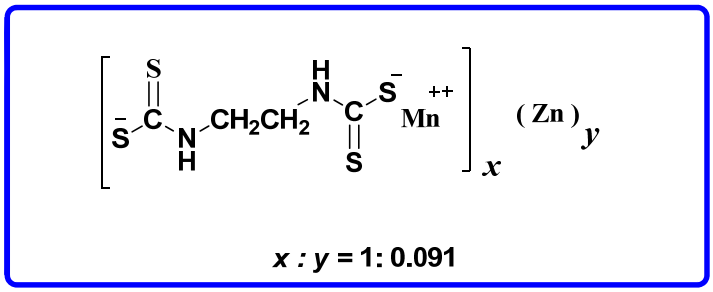
5157/R Mancozeb- Chinese Pesticide Advisory Committee

Collaborative Study for the Determination of Mancozeb in TC and WP by HPLC



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Economic Development Zone, Xinyi, Jiangsu, China

Presented at the CIPAC meeting in Germany
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Content

1. Background

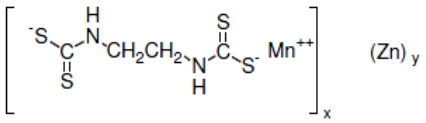
The collaborative study is to test the slightly modified method for the determination of Mancozeb in TC and WP following up on the comments that the difficulty on controlling the room temperature of 17+/-1 ℃ from 2018 CIPAC meeting. In the updated method, the environment temperature has been changed from 17+/-1 ℃ to below 20℃.

2. Participants

The collaborative study was attended by 8 voluntary participants. One participant decided not to contribute in the collaborative study as the samples and reagents were retained in customs in inappropriate conditions for more than 1 month, due to the nature of the molecule it is degraded, they worked with expired samples. The results of 7 participants were presented, and the lab numbers in the result tables were assigned, chronologically, based upon receipt of results.

Participant	**Contacts**
Laboratorio Control de Calidad de Plaguicidas MAG-OIRSA	Elizabeth Carranza de aguila
Cerexagri B.V.	Masha Bos
Jiangsu Provincinal Agro-Product Quality Test Center	Huimin Tang
Bayer CropScience (China) Co., Ltd.	Zhonghua LU
China National Pesticide Quality Supervision Testing Center (Shenyang)	Yu Liang
Institute for the Control of Agrochemicals Ministry of Agriculture (ICAMA)	Peng Wu
Limin Chemical Co., Ltd.	Xu Nuo
Lanfeng Biochemical	Lin Ma

3. Active Ingredient: General Information

ISO common name	Mancozeb
Chemical name(s)	IUPAC Manganese ethylenebis (dithiocarbamate) (polymetic) complex with zinc salt CA [[1,2-3thanediybiscarbamodithioato]] (2-) manganese mixture with [[1,2-ethanediybis [carbamodithioato]] (2-)] zinc
Structure	 x:y = 1:0.091
Molecular formula	$[C_4H_6MnN_2S_4]_xZn_y$
m.p.	Decomposes
Relative molecular mass	271.3g/mol
CAS No	8018-01-7
Solubility	Insoluble in water and most organic solvents
Description	Yellow powder
Stability	Decomposed under acid conditions
Formulation	Wettable powders, water dispersible granules, suspension concentrates and dustable powders

4. Samples

The following samples and reference standard were sent to the participants:

201812001-1# Mancozeb Technical

201812002-2# Mancozeb Technical

2018012003-3# Mancozeb WP

2018012004-4#Mancozeb WP

201812005-5# Mancozeb WP

Mancozeb reference standard (purity 86.0%), calibrated by commercial standard (Dr.Ehrenstorfer, Germany) using CIPAC method.

5. Method

5.1 Scope

Determination of the content of Mancozeb in TC and WP.

5.2Outline of the Method

Mancozeb is determined by reversed phase high performance liquid chromatography using UV detection and external standardization.

5.3 Procedure for the collaborative trial

The samples were analyzed on two different days with duplicate injections of two weighing per sample. Both test and reference solutions were freshly prepared on each day. Each sample bracketing them by single injection of calibration solution (C1 or C2) using the following sequence: C1, S1, S1, C2, S2, S2, C1, etc. The average response factor, used to calculate the amount of Mancozeb in the test solution, was calculated using the injection before and after the test injection.

6. Remarks and Deviations of the Participant.

6.1 Analytical Conditions

Lab	Instrument	Column	Flow Rate	Column Temperature°C
1	Agilent 1200 HPLC With DAD	Agilent Extend C18, 150 mm×4.6 mm, 5µm column	1.0	15
2	Agilent 1100 HPLC with UV	Agilent 773450-902 Zorbox Extend-C18, 150 mm×4.6 mm, 5µm	1.0	15
3	Agilent 1100 HPLC with DAD	Agilent Extend C18, 250 mm×4.6 mm, 5µm	1.3	15
4	Agilent 1100 HPLC with DAD	Agilent Extend C18, 150 mm×4.6 mm, 5µm	1.0	15
5	Thermo Ultimate 3000 with DAD	Agilent Extend C18, 150 mm×4.6 mm, 5µm	1.0	15
6	Agilent 1100 HPLC with UV	Agilent Extend C18, 150 mm×4.6 mm, 5µm	1.0	15
7	Agilent 1260 HPLC with UV	Agilent Extend C18, 150 mm×4.6 mm, 5µm	1.0	15
8	Shimadzu 20A, with UV	Agilent Extend C18, 150 mm×4.6 mm, 5µm	1.0	15

6.2 Remarks

Lab 1: as the samples and reagents were retained in customs in inappropriate conditions for more than 1 month, due to the nature of the molecule it is degraded, we worked with expired samples.

Lab 2:

1. Solutions are not stable, maybe better to calculate with calibrations of the beginning of the day, otherwise prepare fresh calibrations every two hours. We used the same calibration solutions the whole day (more than seven hours).

Response:the stability test showed that the solution is stable up to 8 hrs, which is also supported by the data from the other labs.

2. The peak in the chromatogram is not mancozeb but EBDC. Therefore the method is not specific (also other EBDC's can be detected like maneb and zineb).

Response:For the identity test, CIPAC MT 165 as recommended, can be

added to differentiate Mancozeb and the mixture of Maneb and insoluble zinc.

CIPAC MT 154 could also be considered, in which TLC is used to differentiate different dithiocarbamates containing zinc by different Rf value.

3. It is impossible to buy an accurate mancozeb standard, to use this method such a standard is mandatory. Mancozeb is not stable at room temperature especially not in small amounts. Standards should be kept in freezer and better in a larger amount.

Response:For the standard, Limin purchases from Dr. Ehrenstorfer and re-quantifies them prior to use and finds they are reliable for quantification test.

Deviations:

1. The sequence is slightly different and went as: C1 C1 S1-1 S1-2 S2-1 S2-2 C2 C2….

2. 0.45 µm filters was used instead of 0.22 µm

7. Evaluation and Discussion

The results of 7 labs were included within the statistical assessment. 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.

7.1 Summary of results

The assay results obtained by the collaborators and the statistical evaluation are reported in **Table1-5** and displayed in **Figure 1-5**.

The lab experimental Relative Reproducibility Standard Deviation (% RSDr) is well below the calculated acceptable value based on the Horwitz curve calculation (% RSD(Hor)) for the Mancozeb technical samples 1 and 2, as well as for WP formulation samples 3 to 5 (Table 6).

Table 1: TC-1 (Batch No: 201812001-1#)

Lab	Day 1		Day 2		Mean g/kg	Std. Dev.
	1	2	1	2		
2	865.1	858.0	857.1	860.8	860.3	3.60
3	860.9	862.7	861.3	868.3	863.3	3.42
4	862.9	862.0	859.5	865.7	862.5	2.56
5	863.5	862.5	862.4	866.7	863.8	2.01
6	859.4	863.5	861.7	864.4	862.3	2.21
7	861.8	860.3	860.1	860.6	860.7	0.76
8	862.6	861.8	860.3	859.6	861.1	1.37

Fig.1: TC-1 (Batch No: 201812001-1#)

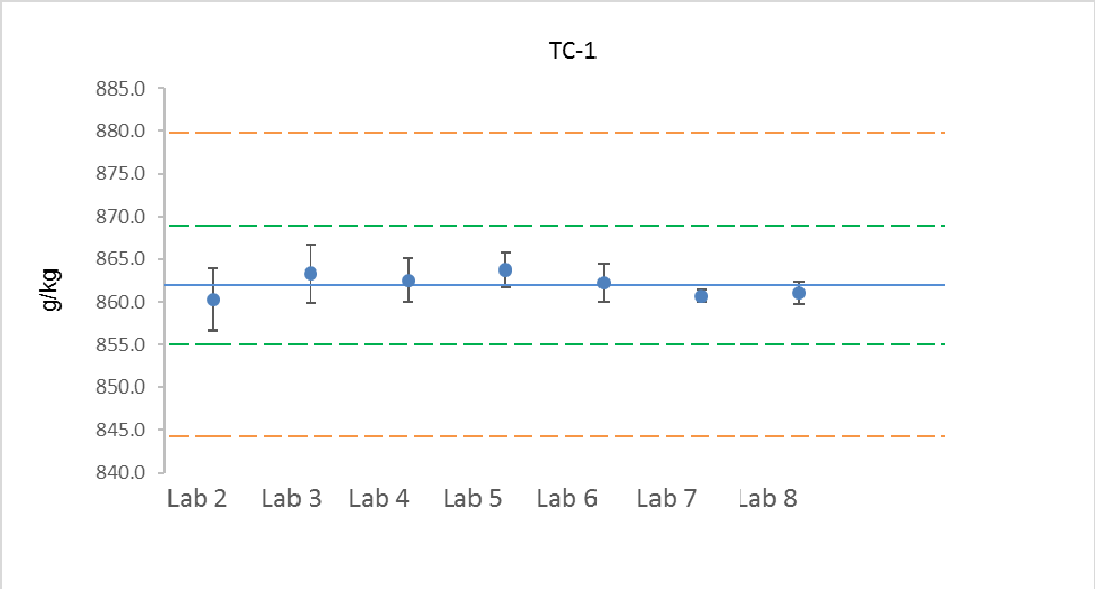


Table 2: TC-2(Batch No: 201812002-2#)

Lab	Day 1		Day 2		Mean g/kg	Std. Dev.
	1	2	1	2		
2	861.6	859.0	862.0	859.0	860.4	1.62
3	854.9	861.3	867.6	860.7	861.1	5.19
4	863.9	864.4	860.8	867.4	864.1	2.70
5	854.4	860.7	861.4	856.9	858.4	3.29
6	861.4	865.8	859.6	857.5	861.1	3.53
7	859.0	861.5	860.9	861.4	860.7	1.16

8	861.0	857.1	859.1	861.7	859.7	2.07

Fig.2: TC-2 (Batch No: 201812002-2#)

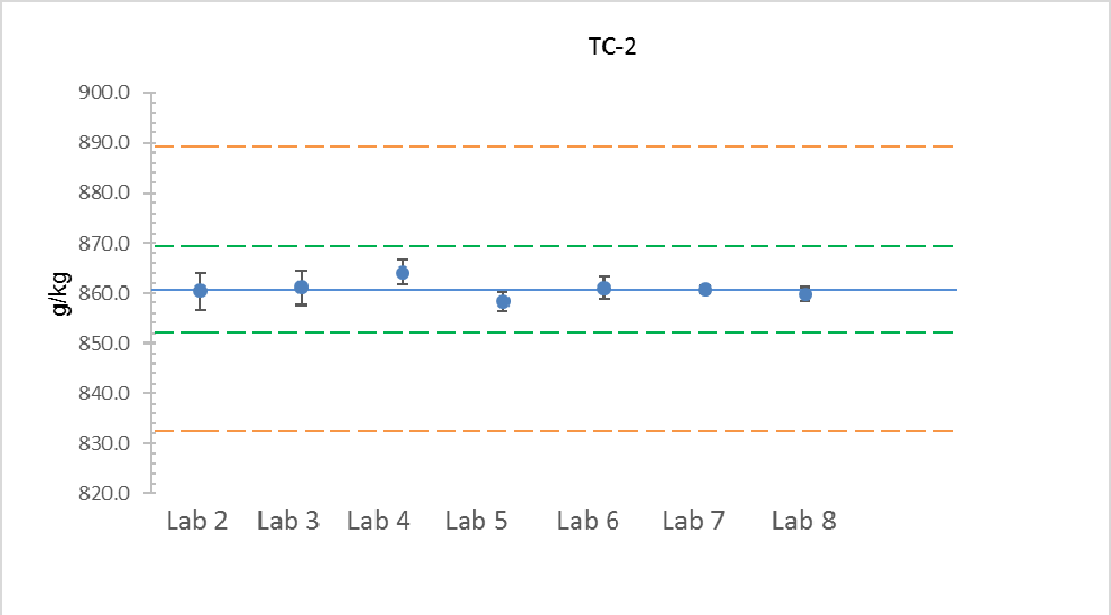


Table 3: WP-1 (Batch No: 2018012003-3#)

Lab	Day 1		Day 2		Mean g/kg	Std. Dev.
	1	2	1	2		
2	828.7	823.5	826.2	819.2	824.4	4.07
3	821.7	823.3	821.9	819.8	821.7	1.44
4	817.6	816.5	814.5	816.0	816.2	1.29
5	824.5	823.9	822.4	816.2	821.8	3.80
6	826.6	814.8	824.8	819.8	821.5	5.31
7	820.3	824.1	819.1	821.4	821.2	2.13
8	817.5	819.6	818.8	820.5	819.1	1.27

Fig.3: WP-1 (Batch No: 2018012003-3#)

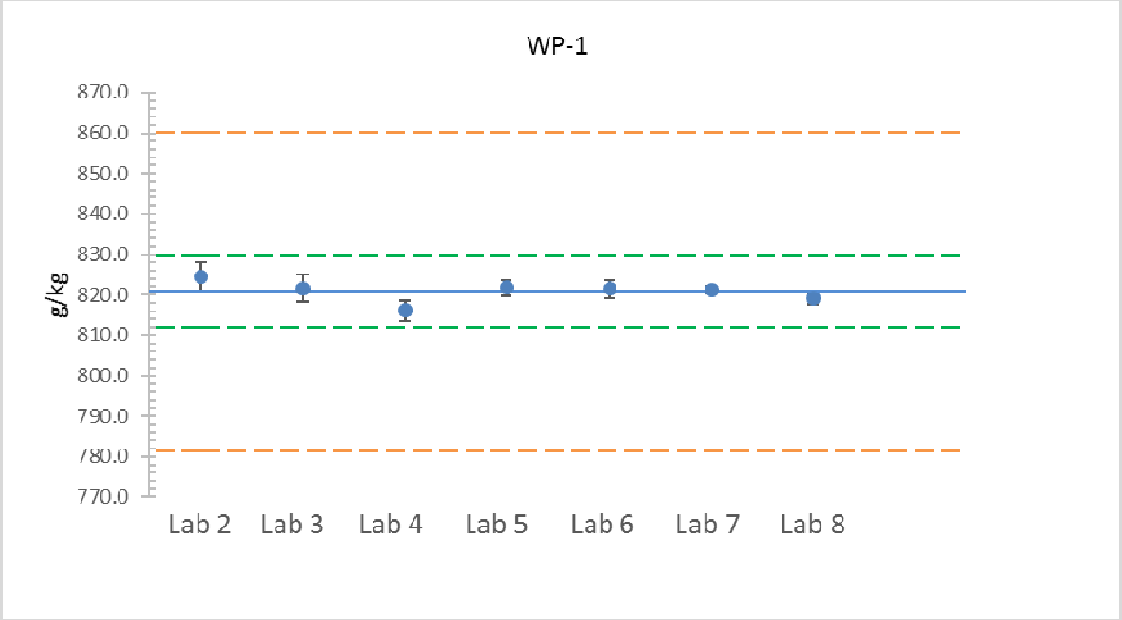


Table 4: WP-2 (Batch No: 2018012004-4#)

Lab	Day 1		Day 2		Mean g/kg	Std. Dev.
	1	2	1	2		
2	832.1	819.2	827.1	820.3	824.7	6.06
3	820.7	819.2	821.5	819.5	820.2	1.07
4	818.1	816.0	818.4	813.3	816.5	2.36
5	820.1	824.6	825.0	824.8	823.6	2.36
6	821.1	823.9	814.7	818.4	819.5	3.92
7	823.0	820.3	819.5	820.6	820.9	1.51
8	821.4	819.1	820.5	820.2	820.3	0.95

Fig.4: WP-2 (Batch No: 2018012004-4#)

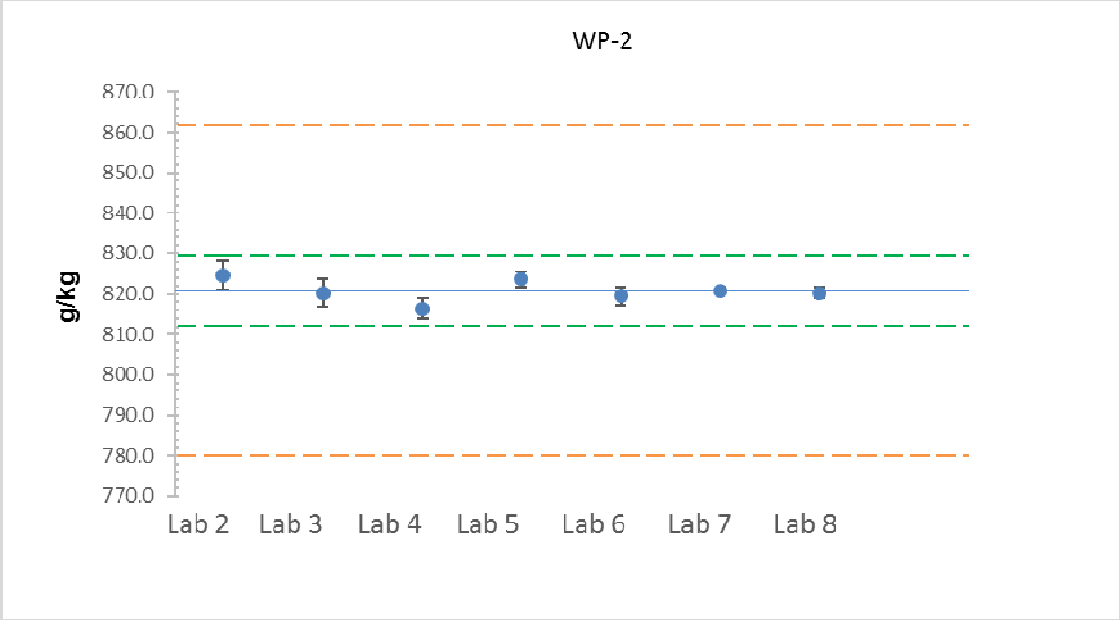


Table 5: WP-3 (Batch No: 201812005-5#)

Lab	Day 1		Day 2		Mean g/kg	Std. Dev.
	1	2	1	2		
2	817.4	817.9	826.5	819.2	820.3	4.24
3	824.1	817.7	820.5	823.6	821.5	2.98
4	816.1	820.3	810.6	814.6	815.4	4.01
5	827.9	824.9	823.9	817.6	823.6	4.33
6	813.7	826.3	811.7	818.3	817.5	6.48
7	817.2	817.7	817.8	818.5	817.8	0.54
8	815.3	815.5	815.7	821.0	816.9	2.75

Fig.5: WP-3 (Batch No: 201812005-5#)

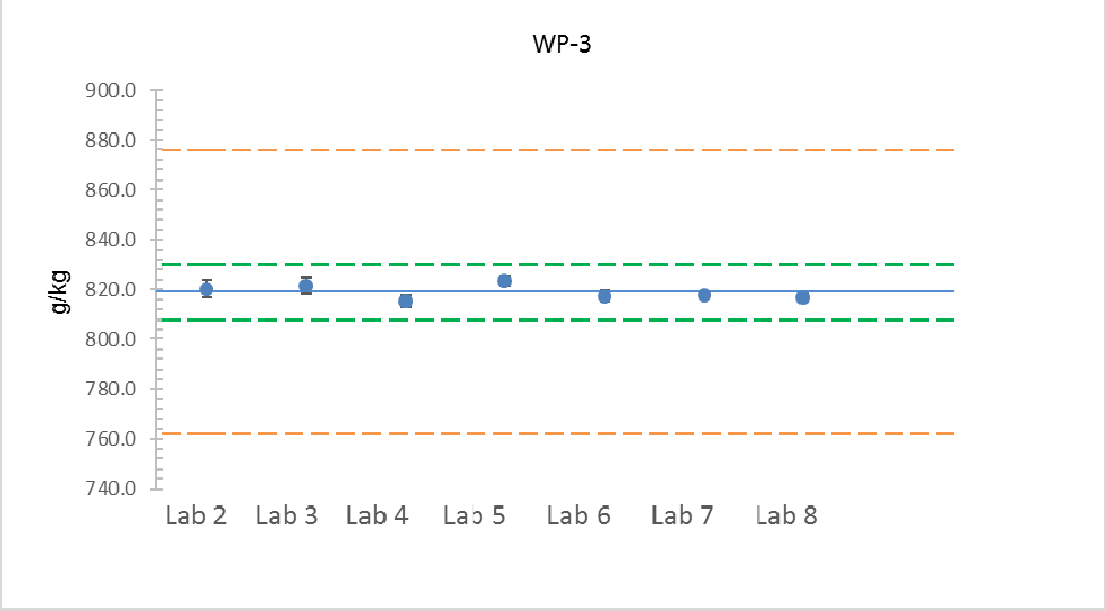


Table 6 Summary of the results of all laboratories

	TC-1	TC-2	WP-1	WP-2	WP-3
Xm	862.0	860.8	820.8	820.8	819.0
L	7	7	7	7	7
S_r	2.47	3.07	3.14	3.11	3.99
S_R	2.52	3.19	3.75	3.82	4.50
r	6.90	8.59	8.80	8.70	11.18
R	17.80	28.41	39.31	40.85	56.70
RSDr	0.29	0.36	0.38	0.38	0.49
RSDR	0.29	0.37	0.46	0.47	0.55
RSDR (Hor)	2.05	2.05	2.06	2.06	2.06

Where:

Xm = average, in unit of g/kg
L = number of laboratories
Sr = repeatability standard deviation
SR = reproducibility standard deviation
r = repeatability (Sr·2.8)
R = reproducibility (SR·2.8)
RSDr = repeatability relative standard deviation (100·Sr/Xm)
RSD_R = reproducibility relative standard deviation (100·SR/Xm)
RSD_R(Hor) = Horwitz value calculated from: 2(1-0.5log c)
where c = the concentration of the analyte as a decimal fraction

7.2 Statistical formulas

Yi = mean of the various laboratories

Si = standard deviation

P = number of laboratories

n = number of measurements (here n=4)

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

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

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

Repeatability and reproducibility were calculated as follows:

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

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

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

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

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

8. Conclusions

For all the samples, the values of RSD_R were smaller than those calculated by Horwitz's equation. The updated method is considered appropriate for the determination of mancozeb in TC and WP.

Limin Chemical Co. Ltd. considers this method to be suitable for the intended purpose, and recommend accepting it as a provisional CIPAC method for the determination of mancozeb in TC and WP.

9. Acknowledgement

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