



Full Scale Collaborative Study for the Determination of Chlorpyrifos in TC and EC formulation by HPLC

Report to CIPAC
by
Shenyang SYRICI Testing Co., Ltd.

Who is SYRICIT?

- OECD GLP inspected by Netherlands
- Certified by Chinese MORA/FDA/EPM
- Host Secretariat of National Standardization Technical Committee of Pesticide and Dyestuff
- 23 National Standards and 156 industry Standards developed during past 5 years

One-Stop solution to both China Market and the rest of the world.

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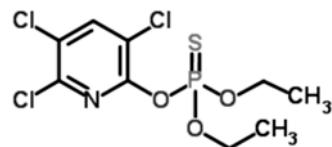
Participants

- In September 2020, Information Sheet No. **327** was sent out by CIPAC inviting members to participate.
- **20** laboratories participated in the trial.

Contact	Lab
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Hou Chunqing	Pesticides Test Lab of Shenyang SYRICI Testing Co., Ltd. No.8, Shenliao East Road, Tiexi District Shenyang 110021, P.R. China
Shirish Bondre	Quality Assurance Dept., Gharda Chemicals Ltd. D-1/2, MIDC, Lote Parshuram, Taluka Khed District: Ratnagari 415722, Maharashtra India
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Judy	Shandong Binnong Technology Co., Ltd. No.518, Yongxin Road, Binbei Town, Binzhou, Shandong, China
Maureen Tian	Test Center of Shandong Weifang Rainbow Chemical Co., Ltd. Binhai Economic Development Area, Weifang, Shandong, China
Vanessa Lecocq	Walloon Agricultural Research Centre (CRA-W) Batiment Rachel Carson Rue du Bordia, 115030 Gembloux Belgique Belgium

AI General Information



Chlorpyrifos

- Chemical name: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate(IUPAC); O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate
- CAS-No.: 2921-88-2
- Molecular mass: 350.6 g/mol

Samples

- TC sample 1 (Batch No: 2020092263)
- TC sample 2 (Batch No: 2020092161)
- TC sample 3 (Batch No: 2020092365)
- EC sample 4 (Batch No : 20200710)
- EC sample 5 (Batch No : 20200705)
- Reference standard (purity 99.2 %)

Method

- **4.1 Scope**

The determination of Chlorpyrifos active ingredient content in TC and EC formulation.

- **4.2 Principle**

Chlorpyrifos is determined by reversed phase HPLC using UV detection at 290 nm and external standardization.

Method Condition

<i>Column temperature</i>	25°C
<i>Flow rate</i>	1.0 ml/min
<i>Detector wavelength</i>	290 nm
<i>Injection volume</i>	5 µl
<i>Mobile phase</i>	acetonitrile/water/glacial acetic acid 820/175/5 (v/v)
<i>Retention time</i>	approximately 9.6 min

Method

- **4.3 Procedure**
- Each sample was analyzed using **four independent determinations**. The samples were analyzed on **two different days**, each day involving **duplicate injections of duplicate weights**. Both test and reference solutions were freshly prepared on each day. The four injections of each test solution were bracketed by double injections of the calibration solution. The average response factor, used to calculate the amount of Chlorpyrifos in the test solution, was calculated using the injection before and after the test injections.

Analytical Condition of Participants

Lab	Instrument	Column	Flow Rate	Column Temp. °C	Wave length	Injection Volume	Mobile phase
1	Thermo Ultimate 3000	Agilent Extend-C18 5um 250 x 4.6mm	1.0	30	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
2	Agilent 1260	ZORBAX SB-C18 5um 250 x 4.6mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
3	Agilent 1260	TC-C18(2), 5um 250 x 4.6mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
4	Agilent 1100	Phenomenex Lunar® 5um C18(2) 100Å	1.2	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
5	Agilent 1260 Infinity II	Agilent Zorbax Extend-C18 / 5um 250 x 3.0mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
6	Waters , Model Alliance e2695 , Detector 2998	Xterra RP18 with 5um 250 x 4.6mm, 123 oA pore diameter	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
7	SHIMADZU LC-2030C 3D Plus	ZORBAX Extend-C18, 5um 250 x 4.6mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
8	Agilent 1200 Series	Zorbax Extend- C18, 5um 250 x 4.6mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
9	Agilent HPLC-DAD 1260 Infinity	Luna C18(2) 100A, 5um 250 x 4.6mm	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
10	Shimadzu Nexera XR with SPD-20A	Zorbax Extend C18 5um 250 x 4.6mm with precolumn	1.0	25	290	5	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)

Analytical Condition of Participants

11	YQ-1-35	Agilent Extend-C18, 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
12	Dionex UltiMate 3000	Agilent - Zorbax Eclipse XDB-C18 , 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
13	Shimadzu 20 AD	Phenomenex Luna C-18, 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
14	Themro, Dionex UltiMate 3000 HPLC	Agilent Extend C18, 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
15	Thermo Scientific Ultimate-3000	Cosmosil C-18, 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
16	Agilent	Agilent Extend-C18, 5um 250 x 4.6mm	1.0	35	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
17	Agilent Technologies Pte. Ltd DAD: DEAEK00202/G7117C	WondaSil C18 Superb 5um 250 x 4.6mm	1.0	35	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
18	Agilent 1260 Infinity II	Agilent Eclipse XDB-C18, 5um 250 x 4.6mm	1.0	35	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
19	Agilent 1100 Series	Agilent Zorbax Extend-C18, 5um 250 x 4.6mm	1.0	25	2905	Acetonitrile/Water/Glacial acetic acid(820/175/5, v/v/v)
20	GC	2021 CIPAC Virtual Meeting				

Remarks of the Participants

Lab No.	Comments
Lab 6	The preparation of TC samples is deviated from the method specified by adding only 80 ml of acetronitrile before being placed in an ultrasonic bath for 5 min, allow to cool at ambient temperature and then fill acetronitrile to the mark.
Lab 8	Each TC sample was one cluster in the container. To weight the required amount the sample has to be scraped with a spatula. The labeling of the samples was not clearly. For identification the charge numbers must be compared (from container and from the report). For the Day2-Z sheet the formulae were missing. Sheet Day1-Z was duplicated and re-named.
Lab 17	DAY1-Z: The test/reference items were injected into HPLC system on 2021-01-12. The data was analyzed on 2021-01-13. DAY2-Z: The test/reference items were injected into HPLC system on 2021-01-13. The data was analyzed on 2021-01-14.
Lab 18	1 Approximately weigh 50mg of Chlorpyrifos standard respectively into 50ml volumetric flask. 2 Approximately weigh containing Chlorpyrifos 50mg of Chlorpyrifos TC or EC into 50ml volumetric flask.
Lab 19	Some particles in the 2 EC formulations

6.1 Data Review

- In summary it can be stated that the method deviations, noted by the participants, were deemed not to affect the analytical results significantly and therefore all data sets from **19 participating laboratories** were included within the statistical assessment, except the one using GC method.

6.2 Determination of Chlorpyrifos

- The statistical evaluation of the data was accomplished following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725. The testing for outliers/stragglers of the laboratory mean values were performed according to Grubbs test on a 1%/5% significance level, respectively.

Table 1 Results of 19 labs

	Chlorpyrifos TC-1		Chlorpyrifos TC-2		Chlorpyrifos TC-3		Chlorpyrifos EC-1		Chlorpyrifos EC-2	
	Day 1	Day 2								
Lab 1	977.9	975.3	977.2	980.6	979.9	978.1	406.3	406.7	405.1	406.5
Lab 2	978.4	977.0	979.0	978.2	979.6	980.2	403.6	403.2	403.2	402.8
Lab 3	975.4	977.4	975.9	978.3	979.1	977.9	404.6	403.7	403.4	403.2
Lab 4	976.7	976.4	979.3	977.4	979.1	971.2	400.6	406.8	400.8	402.7
Lab 5	982.8	981.0	978.9	978.0	984.0	975.2	407.7	407.9	407.0	407.6
Lab 6	963.4	973.6	960.6	971.5	953.4	962.0	397.8	397.4	398.3	399.2
Lab 7	982.4	985.6	982.8	984.8	982.1	983.8	406.4	406.7	405.5	405.8
Lab 8	978.9	973.3	983.2	972.7	983.0	966.2	403.3	398.1	402.4	399.9
Lab 9	935.2	954.6	924.2	963.5	936.5	945.3	395.6	400.8	395.7	400.0
Lab 10	985.7	980.1	989.3	977.3	991.2	977.8	408.5	405.8	410.5	407.4
Lab 11	971.5	971.3	975.4	974.8	980.3	980.8	404.3	405.7	401.3	402.7
Lab 12	976.8	976.1	979.4	979.6	981.6	981.9	402.9	403.3	403.4	403.9
Lab 13	977.5	973.7	979.8	974.8	969.3	970.5	403.2	399.5	399.3	398.6
Lab 14	979.9	980.7	978.6	980.1	977.7	976.5	405.0	405.6	406.0	405.3
Lab 15	977.2	977.5	980.4	975.7	974.4	977.4	406.6	407.1	405.7	410.2
Lab 16	980.0	977.6	978.1	976.1	980.2	979.6	405.8	404.2	406.3	404.2
Lab 17	983.8	978.2	982.6	984.0	983.7	979.2	405.5	403.5	405.3	402.5
Lab 18	981.4	978.1	982.7	981.9	980.6	982.1	403.8	404.6	405.4	405.5
Lab 19	982.6	978.6	962.3	970.3	973.2	965.0	404.1	406.1	404.0	405.7

Table 2 Mean values

	Chlorpyrifos TC-1	Chlorpyrifos TC-2	Chlorpyrifos TC-3	Chlorpyrifos EC-1	Chlorpyrifos EC-2
Lab 1	976.6	978.9	979.0	406.5	405.8
Lab 2	977.7	978.6	979.9	403.4	403.0
Lab 3	976.4	977.1	978.5	404.2	403.3
Lab 4	976.5	978.3	975.1	403.7	401.8
Lab 5	981.9	978.4	979.6	407.8	407.3
Lab 6	968.5	966.0	957.7	397.6	398.7
Lab 7	984.0	983.8	983.0	406.6	405.7
Lab 8	976.1	978.0	974.6	400.7	401.2
Lab 9	944.9	943.9	940.9	398.2	397.8
Lab 10	982.9	983.3	984.5	407.1	408.9
Lab 11	971.4	975.1	980.6	405.0	402.0
Lab 12	976.5	979.5	981.8	403.1	403.6
Lab 13	975.6	977.3	969.9	401.4	398.9
Lab 14	980.3	979.4	977.1	405.3	405.7
Lab 15	977.4	978.1	975.9	406.9	408.0
Lab 16	978.8	977.1	979.9	405.0	405.2
Lab 17	981.0	983.3	981.5	404.5	403.9
Lab 18	979.8	982.3	981.4	404.2	405.5
Lab 19	980.6	966.3	969.1	405.1	404.9

Table 3 Summary of the statistical evaluation (19 Labs included)

	Chlorpyrifos TC-1	Chlorpyrifos TC-2	Chlorpyrifos TC-3	Chlorpyrifos EC-1	Chlorpyrifos EC-2
x_m [g/kg]	976.15	976.02	974.89	404.01	403.75
x_m [% w/w]	97.62	97.60	97.49	40.40	40.38
L	19	19	19	19	19
S_r	4.31	6.68	4.82	2.06	1.57
S_R	9.24	10.79	11.30	3.35	3.39
r	12.07	18.70	13.51	5.76	4.38
R	25.86	30.22	31.65	9.37	9.50
RSD_R	0.95	1.11	1.16	0.83	0.84
$RSD_{R \text{ (Hor)}}$	1.42	1.42	1.42	1.62	1.62
HorRat	0.67	0.78	0.82	0.51	0.52

Fig. 1 Chlorpyrifos tech. sample - 1 (19 labs included)

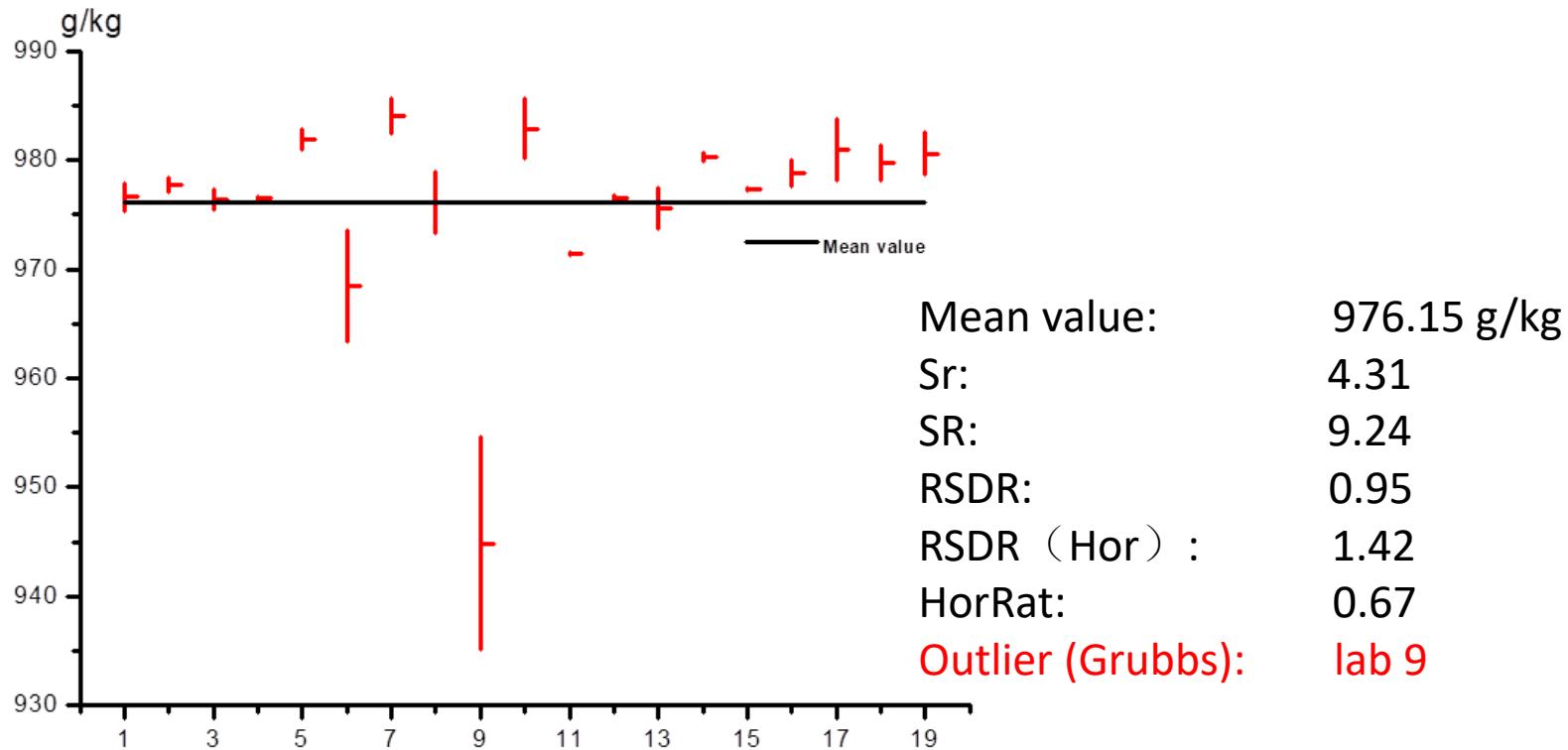


Fig. 2 Chlorpyrifos tech. sample - 2 (19 labs included)

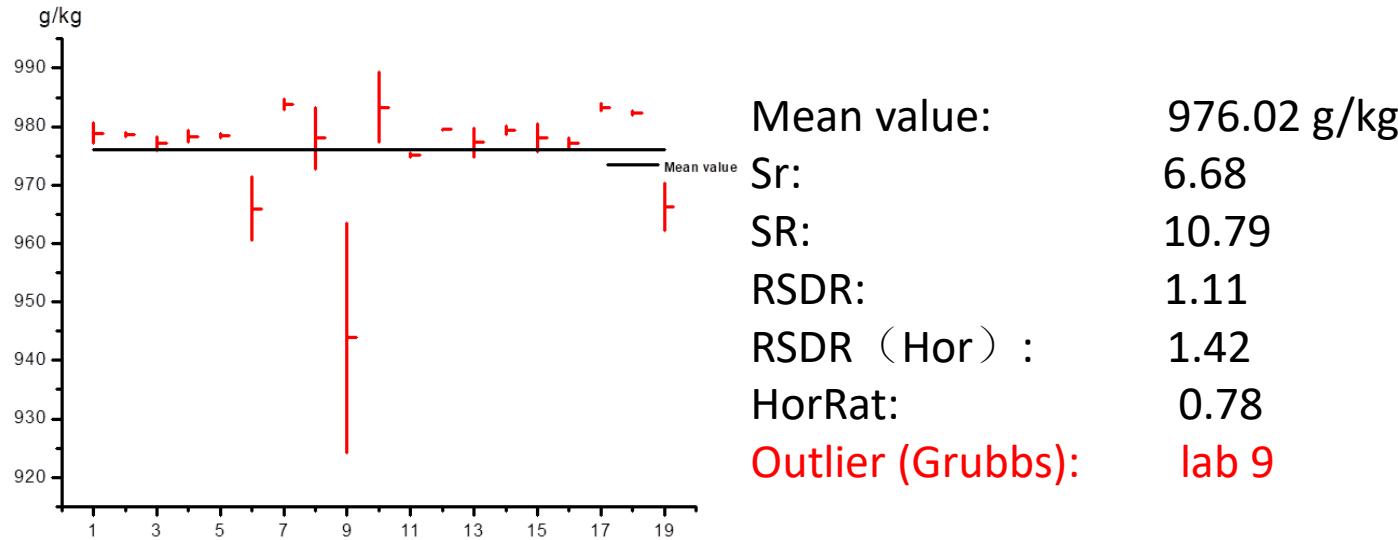


Fig. 3 Chlorpyrifos tech. sample - 3 (19 labs included)

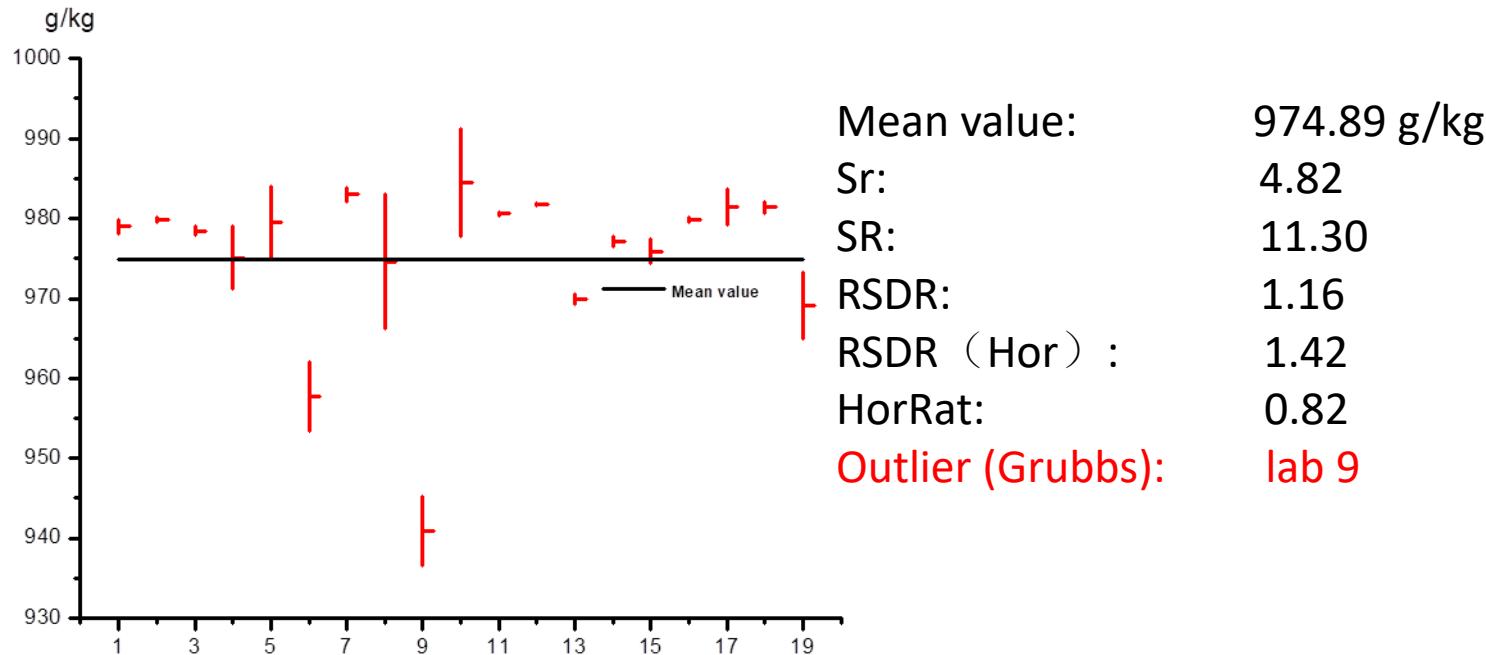


Fig. 4 Chlorpyrifos EC sample – 1 (19 labs included)

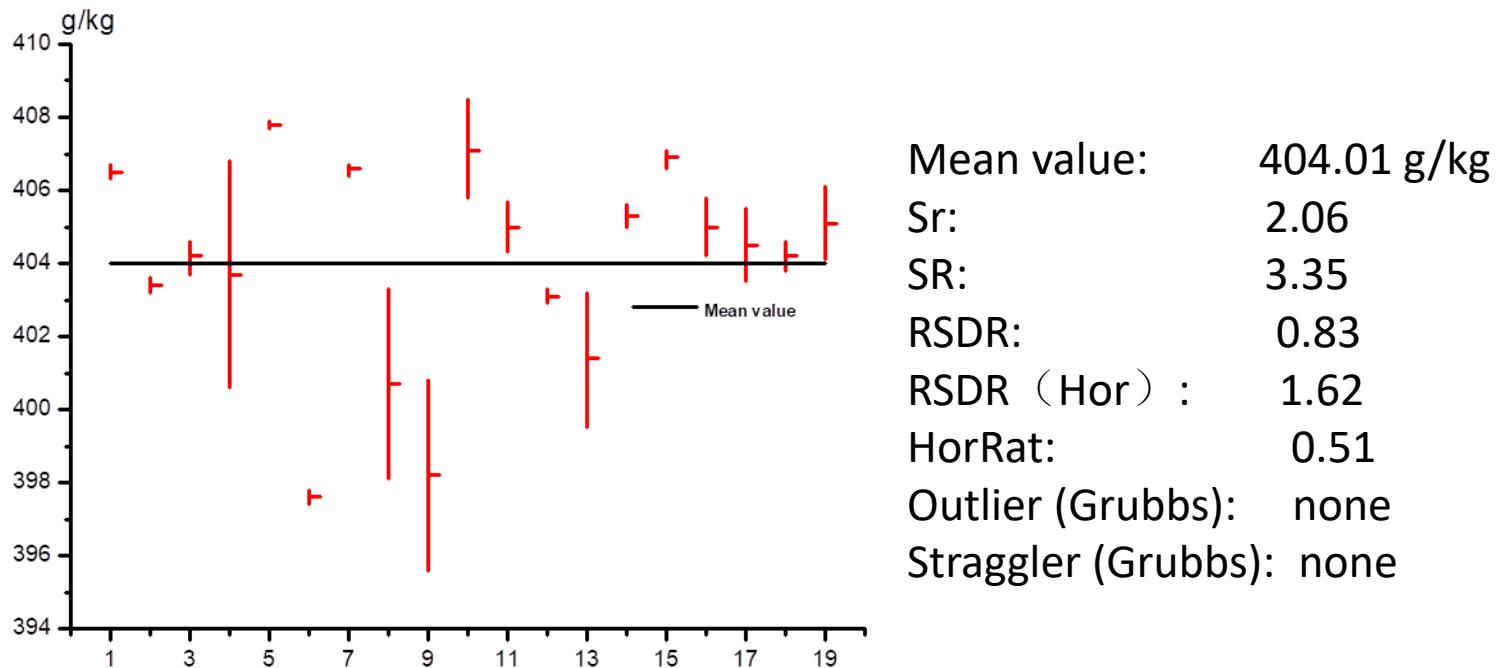
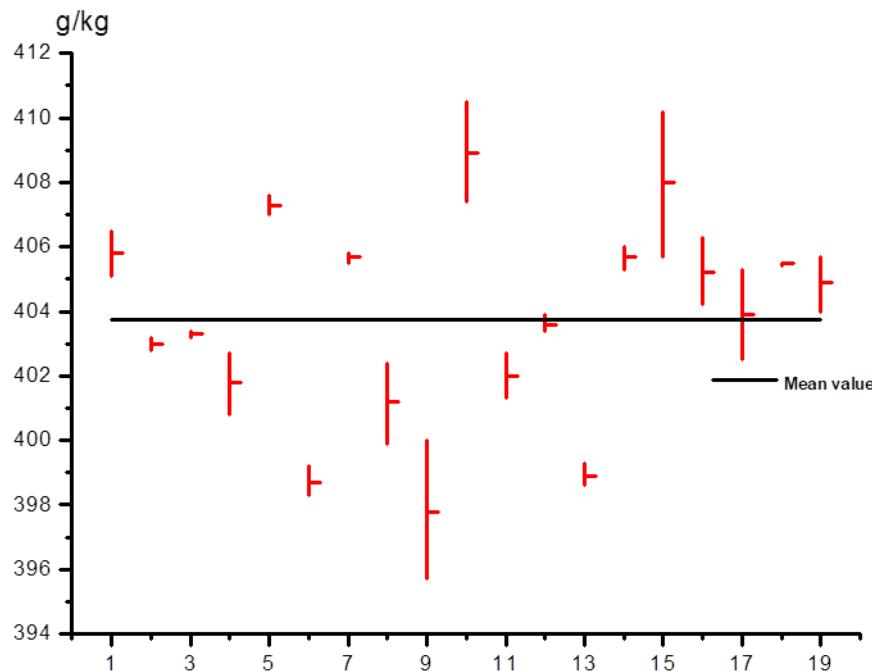


Fig. 5 Chlorpyrifos EC sample – 2 (19 labs included)



Mean value: 403.75 g/kg
Sr: 1.57
SR: 3.39
RSDR: 0.84
RSDR (Hor) : 1.62
HorRat: 0.52
Outlier (Grubbs): none
Straggler (Grubbs): none

Table 4 Results (without lab 9)

	Chlorpyrifos TC-1		Chlorpyrifos TC-2		Chlorpyrifos TC-3	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Lab 1	977.9	975.3	977.2	980.6	979.9	978.1
Lab 2	978.4	977.0	979.0	978.2	979.6	980.2
Lab 3	975.4	977.4	975.9	978.3	979.1	977.9
Lab 4	976.7	976.4	979.3	977.4	979.1	971.2
Lab 5	982.8	981.0	978.9	978.0	984.0	975.2
Lab 6	963.4	973.6	960.6	971.5	953.4	962.0
Lab 7	982.4	985.6	982.8	984.8	982.1	983.8
Lab 8	978.9	973.3	983.2	972.7	983.0	966.2
Lab 10	985.7	980.1	989.3	977.3	991.2	977.8
Lab 11	971.5	971.3	975.4	974.8	980.3	980.8
Lab 12	976.8	976.1	979.4	979.6	981.6	981.9
Lab 13	977.5	973.7	979.8	974.8	969.3	970.5
Lab 14	979.9	980.7	978.6	980.1	977.7	976.5
Lab 15	977.2	977.5	980.4	975.7	974.4	977.4
Lab 16	980.0	977.6	978.1	976.1	980.2	979.6
Lab 17	983.8	978.2	982.6	984.0	983.7	979.2
Lab 18	981.4	978.1	982.7	981.9	980.6	982.1
Lab 19	982.6	978.6	962.3	970.3	973.2	965.0

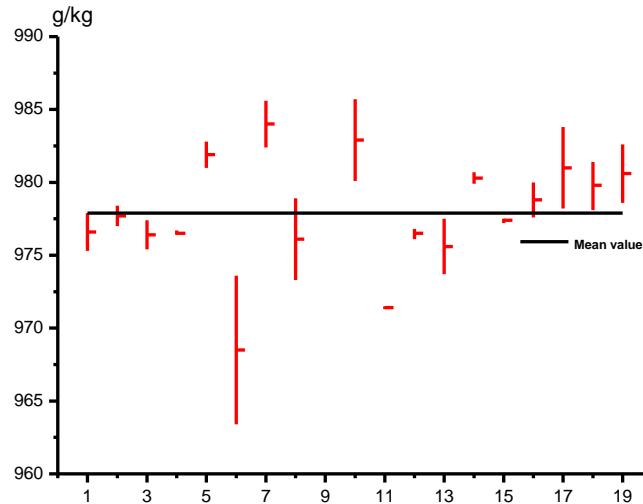
Table 5 Mean values (without lab 9)

	Chlorpyrifos TC-1	Chlorpyrifos TC-2	Chlorpyrifos TC-3
Lab 1	976.6	978.9	979.0
Lab 2	977.7	978.6	979.9
Lab 3	976.4	977.1	978.5
Lab 4	976.5	978.3	975.1
Lab 5	981.9	978.4	979.6
Lab 6	968.5	966.0	957.7
Lab 7	984.0	983.8	983.0
Lab 8	976.1	978.0	974.6
Lab 10	982.9	983.3	984.5
Lab 11	971.4	975.1	980.6
Lab 12	976.5	979.5	981.8
Lab 13	975.6	977.3	969.9
Lab 14	980.3	979.4	977.1
Lab 15	977.4	978.1	975.9
Lab 16	978.8	977.1	979.9
Lab 17	981.0	983.3	981.5
Lab 18	979.8	982.3	981.4
Lab 19	980.6	966.3	969.1

Table 6 Summary of the statistical evaluation (without lab 9)

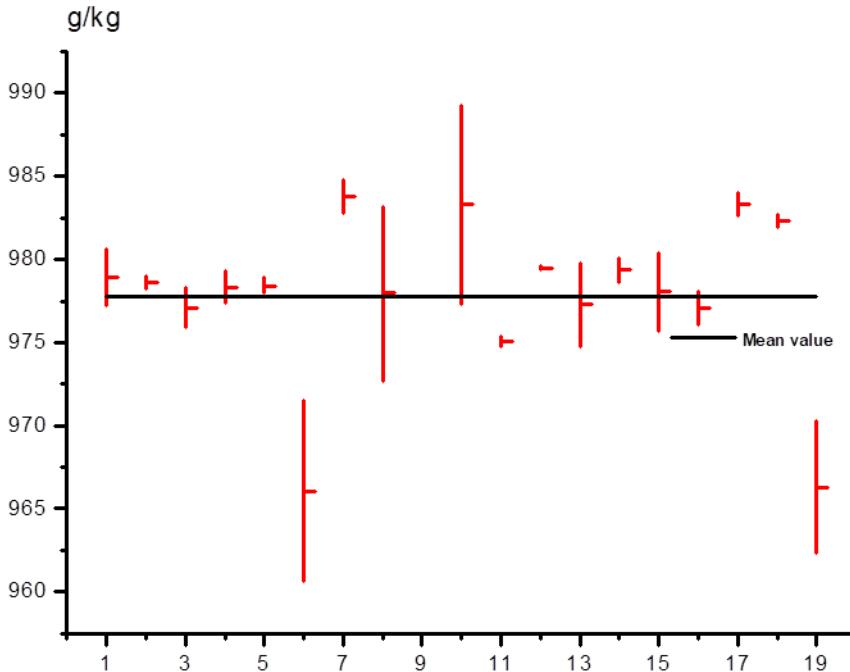
	Chlorpyrifos TC-1	Chlorpyrifos TC-2	Chlorpyrifos TC-3
x_m [g/kg]	977.89	977.81	974.89
x_m [% w/w]	97.79	97.78	97.68
L	18	18	18
S_r	3.56	4.10	4.66
S_R	4.94	6.03	7.83
r	9.96	11.47	13.06
R	13.83	16.88	21.93
RSD_R	0.51	0.62	0.80
$RSD_{R \text{ (Hor)}}$	1.42	1.42	1.42
HorRat	0.36	0.43	0.57

Fig. 6 Chlorpyrifos tech. sample - 1 (without lab 9)



Mean value: 977.89 g/kg
Sr: 3.56
SR: 4.94
RSDR: 0.51
RSDR (Hor) : 1.42
HorRat: 0.36
Outlier (Grubbs): none
Straggler (Grubbs): none

Fig. 7 Chlorpyrifos tech. sample - 2 (without lab 9)



Mean value: 977.81 g/kg
Sr: 4.10
SR: 6.03
RSDR: 0.62
RSDR (Hor) : 1.42
HorRat: 0.43
Outlier (Grubbs): none
Straggler (Grubbs): none

Fig. 8 Chlorpyrifos tech. sample - 3 (without lab 9)

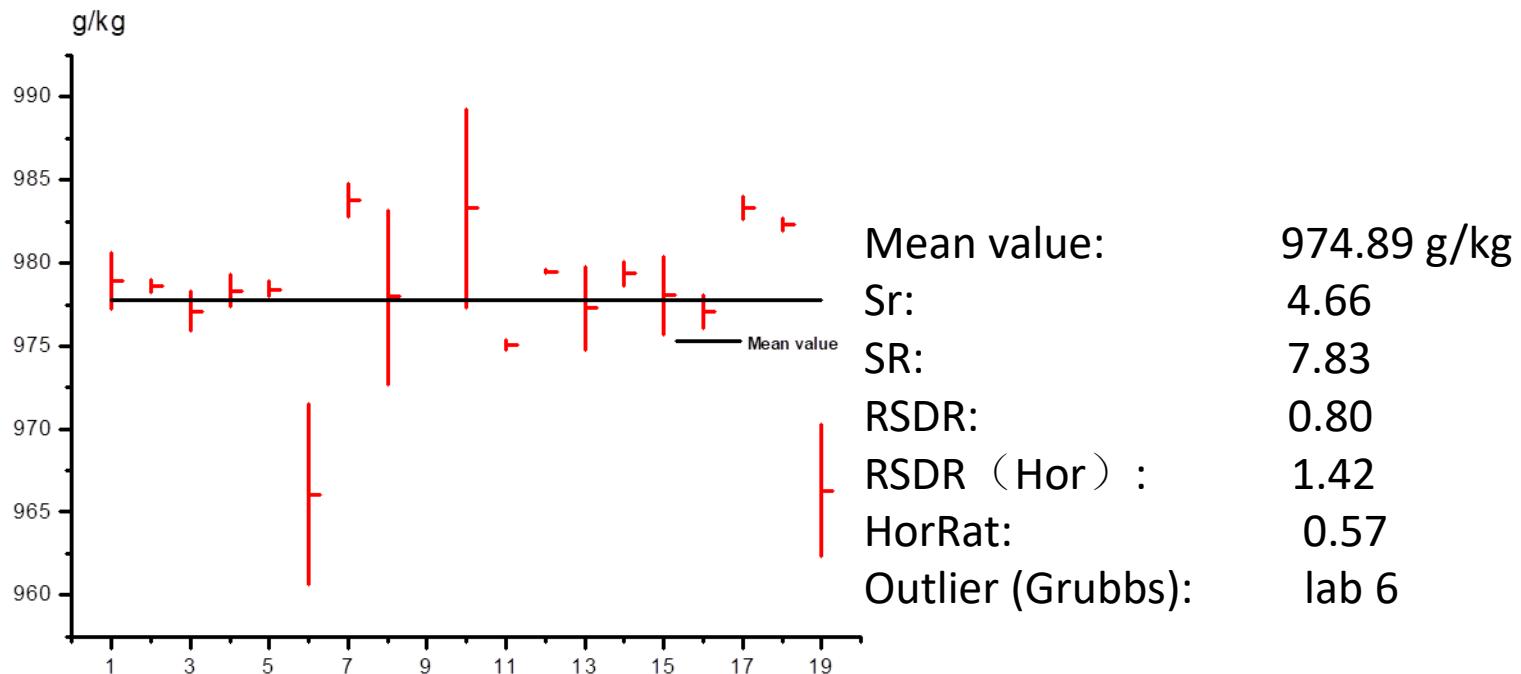


Table 7 Results (without lab 9 and 6)

	Chlorpyrifos TC-3	
	Day 1	Day 2
Lab 1	979.9	978.1
Lab 2	979.6	980.2
Lab 3	979.1	977.9
Lab 4	979.1	971.2
Lab 5	984.0	975.2
Lab 7	982.1	983.8
Lab 8	983.0	966.2
Lab 10	991.2	977.8
Lab 11	980.3	980.8
Lab 12	981.6	981.9
Lab 13	969.3	970.5
Lab 14	977.7	976.5
Lab 15	974.4	977.4
Lab 16	980.2	979.6
Lab 17	983.7	979.2
Lab 18	980.6	982.1
Lab 19	973.2	965.0

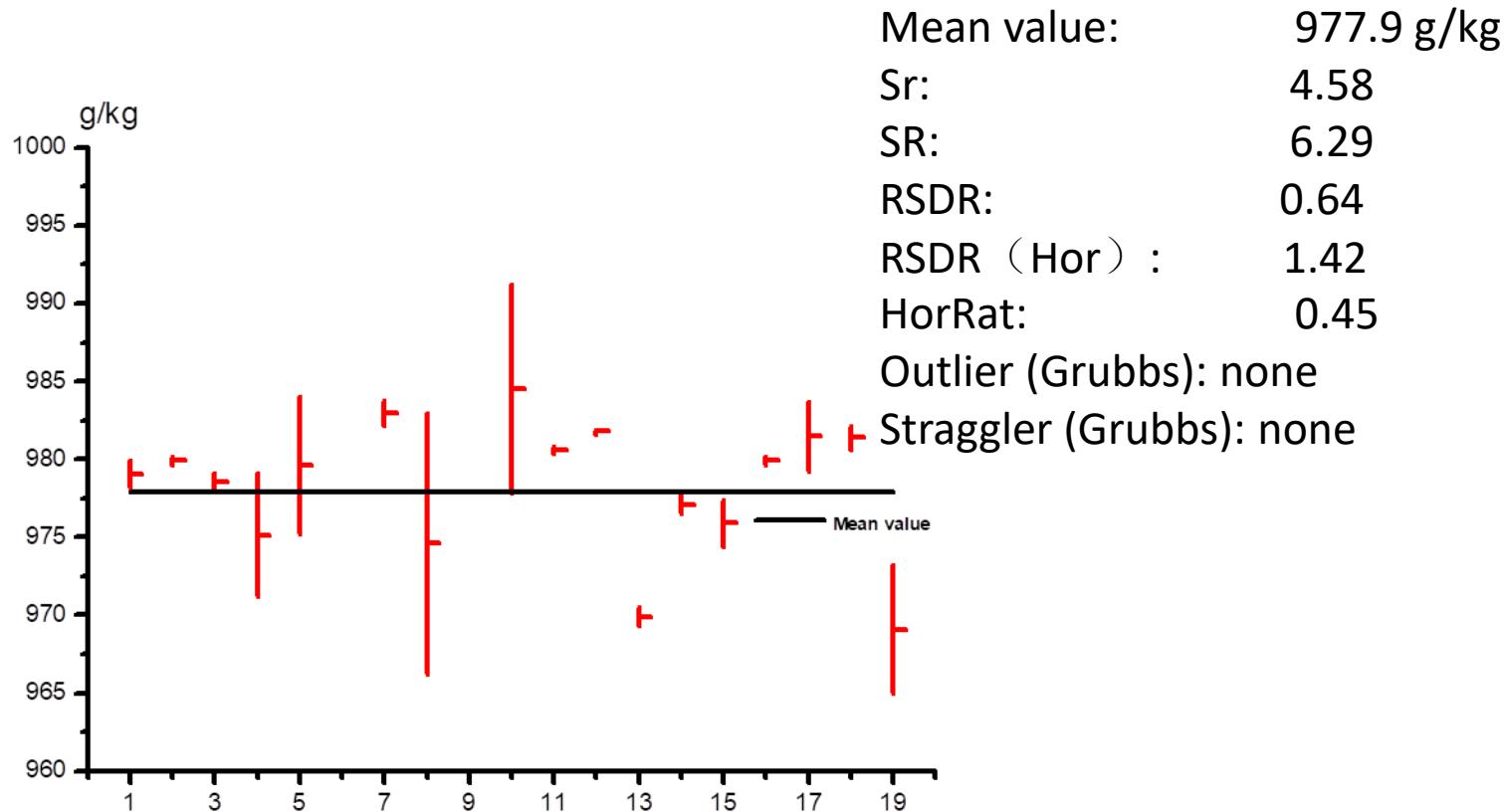
Table 8 Mean values (without lab 9 and 6)

	Chlorpyrifos TC-3
Lab 1	979.0
Lab 2	979.9
Lab 3	978.5
Lab 4	975.1
Lab 5	979.6
Lab 7	983.0
Lab 8	974.6
Lab 10	984.5
Lab 11	980.6
Lab 12	981.8
Lab 13	969.9
Lab 14	977.1
Lab 15	975.9
Lab 16	979.9
Lab 17	981.5
Lab 18	981.4
Lab 19	969.1

Table 9 Summary of the statistical evaluation (without lab 9 and 6)

Chlorpyrifos TC-3	
x_m [g/kg]	977.9
x_m [% w/w]	97.79
L	17
S_r	4.58
S_R	6.29
r	12.82
R	17.62
RSD_R	0.64
$RSD_{R(Hor)}$	1.42
HorRat	0.45

Fig. 9 Chlorpyrifos tech. sample - 3 (without lab 9 and 6)



7. Conclusions

- A total of 20 laboratories have participated in this full scale collaborative study. And data from 19 participants have been included in the final data statistical. The excluded one used GC method instead of HPLC.
- The data presented in the statistical summary show that this method led to HorRat values all within the required range. That is evidence for the fact that the present LC method is acceptable and suitable to produce reproducible results.
- SYRICIT consider this method to be suitable for the intended purpose, without further changes, and recommend accepting it as a provisional CIPAC method for the determination of Chlorpyrifos in technical grade material and in EC formulation.

Thank You

Any Question?

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