Bifenthrin + Chlorfenapyr + Pyriproxyfen + Piperonyl Butoxide

Full scale collaborative trial for the determination of bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide content

in Technical material (TC)
and Long-Lasting Insecticide-treated Nets (LN/ITN), incorporated into filaments

Report to CIPAC

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by

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1. Background

This full scale collaborative trial aims to assess the performance of a new analytical method that is intended for determining bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide content in insecticide-treated nets (LN/ITN), incorporated into filament.

This method was designed to determine the active ingredients and synergist content in long-lasting insecticide-treated nets that contains a combination of the four molecules cited above. Even if an analytical CIPAC method exists for each of these molecules (at least for technical materials), they were not necessary developed for insecticide-treated nets. Furthermore, the new analytical method is capable to determine the content of these active ingredients and synergist at once, in only one single extraction process and in only one chromatographic injection step. The chromatography used is a gas chromatography with a flame ionisation detection (GC-FID).

2. List of participants

Sixteen laboratories agreed to participate in the bifenthrin + chlorfenapyr + pyriproxyfen + piperonyl butoxide full scale collaborative trial, thirteen provided results on due time. A number has been assigned randomly to each of them.

One laboratory did not provide results and two laboratories were unable to perform the trial because they did not receive the technical samples nor the analytical and internal standards. Indeed, the shipment of substances classified as dangerous goods to the destination country requires extremely heavy formalities that could not be solved.

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

Table 1 List of participants to the trial

Agus Salim	**AGRICON**	INDONESIA
Akshay Kant Chaturvedi	**Shivalik Rasayan Limited**	INDIA
Anand Samiappan	**V.K.A. POLYMERS Pvt. Ltd.**	INDIA
Bojana Špirović	**University of Belgrade**	SERBIA
Carmen Riehle	**Syngenta Crop Protection AG**	SWITZERLAND
Elen Karasali	**Benaki Phytopathological Institute**	GREECE

Jia Jian Loo	**TÜV SÜD PSB Pte Ltd**	SINGAPORE
Kamlesh Vishwakarma	**NACL Industries Limited**	INDIA
Marie Baes	**Walloon Agricultural Research Centre (CRA-W)**	BELGIUM
Mary Ellen McNally	**FMC Corporation**	USA
Nam Le	**Vestergaard Sarl**	VIETNAM
Qu Tingsi	**Vaster Testing Technology Co., Ltd**	CHINA
Rolf Foerster	**BASF**	GERMANY
Sunil Khot Ingo	**Syngenta Biosciences Pvt. Ltd.**	INDIA
Woramon Suriyachan	**Department of Medical Sciences (DMSc)**	THAILAND
Zhiyu He	**Guizhou JAD Technology Co., Ltd**	CHINA

3. Active ingredients, general information

BIFENTHRIN

415

ISO common name

Bifenthrin (ISO 1750 published)

Synonyms

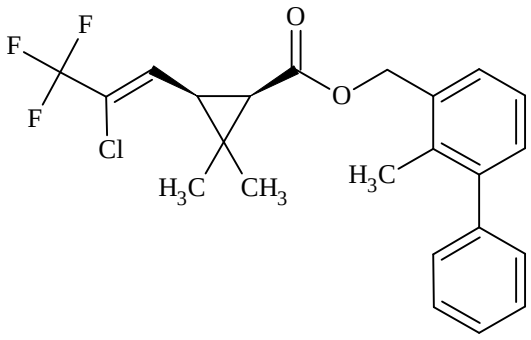
FMC 54800

Chemical names

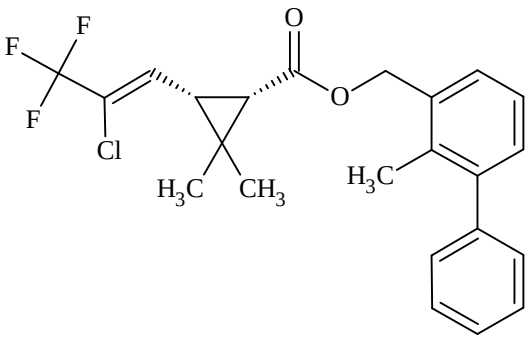
IUPAC: 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CA: (2-methyl[1,1'-biphenyl]-3-yl)methyl 3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1propenyl)-2,2-dimethylcyclopropanecarboxylate

Structural formula



(*Z*)-(1*R*)-*cis*



(*Z*)-(1*S*)-*cis*

Molecular formula

$C_{23}H_{22}ClF_3O_2$

Relative molecular mass

423.0

CAS Registry number

82657-04-3

CIPAC number

415

CHLORFENAPYR
570

Common name

chlorfenapyr (ISO 1750 approved)

Synonyms

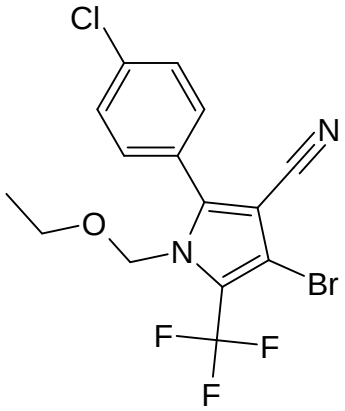
None

Chemical names

IUPAC: 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-*1H*-pyrrole-3-carbonitrile

CA: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-*1H*-pyrrole-3-carbonitrile

Structural formula



Molecular formula

$C_{15}H_{11}BrClF_3N_2O$

Relative molecular mass

407.6

CAS Registry number

122453-73-0

CIPAC number

570

PYRIPROXYFEN
715

Common name

Pyriproxyfen (BSI, E-ISO)

Synonyms

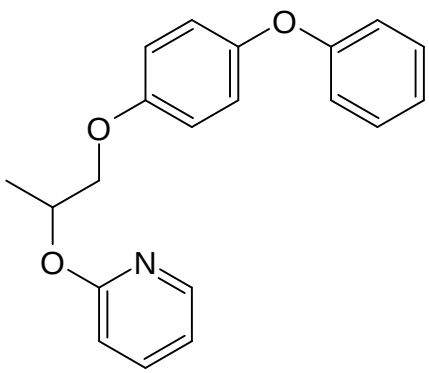
None

Chemical names

IUPAC: 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether

CA: 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine

Structural formula



Molecular formula

$C_{20}H_{19}NO_3$

Relative molecular mass

321.37

CAS Registry number

95737-68-1

CIPAC number

715

PIPERONYL BUTOXIDE

33

Common name

Piperonyl butoxide (BAN; accepted in lieu of a common name by BSI, E-ISO, ESA); piperonyl butoxyde (F-ISO)

Synonyms

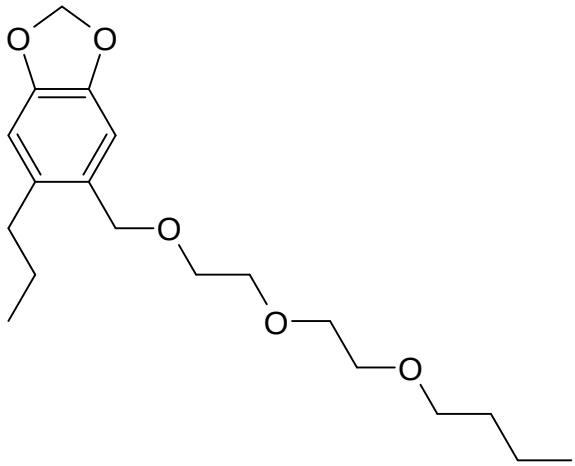
None

Chemical names

IUPAC: 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole

CA: 5-[[2-(2-butoxyethoxy)ethoxy]methyl]-6-propyl-1,3-benzodioxole

Structural formula



Molecular formula

$C_{19}H_{30}O_5$

Relative molecular mass

338.4

CAS Registry number

51-03-6

CIPAC number

33

4. Samples and reagents provided

Eight samples of technical materials (two bifenthrin TC, two chlorfenapyr TC, two pyriproxyfen TC and two piperonyl butoxide TC respectively) and four samples of long-lasting insecticide-treated nets (LN/ITN) were sent by V.K.A. Polymers to the participants, as listed below:

- 2 Bifenthrin TC :

  500 mg of bifenthrin TC, from the batch R-22-92, purity 98.22%, codified as **TC_{BIF} 1**.

  500 mg of bifenthrin TC, from the batch R-22-93, purity 98.24%, codified as **TC_{BIF} 2**.

- 2 Chlorfenapyr TC :

  500 mg of chlorfenapyr TC, from the batch CLF-004/5BA/ST-IV-002, purity 99.10%, codified as **TC_{CFP} 1**.

  500 mg of chlorfenapyr TC, from the batch CLF-004/5BA/ST-IV-003, purity 98.72%, codified as **TC_{CFP} 2**.

- 2 Pyriproxyfen TC :

  500 mg of pyrifproxyfen TC, from the batch PFN041I22 LOT I, purity 98.47%, codified as **TC_{PYR} 1**.

  500 mg of pyrifproxyfen TC, from the batch PFN028H22 LOT II, purity 98.38%, codified as **TC_{PYR} 2**.

- 2 Piperonyl Butoxide TC :

  500 mg of piperonyl butoxide TC, from the batch L202207065, purity 95.57%, codified as **TC_{PBO} 1**.

  500 mg of piperonyl butoxide TC, from the batch L202208091, purity 95.77%, codified as **TC_{PBO} 2**.

- LN/ITN 1

  5 pieces of 25 cm x 25 cm from a bifenthrin 7 g/kg + chlorfenapyr 8 g/kg + piperonyl butoxide 6 g/kg long-lasting (incorporated into filaments) insecticide-treated net (LN / ITN), batch (PNT) VKA-258-1, CRA-W registration number Mo.841.

- LN/ITN 2

  5 pieces of 25 cm x 25 cm from a bifenthrin 7 g/kg + pyriproxyfen 6 g/kg + piperonyl butoxide 6 g/kg long-lasting (incorporated into filaments) insecticide-treated net (LN / ITN), batch IP-2-0, CRA-W registration number Mo.842.

- LN/ITN 3

  5 pieces of 25 cm x 25 cm from a bifenthrin 7 g/kg + chlorfenapyr 8 g/kg long-lasting (incorporated into filaments) insecticide-treated net (LN / ITN), batch (PND) VKA-257-2-1, CRA-W registration number Mo.843.

- LN/ITN 4

  5 pieces of 25 cm x 25 cm from a bifenthrin 7 g/kg + piperonyl butoxide 6 g/kg long-lasting (incorporated into filaments) insecticide-treated net (LN / ITN), batch (PNP) VKA-256-1, CRA-W registration number Mo.844.

The participants also received analytical standards and internal standard:

- 100 mg of bifenthrin certified reference standard,
  batch 15092021, purity 99.11%.
- 100 mg of chlorfenapyr certified reference standard,
  batch PCL3/002, purity 100%.
- 100 mg of pyriproxyfen certified reference standard,
  batch TCIRD/PFN/053, purity 99%.
- 100 mg of piperonyl butoxide certified reference standard,
  batch L202204030, purity 95.13%.

- 500 mg of dicyclohexyl phthalate internal standard,
  batch MKBB1696, purity 99.9%.

5. Preparation of LN/ITN samples

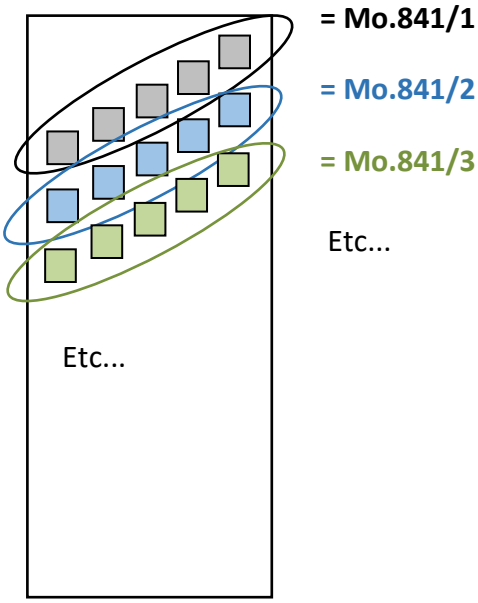
5.1 Preparation of LN/ITN samples by CRA-W

The samples of long-lasting insecticide treated nets were prepared by CRA-W from incorporated netting, according to the Manual on the development and use of FAO and WHO specifications for chemical pesticides – second edition[1], as described hereafter. All samples (TC and LN/ITN) were supplied by V.K.A. Polymers (Karur, India).

For each batch of LN/ITN and for each participating laboratory, 5 pieces of 25 cm x 25 cm were cut with scissors from the bulk material, 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 the CRA-W registration number and the declared active ingredient.s/synergist content.

The procedure is illustrated with the following scheme :

Figure 1 Example of a sampling of a netting



This procedure was performed **20 times** (once for each participating laboratory and four samples Mo.xxx/5, Mo.xxx/10, Mo.xxx/15 and Mo.xxx/20 were kept by CRA-W for additional internal testing purpose) for each of the four type of netting. One sample consists thus of 5 combined net pieces of 25 cm x 25 cm.

The samples (LN/ITN 1, LN/ITN 2, LN/ITN 3 and LN/ITN 4) were sent to each participating laboratory.

[1] 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.

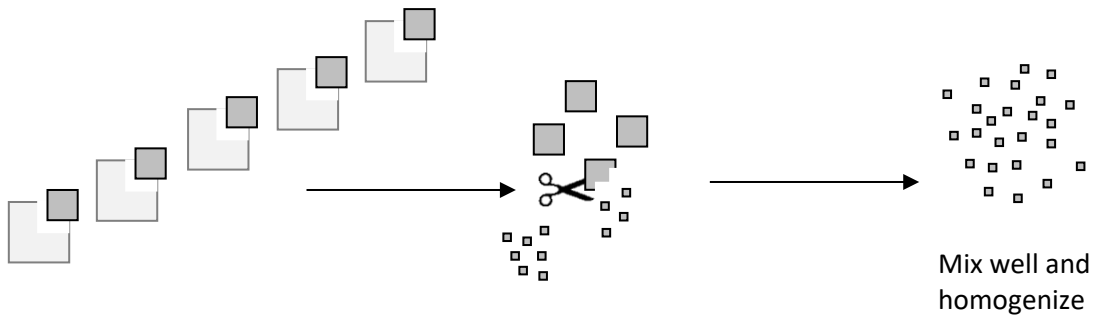
Table 2 Summary of the samples received by each participant

Nature	Batch n° / Sample n°	Sample code	Quantity
Bifenthrin TC	R-22-92	**TC_{BIF} 1**	500 mg
Bifenthrin TC	R-22-93	**TC_{BIF} 2**	500 mg
Chlorfenapyr TC	CLF-004/5BA/ST-IV-002	**TC_{CFP} 1**	500 mg
Chlorfenapyr TC	CLF-004/5BA/ST-IV-003	**TC_{CFP} 2**	500 mg
Pyriproxyfen TC	PFN041I22 LOT I	**TC_{PYR} 1**	500 mg
Pyriproxyfen TC	PFN028H22 LOT II	**TC_{PYR} 2**	500 mg
Piperonyl Butoxide TC	L202207065	**TC_{PBO} 1**	500 mg
Piperonyl Butoxide TC	L202208091	**TC_{PBO} 2**	500 mg
Mosquito net sample	(PNT) VKA-258-1 – Mo.841	**LN/ITN 1**	5 pieces of 25 cm x 25 cm
Mosquito net sample	IP-2-0– Mo.842	**LN/ITN 2**	5 pieces of 25 cm x 25 cm
Mosquito net sample	(PND) VKA-257-2-1 – Mo.843	**LN/ITN 3**	5 pieces of 25 cm x 25 cm
Mosquito net sample	(PNP) VKA-256-1 – Mo.844	**LN/ITN 4**	5 pieces of 25 cm x 25 cm

5.2 Sampling procedure, to be done by each participating laboratory

One quarter of each of the 5 pieces of 25 cm x 25 cm has to be cut with scissors and pooled together. Then, cut all the quarters in small pieces of max 5 mm x 5 mm and mix it carefully to obtain a representative sample of the entire net, to get a homogeneous sample.

Figure 2 Sub-sampling of a netting



6. Analytical method

6.1 Performance of the method

This method had to be done entirely at 2 different days, named ”DAY 1“ and ”DAY 2“.

Each sample had to be analyzed in duplicate, at 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 5347/m.

6.2 Scope

This method is intended for determining bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide content in insecticide-treated net (LN/ITN), incorporated into filament.

6.3 Outline of method

The sample is extracted by heating and sonication with n-heptane using dicyclohexyl phthalate as internal standard. Bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide contents are determined by gas chromatography with flame ionisation detection (GC-FID).

6.4 Sampling

The sampling procedure is detailed in chapters 5.1 and 5.2.

6.5 Identity test

Bifenthrin	**GC.**	Use the GC method below. The retention time of bifenthrin and of the internal standard in the sample solution should not deviate by more than 2 % from that of the calibration solution.
Chlorfenapyr	**GC.**	Use the GC method below. The retention time of chlorfenapyr and of the internal standard in the sample solution should not deviate by more than 2 % from that of the calibration solution.
Pyriproxyfen	**GC.**	Use the GC method below. The retention time of pyriproxyfen and of the internal standard in the sample solution should not deviate by more than 2 % from that of the calibration solution.
Piperonyl butoxide	**GC.**	Use the GC method below. The retention time of piperonyl butoxide and of the internal standard in the sample solution

should not deviate by more than 2 % from that of the calibration solution.

6.6 Bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide content

6.6.1 Reagents

Bifenthrin (BIF), reference standard of known purity

Chlorfenapyr (CFP), reference standard of known purity

Pyriproxyfen (PYR), reference standard of known purity

Piperonyl butoxide (PBO), reference standard of known purity

Dicyclohexyl phthalate, internal standard (*ISTD*) of known purity

n-Heptane, analytical reagent grade

6.6.2 Equipment

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

Volumetric flasks of 25 mL and of suitable volume (to prepare the internal standard stock solution)

Volumetric pipettes of 0.3 mL, 1 mL, 2 mL, 2.5 mL, 4 mL and 5 mL or electronic pipettes able to dispense these volumes

Conical flasks of 50 mL (or of suitable volume)

100 mL cap glass bottles or flasks

Heating ultrasonic bath capable of heating up to 80°C

Thermostatic bath

Solvent filtration unit with 0.45 µm PTFE filters

Capillary column fused silica, coated with (50 % trifluoropropyl)-methylpolysiloxane (e.g. DB-210), 30 m x 0.25 mm i.d., 0.25 µm film thickness or equivalent column with same selectivity.

Gas chromatograph capable to operate with temperature rate, equipped with flame ionisation detector (FID), split / splitless injection and automatic sampler.

Software of integration

6.6.3 Preparation of solutions

a. <u>*Internal standard stock solution*</u>

Weigh, accurately to the nearest 0.1 mg, enough dicyclohexyl phthalate into a suitable volumetric flask to obtain a concentration of about 2.5 mg/mL. Add *n*-heptane 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 *n*-heptane (solution C_{ISTD}). Mix thoroughly.

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

[concentration of about 2.5 mg dicyclohexyl phthalate / mL].

Checking of non-interference

Transfer 1 mL of internal standard stock solution into a 25 mL volumetric flask and and add 24 mL of *n*-heptane. Mix thoroughly before filling an injection vial (solution Blank ISTD).

b. Bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide calibration stock solutions

Weigh in duplicate, accurately to the nearest 0.1 mg, about **25 mg of bifenthrin** (s_{BIF} mg), about **25 mg of chlorfenapyr** (s_{CFP} mg), about **25 mg of pyriproxyfen** (s_{PYR} mg) and about **25 mg of piperonyl butoxide** (s_{PBO} mg) analytical standards into two 25 mL volumetric flasks, each flask containing the four analytical standards. Add *n*-heptane 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 *n*-heptane (Solutions $C_{BIF+CFP+PYR+PBO}$ and $C^*_{BIF+CFP+PYR+PBO}$). Mix thoroughly.

[concentrations of about 1 mg bifenthrin / mL, of 1 mg chlorfenapyr / mL, of 1 mg pyriproxyfen / mL and of 1 mg piperonyl butoxide / mL].

c. *Bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide calibration working solutions*

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

Internal standard (C_{ISTD}) and bifenthrin + chlorfenapyr + pyriproxyfen + piperonyl butoxide ($C_{BIF+CFP+PYR+PBO}$) solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Table 3 Preparation of the calibration working solutions C_1 to C_5 from $C_{BIF+CFP+PYR+PBO}$ stock solution

Code	C_{ISTD}	Volume of $C_{BIF+CFP+PYR+PBO}$ transferred ($V_{BIF+CFP+PYR+PBO\ transferred}$)	Bifenthrin (µg/mL), approx.	Chlorfenapyr (µg/mL), approx.	Pyriproxyfen (µg/mL), approx.	Piperonyl butoxide (µg/mL), approx.	*n*-Heptane	Final volume ($V_{working\ cal\ BIF+CFP+PYR+PBO}$)
C_1	**1 mL**	**0.3 mL**	12	12	12	12	Up to volume	25 mL
C_2	**1 mL**	**1 mL**	40	40	40	40	Up to volume	25 mL
C_3	**1 mL**	**2.5 mL**	100	100	100	100	Up to volume	25 mL
C_4	**1 mL**	**4 mL**	160	160	160	160	Up to volume	25 mL
C_5	**1 mL**	**5 mL**	200	200	200	200	Up to volume	25 mL

$C^*_{BIF+CFP+PYR+PBO}$ is used to control the weighing of $C_{BIF+CFP+PYR+PBO}$: prepare a C^*_3 using the calibration stock solution $C^*_{BIF+CFP+PYR+PBO}$ as described in the below table (= calibration solution C^*_3).

Internal standard (C_{ISTD}) and bifenthrin + chlorfenapyr + pyriproxyfen + piperonyl butoxide ($C^*_{BIF+CFP+PYR+PBO}$) solutions shall be added at 20°C ± 1°C and using a volumetric pipette.

Table 4 Preparation of the calibration working solution C^*_3 from $C^*_{BIF+CFP+PYR+PBO}$ stock solution

Code	C_{ISTD}	Volume of $C^*_{BIF+CFP+PYR+PBO}$ transferred ($V_{BIF+CFP+PYR+PBO\ transferred}$)	Bifenthrin (µg/mL), approx.	Chlorfenapyr (µg/mL), approx.	Pyriproxyfen (µg/mL), approx.	Piperonyl butoxide (µg/mL), approx.	*n*-Heptane	Final volume ($V_{working\ cal\ BIF+CFP+PYR+PBO}$)
C^*_3	**1 mL**	**2.5 mL**	100	100	100	100	Up to volume	25 mL

Stock and working calibration solutions should be stored out of direct sunlight and in a refrigerated (<10°C) zone.

d. <u>Preparation of samples solutions for bifenthrin TC</u>

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add *n*-heptane 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 *n*-heptane. Mix thoroughly.

Transfer precisely by pipette 2.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 21.5 mL of *n*-heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Prepare this solution in duplicate for each batch of bifenthrin TC, named as follows :

- first weighing of bifenthrin TC_{BIF} 1 (batch R-22-92): **TC_{BIF}1A**
- second weighing of bifenthrin TC_{BIF} 1 (batch R-22-92): **TC_{BIF}1B**

- first weighing of bifenthrin TC_{BIF} 2 (batch R-22-93): **TC_{BIF}2A**
- second weighing of bifenthrin TC_{BIF} 2 (batch R-22-93): **TC_{BIF}2B**

e. <u>Preparation of samples solutions for chlorfenapyr TC</u>

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add *n*-heptane 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 *n*-heptane. Mix thoroughly.

Transfer precisely by pipette 2.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 21.5 mL of *n*-heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Prepare this solution in duplicate for each batch of chlorfenapyr TC, named as follows :

- first weighing of chlorfenapyr TC_{CFP} 1 (batch CLF-004/5BA/ST-IV-002): **TC_{CFP}1A**
- second weighing of chlorfenapyr TC_{CFP} 1 (batch CLF-004/5BA/ST-IV-002): **TC_{CFP}1B**

- first weighing of chlorfenapyr TC_{CFP} 2 (batch CLF-004/5BA/ST-IV-003): **TC_{CFP}2A**
- second weighing of chlorfenapyr TC_{CFP} 2 (batch CLF-004/5BA/ST-IV-003): **TC_{CFP}2B**

f. <u>Preparation of samples solutions for pyriproxyfen TC</u>

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add *n*-heptane 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 *n*-heptane. Mix thoroughly.

Transfer precisely by pipette 2.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal

standard stock solution and add 21.5 mL of *n*-heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Prepare this solution in duplicate for each batch of pyriproxyfen TC, named as follows :

- first weighing of pyriproxyfen TC_{PYR} 1 (batch PFN041I22 LOT I): **TC_{PYR}1A**
- second weighing of pyriproxyfen TC_{PYR} 1 (batch PFN041I22 LOT I): **TC_{PYR}1B**

- first weighing of pyriproxyfen TC_{PYR} 2 (batch PFN028H22 LOT II): **TC_{PYR}2A**
- second weighing of pyriproxyfen TC_{PYR} 2 (batch PFN028H22 LOT II): **TC_{PYR}2B**

g. <u>Preparation of samples solutions for piperonyl butoxide TC</u>

Weigh, accurately to the nearest 0.1 mg, about 25 mg of TC sample into a 25 mL volumetric flask. Add *n*-heptane 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 *n*-heptane. Mix thoroughly.

Transfer precisely by pipette 2.5 mL of this solution, at 20°C ± 1°C, into a cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution and add 21.5 mL of *n*-heptane. Mix thoroughly and filter an aliquot of the solution through a Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Prepare this solution in duplicate for each batch of piperonyl butoxide TC, named as follows :

- first weighing of piperonyl butoxide TC_{PBO} 1 (batch L202207065): **TC_{PBO}1A**
- second weighing of piperonyl butoxide TC_{PBO} 1 (batch L202207065): **TC_{PBO}1B**

- first weighing of piperonyl butoxide TC_{PBO} 2 (batch L202208091): **TC_{PBO}2A**
- second weighing of piperonyl butoxide TC_{PBO} 2 (batch L202208091): **TC_{PBO}2B**

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

Samples were cut according to the chapters 5.1 and 5.2.

Weigh, accurately to the nearest 0.1 mg, about 500 mg of LN/ITN sample cut in small pieces into a 100 mL cap glass bottle or flask. Add precisely at 20°C ± 1°C and with a volumetric pipette 1 mL of internal standard stock solution. Add 24 mL of *n*-heptane and put the flask in a heating ultrasonic bath at about 80°C for 60 minutes. 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 Nylon or PTFE filter with maximum 0.45 µm pore size, before filling an injection vial.

Prepare this solution in duplicate for each batch of net, named as follows :

- first weighing of the net sample Mo.841, batch (PNT) VKA-258-1: **LN/ITN 1A**
- second weighing of the net sample Mo.841, batch (PNT) VKA-258-1: **LN/ITN 1B**

- first weighing of the net sample Mo.842, batch IP-2-0: **LN/ITN 2A**
- second weighing of the net sample Mo.842, batch IP-2-0: **LN/ITN 2B**

- first weighing of the net sample Mo.843, batch (PND) VKA-257-2-1: **LN/ITN 3A**
- second weighing of the net sample Mo.843, batch (PND) VKA-257-2-1: **LN/ITN 3B**

- first weighing of the net sample Mo.844, batch (PNP) VKA-256-1: **LN/ITN 4A**
- second weighing of the net sample Mo.844, batch (PNP) VKA-256-1: **LN/ITN 4B**

6.6.4 Operating chromatographic conditions (typical)

Column capillary column Agilent J&W DB-210 ((50 % trifluoropropyl)-methylpolysiloxane), 30 m x 0.25 mm i.d., 0.25 µm film thickness or equivalent column with same selectivity.

Injection system

- Injector : split injection
- Injector temperature : 260°C
- Split ratio : 10 : 1
- Split flow : 12 mL/min
- Total flow : 16.2 mL/min
- Injection volume : 1 µL

Detector system

- Type : Flame Ionization Detection (FID)
- Detector temperature : 300°C

Oven temperature program

- 110°C, hold 0.5 minute
- ramp rate : 15°C / minutes, up to 215°, hold 10 minutes
- ramp rate : 10°C / minutes, up to 240°, hold 2(*) minutes

(*) can be increased in order to completely elute the potential remaining components.

Gas and flow :

- Carrier : helium, 1.2 mL/min in constant flow (with an EPC)
- Makeup : nitrogen, 30 mL/min
- FID : hydrogen, 30 mL/min
  air (clean) : 300 mL/min

Run time 22 minutes

Retention times : piperonyl butoxide : ± 11.3 minutes
bifenthrin : ± 13.4 minutes
pyriproxyfen : ± 13.7 minutes

chlorfenapyr : ± 18.3 minutes
dicyclohexyl phthalate (internal standard) : ± 18.5 minutes

Injection sequence : calibration solution 1, sample weighing 1, sample weighing 2, calibration solution 2, sample weighing 3, ...

Each solution is injected in duplicate.

6.6.5 Determination

Quantitative determination of bifenthrin, of chlorfenapyr, of pyriproxyfen and of piperonyl butoxide in the sample solutions is carried out by comparing the ratio of peaks area of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide to the peak area of dicyclohexyl phthalate in the sample solutions with that of the standard solutions, on basis of a calibration curve calculated with standard solutions (C_1 to C_5) bracketing the sample solutions.

6.6.6 Data handling

a. <u>Response factor of the calibration solutions</u>

After equilibration of the chromatographic system, inject the 2 calibration working solutions C_3 and C^*_3 before analysis to ensure that the relative response factors for C^*_3 ($f_{i\ BIF}$ *vs* $f^*_{i\ BIF}$, $f_{i\ CFP}$ *vs* $f^*_{i\ CFP}$, $f_{i\ PYR}$ *vs* $f^*_{i\ PYR}$ and $f_{i\ PBO}$ *vs* $f^*_{i\ PBO}$) does not deviate by more than 2.0 % from that of solution C_3, for each active ingredient. Otherwise, prepare new calibration solutions.

Calculate the relative response factors using the following formula :

$$f_{i\ BIF\ or\ CFP\ or\ PYR\ or\ PBO} = \frac{I_r \times s_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times V_{BIF+CFP+PYR+PBO\ transferred}}{H_{s\ BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times V_{stock\ BIF+CFP+PYR+PBO} \times V_{working\ cal\ BIF+CFP+PYR+PBO}}$$

Where :

$f_{i\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = individual response factor, for bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide.

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

$H_{s\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide in the calibration working solution (C_3 or C^*_3).

$s_{BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+CFP+PYR+PBO}$ and $C^*_{BIF+CFP+PYR+PBO}$, in mg.

$P_{BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+CFP+PYR+PBO}$ and $C^*_{BIF+CFP+PYR+PBO}$, in g/kg.

$V_{BIF+CFP+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+CFP+PYR+PBO}$ or $C^*_{BIF+CFP+PYR+PBO}$) transferred to prepare the working calibration solution (C_3 or C^*_3), in mL (= 2.5 mL).

$V_{stock\ BIF+CFP+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+CFP+PYR+PBO}$ or $C^*_{BIF+CFP+PYR+PBO}$), in mL (= 25 mL).

$V_{working\ cal\ BIF+CFP+PYR+PBO}$ = total volume of the calibration working solution (C_3 or C^*_3), in mL (= 25 mL).

b. <u>Determination of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide in the sample solutions</u>

Quantitative determination of bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide in the sample solutions is carried out by comparing the ratio of peaks area of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide to the peak area of dicyclohexyl phthalate in the sample solutions with that of the standard working solutions (C_1 to C_5) bracketing the sample solutions.

The calibration curves for bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide are obtained by the internal standard calibration method from the injection of bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide standard solutions containing dicyclohexyl phthalate and plotting the ratio of peaks areas (peak area BIF, CFP, PYR or PBO / peak area ITSD) versus the bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide concentration (in µg/mL) :

$$\text{y- values} = \frac{H_{s\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}}{I_r}$$

$$\text{x- values} = \frac{s_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times P_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times V_{BIF+CFP+PYR+PBO\ transferred}}{V_{stock\ BIF+CFP+PYR+PBO} \times V_{working\ cal\ BIF+CFP+PYR+PBO}}$$

Where :

$H_{s\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide in the calibration working solution (C_1, C_2, C_3, C_4 or C_5).

I_r = peak area of internal standard in the calibration working solution (C_1, C_2, C_3, C_4 or C_5).

$s_{BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = mass of bifenthrin, chlorfenapy, pyriproxyfen or piperonyl butoxide reference standard in the calibration stock solution $C_{BIF+CFP+PYR+PBO}$, in mg.

$P_{BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = purity of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide reference standard used to prepare the calibration stock solution $C_{BIF+CFP+PYR+PBO,}$ in g/kg.

$V_{BIF+CFP+PYR+PBO\ transferred}$ = volume of the calibration stock solution ($C_{BIF+CFP+PYR+PBO}$) transferred to prepare the working calibration solutions (C_1 to C_5), in mL (= 0.3 mL, 1 mL, 2.5 mL, 4 mL and 5 mL, respectively).

$V_{stock\ BIF+CFP+PYR+PBO}$ = volume of the volumetric flask used to prepare the calibration stock solution ($C_{BIF+CFP+PYR+PBO}$), in mL (= 25 mL).

$V_{working\ cal\ BIF+CFP+PYR+PBO}$ = total volume of the calibration working solution (C_1 to C_5), in mL (= 25 mL).

Calculate, for each of the 4 calibration curves, the equation of the linear regression obtained :

$$y = ax + b$$

Where :

a = slope of the linear regression obtained for bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide.

b = intercept of the linear regression obtained for bifenthrin, chlorfenapyr pyriproxyfen or piperonyl butoxide.

The amount of bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide in the samples is obtained using the equation of the linear regression :

$$C_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} = \frac{\frac{H_{w\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}}{I_q}}{a} - b$$

Where :

$C_{BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = concentration of bifenthrin, chlorfenapyr , pyriproxyfen or piperonyl butoxide in the sample solution, in µg/mL.

$H_{w\ BIF\ or\ CFP\ or\ PYR\ or\ PBO}$ = peak area of bifenthrin, chlorfenapyr , pyriproxyfen or piperonyl butoxide in the sample solution.

I_q = peak area of internal standard in the sample solution.

Bifenthrin, chlorfenapyr, pyriproxyfen or piperonyl butoxide content in the samples is expressed in g of bifenthrin, in g of chlorfenapyr, in g of pyriproxyfen and in g of piperonyl butoxide per kg of sample, taking into account of dilution factor and sample weight:

$$\text{BIF or CFP or PYR or PBO content} = \frac{C_{BIF\ or\ CFP\ or\ PYR\ or\ PBO} \times D}{W}\ \text{g/kg}$$

Where :

D = dilution factor of the sample solution (= 25 for LN/ITN,
= 25*25/2.5 for TC).

W = weight of the sample, in mg.

7. Remarks from the participants

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

Sample and calibration solutions preparation

Laboratory 1 : The amount of chlorfenapyr in the calibration solution (C*) on day 2 is about 20 mg due to the analytical standard (100 mg) is not enough to weigh in duplication of 25 mg for 2 days.

R./ 2*100 mg were sent (because analytical standards are quite expensive), when 2*100 mg were necessary. The risk is that the uncertainty of measurement increases inversely with the weight. Users shall ensure that the weighing is within the range of use of the balance that gives a reasonable and defined uncertainty of measurement.

Laboratories 8 & 11: The quantity of reference substance was only 100 mg : the preparation of C_3 and C_3* was not possible on 2 days as piperonyl butoxide could not be weighed being a liquid hence, only C_3 was prepared on 2 days but C_3* which was prepared on day 1 was used for day 2. (Same comment for chlorfenapyr, except that it is a solid).

R./ 2*100 mg were sent (because analytical standards are quite expensive), when 2*100 mg were necessary. The solution proposed by the laboratories is acceptable since the C_3* is not used for the determination of the active ingredients / synergist, but for checking the accuracy of the calibration solution.

Laboratory 11: Ultrasonic bath only reached 64 °C to 74 °C (LN net samples).

R./ It is a significant issue since 80°C are needed to fully extract the active ingredients and/or synergist.

Laboratory 12: Our ultrasonic bath only heats up to 70°C.

R./ See previous remark.

Laboratory 2: The final solution was filtered through a Nylon filter with 0.45 µm pore size.

R./ This is in accordance with the analytical method.

Laboratory 11: Only LN net samples were filtered. All TC samples and analytical standards dissolved without needing an ultrasonic bath to a clear solution without suspended particles.

R./ It has no consequence on the results.

Laboratory 2: Recommend to preparation TC sample in diluting step by filling *n*-heptane to the mark of 25 mL volumetric flask after allow the solution to cool to the room temperature, instead of adding 21.5 ml of *n*-heptane.

R./ This can be done, if desired, but it takes more time and it is not necessary because adding a controlled volume of internal standard allows an approximate volume of solvent to be added.

Laboratory 8: The sample preparation was tedious. (Instead of taking a bottle and adding the solvent by pipette, a volumetric flask of 25 ml was used).

R./ This can be done, if desired, but it takes more time and it is not necessary because adding a controlled volume of internal standard allows an approximate volume of solvent to be added, not necessarily with a pipette.

Laboratory 11: TC-Sample dilution done in two steps, 2.0 mL glass pipette + 0.5 mL Microman capillary piston pipette.

R./ This can lead to a greater variability in the results. However, this laboratory obtained good results.

Laboratory 7: Conditions for testing: 25 ± 3°C.

R./ It has no consequence on the results, provided that the internal standard has been added at 20°C ± 1°C, which is the temperature at which pipettes are calibrated. If not, the volume of internal standard actually added differs with the variation of temperature.

Laboratory 11: Room temperature = 24 °C

R./ See previous remark.

Laboratory 7: The internal standard used for day 2 has a purity of 99.0%

R./ It has no consequence on the results, provided that the same internal standard solution was used for both calibration and sample solutions.

Laboratory 11: $TC_{BIF}1$ was measured in duplicate a 2nd time (day 1), injections 9-12 were replaced with injections 85-88 (due to a handling error in the laboratory, ~130% purity).

R./ -.

Chromatographic injection

Laboratories 1 & 11: Retention times variations

R./ This may be due to an issue with chromatograph (leakage of gas, flow instability, pressure control problem …)

Laboratory 3: In Perkin Elmer GC, the facility for makeup gas is not available, since the complete sample eluting out of column enters the FID detector leading to no loss of sample and signal. This is well evident with the R^2 values observed in the calibration curves platted against different concentrations of the standards for both the days

R./ The R^2 values obtained in the 4 calibration curves are >0.999 for both days. It has no consequence on the quality of the results.

Laboratory 8: There are closely eluting peaks (effects the system suitability test i.e. the resolution between the peaks is not >1.5) of IS, CFP and BIF and PYR.

R./ Chromatographic conditions have been optimized to elute and separate in one single chromatographic injection, all the 4 molecules. Some peaks are indeed closely eluted but the typical chromatographic conditions proposed ensure a full separation of the peaks. No overlap, repeatability of injection issues or integration issues have been observed among the chromatograms provided by the participants.

Calculation

Laboratory 11: Purities were taken directly from the vials containing the analytical standards and not from the Excel sheet (there was a discrepancy).

R./ All Excel sheet have been checked by the coordinator of the trial and have been corrected if needed.

In conclusion, the remarks regarding the heating of the ultrasonic bath can significantly affect the accuracy of the results obtained. See chapter 9.3 “Conclusion” for further discussion.

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 firstly Cochran's test and then using Grubbs' test. Cochran's test is an evaluation of the within-laboratory variance and the Grubbs' test 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

Amongst the sixteen laboratories that agreed to participate for the bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide full scale collaborative trial, thirteen provided results on time (see Chapter 1 for more details).

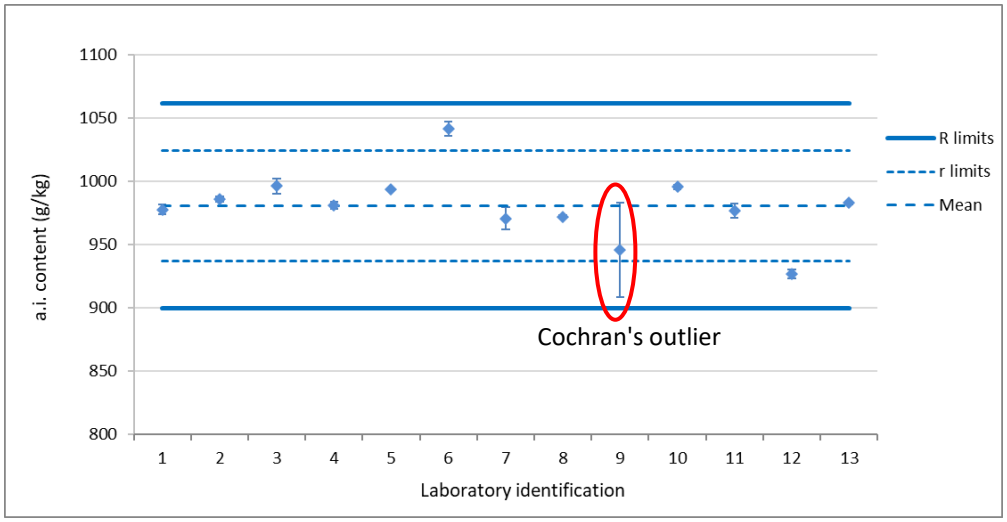
Results of these thirteen laboratories were taken into consideration for statistical treatment.

8.3 Bifenthrin content

Tables 5 and 9 show all results obtained and a summary of the statistical evaluation is given in tables 13 to 16.

8.3.1 Bifenthrin content in technical material (TC)

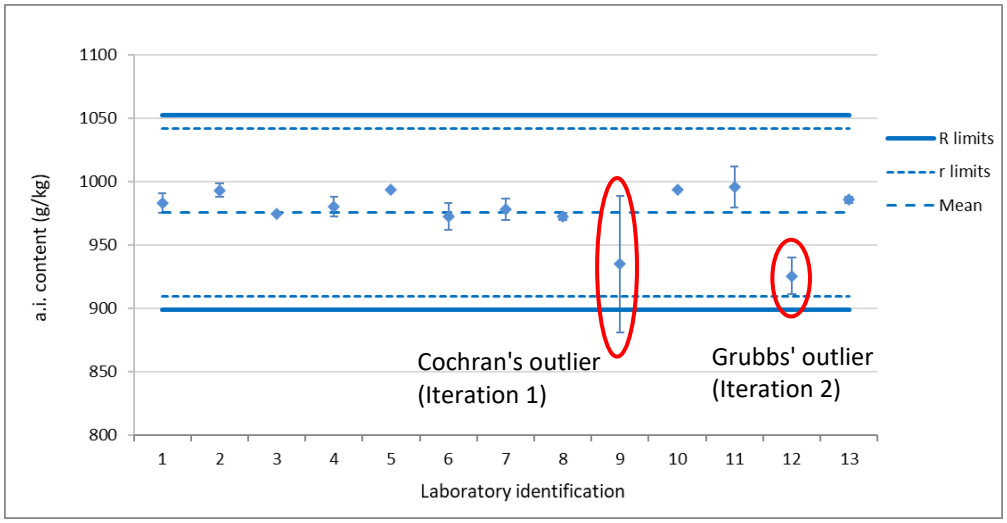
Figure 3 Determination of bifenthrin content in TC_{BIF} 1, batch n° R-22-92



	TC_{BIF} 1
$\bar{x}$	980 g/kg
L	13 labs
s_r	15.71 g/kg
s_L	24.54 g/kg
s_R	29.14 g/kg
r	43.55 g/kg
R	80.78 g/kg
RSD_r	1.60 %
RSD_R	2.97 %
$RSD_{R(Horw)}$	2.01 %
HorRat	**1.48**

HorRat = 1.33 without outlier

Figure 4 Determination of bifenthrin content in TC_{BIF} 2, batch n° R-22-93

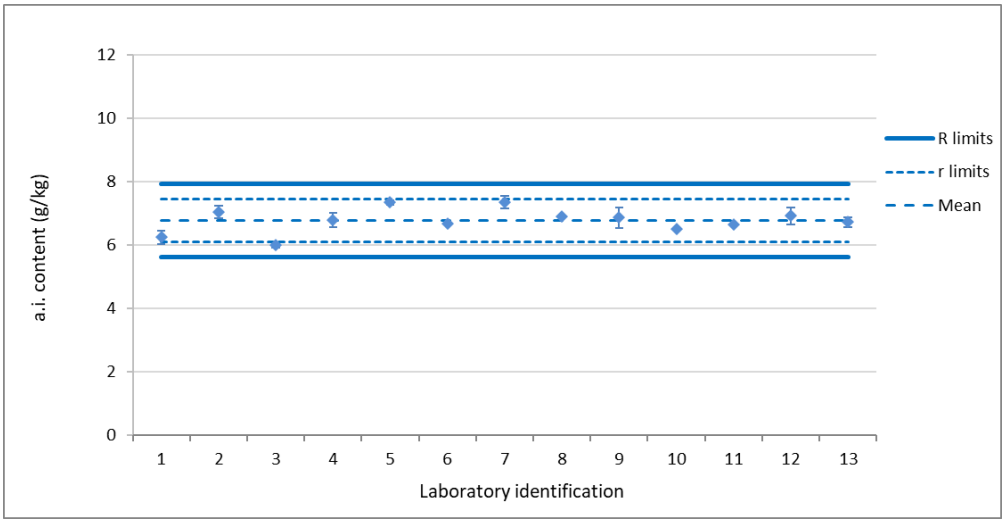


	TC_{BIF} 2
$\bar{x}$	976 g/kg
L	13 labs
s_r	23.91 g/kg
s_L	13.74 g/kg
s_R	27.57 g/kg
r	66.26 g/kg
R	76.43 g/kg
RSD_r	2.45 %
RSD_R	2.83 %
$RSD_{R(Horw)}$	2.01 %
HorRat	**1.41**

HorRat = 1.05 (It.1)
= 0.59 (It.2)
without outliers

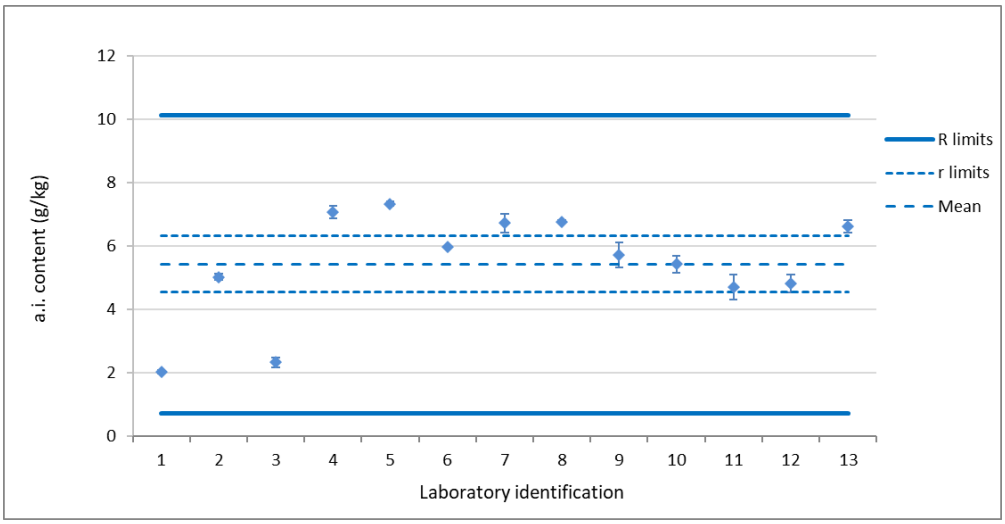
8.3.2 Bifenthrin content in long-lasting (incorporated into filaments) insecticide-treated net (LN/ITN)

Figure 5 Determination of bifenthrin content in LN/ITN 1, batch n° (PNT) VKA-258-1



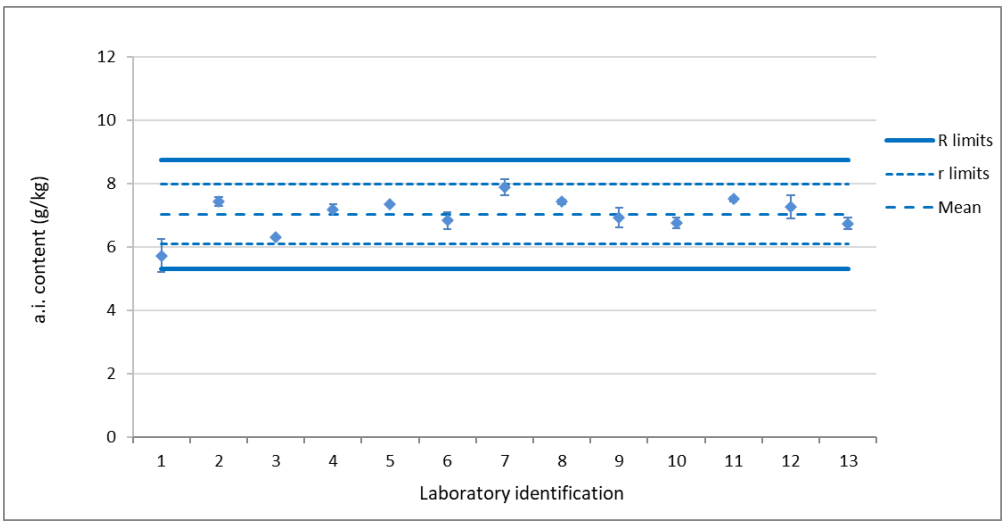
	BIF LN/ITN 1
$\bar{x}$	6.77 g/kg
L	13 labs
s_r	0.24 g/kg
s_L	0.34 g/kg
s_R	0.42 g/kg
r	0.67 g/kg
R	1.16 g/kg
RSD_r	3.58 %
RSD_R	6.20 %
$RSD_{R(Horw)}$	4.24 %
HorRat	**1.46**

Figure 6 Determination of bifenthrin content in LN/ITN 2, batch n° IP-2-0



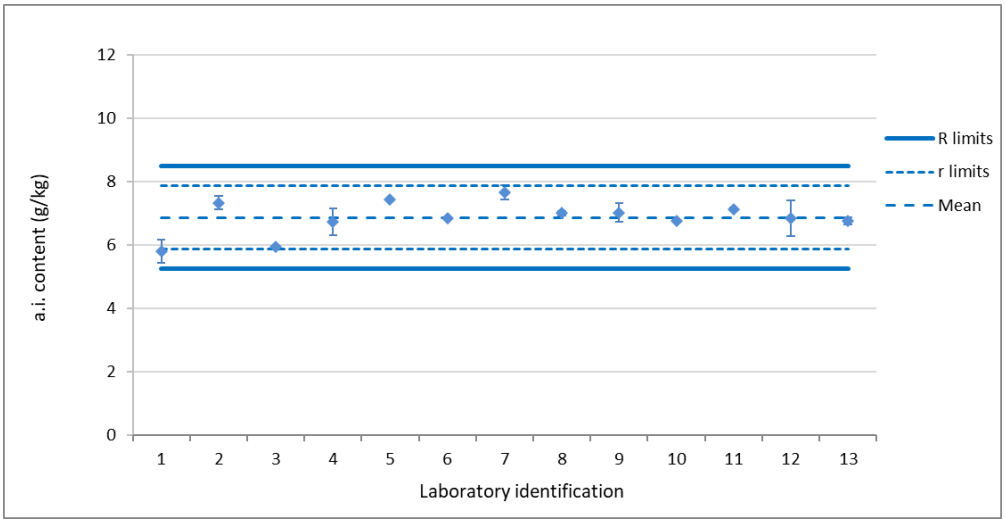
	BIF LN/ITN 2
$\bar{x}$	5.43 g/kg
L	13 labs
s_r	0.32 g/kg
s_L	1.66 g/kg
s_R	1.69 g/kg
r	0.88 g/kg
R	4.69 g/kg
RSD_r	5.88 %
RSD_R	31.20 %
$RSD_{R(Horw)}$	4.39 %
HorRat	**7.12**

Figure 7 Determination of bifenthrin content in LN/ITN 3, batch n° (PND) VKA-257-2-1



	BIF LN/ITN 3
$\bar{x}$	7.03 g/kg
L	13 labs
s_r	0.34 g/kg
s_L	0.52 g/kg
s_R	0.62 g/kg
r	0.94 g/kg
R	1.72 g/kg
RSD_r	4.84 %
RSD_R	8.84 %
$RSD_{R(Horw)}$	4.22 %
HorRat	**2.10**

Figure 8 Determination of bifenthrin content in LN/ITN 4, batch n° (PNP) VKA-256-1



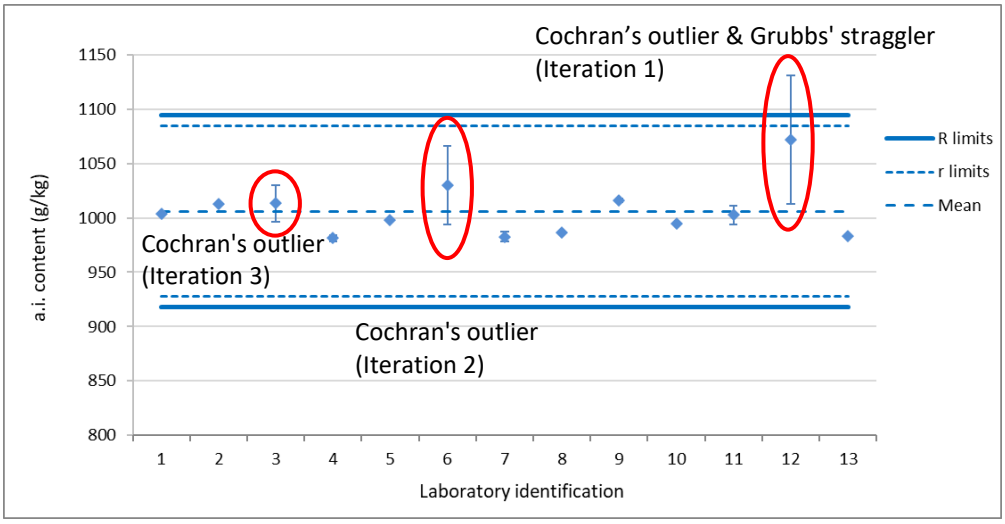
	BIF LN/ITN 4
$\bar{x}$	6.87 g/kg
L	13 labs
s_r	0.36 g/kg
s_L	0.46 g/kg
s_R	0.58 g/kg
r	1.00 g/kg
R	1.61 g/kg
RSD_r	5.24 %
RSD_R	8.47 %
$RSD_{R(Horw)}$	4.23 %
HorRat	**2.00**

8.4 Chlorfenapyr content

Tables 6 and 10 show all results obtained and a summary of the statistical evaluation is given in tables 13 to 16.

8.4.1 Chlorfenapyr content in technical material (TC)

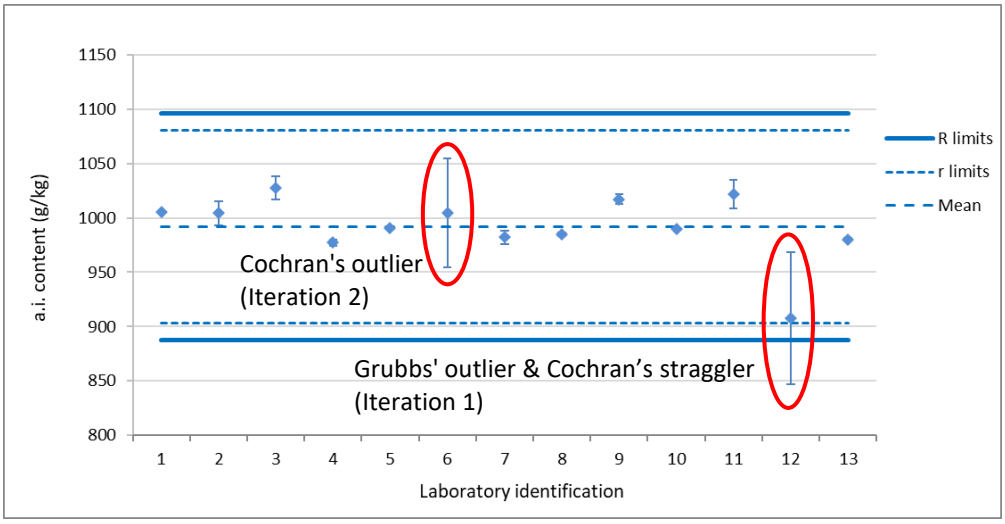
Figure 9 Determination of chlorfenapyr content in TC_{CFP} 1, batch n° CLF-004/5BA/ST-IV-002



	TC_{CFP} 1
$\bar{x}$	1006 g/kg
L	13 labs
s_r	28.27 g/kg
s_L	14.69 g/kg
s_R	31.86 g/kg
r	78.37 g/kg
R	88.32 g/kg
RSD_r	2.81 %
RSD_R	3.17 %
$RSD_{R(Horw)}$	2.00 %
HorRat	**1.59**

HorRat = 0.98 (It .1)
= 0.72 (It.2)
= 0.65 (It.3)
without outliers

Figure 10 Determination of chlorfenapyr content in TC_{CFP} 2, batch n° CLF-004/5BA/ST-IV-003

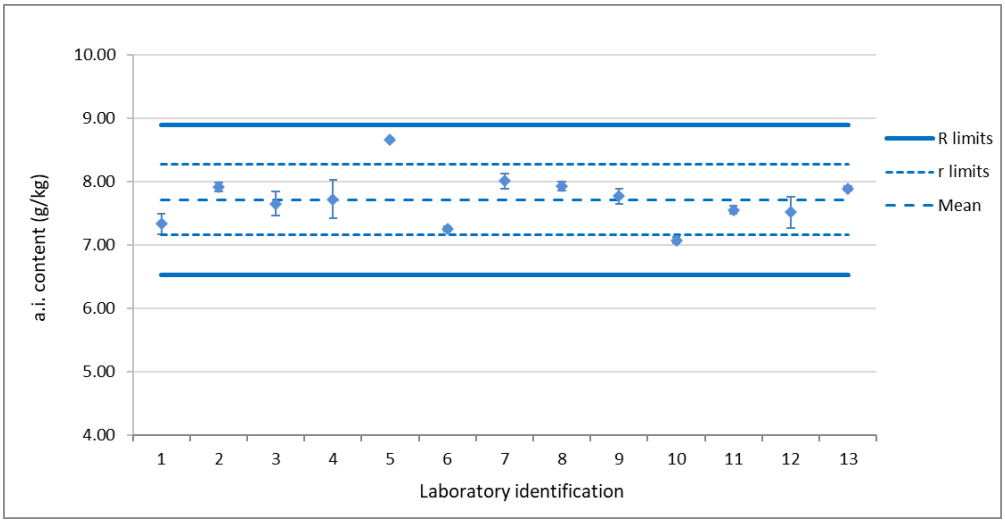


	TC_{CFP} 2
$\bar{x}$	992 g/kg
L	13 labs
s_r	32.02 g/kg
s_L	19.81 g/kg
s_R	37.65 g/kg
r	88.74 g/kg
R	104.35 g/kg
RSD_r	3.23 %
RSD_R	3.80 %
$RSD_{R(Horw)}$	2.00 %
HorRat	**1.90**

HorRat = 1.17 (It .1)
= 0.95 (It.2)
without outliers

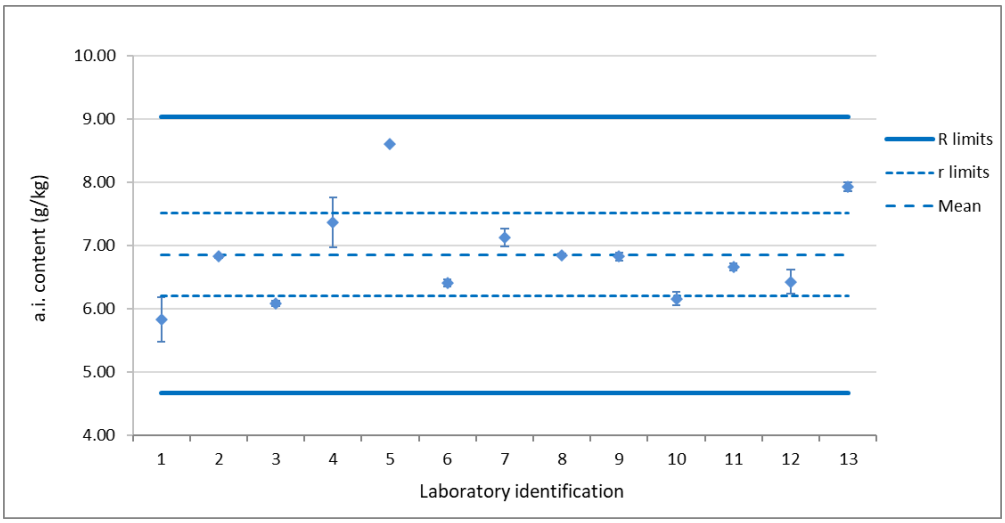
8.4.2 Chlorfenapyr content in long-lasting (incorporated into filaments) insecticide-treated net (LN/ITN)

Figure 11 Determination of chlorfenapyr content in LN/ITN 1, batch n° (PNT) VKA-258-1



	CFP LN/ITN 1
$\bar{x}$	7.71 g/kg
L	13 labs
s_r	0.20 g/kg
s_L	0.38 g/kg
s_R	0.43 g/kg
r	0.56 g/kg
R	1.18 g/kg
RSD_r	2.61 %
RSD_R	5.53 %
$RSD_{R(Horw)}$	4.16 %
HorRat	**1.33**

Figure 12 Determination of chlorfenapyr content in LN/ITN 3, batch n° (PND) VKA-257-2-1



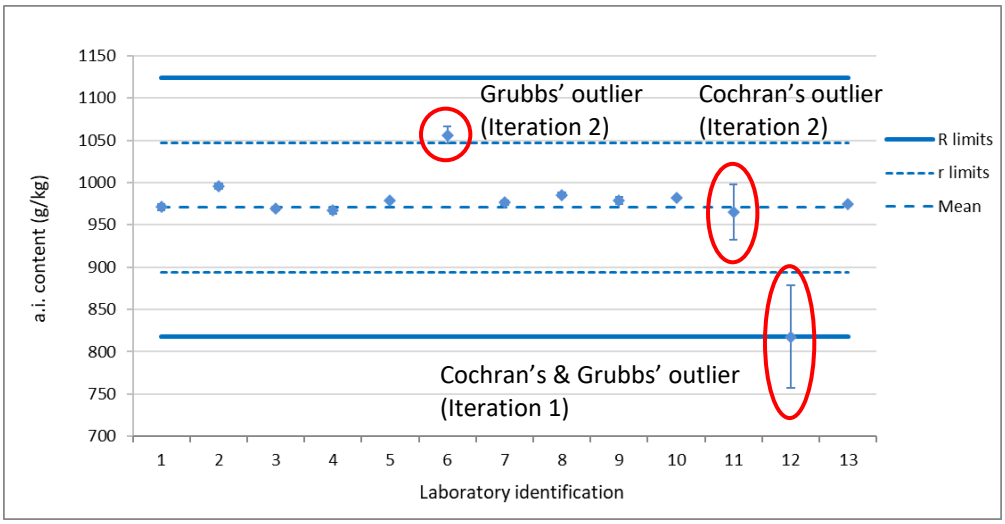
	CFP LN/ITN 3
$\bar{x}$	6.85 g/kg
L	13 labs
s_r	0.24 g/kg
s_L	0.75 g/kg
s_R	0.79 g/kg
r	0.65 g/kg
R	2.18 g/kg
RSD_r	3.44 %
RSD_R	11.47 %
$RSD_{R(Horw)}$	4.23 %
HorRat	**2.71**

8.5 Pyriproxyfen content

Tables 7 and 11 show all results obtained and a summary of the statistical evaluation is given in tables 13 to 16.

8.5.1 Pyriproxyfen content in technical material (TC)

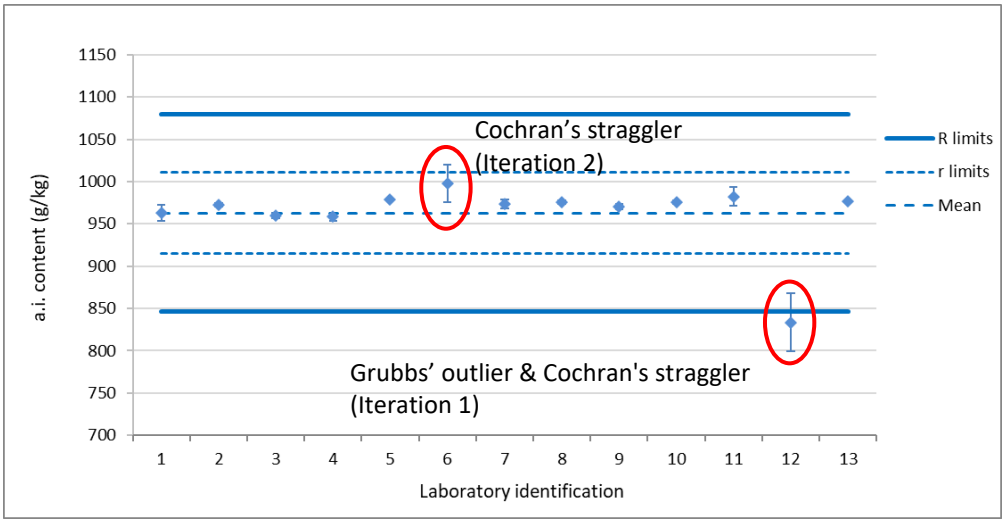
Figure 13 Determination of pyriproxyfen content in TC_{PYR} 1, batch n PFN041I22 LOT I



	TC_{PYR} 1
$\bar{x}$	971 g/kg
L	13 labs
s_r	27.69 g/kg
s_L	47.67 g/kg
s_R	55.13 g/kg
r	76.75 g/kg
R	152.81 g/kg
RSD_r	2.85 %
RSD_R	5.68 %
$RSD_{R(Horw)}$	2.01 %
HorRat	**2.83**

HorRat = 1.34 (It .1)
= 0.46 (It.2)
without outliers

Figure 14 Determination of pyriproxyfen content in TC_{PYR} 2, batch n° PFN028H22 LOT II

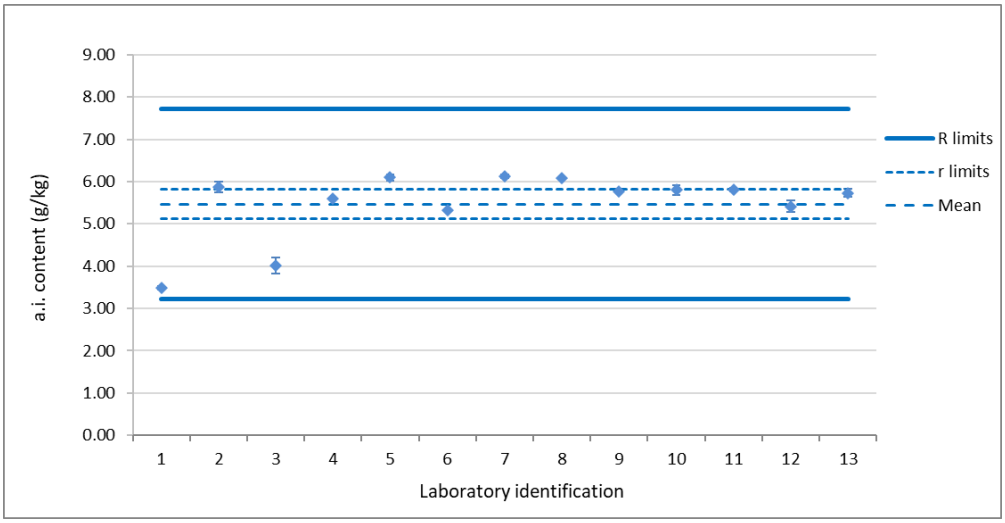


	TC_{PYR} 2
$\bar{x}$	963 g/kg
L	13 labs
s_r	17.32 g/kg
s_L	38.26 g/kg
s_R	42.00 g/kg
r	48.02 g/kg
R	116.42 g/kg
RSD_r	1.80 %
RSD_R	4.36 %
$RSD_{R(Horw)}$	2.01 %
HorRat	**2.17**

HorRat = 0.68
without outlier

8.5.2 Pyriproxyfen content in long-lasting (incorporated into filaments) insecticide-treated net (LN/ITN)

Figure 15 Determination of pyriproxyfen content in LN/ITN 2, batch n° IP-2-0



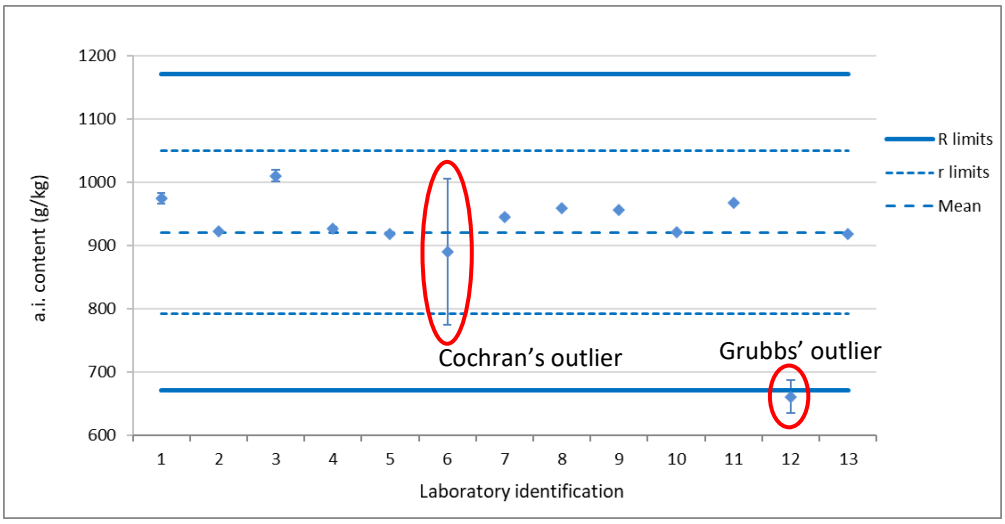
	PYR LN/ITN 2
$\bar{x}$	5.47 g/kg
L	13 labs
s_r	0.13 g/kg
s_L	0.80 g/kg
s_R	0.81 g/kg
r	0.35 g/kg
R	2.25 g/kg
RSD_r	2.33 %
RSD_R	14.82 %
$RSD_{R(Horw)}$	4.38 %
HorRat	**3.38**

8.6 Piperonyl butoxide content

Tables 8 and 12 show all results obtained and a summary of the statistical evaluation is given in tables 13 to 16.

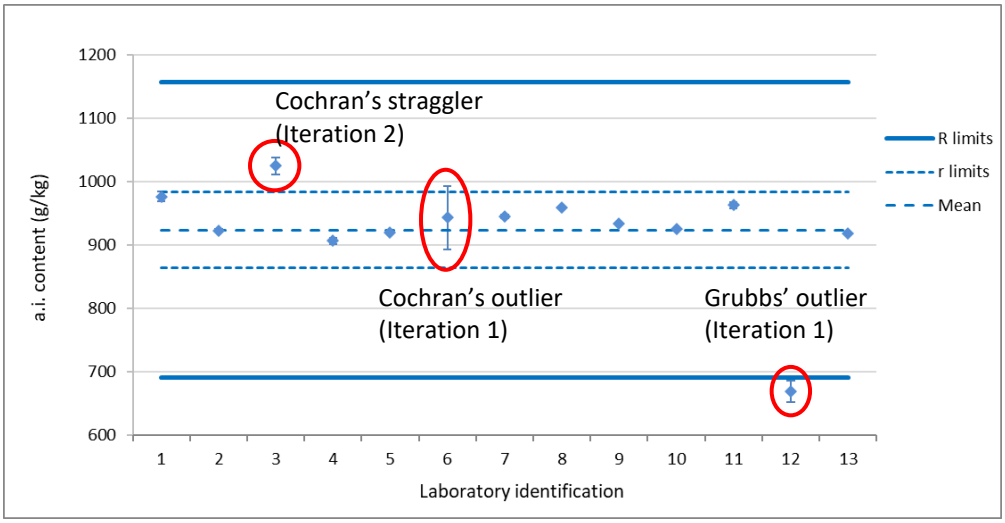
8.6.1 Piperonyl butoxide content in technical material (TC)

Figure 16 Determination of piperonyl butoxide content in TC_{PBO} 1, batch n L202207065



	TC_{PBO} 1
$\bar{x}$	921 g/kg
L	13 labs
s_r	46.52 g/kg
s_L	77.34 g/kg
s_R	90.25 g/kg
r	128.93 g/kg
R	250.16 g/kg
RSD_r	5.05 %
RSD_R	9.80 %
$RSD_{R(Horw)}$	2.02 %
HorRat	**4.84**
HorRat without outliers	= 1.57

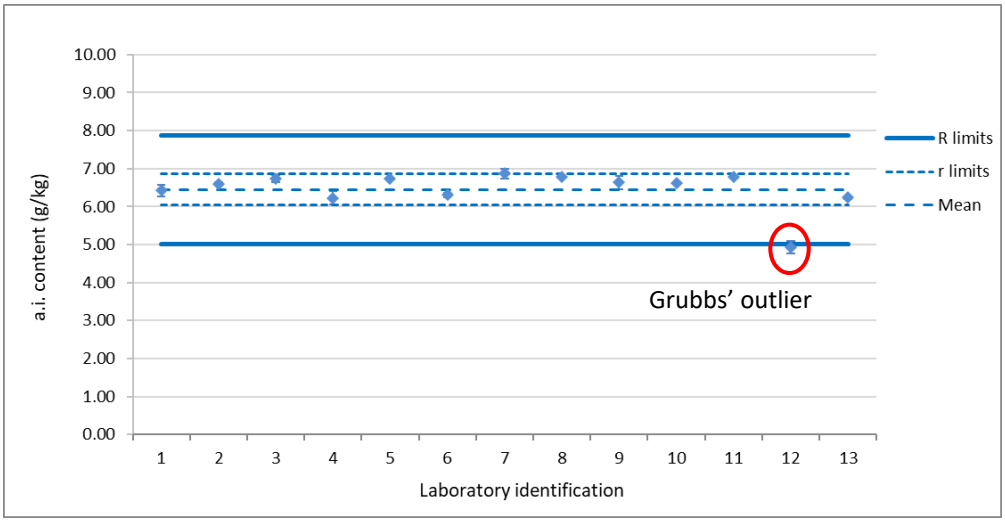
Figure 17 Determination of piperonyl butoxide content in TC_{PYR} 2, batch n° L202208091



	TC_{PBO} 2
$\bar{x}$	924 g/kg
L	13 labs
s_r	21.63 g/kg
s_L	81.21 g/kg
s_R	84.05 g/kg
r	59.96 g/kg
R	232.96 g/kg
RSD_r	2.34 %
RSD_R	9.10 %
$RSD_{R(Horw)}$	2.02 %
HorRat	**4.50**
HorRat without outlier	= 1.81

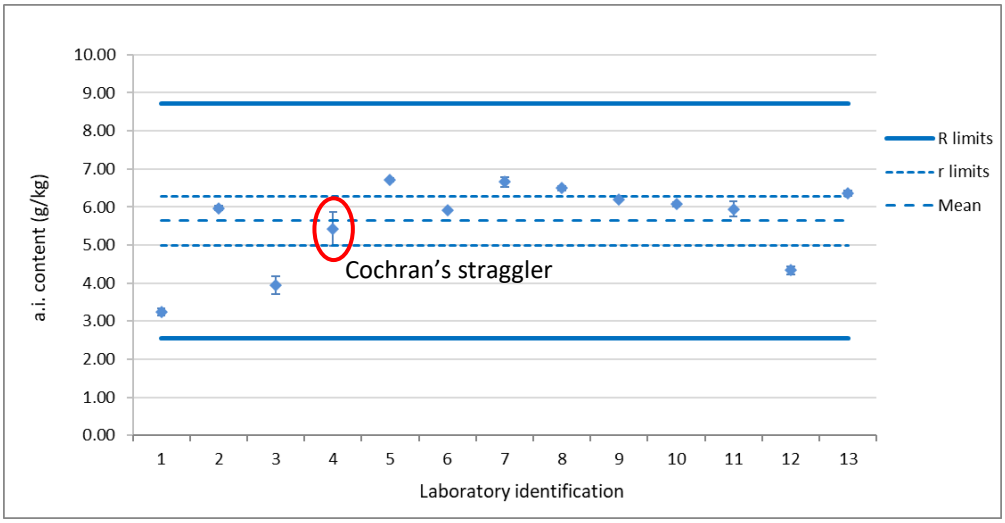
8.6.2 Piperonyl butoxide content in long-lasting (incorporated into filaments) insecticide-treated net (LN/ITN)

Figure 18 Determination of piperonyl butoxide content in LN/ITN 1, batch n° (PNT) VKA-258-1



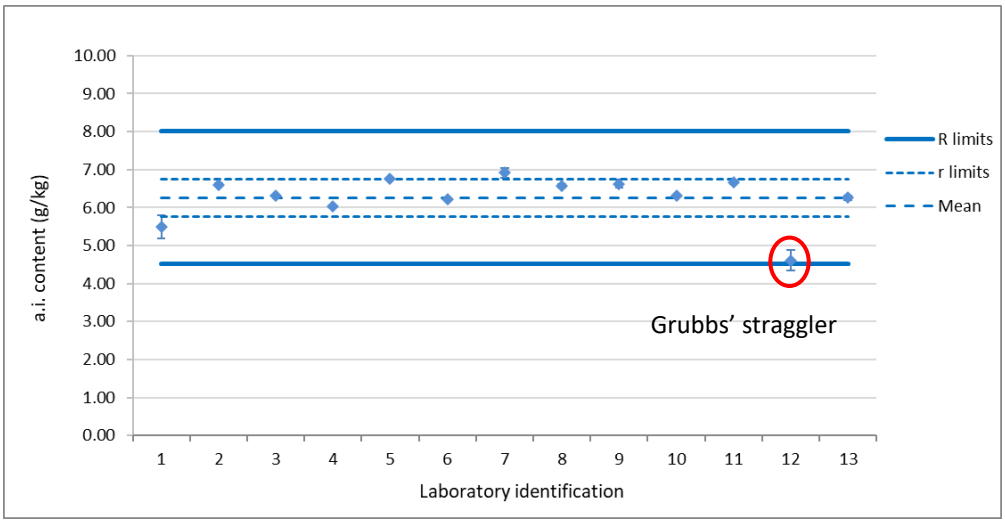
	PBO LN/ITN 1
$\bar{x}$	6.45 g/kg
L	13 labs
s_r	0.15 g/kg
s_L	0.49 g/kg
s_R	0.51 g/kg
r	0.41 g/kg
R	1.43 g/kg
RSD_r	2.28 %
RSD_R	7.99 %
$RSD_{R(Horw)}$	4.27 %
HorRat	**1.87**
HorRat without outlier	= 0.87

Figure 19 Determination of piperonyl butoxide content in LN/ITN 2, batch n° IP-2-0



	PBO LN/ITN 2
$\bar{x}$	5.63 g/kg
L	13 labs
s_r	0.23 g/kg
s_L	1.09 g/kg
s_R	1.11 g/kg
r	0.64 g/kg
R	3.09 g/kg
RSD_r	4.10 %
RSD_R	19.77 %
$RSD_{R(Horw)}$	4.36 %
HorRat	**4.53**

Figure 20 Determination of piperonyl butoxide content in LN/ITN 4, batch n° (PNP) VKA-256-1



	PBO LN/ITN 4
$\bar{x}$	6.26 g/kg
L	13 labs
s_r	0.18 g/kg
s_L	0.60 g/kg
s_R	0.63 g/kg
r	0.49 g/kg
R	1.74 g/kg
RSD_r	2.83 %
RSD_R	10.03 %
$RSD_{R(Horw)}$	4.29 %
HorRat	**2.34**

Table 5 Determination of bifenthrin content in technical material, in g/kg

	TC_{BIF} 1			TC_{BIF} 2		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	974	982	**978**	975	991	**983**
Lab 2	988	984	**986**	998	988	**993**
Lab 3	1002	990	**996**	974	976	**975**
Lab 4	978	984	**981**	988	973	**980**
Lab 5	993	994	**993**	993	994	**993**
Lab 6	1047	1036	**1042**	962	983	**973**
Lab 7	962	979	**971**	970	987	**978**
Lab 8	973	971	**972**	970	975	**973**
Lab 9	909	983	**946****	881	989	**935****
Lab 10	997	994	**995**	994	993	**993**
Lab 11	971	982	**977**	1012	979	**996**
Lab 12	930	923	**927**	911	940	**926°°**
Lab 13	982	984	**983**	988	983	**986**

***Cochran's straggler** ****Cochran's outlier**
°Grubbs' straggler **°°Grubbs' outlier**

Table 6 ***Determination of chlorfenapyr content in technical material, in g/kg***

	TC_{CFP} 1			TC_{CFP} 2		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	1004	1004	**1004**	1005	1006	**1005**
Lab 2	1013	1013	**1013**	1015	993	**1004**
Lab 3	1030	996	**1013****	1038	1017	**1028**
Lab 4	984	978	**981**	980	975	**977**
Lab 5	998	998	**998**	991	991	**991**
Lab 6	994	1066	**1030****	954	1055	**1005****
Lab 7	978	987	**983**	976	989	**982**
Lab 8	987	986	**987**	986	983	**985**
Lab 9	1014	1018	**1016**	1022	1013	**1017**
Lab 10	995	994	**994**	989	991	**990**
Lab 11	994	1012	**1003**	1035	1008	**1021**
Lab 12	1131	1013	**1072**°**	847	968	**908**°°*
Lab 13	984	983	**984**	981	979	**980**

***Cochran's straggler** ****Cochran's outlier**
°Grubbs' straggler **°°Grubbs' outlier**

Table 7 ***Determination of pyriproxyfen content in technical material, in g/kg***

	TC_{PYR} 1			TC_{PYR} 2		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	975	968	**971**	953	972	**963**
Lab 2	999	993	**996**	975	970	**972**
Lab 3	967	972	**969**	956	963	**960**
Lab 4	963	971	**967**	963	954	**959**
Lab 5	978	980	**979**	978	979	**979**
Lab 6	1066	1046	**1056**°°	976	1020	**998***
Lab 7	979	973	**976**	968	979	**973**
Lab 8	983	988	**985**	978	973	**976**
Lab 9	983	975	**979**	973	967	**970**
Lab 10	981	982	**982**	978	974	**976**
Lab 11	933	998	**965****	971	993	**982**
Lab 12	879	757	**818**/°°	868	799	**834**°/*
Lab 13	976	973	**974**	977	977	**977**

***Cochran's straggler** ****Cochran's outlier**
°Grubbs' straggler **°°Grubbs' outlier**

Table 8 ***Determination of piperonyl butoxide content in technical material, in g/kg***

	TC_{PBO} 1			TC_{PBO} 2		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	983	967	**975**	984	968	**976**
Lab 2	923	922	**922**	921	925	**923**
Lab 3	1020	1001	**1010**	1011	1038	**1025***
Lab 4	923	930	**926**	911	902	**907**
Lab 5	918	918	**918**	921	918	**920**
Lab 6	775	1005	**890****	893	992	**943****
Lab 7	943	947	**945**	947	943	**945**
Lab 8	959	958	**959**	959	960	**959**
Lab 9	958	955	**956**	936	933	**934**
Lab 10	921	921	**921**	927	925	**926**
Lab 11	969	966	**968**	958	968	**963**
Lab 12	688	635	**661°°**	686	652	**669°°**
Lab 13	916	919	**918**	919	918	**919**

***Cochran's straggler** ****Cochran's outlier**
°Grubbs' straggler **°°Grubbs' outlier**

Table 9 Determination of bifenthrin content in LN/ITN formulations, in g/kg

	LN/ITN 1			LN/ITN 2			LN/ITN 3			LN/ITN 4		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	6.04	6.45	**6.25**	2.08	1.98	**2.03**	5.20	6.24	**5.72**	5.44	6.18	**5.81**
Lab 2	6.85	7.23	**7.04**	5.13	4.92	**5.02**	7.30	7.58	**7.44**	7.13	7.53	**7.33**
Lab 3	6.09	5.92	**6.00**	2.48	2.19	**2.33**	6.29	6.31	**6.30**	5.95	5.94	**5.94**
Lab 4	7.02	6.56	**6.79**	7.26	6.88	**7.07**	7.34	7.04	**7.19**	7.16	6.30	**6.73**
Lab 5	7.41	7.28	**7.35**	7.26	7.41	**7.34**	7.36	7.37	**7.36**	7.44	7.41	**7.42**
Lab 6	6.69	6.65	**6.67**	5.95	6.01	**5.98**	7.11	6.55	**6.83**	6.85	6.83	**6.84**
Lab 7	7.56	7.16	**7.36**	7.02	6.43	**6.72**	8.15	7.65	**7.90**	7.87	7.43	**7.65**
Lab 8	6.92	6.88	**6.90**	6.81	6.73	**6.77**	7.36	7.49	**7.42**	6.93	7.08	**7.01**
Lab 9	6.54	7.19	**6.86**	6.12	5.34	**5.73**	6.61	7.24	**6.93**	6.72	7.33	**7.03**
Lab 10	6.52	6.49	**6.51**	5.68	5.17	**5.43**	6.92	6.59	**6.76**	6.78	6.73	**6.75**
Lab 11	6.64	6.67	**6.66**	5.11	4.32	**4.71**	7.43	7.58	**7.51**	7.09	7.18	**7.14**
Lab 12	6.66	7.19	**6.92**	4.54	5.10	**4.82**	6.90	7.63	**7.26**	6.29	7.42	**6.85**
Lab 13	6.87	6.58	**6.72**	6.81	6.41	**6.61**	6.92	6.56	**6.74**	6.88	6.64	**6.76**

***Cochran's straggler **Cochran's outlier**
°Grubbs' straggler °°Grubbs' outlier

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

	LN/ITN 1			LN/ITN 3		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	7.17	7.49	**7.33**	5.48	6.18	**5.83**
Lab 2	7.84	7.99	**7.92**	6.80	6.85	**6.82**
Lab 3	7.84	7.46	**7.65**	6.12	6.05	**6.09**
Lab 4	8.03	7.42	**7.72**	7.75	6.97	**7.36**
Lab 5	8.69	8.63	**8.66**	8.59	8.61	**8.60**
Lab 6	7.21	7.30	**7.26**	6.47	6.35	**6.41**
Lab 7	8.12	7.89	**8.01**	7.27	6.99	**7.13**
Lab 8	8.00	7.86	**7.93**	6.87	6.82	**6.85**
Lab 9	7.65	7.89	**7.77**	6.77	6.89	**6.83**
Lab 10	7.03	7.12	**7.07**	6.27	6.05	**6.16**
Lab 11	7.49	7.62	**7.55**	6.61	6.71	**6.66**
Lab 12	7.27	7.76	**7.51**	6.24	6.62	**6.43**
Lab 13	7.93	7.86	**7.89**	8.00	7.86	**7.93**

***Cochran's straggler **Cochran's outlier**
°Grubbs' straggler °°Grubbs' outlier

Table 11 Determination of pyriproxyfen content in LN/ITN formulations, in g/kg

	LN/ITN 2		
	DAY-1	**DAY-2**	**Mean**
Lab 1	3.54	3.44	**3.49**
Lab 2	5.74	5.99	**5.87**
Lab 3	4.21	3.82	**4.02**
Lab 4	5.62	5.58	**5.60**
Lab 5	6.03	6.16	**6.09**
Lab 6	5.34	5.31	**5.32**
Lab 7	6.16	6.08	**6.12**
Lab 8	6.05	6.11	**6.08**
Lab 9	5.74	5.77	**5.76**
Lab 10	5.68	5.92	**5.80**
Lab 11	5.85	5.76	**5.80**
Lab 12	5.28	5.55	**5.42**
Lab 13	5.82	5.63	**5.73**

***Cochran's straggler **Cochran's outlier**
°Grubbs' straggler °°Grubbs' outlier

Table 12 Determination of piperonyl butoxide content in LN/ITN formulations, in g/kg

	LN/ITN 1			LN/ITN 2			LN/ITN 4		
	DAY-1	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**	**DAY-1**	**DAY-2**	**Mean**
Lab 1	6.28	6.56	**6.42**	3.33	3.15	**3.24**	5.20	5.79	**5.49**
Lab 2	6.63	6.58	**6.60**	6.02	5.91	**5.97**	6.63	6.56	**6.60**
Lab 3	6.83	6.63	**6.73**	4.18	3.70	**3.94**	6.34	6.30	**6.32**
Lab 4	6.05	6.40	**6.23**	5.86	4.98	**5.42***	6.00	6.06	**6.03**
Lab 5	6.72	6.74	**6.73**	6.66	6.76	**6.71**	6.78	6.73	**6.76**
Lab 6	6.25	6.36	**6.30**	5.91	5.93	**5.92**	6.18	6.28	**6.23**
Lab 7	6.99	6.74	**6.86**	6.79	6.53	**6.66**	7.05	6.78	**6.91**
Lab 8	6.79	6.75	**6.77**	6.42	6.56	**6.49**	6.52	6.63	**6.57**
Lab 9	6.46	6.80	**6.63**	6.19	6.19	**6.19**	6.51	6.75	**6.63**
Lab 10	6.60	6.64	**6.62**	6.13	6.00	**6.07**	6.33	6.31	**6.32**
Lab 11	6.76	6.78	**6.77**	6.14	5.75	**5.95**	6.61	6.70	**6.66**
Lab 12	4.76	5.10	**4.93**$^{°°}$	4.24	4.45	**4.34**	4.35	4.88	**4.61**$^{°}$
Lab 13	6.27	6.20	**6.24**	6.44	6.28	**6.36**	6.32	6.20	**6.26**

***Cochran's straggler **Cochran's outlier**
°Grubbs' straggler °°Grubbs' outlier

Table 13 Summary of the statistical evaluation for technical material (TC) – with all data

	Bifenthrin content		Chlorfenapyr content		Pyriproxyfen content		Piperonyl Butoxide content	
	TC_{BIF} 1	TC_{BIF} 2	TC_{CFP} 1	TC_{CFP} 2	TC_{PYR} 1	TC_{PYR} 2	TC_{PBO} 1	TC_{PBO} 2
$\bar{x}$	980	976	1006	992	971	963	921	924
L	13	13	13	13	13	13	13	13
s_r	15.71	23.91	28.27	32.02	27.69	17.32	46.52	21.63
s_L	24.54	13.74	14.69	19.81	47.67	38.26	77.34	81.21
s_R	29.14	27.57	31.86	37.65	55.13	42.00	90.25	84.05
r	43.55	66.26	78.37	88.74	76.75	48.02	128.93	59.96
R	80.78	76.43	88.32	104.35	152.81	116.42	250.16	232.96
RSD_r	1.60	2.45	2.81	3.23	2.85	1.80	5.05	2.34
RSD_R	2.97	2.83	3.17	3.80	5.68	4.36	9.80	9.10
$RSD_{R(Horw)}$	2.01	2.01	2.00	2.00	2.01	2.01	2.02	2.02
HorRat	**1.48**	**1.41**	**1.59**	**1.90**	**2.83**	**2.17**	**4.84**	**4.50**

0.3 ≤ HorRat ≤ 1 : acceptable

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

HorRat > 2 : not acceptable

Table 14 Summary of the statistical evaluation for insecticide-treated nets (LN/ITN) – with all data

	Bifenthrin content				Chlorfenapyr content		Pyriproxyfen content	Piperonyl Butoxide content		
	LN/ITN 1	LN/ITN 2	LN/ITN 3	LN/ITN 4	LN/ITN 1	LN/ITN 4	LN/ITN 2	LN/ITN 1	LN/ITN 2	LN/ITN 4
$\bar{x}$	6.77	5.43	7.03	6.87	7.71	6.85	5.47	6.45	5.63	6.26
L	13	13	13	13	13	13	13	13	13	13
s_r	0.24	0.32	0.34	0.36	0.20	0.24	0.13	0.15	0.23	0.18
s_L	0.34	1.66	0.52	0.46	0.38	0.75	0.80	0.49	1.09	0.60
s_R	0.42	1.69	0.62	0.58	0.43	0.79	0.81	0.51	1.11	0.63
r	0.67	0.88	0.94	1.00	0.56	0.65	0.35	0.41	0.64	0.49
R	1.16	4.69	1.72	1.61	1.18	2.18	2.25	1.43	3.09	1.74
RSD_r	3.58	5.88	4.84	5.24	2.61	3.44	2.33	2.28	4.10	2.83
RSD_R	6.20	31.20	8.84	8.47	5.53	11.47	14.82	7.99	19.77	10.03
$RSD_{R(Horw)}$	4.24	4.39	4.22	4.23	4.16	4.23	4.38	4.27	4.36	4.29
HorRat	**1.46**	**7.12**	**2.10**	**2.00**	**1.33**	**2.71**	**3.38**	**1.87**	**4.53**	**2.34**

0.3 ≤ HorRat ≤ 1 : acceptable

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

HorRat > 2 : not acceptable

Table 15 Summary of the statistical evaluation for technical material (TC) – after elimination of Cochran's and Grubbs' outliers

	Bifenthrin content		Chlorfenapyr content		Pyriproxyfen content		Piperonyl Butoxide content	
	TC_{BIF} 1	TC_{BIF} 2	TC_{CFP} 1	TC_{CFP} 2	TC_{PYR} 1	TC_{PYR} 2	TC_{PBO} 1	TC_{PBO} 2
$\bar{x}$	983	984	996	998	978	974	947	945
L	12	11	10	11	10	12	11	11
s_r	6.12	10.48	4.77	9.35	3.98	11.42	5.67	7.36
s_L	25.60	4.86	12.13	16.52	8.08	7.00	29.46	33.63
s_R	26.32	11.55	13.04	18.98	9.01	13.39	30.00	34.42
r	16.97	29.05	13.23	25.90	11.04	31.65	15.72	20.39
R	72.95	32.02	36.14	52.60	24.96	37.12	83.16	95.41
RSD_r	0.62	1.07	0.48	0.94	0.41	1.17	0.60	0.78
RSD_R	2.68	1.17	1.31	1.90	0.92	1.38	3.17	3.64
$RSD_{R(Horw)}$	2.01	2.00	2.00	2.00	2.01	2.01	2.02	2.02
HorRat	**1.33**	**0.59**	**0.65**	**0.95**	**0.46**	**0.68**	**1.57**	**1.81**

0.3 ≤ HorRat ≤ 1 : acceptable

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

HorRat > 2 : not acceptable

Table 16 *Summary of the statistical evaluation for insecticide-treated nets (LN/ITN) – after elimination of Cochran's and Grubbs' outliers (2/2)*

	Bifenthrin content				Chlorfenapyr content		Pyriproxyfen content	Piperonyl Butoxide content		
	LN/ITN 1	LN/ITN 2	LN/ITN 3	LN/ITN 4	LN/ITN 1	LN/ITN 3	LN/ITN 2	LN/ITN 1	LN/ITN 2	LN/ITN 4
$\bar{x}$	6.77	5.43	7.03	6.87	7.71	6.85	5.47	6.58	5.63	6.26
L	13	13	13	13	13	13	13	12	13	13
s_r	0.24	0.32	0.34	0.36	0.20	0.24	0.13	0.14	0.23	0.18
s_L	0.34	1.66	0.52	0.46	0.38	0.75	0.80	0.20	1.09	0.60
s_R	0.42	1.69	0.62	0.58	0.43	0.79	0.81	0.24	1.11	0.63
r	0.67	0.88	0.94	1.00	0.56	0.65	0.35	0.38	0.64	0.49
R	1.16	4.69	1.72	1.61	1.18	2.18	2.25	0.67	3.09	1.74
RSD_r	3.58	5.88	4.84	5.24	2.61	3.44	2.33	2.07	4.10	2.83
RSD_R	6.20	31.20	8.84	8.47	5.53	11.47	14.82	3.70	19.77	10.03
$RSD_{R(Horw)}$	4.24	4.39	4.22	4.23	4.16	4.23	4.38	4.26	4.36	4.29
HorRat	**1.46**	**7.12**	**2.10**	**2.00**	**1.33**	**2.71**	**3.38**	**0.87**	**4.53**	**2.34**

0.3 ≤ HorRat ≤ 1 : acceptable

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

HorRat > 2 : not acceptable

Table 17 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	$RSD_R(Hor) = 2^{(1 - 0.5\log c)}$
HorRat	Horwitz ratio	$HorRat = RSD_R/RSD_{R(Horw)}$

9. Evaluation and discussion

Sixteen laboratories participated to this full scale collaborative trial and thirteen provided results on time. Cochran's and Grubbs' tests were applied for statistical evaluation of the results, outliers are identified with 99% confidence and stragglers with 95 % confidence.

9.1 Identification of outliers and stragglers

The tables 5 to 12 on pages 38 to 45 contain all data obtained from the thirteen participants.

9.1.1 Cochran's test (within laboratory reproducibility)

For samples of technical material (TC)

For bifenthrin content, the laboratory 9 has been identified as outlier with Cochran's test for the 2 samples $TC_{BIF}1$ and $TC_{BIF}2$.

For chlorfenapyr content, the laboratory 12 has been identified as outlier for $TC_{CFP}1$ and as straggler for $TC_{CFP}2$. A second iteration of Cochran's test identified the laboratory 6 as outlier for $TC_{CFP}1$ and $TC_{CFP}2$ and to the third iteration, the laboratory 3 has been found as outlier for $TC_{CFP}1$.

For pyriproxyfen content, the laboratory 12 has been identified as outlier for $TC_{PYR}1$ and as straggler for $TC_{PYR}2$. To the second iteration of the test, the laboratory 11 is pointed out as outlier for $TC_{PYR}1$ and the laboratory 6 as straggler for $TC_{PYR}2$.

For piperonyl butoxide content, Cochran's test identified the laboratory 6 as outlier for the 2 samples $TC_{PBO}1$ and $TC_{PBO}2$. In the second iteration, the laboratory 3 is found as straggler for $TC_{PBO}2$.

For samples of insecticide-treated nets (LN/ITN)

For piperonyl butoxide content in the sample LN/ITN 2, Cochran's test identified the laboratory 4 as straggler.

An explanation for the laboratory 6 is that we noticed that the weighing of the 4 molecules in calibration stock solution are the same for Day 1 and Day 2 suggesting a encoding error, even if the laboratory did not confirm it.

9.1.2 Grubbs' test (between laboratory reproducibility)

For samples of technical material (TC)

For chlorfenapyr content in $TC_{CFP}2$, for pyriproxyfen content in $TC_{PYR}1$ and in $TC_{PYR}2$, and for piperonyl butoxide content in $TC_{PBO}1$ and $TC_{PBO}2$, the laboratory 12 has been identified as outlier, and as straggler for chlorfenapyr content in $TC_{CFP}1$. In the second iteration of the test, this laboratory is found as outlier for $TC_{BIF}2$.

For pyriproxyfen content in $TC_{PYR}1$, the laboratory 6 is found as outlier at the second iteration of Grubbs' test.

For samples of insecticide-treated nets (LN/ITN)

For piperonyl butoxide content in the sample LN/ITN 1, Grubbs' test identified the laboratory 12 as outlier and as straggler for the sample LN/ITN 4.

We have noted that the laboratories 1 and 3 were not identified as outliers after Grubbs's test for all the 3 molecules (bifenthrin, pyriproxyfen and piperonyl butoxide) contained in the sample LN/ITN 2 even if these laboratory are visually significantly lower than the other laboratories: see figure 6 pg.31, figure 15 pg. 35 and figure 19 pg. 37.

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 13 to 16 on pages 46 to 49 show the statistical evaluation with all data and after discarding the outliers. Below is what we noticed that from these values.

9.2.1 For samples of technical material (TC)

For bifenthrin TC

The HorRat ratio is between 1 and 2 for bifenthrin content in TC before discarding the outliers and is below 1 for $TC_{BIF}2$ after discarding the outliers, but remains at 1.33 for $TC_{BIF}1$, what is acceptable only if a reasonable explanation is given.

For chlorfenapyr TC

The HorRat ratio is between 1 and 2 for chlorfenapyr content in TC before discarding the outliers and is below 1 after discarding the outliers, what is acceptable.

For pyriproxyfen TC

The HorRat ratio is higher than 2 for pyriproxyfen content in TC before discarding the outliers and is below 1 after discarding the outliers, what is acceptable.

For piperonyl butoxide TC

The HorRat ratio is higher than 4 for piperonyl butoxide content in TC before discarding the outliers and remains between 1 and 2 after discarding the outliers, what is acceptable provided that a reasonable explanation is given.

See chapter 9.3 for further discussion and explanations.

9.2.2 For samples of insecticide-treated nets (LN/ITN)

For bifenthrin in LN/ITN

The HorRat ratio is between 1 and 2 for bifenthrin content in LN/ITN 1, close to 2 in LN/ITN 3 and 4 but higher than 7 for LN/ITN 4, what is not acceptable.

For chlorfenapyr in LN/ITN

The HorRat ratio is between 1 and 2 for chlorfenapyr content in LN/ITN 1 and higher than 2 in LN/ITN 3, what is not acceptable.

For pyriproxyfen in LN/ITN

The HorRat ratio is higher than 3 for pyriproxyfen content in LN/ITN 2, what is not acceptable.

For piperonyl butoxide in LN/ITN

The HorRat ratio is between 1 and 2 for piperonyl butoxide content in LN/ITN 1 before discarding the outliers and is below 1 after discarding the outliers, what is acceptable. In LN/ITN 2, the HorRat ratio is between 1 and 2 for piperonyl butoxide content, what is acceptable only if a reasonable explanation is given. However, in LN/ITN 2, the HorRat ratio is higher than 4, what is not acceptable.

See chapter 9.3 for further discussion and explanations.

9.3 Conclusion

The HorRat ratios obtained in this full scale collaborative trial does not allow the analytical method to be accepted as provisional CIPAC method for determining bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide content in insecticide-treated nets (LN/ITN), incorporated into filament. The method has nevertheless been fully and successfully validated according to the EU document SANCO/3030/99 rev.5, for the non analyte interference, the specificity, the precision (repeatability), the linearity of detector response and the accuracy (with recoveries). A kinetic of extraction showed that the active ingredients/synergist are fully extracted. The range of the method and limit of quantification has been established for LN/ITN samples to which the test samples of this trial correspond.

We recommend to conduct a second full scale trial, with the following suggestions of improvement of the method :

- For samples of technical material as well as for calibration stock solution, we suggest to increase the weighing from 25 mg to at least 50 mg, to reduce the

uncertainty of measurement that could significantly affect the results if the weighing is too low.

- For samples of insecticide-treated nets, the coordinator asked to the participants if the sonication has been done 1) at 80°C 2) during 60 minutes. Among the responses provided, 5 laboratories confirmed that their ultrasonic bath cannot reach the 80°C requested and 1 of these laboratories heated up to 80°C but without sonication.

  This is a major deviation to the method because during the development and validation of the method, the extraction using ultrasonication and a temperature of 80°C are key points to obtain consistent and accurate results. The net samples are indeed incorporated nets, which means that the active ingredients/ synergist need to be extracted out of filaments of a polymer (polyethylene), what is not easy and requires strong extraction conditions. We intend to add in the analytical method that the temperature of the ultrasonic bath must reach at least 80°C and shall be verified with an external sensor before and after sonication, to ensure that the temperature displayed on the ultrasonic bath has be reached actually during all the sonication.

- Additionally to the temperature, the power of the sonication must be mentioned in the analytical method, for the same reasons cited above.

- Since the method has been designed for insecticide-treated nets and not for technical materials, the coordinator ask to the CIPAC authorities, if a second trial is conducted, the possibility not to test the technical materials. Indeed, standardized analytical methods exists for the 4 molecules, that are referee methods. That would make the trial easier to manage for the participants as well (8 samples of technical material were tested, i.e. 2 samples par type of TC additionally to the net samples).

- If the full scale trial is repeated, we suggest that the coordinator cut the net samples in small pieces of max 5 mm x 5 mm and homogenize appropriately the sample before sending aliquots to the participants. This is the only way to ensure an appropriate homogenization of net samples and to ensure that all the participants receive the same sample.

  Indeed, the coordinator kept 4 series of net sample per type of net as explained in the chapter 5.1 on pg.13. These four series of sample per type of net were tested under repeatability conditions and a statistical evaluation has been made :

	Bifenthrin content				Chlorfenapyr content		Pyriproxyfen content	Piperonyl Butoxide content		
	LN/ITN 1	**LN/ITN 2**	**LN/ITN 3**	**LN/ITN 4**	**LN/ITN 1**	**LN/ITN 3**	**LN/ITN 2**	**LN/ITN 1**	**LN/ITN 2**	**LN/ITN 4**
$\bar{x}$	6.56	6.14	6.78	6.76	7.84	6.73	5.79	6.54	6.24	6.50
$n_{samples}$	4	4	4	4	4	4	4	4	4	4
RSD_R	0.54	3.10	3.09	0.79	3.37	1.34	3.55	1.53	2.59	0.44
$RSD_{R(Horw)}$	4.26	4.30	4.24	4.24	4.15	4.25	4.34	4.26	4.29	4.27
HorRat	**0.13**	**0.72**	**0.73**	**0.19**	**0.81**	**0.32**	**0.82**	**0.36**	**0.60**	**0.10**

The HorRat ratio obtained are often higher than 0.6 – 0.7: it shows that the inherent inhomogeneity, which is unavoidable with insecticide-treated nets, affects significantly the HorRat ratio. That is why we suggest to sub-sample (cutting into small pieces) the nets in 1 single laboratory and send aliquots to the participants after all the small piece have been homogenized. This is to minimize as much as possible an increase of the HorRat ratio due to the unhomogeneity of the sample and not because of the analytical method.