FLUPYRADIFURONE

Collaborative Study

Full Scale Collaborative Study
for the
Determination of Flupyradifurone AI and formulations, by
High Performance Liquid Chromatography

Report to CIPAC
by
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1. Participants

In December 2016, Information Sheet No. 308 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Flupyradifurone, by gradient reversed phase High Performance Liquid Chromatography.

By end of March 2017, 22 of the 24 respondents provided their results.

- One participant decided not to contribute in the collaborative study as the column was not available and the gradient has to be significantly modified.
- Another participant did not reply in time.

The results of one participant were rejected as a number of major changes regarding the chromatographic conditions were introduced:

Of the remaining 21 laboratories, only 13 participants used the column material described in the CIPAC collaboration trial, 8 laboratories used a different column material.

The results of 13 participants were presented, in addition the full set of all 21 participants were evaluated.

The remaining 21 laboratories are listed in alphabetical order whereas lab numbers in the result tables were assigned, chronologically, based upon receipt of results.

Benke, Lajos	National Food Chain Safety Office Directorate of Plant Protection, Soil Conservation and Agri-environment Pesticide Analytical Laboratory, Velence H-2481,Velence, Ország u. 23 Hungary
Csicsay, Frantisek	Central Control and Testing Institute in Agriculture Matúškova 21, 833 16 Bratislava Slovakia
de Rijk, Theo	RIKILT Wageningen University & Research PO Box 230, 6700 AE Wageningen Netherlands
de Ryckel, Bernard	Centre wallon de Recherches agronomiques Wallon Agricultural Research Center Bâtiment Rachel Carson Rue du Bordia 11, 5030 Gembloux Belgium
Etienne-Thewissen, Fabian Monisse, Isabelle	Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (AFSCA) Rue de Visé 495, 4000 Liège Belgium

Foerster, Rolf	BASF SE APR/DP 67117 Limburgerhof Germany
Garvey, Jim	Pesticide Control Laboratory, Department of Agriculture, Food and the Marine Backweston Laboratory Complex, Backweston, Celbridge, Co. Kildare, Ireland
Grecu, Cornel	ALCHIMEX S.A. 63-Alexandru Constantinescu, sector 1 011472 Bucharest Romania
Haustein, Michael	Currenta GmbH & Co. OHG ANT-PDA-PÜ3 HB 41538 Dormagen Germany
Jacobsen, Eva	Danish Technological Institute, Laboratory for Chemistry and Microbiology Life Science Kongsvang Allé 29, 8000 Aarhus Denmark
Joseph, Rachel	Jiangsu Rotam Chemistry Co. Ltd. Rotam Research Laboratory (RRL-GLP) No. 88, Long Deng Road, ETDZ, Kunshan, 215300 Jiangsu China
Karasali, Helen	Laboratory of Chemical Control of Pesticides/Benaki Phytopathological Institute 8 Stefanou Delta street, 14561 Kifissia, Athens Greece
Kettner, Robert	Syngenta Crop Protection AG Breitenloh 5, 4333 Münchwilen Switzerland
Manso, Luis	Laboratorio Arbitral Agroalimentario Ministerio de Agricultura, Alimentación y Medio Ambiente Aguarón, 13. Aravaca, 28023 Madrid Spain

Michel, Alexandra	Bayer AG, Crop Science Division Formulation Technology Alfred-Nobel-Str. 50, 40789 Monheim am Rhein Germany
Mykhaylov, Volodymyr	L.I.Medved`s Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health 6, Heroiv Oborony st., Kiev, 03680 Ukraine
Orgei, Iwan	Frandesa Co., LTD. 1, Bereza District, 225209 Brest Region Republic of Belarus
Tashev, Krste	State Phytosanitary Laboratory Ministry of Agriculture, Forestry and Water Economy blvd. Aleksandar The Great bb, pfah: 18 Post 1130, 1000 Skopje Republic of Macedonia
Wagner, Silke	Bayer AG, Crop Science Division Product Chemistry Analytics Alfred-Nobel-Str. 50, 40789 Monheim am Rhein Germany
Wang, Yue	Nutrichem Laboratory Co., Ltd. Beijing China
Watanabe, Takashi	Agricultural Chemicals Inspection Station (ACIS), Food and Agricultural Materials Inspection Center (FAMIC) 2-772, Suzuki-Cho, Kodaira-Shi,Tokyo Japan

2. Active Ingredient: General Information

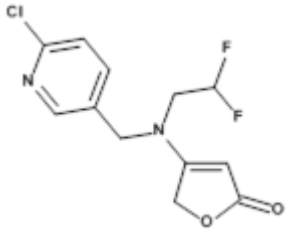
Chemical name: IUPAC 4-[(6-chloropyridin-3-ylmethyl)(2,2-difluoroethyl)amino]furan-2(5H)-one

CAS 2(5H)-furanone, 4-[[(6-chloro-3-pyridinyl)methyl](2,2-difluoroethyl)amino]-

ISO common name: Flupyradifurone

CAS-No.: 951659-40-8

Structure:



Molecular mass: 288.7 g/mol

Empirical formula: C_{12} H_{11} Cl F_2 N_2 O_2

Activity: Insecticide

3. Samples

Seven test samples and one analytical standard were sent to the participants:
1. Flupyradifurone tech. sample – 1
2. Flupyradifurone AL 0.08 g/L
3. Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)
4. Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)
5. Flupyradifurone FS 480 g/L
6. Flupyradifurone SL 200 g/L
7. Flupyradifurone WG 240 g/kg (120 g/kg Flupyradifurone)

Flupyradifurone, reference standard (purity 99.4%w/w)

4. Method

4.1 Scope

The determination of Flupyradifurone active ingredient content contained within Technical Grade Active Ingredient (TGAI) and AL, EC, EW, FS, SL and WG formulations.

4.2 Principle

Flupyradifurone content is determined using gradient reversed phase High Performance Liquid Chromatography incorporating UV detection at 280 nm with an external standard calibration.

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 single injections of the calibration solution. The average response factor, used to calculate the amount of Flupyradifurone in the test solution, was calculated using the injection before and after the test injections.

5. Remarks of the Participants

Several participants provided comments about the method performance and also made a note of any deviations from the method:

Laboratory 1 Column: Kinetex C18 (50 x 4.6 mm, 2.6 µm, 100 Å)
Remarks: Type of Integrator: DIONEX Chromeleon Vers. 6.80;
EW- and WG-formulations are filtrated by Chromafil Xtra 0,45µm filters to clarify the sample solutions.

Laboratory 2 Column: Kinetex C18 (50 x 4.6 mm, 2.6 µm, 100 Å)
Remarks: None

Laboratory 3 Column: Allsphere ODS-1 (250 x 2.1 mm, 5 µm)
Remarks: Flow rate: 1.0 mL/min
Injection volume: 10 µL

Laboratory 4 Column: Kinetex C18 (100 x 4.6 mm, 2.6 µm, 100 Å)
Remarks: The system is not capable of changing solvent composition within 0.1 min as described in the method. The gradient has therefore been altered. See below.
Retention time approximately 6.2 min

Gradient		
Time	**A%**	**B%**
0 min	90	10
5 min	85	15
9 min	5	95
10 min	5	95
11 min	90	10
14 min	90	10

Laboratory 5 Column: Kinetex C18 (50 x 4.6 mm, 2.6 µm)
Remarks:
- It was difficult to fill up exactly because many bubbles and insoluble matters remained in the solution of EW sample.
- Since the solution of WG contained insoluble matters as precipitation, I would recommend to fill up after removing these matters by filteration.
- Otherwise, I would recommend that EW and WG sample were determined by internal standard method.

Laboratory 6 Column: Kinetex C18 (50 x 4.6 mm, 2.6 µm)
Remarks: None

Laboratory 7 Column: Kinetex C18 (50 x 4.6 mm, 2.6 µm)
Remarks: None

Laboratory 8	Column:	Kinetex C18 (50 x 4.6 mm, 2.6 µm)
	Remarks:	In the method the flow rate is missing In method Flupyradifurone technical under (d) sample preparation is stated that the sample should contain approximately 50mg Flupyradifurone reference standard. Reference standard should be deleted. The same is true for method "any other liquid" and "emulsifiable concentrate".
Laboratory 9	Column:	Chromolith RP-18e (100 x 4.6mm)
	Remarks:	The method followed exactly as described in the information sheet. It was used a monolithic RP-HPLC column -chromolith (100 x 4.6 mm) and flowrate 3.3 mL/min. The back pressure was 104 bar.
Laboratory 10	Column:	Kinetex C18 (50 x 4.6 mm, 2.6 µm)
	Remarks:	All conditions of method were used. However, the active ingredient peak elutes at 3.2 min. The same column was used in the DAPA collaborative trial.
Laboratory 11	Column:	Kinetex C18 (100 x 4.6 mm, 2.6 µm, 100 Å)
	Remarks:	Some recovery tests have been done and the results of the EW formulation are not as good as the ones of the technical product and the other formulations
Laboratory 12	Column:	Purospher RP-18e (125 x 4 mm, 5 µm)
	Remarks:	Flow rate: 1.5 mL/min
Laboratory 13	Column:	Waters BEH C18 (50 x 2,1 mm, 1,7µm)
	Remarks:	Flow rate: 0.5 mL/min Injection volume: 1 µL I worked with a flow rate of 0,5ml/min in order to have the pic of Flupyradifurone with a retention time of about 4 min. I have respected the gradient elution described in the method as my pic comes out under the same conditions. In order to have a pic well symmetrical, I injected 1µl instead of 5µl as described in the method. I have a problem with the results for the EW formulation reproductibilte. I did 2 tests on 2 different days (3 and 4) and my results fluctuate so much. The chromato are beautiful. I don't have any explanation…

Laboratory 14	Column:	Zorbax SB-C18 (30 x 2.1 mm, 3.5 µm)
	Remarks:	Flow rate: 0.5 mL/min Injection volume: 1 µL Different column was used and the gradient was adjusted (time and flow rate) according to it's parameters, to achieve similar separation. The composition of mobil phase was unchanged.
Laboratory 15	Column:	Kinetex C18 (50 x 4.6 mm, 2.6 µm, 100 Å)
	Remarks:	No changes to the proposed method
Laboratory 16	Column:	Kinetex C18 (100 x 3 mm, 2.6 µm, 100 Å)
	Remarks:	Flow rate: 1.5 mL/min Injection volume: 2 µL The EW 78.8 g/L sample was full of crystal growth and from this reason was impossible to be analyzed. The sample was kept in the lab at temperature between 17-22 degree.
Laboratory 17	Column:	Kinetex C18 (50 x 4.6 mm, 2.6 µm)
	Remarks:	Security Guard ULTRA Cartridge UHPLC C18 (Phenomenex # AJO-8768)
Laboratory 18	Column:	Agilent Poroshell 120 EC-C18 (50 x 4.6 mm, 2.7 µm)
	Remarks:	Stop time: 9 min Post time: 5 min The flow rate was changed to 1.7 ml/min because the separation between active ingredient and other ingredient are not quite good for 2 ml/min. Therefore, we adjust to 1.7. For EW, the RSD% for 2 days's result are higher than the requirement. We re-test it in the 3rd and 4th day. The result are still not good. Please check in the attached email. In the 4th day, we prolong the sonic time, the RSD% was still not good. Do you have any comments on it?
Laboratory 19	Column:	Kinetex C18 (50 x 4.6 mm, 2.6 µm)
	Remarks:	None

Laboratory 20 Column: Merck LiChrospher C18 (150 x 4,6 mm, 5 µm)

Remarks: Flow rate: 1.5 mL/min

Injection volume: 10 µL

Gradient		
Time	**A%**	**B%**
0 min	90	10
3 min	85	15
7 min	5	95
9 min	5	95
11 min	90	10
12 min	90	10

Laboratory 21 Column: Poroshell 120 EC C18 (50 x 4.6, 2.7 µm)

Remarks:

1. The analysis was made on different columns Poroshell 120 EC C18 in different two days
2. Overpressure can be arised in the flask because of intensive manual mixing due to the separate adding acetonitrile and water. Air bubbles as a result of this process. We recommend to degas the solution in ultrasonic bath after make up the flasks with purified water to the calibration mark. Than correct the volume by aditional portions of water.
3. The degradation of the column stationary phase was observed under these chromatographic conditions. The result is deterioration of the AIs peaks symmetry.
4. It is recommended to use the pipette dispenser with the more wide tip when sampling EW formulation. If to use the tip with small diameter than crystallization of solid particles was observed at the end of tip instead of Pellet pipette applying. This is the reason why Flupyradifurone EW analysis result at the first day first sample (23,0 g/kg) was less than sample 2 this day and the results in a Day 2 (26,0 g/kg ; 27,3 g/kg).
5. It is recommended to use wave length 262 nm for the analysis of this substance in accordance with UV-spectra below.

6. Evaluation and Discussion

6.1 Data Review

The data obtained from each laboratory was visually reviewed to determine if there were any significant chromatography differences, from what was expected, which might affect the analytical results.
In summary it can be stated that the method deviations, noted by the remaining 13 participants, who used the Kinetex C18 column, were deemed not to affect the analytical results significantly and therefore all data sets were included within the statistical assessment.
The EW formulation showed crystallization due to insufficient cold stability of the formulation. The crystallization has been reported by several participants, see comments of the participants, but it was not observed in all samples.
8 results of the participants are reported separately, which were generated by using the correct column (Kinetex C18) and gaining plausible results with respect to result level or day to day consistency, respectively.

The full set of all results of the 21 participants are reported in addition.

6.2 Determination of Flupyradifurone

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. All results reported by the 13 laboratories, which used the column mentioned in the CIPAC collaboration trial, are reported and the statistical evaluation of these are listed in Tables 1-6 and displayed in Figures 1-6. These results are reported without any exclusion of outliers and/or stragglers.
The results of the EW formulation are reported in Tables 7-8 and displayed in Figure 7.
The full set of all results of the 21 participants are reported in addition in Tables 9-12 and displayed in Figure 8-14.
The statistical evaluation in Table 9 and Table 10 shows outliers or stragglers for the TC, EC, SL and WG samples. These results were not generated with the Kinetex C18 column.
A separate evaluation with 13 participants in Table 1 and 2 shows that no elimination of any outliers or stragglers is needed, as acceptable Horwitz ratio are obtained for all samples.

Determination of Flupyradifurone – elimination of labs who used a different column material, results of 13 labs

All results tabulated in table 1 to table 4 are given in g/kg

Table 1 Results

	Flupyradifurone tech. sample		Flupyradifurone AL 0.08 g/L		Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Laboratory 1	991	991	0.0757	0.0751	64.0	63.7
Laboratory 2	983	994	0.0748	0.0744	63.5	63.3
Laboratory 4	998	1019	0.0759	0.0755	63.2	64.1
Laboratory 5	991	990	0.0811	0.0808	64.2	64.1
Laboratory 6	987	990	0.0809	0.0804	63.4	64.0
Laboratory 7	1002	983	0.0813	0.0800	64.9	64.1
Laboratory 8	990	981	0.0800	0.0803	63.0	62.9
Laboratory 10	983	983	0.0750	0.0757	63.0	63.1
Laboratory 11	987	988	0.0751	0.0757	63.4	63.4
Laboratory 15	992	996	0.0810	0.0799	63.7	63.6
Laboratory 16[1]	995	994	0.0753	0.0752	63.5	63.8
Laboratory 17	997	995	0.0749	0.0761	63.3	64.0
Laboratory 19	994	997	0.0761	0.0782	64.1	63.5

[1] Due to crystal growth EW formulation was not analyzed

Table 2 Results

	Flupyradifurone FS 480 g/L		Flupyradifurone SL 200 g/L		Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Laboratory 1	417	414	171	171	121	121
Laboratory 2	410	410	170	169	118	118
Laboratory 4	408	417	169	172	121	119
Laboratory 5	419	414	173	171	120	120
Laboratory 6	410	413	170	171	121	122
Laboratory 7	417	417	172	171	121	121
Laboratory 8	418	414	171	169	120	117
Laboratory 10	411	414	169	169	119	119
Laboratory 11	412	414	171	171	120	120
Laboratory 15	415	413	171	170	120	119
Laboratory 16	409	412	170	170	120	120
Laboratory 17	410	416	170	173	120	122
Laboratory 19	407	400	171	169	121	118

Table 3 Mean values

	Flupyradifurone tech. sample	Flupyradifurone AL 0.08 g/L	Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)
Laboratory 1	991.0	0.07540	63.85
Laboratory 2	988.5	0.07460	63.40
Laboratory 4	1008.5	0.07570	63.65
Laboratory 5	990.5	0.08095	64.15
Laboratory 6	988.5	0.08065	63.70
Laboratory 7	992.5	0.08065	64.50
Laboratory 8	985.5	0.08015	62.95
Laboratory 10	983.0	0.07535	63.05
Laboratory 11	987.5	0.07540	63.40
Laboratory 15	994.0	0.08045	63.65
Laboratory 16[2]	994.5	0.07525	63.65
Laboratory 17	996.0	0.07550	63.65
Laboratory 19	995.5	0.07715	63.80

[2] Due to crystal growth EW formulation was not analyzed

Table 4 Mean values

	Flupyradifurone FS 480 g/L	Flupyradifurone SL 200 g/L	Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)
Laboratory 1	415.5	171.0	121.0
Laboratory 2	410.0	169.5	118.0
Laboratory 4	412.5	170.5	120.0
Laboratory 5	416.5	172.0	120.0
Laboratory 6	411.5	170.5	121.5
Laboratory 7	417.0	171.5	121.0
Laboratory 8	416.0	170.0	118.5
Laboratory 10	412.5	169.0	119.0
Laboratory 11	413.0	171.0	120.0
Laboratory 15	414.0	170.5	119.5
Laboratory 16	410.5	170.0	120.0
Laboratory 17	413.0	171.5	121.0
Laboratory 19	403.5	170.0	119.5

Table 5 Summary of the statistical evaluation (data of 13 labs included)

	Flupyradifurone tech. sample	Flupyradifurone AL 0.08 g/L	Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)
x_m [g/kg]	991.96	0.07748	63.65
x_m [% w/w]	99.20	0.007748	6.365
L	13	13	13
s_r	6.34	0.00064	0.34
s_R	7.76	0.00265	0.47
r	17.75	0.00180	0.94
R	21.74	0.00742	1.32
RSD_R	0.78	3.42	0.743
$RSD_{R\ (Hor)}$	2.00	8.31	3.027
$RSD_R/RSD_{R(Hor)}$	0.39	0.41	0.24

Table 6 Summary of the statistical evaluation (data of 13 labs included)

	Flupyradifurone FS 480 g/L	Flupyradifurone SL 200 g/L	Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)
x_m [g/kg]	412.73	170.54	119.92
x_m [% w/w]	41.27	17.05	11.99
L	13	13	13
s_r	3.11	1.14	1.04
s_R	4.18	1.18	1.27
r	8.70	3.20	2.91
R	11.71	3.29	3.56
RSD_R	1.01	0.69	1.06
$RSD_{R\ (Hor)}$	2.28	2.61	2.75
$RSD_R/RSD_{R(Hor)}$	0.44	0.26	0.39

x_m	=	overall sample mean
L	=	number of laboratories
s_r	=	repeatability standard deviation
s_R	=	reproducibility standard deviation
r	=	repeatability limit
R	=	reproducibility limit
RSD_r	=	relative repeatability standard deviation
RSD_R	=	relative reproducibility standard deviation

Determination of Flupyradifurone – EW formulation, elimination of labs which used a different column material and elimination of outliers, results of 8 labs

The EW formulation showed crystallization due to insufficient cold stability of the formulation. The crystallization has been reported by several participants, see comments from the participants.
To demonstrate the validity of the analytical method for EW formulations, the results of the 8 participants are reported separately, which:

- used the Kinetex C18 column
- show no significant deviations
- the result level is acceptable

All results tabulated in table 7 are given in g/kg

Table 7 Results EW

	Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)		Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)
	Day 1	Day 2	Mean values EW
Laboratory 1	26.0	26.3	26.15
Laboratory 2	25.5	25.6	25.55
Laboratory 6	25.9	26.1	26.00
Laboratory 8	25.4	25.3	25.35
Laboratory 10	25.8	26.6	26.20
Laboratory 11	27.2	27.4	27.30
Laboratory 15	26.3	25.4	25.85
Laboratory 19	24.7	24.3	24.50

Table 8 Summary of the statistical evaluation (data of 8 labs included)

	Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)
x_m [g/kg]	25.86
x_m [% w/w]	2.586
L	8
s_r	0.34
s_R	0.84
r	0.94
R	2.34
RSD_R	3.223
$RSD_{R\ (Hor)}$	3.467
$RSD_R/RSD_{R(Hor)}$	0.93

x_m = overall sample mean
L = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation
RSD_R = relative reproducibility standard deviation

Determination of Flupyradifurone – full set of 21 participants

All results tabulated in table 9 to table 10 are given in g/kg

Table 9 Results

	Flupyradifurone tech. sample		Flupyradifurone AL 0.08 g/L		Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)		Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)			
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1		Day 2	
Laboratory 1	991	991	0.0757	0.0751	64.0	63.7	26.0		26.3	
Laboratory 2	983	994	0.0748	0.0744	63.5	63.3	25.5		25.6	
Laboratory 3	949	943	0.0756	0.0766	66.8	67.3	26.6		25.6	
Laboratory 4	998	1019	0.0759	0.0755	63.2	64.1	20.5		24.7	
Laboratory 5	991	990	0.0811	0.0808	64.2	64.1	14.4		13.0	
Laboratory 6	987	990	0.0809	0.0804	63.4	64.0	25.9		26.1	
Laboratory 7	1002	983	0.0813	0.0800	64.9	64.1	14.2		15.1	
Laboratory 8	990	981	0.0800	0.0803	63.0	62.9	25.4		25.3	
Laboratory 9	993	994	0.0832	0.0816	66.6	64.1	22.5		22.0	
Laboratory 10	983	983	0.0750	0.0757	63.0	63.1	25.8		26.6	
Laboratory 11	987	988	0.0751	0.0757	63.4	63.4	27.2		27.4	
Laboratory 12	987	986	0.0782	0.0797	64.0	63.9	23.1		23.0	
Laboratory 13[3]	966	971	0.0727	0.0759	63.5	63.6	14.7	27.5	20.8	14.1
Laboratory 14	992	985	0.0769	0.0704	64.0	64.1	26.8		28.5	
Laboratory 15	992	996	0.0810	0.0799	63.7	63.6	26.3		25.4	
Laboratory 16[4]	995	994	0.0753	0.0752	63.5	63.8	-		-	
Laboratory 17	997	995	0.0749	0.0761	63.3	64.0	14.0		14.7	
Laboratory 18[5]	985	988	0.0789	0.0795	63.0	62.2	20.9	25.3	27.7	
Laboratory 19	994	997	0.0761	0.0782	64.1	63.5	24.7		24.3	
Laboratory 20	964	968	0.0700	0.0808	64.3	67.3	23.4		26.7	
Laboratory 21	987	985	0.0806	0.0804	63.9	63.8	24.5		27.3	

[3] Analysis was repeated and four results have been reported

[4] Due to crystal growth EW formulation was not analyzed

[5] Analysis was repeated and three results have been reported

Table 10 Results

	Flupyradifurone FS 480 g/L		Flupyradifurone SL 200 g/L		Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Laboratory 1	417	414	171	171	121	121
Laboratory 2	410	410	170	169	118	118
Laboratory 3	414	404	174	172	134	135
Laboratory 4	408	417	169	172	121	119
Laboratory 5	419	414	173	171	120	120
Laboratory 6	410	413	170	171	121	122
Laboratory 7	417	417	172	171	121	121
Laboratory 8	418	414	171	169	120	117
Laboratory 9	430	418	179	174	127	122
Laboratory 10	411	414	169	169	119	119
Laboratory 11	412	414	171	171	120	120
Laboratory 12	414	413	171	171	120	119
Laboratory 13	418	410	169	172	119	120
Laboratory 14	413	423	173	169	118	118
Laboratory 15	415	413	171	170	120	119
Laboratory 16	409	412	170	170	120	120
Laboratory 17	410	416	170	173	120	122
Laboratory 18	409	404	169	167	120	117
Laboratory 19	407	400	171	169	121	118
Laboratory 20	421	416	174	172	112	105
Laboratory 21	418	421	171	178	120	121

Table 11 Mean values

	Flupyradifurone tech. sample	Flupyradifurone AL 0.08 g/L	Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)	Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)
Laboratory 1	991.0	0.07540	63.85	26.15
Laboratory 2	988.5	0.07460	63.40	25.55
Laboratory 3	946.0	0.07610	67.05	26.10
Laboratory 4	1008.5	0.07570	63.65	22.60
Laboratory 5	990.5	0.08095	64.15	13.70
Laboratory 6	988.5	0.08065	63.70	26.00
Laboratory 7	992.5	0.08065	64.50	14.65
Laboratory 8	985.5	0.08015	62.95	25.35
Laboratory 9	993.5	0.08240	65.35	22.25
Laboratory 10	983.0	0.07535	63.05	26.20
Laboratory 11	987.5	0.07540	63.40	27.30
Laboratory 12	986.5	0.07895	63.95	23.05
Laboratory 13	968.5	0.07430	63.55	19.28
Laboratory 14	988.5	0.07365	64.05	27.65
Laboratory 15	994.0	0.08045	63.65	25.85
Laboratory 16[6]	994.5	0.07525	63.65	-
Laboratory 17	996.0	0.07550	63.65	14.35
Laboratory 18	986.5	0.07920	62.60	24.63
Laboratory 19	995.5	0.07715	63.80	24.50
Laboratory 20	966.0	0.07540	65.80	25.05
Laboratory 21	986.0	0.08050	63.85	25.90

[6] Due to crystal growth EW formulation was not analyzed

Table 12 Mean values

	Flupyradifurone FS 480 g/L	Flupyradifurone SL 200 g/L	Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)
Laboratory 1	415.5	171.0	121.0
Laboratory 2	410.0	169.5	118.0
Laboratory 3	409.0	173.0	134.5
Laboratory 4	412.5	170.5	120.0
Laboratory 5	416.5	172.0	120.0
Laboratory 6	411.5	170.5	121.5
Laboratory 7	417.0	171.5	121.0
Laboratory 8	416.0	170.0	118.5
Laboratory 9	424.0	176.5	124.5
Laboratory 10	412.5	169.0	119.0
Laboratory 11	413.0	171.0	120.0
Laboratory 12	413.5	171.0	119.5
Laboratory 13	414.0	170.5	119.5
Laboratory 14	418.0	171.0	118.0
Laboratory 15	414.0	170.5	119.5
Laboratory 16	410.5	170.0	120.0
Laboratory 17	413.0	171.5	121.0
Laboratory 18	406.5	168.0	118.5
Laboratory 19	403.5	170.0	119.5
Laboratory 20	418.5	173.0	108.5
Laboratory 21	419.5	174.5	120.5

Table 13 Summary of the statistical evaluation (all data included)

	Flupyradifurone tech. sample	Flupyradifurone AL 0.08 g/L	Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)	Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)
x_m [g/kg]	986.52	0.07751	63.98	23.15
x_m [% w/w]	98.65	0.007751	6.398	2.315
L	21	21	21	20
s_r	5.31	0.00210	0.67	2.67
s_R	13.40	0.00313	1.11	4.72
r	14.88	0.00589	1.89	7.48
R	37.51	0.00875	3.11	13.23
RSD_R	1.36	4.03	1.74	20.406
$RSD_{R\ (Hor)}$	2.00	8.31	3.03	3.525
$RSD_R/RSD_{R(Hor)}$	0.68	0.48	0.57	5.78

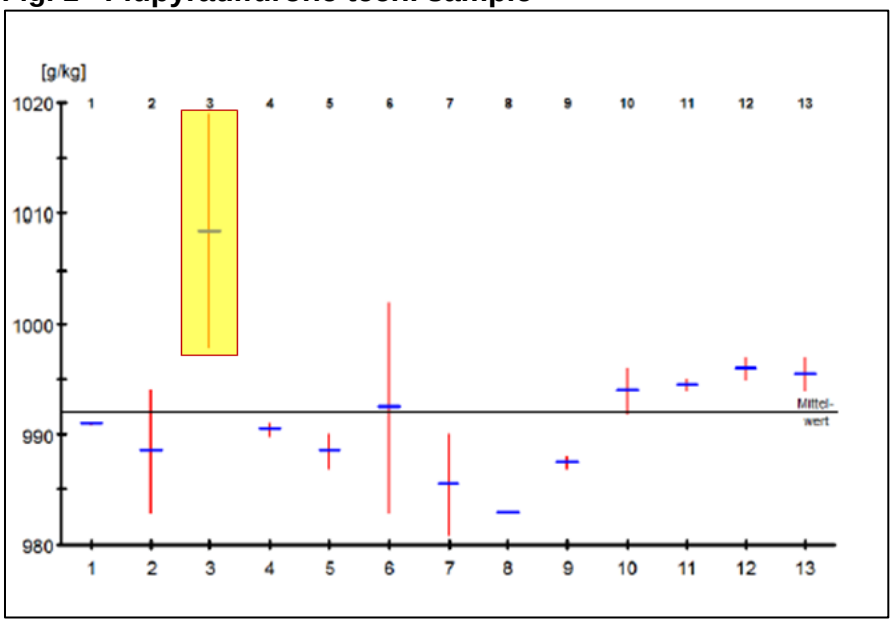
Table 14 Summary of the statistical evaluation (all data included)

	Flupyradifurone FS 480 g/L	Flupyradifurone SL 200 g/L	Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)
x_m [g/kg]	413.74	171.17	120.12
x_m [% w/w]	41.37	17.12	12.01
L	21	21	21
s_r	4.14	1.86	1.65
s_R	5.43	2.29	4.53
r	11.59	5.20	4.63
R	15.21	6.42	12.67
RSD_R	1.31	1.34	3.77
$RSD_{R\ (Hor)}$	2.28	2.61	2.75
$RSD_R/RSD_{R(Hor)}$	0.57	0.51	1.37

x_m	=	overall sample mean
L	=	number of laboratories
s_r	=	repeatability standard deviation
s_R	=	reproducibility standard deviation
r	=	repeatability limit
R	=	reproducibility limit
RSD_r	=	relative repeatability standard deviation
RSD_R	=	relative reproducibility standard deviation

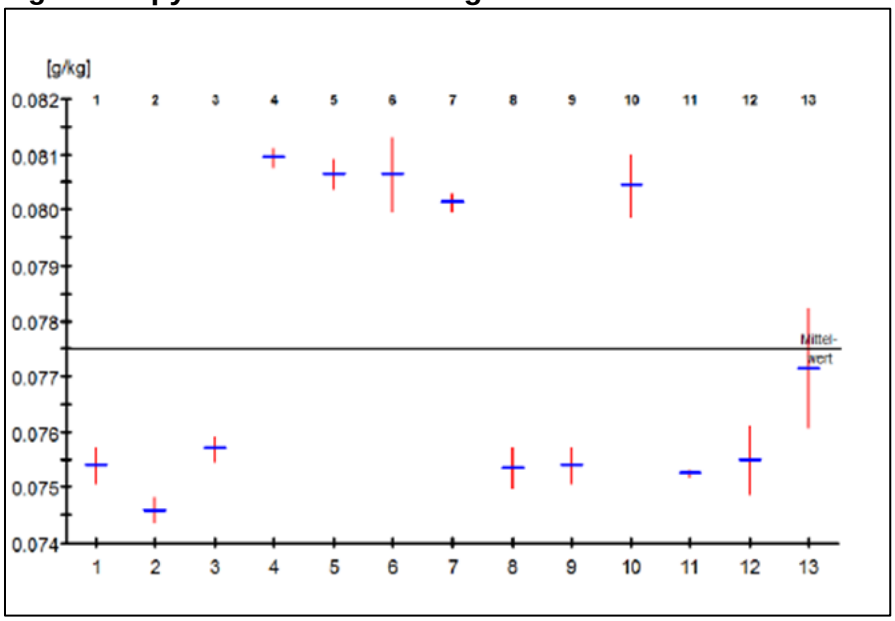
Results of 13 participants:

Fig. 1 - Flupyradifurone tech. sample



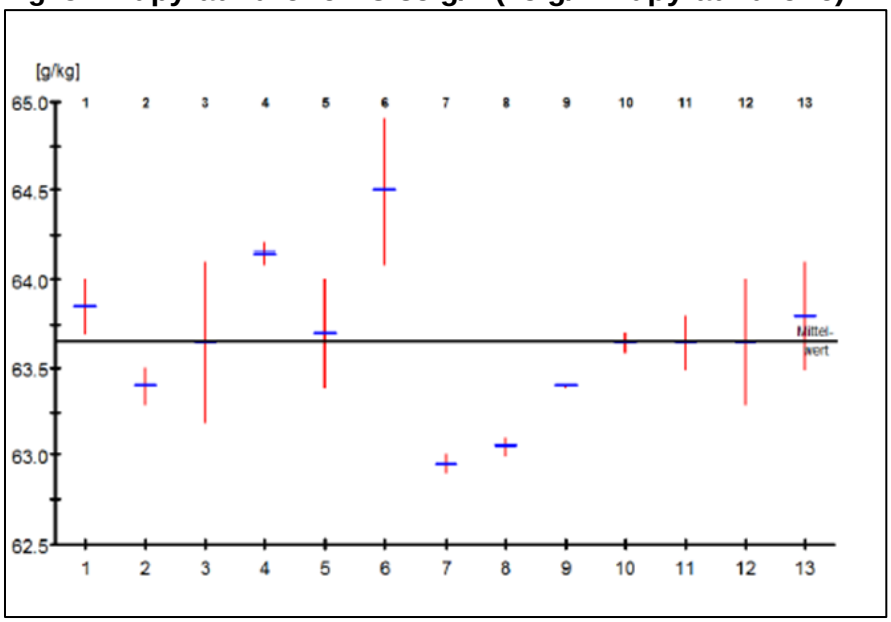
Mean value:	**991.96 g/kg**
s_r:	**6.34**
s_R:	**7.76**
RSD_R:	**0.78**
$RSD_{R\ (Hor)}$:	**2.00**
HorRat:	**0.39**
Outlier (Grubbs):	**upper**

Fig. 2 - Flupyradifurone AL 0.08 g/L



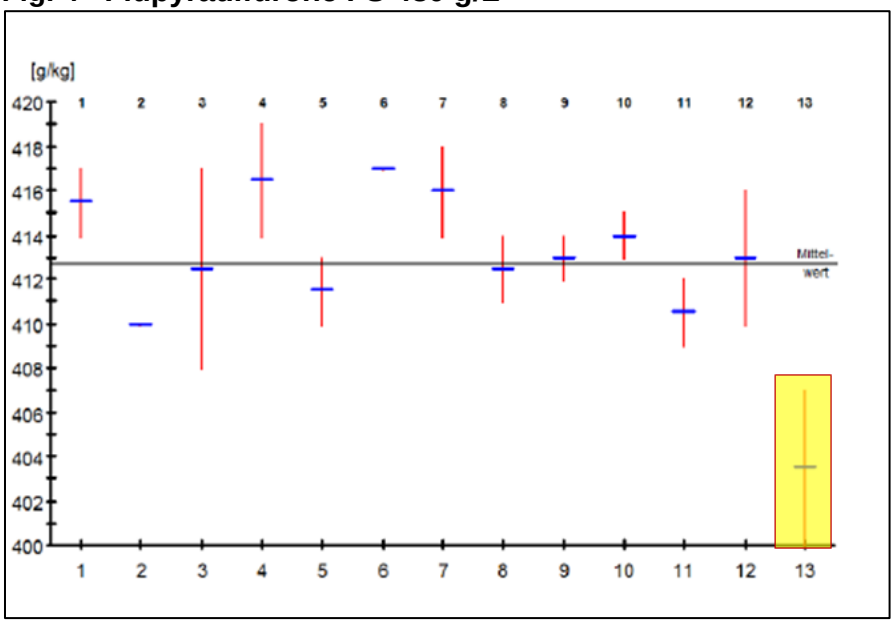
Mean value:	**0.07748 g/kg**
s_r:	**0.00064**
s_R:	**0.00265**
RSD_R:	**3.42**
$RSD_{R\ (Hor)}$:	**8.31**
HorRat:	**0.41**
Outlier (Grubbs):	**no**

Fig. 3 - Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)



Mean value:	**63.65 g/kg**
s_r:	**0.34**
s_R:	**0.47**
RSD_R:	**0.74**
$RSD_{R\ (Hor)}$:	**3.03**
HorRat:	**0.24**
Outlier (Grubbs):	**no**

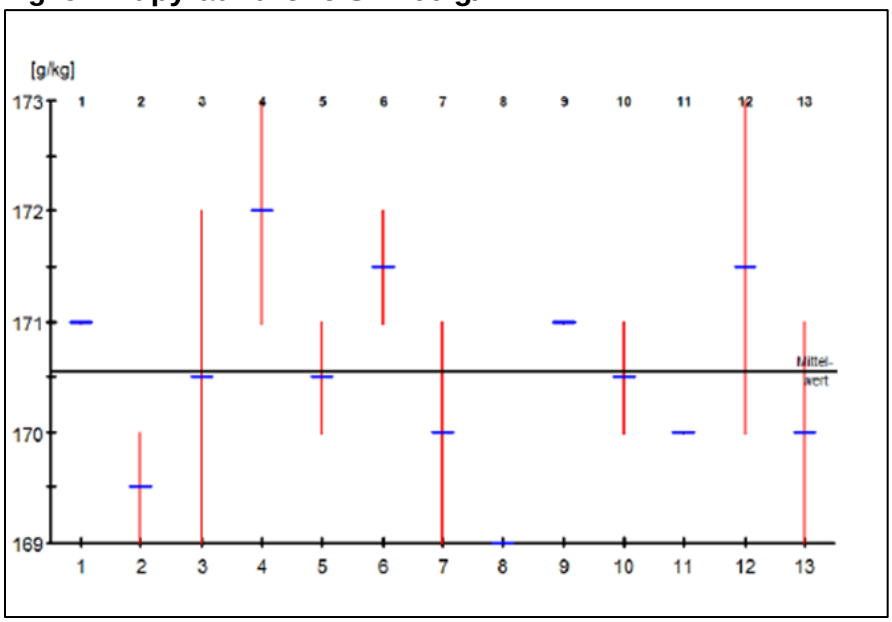
Fig. 4 - Flupyradifurone FS 480 g/L



Mean value:	**412.73 g/kg**
s_r:	**3.11**
s_R:	**4.18**
RSD_R:	**1.01**
$RSD_{R\ (Hor)}$:	**2.28**
HorRat:	**0.44**
Outlier (Grubbs):	**yes***

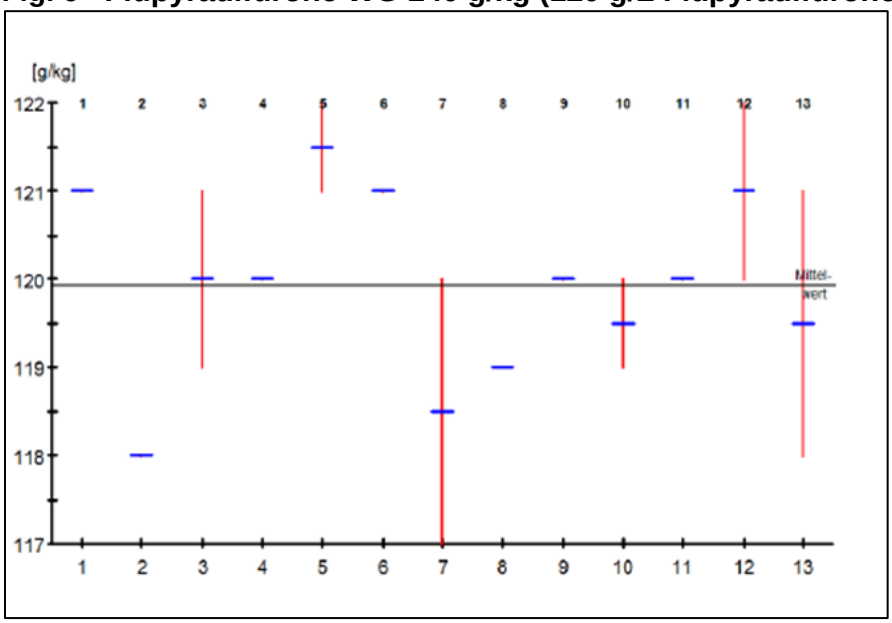
* Lower Grubbs Straggler

Fig. 5 - Flupyradifurone SL 200 g/L



Mean value:	**170.54 g/kg**
s_r:	**1.14**
s_R:	**1.18**
RSD_R:	**0.69**
$RSD_{R\ (Hor)}$:	**2.61**
HorRat:	**0.26**
Outlier (Grubbs):	**no**

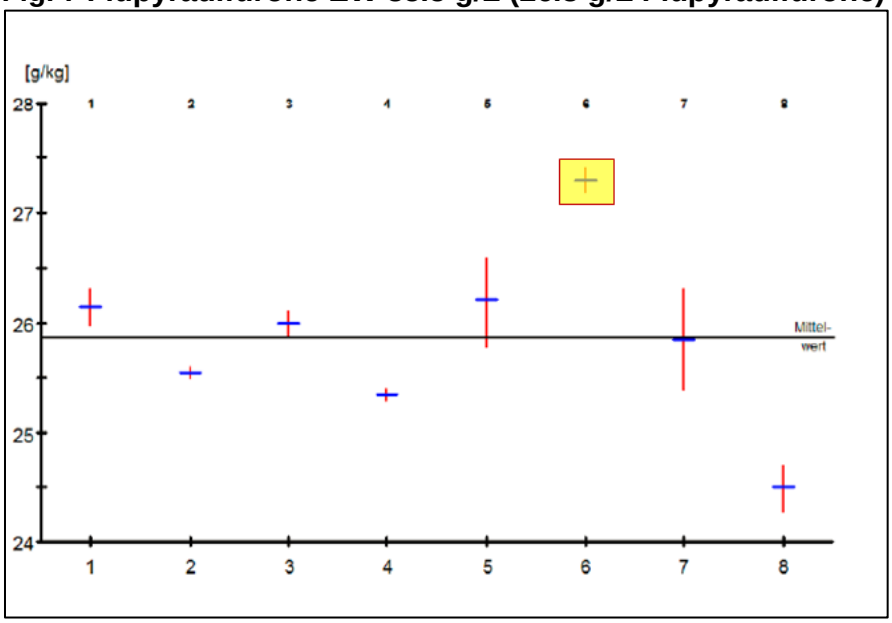
Fig. 6 - Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)



Mean value:	**119.92 g/kg**
s_r:	**1.04**
s_R:	**1.27**
RSD_R:	**1.06**
$RSD_{R\ (Hor)}$:	**2.75**
HorRat:	**0.39**
Outlier (Grubbs):	**no**

EW Results of 8 participants:

Fig. 7 Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)

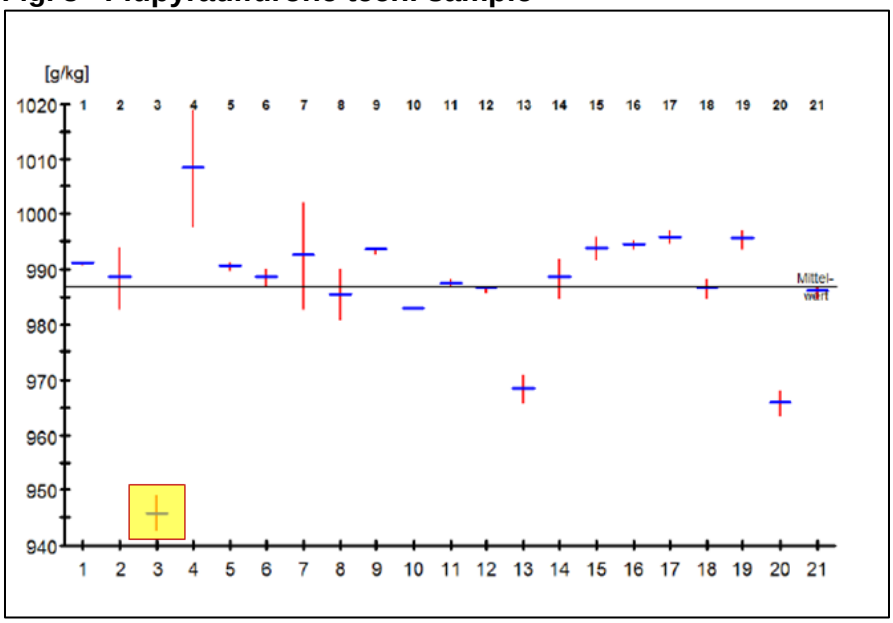


Mean value:	**25.86 g/kg**
s_r:	**0.34**
s_R:	**0.84**
RSD_R:	**3.22**
$RSD_{R\ (Hor)}$:	**3.47**
HorRat:	**0.93**
Outlier (Grubbs):	**yes***

* Upper Grubbs Straggler

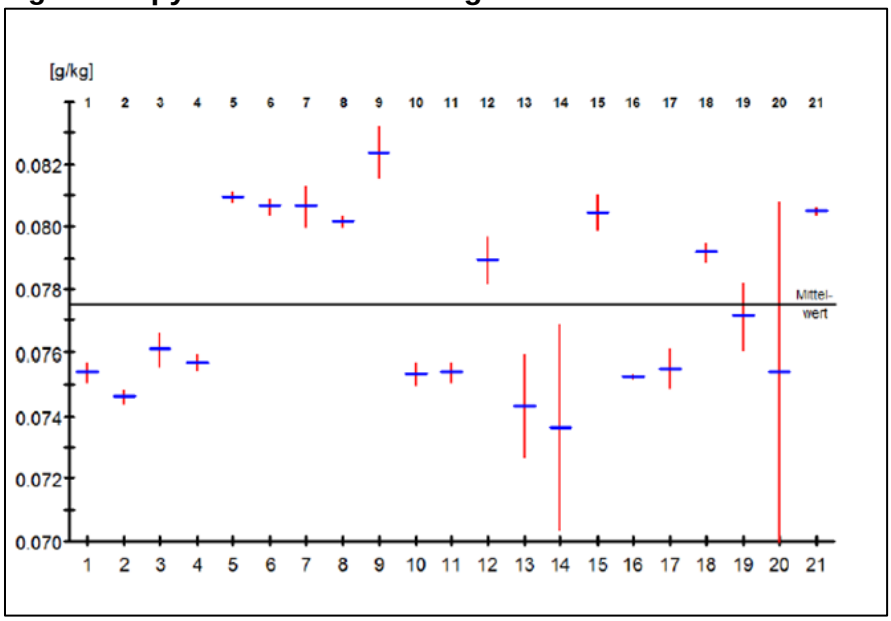
Results of 21 participants:

Fig. 8 - Flupyradifurone tech. sample



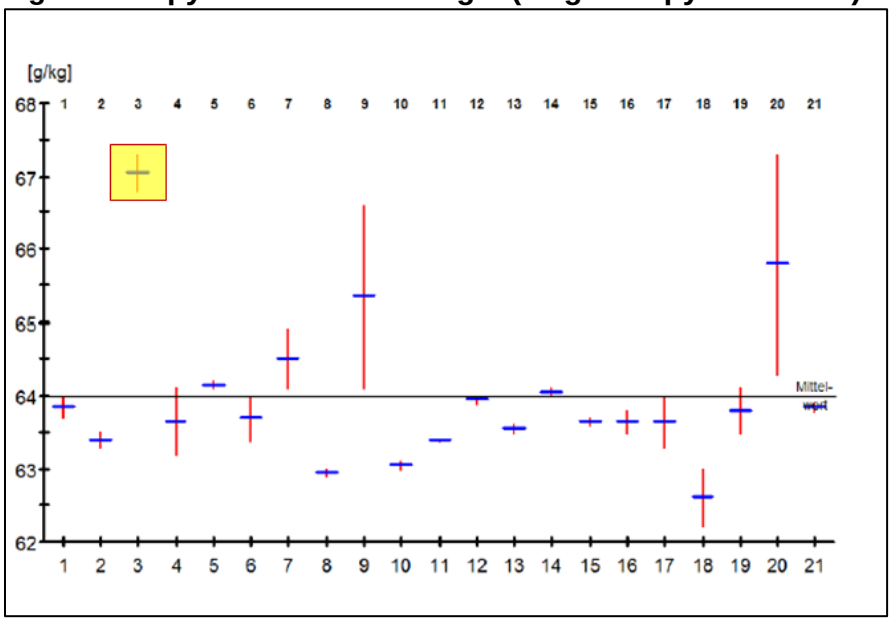
Mean value:	**986.52 g/kg**
s_r:	**5.31**
s_R:	**13.40**
RSD_R:	**1.36**
$RSD_{R\ (Hor)}$:	**2.00**
HorRat:	**0.68**
Outlier (Grubbs):	**lower**

Fig. 9 - Flupyradifurone AL 0.08 g/L



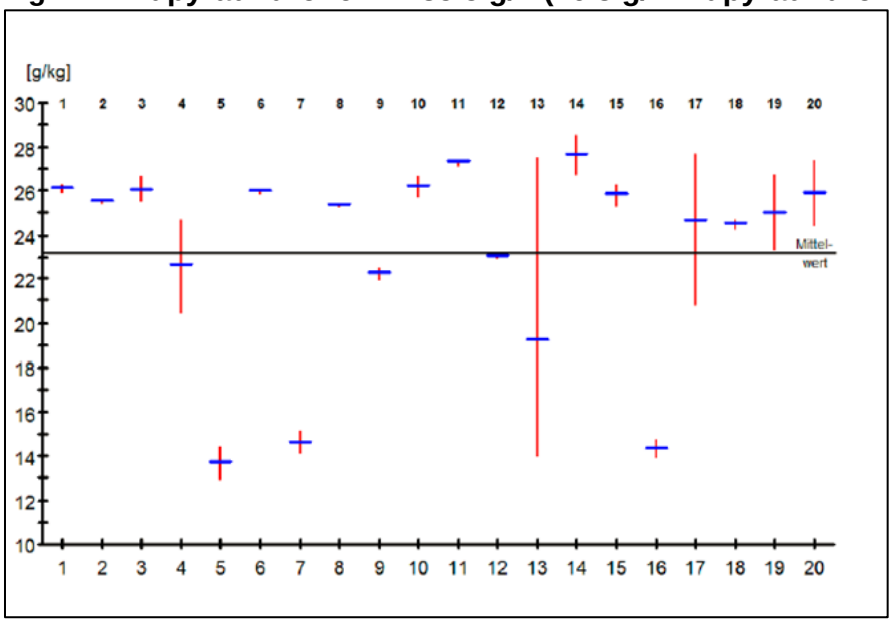
Mean value:	**0.07751 g/kg**
s_r:	**0.00210**
s_R:	**0.00313**
RSD_R:	**4.03**
$RSD_{R\ (Hor)}$:	**8.31**
HorRat:	**0.48**
Outlier (Grubbs):	**no**

Fig. 10 - Flupyradifurone EC 85 g/L (75 g/L Flupyradifurone)



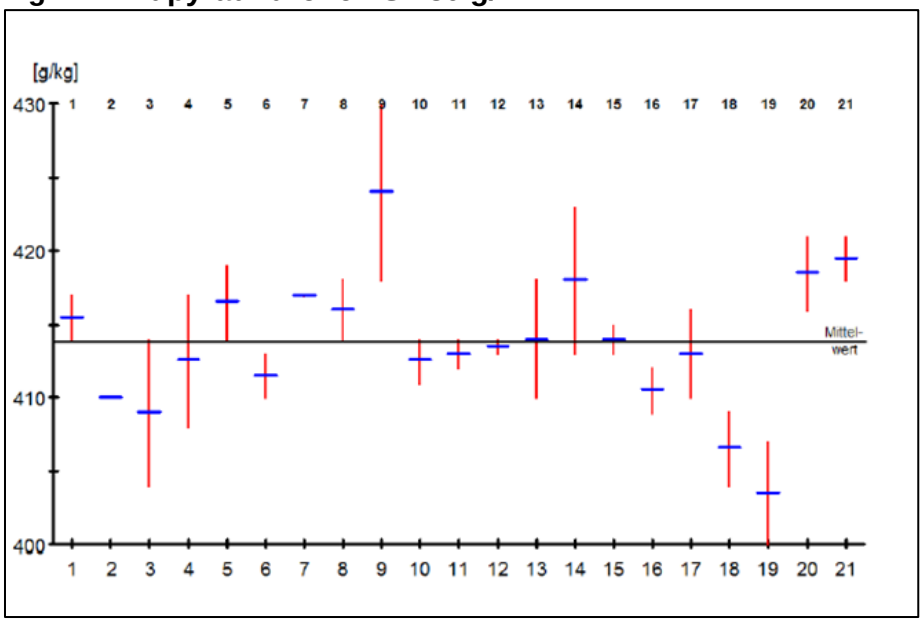
Mean value:	**63.98 g/kg**
s_r:	**0.67**
s_R:	**1.11**
RSD_R:	**1.74**
$RSD_{R\ (Hor)}$:	**3.03**
HorRat:	**0.57**
Outlier (Grubbs):	**upper**

Fig. 11 - Flupyradifurone EW 85.5 g/L (26.3 g/L Flupyradifurone)



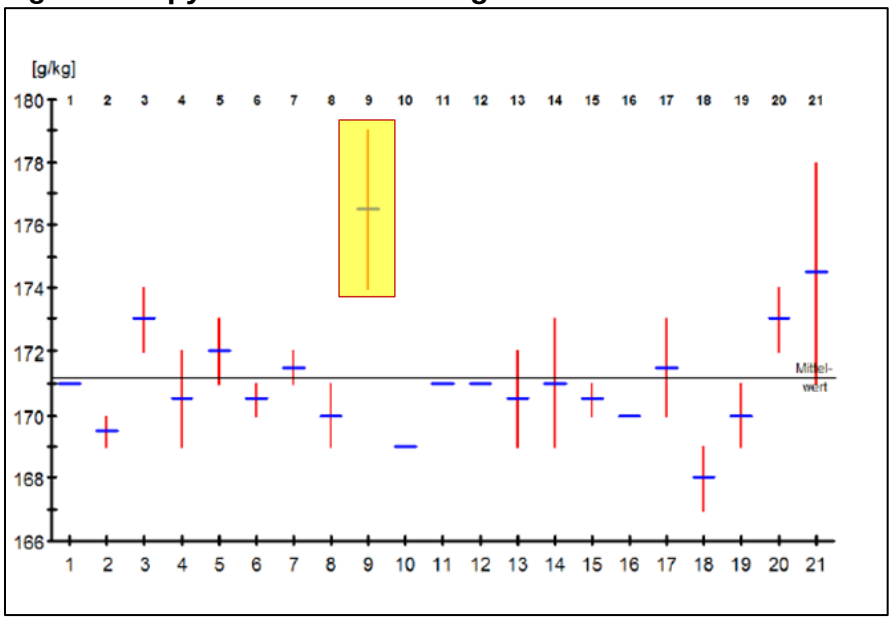
Mean value:	**23.15 g/kg**
s_r:	**2.67**
s_R:	**4.72**
RSD_R:	**20.41**
$RSD_{R\ (Hor)}$:	**3.53**
HorRat:	**5.78**
Outlier (Grubbs):	**no**

Fig. 12 - Flupyradifurone FS 480 g/L



Mean value:	**413.74 g/kg**
s_r:	**4.14**
s_R:	**5.43**
RSD_R:	**1.31**
$RSD_{R\ (Hor)}$:	**2.28**
HorRat:	**0.57**
Outlier (Grubbs):	**no**

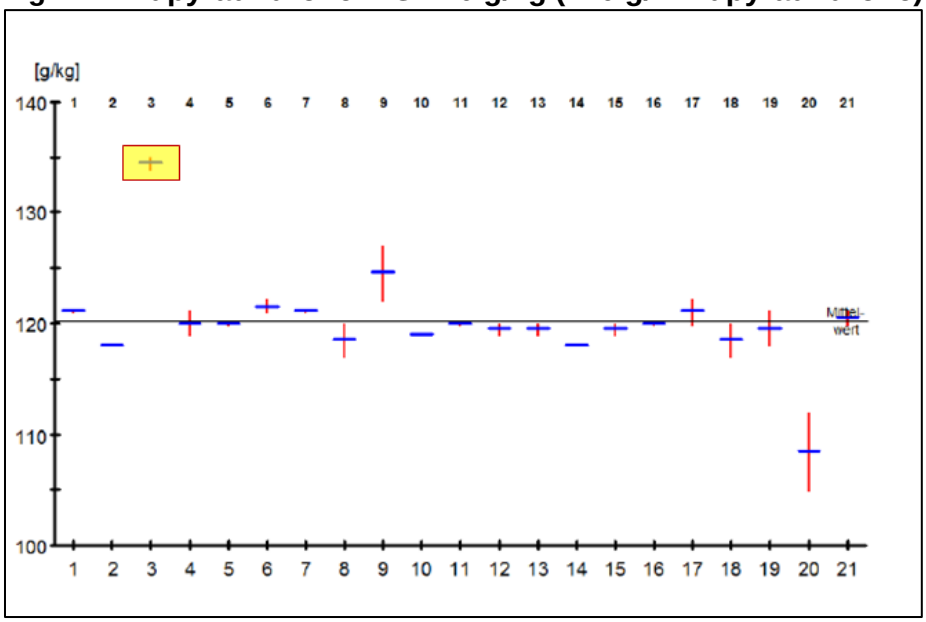
Fig. 13 - Flupyradifurone SL 200 g/L



Mean value:	**171.17 g/kg**
s_r:	**1.86**
s_R:	**2.29**
RSD_R:	**1.34**
$RSD_{R\ (Hor)}$:	**2.61**
HorRat:	**0.51**
Outlier (Grubbs):	**yes***

* Upper Grubbs Straggler

Fig. 14 - Flupyradifurone WG 240 g/kg (120 g/L Flupyradifurone)



Mean value:	**120.12 g/kg**
s_r:	**1.65**
s_R:	**4.53**
RSD_R:	**3.77**
$RSD_{R\ (Hor)}$:	**2.75**
HorRat:	**1.37**
Outlier (Grubbs):	**upper**

7. Conclusions

A total of 22 different laboratories have participated in this full scale collaborative study.
The data of one participant were discarded due to significant changes applied.
The statistical evaluation is reported of 13 laboratories which used the column described in the CIPAC method. Due to cold stability problems of the EW formulation the statistical evaluation of 8 laboratories is reported separately.
In addition the data of the 21 laboratories participating in the CIPAC collaboration trial have been used for the statistical evaluation.
The data presented in the statistical summary show that the method is suitable to gain acceptable and reproducible results for all samples tested and is therefore regarded to be robust.

Bayer AG, Crop Science Division 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 Flupyradifurone in TGAI and associated formulations: AL, EC, EW, FS, SL and WG.