 2.

Table 11 Summary of the statistical evaluation deltamethrin content in LN/ITN formulations – with all data (no Grubb's outliers have been identified)

	Broflanilide content				
	LN/ITN 1	**LN/ITN 2**	**LN/ITN 3**	**LN/ITN 4**	**LN/ITN 5**
$\bar{x}$	4.08	4.13	4.12	4.16	4.16
L	3	3	3	3	3
s_r	0.02	0.01	0.03	0.02	0.06
s_L	0.06	0.20	0.11	0.13	0.16
s_R	0.07	0.20	0.12	0.14	0.18
r	0.06	0.03	0.08	0.07	0.17
R	0.18	0.56	0.33	0.38	0.49
RSD_r	0.53	0.27	0.68	0.59	1.49
RSD_R	1.60	4.87	2.87	3.26	4.23
$RSD_{R(Horw)}$	4.58	4.57	4.57	4.56	4.56
HorRat	**0.35**	**1.07**	**0.63**	**0.71**	**0.93**

0.3 ≤ HorRat ≤ 1 : acceptable

0.3 > HorRat or 1 < HorRat ≤ 2 : acceptable in case of a reasonable explanation

HorRat > 2 : not acceptable

Table 12 Definition and formula of the parameters used for the statistical evaluation

Parameter	Definition	Formula
$\bar{x}$	Overall mean	$\bar{x} = \Sigma\bar{x}_i/L$
L	number of laboratories	$1 \leq i \leq L$
s_r	repeatability standard deviation	$s_r^2 = \Sigma s_i^2 / L$
s_L	"pure" between laboratory standard variation	$s_L^2 = \frac{\sum\left(\bar{x}_i - \frac{\sum\bar{x}_i}{L}\right)^2}{L-1} - \frac{s_r^2}{2} = \frac{L.\Sigma(\bar{x}_i)^2 - (\Sigma\bar{x}_i)^2}{L(L-1)} - \frac{s_r^2}{2}$
s_R	reproducibility standard deviation	$s_R^2 = s_L^2 + s_r^2$
r	repeatability	$r = s_r * 2.8$
R	reproducibility	$R = s_R * 2.8$
RSD_r	repeatability relative standard deviation	$RSD_r = s_r / \bar{x} * 100\%$
RSD_R	reproducibility (inter-laboratory) relative standard deviation	$RSD_R = s_R / \bar{x} * 100\%$
$RSD_{R(Horw)}$	Horwitz value for concentration c with c = concentration of the analyte as a decimal fraction	$RSD_R(Horw) = 2^{(1 - 0.5\log c)}$
HorRat	Horwitz ratio	$HorRat = RSD_R/RSD_{R(Horw)}$

9. Evaluation and discussion

Five laboratories participated to this small scale collaborative trial and three provided results on time. Grubbs' tests was applied for statistical evaluation of the results, outliers are identified with 99% confidence and stragglers with 95 % confidence.

Tables 8 and 10 on pages 17 and 21 contain all data obtained from the three participants.

9.1 Identification of outliers and stragglers with Grubbs' test (between laboratory reproducibility)

No outliers nor stragglers were identified by this test.

9.2 HorRat values

The HorRat value is used as a criterion of acceptance for methods collaboratively tested by CIPAC, with the following guidelines :

0.3 ≤ HorRat ≤ 1	=> fully acceptable
HorRat < 0.3 or 1 < HorRat ≤ 2	=> acceptable, but reasonable explanation required
HorRat > 2	=> not acceptable

Tables 9 to 11 on pages 20 and 24 show the statistical evaluation with all data. Below is what we noticed from these values.

- for **deltamethrin content in LN/ITN**, the HorRat ratio is
  - between 0.3 and 1 for 2 samples and
  - between 1 and 2 for 3 samples.
- for **broflanilide content in LN/ITN**, the HorRat ratio is
  - between 0.3 and 1 for 4 samples and
  - between 1 and 2 for 1 sample.

See Chapter 9.3 for further discussion and explanations.

9.3 Conclusion

For deltamethrin content, and reasonable explanation need to be provided for HorRat ratios between 1 and 2. LN/ITN formulations being inherently inhomogeneous samples; HorRat value between 1 and 2 remains acceptable in consideration of this. Furthermore, it is important to bear in mind that the statistics were conducted based on three data sets only, thus the results obtained are indicative.

For broflanilide content, all HorRat ratios are close or lower than 1, what is acceptable. Indeed, the only ratio above 1 is 1.07.

Based on the results of the collaborative trial and considering what is mentioned above, we recommend proceeding with a full-scale collaborative trial.