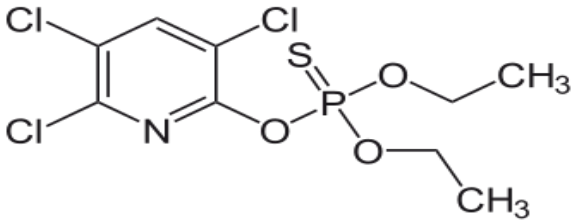
Yorkool

Chlorpyrifos 221.202



CIPAC Collaborative Trial

CIPAC 5081/R, full scale study

CIPAC Collaborative Study of a High Performance Liquid Chromatographic Analysis of Chlorpyrifos in Long Lasting Insecticidal Net

By
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1. List of Participants

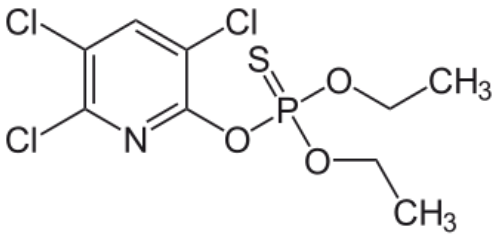
14 laboratories took part in the collaborative study:

NAME	ORGANIZATION	COUNTRY
Eva Jacobsen	Danish Technological Institute	Denmark
Peter Wagener	Bayer AG Cropscience Division RT Analytics Quality Control	Germany
Enache Preoteasa Cristian	National Phytosanitary Laboratory	Romania
Ahmad Rezvani, Kenneth McManus	Maryland Department of Agriculture, SCS	America
Nongpanga Olsen	Office of Agricultural Research and Development Region 1	Thailand
Kolesnikova T	Ukrainian Laboratory of Quality and Safety of Agricultural Products (hereinafter referred to as ULQSAP) of the National University of Life and Environmental Sciences of Ukraine (NUBiP of Ukraine)	Ukraine
Luis Manso	Arbitral Agroalimentario	Spain
Qibo Jiang	Jiangsu Yangnong Chemical Co., Ltd. Yangnong GLP Laboratory	China
Dr.A.Ramesh	International institute of biotechnology and toxicology	India
Agus Salim	PT Agricon	Indonesia
Robin Zou	Jiang Su Rotam Chemistry Co., Ltd. Analytical Chemistry Department	China
Ivan Orgei	Frandesa Co. Ltd Research laboratory	The Republic of Belarus
Ji Hua	Testing Center of Nanjing Limin Chemical Co., Ltd.	China
Dr. ir.Olivier PIGEON	Walloon Agricultural Research Centre (CRA-W)	Belgium

2. General Information

Chlorpyrifos

221.202/ LN /M/-



ISO Common name	Chlorpyrifos
Chemical name	*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (IUPAC, CA; *2921-88-2*)
Empirical formula	$C_9H_{11}Cl_3NO_3PS$
RMM	350.6
m.p.	42.5 to 43 ℃
v.p.	2.49 mPa (1.87×10^{-5} mm Hg) at 25 ℃
Solubility	At 35 ℃, 2 mg/l water, 790 g/kg octanes, 430 g/kg methanol. Readily soluble in most other organic solvents.
Description	Colourless crystals with a mild mercaptan odour.
Stability	Stable under normal storage conditions.
Formulations	As long lasting insecticidal nets.

3. Distribution of Samples

The following samples were provided to the partcipants:

Chlorpyrifos standard 0.25 g
Lot No. 91441, purity: 990 g/kg

1,4-dibromonaphthalene internal standard 0.2 g
Lot No. 6018800, purity: 995 g/kg

Chlorpyrifos long lasting insecticidal net sample (SA) 5.5 g
Batch No. 20161101, approx. 12.4 g/kg

Chlorpyrifos long lasting insecticidal net sample (SB) 5.5 g
Batch No. 20161102, approx. 12.4 g/kg

4. Procedure

4.1. Outline of Method

The chlorpyrifos in long lasting insecticidal net is extracted by acetonitrile and determined by reverse phase high performance liquid chromatography using UV detector, at detection wavelength of 290 nm with 1,4-dibromonaphthalene as internal standard. The analyte solution contains about 10 mg of chlorpyrifos and 10 mg of 1,4-dibromonaphthalene in 50 mL solution.

4.2. Program of Work

We requested the collaborators to:

1) conduct triplicate determinations on two different days for each of the two samples;
2) inject each sample solution in duplicate and calculate the mean value;
3) check equilibration of the system before the determination;
4) describe operating conditions in detail; and
5) attach the typical chromatograms for the two samples.

5. Analytical Methods

5.1. Analytical Conditions

Lab	Liquid chromatograph integrator	Column	Mobile phase	Flow rate (mL/min)	Column temp(℃)
Proposed Conditions		Inertsil ODS-3, C18, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
1	Agilent 1260 med, DAD detecktor	Phenomenex Kinetex, EVO C18, 5µm 150×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	0.8	30
2	Agilent 1260 Infinity	Hypersil ODS C18, 5µm 250×4mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	0.76	30
3	Dionex Ultimate 3000	Phenomenex Kinetex, XB C18, 2.6µm 50×4.6mm (i.d.)	methanol – water, 800+200 (v/v)	1.0	40
4	HPLC-waters 2695, Detector-waters 2487, Empower 3	Phenomenex Luna, C18, 100 A, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	D1:30±5 ℃ D2:30±15 ℃
5	Agilent 1100	Thermo Hypersil BDS, C18, 5µm 150×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
6	Dionex 3000 HPLC/UV/DAD	Hypersil Gold, 6µm 50×4mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
7	Agilent 1100	Phenomenex Luna, C18, 100 A, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 800+195+5 (v/v/v)	1.0	30
8	Agilent 1200	Inertsil ODS-3, C18, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
9	Agilent 1290 Infinity	Inertsil ODS-3, C18, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
10	Shimadzu 20AT, detector UV, SPD 20A	Agilent Eclipse XDB-C18, 5µm 150×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
11	Agilent 1200 HPLC	Shim-pack VP-ODS,5um	acetonitrile – water – acetic acid,	1.0	35

		250×4.6mm (i.d.)	820+175+5 (v/v/v)		
12	Agilent 1260	Agilent Zorbax, SB-C18, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
13	Agilent 1260	XDB-C18, 5µm 250×4.6mm (i.d.)	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30
14	Agilent G1312A (1100 series)	Phenomenex, Prodigy ODS-3, 250 mm x 4.6 mm (id), 5 µm	acetonitrile – water – acetic acid, 820+175+5 (v/v/v)	1.0	30

5.2. Deviations from the Analytical Method

Lab 1: Column: Phenomenex Kinetex, EVO C18, 5µm,150×4.6mm (i.d.).
Flow rate: 0.8 mL/min;

Lab 2: Column: Hypersil, ODS C18, 5µm, 250×4mm (i.d.).
Flow rate: 0.76mL/min;

Lab 3: Column: Phenomenex Kinetex, XB C18, 2.6µm, 50×4.6mm (i.d.).
Column temperature: 40 ℃;
Injection volume: 1µL;
Mobile phase: 80% methanol and 20% water.

Lab 4: Column: Phenomenex Luna, C18, 100 A, 5µm, 250×4.6mm (i.d.).
Column temperature: D1:30±5 ℃; D2:30±15 ℃.

Lab 5: Column: Thermo Hypersil BDS, C18, 5µm, 150×4.6mm (i.d.).

Lab 6: Column: Hypersil Gold, 6µm, 50×4mm (i.d.).

Lab 7: Column: Phenomenex Luna, C18, 100 A, 5µm, 250×4.6mm (i.d.)
Mobile phase: 80% acetonitrile, 19.5% water and 0.5% acetic acid

Lab 8: No deviations.

Lab 9: No deviations.

Lab 10: Column: Agilent Eclipse XDB-C18, 5µm, 150×4.6mm (i.d.)
purely manual injector with loop injector 20 µL. the Injection volume was 10 µL (used syringe volume 25 µL) same as reference method

Lab 11: Column: Shim-pack VP-ODS, 5µm 250×4.6mm (i.d.)
Column temperature: 35 ℃;
Injection volume: 5µL;

Lab 12: Column: Shim-pack VP-ODS, 250×4.6mm (i.d.)

Lab 13: Column: XDB-C18, 5µm 250×4.6mm (i.d.)

Lab 14: Column: Phenomenex, Prodigy ODS-3, 5µm 250 mm x 4.6 mm (id)

5.3. Remarks about the Analytical Method

Lab 1: No loss of solvent (<1%).

Lab 2: Due to the use of a 4mm column we had to adapt the flow. We reduced the flow to 0.76 mL/min (relationship cross-sectional area).

Lab 3: Injection volume 1 µl.

Lab 4: is a straight forward and robust method.

Lab 5: This method is ok.

Lab 6: No remarks.

Lab 7: Acetonitrile/water/acetic acid, 80+19.5+0.5 was used as mobile phase. In the first day, as we did some previous essays, less amount of internal standard remained to prepare subsample B3 and because of that its weight was corrected accordingly.

Lab 8: No remarks.

Lab 9: No remarks.

Lab 10: We use column from Phenomenex 250 x 4.6 mm (i.d), Lux® 5 µm C18, different with reference method (Inertsil ODS-3, 250

mm x 4.6 mm (id) x 5 µm, C18 column), but both of columns have a same content. The column give the retention time for chlorpyrifos approx. 9.4 ± 0.4 min faster than the retention time approx. 11 min stated in the reference method and 1,4-dibromonaphthalene approx. 12.8 ± 0.5 min faster than the retention time approx. 17 min stated in the reference method and the running time we set at 16 min.

Lab 11: Difference for operating conditions compared to the given:
1. HPLC column:Shim-pack VP-ODS , 5µm 250x 4.6mm(i.d.).
2. Injection volume:5 µL
3. Column temperature:35 ℃
4. Retention time:Chlorpyrifos about 7.7 min and 1,4-dibromonaphthalene about 11.7 min

Lab 12: No remarks.

Lab 13: No remarks.

Lab 14: chlorpyrifos retention time : 9.2 min
1,4-dibromonaphtalene retention time : 13.2 min

6. Statistical Evaluation

Samples were sent to 14 laboratories. All of them sent back results.

The statistical evaluation of the data was done following DIN ISO 5725 and “Guidelines for CIPAC Collaborative Study Procedure for Assessment of Performance of Analytical Methods”.

The assay results obtained by the collaborators and the statistical evaluation are reported in Tables 1 through 2, and in Figures 1-1 through 2-2. Formulas used are listed in sector 9, page 17.

7. Results

Table 1: Chlorpyrifos mosquito net sample (SA) (Batch No: 20161101)

Lab	Day 1			Day 2			Mean	Std.
	A	B	C	A	B	C	g/kg	Dev.
1	12.445	12.368	12.408	12.209	12.171	12.184	12.298	1.000
2	12.336	12.266	12.315	12.377	12.345	12.334	12.329	0.299
3	**12.780**	**12.836**	**12.808**	**12.015**	**11.862**	**11.951**	**12.375**	**3.852**
4	12.159	12.070	12.162	12.258	12.159	12.148	12.159	0.492
5	**11.693**	**11.720**	**12.028**	**12.159**	**12.087**	**12.120**	**11.968**	**1.728**
6	11.984	12.080	12.049	12.037	12.095	11.985	12.038	0.386
7	12.283	12.350	12.372	12.252	12.343	12.339	12.323	0.371
8	12.477	12.441	12.493	12.677	12.609	12.635	12.555	0.008
9	12.535	12.515	12.415	12.453	12.513	12.524	12.492	0.381
10	12.038	12.013	12.005	11.983	11.993	11.943	11.996	0.269
11	12.267	12.255	12.349	12.277	12.308	12.363	12.303	0.004
12	12.219	12.215	12.212	12.339	12.304	12.262	12.259	0.053
13	12.235	12.188	12.155	12.261	12.232	12.229	12.217	0.313
14	12.178	12.314	12.406	12.132	12.061	12.010	12.183	1.240

Lab 3 and Lab 5: Outlier according to Cochran Test.

Fig.1-1 : Chlorpyrifos mosquito net sample (SA) (Batch No: 20161101) All labs

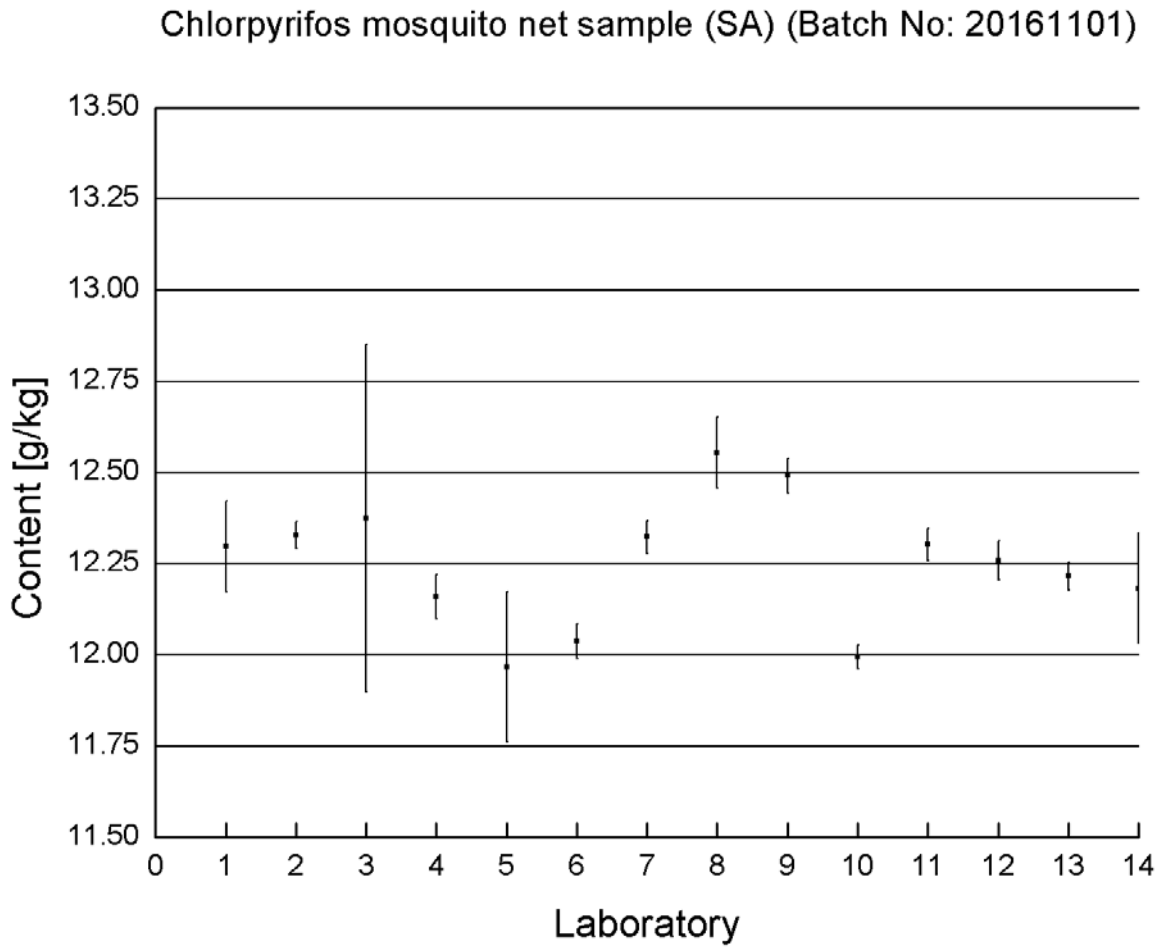


Fig.1-2: Chlorpyrifos mosquito net sample (SA) (Batch No: 20161101) Labs 3 and 5 excluded

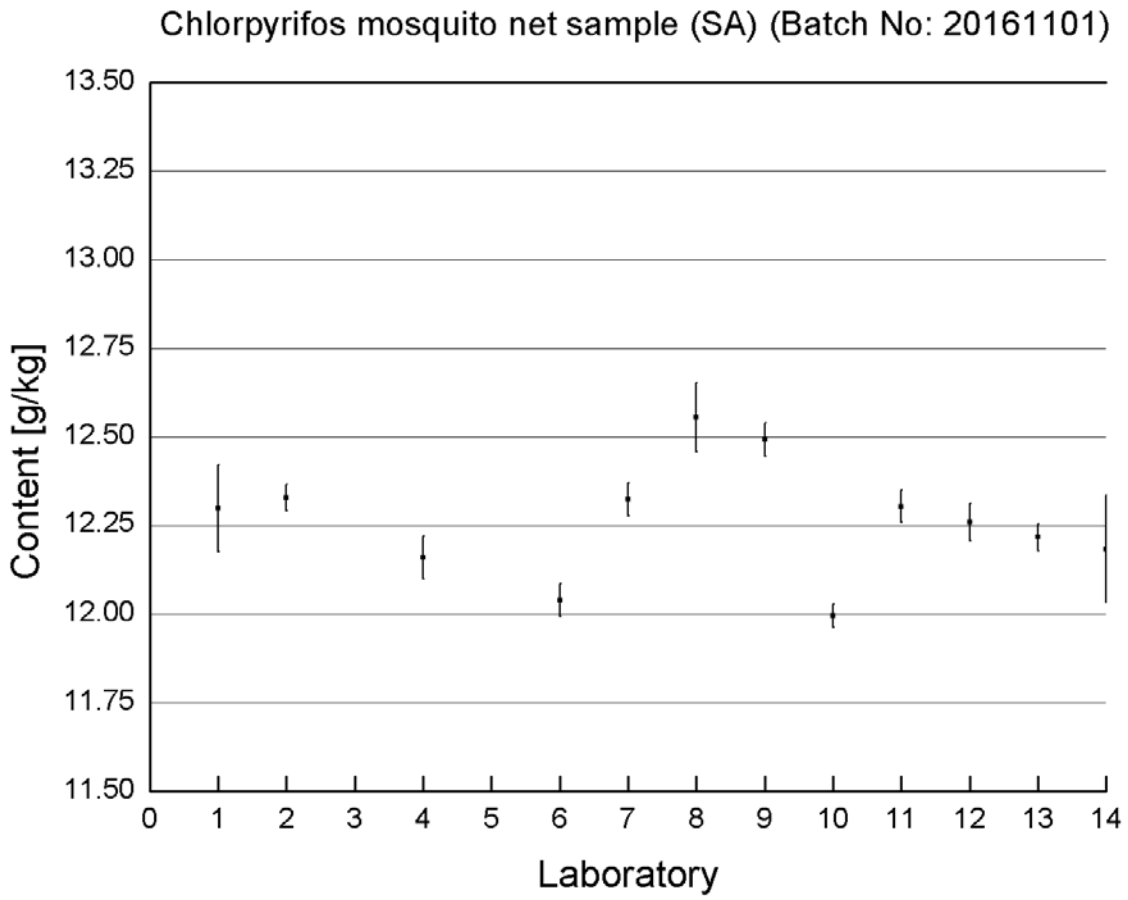


Table 2: Chlorpyrifos mosquito net sample (SB) (Batch No: 20161102)

Lab	Day 1			Day 2			Mean	Std.
	A	B	C	A	B	C	g/kg	Dev.
1	12.474	12.419	12.464	12.273	12.347	12.291	12.378	0.703
2	12.333	12.339	12.367	12.325	12.263	12.343	12.328	0.285
3	**12.607**	**12.608**	**12.486**	**11.985**	**12.084**	**11.998**	**12.295**	**2.469**
4	12.217	12.067	12.201	12.232	12.177	12.155	12.175	0.489
5	11.797	11.826	11.785	12.108	12.164	12.003	11.947	1.397
6	11.900	12.064	12.058	12.076	12.135	12.026	12.043	0.652
7	12.318	12.377	12.354	12.382	12.370	12.346	12.358	0.194
8	12.426	12.652	12.601	12.563	12.788	12.716	12.624	0.010
9	12.484	12.485	12.499	12.523	12.424	12.479	12.483	0.264
10	12.104	12.130	12.055	12.007	12.036	12.031	12.060	0.388
11	12.220	12.273	12.293	12.277	12.308	12.363	12.289	0.004
12	12.114	12.123	12.141	12.192	12.298	12.151	12.170	0.068
13	12.090	12.166	12.187	12.298	12.314	12.336	12.232	0.805
14	**12.485**	**12.443**	**12.390**	**12.057**	**12.033**	**11.991**	**12.233**	**1.869**

Lab 3 and Lab 14: Outlier according to Cochran Test.

Fig.2-1: Chlorpyrifos mosquito net sample (SB) (Batch No: 20161102) All labs

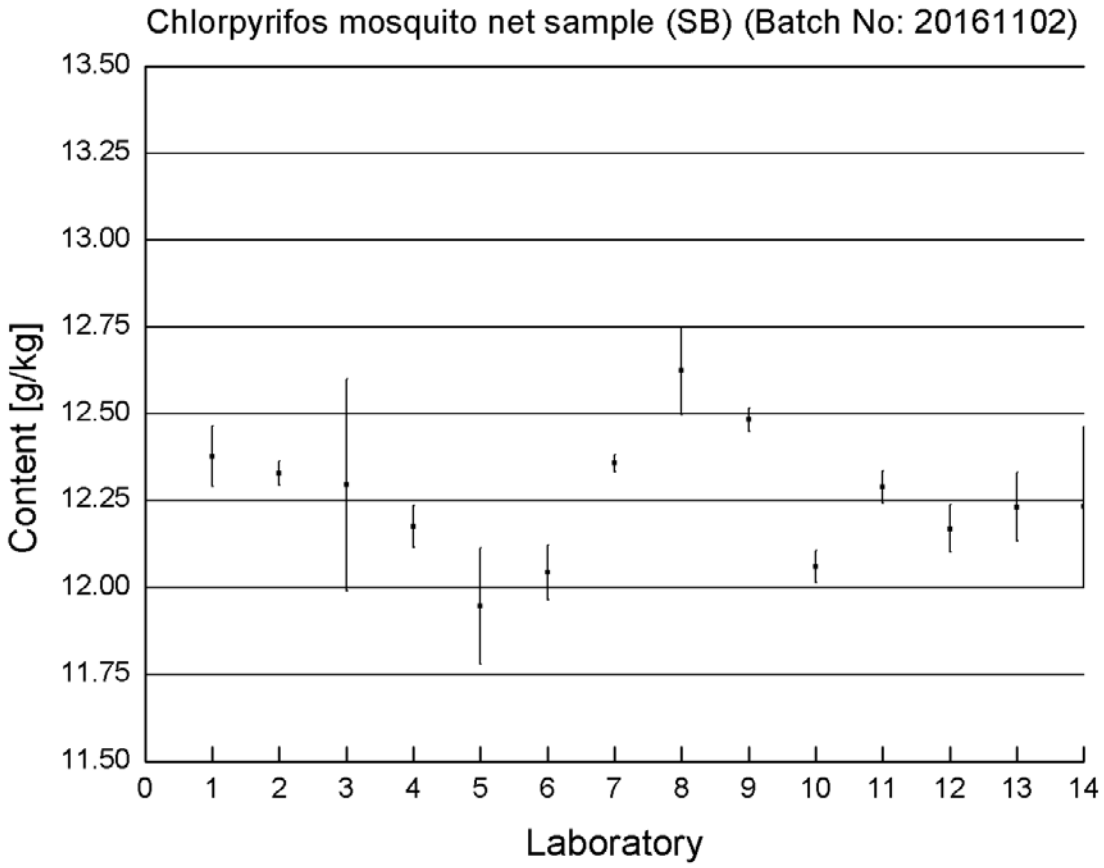
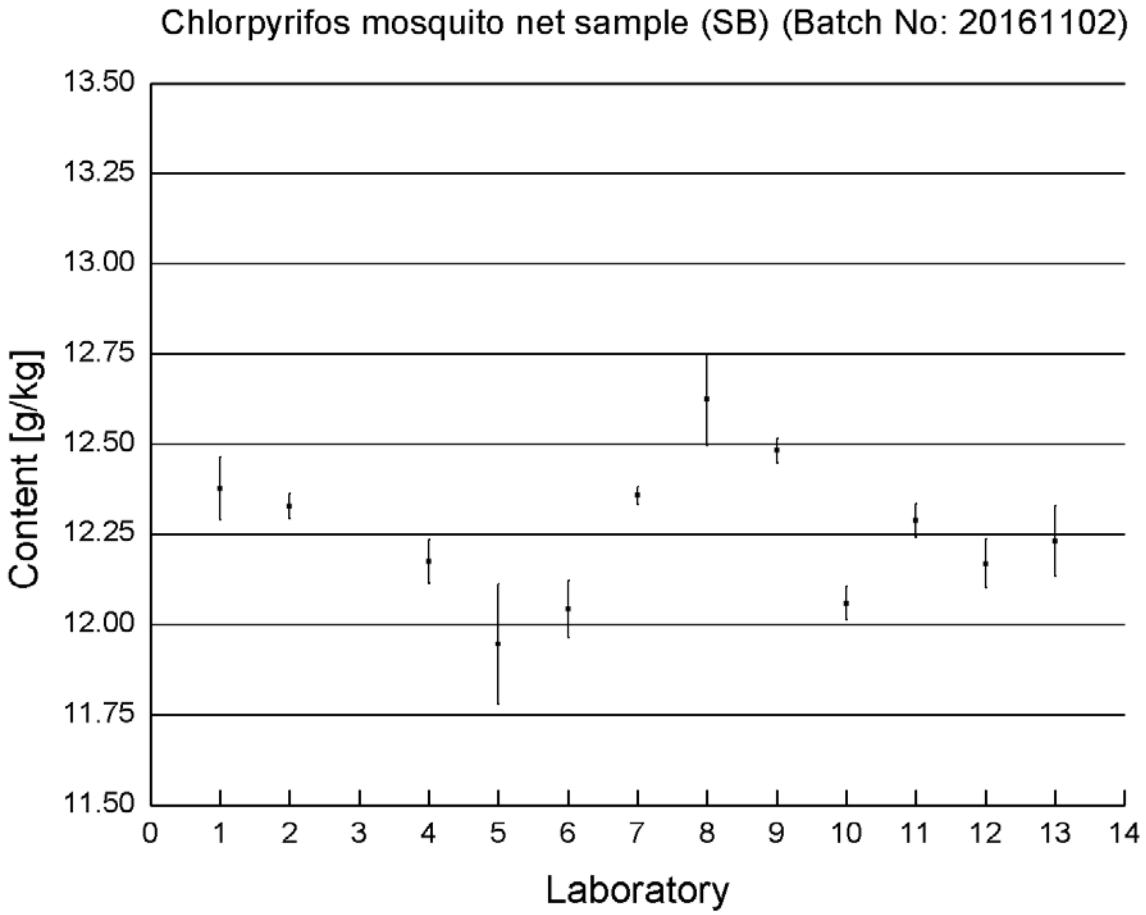


Fig.2-2: Chlorpyrifos mosquito net sample (SB) (Batch No: 20161102) Labs 3 and 14 excluded



8. Summary of the results

Table 3 Summary of the results of all laboratories

	SA	SB
x	12.250	12.258
L	14	14
S_r	0.155	0.127
S_R	0.223	0.213
r	0.434	0.357
R	0.625	0.596
RSD_r	1.265	1.039
RSD_R	1.822	1.737
$RSD_{R(Hor)}$	3.873	3.873

(values given in units of g/kg)

Table 4 Summary of the results after elimination of outlier values

	SA	SB
x	12.263	12.257
L	12	12
S_r	0.074	0.083
S_R	0.176	0.208
r	0.208	0.232
R	0.493	0.582
RSD_r	0.606	0.677
RSD_R	1.435	1.696
$RSD_{R(Hor)}$	3.873	3.873

(values given in units of g/kg)

Where:

x	= average, in unit of g/kg
L	= number of laboratories
S_r	= repeatability standard deviation
S_R	= reproducibility standard deviation $= \sqrt{(S_r^2 + S_L^2)}$
r	= repeatability (S_r • 2.8)
R	= reproducibility (S_R • 2.8)
RSD_r	= repeatability relative standard deviation (100 • S_r/x)
RSD_R	= reproducibility relative standard deviation (100 • S_R/x)
RSD_R(Hor)	= Horwitz value calculated from: $2^{(1-0.5\log c)}$
where c	= the concentration of the analyte as a decimal fraction

9. Statistical formulas

Yi = mean of the various laboratories
Si = standard deviation
P = number of laboratories
n = number of measurements (here n=6)

$$T_1 = \sum_{i=1}^{p} Yi$$

$$T_2 = \sum_{i=1}^{p} Yi^2$$

$$T_3 = \sum_{i=1}^{p} Si^2$$

Repeatability and reproducibility were calculated as follows:

$$S_r^2 = \frac{T_3}{P}$$

$$S_L^2 = \frac{PT_2 - T_1^2}{P(P-1)} - \frac{S_r^2}{n}$$

$$S_R^2 = S_r^2 + S_L^2$$

$$r = 2.8 * \sqrt{S_r^2}$$

$$R = 2.8 * \sqrt{S_R^2}$$

10. Discussion

Following the successful outcome of the full scale collaborative study organized by Yorkool, an international CIPAC collaborative study was initiated in January 2017 to test a specific HPLC method for the determination of chlorpyrifos in long lasting insecticidal net.

14 laboratories had announced to participate the CIPAC trial and sent back results.

The data from each of the laboratories were reviewed to determine if there were any problems with analysis procedure used, chromatography or reporting results, which might affect the analyses results. The changes, deviations, and observations which were noted will not be expected to affect the analyses results significantly.

If the results of 14 laboratories participated in the collaborative trial are taken into account for the statistical evaluation, i.e. all stragglers and outliers according to Cochran test and Grubbs test are left in the evaluation and no data are rejected, the Horwitz criteria are fulfilled in the case of SA and SB. The results are shown in the table 3.

The Horwitz criterion is improved for SA after elimination of three outliers according to Cochran test (Lab 3 and Lab 5). The result is shown in the table 4.

The Horwitz criterion is improved for SB after elimination of two outliers according to Cochran test (Lab 3 and Lab 14). The result is shown in the table 4.

Overview: outliers and stragglers identified and allocated to the participant

Sample No.	Lab ID No. Identification of outliers and stragglers
SA	3, 5
SB	3, 14

Conclusion:

We would like to propose the analytical method for chlorpyrifos in long lasting insecticidal net to become provisional.

Acknowledgements:

The organizer wishes to thank all laboratories and their staff who participated in this study.