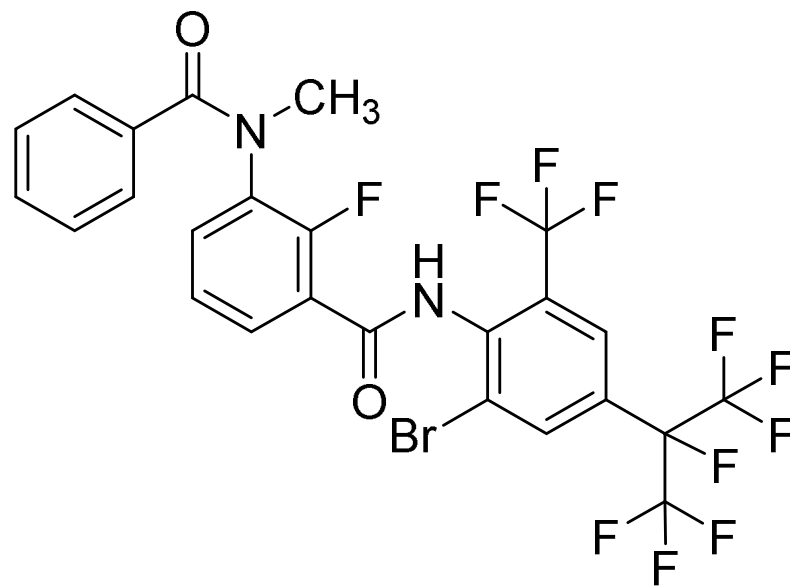


Broflanilide (No.994)

Full Scale Collaborative Trial



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Mitsui Chemicals Agro, Inc.

Content

1. Introduction of Full Scale Collaborative Trial
2. Analytical Method
3. Participants
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5. Results and Discussion
6. Conclusion

Introduction of Full Scale Collaborative Trial

◆ Physicochemical Properties of Broflanilide

N-[2-bromo-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-2-fluoro-3-(*N*-methylbenzamido)benzamide

Empirical formula : $C_{25}H_{14}BrF_{11}N_2O_2$ RMM : 663.29

Solubility : In water: 0.71 mg/l, *n*-heptane: 0.096 g/l, xylene: 6.0 g/l,
1,2-dichloroethane: 110 g/l, acetone, ethyl acetate and
methanol: >250 g/l, *n*-octanol: 7.4 g/l, acetonitrile: 77 g/l
; all at 20°C

◆ Samples

- 1) Broflanilide Technical Material (TC-1)
- 2) Broflanilide Technical Material (TC-2)
- 3) Broflanilide Wettable Powder (WP-1)
- 4) Broflanilide Wettable Powder (WP-2)
- 5) Broflanilide Wettable Powder (WP-3)

◆ Application Field : Vector Control

Analytical Method (1/3)

◆ Outline of Method

Broflanilide is determined by reversed phase high performance liquid chromatography using UV detection at 254 nm and external standardization.

◆ Procedure for Preparing Sample Solutions(Main Points)

- Sample solutions are prepared in duplicate
- Weigh (to the nearest 0.01 mg) enough sample to contain 50 mg of broflanilide into separate 100 ml volumetric flasks.

Technical Material (TC)

- Fill it up to the mark with dilution solution (mobile phase)
(sample solutions TC-1-A, TC-1-B, TC-2-A and TC-2-B).

Wetable Powder (WP)

- About 70 ml of dilution solution (mobile phase) is added into the flask and place the flasks in an ultrasonic bath for about 5 min.
- Fill it up to the mark with dilution solution.
- Filter the supernatant through 0.45 μ m filter.
(sample solutions WP-1-A, WP-1-B, WP-2-A, WP-2-B, WP-3-A and WP-3-B).

Analytical Method (2/3)

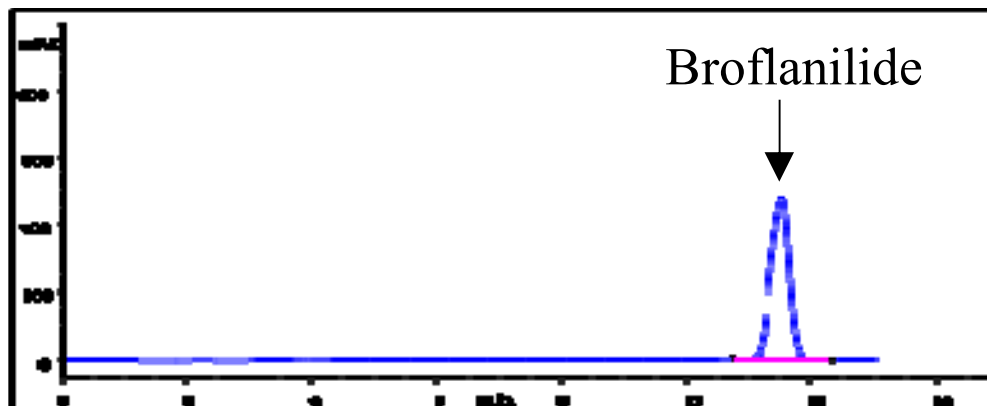
◆ HPLC Operating Conditions (Proposed Method)

Column	:	XSelect CSH C ₁₈ 250 x 4.6 mm (i.d.) 5 μm
Mobile phase	:	Acetonitrile - Water, 65 + 35 (v/v)
Flow rate	:	1.0 ml/min
Column temperature	:	40 °C
Detector wavelength	:	254 nm
Injection volume	:	20 μl
Retention time	:	Broflanilide ; approximately 11.5 min

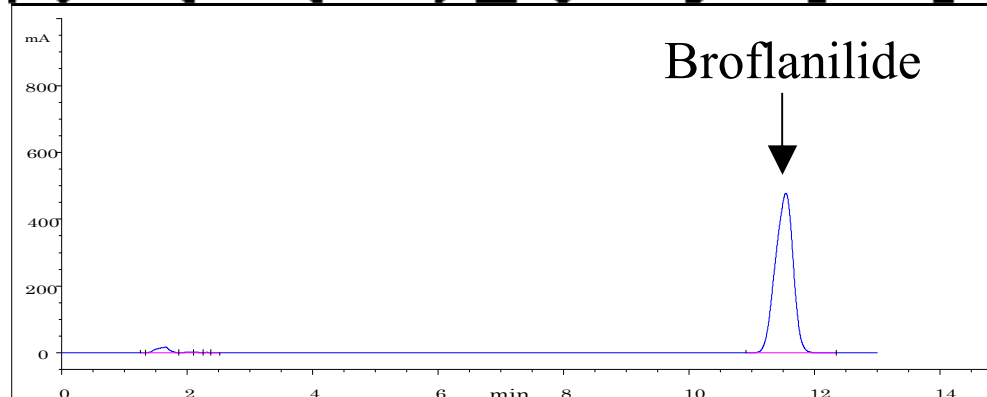
Analytical Method (3/3)

◆ Example of Chromatogram

Broflanilide TC

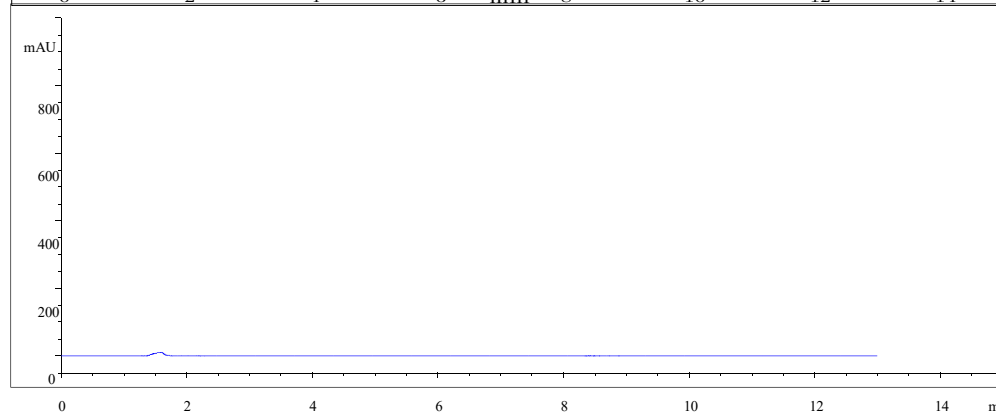


Broflanilide WP



Reference*

*Chromatogram of the WP not including broflanilide



Participants* (1/2)

In August 2018, Information Sheet No 315 was sent out by the CIPAC Secretary. The trial was limited to maximum 20 participating laboratories. However, we accepted all laboratories who applied because the maximum number of 20 laboratories applied within one working day.

We sent samples to 23 laboratories and received examination results from all the laboratories.

Isabelle Monisse	AFSCA (Belgium)
Veronika Kmecl	AGRICULTURAL INSTITUTE OF SLOVENIA (KMETIJSKI INSTITUT ŠLOVENIJE) (Slovenia)
Astrid Bächli	Agroscope (Switzerland)
Olivera Gogic	Center for Ecotoxicological Research Podgorica (Montenegro)
Frantisek Csicsay	Central Control and Testing Institute in Agriculture (Slovakia)
Kevin King	Clarke Mosquito Control (USA)
F. Güdel	CURRENTA GmbH&Co OHG, ANT-PDA3, A559 (Germany)
Ivan Orgei	FRANDESA Co. LTD (Belarus)
Ana B. Estebanez	Laboratorio Arbitral Agroalimentario (Spain)
Eva Jacobsen	Laboratory of chemistry and microbiology, Danish Technological Institute (Denmark)
Volodymyr Mykhaylov	Laboratory of pesticides analytical chemistry of L.I. Medved's Reseach Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine (Ukraine)

*Participants are listed in alphabetical sequence.

Participants* (2/2)

Juliana Pereira, Estela Bonilha	Lanagro/SP (Brazil)
Ahmad Rezvani	Maryland Department of Agriculture, State Chemist Section (USA)
Kaiwei Shi	National Center for Pesticide Quality Supervision and Inspection (Beijing), Institute for the Control of Agrochemicals, Ministry of Agriculture and Rural Affairs , P. R. China (China)
Márió Molnár	National Food Chain Safety Office, Pesticide Analytical National Reference Laboratory, Velence (Hungary)
Ileana Ionica	National Phytosanitary Laboratory (Romania)
Denis Carr	Pesticides Control Laboratory (Ireland)
Meilinda Pramleonita	PT Agricon – Indonesia (Indonesia)
Cornel Grecu	Quality Control Laboratory (Romania)
Kailas Gore, Satish Patel	RALLIS INDIA LIMITED (India)
Ovsanna Tshakatyan	Republican Veterinary-Sanitary and Phyto-Sanitary Centre of Laboratory Services SNCO (Armenia)
Yukiko Koma	The Institute of Environmental Toxicology (Japan)
Régis De Bruyne, Laurent Soquette	Walloon Agricultural Research Centre (CRA-W) Agriculture and Natural Environment Department (D3) Plant Protection Products and Biocides Physico-chemistry and Residues Unit (U10) (Belgium)

*Participants are listed in alphabetical sequence.

Remarks of Participants (1/6)

◆ Analytical Conditions

Lab. No.*	High Performance Liquid Chromatography Integrator	Column	Mobile phase	Column temp. (°C)	Flow rate (ml/min)
				Injection volume(μl)	Retention time(min)
Proposed Method		250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.5
1	Agilent 1260 Infinity II ChemStation	250 x 4.6 mm (i.d.) Waters XBridge C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	0.92
				20	11.6
2	Thermo Ultimate 3000 Chromeleon Version 7.2.7	250 x 4.6 mm (i.d.) Inertsil 5 ODS-2 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.2
				20	11.8
3	PerkinElmer Altus Empower 3	250 x 4.6 mm (i.d.) Phenomenex Prodigy ODS3 , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				5	14.4
4	Waters UPLC Acquity Empower	250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.6
5	Shimadzu Prominence LabSolutions	250 x 4.6 mm (i.d.) Cosmosil C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	13.0
6	Dionex Chromeleon	250 x 4.6 mm (i.d.) Zorbax SB C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.2
				20	12.0

*Laboratory numbers in the result tables were assigned in the sequence of results receipt. **MITSUI CHEMICALS AGRO, INC.**
Research & Development Division

Remarks of Participants (2/6)

◆ Analytical Conditions

Lab. No.*	High Performance Liquid Chromatography Integrator	Column	Mobile phase	Column temp. (°C)	Flow rate (ml/min)
				Injection volume(μl)	Retention time(min)
Proposed Method		250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.5
7	Agilent 1100 Series Agilent ChemStation for LC 3D systems	250 x 4.6 mm (i.d.) Zorbax SB C18, 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	13.0
8	Shimadzu Nexera XR with SPD-20A Shimadzu LC Solution	250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.2
9	Agilent 1260 Infinity series Chemstation	250 x 4.6 mm (i.d.) Inertsil ODS-3V 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	16.6
10	Shimadzu LC-20AD Prominence Shimadzu LabSolutions	250 x 4.6 mm (i.d.) Zorbax Eclipse XDB-C18 , 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	unknown
11	Agilent 1260 Infinity II Chromeleon 6.80	250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.7
12	Agilent Infinity 1200 Open Lab CDS ChemStation	250 x 4.6 mm (i.d.) Zorbax Eclipse Plus C18 , 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.1
				20	11.8

*Laboratory numbers in the result tables were assigned in the sequence of results receipt. **MITSUI CHEMICALS AGRO, INC.**
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Remarks of Participants (3/6)

◆ Analytical Conditions

Lab. No.*	High Performance Liquid Chromatography Integrator	Column	Mobile phase	Column temp. (°C)	Flow rate (ml/min)
				Injection volume(μl)	Retention time(min)
Proposed Method		250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.5
13	Dionex UltiMate 3000 Chromeleon 6.88	250 x 4.6 mm (i.d.) Zorbax Eclipse XDB-C18 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	13.1
14	Agilent 1260 Infinity Chemstation	250 x 4.6 mm (i.d.) Luna C18(2) 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	15.3
15	Agilent 1200 Series Agilent, Chemstation	250 x 4.6 mm (i.d.) Phenomenex, Gemini C18, 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.7
16	Thermo UltiMate 3000 Chromeleon Version 7.2.9	250 x 4.6 mm (i.d.) Kromasil 100-5C18 , 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.4
				5	11.6
17	Agilent 1100 Series Agilent ChemStation for LC 3D	250 x 4.6 mm (i.d.) Phenomenex Luna C18 (2) , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	15.6
18	Waters Acquity UPLC H- Class Series Waters Empower 3	250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				10	11.5

*Laboratory numbers in the result tables were assigned in the sequence of results receipt. **MITSUI CHEMICALS AGRO, INC.**
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Remarks of Participants (4/6)

◆ Analytical Conditions

Lab. No.*	High Performance Liquid Chromatography Integrator	Column	Mobile phase	Column temp. (°C)	Flow rate (ml/min)
				Injection volume(μl)	Retention time(min)
Proposed Method		250 x 4.6 mm (i.d.) Waters XSelect CSH C ₁₈ , 5 μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.5
19	Shimadzu 20 AD Shimadzu LabSolutions	250 x 4.6 mm (i.d.) Hypersil ODS 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	0.9
				20	11.4
20	Thermo UltiMate 3000 Chromeleon (Cobra Wizard)	250 x 4.0 mm (i.d.) Purospher , 5um	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	11.6
21	UPLC Hclass Waters Empower 3	100 x 2.1mm (i.d) Kinetex Evo C18 2.6μm	Acetonitrile – Water, 65 + 35 (v/v)	40	0.3
				1	unknown
22	Agilent 1100 Series Chemstation	250 x 4.6 mm (i.d.) Zorbax Eclipse XDB-C18 , 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	13.2
23	Agilent 1260 Infinity ChemStation for LC 3D Systems	250 x 4.6 mm (i.d.) Zorbax SB-C18 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	1.0
				20	12.5
19	Shimadzu 20 AD Shimadzu LabSolutions	250 x 4.6 mm (i.d.) Hypersil ODS 5μm	Acetonitrile – Water, 65 + 35 (v/v)	40	0.9
				20	11.4

*Laboratory numbers in the result tables were assigned in the sequence of results receipt. [MITSUI CHEMICALS AGRO, INC.](#)
Research & Development Division

Remarks of Participants (5/6)

◆ Remarks

Laboratory 1:

- Flow rate was changed to 0.92 ml/min to adjust the retention time to 11.5 min.

Laboratory 2:

- C3: There were some particles left after shaking, therefore 15s in ultrasonic bath. Since C3 and C4 deviated finally too much, new calibrations (C5 and C6) were prepared
- We used 0.20 µm RC (regenerated cellulose) filter (instead of 0.45 µm filter).
- Flow 1.2 ml/min (instead of 1.0 ml/min), RT: 11.84 min.

Laboratory 3:

- Reduced injection volume to 5µL.

Laboratory 9:

- We have used comparable HPLC column, but retention time of Broflanilide is 16.6 min. As per test method shared with us retention time should be 11.5 min. No change in the test parameters.

Laboratory 10:

- I have placed the calibration and technical concentrate solutions in ultrasonic bath for about 1 min.

Laboratory 11:

- WP-formulations are filtrated by Chromafil Xtra 0.45 µm filters to clarify the sample solutions.

Remarks of Participants (6/6)

Laboratory 12:

- Flow rate was changed to 1.1 ml/min. In the WP sample preparation the flasks were placed in the ultrasonic bath for about 15 minutes.

Laboratory 16:

- I injected just 5 μ l because is the maximum volume that can be injected with this type of autosampler.

Laboratory 18:

- The injection volume was set at 10 μ L because the HPLC is not able to inject a greater volume.

Laboratory 19:

- Flow rate was changed to 0.90 ml/min to adjust the retention time about to 11.4 min.

Laboratory 21:

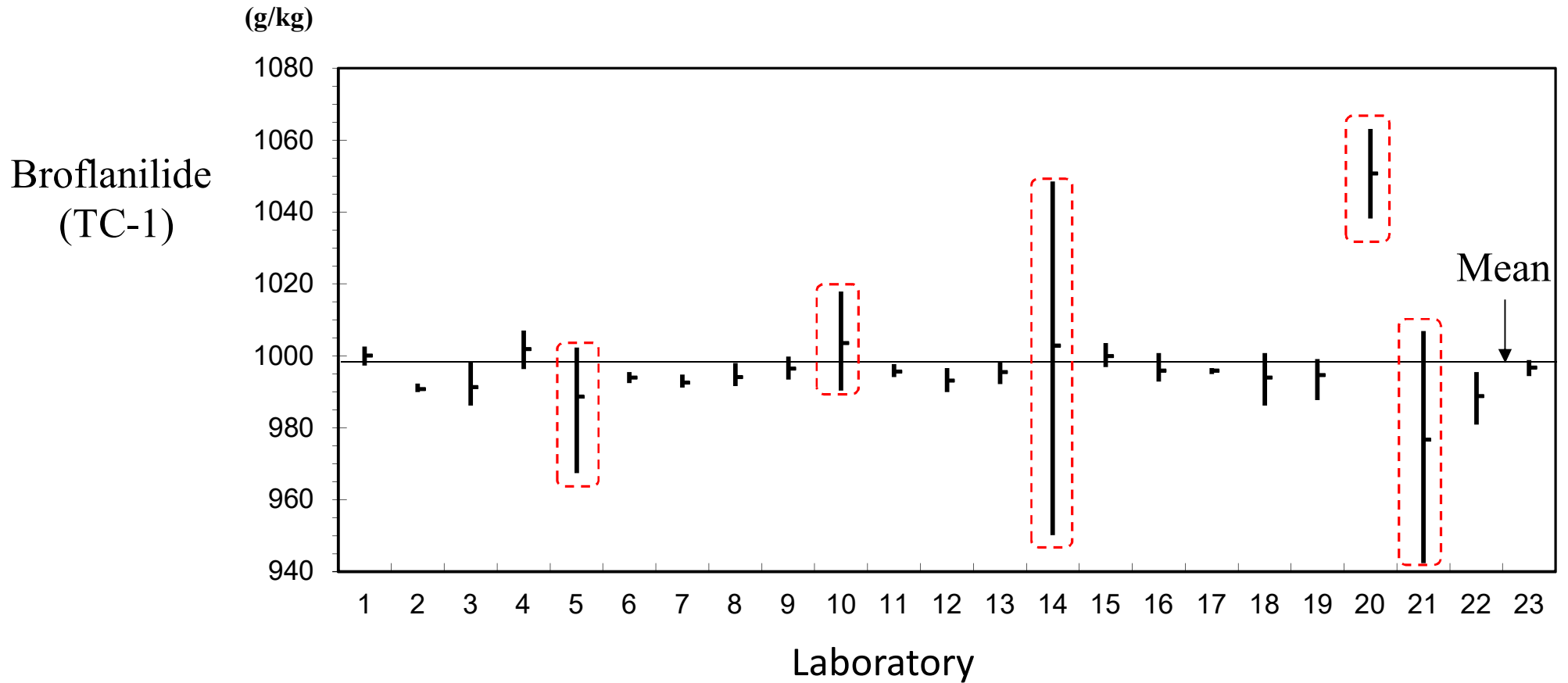
- I observe variations between 2 test samples of the same sample, despite great attention to the homogenization of the sample before sampling. The system suitability was done on the 2nd day.

Laboratory 22:

- Calibration solution and sample preparation: 25mg broflanilide was weighed into 50ml volumetric flasks.

Results and Discussion (1/7)

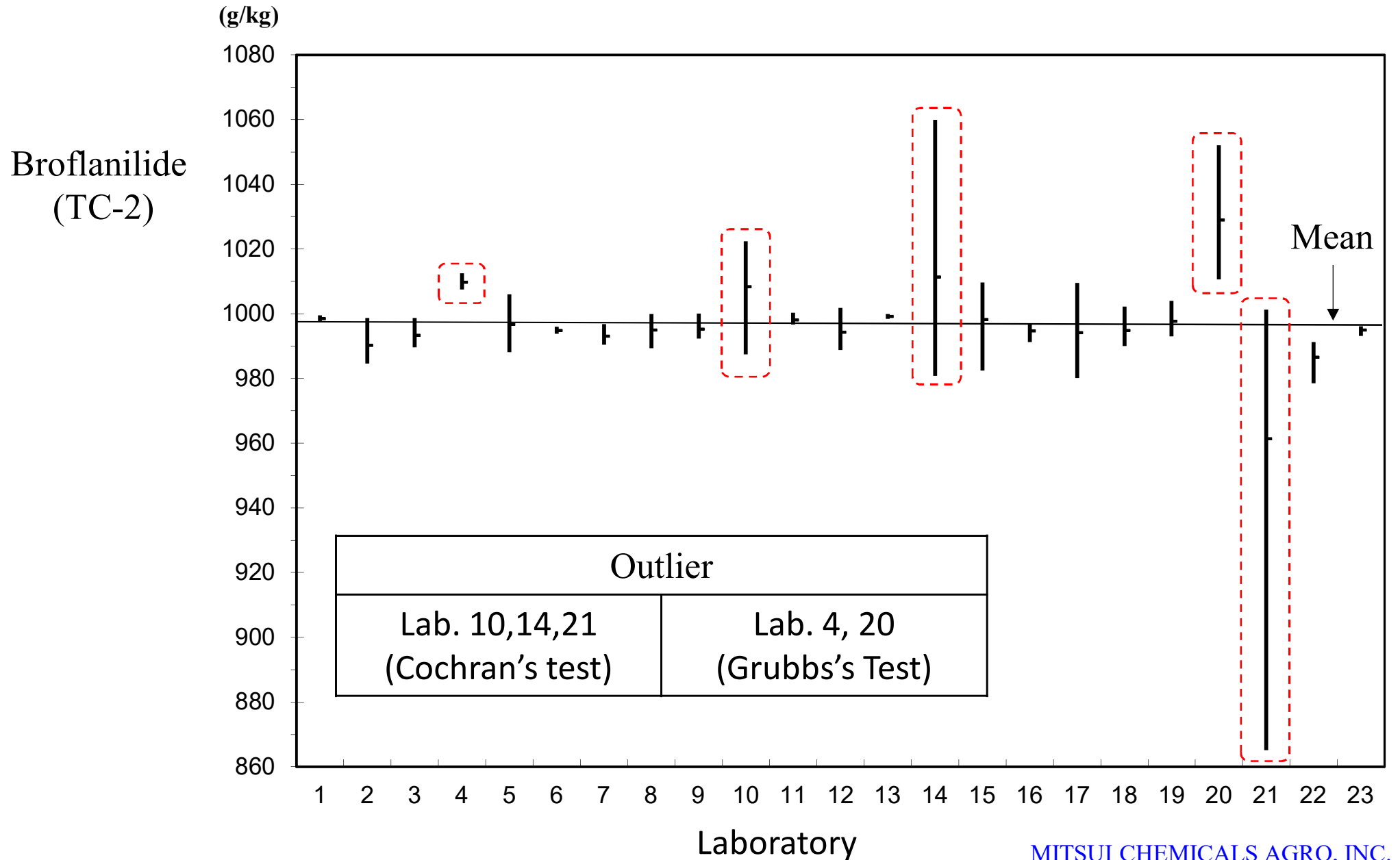
◆ Graphical Presentation of TC-1 Data



Outlier	
Lab. 5,10,14,21 (Cochran's test)	Lab.20,21 (Grubbs's Test)

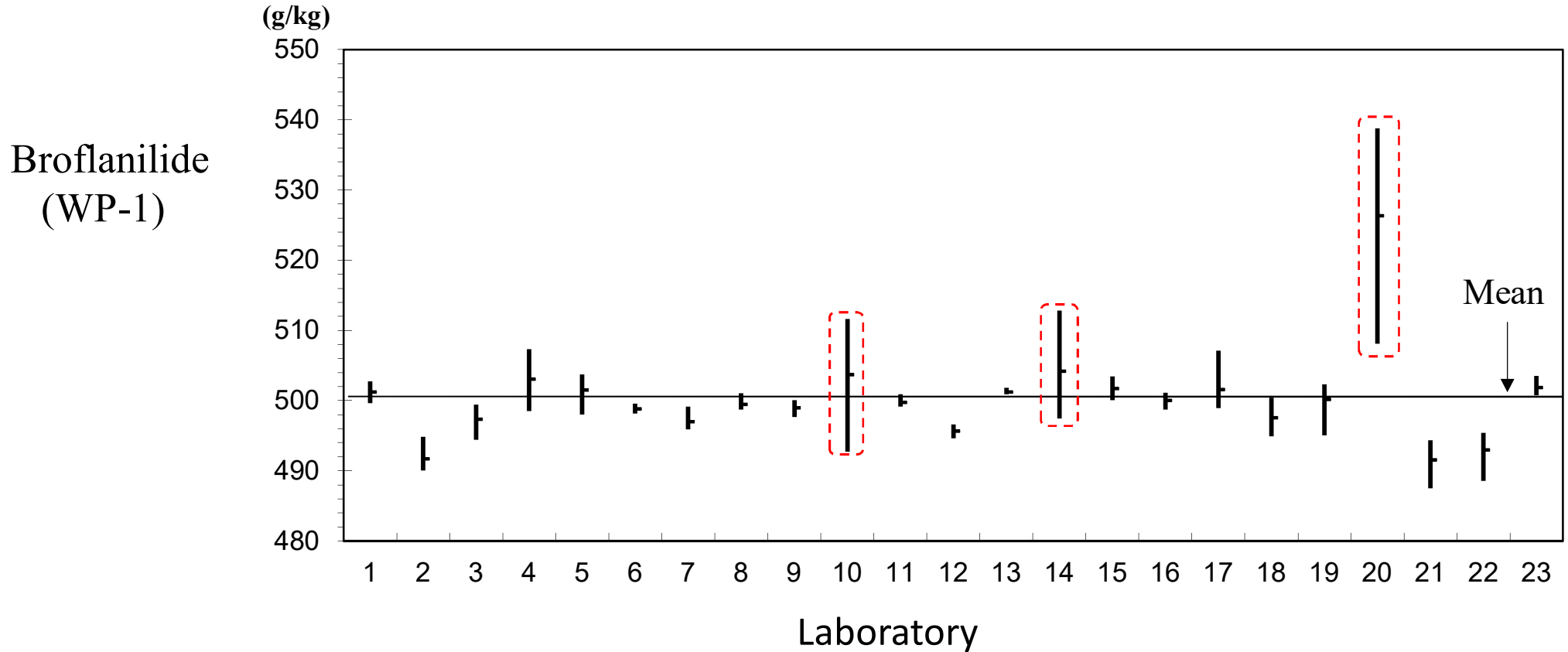
Results and Discussion (2/7)

◆ Graphical Presentation of TC-2 Data



Results and Discussion (3/7)

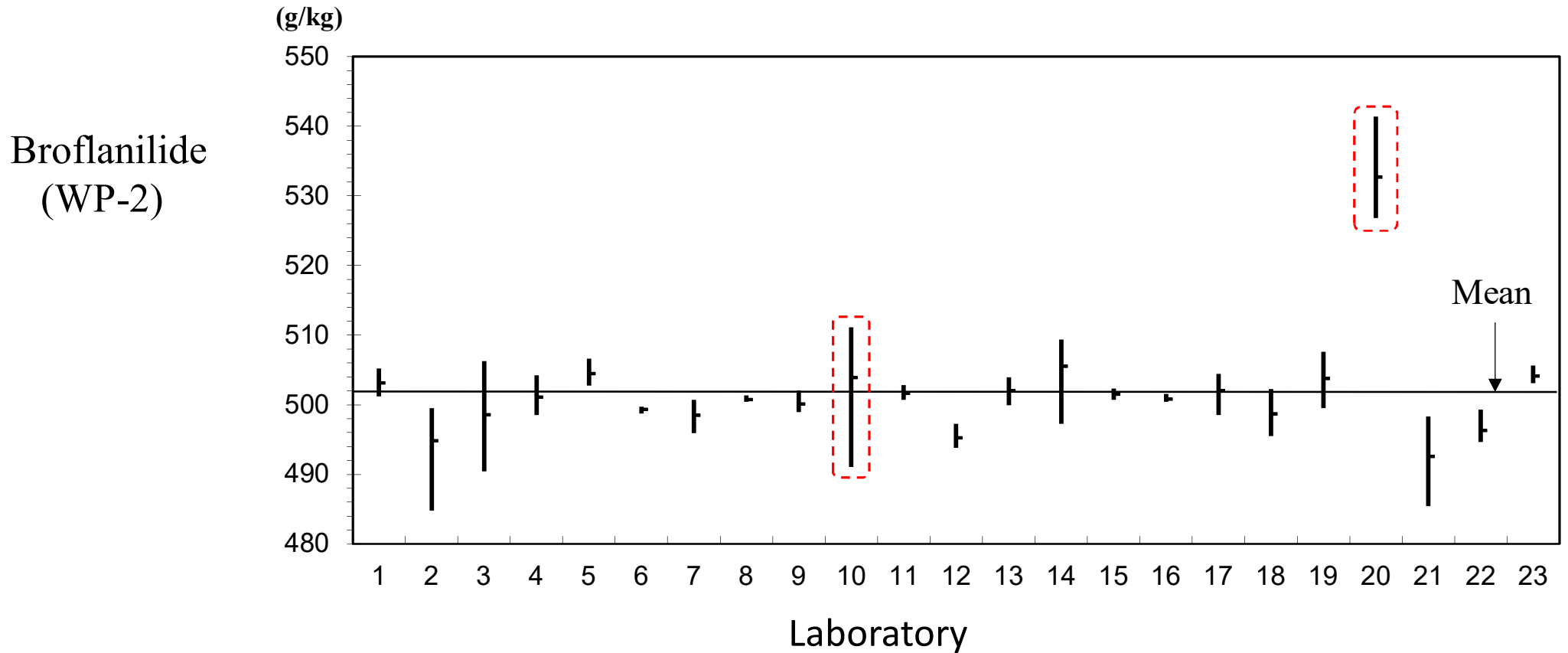
◆ Graphical Presentation of WP-1 Data



Outlier	
Lab. 10,14,20 (Cochran's test)	Lab. 20 (Grubbs's Test)

Results and Discussion (4/7)

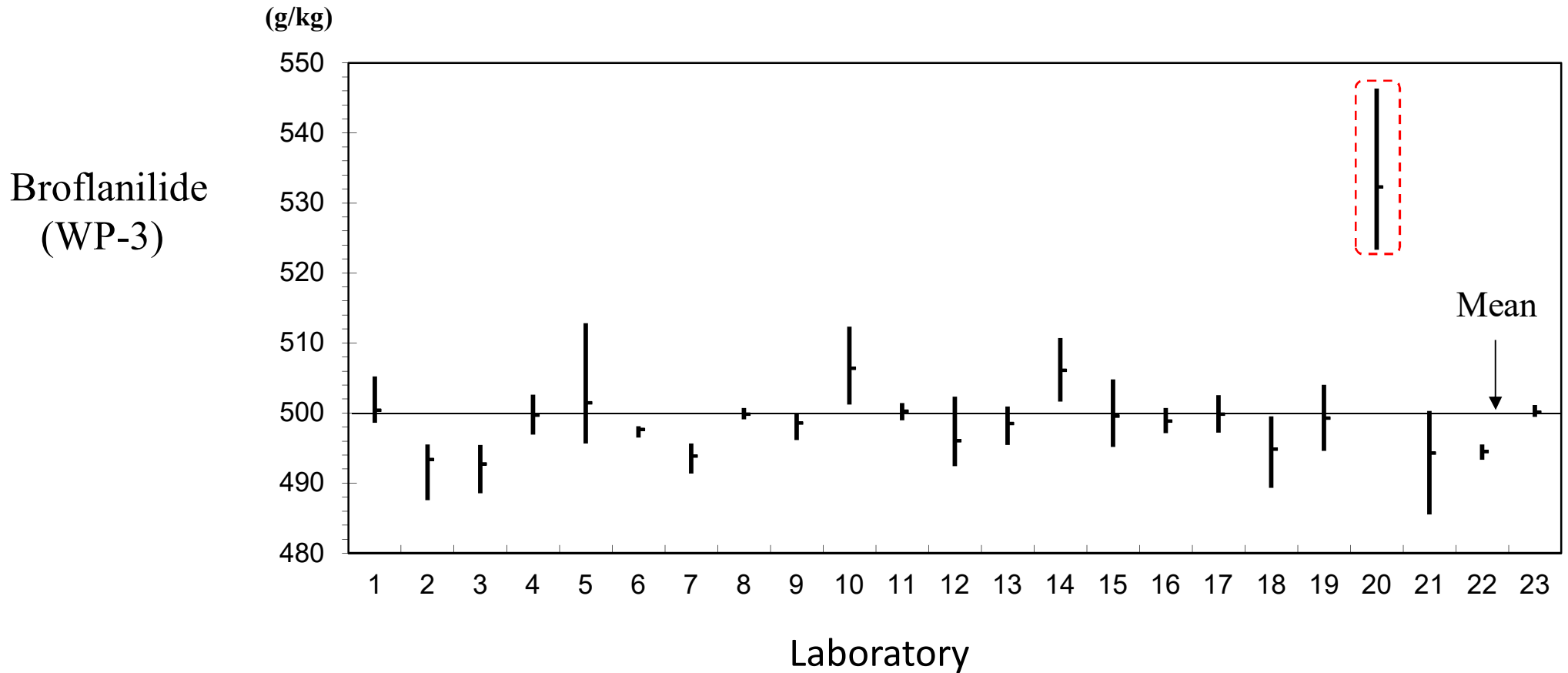
◆ Graphical Presentation of WP-2 Data



Outlier	
Lab. 10 (Cochran's test)	Lab. 20 (Grubbs's Test)

Results and Discussion (5/7)

◆ Graphical Presentation of WP-3 Data



Outlier
Lab. 20 (Cochrans's test)

Results and Discussion (6/7)

◆ Outliers

TC-1

The variance of Lab.5, 10, 14, 21 was identified as an outlier by Cochran's test. The mean of Lab.20, 21 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

TC-2

The variance of Lab.10, 14, 21 was identified as an outlier by Cochran's test. The mean of Lab.21 was identified as a straggler by Grubbs's test. The mean of Lab.4, 20 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

WP-1

The variance of Lab.10, 14, 20 was identified as an outlier by Cochran's test. The mean of Lab.20 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

WP-2

The variance of Lab.10 was identified as an outlier by Cochran's test. The mean of Lab.20 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

WP-3

The variance of Lab.20 was identified as an outlier by Cochran's test. The mean of Lab.20 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

Results and Discussion (7/7)

◆ Statistical Evaluation (Full set of 23 participants)

	TC-1	TC-2	WP-1	WP-2	WP-3
Average (g/kg)	997.0	996.9	500.3	501.8	499.9
Number of Labs.	23	23	23	23	23
S _r	12	17	4.0	3.8	4.1
S _L	11	8.2	6.4	7.3	7.6
S _R	17	19	7.5	8.2	8.7
Repeatability	35	47	11	11	12
Reproducibility	47	52	21	23	24
RSD _r	1.2	1.7	0.80	0.77	0.83
RSD _R	1.7	1.9	1.5	1.6	1.7
Horwitz's value	2.0	2.0	2.2	2.2	2.2
HorRat value (reference)	0.85	0.94	0.68	0.74	0.78

Conclusion

All data of 23 different laboratories, who participated in this full scale CIPAC collaboration trial, have been used for the statistical evaluation. The values of RSD_R (reproducibility relative standard deviation) were less than Horwitz's value even if outliers on Cochran's and Grubbs's tests were included.

The proposed method is considered to be appropriate for the determination of broflanilide in technical material and wettable powder.

JAPAC proposes that the method is accepted as a provisional CIPAC method.

Reference

◆ Statistical Evaluation (after elimination of outliers)

	TC-1	TC-2	WP-1	WP-2	WP-3
Average (g/kg)	995.0	995.0	498.7	500.2	498.5
Number of Labs.	18	18	20	21	22
S _r	3.7	5.7	2.2	3.2	3.7
S _L	2.7	1.2	3.2	3.0	3.2
S _R	4.6	5.8	3.9	3.0	4.8
Repeatability	10	16	6.1	9.0	10
Reproducibility	13	16	11	12	14
RSD _r	0.38	0.57	0.44	0.64	0.74
RSD _R	0.47	0.58	0.78	0.88	1.0
Horwitz's value	2.0	2.0	2.2	2.2	2.2
HorRat value (reference)	0.23	0.29	0.35	0.39	0.44