TEMBOTRIONE

Collaborative Study

Full Scale Collaborative Study for the Determination of Tembotrione in TC, OD and SC by HPLC

Report to CIPAC by
Chinese Pesticide Analytical Committee（CHIPAC）

Method Developed by
Jiangxi Tianyu Chemical Co., Ltd

May 2024

Content

1. Participants

By mid of March 2024, all of the 21 laboratories provided their results on the determination of Tembotrione according to CIPAC Information Sheet No. 339. The results for the 21 participants are presented in the following section.

Participating laboratories are listed in the table below.

Contact person	Participating Laboratory	Country
Milan Damjanovic	Agrosava-Agrochemical testing laboratory	Serbia
Moustafa Khalifa	Al - Sharhan Laboratory for Chemical Testing, Al Sharhan Industries ,Kuwait	Kuwait
Liang Huang	Anhui Fengle Agrochemical Co., Ltd. Product Testing Center	China
Peter Wagener, Simon Doerner-Rieping	Bayer AG, Research & Development, Crop Science, Chemical Quality Control	Germany
Cornel Grecu	Biochem - ROMANIA Quality Control Laboratory	Romania
Dipl.-Ing. Dagmar Julínková	Central institute for supervising and testing in agriculture National Reference Laboratory, Department of testing plant protection products	The Czech Republic
Hongfeng Sun, Chunqing Hou	China National Pesticide Quality Inspection and Testing Center (Shenyang)	China
Lisha Zhang	Chongqing Chemical Pesticide Quality Supervision and Inspection Station	China
Claudia Vinke	Federal Office of Consumer Protection and Food Safety	Germany
Nannan Li, Rongmei Chen	GreenTech Laboratory Co., Ltd.	China
Lu Huang	Hunan Research Institute of Chemical Industry Testing Technology Co., Ltd.	China
Jianzhong Yu	Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences	China
Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.	China
Rong Xie	Jiangsu Authority Testing Co., Ltd.	China

Volodymyr Mykhaylov	L.I.medved`s Reseach Center of Preventive Toxycology, Food and Chemical Safety, Ministry of Heath, Ukraine	Ukraine
Hongxia Li	Nutrichem Laboratory Co., Ltd	China
Daifeng Wang, Yuying Wang	Pesticide Quality Supervision, Inspection and Testing Center in Shenyang, MOA	China
Agus Salim	PT Agriculture Construction (AGRICON)	Indonesia
Yongfei Guo	Testing Technology Center of Sino-Agri Leading Bioscience Co., Ltd.	China
Peize Li	The National Center for the Pesticide Quality Inspection and Testing (Beijing)	China
Hucorne Pierre	Walloon Agricultural Research Centre (CRA-W) Protection, control products and residues Unit (U10)	Belgium

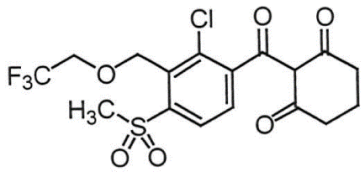
2. Tembotrione, General Information

Chemical name: 2-{2-chloro-4-mesyl-3[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dionetrifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione

Common name: Tembotrione

CAS-Number: 335104-84-2

Structure:



Molecular mass: 440.8 g/mol

Empirical formula: $C_{17}H_{16}ClF_3O_6S$

3. Samples

In October 2023, Information Sheet No. 343 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Tembotrione by HPLC.

Six test samples (described below), including the Tembotrione analytical reference standard were shipped to the participants:

A) Tembotrione TC-1
B) Tembotrione TC-2
C) Tembotrione OD-1
D) Tembotrione OD-2
E) Tembotrione SC-1
F) Tembotrione SC-2

Tembotrione analytical reference standard (98.6% purity)

All participants sent back their results in time.

4. Method

4.1 Scope

The contents of Tembotrione in technical materials and in formulated products (suspension concentrate, oil dispersion) were determined.

4.2 Principle

The content of Tembotrione in the sample is determined by high performance liquid chromatography on a reversed phase column (C_{18}) with UV detection at 284 nm and external standardization.

4.3 Procedure

Samples should be analyzed in duplicate at two different days resulting in a total of four individual test results for each sample. All test solutions should be prepared freshly on Day 2.

5. Remarks of the Participants

Participants made comments about the performance of the method and noted deviations from the method. Below is a summary of specific method conditions provided by the participating laboratories.

Lab Number	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (µL)	Mobile phase (v/v)
1	Watters 2695, Alliance	Agilent Zorbax SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
2	Thermo Vanquish Flex	Zorbax Eclipse XDB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
3	Shimadzu Prominence PDA	Waters Xselect HSS C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
4	Dionex UltiMate 3000 UHPLC	Zorbax Eclipse XDB-C_{18} (150×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
5	Agilent Technologies, Infinity II, 1260	Kromasil C_{18} (300×4.0 mm, 5 µm)	0.8	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
6	Shimadzu LC 2050 with SPD-M20A	Kromasil C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
7	HPLC 1200 binary	LiCrospher 100 RP 18 (250×4.0 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)

8	Agilent 1100	Agilent Zorbax SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
9	Agilent 1260	Agilent Zorbax SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
10	Agilent 1260 infinity II	Agilent SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
11	Agilent 1260	Zorbax SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
12	Agilent 1260 II	Eclipse Plus C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
13	Agilent 1260 infinity II	Agilent Zorbax Eclipse plus C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
14	Shimadzu 20AT	Phenomenex Luna C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
15	Shimadzu, LC-2050C 3D	SHIMADZU, ShimNex CS C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
16	LC-20AD	Intertsustain C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
17	Agilent 1260 infinity	Agilent SB-C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
18	Agilent 1260 infinity II	ZORBAX Eclipse Plus C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
19	Agilent 1260 infinity II	ODS2 WATERS (150×4.6 mm, 5 µm)	1	30	284	10	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)

20	Agilent 1200	Venusil XBP C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)
21	Agilent 1260 infinity II	ZORBAX Eclipse Plus C_{18} (250×4.6 mm, 5 µm)	1	30	284	5	Acetonitrile : 0.1 % H_3PO_4 60 : 40 (v/v)

Remark and Comment

No.	Remark and Comment
Lab 1	**Comment:** Missing requirement for filter type (e.g. PTFE) in method information. **Recommendation:** Add sample filtration for all formulations. Especially for the SC formulation filtration is necessary due to the presence of insoluble residue after sonicaton
Lab 7	**Remark:** The standard material was stuck to the funnel and was flushed with solvent after weighting, the samples were weighted without funnel directly into the flask. The OD samples were filtered through a 0.45 µm filter. **Recommendation:** The ultrasound pre-treatment be modified to 15 min.
Lab 11	**Remark:**The ultrasonic pre-treatment was prolonged to 10 min for OD samples.
Lab 19	**Remark:** For TC and OD, the preparation concentrations of calibration and sample solution were changed by weighing samples containing about 25 mg of tembotrione into 25 ml volumetric flasks, which is twice the solution concentration as specified in the provided method. For SC, the sample solution was prepared the same as described in the provided method. But the preparation of calibration solution is the same as that of TC and OD and the concentration is the twice as described in the provided method.

Note:

1. The recommendation from Lab 1 was accepted. Adding filtration in the preparation of SC sample solution in the method.
2. Lab 7 realized that the results, especially for the TC samples, were not satisfactory due to significant spreading and low values. They made an attempt to extend the ultrasonic pre-treatment time to 15 minutes, using 2 ml water after weighing for the SC samples, and repeated the test for one day with minor adjustments to the method as mentioned above. The results showed that the TC data improved after prolonged ultrasound time, but 2 ml water seems not to make a big difference for the SC samples. Therefore, Lab 7 recommend to change the method to using 15 min ultrasonic pre-treatment instead of 5 min. The suggestion was partially accepted and the method was revised.
3. Considering the method deviations from Lab 19 included column length, injection volume and standard preparation and sample solution preparation, the method used in Lab 19 was quite different from the proposed method especially for TC and OD samples.

6. Evaluation and Discussion

6.1 Evaluation of the Quality of Data and Chromatograms

The data obtained from each of the laboratories were reviewed to determine if there were any

significant deviations regarding the chromatography which might affect the analysis results.
Visual examination of the chromatograms showed no evidence for invalid data.
All other changes and observations noted by the 20 participants were not expected to affect the analysis results significantly except Lab 19.

6.2 Determination of Tembotrione

All laboratories provided duplicate data for two different days, except Lab 7, which carried out the 3rd day's replicate analyses after modifying the method. As shown in tables 1-4 and figures 1-6, all results have been reported, and were taken into account by a statistical procedure after evaluating the validity of the results.

The statistical evaluation of the data was done following the "Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods", according to DIN ISO 5725. The data were examined for outliers and stragglers using the Grubb`s test, and the tests were performed at an alpha level of 0.01 for outlier (marked with **), and 0.05 for straggler (marked with *).

After Grubb`s test, outliers and stragglers were identified in the TC1 and TC2 data from Lab 7 and 19, as well as outliers in the OD1 data from Lab 19. The data were re-analyzed after the outliers were omitted (see Table 4). The minimum number of considered results after the elimination of outliers was 19 for TC and 20 for OD1.

For TC1 and TC2, the between lab experimental Relative Reproducibility Standard Deviation (RSD_R) is below the Horwitz value after eliminating the outliers of Lab 7 and 19, and the HorRat was found within the desired range (lower than 1.0).

Regarding OD1, OD2, SC1 and SC2, the RSD_R were below the Horwitz value without the elimination of any outliers or stragglers, the HorRat values were between 0.3-1.0, and the RSD_R was further improved for OD1 after eliminating the outliers of Lab 19.

Due to the universal applicability of the method, this collaborative trial is acceptable.

Table 1: Tembotrione (g/kg); Results for each laboratory on day 1 and day 2

	Tembotrione TC-1			Tembotrione TC-2			Tembotrione OD-1			Tembotrione OD-2			Tembotrione SC-1			Tembotrione SC-2		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
Lab 1	978.2	971.7	/	977.3	974.3	/	82.97	82.24	/	82.73	81.92	/	357.7	360.2	/	359.5	357.7	/
Lab 2	973.8	978.7	/	977.1	975.7	/	83.63	83.13	/	83.35	83.26	/	356.6	358.8	/	357.2	359.2	/
Lab 3	982.8	971.1	/	978.0	978.9	/	79.80	79.22	/	81.60	79.01	/	355.0	352.6	/	354.3	352.1	/
Lab 4	974.8	973.2	/	976.0	981.6	/	83.15	83.52	/	82.74	84.09	/	357.7	361.7	/	357.3	361.1	/
Lab 5	979.2	977.0	/	980.5	978.7	/	82.08	82.36	/	82.32	82.09	/	355.1	353.3	/	354.5	351.8	/
Lab 6	986.6	975.1	/	992.2	969.2	/	80.90	81.29	/	78.73	80.29	/	351.8	345.5	/	352.9	346.8	/
Lab 7	851.3	857.1	964.8	974.6	825.0	967.8	78.45	81.49	81.21	79.29	75.08	81.22	357.4	339.3	360.4	356.8	356.1	354.7
Lab 8	980.8	978.8	/	975.1	976.8	/	81.08	83.10	/	80.37	83.58	/	358.2	357.8	/	357.3	356.5	/
Lab 9	975.1	975.7	/	977.6	973.2	/	77.83	77.51	/	78.76	79.05	/	355.1	359.4	/	354.2	357.7	/
Lab 10	976.3	979.8	/	978.8	980.0	/	84.43	81.97	/	87.79	83.66	/	359.6	360.9	/	359.2	356.8	/
Lab 11	980.3	979.9	/	980.0	979.8	/	82.06	82.65	/	82.19	82.29	/	351.3	350.6	/	350.5	350.2	/
Lab 12	969.6	974.0	/	980.8	976.5	/	80.85	81.01	/	79.71	80.31	/	358.3	355.7	/	358.3	357.0	/
Lab 13	976.8	974.0	/	975.8	979.7	/	80.49	81.13	/	80.53	81.20	/	351.1	354.7	/	353.2	355.5	/
Lab 14	974.4	972.3	/	978.9	978.3	/	82.34	82.47	/	85.82	81.54	/	353.9	357.8	/	357.3	352.1	/
Lab 15	974.3	975.4	/	974.6	973.7	/	82.14	82.66	/	81.68	82.15	/	353.6	353.2	/	351.9	353.6	/
Lab 16	973.0	975.2	/	970.4	977.7	/	79.52	80.27	/	80.27	79.63	/	350.3	352.4	/	350.1	351.8	/
Lab 17	973.6	979.9	/	975.5	971.3	/	82.73	82.99	/	83.07	82.50	/	354.5	354.2	/	353.8	352.9	/
Lab 18	973.0	971.3	/	974.3	973.5	/	82.31	82.25	/	82.10	82.31	/	354.1	354.5	/	355.2	353.0	/
Lab 19	978.8	1004.0	/	984.2	1014.0	/	87.82	87.96	/	82.48	83.48	/	351.2	359.9	/	349.7	361.9	/
Lab 20	976.8	977.2	/	981.1	980.8	/	81.45	80.27	/	81.60	80.47	/	353.2	353.7	/	353.2	351.4	/
Lab 21	971.7	971.0	/	972.0	971.6	/	82.45	80.57	/	81.86	80.85	/	352.1	352.0	/	351.5	351.8	/

Table 2: Mean values

	Tembotrione TC-1	Tembotrione TC-2	Tembotrione OD-1	Tembotrione OD-2	Tembotrione SC-1	Tembotrione SC-2
Lab 1	974.9	975.8	82.60	82.32	358.9	358.6
Lab 2	976.3	976.4	83.38	83.31	357.7	358.2
Lab 3	976.9	978.4	79.51	80.30	353.8	353.2
Lab 4	974.0	978.8	83.33	83.41	359.7	359.2
Lab 5	978.1	979.6	82.22	82.21	354.2	353.1
Lab 6	980.8	980.7	81.09	79.51	348.6	349.8
Lab 7	891.0**	922.5**	80.38	78.53	352.4	355.8
Lab 8	979.8	975.9	82.09	81.97	358.0	356.9
Lab 9	975.4	975.4	77.67	78.91	357.2	355.9
Lab 10	978.0	979.4	83.20	85.72	360.2	358.0
Lab 11	980.1	979.9	82.35	82.24	351.2	350.3
Lab 12	971.8	978.6	80.93	80.01	357.0	357.6
Lab 13	975.4	977.8	80.81	80.87	352.9	354.3
Lab 14	973.4	978.6	82.40	83.68	355.8	354.7
Lab 15	974.8	974.1	82.40	81.91	353.4	352.7
Lab 16	974.1	974.1	79.90	79.95	351.3	351.0
Lab 17	976.8	973.4	82.86	82.78	354.4	353.4
Lab 18	972.1	973.9	82.28	82.21	354.3	354.1
Lab 19	991.4*	999.1**	87.89**	82.98	355.5	355.8
Lab 20	977.0	980.9	80.86	81.03	353.4	352.3
Lab 21	971.4	971.8	81.51	81.35	352.1	351.7

* Grubbs test straggler ** Grubbs test outlier

Table3: Summary of the statistical evaluation - no elimination of any outliers /stragglers

	Tembotrione TC-1	Tembotrione TC-2	Tembotrione OD-1	Tembotrione OD-2	Tembotrione SC-1	Tembotrione SC-2
xm [g/kg]	972.5	975.5	81.89	81.68	354.8	354.6
L	21	21	21	21	21	21
S_r	25.65	22.37	1.293	1.424	4.324	3.648
S_R	31.91	24.90	2.267	2.186	4.849	4.228
S_L	18.98	10.95	1.862	1.658	2.194	2.138
r	71.81	62.63	3.621	3.988	12.11	10.21
R	89.34	69.73	6.347	6.121	13.58	11.84
RSD_r	2.637	2.293	1.579	1.744	1.219	1.029
RSD_R	3.281	2.553	2.768	2.676	1.367	1.192
RSD_R (Hor)	2.008	2.008	2.915	2.916	2.338	2.338
HorRat	1.6	1.3	0.9	0.9	0.6	0.5

Table4: Summary of the statistical evaluation with elimination Grubbs test Stragglers /Outliers

	Tembotrione TC-1	Tembotrione TC-2	Tembotrione OD-1
xm [g/kg]	975.8	977.0	81.59
L	19	19	20
S_r	4.058	4.336	1.014
S_R	4.429	4.592	1.695
S_L	1.773	1.511	1.359
r	11.36	12.14	2.838
R	12.40	12.86	4.747
RSD_r	0.4159	0.4438	1.243
RSD_R	0.4538	0.4700	2.078
RSD_R (Hor)	2.007	2.007	2.916
HorRat	0.2	0.2	0.7

TC-1: Results of Lab 7 and 19 were eliminated; TC-2: Results of Lab 7and 19 were eliminated; OD-1: Results of Lab 19 were eliminated.

X_m = overall sample mean

L = number of laboratories

S_r = repeatability standard deviation

RSD_r = relative repeatability standard deviation

r = repeatability limit

S_R = reproducibility standard deviation

RSD_R = relative reproducibility standard deviation

R = reproducibility limit

S_L = “pure” between laboratory standard deviation

RSD_R (Hor) = relative reproducibility standard deviation (Horwitz equation)

Figures 1 – 6 (all results)

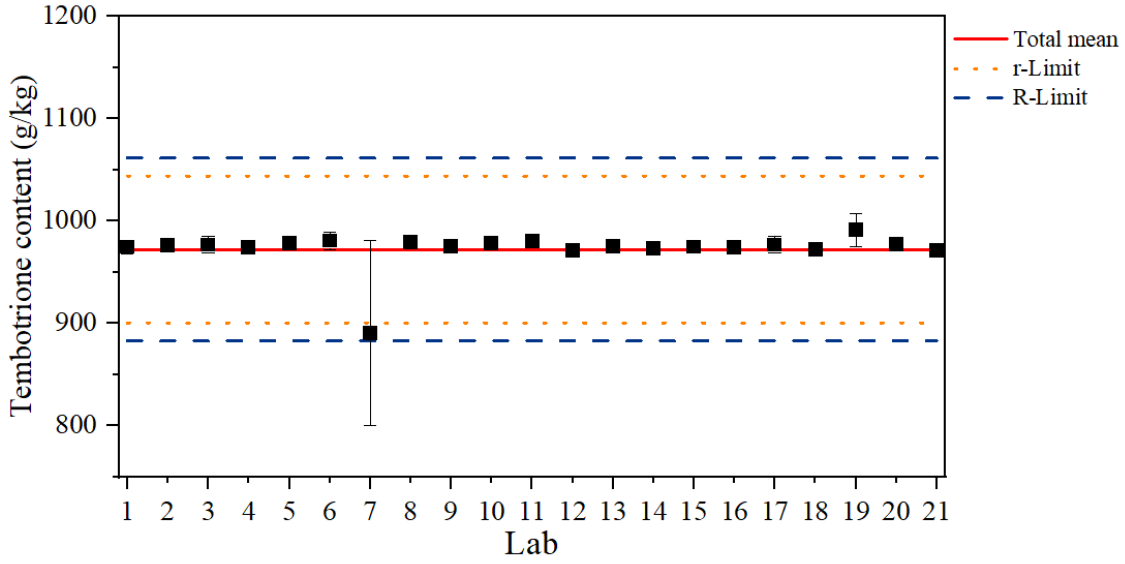


Figure 1. Graphical presentation of TC1 data

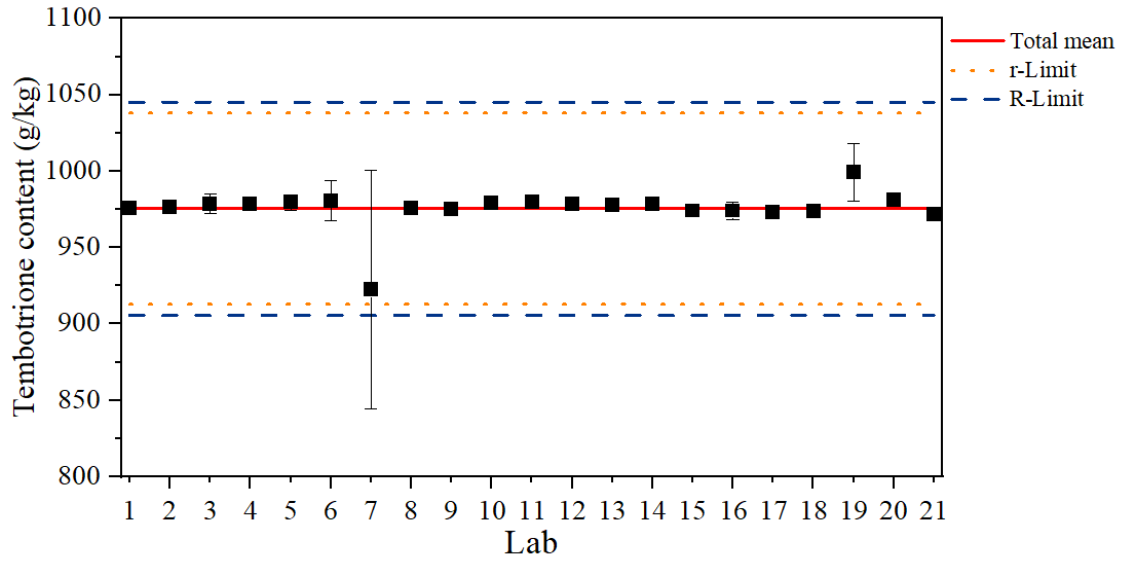


Figure 2. Graphical presentation of TC2 data

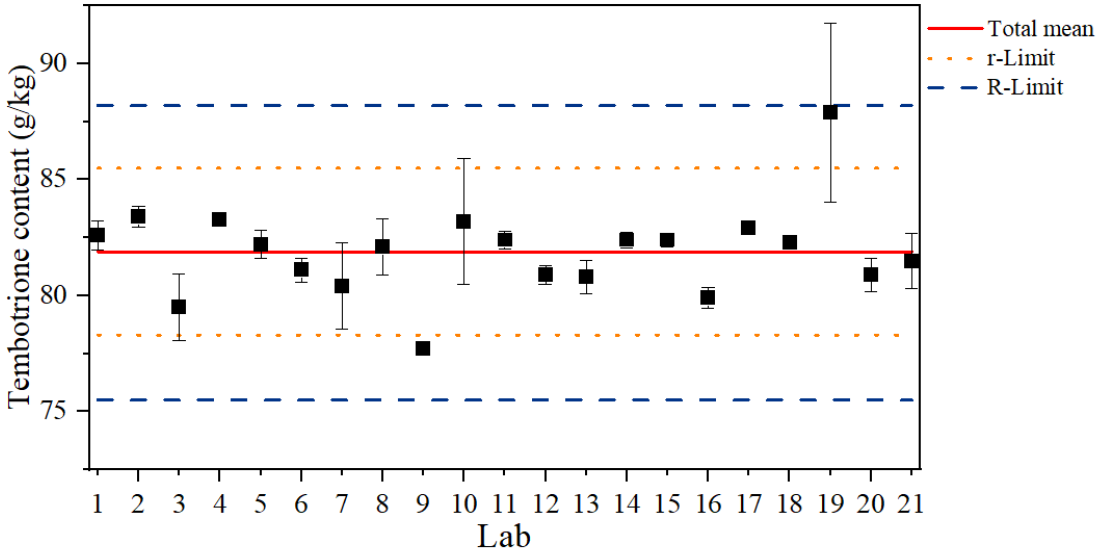


Figure 3. Graphical presentation of OD1 data

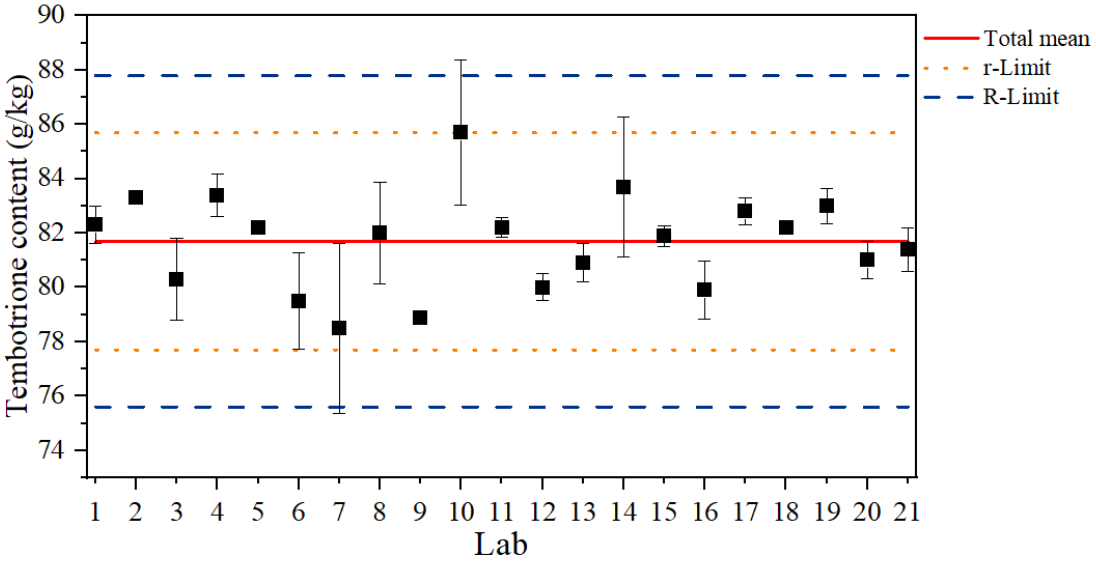


Figure 4. Graphical presentation of OD2 data

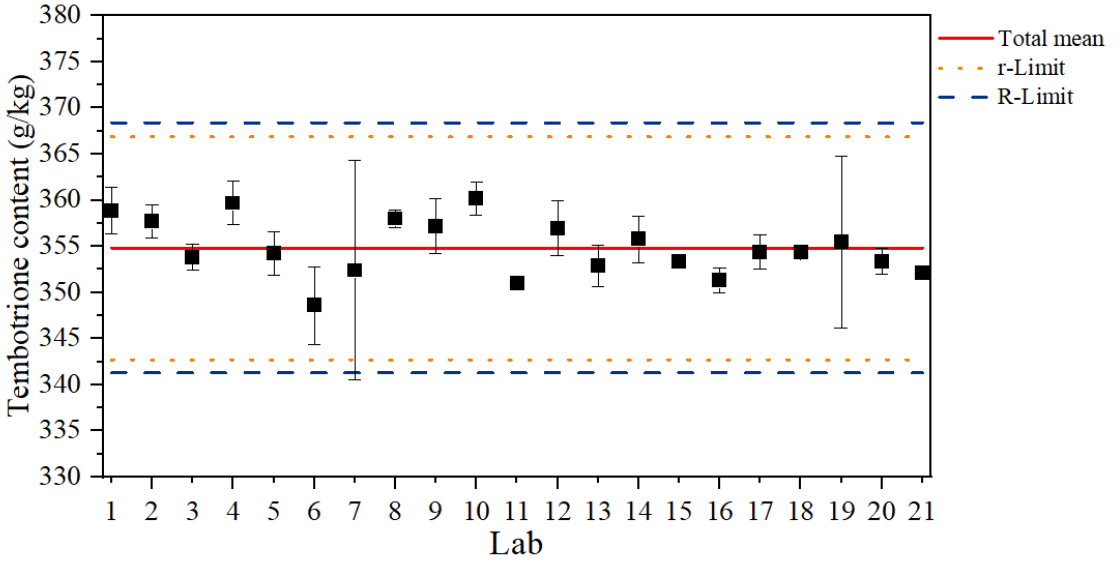


Figure 5. Graphical presentation of SC1 data

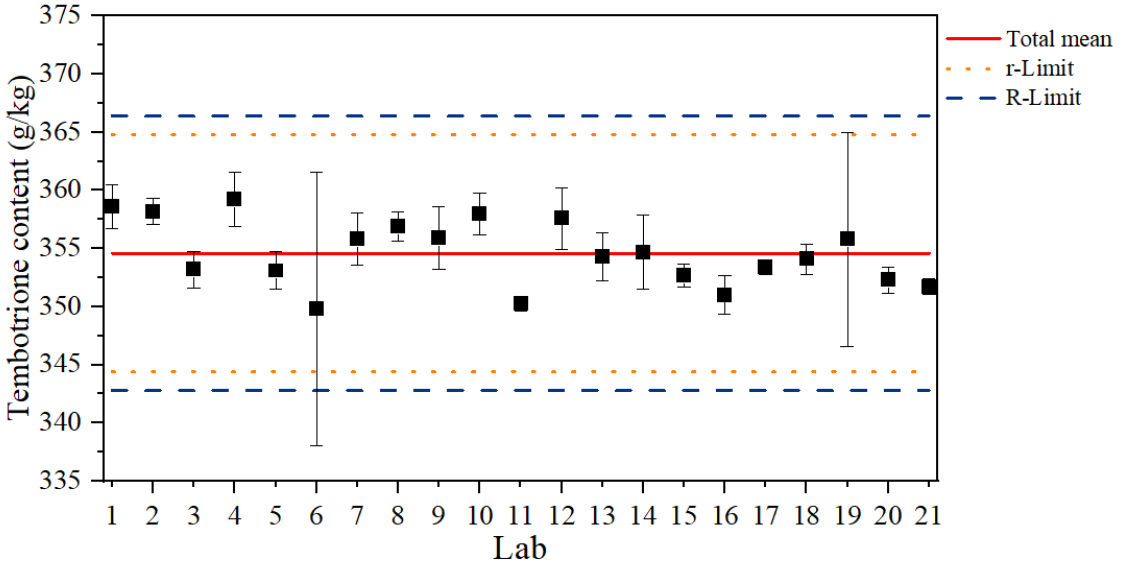


Figure 6. Graphical presentation of SC2 data

7. Conclusions

21 different laboratories participated in this collaborative study. The results of the labs are given in Table 1-2, the statistical summary is given in Table 3-4. The results are illustrated in figures 1–6.

For TC1 and TC2, the between lab experimental Relative Reproducibility Standard Deviation (%RSD_R) is below the calculated acceptable value based on the Horwitz curve calculation (RSD_R (Hor)) after eliminating stragglers and outliers, and the HorRat values were lower than1.0 by employing this method. The minimum number of considered results after elimination of stragglers and outliers was 19.

For OD1, OD2, SC1, and SC2, the RSD_R were below the RSD_R (Hor) without the elimination of any outliers or stragglers for above samples, and the HorRat values all within required range (0.3-1.0). the minimum number of considered results before elimination of stragglers and outliers was 21.The RSD_R was further improved for OD1 after eliminating the outliers of Lab 19, and the minimum number of considered results after elimination of stragglers and outliers was 20.

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Taking into account the relatively high number of participating laboratories, a broad basis was given even after elimination of the outliers. Therefore, CHIPAC considers this method to be suitable and recommend accepting it as a provisional CIPAC method for the determination of Tembotrione in both technical materials and its associated formulated products.