BASF SE

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Boscalid

CIPAC Collaborative Trial

CIPAC 4612/R, full scale study

COLLABORATIVE STUDY OF A HPLC ANALYSIS OF BOSCALID TECHNICAL MATERIAL AND FORMULATED PRODUCTS

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1 List of participants

17 Laboratories took part at the collaborative study (in randomized order)

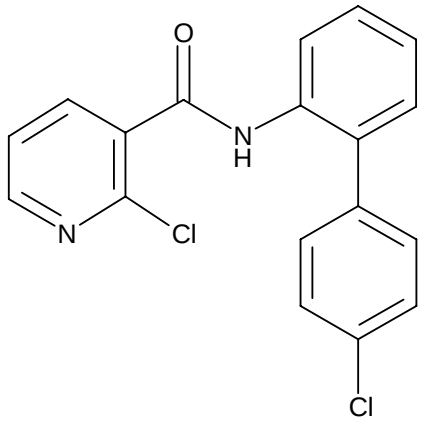
ORGANIZATION	NAME	COUNTRY
Federal Laboratory for Food Safety	Alain Dubois	Belgium
Agricultural Institute of Slovenia	Ana Gregorcic	Slovenia
Food Science Group, Central Science Laboratory, Cork UK	Andrew Plumb	United Kingdom
Istituto Superiore di Sanità (National Institute of Health)	Angela Santilio	Italy
FAW, Wädenswil	Bruno Patrian	Switzerland
Federal Office of Consumer Protection and Food Safety, Braunschweig	Claudia Vinke	Germany
BASF SE, Limburgerhof	Jürgen Fries	Germany
Bayer Crop Science Aktiengesellschaft, Monheim	Gerhard Thielking	Germany
Pesticide Control Laboratory	Jim Garvey	Ireland
BASF Corporation, RTP Raleigh	Tacheng Hsieh	USA
Institute of Industrial Organic Chemistry	Hanna Nowacka-Krukowska	Poland
Pesticide Chemistry Division	Hei-tung Feng	Taiwan
BASF Agro Research, Tahara	Kaori Ohba	Japan
Ministerio de Agricultura. Pesca Alimentación	Luis Manso	Spain
Finnish Food Safety Authority Evira Chemistry and Toxicology Unit	Ritva Mutanen	Finland
Central Laboratory for Phytosanitary Quarantine, Romania	Teodora Iurascu	Romania
Walloon Agricultural Research Centre (CRA-W)	Vanessa Lecocq	Belgium

Additional remarks

- 19 Laboratories offered to participate at the collaborative trial
- Samples had been sent to 18 laboratories
- Sample dispatch to one participant failed
- 17 Laboratories reported results

2 General information on Boscalid

Structucture



ISO common name	Boscalid
Chemical name	2-Chlor-N-(4'-chlor-biphenyl-2-yl)nicotinamid (IUPAC)
CAS.No.	188425-85-6
Empirical formula	$C_{18}H_{12}Cl_2N_2O$
RMM	343.2
m.p.	143 to 145 °C
Solubility	In water: 4.6 mg/l at 20 °C
Description	white odourless powder
Formulations	Water Dispersible Granules Suspension Concentrates Suspo-Emulsions
Indication	Fungicide

3 Distribution of samples

The following samples were provided to the participants:

Boscalid, reference substance	about 1.0 g	991 g/kg
Boscalid, technical material TC1	about 1.5 g	approx. 987 g/kg
Boscalid, technical material TC2	about 1.5 g	approx. 975 g/kg
Boscalid, dispersible granule WG	about 40 g	approx. 500 g/kg
Boscalid, suspension concentrate SC	about 50 g	approx. 500 g/kg
Boscalid, suspo-emulsion SE	about 40 g	approx. 200 g/kg

Together with the samples of the collaborative trial study procedure instructions were forwarded to the participants.

4 Study procedure instructions

1. TC1, TC2, WG, SC and SE can be stored at ambient temperature (typically +25°C) or cooler.

2. The study design is based on two series of determinations performed at two different days. Please prepare two calibration solutions for each day of determination. Each sample is weighed once and analyzed once (twofold injection), and the procedure is repeated at a later date using calibration solutions C1 and C2 freshly prepared. Bracketing calibration is suggested.

3. Once the performance of the HPLC-system has been checked by five consecutive injections of e.g. calibration solution C1 the sequence of injections to be followed is given as: C1, TC1, TC1, C2, TC2, TC2, C1, WG, WG and so on. Please refer to the result tables 1 and 2 attached for details about the complete injection sequence.
   Typical examples of chromatograms are given in the method provided.

4. Tabulate all your results, (please consider the purity of the analytical standard) deviations of the method and any comments you may have on the result sheets provided, including details of your analytical equipment used and your chromatographic parameters.

5 Method

5.1 The aim of the collaborative study was to test a new reversed phase HPLC method which allows determining the content of Boscalid in technical material, WG, SC and SE formulations.

5.2 Principle
The sample is dissolved in a mixture of acetonitrile/water and the content of the active ingredient is determined by HPLC on a J´sphere ODS column (suggested column type). The mobile phase is composed of an acetonitrile/water/ammonium acetete solution. Quantitation is achieved by UV detection with external calibration using Boscalid as reference substance.

6 Deviations from the analytical method reported by the participants

Laboratory 1: No deviations reported

Laboratory 2: Column: Inertsil ODS 2, 5µm, 250 x 4.6 mm,
total run time prolonged to 30 min.

Laboratory 3: Column: Nucleodur Sphinx RP, 5µm, 250 x 4 mm

Laboratory 4: No deviations reported

Laboratory 5: Column: Phenomenex Luna C18, 100A, 5µm, 250 x 4.6 mm,
different gradient to shorten the run time

Laboratory 6: Column: J´sphere ODS-H80, 4µm, 250 x 3.0 mm,
so needed 0.425 ml/min instead of 1 ml/min and
0.850 ml/min instead of 2 ml/min

Laboratory 7: No deviations reported

Laboratory 8: Column: ALLTIMA HP C18 HL, 5µm, 250 x 4.6 mm,
10µl instead of 5µl loop

Laboratory 9: No deviations reported

Laboratory 10: Column: LiChrospher 100 RP-18, 5µm, 250 x 4.0 mm

Laboratory 11: Column: Allure C18, 5µm, 250 x 4.6 mm

Laboratory 12: No deviations reported

Laboratory 13: No deviations reported

Laboratory 14: Column: J´sphere ODS-M80, 4µm, 250 x 4.6 mm,
5 ml water was added to WG, SC and SE before adding acetonitrile

Laboratory 15: Column: Inertsil ODS 2, 5µm, 250 x 4.6 mm

Laboratory 16: No deviations reported

Laboratory 17: Column: Phenomenex ODS3 100A, 5µm, 250 x 4.6 mm

7 Remarks reported by the participants

Laboratory 1: Mean retention time of the three successive injections is 7.067 min. Range is +/- 0.035 min. The three successive injections are within the range.
Day 1 and Day 2 were carried out on different instruments. Day 1 just before maintenance and Day 2 just after. This might account for the differences observed. Some retention time drift was observed on Day 2.

Laboratory 2: We expanded the run time to 30 min, because we observed a “ghost peak” from the eluent/flow change after 26.5 min.
We encountered a problem with the SE formulation as follows: After shaking, the sample became thick like whipped cream or honey. Therefore it was difficult to weigh in the sample and the homogeneity of the sample is questionable. The results of this sample possibly need to be eliminated in the statistical evaluation.

Laboratory 3: The SE-sample was viscous; therefore a representative weigh in was not possible.

Laboratory 4: It is difficult to weigh in the SE-formulation because the product is very viscous.

Laboratory 5: Concerning the mobile phase, a different gradient was used to make the run time shorter.

Laboratory 6: The column used had a smaller i.d. than the one specified in the method (i.e. 3.0 mm instead of 4.6 mm). The flow rate was adjusted accordingly.

Laboratory 7: No comments

Laboratory 8: No comments

Laboratory 9: No comments

Laboratory 10: No comments

Laboratory 11: No comments

Laboratory 12: In the dilution step for both the calibration and sample solutions, the flasks needed to equilibrate to ambient temperature before making the volume with acetonitrile. This should perhaps be added to the final method.

Laboratory 13: TC2, Day2, second injection: Peak area is relatively low, compared to first injection and Day1 injections. But the difference is only about 2 percent, so we think it was close enough and there was no reason to repeat Day2 analyses.

Laboratory 14: No comments

Laboratory 15: No comments

Laboratory 16: No comments

Laboratory 17: No comments

8 Statistical evaluation

The statistical evaluation is based on that outlined in DIN ISO 5725. The formulas used for calibration of reproducibility and repeatability are listed in section 12 of the present report.

9 Results

Table 1: Boscalid technical material TC1

Laboratory	Day 1 [g/kg]	Day 2 [g/kg]	Mean Value y_i [g/kg]	Spread w_i [g/kg]
1	1020.6	981.0	1000.8	39.6
2	986.6	982.9	984.7	3.7
3	983.6	984.7	984.1	1.1
4	984.1	979.2	981.6	4.9
5	987.4	990.0	988.7	2.6
6	976.7	984.1	980.4	7.4
7	975.5	981.8	978.7	6.3
8	976.9	979.8	978.4	3.0
9	980.7	982.4	981.5	1.7
10	982.4	983.4	982.9	1.0
11	989.1	967.0	978.1	22.1*
12	977.2	969.6	973.4	7.6
13	983.2	984.4	983.8	1.2
14	984.9	990.5	987.7	5.5
15	968.3	974.8	971.5	6.5
16	989.2	975.0	982.1	14.2
17	975.5	975.9	975.7	0.4

Lab 1: Straggler according to Dixon-Test and outlier according to Cochran-Test.

Table 1: Results Boscalid TC 1

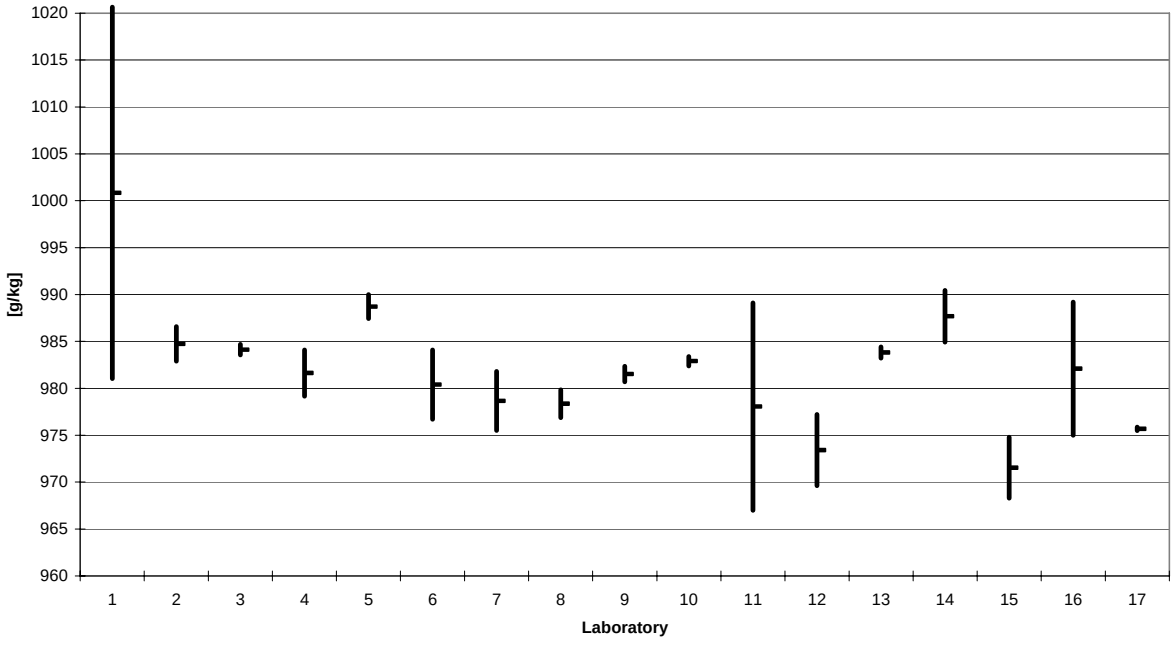


Table 2: Boscalid technical material TC2

Laboratory	Day 1 [g/kg]	Day 2 [g/kg]	Mean Value y_i [g/kg]	Spread w_i [g/kg]
1	995.8	969.3	982.5	26.5
2	997.1	986.6	991.9	10.5
3	985.3	987.2	986.3	1.9
4	980.1	981.5	980.8	1.4
5	989.7	988.5	989.1	1.1
6	981.1	984.9	983.0	3.8
7	976.4	976.5	976.4	0.0
8	985.2	984.5	984.8	0.7
9	969.4	974.2	971.8	4.8
10	967.6	984.8	976.2	17.2
11	981.0	969.1	975.0	12.0
12	977	971.8	974.4	5.1
13	981.4	969.2	975.3	12.2
14	990.6	986.9	988.7	3.7
15	983.8	975.1	979.4	8.7
16	990.9	977.4	984.2	13.5
17	974.5	974.3	974.4	0.1

Table 2: Results Boscalid TC 2

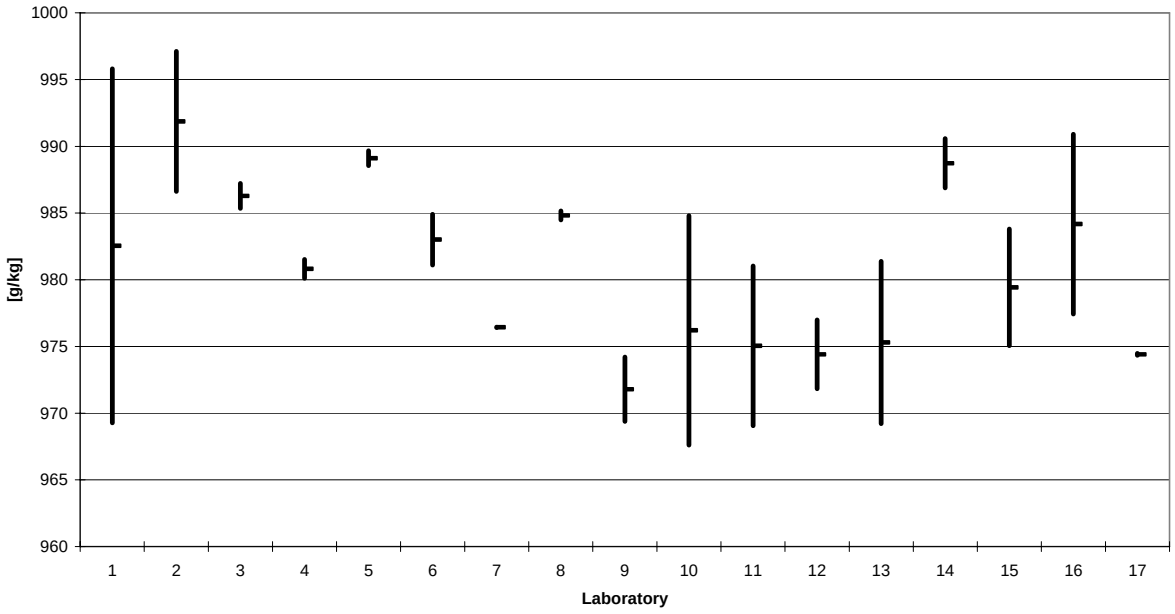


Table 3: Boscalid water dispersible granule WG

Laboratory	Day 1 [g/kg]	Day 2 [g/kg]	Mean Value y_i [g/kg]	Spread w_i [g/kg]
1	490.2	511.5	500.8	21.3
2	534.3	522.9	528.6	11.4
3	519.3	517.1	518.2	2.2
4	516.3	517.9	517.1	1.5
5	517.7	517.4	517.6	0.3
6	516.3	518.9	517.6	2.6
7	519.7	514.0	516.9	5.8
8	518.9	519.5	519.2	0.5
9	504.8	501.9	503.3	3.0
10	520.6	524.0	522.3	3.4
11	519.2	522.9	521.0	3.6
12	516.1	518.0	517.0	2.0
13	517.8	504.6	511.2	13.1
14	518.9	516.4	517.6	2.4
15	517.0	520.2	518.6	3.2
16	501.3	510.9	506.1	9.7
17	516.1	511.6	513.9	4.5

Lab 1: Straggler according to Cochran-Test.

Table 3: Results Boscalid WG

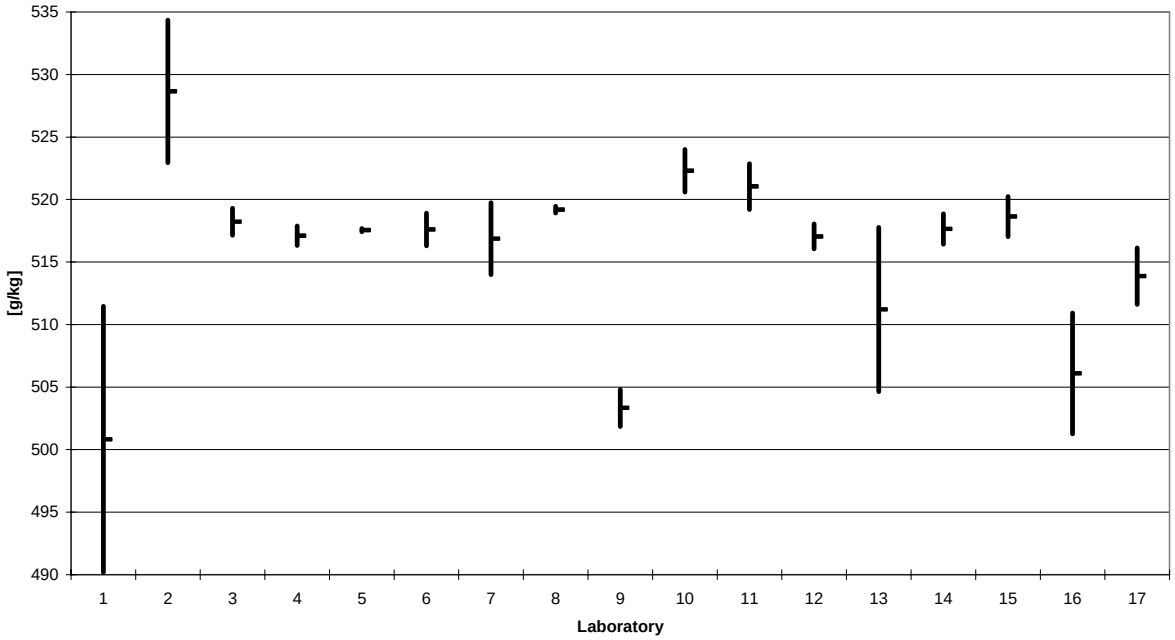


Table 4: Boscalid suspension concentrate SC

Laboratory	Day 1 [g/kg]	Day 2 [g/kg]	Mean Value y_i [g/kg]	Spread w_i [g/kg]
1	445.0	432.4	438.7	12.5
2	447.4	442.0	444.7	5.3
3	440.7	438.4	439.5	2.2
4	436.8	437.1	437.0	0.3
5	440.8	438.7	439.7	2.1
6	438.2	439.3	438.8	1.1
7	436.2	435.2	435.7	1.0
8	442.6	437.9	440.3	4.7
9	431.0	435.1	433.0	4.1
10	437.7	437.1	437.4	0.6
11	438.0	442.8	440.4	4.8
12	435.7	436.5	436.1	0.8
13	432.3	437.0	434.6	4.7
14	441.4	440.2	440.8	1.2
15	439.5	438.6	439.0	0.9
16	434.3	428.7	431.5	5.5
17	436.0	430.9	433.4	5.1

Lab 1: Straggler according to Cochran-Test.

Table 4: Results Boscalid SC

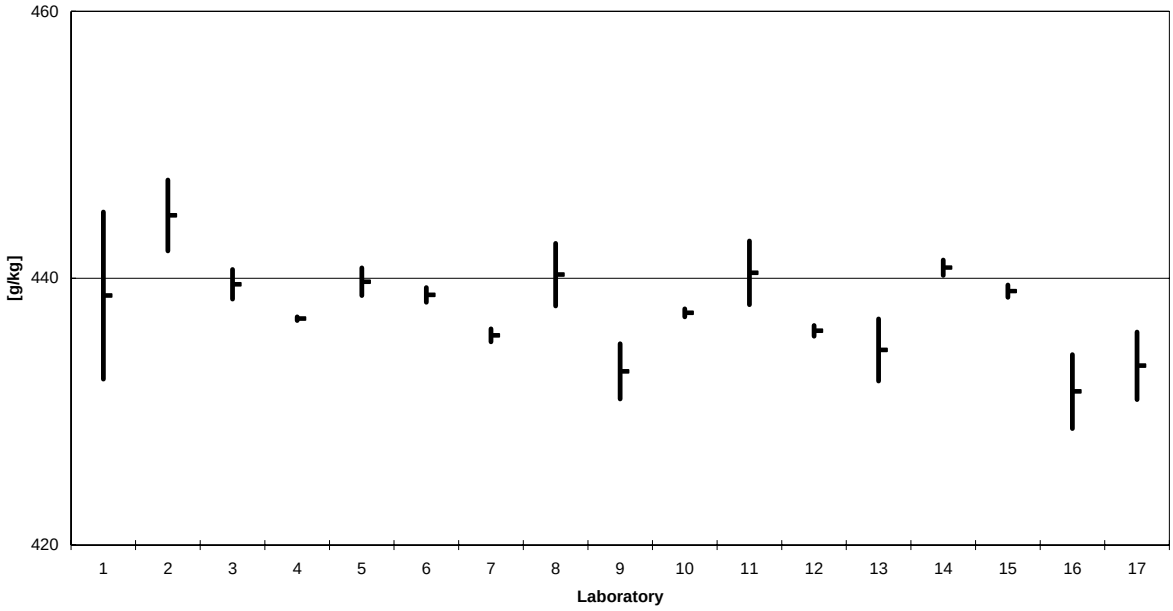
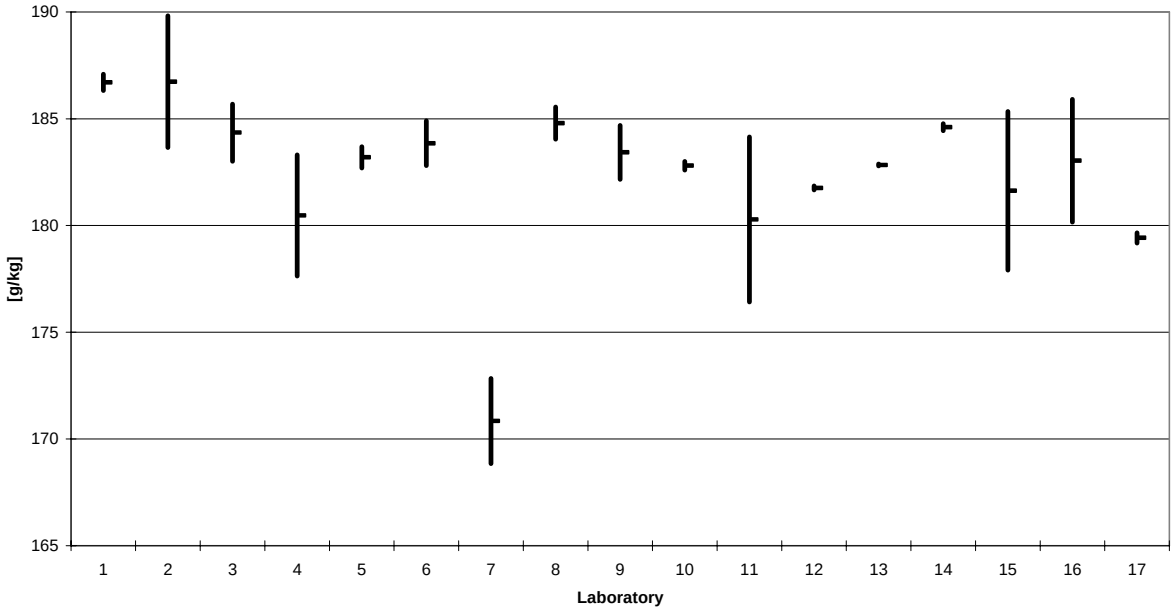


Table 5: Boscalid suspo-emulsion SE

Laboratory	Day 1 [g/kg]	Day 2 [g/kg]	Mean Value y_i [g/kg]	Spread w_i [g/kg]
1	187.1	186.3	186.7	0.8
2	189.8	183.7	186.7	6.2
3	183.0	185.7	184.4	2.7
4	183.3	177.6	180.5	5.7
5	182.7	183.7	183.2	1.0
6	182.8	184.9	183.9	2.1
7	168.9	172.8	170.8	4.0
8	185.6	184.0	184.8	1.5
9	184.7	182.2	183.4	2.5
10	182.6	183.0	182.8	0.4
11	184.2	176.4	180.3	7.7
12	181.9	181.7	181.8	0.2
13	182.8	182.9	182.8	0.1
14	184.4	184.8	184.6	0.3
15	185.3	177.9	181.6	7.4
16	185.9	180.2	183.0	5.8
17	179.7	179.2	179.4	0.5

Lab 7: Outlier according to Dixon-Test.

Table 5: Results Boscalid SE



10 Summary of the results

Table 6: Summary of the results of all laboratories

	TC1	TC2	WG	SC	SE
x	982.0	980.8	515.7	437.7	182.4
L	17	17	17	17	17
S_r	8.65	7.16	5.34	3.16	2.75
S_R	9.06	7.90	7.91	4.02	4.10
RSD_r	0.88	0.73	1.04	0.72	1.51
RSD_R	0.92	0.81	1.53	0.92	2.25
r	24.21	20.06	14.96	8.85	7.69
R	25.36	22.12	22.14	11.26	11.47
$RSD_{R(Hor)}$	2.01	2.01	2.21	2.26	2.58

Table 7: Summary of the results excluding outliers

	TC1	TC2	WG	SC	SE
x	980.8	980.8	515.7	437.7	183.1
L	16	17	17	17	16
S_r	5.52	7.16	5.34	3.16	2.74
S_R	6.14	7.90	7.91	4.02	2.86
RSD_r	0.56	0.73	1.04	0.72	1.50
RSD_R	0.63	0.81	1.53	0.92	1.56
r	15.45	20.06	14.96	8.85	7.68
R	17.21	22.12	22.14	11.26	8.00
$RSD_{R(Hor)}$	2.01	2.01	2.21	2.26	2.58

Table 8: Summary of the results excluding outliers and stragglers

	TC1	TC2	WG	SC	SE
x	980.8	980.8	516.6	437.6	183.1
L	16	17	16	16	16
S_r	5.52	7.16	4.02	2.39	2.74
S_R	6.14	7.90	6.62	3.84	2.86
RSD_r	0.56	0.73	0.78	0.55	1.50
RSD_R	0.63	0.81	1.28	0.88	1.56
r	15.45	20.06	11.26	6.70	7.68
R	17.21	22.12	18.55	10.74	8.00
$RSD_{R(Hor)}$	2.01	2.01	2.21	2.26	2.58

Where:

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

11 Discussion

The results of all 17 laboratories participated in the trial have been taken into account for the statistical evaluation (table 6, page 14), i.e. all stragglers and outliers according to Dixon-Test and Cochran-Test were left in the evaluation. No data were rejected.
The Horwitz-criterion is fulfilled.

The results after exclusion of outliers as well as of outliers and stragglers are reported in tables 7 and 8 (pages 14 and 15). The Horwitz-criterion is fulfilled.

For the Boscalid, technical material TC1, ‘Laboratory 1’ is indicated as a significant straggler by Dixon-Test and as a significant outlier by Cochran-Test.

For the Boscalid, suspo-emulsion SE, ‘Laboratory 7’ is indicated as an outlier by Dixon-Test.

For the Boscalid WG- and SC-formulations, ‘Laboratory 1’ is indicated as a straggler by Cochan-Test.

We would like to propose the analytical method for Boscalid to become provisional

12 Statistical formulae

Y_i = mean of the various laboratories
W_i = spread of the individual values
L = number of laboratories

$$T_1 = \sum_{i=1}^{L} Y_i$$

$$T_2 = \sum_{i=1}^{L} Y_i^2$$

$$T_3 = \sum_{i=1}^{L} W_i^2$$

Repeatability and reproducibility were calculated as follows:

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

$$S_L^2 = \frac{LT_2 - T_1^2}{L(L-1)} - \frac{S_r^2}{2}$$

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