Bayer CropScience

STUDY TITLE

CIPAC Collaborative Trial

Mefenpyr-diethyl

Report to CIPAC

Data Requirement

Collaborative Trial 651.229 (information sheet 274) for the Determination of Mefenpyr-diethyl in the Technical Active Substance and in Formulations by HPLC

AUTHOR

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STUDY COMPLETION DATE

2008-05-16

PERFORMING LABORATORY

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Certification of Authenticity

PRODUCT RESPONSIBLE SCIENTIST

BAYER CROPSCIENCE AG

Dr. U. Doeller: _____

Date: 2008-05-16

HEAD OF TEST FACILITY (BCS-R-PT-AF)

BAYER CROPSCIENCE AG

Dr. M. Feyerabend: _____

Date: 2008-05-19

Archiving

The original report as well as all raw data relevant to this study will be stored in the archive of BCS-R-PT-AF.

Table of Contents

1 List of Participants

1	Cornel Grecu	ALCHIMEX	Bucharest	Romania
2	Dr. Héctor Di Loreto	Gerente de Desarrollo IPESA S.A.	Buenos Aires	Argentina
3	Dr Jim Garvey	Pesticide Control Laboratory Backweston Laboratory Campus	Backweston	Ireland
4	Alexandra Michel	Bayer Cropscience AG, FT Analytics	Frankfurt	Germany
5	Dr. Tillmann Werner	Bayer Industry Services GmbH & Co. OHG	Dormagen	Germany
6	Andrew Plumb	Food Science Group Central Science Laboratory	York	England
7	Nunchana Luetrakool	Agricultural Production Science Research, Department of Agriculture	Bangkok	Thailand
8	Dipl-Ing.Olga Novakova	State Phytosanitary Administration Department of Chemical Laboratories	Bruno	Czech Republic
9	Dr Helen Karasali	Laboratory of Chemical Analysis of Pesticides, Benaki Phytopathological Institute	Athens	Greece
10	Ping Wan	Chair, AAPCO Check Sample Program, Pesticide Formulations Laboratory, Office of Indiana State Chemist	West Lafayette	USA
11	Bruno Patrian	Eidgenössische Forschungsanstalt für Obst-, Wein-, und Gartenbau	Wädenswil	Switzerland
12	Dr. Leonhard	BASF AG	Limburgerhof	Germany
13	Luis Manso	Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura,Pesca y Alimentación	Madrid	Spain

14	Dr. Uwe Doeller	Bayer Cropscience AG, PT Analytics	Frankfurt	Germany
15	Peter Wagener	Bayer Cropscience AG, PT QK	Frankfurt	Germany
16	Teodora Iurascu	Central Laboratory for Phytosanitary Quarantine Laboratory for Quality Control of Pesticides	Bucharest	Romania

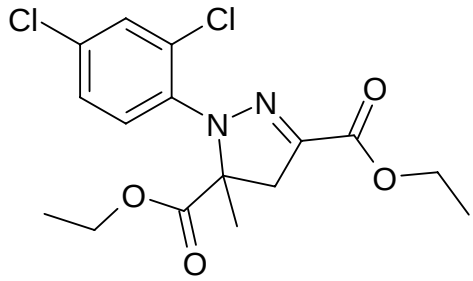
2 Test substance

Common name: MEFENPYR-DIETHYL

Company code: AE F107892

Trade names: none

Structure:



Molecular Formula: $C_{16}H_{18}Cl_2N_2O_4$

Molar Mass: 373.26 g/mol

Chemical name: (RS)-diethyl-1-(2,4-dichlorophenyl)-5-methyl-2-pyrazoline-3, 5-dicarboxylate (IUPAC)

CAS No.: 135590-91-9

Activity: Safener

On the 51th meeting of the “Collaborative International Pesticides Analytical Council” CIPAC in Durban, South-Africa it was decided to perform this collaborative trial, organized by Bayer CropScience.

3 Samples

In December 2007 the following samples and reference items were sent to the participants. On request, some participants were also supplied with the respective analytical coloums:

- Approx. 1 g of Mefenpyr-diethyl calibration substance, CoA 13697
  Product code AE F107892 00 1B99 0002 **content 994 g/kg**

- Approx. 15 g of technical Mefenpyr-diethyl AE F107892
  Batch EK2 M000122 PV2 CoA 14574 (TC) **approx. 950 g/kg**

- Approx. 15 g of AE F075032 08 WG19 A3 Master-ID 0002612-001
  Sekator new batch 2006-000195 (Formulation 1) **approx. 125 g/kg**

- Approx. 15 g of AE F046360 52 1L09 B1 (Solvesso 200 ND)
  Hussar OF batch EFKM001297 (Formulation 2) **approx. 25 g/kg**

- Approx. 15 g of Fenoxaprop-P-ethyl/Mefenpyr-diethyl
  EW 69 + 75 g/l Ralon Super batch EFKM001272 (Formulation 3)
  approx. 71 g/kg

- Approx. 15 g of Fenoxaprop-P-ethyl + Mefenpyr-diethyl EC 120 + 33 g/l, Puma Wheat batch AAKI00885 (Formulation 4)
  approx. 32 g/kg

4 Methods

For MEFENPYR-DIETHYL a draft CIPAC method based on the analytical methods AM002804FF3 and AM003404FP1 supplied by Bayer CropScience was tested. For the technical active substance and the formulated products the two different method parts A and B have to be used.

4.1 Scope

The determination of the active substance content in the technical material and in the formulations was performed in a range between 19.7 and 969.0 g/kg.

4.2 Principle

Analytical method for the TGAS based on AM003404FP1 (part A):
The samples were homogeneously melted at approx. 80°C for approx. 0.5 h followed by shaking of the sample container prior to the sample weighing. The samples were dissolved in acetonitrile and water. Separation was achieved by reversed phase high performance liquid chromatography and detection with a UV spectral photometric detector. The quantitative results were obtained by comparison with a certified external standard.

Analytical method for formulated products based on AM002804FF3 (part B):
The samples were dissolved in 1, 4-Dioxane, treated in an ultrasonic bath for 10 minutes and then Isooctane was added. The samples were separated by normal phase high performance liquid chromatography and detected with a UV spectral photometric detector. The quantitative results were obtained by comparison with a certified external standard.

4.3 Procedure

Each sample had to be prepared and measured twice at two different days. The HPLC determinations should be performed in duplicate injections each in the following sequence:

1st Day:

Calibration 1
Calibration 1
Calibration 2
Calibration 2
Sample 1 (1st preparation run 1)
Sample 1 (1st preparation run 2)
Sample 1 (2nd preparation run 1)
Sample 1 (2nd preparation run 2)
Sample 2 (1st preparation run 1)
Sample 2 (1st preparation run 2)
Sample 2 (2nd preparation run 1)
Sample 2 (2nd preparation run 2)
Calibration 1
Calibration 2
Sample 3 (1st preparation run 1)
etc.

2nd Day:

Calibration 1
Calibration 1
Calibration 2
Calibration 2
Sample 1 (1st preparation run 1)
Sample 1 (1st preparation run 2)
Sample 1 (2nd preparation run 1)
Sample 1 (2nd preparation run 2)
Sample 2 (1st preparation run 1)
Sample 2 (1st preparation run 2)
Sample 2 (2nd preparation run 1)
Sample 2 (2nd preparation run 2)
Calibration 1
Calibration 2
Sample 3 (1st preparation run 1)
etc.

For the quantification the mean of the calibration factors bracketing a sample should be used.

5 Remarks of the Participants for part A (TC)

Lab 1, 2, 6, 10, 12, 13, 14, 16: no comments/no problems encountered.

Lab 3: Small changes in dimension of the column used (see methods and deviations). Method is satisfactory.

Lab 4: Only 50 mg of TC sample used in 100 ml/ dilution 10 => 100 ml.

Lab 5: The method was performed without problems.

Lab 7: Relative Difference of mean and RT are good (< 0.5 %).

Lab 8: Calibration curve measured twice at the same concentration due to lack of standard.

Lab 9: Manual injection 5 µl, different HPLC system used (see methods and deviations).

Lab 11: Procedure easy to follow. Shift of retention time at day 2 may be due to ODS column or due to un-buffered eluent. No impact on peak areas.

Lab 15: No sufficient amount of TC sample available, label not stable after melting, retention time in example chromatogram not in line with written details in the method.

6 Remarks of the Participants for part B (Formulations)

Lab 1: Volatility of the sample preparation solvent may cause problems.

Lab 2: Dilution factors have not been considered in the reporting excel sheet.

Lab 3: Satisfactory. Small changes in the column dimension (methods and deviations).

Lab 4: No dilution for EW, EC and OD formulation samples.

Lab 5: No dilution of formulation samples. Sample 1 filtered through PTFE filter. Different column used.

Lab 6: Different flow rate due to deviating column size (see methods and deviations).

Lab 7: Only solvent A used due to base line drift. Use of normal phase HPLC questioned.

Lab 8: Serious problems with the HPLC. Column did not separate the analytes well, mistake in the sample preparation procedure of the WG identified (draft method). Own mistakes claimed in performing the normal phase HPLC analysis (first time for the respective lab).

Lab 9, 13, 14, 16: No comments/no problems encountered.

Lab 10: Method exactly followed, 300 nm would work better than 227 nm for them on old silica column.

Lab 11: Sample preparation procedure in the formulation method needs to be edited (dilution step).

Lab 12: Poor solubility of the 0.15% water in the eluents.

Lab 13: No dilution for EW, EC and OD formulation samples.

Lab 15: Container of EW sample only partly sealed. Sample preparation procedure in the formulation method needs to be edited (dilution step). Not sufficient amount of samples available.

7 Results and Discussion

The statistical evaluation of the collaborative trial was performed according to DIN ISO 5725. Samples were sent to 17 laboratories. By start of the evaluation, 16 had sent back their results. One laboratory had to stop the trial due to illness of the operator. The results obtained by the collaborators as well as the statistical evaluation are reported in the tables and figures in the appendix. The results of the TC, the WG, OF, EW and EC formulations were analyzed with the Cochran test of

variance homogeneity. In all groups a straggler and an outliers was detected. The outliers are related to laboratories 3(*2*), 6, 10 and 19. .

Two outliers were found by the Grubbs test for the TC and the OD formulation (lower limit).

For all groups the reproducibility relative standard deviation RSD_R was clearly below the Horwitz criterion. Therefore no elimination of outliers was applied to the final statistical evaluation.

8 Conclusions

When applied to the technical active substance and to the formulations the two liquid chromatographic methods parts which were tested in this CIPAC collaborative trial gave results within the usually accepted range of variations for technical substance, WG, OD, EW and EC formulations.

It is recommended to accept this methods (part A and B) as full CIPAC method for Mefenpyr-diethyl and the formulations tested.

9 Appendix

Table 1: Equipment and deviations from part A (TC)

Lab. No.	HPLC-System	column type	length (mm)	int. Diameter (mm)	particle Size (µ)	column temperature	flow rate ml/min	injection Volume (µL)	wavelength	eluent	remarks
1	**Waters 1525 Binary HPLC Pump**	*	*	*	*	*	*	*	*	*	none
2	**SHIMADZU 10 VP**	*	*	*	*	*	*	*	*	*	none
3	**Agilent 1100 Series HPLC G1313A**	Thermo Scientific, ODS Hypersil	150	4.6	5					*	Small differences in column dimensions
4	**Hewlett Packard G1311A**	*	*	*	*	*	*	*	*	*	none
5	**Agilent 1100:**	*	*	*	*	*	*	*	*	*	none
6	**Hewlett Packard HP 1050 pump**	HyperClone ODS, Phenomenex Part No. 00E-43361-DO	125	4	5	*	*	*	*	*	none
7	**Agilent 1100 Series: Quat Pump**	LiChroCART: Lichrospher 100 RP-18	125	4	5						none
8	**Waters Alliance 2695 separation module**	*	*	*	*	*	*	*	*	*	none

*according to the method

Lab. No.	HPLC-System	column type	length (mm)	int. Diameter (mm)	particle Size (µ)	column temperature	flow rate ml/min	injection Volume (µL)	wavelength	eluent	remarks
9	**Shimadzu LC 10AD**	*	*	*	*	*	*	5	*	*	Loop 5µl (instead of 20 µl)
10	**Waters 2695 Separations Module**	Alltech Hypersil ODS	250	4.6	5	*	*	*	*	*	none
11	**Dionex P680 HPLC Pump**	Infochroma, Relia Sil ODS	125	4	5	*	*	*	*	*	none
12	**Agilent 1100**	*	*	*	*	*	*	*	*	*	none
13	**Agilent 1100**	Phenomenex Gemini C18 110A 388217-15	150	3	**	*	*	*	*	*	Less amount of calibration solutions and samples have been weighted to avoid dilutions
14	**Hewlett Packard HP 1100 Series**	*	*	*	*	*	*	*	*	*	none
15	**Hewlett Packard HP 1100 Series**	*	*	*	*	*	*	*	*	*	none
16	**VARIAN PROSTAR**	Thermo, ODS Hypersil	125	4	3	*	*	*	*	*	none

*according to the method

** no data given

Table 2: Equipment and deviations from part B (formulations)

Lab. No.	HPLC-System	column type	length (mm)	int. Diameter (mm)	particle Size (µ)	column temperature	flow rate ml/min	injection Volume (µL)	wavelength	eluent	remarks
1	**Waters 1525 Binary HPLC Pump**	*	*	*	*	*	*	*	*	*	Dioxane p.a. total run time 30 min. A short time is not enough for column equilibration with mobile phase A.
2	**Hewlett Packard; 1100**	*	*	*	*	*	*	*	*	*	none
3	**Agilent 1100 Series HPLC G1313A**	Thermo Scientific, Hypersil	150	4.6	3					*	Small differences in column dimensions
4	**Hewlett Packard G1311A**	*	*	*	*	*	*	*	*	*	none
5	**Agilent 1100:**	Luna Silica	150	4.6	3	*	2.1	*	*	A: Isooctane B: 1,4-dioxane (+ 0,15 % water)	Other flow rate because other column
6	**Hewlett Packard HP 1050 pump**	HyperClone Silica, Phenomenex Part No. 00F-4353-EO	150	4.6	3	*	2.0	*	*	*	Flow rate increased to 2,0 ml/min to take into account different dimensions to those given in method
7	**Agilent 1100 Series: Quat Pump**	PhaseSep: Spherisopb S5W, 167588	150	4	**	*	*	*	300	A: iso-Octane / 1,4-Dioxane(+ 0.15% water) (97:3)	Used only solv. A because the baseline was drifted when used both solv. A and B
8	**No info**	*									No information given

*according to the method

** no data given

Lab. No.	HPLC-System	column type	length (mm)	int. Diameter (mm)	particle Size (µ)	column temperature	flow rate ml/min	injection Volume (µL)	wavelength	eluent	remarks
9	**Varian PROStar**	*	*	*	*	*	*	*	*	*	*
10	**Waters 2695 Separations Module**	Alltech Econosphere Silica	150	4.6	5	*	*	*	300	*	Used 300 nm instead 227 nm. Total dilution of calibration solution was 1000 mL.Total dilution of samples was 100mL. Excel calculations corrected by a factor of 10.
11	**Dionex P680 HPLC Pump**	Infochroma, Relia sil SI, 3µm, 125 x 4 mm	125	4	3	*	*	*	*	*	In the sample preparation process we diluted all samples 1:10 with the mobile phase B as with the calibration samples.
12	**Agilent 1100**	*	*	*	*	*	*	*	*	*	none
13	**Agilent 1100**	Hypersil Silica 120A	150	4	3	*	*	10	*	A: 97% 2,2,4-trimethylpentane B: 3% 1,4-dioxane (+ 0,15% water)	Less amount of calibration solutions and samples have been weighted to avoid dilutions. Injection volume 10µL.
14	**Hewlett Packard HP 1200 Series**	*	*	*	*	*	*	*	*	*	none
15	**Hewlett Packard HP 1100 Series**	*	*	*	*	*	*	*	*	*	none
16	**VARIAN PROSTAR**	Thermo, Hypersil Silica	150	4	3	*	*1.2	*	*	*	The flow rate was changed at 1.2 ml/min because the dimensions of the column are 150 x4 mm i.d..

*according to the method

Table 2: Results TC

Laboratory	1	2	3	4	5	6	7	8	9	10**	11	12	13	14	15	16
Day 1	963,1	963,5	958,8	960,6	954,3	950,8	957,8	956,8	955,7	**922,4**°°	949,6	956,4	957,8	957,6	958,2	954,4
Day 2	957,9	960,2	965,6	961,6	957,5	958,9	953,8	958,2	955,6	956,3	954,1	957,2	957,1	958,6	957,1	954,4
Mean	960,5	961,8	962,2	961,1	955,9	954,9	955,8	957,5	955,7	**939,3**	951,9	956,8	957,5	958,1	957,7	954,4
Std.dev. sj	3,719	3,615	4,828	1,413	3,350	5,925	3,915	1,984	2,021	**19,633**	3,777	1,415	1,810	1,133	2,578	1,828

Table 3: Results WG A13

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Day 1	133,7	132,2	131,5	131,6	131,4	130,1	129,9	135,2	125,4	135,9	132,7	132,4	134,8	133,9	133,5	127,5
Day 2	133,1	133,0	**140,2**°°	131,9	133,9	134,7	131,5	134,7	125,5	134,1	132,2	129,3	137,3	133,1	132,0	127,3
Mean	133,4	132,6	135,9	131,8	132,6	132,4	130,7	135,0	125,5	135,0	132,4	130,9	136,1	133,5	132,7	127,4
Std.dev. sj	0,356	0,792	**5,241**	0,335	1,482	2,929	1,029	1,093	0,677	1,353	0,344	4,718	1,549	0,521	0,926	0,309

Table 4: Results OD 1LB09 B1

Laboratory	1	2	3	4	5	6**	7	8	9	10	11	12	13	14	15	16
Day 1	24,8	24,5	24,6	24,8	24,6	**21,0**°°	24,7	25,0	25,0	24,7	25,1	24,5	25,0	24,8	25,1	24,6
Day 2	24,7	24,5	25,2	24,7	25,0	24,1	24,7	24,5	24,8	24,0	24,9	24,9	24,8	24,7	24,8	24,0
Mean	24,8	24,5	24,9	24,7	24,8	**22,5**	24,7	24,7	24,9	24,3	25,0	24,7	24,9	24,7	24,9	24,3
Std.dev. sj	0,068	0,041	0,402	0,086	0,219	**2,072**	0,146	0,336	0,111	0,732	0,105	0,284	0,120	0,040	0,238	0,375

Table 5 Results EW 69 +75 g/L

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Day 1	71,1	70,9	**66,4**°°	70,8	70,7	69,7	70,0	71,4	71,2	71,5	71,4	69,5	71,5	71,2	72,8	70,6
Day 2	70,5	71,2	73,1	70,4	71,9	71,9	70,5	71,8	71,8	66,4	71,7	71,7	71,2	72,0	71,7	70,4
Mean	70,8	71,0	**69,8**	70,6	71,3	70,8	70,2	71,6	71,5	69,0	71,6	70,6	71,4	71,6	72,3	70,5
Std.dev. sj	0,393	0,279	**5,398**	0,335	0,750	1,271	0,319	0,634	0,324	3,314	0,274	1,666	0,236	0,463	0,645	0,196

Table 6: Results EC 120 + 33 g/l

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Value 1	34,1	34,0	34,1	34,1	33,8	32,8	32,7	35,2	33,0	34,0	34,3	34,0	34,3	34,0	34,9	**35,5**°°
Value 2	34,1	34,5	34,8	34,3	34,5	33,6	34,6	35,4	33,0	34,3	34,2	34,2	34,1	33,9	34,5	**33,0**
Mean	34,1	34,2	34,5	34,2	34,1	33,2	33,6	35,3	33,0	34,1	34,2	34,1	34,2	34,0	34,7	34,2
Std.dev. sj	0,039	0,274	0,543	0,183	0,386	0,512	1,104	0,561	0,034	0,711	0,107	0,227	0,110	0,093	0,250	**1,425**

**outlier according to Grubbs test (1% one sided), lower limit

*straggler according to Grubbs test (5% one sided)

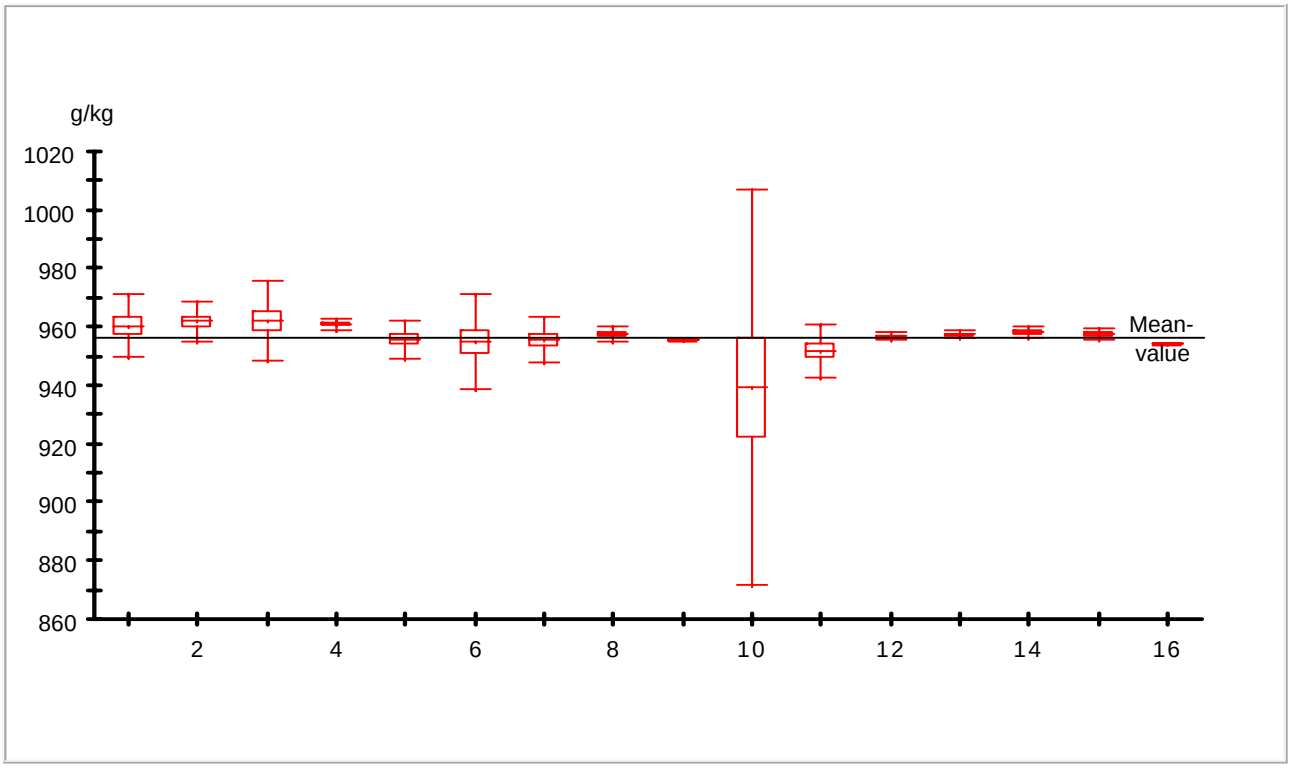
°°outlier according to Cochran test (1% one sided)

°straggler according to Cochran test (5% one sided)

Table 3: Final statistical evaluation

Sample	TC	WG 19 A3	OD 1L09 B1	EW 69+75	EC 120+33
X	956,3	132,4	24,6	70,9	34,1
N	16	16	16	16	16
L	16	16	16	16	16
sr	5,799	2,086	0,586	1,711	0,561
sL	4,483	2,625	0,510	0,000	0,456
sR	7,330	3,353	0,777	1,711	0,723
RSDr	0,606	1,576	2,385	2,413	1,644
RSDR	**0,767**	**2,533**	**3,160**	**2,413**	**2,120**
r	16,238	5,842	1,642	4,790	1,570
R	20,525	9,389	2,175	4,790	2,024
RSDR (Horwitz)	**2,013**	**2,712**	**3,493**	**2,979**	**3,325**

Figure 1: Mefenpyr-diethyl TC



x-axis : laboratory number each
y-axis: content in g/kg each

Figure 2: WG 19 A3

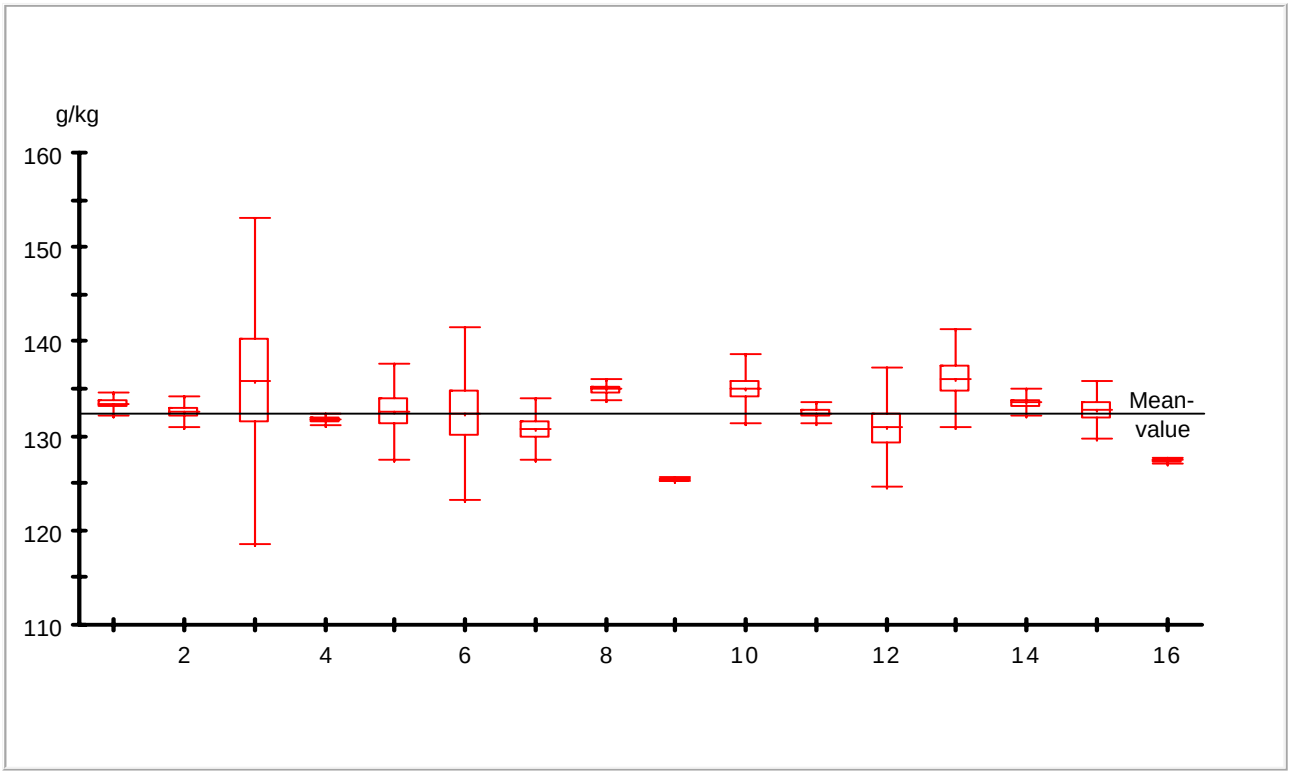


Figure 3: OD 1L09 B1

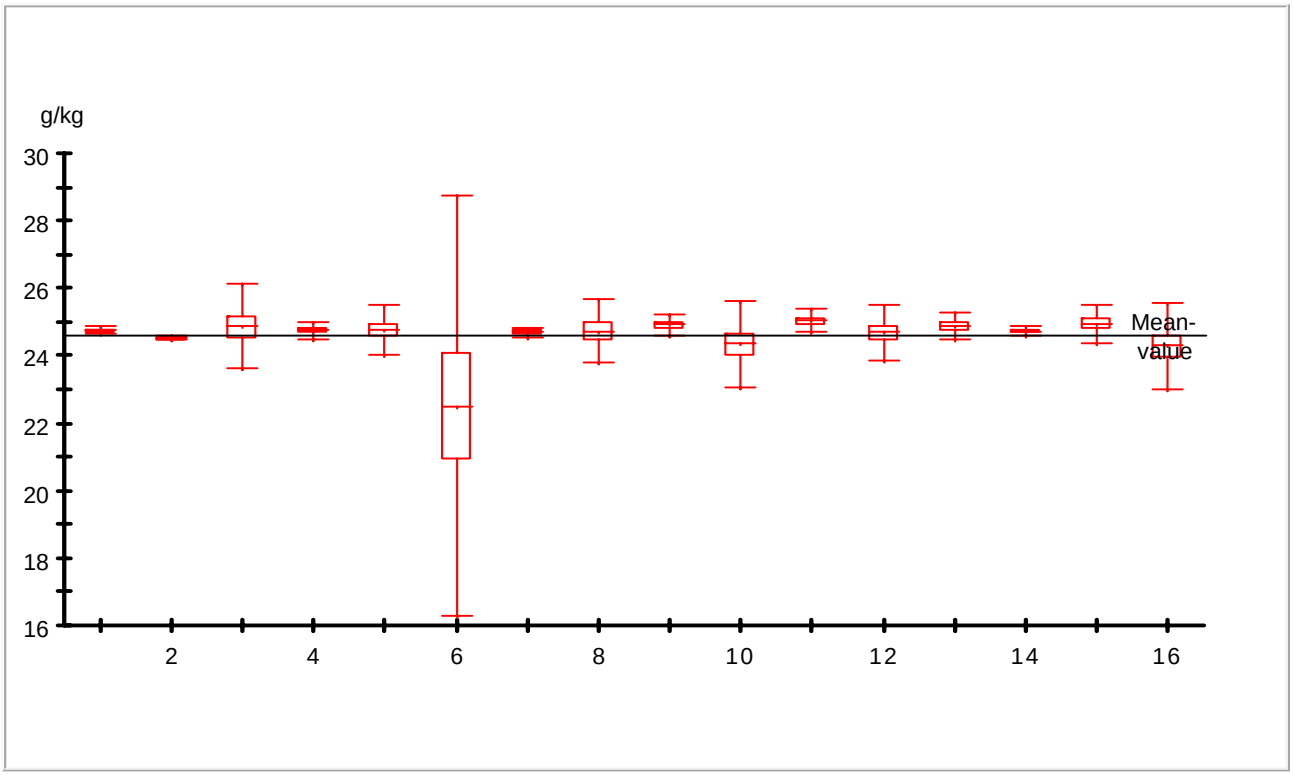


Figure 4: EW 69 + 33 g/L

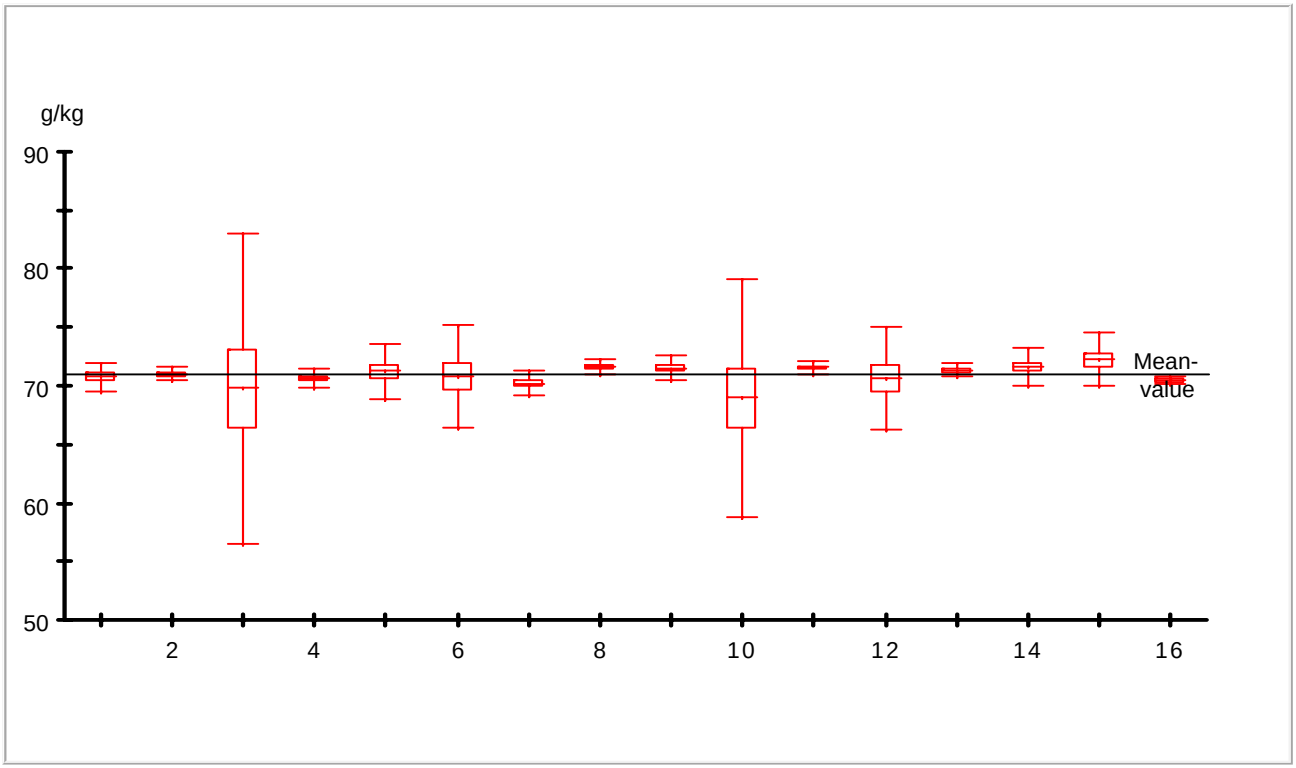


Figure 5: EC 120 + 33 g/L