



## ***Laboratory Performance Assessment***

### ***Analysis of Pesticides in Amaranth***

#### ***Report***

October 2014

## Summary

The laboratory performance assessment related to pesticides in amaranth was designed and organised by Lach & Bruns in cooperation with PROOF-ACS in October 2014 on behalf of BNN e.V. (Bundesverband Naturkost Naturwaren).

The test material was prepared of organic amaranth. Seven pesticides were spiked to the surface of the whole, unmilled amaranth:

*Chlorotoluron, Dieldrin, Epoxiconazole, Piperonyl butoxide (PBO), Pencycuron, Pirimiphos-methyl, and Pyrethrins.*

The test material was distributed to thirteen participants across four European countries (Germany, Italy, Spain, the Netherlands). Each laboratory received 200 g of whole, unmilled amaranth. No information with respect to the identity or the number of spiked pesticides was provided to the laboratories in advance. The laboratories were requested to identify and quantify seven pesticides in the test sample. All participants kept the term of submission of results and were considered for evaluation.

The performance assessment considers the following test criteria:

- No *false positive results*.
- Correct *identification* of seven pesticides.
- Correct *quantification* of six pesticides (Chlorotoluron, Dieldrin, Epoxiconazole, Piperonyl butoxide, Pencycuron, Pirimiphos-methyl) in terms of 70 to 120 % recovery of the spiked value.

Summary of the performance of the laboratories with respect to the identification and quantification of the pesticides:

<b>Criterion</b>	<b>Criterion passed</b>
Identification of the seven spiked pesticides	11 out of 13 laboratories (85 %)
Quantification of six pesticides - Chlorotoluron, Dieldrin, Epoxiconazole, PBO, Pencycuron, Primiphos-methyl - within 70 to 120 % recovery of the spiked level	5 out of 13 laboratories (38 %)

## Assessment of quantification

Analytical results between 70 and 120 % recovery of the spiked levels are considered satisfying for the assessment of the correct quantification of the pesticides.

Pesticide	Spiked level [µg/kg]	Assigned value [µg/kg]	Number of results	Correct quantification
Chlorotoluron	42	32.6	13	8 out of 13 labs (62 %)
Dieldrin	24	21.6	13	11 out of 13 labs (85 %)
Epoxiconazole	27	24.9	13	10 out of 13 labs (77 %)
Piperonyl butoxide	130	109	13	11 out of 13 labs (85 %)
Pencycuron	37	31.1	13	12 out of 13 labs (92 %)
Pirimiphos-methyl	45	40.7	12	12 out of 13 labs (92 %)

Pyrethrins were spiked to the test material as a technical mixture of Pyrethrine I&II, Cinerin I&II, and Jasmoline I&II. Since pyrethrins are known to be unstable under certain conditions (f.ex. UV light) they were assessed with respect to the correct identification only.

Pesticide	Spiked level [µg/kg]	Assigned value [µg/kg]	Correct identification
Pyrethrins (sum)	540	331	12 out of 13 labs (92 %)

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## 1. Test material preparation and design

Amaranth is one of the upcoming products, especially in the organic sector. It is widely used for example in mueslis and cereal bars.

Ideal laboratory performance assessments should test the entire analytical procedure, including sample pretreatment, sample preparation and analysis. To achieve this goal, test materials should be provided to the participants which are designed as close as possible to routine samples. Whole, unmilled amaranth was used in this laboratory performance assessment. Thus, the critical step with respect to the sample preparation, the uniform milling of the amaranth, is included in the test.

Amaranth is well suitable for the preparation of a spiked but whole and unmilled test material. The surface is smooth and the individual grains are small in size.

An external laboratory prepared the test material under supervision of PROOF-ACS.

10 kg of whole, unmilled organic amaranth was used as raw material. The absence of pesticides was confirmed at a level of 10 ppb. The raw material was thoroughly homogenised and subsamples of 200 g of each were bottled as blank materials thereafter.

The amaranth grains were coated applying a solution of 7 pesticides to give the final concentrations:

42 µg/kg Chlorotoluron, 24 µg/kg Dieldrin, 27 µg/kg Epoxiconazole, 130 µg/kg Piperonyl butoxide, 37 µg/kg Pencycuron, 45 µg/kg Pirimiphos-methyl, and 540 µg/kg of a technical mix of Pyrethrins.

For that purpose the pesticide mix was prepared in acetone and added gradually to 6 kg of amaranth while stirring carefully and continuously. Stirring was continued after spiking in order to ensure the homogeneity of the test material. The spiked test material was distributed into PE-bottles with at least 200 g in each. The subsamples were stored at -20°C in the dark until shipment.

The results of the homogeneity and stability testing confirm the uniform distribution of the pesticides in the test material and the stability throughout the whole testing period (section 4 and 5).

## 2. Statistical evaluation of results

### 2.1. Trueness of results

The trueness criterion considers the correct quantification of the actual analyte concentration in the sample. The trueness of the results was assessed as the level of congruence with the spiked level. The coverage of the spiked level was calculated according to the equation below:

$$\text{coverage of the spiked level} = \frac{x}{sl} * 100$$

where x = reported result and sl = spiked level.

## 2.2. Accepted ranges – Trueness criterion

Results within the range of 70-120 % of the spiked level are accepted as satisfying in this laboratory performance assessment in accordance with the guidelines of the BNN<sup>1</sup>. The calculated values which correspond to 70 % recovery are rounded to the next lowest integer (no commercial rounding). For example 18.9 µg/kg (70 % recovery of Epiconazole) is rounded to 18 µg/kg. The calculated values which correspond to 120 % recovery are rounded to the next higher integer. For example 44.4 µg/kg (120 % recovery of Pencycuron) is rounded to 45 µg/kg.

## 2.3. Assigned value

The assigned value  $\hat{X}$  is derived as a robust mean of the results of all participants and represents the consensus of participants' results. The Winsorisation algorithm is applied to minimise the influence of outliers. As a first step the mean and the standard deviation of the results are calculated. After that, all results higher than the mean + 1.5-fold standard deviation are set to the mean + 1.5-fold standard deviation and results lower the mean - 1.5-fold standard deviation are set to the mean - 1.5-fold standard deviation. This procedure is repeated several times, until the robust mean remains the same<sup>2</sup>.

The assigned values are subjected to commercially rounding and are reported with an accuracy of three significant digits.

## 2.4. z-score

The z-score compares the analytical result to the assigned value and can be used to describe the comparability of results. The z-score is derived of the result  $x$  of each participant according to the equation below:

$$z - score = \frac{x - \hat{X}}{\hat{\sigma}_H}$$

The z-scores are provided for information purpose only in this laboratory performance assessment. The criterion for the evaluation of the performance of the laboratories is the trueness criterion only.

## 3. Results

Thirteen laboratories across four European countries (Germany, Italy, Spain, the Netherlands) took part in the laboratory performance assessment. All participants kept the term of submission and were considered for evaluation. Each laboratory was given a randomly selected identifier, hereinafter referred to as laboratory code.

The participants were not informed about the identity or the number of the spiked pesticides. Thus, they were requested to report all sought and found pesticides together with the corresponding recoveries and the reporting limits (RL). The recovery rates were

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<sup>1</sup> BNN, Guidelines for laboratory approval by Bundesverband Naturkost Naturwaren (BNN) e. V. (Federal Association for Natural Foods and Natural Products inc. soc.)

<sup>2</sup> Analytical Methods Committee, "Robust Statistics - How Not to Reject Outliers. Part 1. Basic Concepts," *The Analyst*, vol. 114, no. 12, p. 1693, 1989.

not considered during the results reporting, unless they were lower than 70 % or higher than 120 %.

The results of the participants are presented in tables 1 to 3 (pp. 8-11) and in figures 1 to 6 (pp. 12-17) in relation to the accepted range of 70 to 120 % recovery of the spiked level. Results of the individual pyrethrins and the sum of pyrethrins are presented in table 4 (p. 11) and figure 7 (p. 18). Pyrethrins were assessed with respect to the correct identification in the sample only since they are known to be unstable under certain circumstances.

Lab 9 reported traces of Chlorpyrifos (2 µg/kg) below their reporting limit of 10 µg/kg in the blank material and in the spiked material in addition to the spiked pesticides.

All laboratories reported RLs of 10 µg/kg or lower for all tested pesticides except pyrethrins. For pyrethrins, RLs are in the range of 10 µg/kg up to 100 µg/kg. Reporting limits of 0.01 µg/kg as reported by lab 12 for all pesticides are unrealistically low and must be considered as submitted in mg/kg instead of µg/kg.

**To summarise, the overall performance of the laboratories was quite good:**

- **All in all, nine out of thirteen laboratories (70 %) participated with good or excellent results.**
- **Eleven out of thirteen laboratories identified all pesticides correctly in the test sample.**
- **No false positive results were reported.**
- **Five out of 13 laboratories quantified the six pesticides Chlorotoluron, Dieldrin, Epoxiconazole, Piperonyl butoxide, Pencycuron, and Pirimiphos-methyl within 70 to 120 % recovery of the spiked level.**
- **Four more laboratories failed with just one of the six pesticides to be quantified within 70 to 120 % of the spiking level.**
- **Only four laboratories reported more than one result outside the specification range, while two of these labs reported false negative results, too.**

Table 1. Results of Chlorotoluron and Dieldrin

Laboratory code	Chlorotoluron spiked level: 42 µg/kg assigned value: 32.6 µg/kg					Dieldrin spiked level: 24 µg/kg assigned value: 21.6 µg/kg				
	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 29-51 µg/kg	z-score	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 16-29 µg/kg	z-score
1	26	92	10	no	-0.9	30	70	10	no	1.8
2	36	78	10	yes	0.5	22	103	5	yes	0.1
3	33	95	10	yes	0.1	24	95	2	yes	0.5
4	43	107	10	yes	1.5	16	106	10	yes	-1.2
5	22	94	10	no	-1.5	17	73	10	yes	-1.0
6	22	76	10	no	-1.5	23	89	10	yes	0.3
7	32	95	10	yes	-0.1	25	99	10	yes	0.7
8	23.0	91.0	10	no	-1.3	22.3	96.3	10	yes	0.2
9	44	95	10	yes	1.6	24	95	10	yes	0.5
10	36	85	5	yes	0.5	21	80	5	yes	-0.1
11	34	95	10	yes	0.2	25	100	10	yes	0.7
12	44.2	75.6	0.01	yes	1.6	15.5	83.6	0.01	no	-1.3
13	28	92	10	no	-0.6	18	100	10	yes	-0.8

RL: reporting limit



Table 2. Results of Epoxiconazole and Pencycuron

	<b>Epoxiconazole</b> spiked level: 27 µg/kg assigned value: 24.9 µg/kg					<b>Pencycuron</b> spiked level: 37 µg/kg assigned value: 31.1 µg/kg				
Laboratory code	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 18-33 µg/kg	z-score	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 25-45 µg/kg	z-score
1	26	97	10	yes	0.2	33	92	10	yes	0.3
2	24	108	10	yes	-0.2	36	99	10	yes	0.7
3	22	96	10	yes	-0.5	28	92	10	yes	-0.5
4	26	101	10	yes	0.2	29	116	10	yes	-0.3
5	18	100	10	yes	-1.3	26	108	10	yes	-0.7
6	24	89	10	yes	-0.2	31	79	10	yes	0.0
7	55	98	10	no	<b>5.5</b>	35	100	10	yes	0.6
8	16.0	81.0	10	no	-1.6	29.7	84.0	10	yes	-0.2
9	25	95	10	yes	0.0	32	95	10	yes	0.1
10	24	90	5	yes	-0.2	30	90	5	yes	-0.2
11	51	98	10	no	<b>4.8</b>	35	100	10	yes	0.6
12	30.8	89.6	0.01	yes	1.1	13	78.4	0.01	no	<b>-2.6</b>
13	22	106	10	yes	-0.5	33	109	10	yes	0.3

RL: reporting limit

Table 3. Results of Piperonyl butoxide and Pirimiphos-methyl

	Piperonyl butoxide spiked level: 130 µg/kg assigned value: 109 µg/kg					Pirimiphos-methyl spiked level: 45 µg/kg assigned value: 40.7 µg/kg				
Laboratory code	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 91-156 µg/kg	z-score	Result [µg/kg]	Recovery [%]	RL [µg/kg]	Accepted range: 31-54 µg/kg	z-score
1	147	85	10	yes	1.6	n.r.	-	-	no	-4.5
2	120	91	10	yes	0.5	42	103	10	yes	0.1
3	100	92	10	yes	-0.4	36	85	10	yes	-0.5
4	109	82	10	yes	0.0	42	71	10	yes	0.1
5	87	87	10	no	-0.9	33	97	10	yes	-0.9
6	126	100	10	yes	0.7	39	87	10	yes	-0.2
7	109	102	10	yes	0.0	45	100	10	yes	0.5
8	99.2	99.6	10	yes	-0.4	40.9	101.1	10	yes	0.0
9	125	95	10	yes	0.7	45	95	10	yes	0.5
10	110	90	5	yes	0.0	42	90	5	yes	0.1
11	110	90	10	yes	0.0	41	100	10	yes	0.0
12	76.6	97.8	0.01	no	-1.3	38.6	100	0.01	yes	-0.2
13	100	99	10	yes	-0.4	40	92	10	yes	-0.1

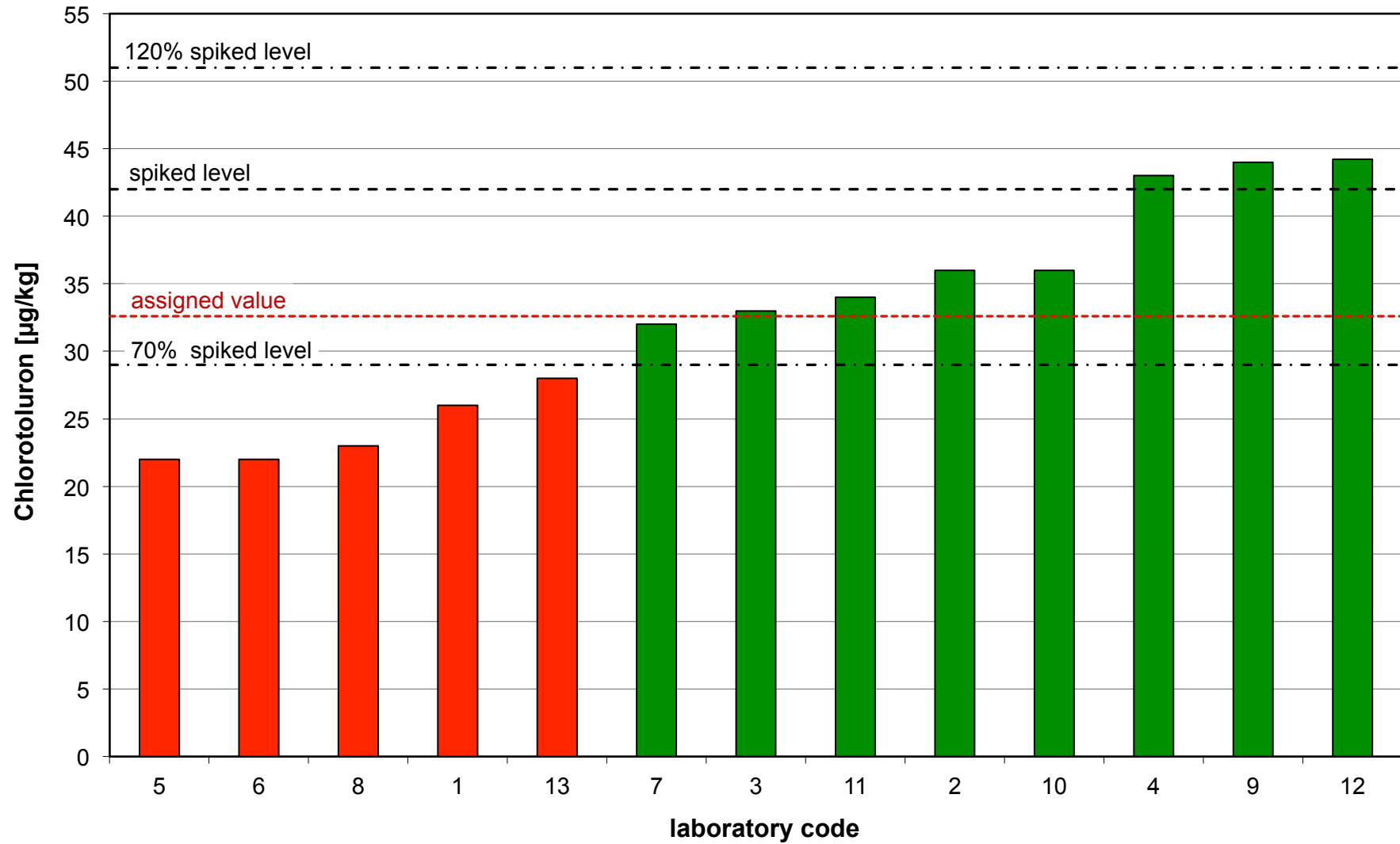
RL: reporting limit  
n.r.: not reported

Table 4. Results of Pyrethrins

Laboratory code	Pyrethrins (sum) spiked level: 540 µg/kg assigned value: 331 µg/kg				Results of the individual Pyrethrins					
	Result [µg/kg]	Recovery [%]	RL [µg/kg]	z-score	Pyrethrine I [µg/kg]	Pyrethrine II [µg/kg]	Cinerin I [µg/kg]	Cinerin II [µg/kg]	Jasmoline I [µg/kg]	Jasmoline I [µg/kg]
1	436	-	-	1.7	217	149	22	25	10	13
2	150	106	50	-2.9	71	54	8	9	9	5
3	330	92	100	0.0	162	110	22	14.5	7	14.5
4	320	95	100	-0.2	182	89	15	16	8	10
5	295	126	10	-0.6	143	95	17	20	12	7
6	358	112	10	0.4	172	119	22	21	13	10
7	380	101	10	0.8	203	129	18	18	12	<RL
8	n.r.	-	-	-5.3	-	-	-	-	-	-
9	338	-	-	0.1	165	110	20	20	13	10
10	300	90	10	-0.5	136	106	18	19	11	10
11	349	101	10	0.3	187	118	17	17	10	<RL
12	139.5	100	0.01	-3.1	68.4	29.0	10.3	10.5	11.2	10.1
13	375	98	50	0.7	178	132	18	22	13	12

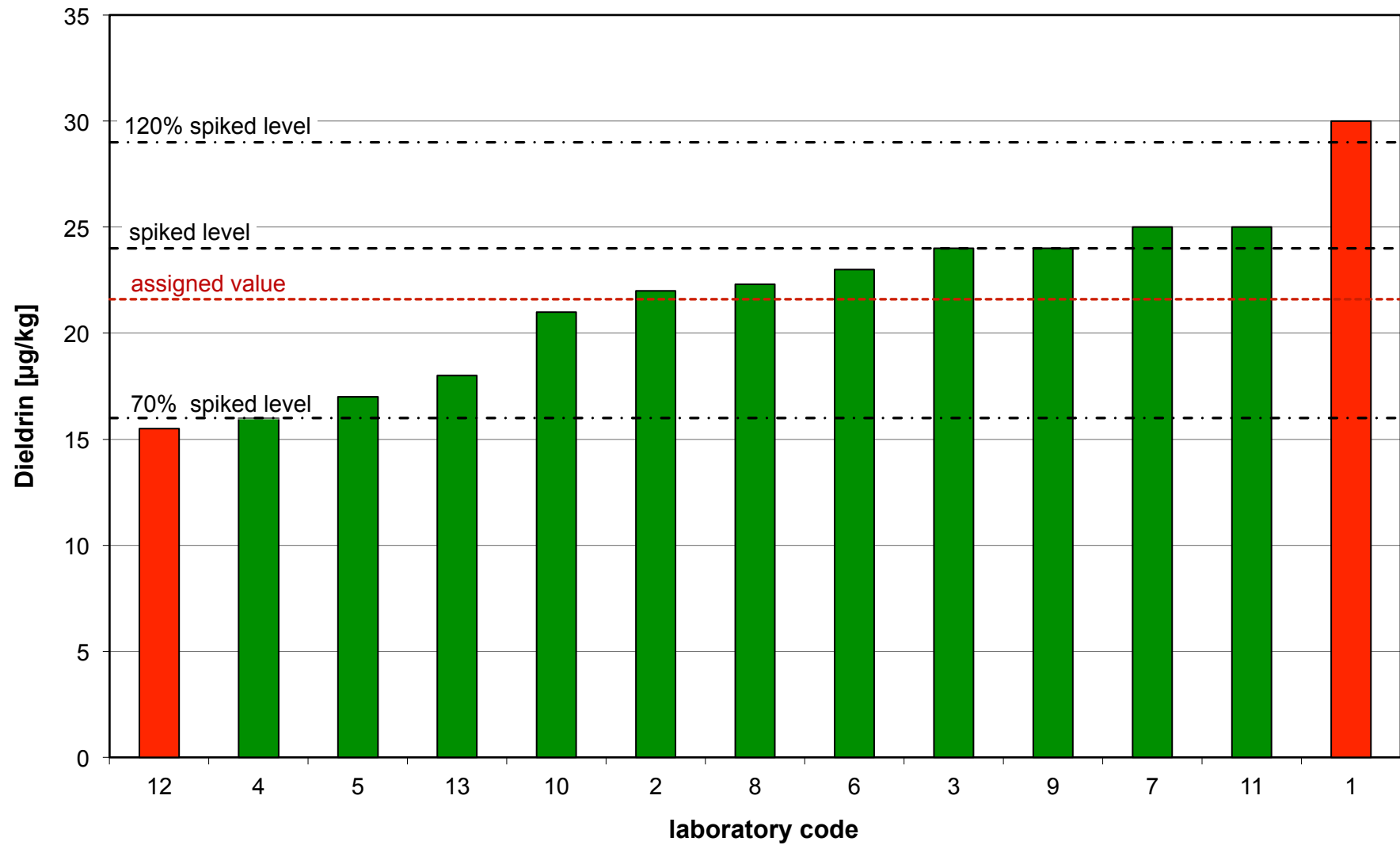
RL: reporting limit  
n.r.: not reported

Figure 1. Assessment of Chlorotoluron (spiked level: 42 µg/kg)



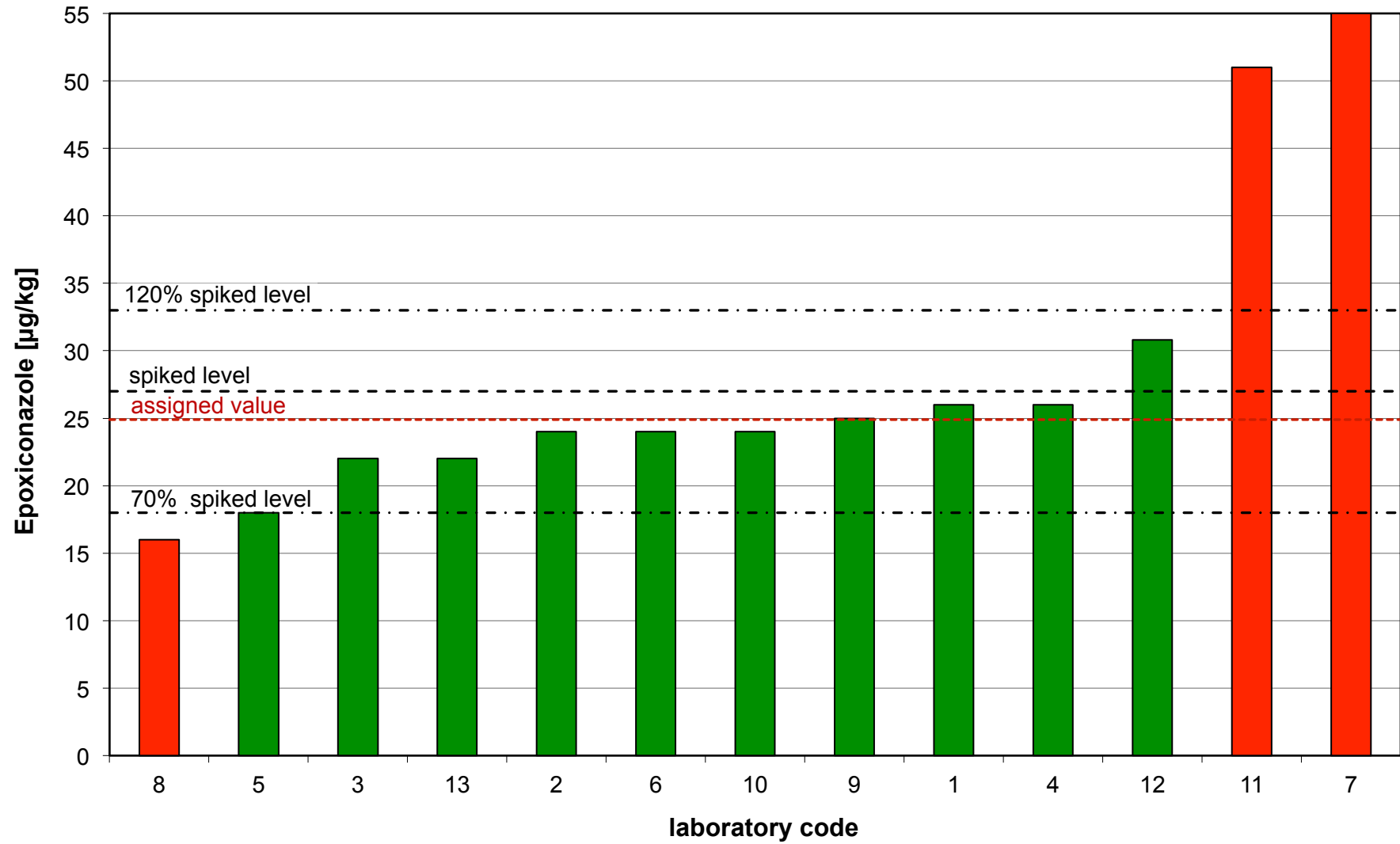
Green: satisfactory results, red: dissatisfactory results

Figure 2. Assessment of Dieldrin (spiked level: 24 µg/kg)



Green: satisfactory results, red: dissatisfactory results

Figure 3. Assessment of Epoxiconazole (spiked level: 27 µg/kg)



Green: satisfactory results, red: dissatisfactory results

Figure 4. Assessment of Pencycuron (spiked level: 37 µg/kg)

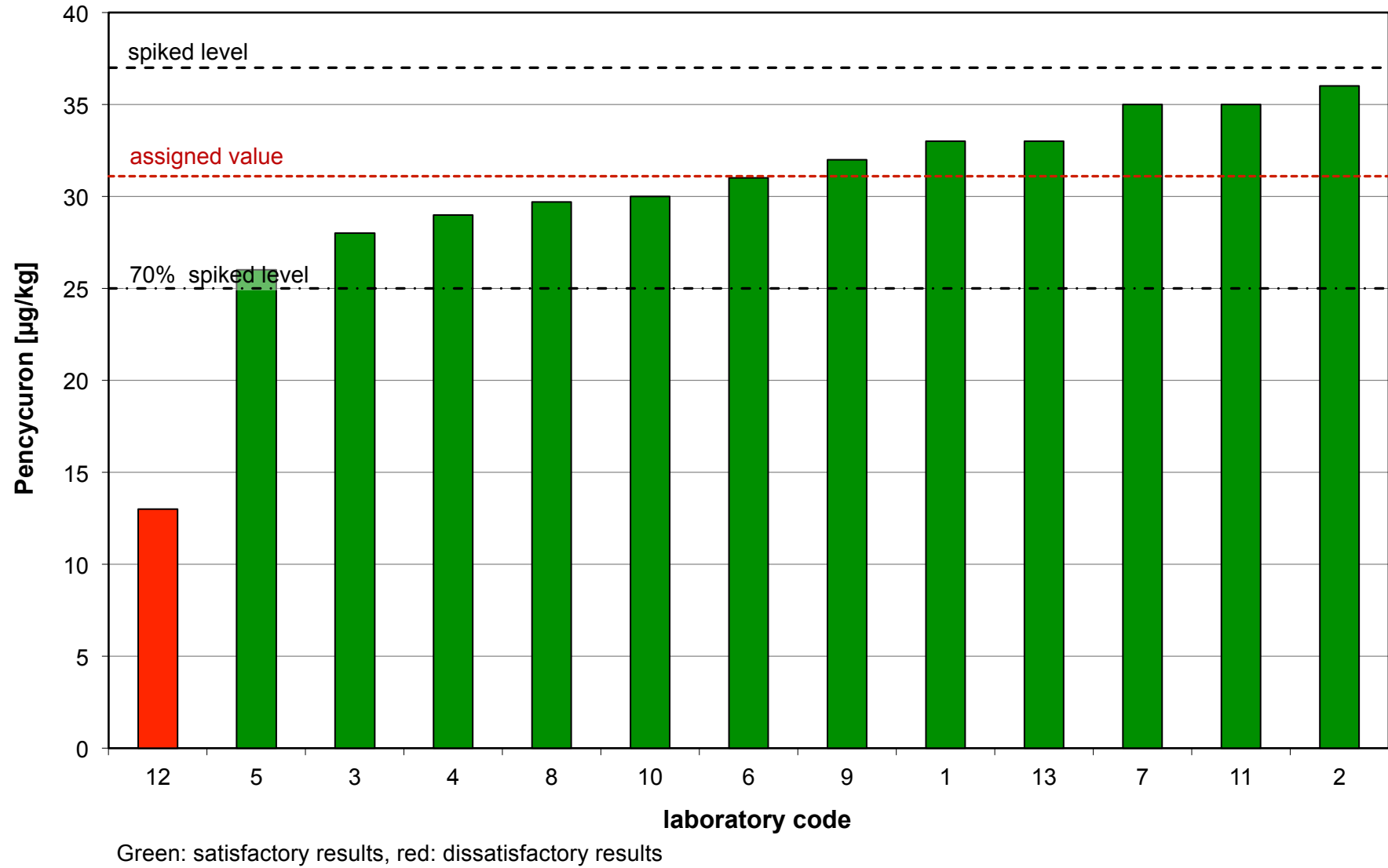
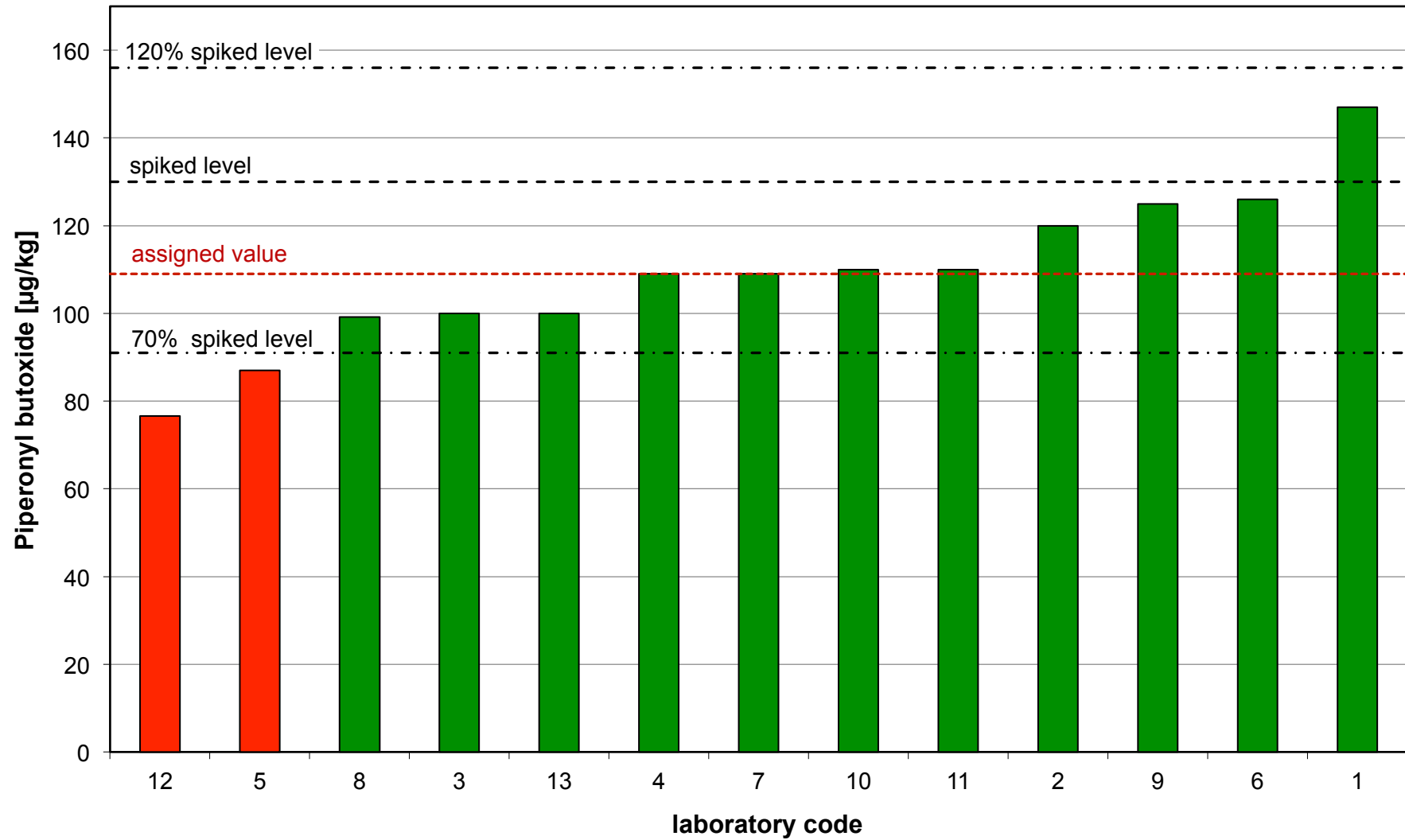


Figure 5. Assessment of Piperonyl butoxide (spiked level: 130 µg/kg)



Green: satisfactory results, red: dissatisfactory results



Figure 6. Assessment of Pirimiphos-methyl (spiked level: 45 µg/kg)

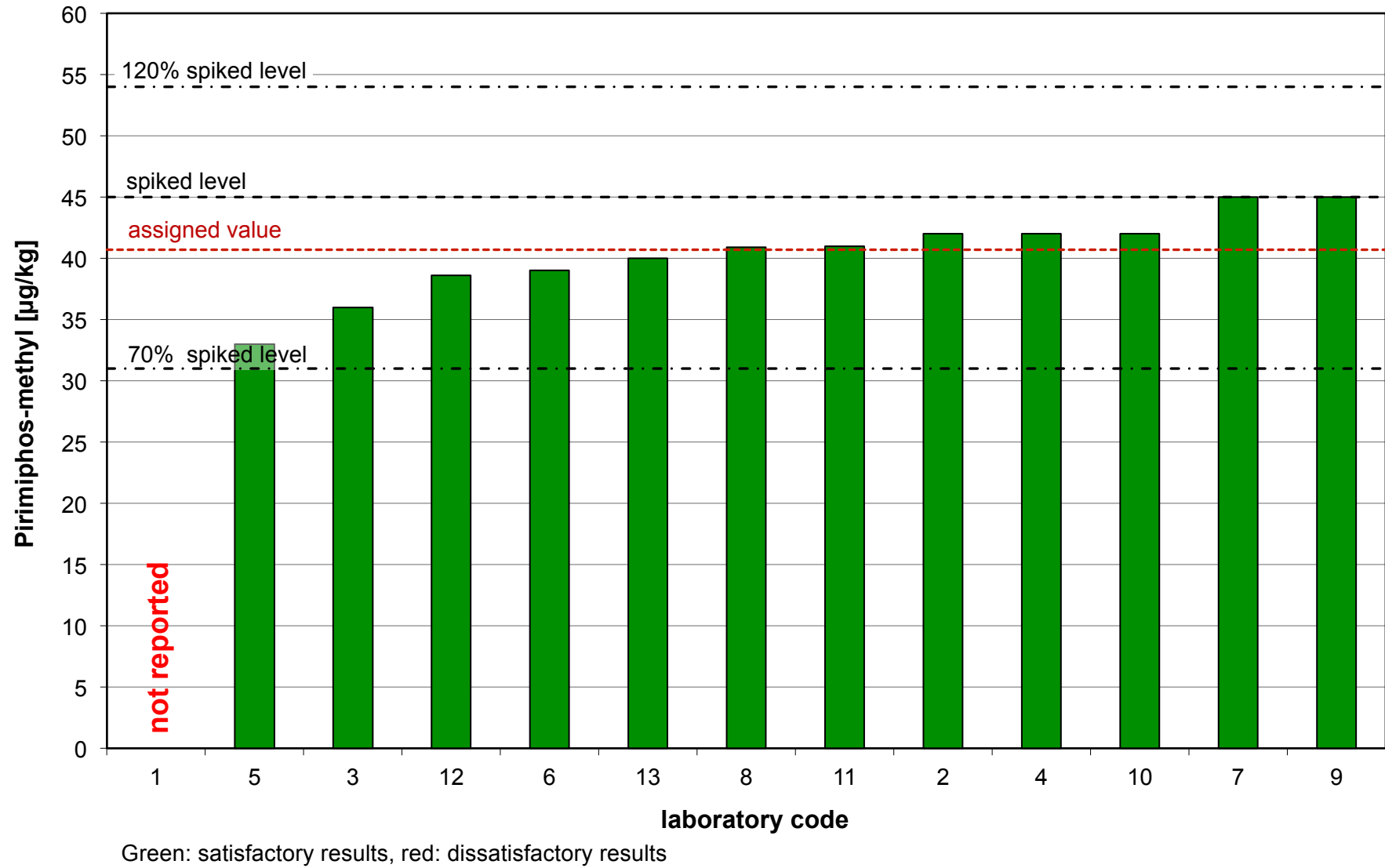
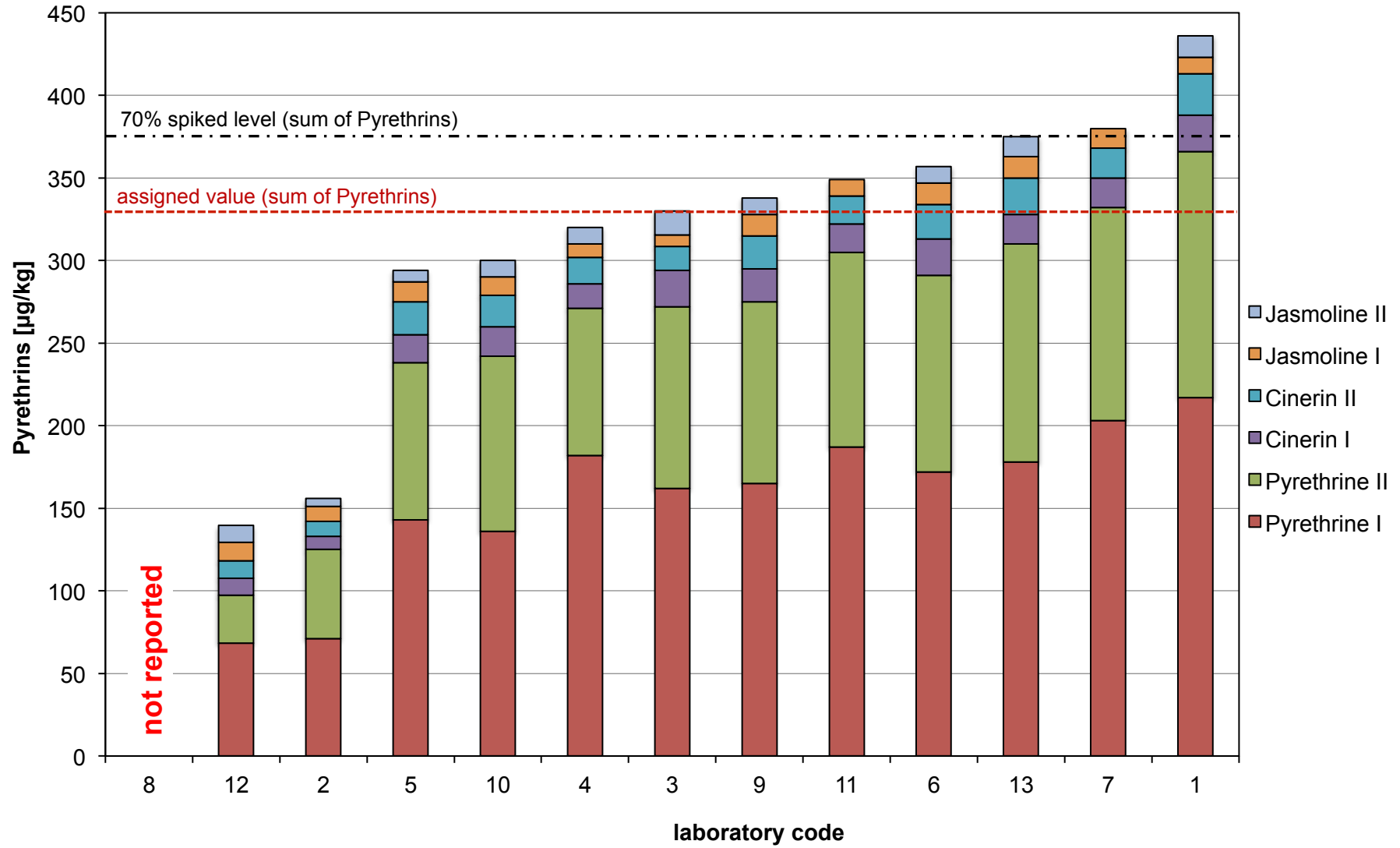


Figure 7. Assessment of Pyrethrins (spiked level: 540 µg/kg)



#### 4. Homogeneity testing

Seven randomly chosen test samples were used for homogeneity testing. Each subsample was analysed for all spiked pesticides in duplicate. The results confirm the homogeneous distribution of the pesticides in the test material (table 5) and the spiked levels.

*Table 5. Results of the homogeneity testing*

Subsample No.	Extraction No.	Chlorotoluron [µg/kg]	Dieldrin [µg/kg]	Epoxi-conazole [µg/kg]	Piperonyl butoxide [µg/kg]	Pencycuron [µg/kg]	Pirimiphos-methyl [µg/kg]
1	1	34.0	18.6	24.4	111	30.7	44.3
	2	34.0	20.2	24.7	112	30.7	44.2
2	1	35.3	19.1	24.8	115	31.1	45.2
	2	34.9	21.2	25.0	114	30.9	45.8
3	1	34.6	18.5	24.0	108	29.3	43.2
	2	33.4	20.2	23.4	106	29.1	42.6
4	1	34.6	22.0	23.8	109	29.0	43.9
	2	33.6	20.4	23.5	107	28.8	43.0
5	1	36.4	23.1	25.1	113	30.4	46.0
	2	35.9	21.2	24.5	111	30.9	44.7
6	1	33.4	19.0	23.1	105	29.2	43.0
	2	33.1	19.5	22.2	101	28.1	41.4
7	1	34.4	21.1	24.1	109	30.7	44.3
	2	38.3	20.1	26.1	119	32.5	47.1
Mean [mg/kg]		34.7	20.3	24.2	110	30.1	44.2
Standard deviation [mg/kg]		1.4	1.3	1.0	4.6	1.2	1.5
Coefficient of variation [%]		4.1	6.5	4.0	4.2	4.0	3.4
Spiked level [mg/kg]		42	24	27	130	37	45
Recovery [%]		83	85	90	85	81	98

## 5. Stability testing

One randomly chosen test sample was stored for stability testing at -18 °C in the dark. After the deadline of results reporting, the test sample was analysed for all pesticides in duplicate (table 9). Recoveries of 93 to 102 % with respect to the mean value of the homogeneity testing confirm the stability of the pesticides throughout the whole testing period.

*Table 9. Results of the stability testing*

Pesticide	Mean result from homogeneity testing [µg/kg]	Mean level at closure of transmission of results [µg/kg]	Recovery compared to the mean of the homogeneity testing [%]
Chlorotoluron	34.7	35.5	102
Dieldrin	20.3	18.9	93
Epoxiconazole	24.2	24.1	100
Piperonyl butoxide	110	106	96
Pencycuron	30.1	29.3	97
Pirimiphos-methyl	44.2	42.7	97
Pyrethrins (sum)	310	302	97