



## ***Laboratory Performance Assessment***

### ***Report***

## ***Analysis of Pesticides and Anthraquinone in Black Tea***

May 2013

## Summary

This laboratory performance assessment on pesticides in black tea was designed and organised by Lach & Bruns in cooperation with PROOF-ACS in April-May 2013 on behalf of the BNN e.V. (Bundesverband Naturkost Naturwaren).

The Test Material was prepared from homogenised organic black tea. The homogenised tea was spiked with eight pesticides plus anthraquinone. Anthraquinone is a widely spread contaminant in tea samples, possibly migrating from the packing to the tea, besides other currently unknown sources. The eight pesticides were acetamiprid, bifenthrin, carbendazim, chlorfenapyr, clothianidin, endosulfan sulfate, p,p'-DDT and its' main metabolite p,p'-DDE.

The Test Material was distributed to sixteen participants across four European countries (Germany, Italy, Spain, the Netherlands). Each laboratory received minimum 50 g homogenised black tea. No information on the identity or the number of the spiked pesticides was provided to the laboratories. 14 participants kept the term for the submission of results. Two laboratories (laboratory 3 and laboratory 10) submitted the results with a delay of one working day resp. six working days.

The laboratories were requested to identify and quantify all eight spiked pesticides plus anthraquinone.

The performance assessment considers the following test criteria:

- No *false negative or false positive results* have to be reported.
- *Correct* quantification related to the 70-120% recovery criteria (of the spiked value) for all pesticides.

*An informational part* on anthraquinone is included in this report since anthraquinone in tea originates from a contamination rather than from pesticide applications.

The overall performance assessment of the competence test on pesticides in black tea is summarised in the following table:

criteria	number of satisfactory participants	total number of participants	satisfactory (%)
correctly identified all eight pesticides	11	16	<b>69</b>
correctly identified and quantified all eight pesticides	2	16	<b>13</b>
correctly identified anthraquinone	8	16	<b>50</b>

## Assessment of quantification

Analytical results between 70 and 120% of the spiked levels are considered satisfying for the assessment of the quantification of the following parameters.

parameter	Spiked level (µg/kg)	number of satisfactory results	number of participants	satisfactory (%)
acetamiprid	42	14	16	88
bifenthrin	58	14	16	88
carbendazim	33	13	16	81
chlorfenapyr	145	12	16	75
clothianidin	145	14	16	88
endosulfan sulfate	27	11	16	69
p,p'-DDE	25	14	16	88
p,p'-DDT	95	5	16	31

## Table of contents

	Page
<b>Summary</b>	<b>2</b>
<b>1. Test material preparation and design</b>	<b>5</b>
<b>2. Statistical Evaluation of Results</b>	<b>5</b>
2.1. Trueness of results	5
2.2. Assigned value	5
<b>3. Results</b>	<b>5</b>
Table 1. Results of anthraquinone in black tea	7
Table 2. Results of acetamiprid and bifenthrin in black tea	8
Table 3. Results of carbendazim and chlorfenapyr in black tea	9
Table 4. Results of clothianidin and endosulfan sulfate in black tea	10
Table 5. Results of p,p'-DDE and p,p'-DDT in black tea	11
Figure 1. Assessment of acetamiprid (spiked level: 42 µg/kg) in black tea	12
Figure 2. Assessment of bifenthrin (spiked level: 58 µg/kg) in black tea	13
Figure 3. Assessment of carbendazim (spiked level: 33 µg/kg) in black tea	14
Figure 4. Assessment of chlorfenapyr (spiked level: 145 µg/kg) in black tea	15
Figure 5. Assessment of clothianidin (spiked level: 145 µg/kg) in black tea	16
Figure 6. Assessment of endosulfan sulfate (spiked level: 27 µg/kg) in black tea	17
Figure 7. Assessment of p,p'-DDE (spiked level: 25 µg/kg) in black tea	18
Figure 8. Assessment of p,p'-DDT (spiked level: 95 µg/kg) in black tea	19
Figure 9. Assessment of anthraquinone (spiked level: 28 µg/kg) in black tea	20
<b>4. Homogeneity testing</b>	<b>21</b>
Table 6. Results of Homogeneity Testing for acetamiprid bifenthrin, carbendazim and chlorfenapyr	21
<b>5. Stability testing</b>	<b>23</b>
Table 9. Results of Stability Testing for all pesticides plus anthraquinone in black tea Test Material	23

## 1. Test material preparation and design

The Test Material was based on organic black tea. An analysis of this tea confirmed the absence of pesticides in the organic black tea at a level of 10 µg/kg. Low levels of anthraquinone were unpreventable in the unspiked black tea, since anthraquinone is a widely spread contaminant in almost all tea samples.

The following pesticides were spiked to give the approximate final concentrations:

42 µg/kg acetamiprid, 58 µg/kg bifenthrin, 33 µg/kg carbendazim, 145 µg/kg chlorfenapyr, 145 µg/kg clothianidin, 27 µg/kg endosulfan sulfate, 25 µg/kg p,p'-DDE and 95 µg/kg p,p'-DDT. Anthraquinone was spiked at 28 µg/kg.

The homogenised and spiked black tea was subdivided into labelled PE-bottles with at least 50 g in each. The bottles were stored at -20°C in the dark until shipment.

The results of the Homogeneity Testing are presented in table 6 to 8 (pp. 21-22), the results of the Stability Testing in table 9 (p. 23).

## 2. Statistical Evaluation of Results

### 2.1. Trueness of results

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:

$$\text{coverage of the spiked level} = \frac{x}{sl} * 100 \quad (x = \text{reported result, sl} = \text{spiked level})$$

The coverage should be at least between 70 and 120% of the Spiked Level. The evaluation related to the coverage of the Spiked Levels, shown as accepted range, is given in tables 2 to 5 on page 8 to 11.

### 2.2. Assigned value

The assigned value  $\hat{X}$  is derived as a robust mean from the results of all participants and represents the consensus of the participants' results. The influence of outliers is minimised by application of the Winsorisation algorithm. As a first step the mean and the standard deviation of the results is 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 than 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 stable<sup>1</sup>.

## 3. Results

Since the participants were not informed about the identity or number of the spiked pesticides, they were requested to report all sought and found pesticides. The results had to be reported together with the corresponding recoveries and the reporting limits (RL). The recovery did not have to be considered for the results reporting, unless it was lower than 70% or higher than 120%.

Each laboratory was given a number (laboratory code). The results of all participants are presented in tables 2 to 5 (pp. 8-11) and subsequently in relation to the accepted range of 70-120% of the spiked level in figures 1 to 9 (pp. 12-20).

---

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

The evaluation of the reported RLs revealed shortcomings for laboratory 2 and 12. Compared to the BNN guideline value of 0.01 mg/kg and compared to the data of the other participants these two laboratories reported too high RLs for all parameters, which are inappropriate to check organic samples for pesticides.

The reported false positive results (> 10 µg/kg) for o,p'-DDT and p,p'-DDD are summarised in the following table:

laboratory code	not spiked! o,p'-DDT		not spiked! p,p'-DDD	
	results ≥ 10 µg/kg	RL (µg/kg)	results ≥ 10 µg/kg	RL (µg/kg)
1			<b>13</b>	5
2	<b>&lt;20</b>	20	<b>&lt;20</b>	20
3			<b>13</b>	5
8			<b>17</b>	5
9			<b>22,1</b>	5
10			<b>18*</b>	
12			<b>11</b>	10
13	<b>34</b>	10	<b>31</b>	10
14			<b>35</b>	10
15	<b>10</b>	5		
16	<b>30</b>	10	<b>35</b>	10

RL: reporting level

\* sum of p,p'-DDD and o,p'-DDT

A check for plausibility of the analytical results is one important step in analytical quality assurance. In order to achieve robust and reliable results related to DDT and its' metabolites and the corresponding isomers it is necessary to compare the individual results of the isomers and to put them into context: technical DDT consists to 65 up to 80% of p,p'-DDT and to 15 up to 21% of o,p'-DDT. This information in mind allows an appropriate plausibility check as the Test Material in BNN Ring Tests should always reflect the composition of real samples as far as possible. The reporting of o,p'-DDT of 30 resp. 34 µg/kg in combination with p,p'-DDT levels of 20 resp. 25 µg/kg (see table above and table 5) is not meaningful. In addition, the formation of the DDD isomer is not likely in biological material (like plant material). Metabolism of DDT to DDD is related to abiotic degradation processes in environmental compartments (f.ex. soil, sludge etc.). The participants of this test who reported certain levels of o,p'-DDT and/or p,p'-DDD (see table above) are requested to investigate the reasons for these false positive findings and to improve the related analytical steps.

Anthraquinone is currently not specifically regulated in food neither as a residue nor as a contaminant. Hence the default MRL of 0.01 mg/kg as defined by Regulation (EC) No 396/2005 applies, independent of the source (unauthorised use as pesticide, migration from packing,...).

Anthraquinone has been repeatedly under discussion in several expert committees. It is a known carcinogen in animal experiments and it is classified in group 2b by the International Agency for Research on Cancer (IARC). According to the ALARA principle ("as low as reasonable achievable") the content of contaminants in food should be lowered wherever feasible.

Today the use of anthraquinone as a pesticide is not authorised worldwide. On the other hand, anthraquinone is used in the production of paperback containers and it is known to migrate from the container towards the contained foodstuff.

Due to the widespread use and the carcinogenic potential analytical laboratories should be able to quantify anthraquinone down to levels of 10 µg/kg or lower. For that reason anthraquinone was included in this Ring Test as an important contaminant.

Out of 16 laboratories only 8 laboratories identified anthraquinone in the Test Material. The reported results are summarised in table 1. Since anthraquinone is a widely spread contaminant the provided Blank Material contained low levels of anthraquinone as well (see table 1). If laboratories reported levels of anthraquinone in the Test Material that have not been corrected for the results in the Blank Material (blank value), the results of the Blank Material were subtracted during evaluation.

Table 1. Results of anthraquinone in black tea

	<b>anthraquinone</b> spiked level: 28 µg/kg assigned value: 22 µg/kg				
<b>laboratory code</b>	Blank Material result (µg/kg)	result corrected for blank value (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 20-34 µg/kg
<b>1</b>	12	<b>28</b>		5	yes
<b>2</b>	enthalt (=present)	<b>10</b>	90	10	<b>no</b>
<b>3</b>		<b>36</b>	87	10	<b>no</b>
<b>4</b>	10	<b>10</b>	94	10	<b>no</b>
<b>8</b>	23	<b>20</b>	n.r.	10	yes
<b>9</b>		<b>16</b>	92	10	<b>no</b>
<b>11</b>	22	<b>23</b>	102	10	yes
<b>15</b>	6	<b>11</b>	80	10	<b>no</b>

The following colours were used in figures 1 to 9 to highlight the results with respect to their quality: Bars in green are satisfactory results, bars in red are non satisfactory results (not reported results are additionally marked as “n.r.”). Yellow bars are used for results which are inappropriate for quantification assessments.

Table 2. Results of acetamiprid and bifenthrin in black tea

laboratory code	acetamiprid spiked level: 42 µg/kg assigned value: 35 µg/kg				bifenthrin spiked level: 58 µg/kg assigned value: 53 µg/kg			
	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 29-50 µg/kg	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 41-70 µg/kg
1	40	95	5	yes	65	92	10	yes
2	37	93	20	yes, #1	26	73	20	no, #1
3	37	101	10	yes	61	88	5	yes
4	20	98	10	no	41	95	10	yes
5	33,4	94	10	yes	64,7	98	10	yes
6	36	110	5	yes	56	96	10	yes
7	28	90	10	no	52	107	10	yes
8	40	107	10	yes	55	89	10	yes
9	36,7	86	10	yes	55,5	79	10	yes
10	40	90-100	5	yes	43	90-105	5	yes
11	35	97	10	yes	45	101	10	yes
12	32	93	15	yes, #1	72	109	15	no, #1
13	35	86	10	yes	56	83	10	yes
14	35	82	10	yes	45	97	10	yes
15	32	78	10	yes	51	92	10	yes
16	34	89	10	yes	55	85	10	yes

RL: reporting level

#1: RL too high, not appropriate to check organic samples



Table 3. Results of carbendazim and chlorfenapyr in black tea

laboratory code	carbendazim spiked level: 33 µg/kg assigned value: 27 µg/kg				chlorfenapyr spiked level: 145 µg/kg assigned value: 129 µg/kg			
	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 23-40 µg/kg	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 102-174 µg/kg
1	31	87	5	yes	136	103	10	yes
2	29	100	20	yes, #1	67	96	20	no, #1
3	28	120	10	yes	104	82	5	yes
4	110	n.r.	5	no	130	105	10	yes
5	22,5	89	10	yes	n.r.			n.r.
6	24	111	5	yes	122	92	10	yes
7	21	100	10	no	119	91	10	yes
8	31	97	10	yes	161	90	10	yes
9	22,6	93	10	yes	149	76	10	yes
10	30	70-90	5	yes	60	90-105	5	no
11	27	92	10	yes	132	98	10	yes
12	28*	61	15	yes, #1	n.r.			n.r.
13	25	76	10	yes	149	85	10	yes
14	27	80	10	yes	128	97	10	yes
15	21	82	10	no	130	91	5	yes
16	26	78	10	yes	145	82	10	yes

RL: reporting level

#1: RL too high, not appropriate to check organic samples

n.r. not reported

\* result corrected for recovery

Table 4. Results of clothianidin and endosulfan sulfate in black tea

laboratory code	clothianidin spiked level: 145 µg/kg assigned value: 129 µg/kg				endosulfan sulfate spiked level: 27 µg/kg assigned value: 23 µg/kg			
	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 102-174 µg/kg	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 19-32 µg/kg
1	137	90	10	yes	18	85	5	no
2	142	101	40	yes, #1	<20	80	20	#1
3	84	111	10	no	19	100	5	yes
4	76	97	10	no	25	103	10	yes
5	119	97	10	yes	22,5	87	10	yes
6	129	116	10	yes	n.r.			n.r.
7	114	82	10	yes	27	88	10	yes
8	143	106	10	yes	30	98	10	yes
9	163	79	10	yes	25,6	79	5	yes
10	125	90-100	5	yes	9	90-105	5	no
11	115	100	10	yes	28	93	10	yes
12	123	90	15	yes, #1	<BG (=RL)		20	#1
13	145	85	10	yes	23	82	10	yes
14	150	81	10	yes	21	99	10	yes
15	120	69	10	yes	23	95	10	yes
16	140	84	10	yes	21	80	10	yes

RL: reporting level

#1: RL too high, not appropriate to check organic samples

n.r.: not reported

Table 5. Results of p,p'-DDE and p,p'-DDT in black tea

laboratory code	p,p'-DDE spiked level: 25 µg/kg assigned value: 24 µg/kg				p,p'-DDT spiked level: 95 µg/kg assigned value: 56 µg/kg			
	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 18-30 µg/kg	result (µg/kg)	recovery (%)	RL (µg/kg)	accepted range: 67-114 µg/kg
1	19	94	5	yes	40	102	5	no
2	30	109	20	yes, #1	52	95	20	no, #1
3	20	87	5	yes	31	87	5	no
4	21	96	5	yes	53	104	5	no
5	27,5	98	10	yes	81,2	98	10	yes
6	n.r.			n.r.	n.r.			n.r.
7	24	110	10	yes	72	108	10	yes
8	21	90	5	yes	76	94	5	yes
9	25	76	5	yes	75	86	5	yes
10	19	n.r.	n.r.	yes	n.r.			n.r.
11	25	93	10	yes	58	95	10	no
12	38	75	15	no, #1	53	83	20	no, #1
13	26	82	10	yes	25	82	10	no
14	21	95	10	yes	n.r.			n.r.
15	21	95	5	yes	88	92	5	yes
16	29	88	10	yes	20	82	10	no

RL: reporting level

#1: RL too high, not appropriate to check organic samples

n.r.: not reported

Figure 1. Assessment of acetamiprid (spiked level: 42 µg/kg) in black tea

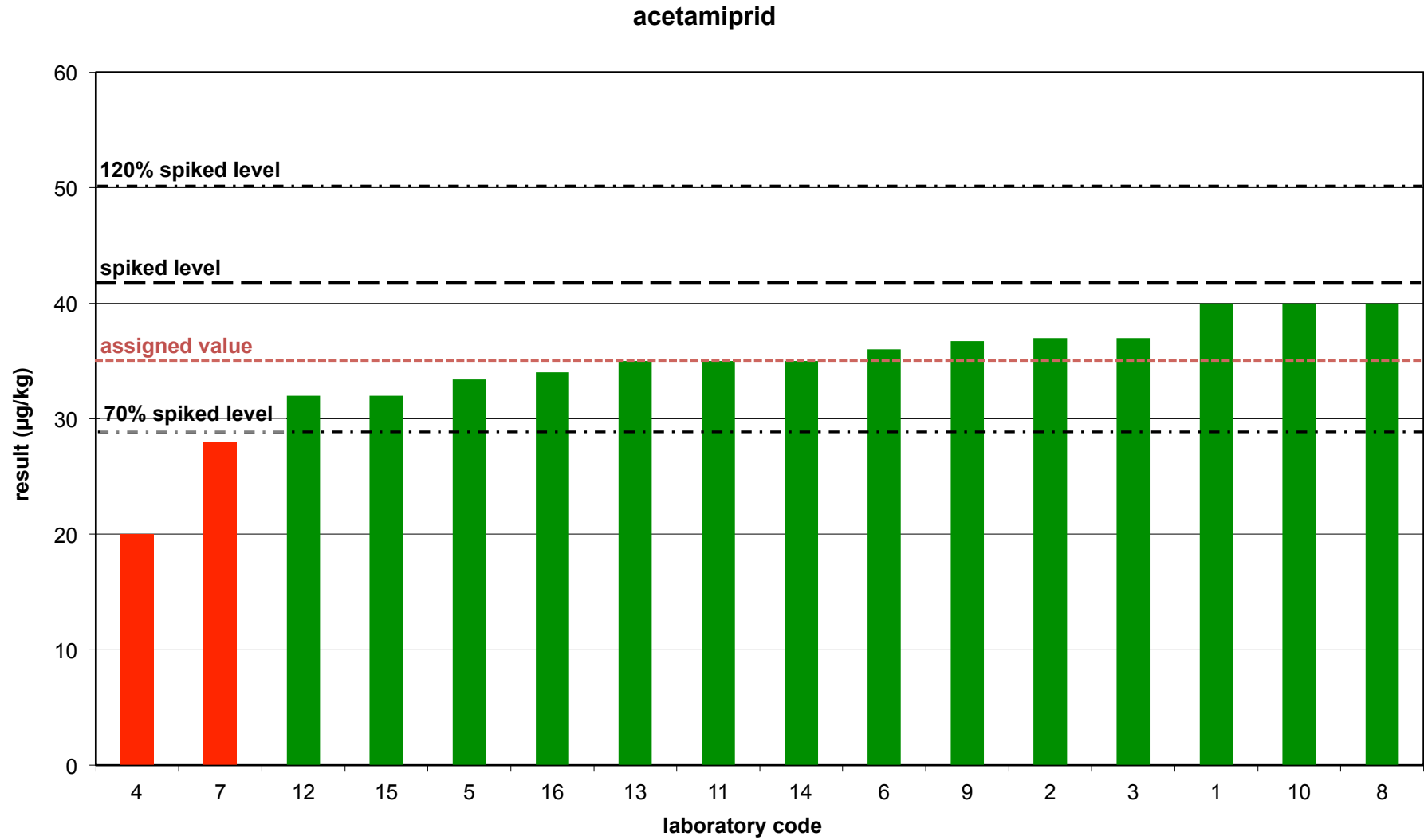


Figure 2. Assessment of bifenthrin (spiked level: 58 µg/kg) in black tea

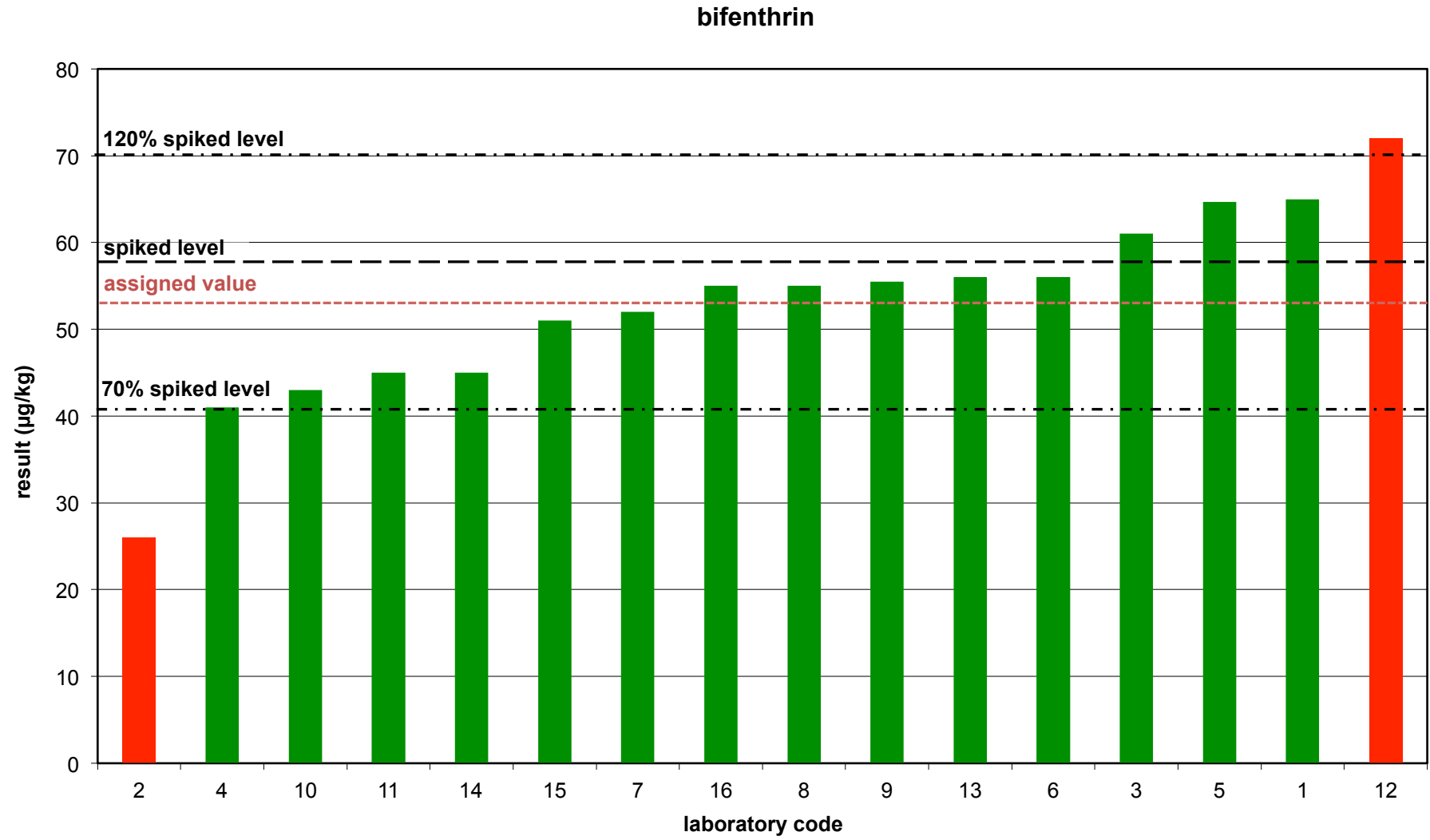


Figure 3. Assessment of carbendazim (spiked level: 33 µg/kg) in black tea

**carbendazim**

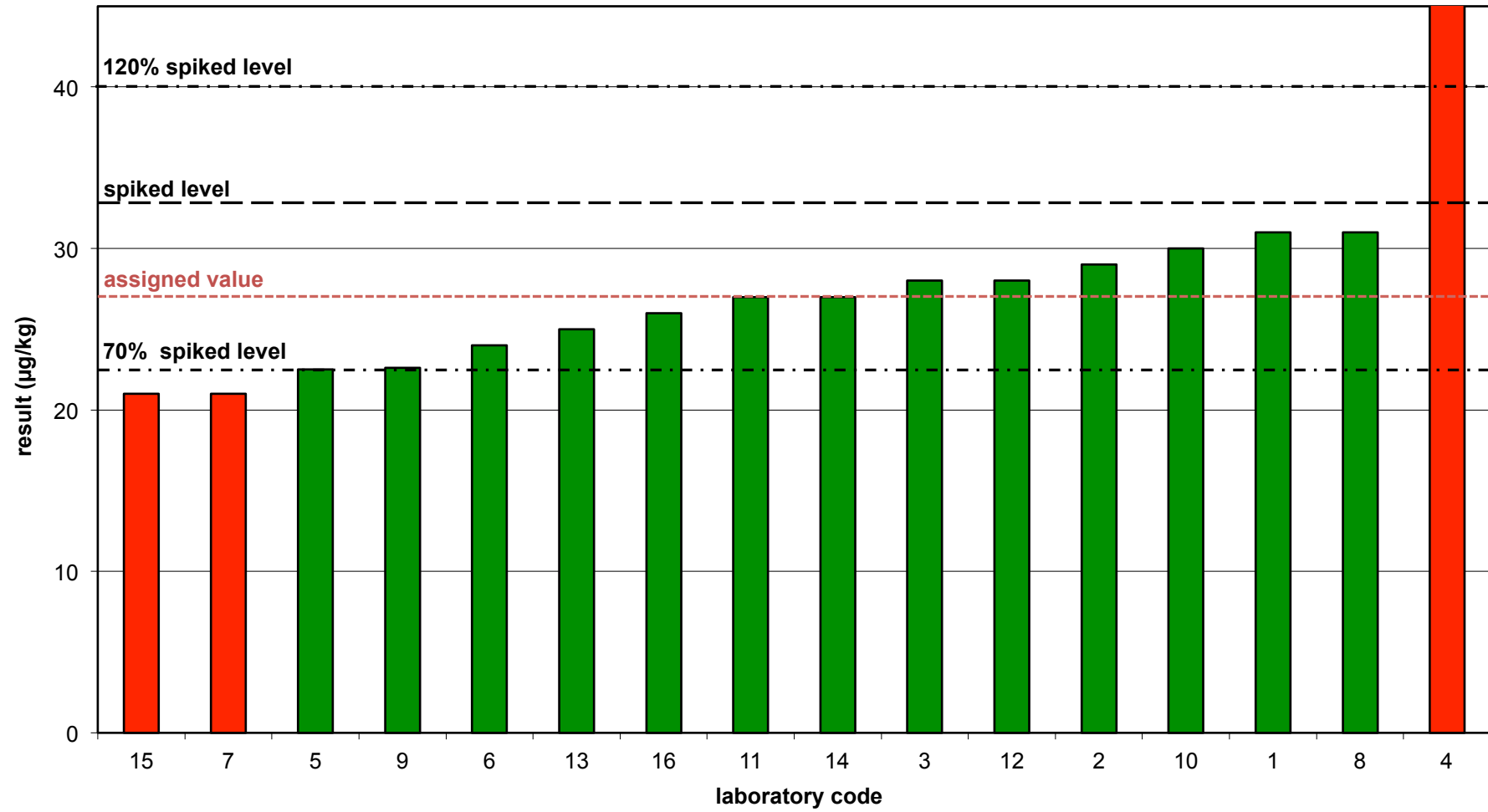


Figure 4. Assessment of chlorfenapyr (spiked level: 145 µg/kg) in black tea

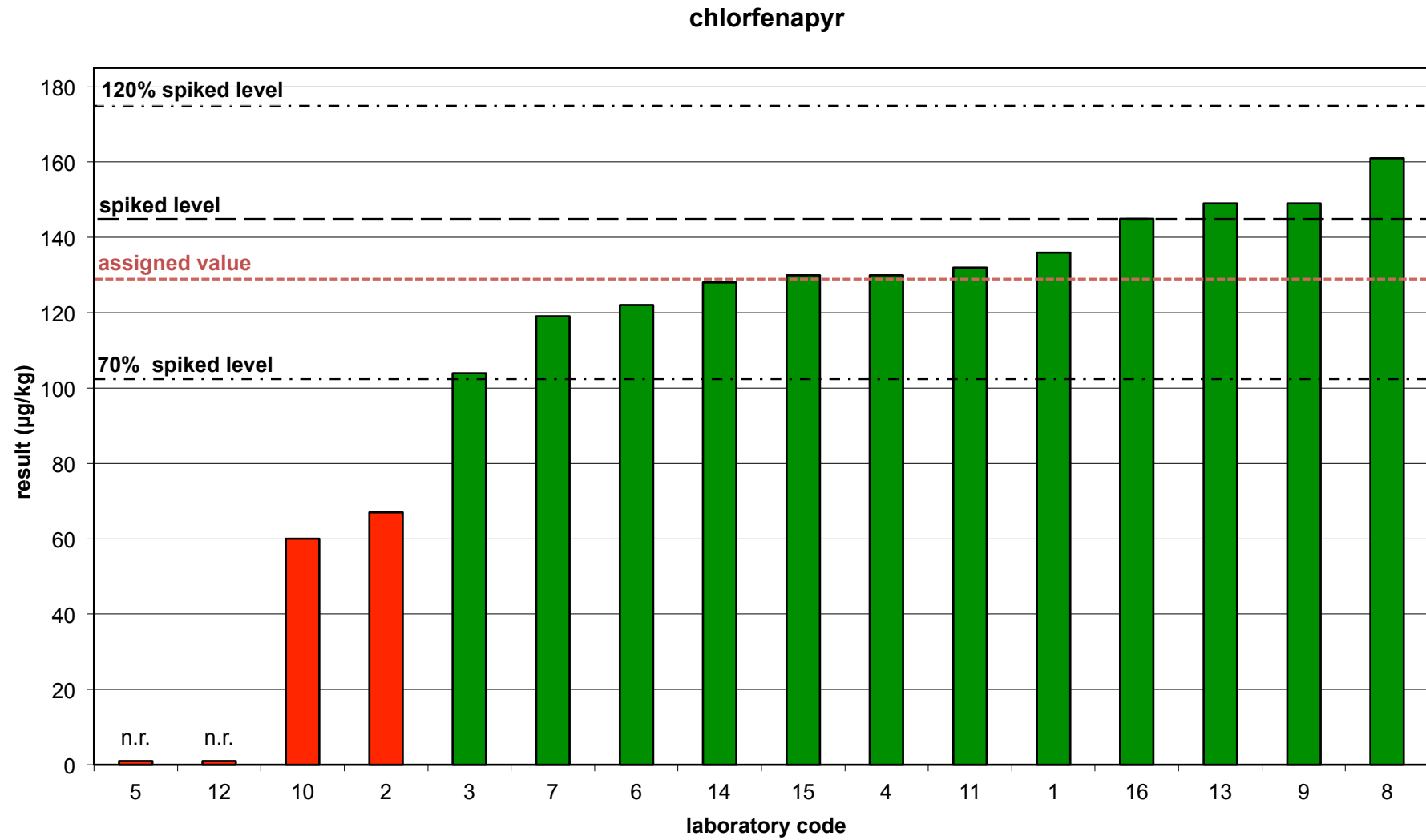


Figure 5. Assessment of clothianidin (spiked level: 145 µg/kg) in black tea

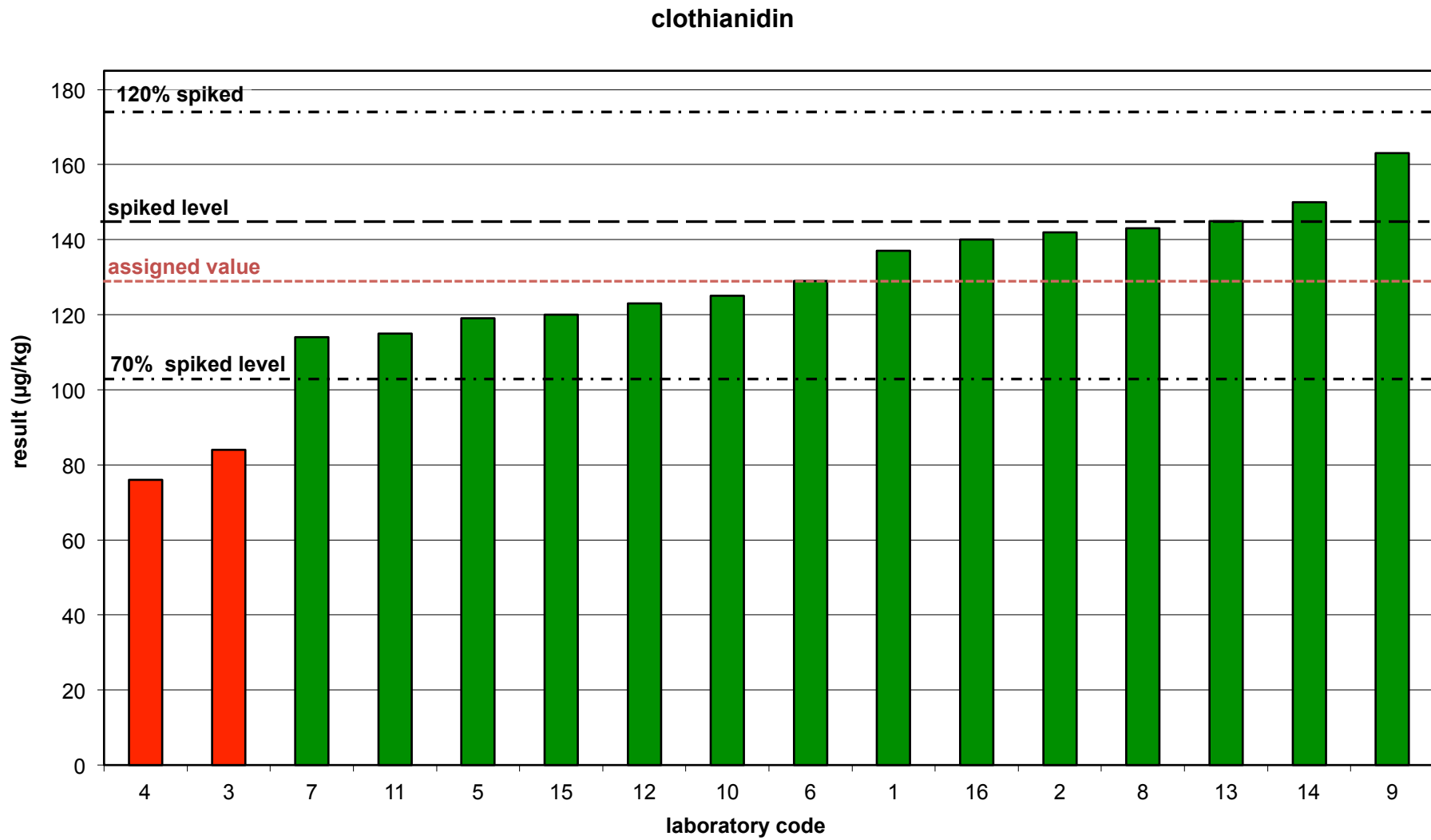




Figure 6. Assessment of endosulfan sulfate (spiked level: 27 µg/kg) in black tea

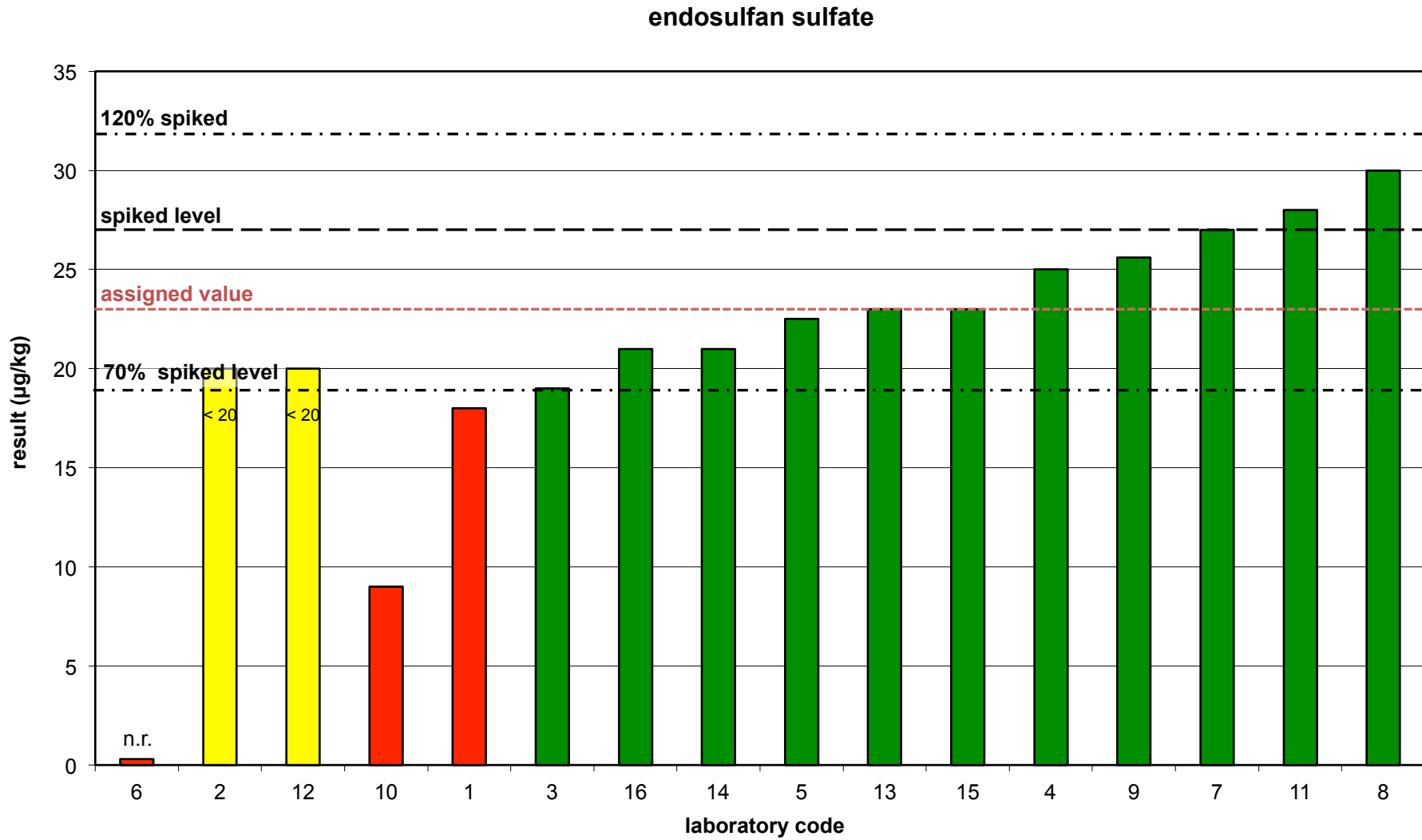


Figure 7. Assessment of p,p'-DDE (spiked level: 25 µg/kg) in black tea

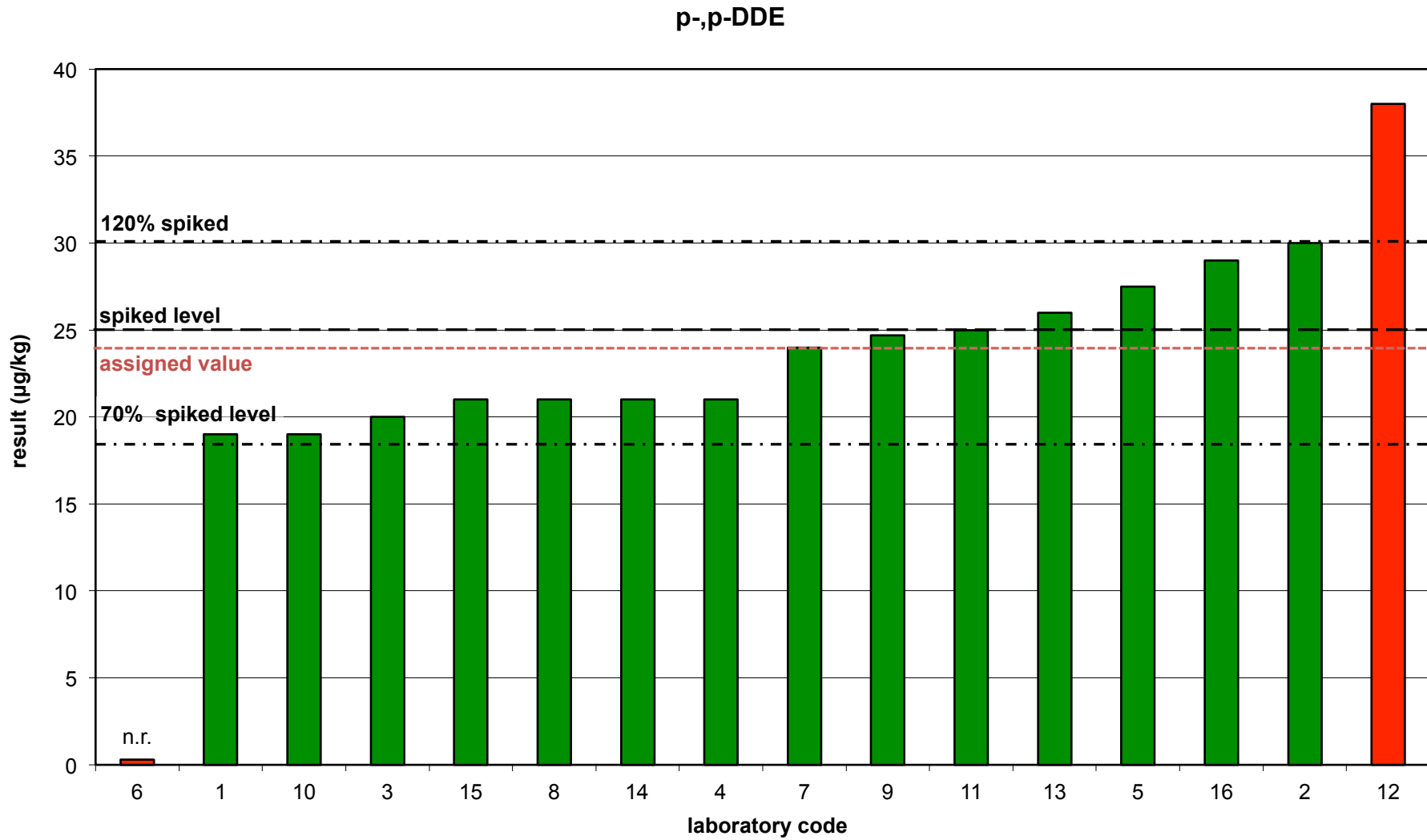


Figure 8. Assessment of p,p'-DDT (spiked level: 95 µg/kg) in black tea

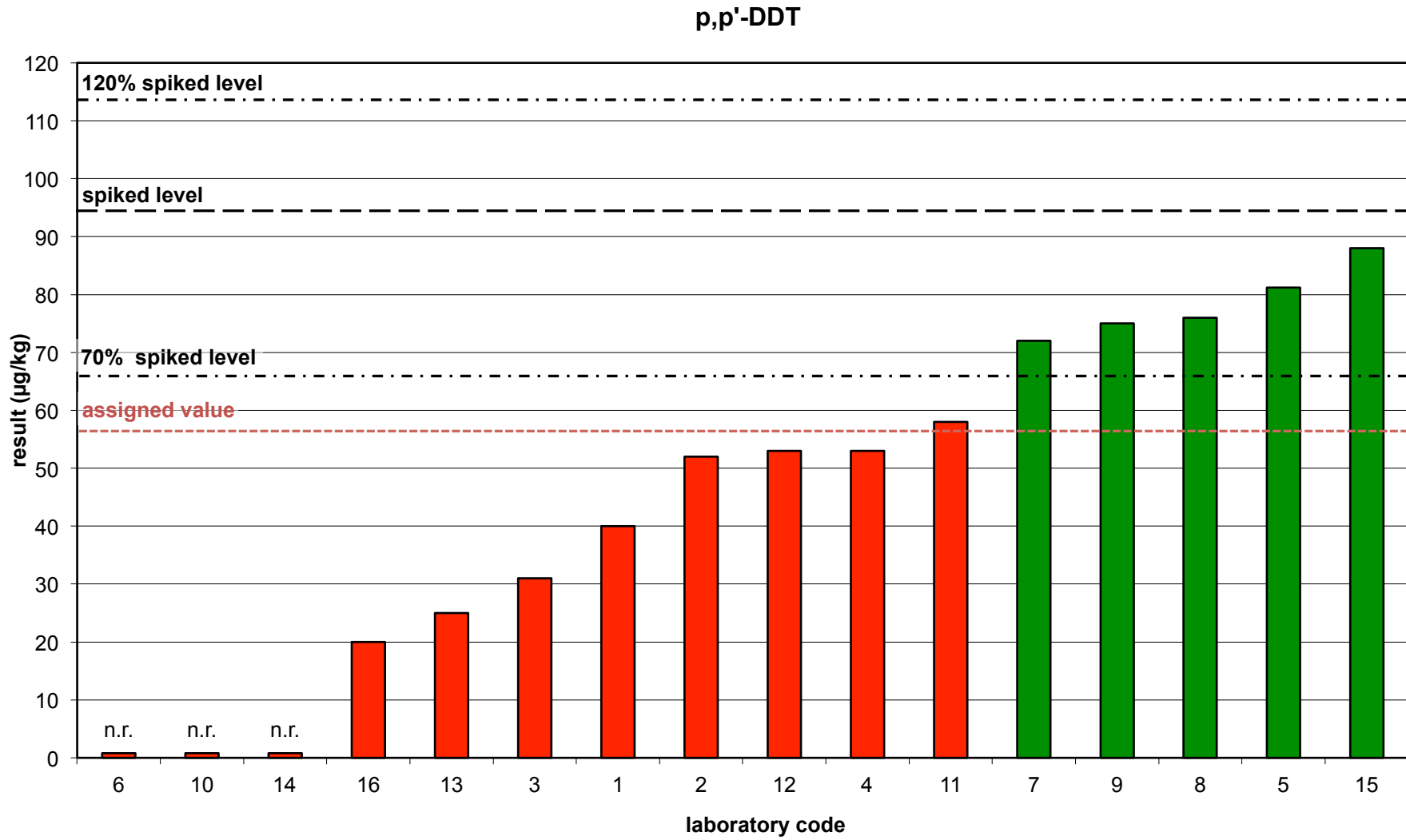
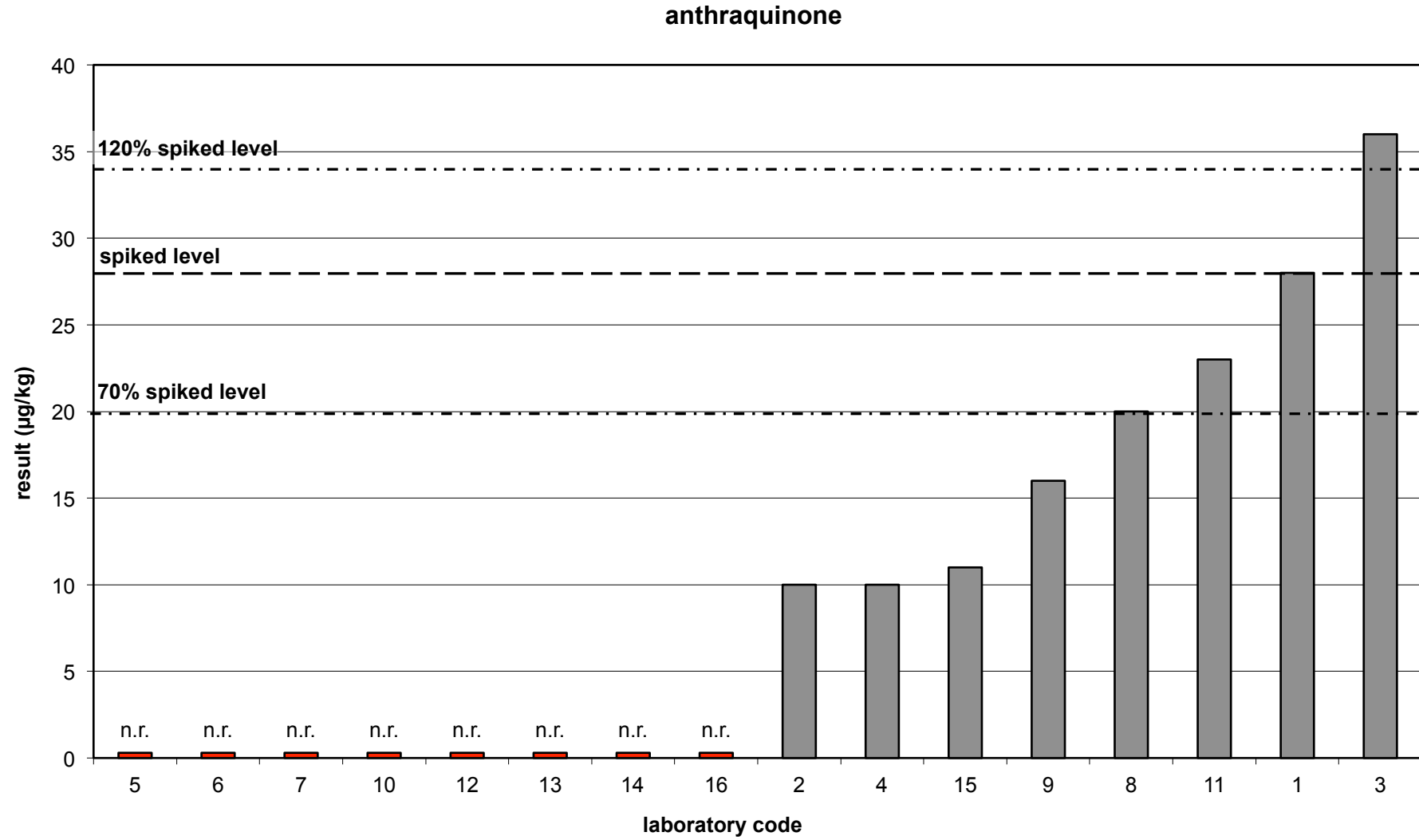


Figure 9. Assessment of anthraquinone (spiked level: 28 µg/kg) in black tea



## 4. Homogeneity testing

Seven randomly chosen Test Material samples were analysed for Homogeneity Testing. The mean of the results was calculated and used for statistics to check the homogeneity of each of the eight spiked pesticides plus anthraquinone in the Test Material.

*Table 6. Results of Homogeneity Testing for acetamiprid bifenthrin, carbendazim and chlorfenapyr*

<b>acetamiprid</b>		<b>bifenthrin</b>	
sample no.	Results [µg/kg]	sample no.	Result [µg/kg]
1	39.3	1	52.7
2	40.8	2	56.4
3	41.5	3	55.4
4	41.1	4	55.5
5	42.6	5	54.2
6	42.9	6	55.6
7	42.3	7	55.3
mean [µg/kg]	41.5	mean [µg/kg]	55.0
standard deviation [µg/kg]	1.24	standard deviation [µg/kg]	1.19
coefficient of variation [%]	3.0	coefficient of variation [%]	2.2
target value (spiked level) [µg/kg]	42	target value (spiked level) [µg/kg]	58
recovery [%]	99	recovery [%]	95

<b>carbendazim</b>		<b>chlorfenapyr</b>	
sample no.	Result [µg/kg]	sample no.	Result [µg/kg]
1	33.0	1	137.6
2	33.7	2	149.2
3	33.6	3	149.9
4	34.2	4	146.2
5	31.7	5	145.3
6	34.3	6	147.1
7	33.6	7	150.1
mean [µg/kg]	33.4	mean [µg/kg]	146.5
standard deviation [µg/kg]	0.88	standard deviation [µg/kg]	4.3
coefficient of variation [%]	2.6	coefficient of variation [%]	3.0
target value (spiked level) [µg/kg]	33	target value (spiked level) [µg/kg]	145
recovery [%]	101	recovery [%]	101

Table 7. Results of Homogeneity Testing for clothianidin, endosulfan sulfate, p,p'-DDE and p,p'-DDT

<b>clothianidin</b>		<b>endosulfan sulfate</b>	
sample no.	Results [µg/kg]	sample no.	Result [µg/kg]
1	147.4	1	23.3
2	144.4	2	25.6
3	149.0	3	26.0
4	147.7	4	26.3
5	153.8	5	26.7
6	151.5	6	24.8
7	153.1	7	23.0
mean [µg/kg]	149.5	mean [µg/kg]	25.1
standard deviation [µg/kg]	3.4	standard deviation [µg/kg]	1.5
coefficient of variation [%]	2.3	coefficient of variation [%]	5.9
target value (spiked level) [µg/kg]	145	target value (spiked level) [µg/kg]	27
recovery [%]	103	recovery [%]	93

<b>p,p'-DDE</b>		<b>p,p'-DDT</b>	
sample no.	Result [µg/kg]	sample no.	Result [µg/kg]
1	23.4	1	78.6
2	24.5	2	84.7
3	24.4	3	85.3
4	23.7	4	87.2
5	23.7	5	87.0
6	24.2	6	91.8
7	24.3	7	93.3
mean [µg/kg]	24.1	mean [µg/kg]	86.9
standard deviation [µg/kg]	0.4	standard deviation [µg/kg]	4.9
coefficient of variation [%]	1.7	coefficient of variation [%]	5.6
target value (spiked level) [µg/kg]	25	target value (spiked level) [µg/kg]	95
recovery [%]	96	recovery [%]	91

Table 8. Results of Homogeneity Testing for anthraquinone

<b>anthraquinone</b>	
sample no.	Results [µg/kg]
1	30.5
2	31.2
3	27.2
4	28.0
5	25.8
6	28.5
7	29.3
mean [µg/kg]	29.6
Mean blank value	28.0
standard deviation [µg/kg]	1.9
coefficient of variation [%]	6.3
target value (spiked level) [µg/kg]	28
recovery [%]	106

## 5. Stability Testing

Stability was tested after storage at -20°C from April 9th (preparation of test material) to May 24th, 2013. The mean values from homogeneity testing (7 samples, see paragraph 4) were used as reference. For Stability Testing an extract was prepared and analysed from one randomly selected Test Material. The results of the Stability testing for all pesticides plus anthraquinone in black tea Test Material are shown in table 9.

*Table 9. Results of Stability Testing for all pesticides plus anthraquinone in black tea Test Material*

Parameter	Mean result from Homogeneity Testing	Result from Stability Testing
acetamiprid	41.5	41.3
bifenthrin	55.0	52.7
carbendazim	33.4	33.8
chlorfenapyr	146.5	141.2
clothianidin	149.5	144.6
endosulfan sulfate	25.1	21.1
p,p'-DDE	24.1	23.9
p,p'-DDT	86.9	93.8
anthraquinone	29.6	27.5