Bundesverband
Naturkost Naturwaren

Laboratory Performance Assessment

Analysis of Pesticides in Lemons

Report

June 2015

Summary

The laboratory performance assessment related to pesticides in lemons was designed and organised by Lach & Bruns in cooperation with PROOF-ACS in June 2015 on behalf of BNN e.V. (Bundesverband Naturkost Naturwaren).

The performance assessment was conducted without prior announcement. The laboratories were informed about the test character of the samples upon arrival of the samples in the lab.

Whole, non-homogenised lemons were provided as test samples. A solution of eight substances was injected to each of the lemons in order to prepare a well-defined spiked test material:

Chlorpyrifos-methyl, Fenbutatin oxide, 2-Phenylphenol, Prochloraz, Pyriproxyfen, Tebufenpyrad, Terbuthylazine, and Phosphonic acid.

The test samples were distributed to twenty participants across six European countries (Germany, Belgium, France, Italy, Spain, the Netherlands). Each laboratory received seven lemons (about 1.8 kg).

The laboratories were instructed to quantify all pesticides, which are common for conventionally agricultured lemons (pesticide multi-methods plus additional specific packages if necessary). No additional information was provided with respect to the identity or the number of spiked pesticides. The quantification of phosphonic acid was ordered specifically.

The performance assessment considers the following test criteria:

- No *false positive results*.
- Correct *identification* of seven pesticides.
- Correct *quantification* of seven pesticides and phosphonic acid in terms of 70 to 120 % recovery of the spiked value.

Summary of the performance of the laboratories:

Criterion	Criterion passed
Identification: All seven <u>pesticides</u> correctly identified	19 out of 20 laboratories (95 %)
Quantification (Pesticides): All seven pesticides correctly quantified (within 70 to 120 % recovery of the spiked level)	6 out of 20 laboratories (30 %)
Quantification (Phosphonic acid): Correctly quantified (within 70 to 120 % recovery of the spiked level)	16 out of 19 laboratories (84 %)
Successful participation according to the guidelines for laboratory approval of BNN	16 out of 20 laboratories (80 %)

Assessment of quantification

Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Assigned value in % of the spiked level	Number of results	Correct quantification
Chlorpyrifos-methyl	0.024	0.016	67	20	13 out of 20 laboratories (65 %)
Fenbutatin oxide	0.032	0.027	84	19	13 out of 19 laboratories (68 %)
2-Phenylphenol	0.34	0.29	85	20	16 out of 20 laboratories (80 %)
Prochloraz	0.052	0.039	75	20	14 out of 20 laboratories (70 %)
Pyriproxyfen	0.060	0.050	83	20	15 out of 20 laboratories (75 %)
Tebufenpyrad	0.014	0.011	79	20	19 out of 20 laboratories (95 %)
Terbuthylazine	0.026	0.022	85	20	18 out of 20 laboratories (90 %)
Phosphonic acid	0.25	0.23	92	19	17 out of 19 laboratories (89 %)

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

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1. Introduction

Unannounced laboratory performance assessments are an appropriate approach to test laboratories under routine-like conditions. A limited turn around time as well as an unannounced arrival of the samples do not allow for "special care analysis" as opposed to announced ring test samples. Unannounced tests help to get knowledge about the daily performance under routine conditions and to identify possible shortcomings, deficiencies and thus areas of improvement.

Comprehensive laboratory performance assessments should include the entire analytical procedure, the sample pretreatment, the sample preparation and the analysis. To meet this objective, which are designed as close as possible to routine samples test materials should be provided to the participants.

For both reasons, BNN decided to perform an unannounced laboratory performance assessment with spiked but non-homogenised lemons.

The laboratories are faced with the following challenges:

1. The test samples did arrive in the laboratories **without any prior announcement**. The laboratories are informed about the test by an enclosed cover letter.

2. The scope of the test is defined as:
   All **pesticides, which are common for conventionally agricultured lemons** (usually covered by pesticide multi-methods – LC, GC). The labs are informed that some of them might require specific packages in order to cover the full scope of possible pesticides. All in all seven pesticides had to be identified and quantified.

3. The quantification of phosphonic acid is ordered specifically.

4. The turn around time is limited to **72 hours** after arrival of the sample in the lab (based on the time of delivery reported by the express courier service).

5. The laboratories have to prepare a **homogenate** of the lemons, like with routine samples. The laboratories are instructed to use all provided lemons for the preparation of the homogenate and not to subsample in order to avoid losses of the spiked pesticides.

2. Test design

In order to ensure a realistic but challenging test design the following sources of information were considered:

- Different data bases (f. ex. pesticides-online.de, publications of state laboratories, outcome of monitoring programmes of commercial companies),

- MRLs laid down in Regulation (EC) No. 396/2005, and

- Analytical aspects.

The choice of the pesticides is linked to the following principles:

- Pesticides, which are common for conventionally grown lemons or possible contaminations due to driftage from conventionally agricultured crops nearby (Terbuthylazine).

- All pesticides, except Fenbutatin oxide, are covered by the scope of common multi-methods. A special hint ("specific packages may be required") was added in the cover letter to make the laboratories analyse for Fenbutatin oxide in case it is not part of their pesticide multi-method.

- Phosphonic acid is a relevant parameter in organic samples. It is ordered specifically, since it is usually determined by means of specific analytical methods (f. ex. QuPPe method).

The final design of the spiking is listed in the table below:

Matrix: Lemons 7 lemons per laboratory		
Parameter	Spiking level [mg/kg]	Maximum Residue Level [mg/kg]
Chlorpyrifos-methyl	0.024	0.3
Fenbutatin oxide	0.032	5.0
2-Phenylphenol	0.34	5.0
Prochloraz	0.052	10.0
Pyriproxyfen	0.060	0.6
Tebufenpyrad	0.014	0.5
Terbuthylazine	0.026	0.1
Phosphonic acid	0.25	75.0*

*Sum of Fosetyl, phosphonic acid and their salts, expressed as fosetyl

3. Test material preparation

A set of seven lemons was prepared for each laboratory as a test sample. All in all 23 sets of lemons (in total 161 lemons) were treated according to the procedure described below:

A subsample of the untreated organic lemons is analysed to confirm the absence of pesticides at a level of 10 ppb. The lemons are sorted according to their individual weight and are arranged to end up at a similar overall weight of each set of lemons (7 pieces) thereafter (see table 9, annex). A unique sample identifier is allocated to each set of lemons.

A pesticide mix is prepared of Chlorpyrifos-methyl, Fenbutatin oxide, 2-Phenylphenol, Prochloraz, Pyriproxyfen, Tebufenpyrad, Terbuthylazine, and Phosphonic acid at different concentration levels (see tables 1 to 4). A volume of 370 µl of the pesticide mix is injected into the peel of each lemon. The lemons are stored at +7°C in the dark until shipment.

Two sets of lemons are randomly selected out of the prepared samples. One is used to confirm the spiked pesticide levels (see section 7). The other one is stored at +7°C for one week and is analysed to confirm the stability of the parameters thereafter (see section 8).

4. Statistical evaluation of results

4.1. Trueness of results

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

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

where x = reported result and sl = spiked level.

The accepted ranges (70 to 120 % of the spiking level) are subjected to non-commercial rounding and are reported with an accuracy of two significant figures:

- The calculated values, which correspond to 70 % recovery of the spiked level, are rounded down. For example 0.0168 mg/kg (70 % recovery of Chlorpyrifos-methyl) is rounded down to 0.016 mg/kg.
- The calculated values, which correspond to 120 % recovery of the spiked level, are rounded up. For example 0.408 mg/kg (120 % recovery of 2-Phenylphenol) is rounded up to 0.41 mg/kg.

4.2. 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[1].

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

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

4.3. 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, the assigned value $\hat{X}$ and the target standard deviation according to Horwitz $\hat{\sigma}_H$ [2]:

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

5. Results

All in all twenty (20) BNN approved laboratories across six (6) European countries (Germany, Belgium, France, Italy, Spain, and the Netherlands) took part in the unannounced performance test. Each laboratory is given a randomly selected identifier, hereinafter referred to as laboratory code.

Seven lemons are provided to each of the labs as a test sample. The overall weight of each sample (7 lemons) varies naturally due to differences in the size of the lemons. The mean overall sample weight is 1836 g with a narrow range of 1814 to 1849 g (see table 9, annex). Each lemon is spiked with a constant volume of a solution of eight different analytes (see section 3). Hence, the laboratories are instructed to homogenise all of the provided lemons and to avoid subsampling before the homogenisation (see section 1).

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 and phosphonic acid together with the corresponding recovery rates and the reporting limits (RL).

The spiking levels, which are used for the evaluation of the results, are calculated based on the mean overall sample weight of 1836 g. Even though the exact spiking levels of the test samples do slightly deviate from each other, the effect with respect to the outcome of the performance test is negligible. However, the results of those laboratories, which received test samples with significantly high or significantly low overall sample weights are double-checked for any possible influence of the sample weight with regard to the results to avoid any misjudgement. None of the results of the laboratories are affected.

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[2] Horwitz W. Evaluation of Analytical Methods Used for Regulation of Foods and Drugs. Anal Chem. 1982;54(1):67A – 76A.

The results of the unannounced test are evaluated according to
- *false-positive* findings,
- *false-negative* findings, and
- according to the *trueness criterion*.

Trueness criterion: Results within a 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[3] (section 4.1).

The assigned values are derived as robust means of the results of all participants and represent the consensus of participants` results (section 4.2). The assigned values are at 67 to 92 % of the spiked levels and thus tend towards lower levels compared to the spiked levels (see table in the summary for details). This might be explained by an insufficient homogenisation of the lemons and an insufficient extraction as a consequence thereof.

The z-scores (section 4.3 and tables 1 to 4) 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.

Summary of the evaluation of results (see table 1 and summary):

- **All in all, sixteen (16) out of twenty (20) laboratories participated with success according to the guidelines for laboratory approval of BNN[2] (80 %).**

- **Six (6) out of twenty (20) laboratories quantified all 8 parameters correctly within a recovery of 70 to 120 % of the spiked level.**

- **Three (3) more laboratories failed with just one of the eight parameters to be quantified within 70 to 120 % of the spiking level.**

- **Nineteen (19) out of twenty (20) laboratories identified all seven pesticides correctly in the test sample.**

- **One laboratory (lab 5) reported a false negative result of phosphonic acid.**

- **Eighteen (18) laboratories quantified phosphonic acid in the sample.**

The individual results of the participants with respect to the eight parameters are presented in tables 2 to 5 (page 13 to 16). Figures 1 to 8 (page 17 to 24) present graphically the results in relation to the accepted range of 70 to 120 % recovery of the spiked level. Bars in green represent satisfactory results, while bars in red represent dissatisfactory results.

The reporting limits (RLs) of 10 ppb or lower, which are reported by the laboratories in the test for all tested pesticides, are suitable for the quantification of low levels of pesticides. With

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[3] BNN, Guidelines for laboratory approval by Bundesverband Naturkost Naturwaren (BNN) e. V. (Federal Association for Natural Foods and Natural Products inc. soc.)

respect to phosphonic acid, the laboratories reported RLs of 0.1 mg/kg or lower, except lab 15, with a RL of 0.2 mg/kg.

Table 1. Summary of the evaluation of results

Laboratory code	All parameters correctly identified	No. of correctly quantified parameters[#]	Participation with success[##]
1	no, Fenbutatin oxide not in scope	5	**yes**[*]
2	**yes**	6	**yes**
3	**yes**	3	no
4	**yes**	5	no
5	no, 1 false negative result (Phosphonic acid)	3	no
6	**yes**	7	**yes**
7	**yes**	**8**	**yes**
8	**yes**	6	**yes**
9	**yes**	6	**yes**
10	**yes**	6	**yes**
11	**yes**	**8**	**yes**
12	**yes**	**8**	**yes**
13	**yes**	**8**	**yes**
14	**yes**	**8**	**yes**
15	**yes**	7	**yes**
16	**yes**	6	**yes**
17	**yes**	**8**	**yes**
18	**yes**	7	**yes**
19	**yes** (Phosphonic acid is currently subcontracted)	4	no[*]
20	**yes**	6	**yes**

[#]: According to the trueness criterion a laboratory's results is considered as correctly quantified if it is within the accepted range of 70 to 120 % recovery of the spiked level. Maximum number of correct results in the test is 8 out of 8 parameters.

[##]: According to the guidelines for laboratory approval [4] a laboratory's result in a competence test is considered satisfying if 75 % of the reported results are within 70 to 120 % recovery of the spiked level and no false positive and no false negative results are reported. Laboratories with 6 out of 8 correctly quantified parameters (* resp. 5 out of 7 parameters) are considered satisfying in this performance assessment.

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[4] BNN, Guidelines for laboratory approval by Bundesverband Naturkost Naturwaren (BNN) e. V. (Federal Association for Natural Foods and Natural Products inc. soc.)

Chlorpyrifos-methyl, success rate 65 % (13 out of 20 laboratories)

All laboratories identified Chlorpyrifos-methyl correctly. Thirteen (13) laboratories reported results between 67 and 104 % recovery of the spiked level and are considered as satisfying. The acceptance of results, which correspond to a recovery of 67 % as satisfying is due to the non-commercial rounding of the accepted ranges (see section 4.1 for explanation). The assigned value (0.016 mg/kg) is equal to 70 % recovery and thus to the lower bound of the accepted range.

Fenbutatin oxide, success rate 68 % (13 out of 19 laboratories)

Fenbutatin oxide is frequently detected in citrus fruits and thus of high relevance for this matrix. It is either within the scope of the pesticide multi-method or analysed by a separate analytical method. For that reason, the laboratories were informed in the cover letter that specific packages of analytical methods might be required to cover the full scope of pesticides in the test.

All but one laboratory (lab 1) identified fenbutatin oxide correctly. Lab 1 does not offer the analysis of fenbutatin oxide according to the provided scope of their analytical methods.

Thirteen (13) labs identified and quantified Fenbutatin oxide correctly within 70 to 120 % recovery of the spiked level. Five (5) labs reported results slightly (lab 3, 10, 19) or significantly (lab 6) below 70 % or even only traces below the reporting limit (<0.005 mg/kg, lab 5). One lab (lab 16) reported a result, which is significantly too high (163 % recovery).

2-Phenylphenol, success rate 80 % (16 out of 20 laboratories)

Sixteen (16) out of twenty (20) labs quantified 2-Phenylphenol correctly. The results of lab 5 (68 % recovery) and lab 15 (121 % recovery) are considered satisfying due to the non-commercial rounding of the accepted ranges (see section 4.1 for explanation).

Prochloraz, success rate 75 % (14 out of 20 laboratories)

Out of twenty (20) laboratories, which identified Prochloraz correctly, fourteen (14) quantified Prochloraz within 70 to 120 % recovery of the spiked level, while six (6) laboratories (labs 2, 3, 4, 5, 9, and 18) reported results slightly below 70 % recovery of the spiked level (62 to 67 % recovery).

Pyriproxyfen, success rate 75 % (15 out of 20 laboratories)

75 % (15 labs) of the laboratories in the test reported satisfying results related to Pyriproxyfen. Five laboratories (labs 3, 5, 10, 19, and 20) failed to achieve the lower bound of the accepted range and reported results showing 60 to 67 % recovery of the spiked level.

Tebufenpyrad, success rate 95 % (19 out of 20 laboratories)

The results related to Tebufenpyrad are satisfying. All but one laboratory (lab 3) reported satisfying results within 70 to 120 % recovery of the spiked level.

Terbuthylazine, success rate 90 % (18 out of 20 laboratories)

The performance of the laboratories is satisfying with respect to the quantification of Terbuthylazine in the test. Eighteen (18) out of twenty (20) laboratories reported satisfying results.

Phosphonic acid, success rate 84 % (16 out of 19 laboratories)

The labs were explicitly instructed to analyse for Phosphonic acid. All laboratories reported results of phosphonic acid with the exception of one lab (lab 19), which currently subcontracts phosphonic acid to another lab. Lab 5 reported a false negative result of phosphonic acid even though the spiked level (0.25 mg/kg) is by far higher than the corresponding reporting limit (0.005 mg/kg).

Seventeen (17) out of nineteen (19) laboratories reported satisfying results within 70 to 120 % recovery of the spiked level.

Table 2. Results of Chlorpyrifos-methyl and Fenbutatin oxide

Laboratory code	Chlorpyrifos-methyl spiked level: 0.024 mg/kg assigned value: 0.016 mg/kg Accepted range (trueness criterion): 0.016-0.029 mg/kg						Fenbutatin oxide spiked level: 0.032 mg/kg assigned value: 0.027 mg/kg Accepted range (trueness criterion): 0.022-0.039 mg/kg					
	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score
1	0.018	104	0.005	75	yes	0.5	n.r.*	-	-	-	**no**	-
2	0.014	95	0.005	**58**	**no**	-0.6	0.024	109	0.005	75	yes	-0.6
3	0.014	70-120	0.005	**58**	**no**	-0.6	0.020	70-120	0.005	**63**	**no**	-1.2
4	0.013	101	0.01	**54**	**no**	-0.9	0.026	71	0.01	81	yes	-0.2
5	0.013	81	0.005	**54**	**no**	-0.9	D<0.005	92	0.005	-	**no**	-4.5
6	0.018	80	-	75	yes	0.5	0.018	95	-	**56**	**no**	-1.6
7	0.017	97	0.010	71	yes	0.2	0.028	99	0.010	88	yes	0.1
8	0.013	101	0.01	**54**	**no**	-0.9	0.024	71	0.01	75	yes	-0.6
9	0.017	-	0.010	71	yes	0.2	0.034	-	0.010	106	yes	1.1
10	0.018	95	0.010	75	yes	0.5	0.020	95	0.010	**63**	**no**	-1.2
11	0.022	79	0.01	92	yes	1.6	0.032	120	0.01	100	yes	0.8
12	0.021	98	0.010	88	yes	1.3	0.031	72	0.010	97	yes	0.6
13	0.016	91	0.01	67	yes	-0.1	0.035	81	0.01	109	yes	1.3
14	0.019	100	0.01	79	yes	0.8	0.032	78	0.01	100	yes	0.8
15	0.016	81	0.01	67	yes	-0.1	0.030	112.60	0.01	94	yes	0.4
16	0.016	90	0.01	67	yes	-0.1	0.052	96	0.01	**163**	**no**	4.1
17	0.025	-	0.005	104	yes	2.5	0.030	-	0.005	94	yes	0.4
18	0.017	110	0.01	71	yes	0.2	0.023	115	0.01	72	yes	-0.7
19	0.012	95	0.01	**50**	**no**	-1.2	0.021	69	0.01	**66**	**no**	-1.1
20	0.011	87	0.01	**46**	**no**	-1.5	0.028	93	0.01	88	yes	0.1

RL: reporting limit; D: detected

* not reported. Fenbutatin oxide is not within the scope of laboratory 1

Table 3. Results of 2-Phenylphenol and Prochloraz

	2-Phenylphenol spiked level: 0.34 mg/kg assigned value: 0.29 mg/kg Accepted range (trueness criterion): 0.23-0.41 mg/kg						Prochloraz spiked level: 0.052 mg/kg assigned value: 0.039 mg/kg Accepted range (trueness criterion): 0.036-0.063 mg/kg					
Laboratory code	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score
1	0.44	106	0.010	**129**	**no**	2.7	0.060	95	0.005	115	yes	2.5
2	0.240	99	0.005	71	yes	-0.9	0.035	96	0.005	**67**	**no**	-0.5
3	0.24	70-120	0.005	71	yes	-0.9	0.032	70-120	0.032	**62**	**no**	-0.8
4	0.17	101	0.01	**50**	**no**	-2.2	0.034	99	0.01	**65**	**no**	-0.6
5	0.23	80	0.005	68	yes	-1.1	0.033	86	0.005	**63**	**no**	-0.7
6	0.240	76	-	71	yes	-0.9	0.036	92	-	69	yes	-0.3
7	0.31	96	0.010	91	yes	0.3	0.042	85	0.010	81	yes	0.4
8	0.20	101	0.01	**59**	**no**	-1.6	0.050	99	0.01	96	yes	1.3
9	0.33	-	0.010	97	yes	0.7	0.035	-	0.010	**67**	**no**	-0.5
10	0.26	95	0.010	76	yes	-0.5	0.036	95	0.010	69	yes	-0.3
11	0.27	87	0.01	79	yes	-0.4	0.043	99	0.01	83	yes	0.5
12	0.254	98	0.010	75	yes	-0.7	0.042	97	0.010	81	yes	0.4
13	0.37	91	0.01	109	yes	1.4	0.040	102	0.01	77	yes	0.1
14	0.29	108	0.01	85	yes	0.0	0.037	100	0.01	71	yes	-0.2
15	0.41	69	0.01	121	yes	2.1	0.039	93.10	0.01	75	yes	0.0
16	0.44	95	0.01	**129**	**no**	2.7	0.042	95	0.01	81	yes	0.4
17	0.35	-	0.005	103	yes	1.1	0.050	-	0.005	96	yes	1.3
18	0.34	122	0.01	100	yes	0.9	0.035	100	0.01	**67**	**no**	-0.5
19	0.25	116	0.01	74	yes	-0.7	0.037	109	0.01	71	yes	-0.2
20	0.29	90	0.01	85	yes	0.0	0.043	103	0.01	83	yes	0.5

RL: reporting limit

Table 4. Results of Pyriproxyfen and Tebufenpyrad

Laboratory code	Pyriproxyfen spiked level: 0.060 mg/kg assigned value: 0.050 mg/kg Accepted range (trueness criterion): 0.042-0.072 mg/kg						Tebufenpyrad spiked level: 0.014 mg/kg assigned value: 0.011 mg/kg Accepted range (trueness criterion): 0.009-0.017 mg/kg					
	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score
1	0.060	98	0.005	100	yes	0.9	0.012	102	0.005	86	yes	0.5
2	0.048	95	0.005	80	yes	-0.2	0.011	94	0.005	79	yes	0.0
3	0.040	70-120	0.005	**67**	**no**	-0.9	0.008	70-120	0.005	**57**	**no**	-1.2
4	0.050	99	0.01	83	yes	0.0	0.010	100	0.01	71	yes	-0.4
5	0.038	87	0.005	**63**	**no**	-1.1	0.010	86	0.005	71	yes	-0.4
6	0.042	93	-	70	yes	-0.7	0.0090	86	-	64	yes	-0.8
7	0.058	89	0.010	97	yes	0.7	0.011	103	0.010	79	yes	0.0
8	0.055	99	0.01	92	yes	0.5	0.012	100	0.01	86	yes	0.5
9	0.049	-	0.010	82	yes	-0.1	0.011	-	0.010	79	yes	0.0
10	0.040	95	0.010	**67**	**no**	-0.9	0.011	95	0.010	79	yes	0.0
11	0.056	83	0.01	93	yes	0.6	0.013	83	0.01	93	yes	0.9
12	0.059	98	0.010	98	yes	0.8	0.013	95	0.010	93	yes	0.9
13	0.060	97	0.01	100	yes	0.9	0.010	94	0.01	71	yes	-0.4
14	0.052	104	0.01	87	yes	0.2	0.011	100	0.01	79	yes	0.0
15	0.049	80	0.01	82	yes	-0.1	0.011	92.40	0.01	79	yes	0.0
16	0.049	102	0.01	82	yes	-0.1	0.010	100	0.01	71	yes	-0.4
17	0.065	-	0.005	108	yes	1.4	0.015	-	0.005	107	yes	1.7
18	0.055	103	0.01	92	yes	0.5	0.010	90	0.01	71	yes	-0.4
19	0.037	87	0.01	**62**	**no**	-1.2	0.012	117	0.01	86	yes	0.5
20	0.036	87	0.01	**60**	**no**	-1.3	0.010	90	0.01	71	yes	-0.4

RL: reporting limit

Table 5. Results of Terbuthylazine and Phosphonic acid

| Laboratory code | Terbuthylazine spiked level: 0.026 mg/kg assigned value: 0.022 mg/kg Accepted range (trueness criterion): 0.018-0.032 mg/kg | | | | | | Phosphonic acid spiked level: 0.25 mg/kg assigned value: 0.23 mg/kg Accepted range (trueness criterion): 0.17-0.30 mg/kg | | | | | |
	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score	Result [mg/kg]	Recovery [%]	RL [mg/kg]	Result in % of the spiked level	Trueness criterion passed	z-score
1	0.025	94	0.005	96	yes	0.5	0.32	73	0.05	128	**no**	1.9
2	0.024	111	0.005	92	yes	0.3	0.228	106	0.005	91	yes	-0.1
3	0.020	70-120	0.005	77	yes	-0.5	0.23	70-120	0.05	92	yes	-0.1
4	0.018	96	0.01	69	yes	-0.9	0.23	111	0.01	92	yes	-0.1
5	0.018	74	0.005	69	yes	-0.9	ND	-	0.005	-	**no**	-5.0
6	0.018	81	-	69	yes	-0.9	0.28	92	-	112	yes	1.0
7	0.023	100	0.010	88	yes	0.1	0.26	94	0.10	104	yes	0.6
8	0.020	96	0.01	77	yes	-0.5	0.23	111	0.01	92	yes	-0.1
9	0.016	-	0.010	**62**	**no**	-1.3	0.23	-	0.1	92	yes	-0.1
10	0.022	95	0.010	85	yes	-0.1	0.26	95	0.10	104	yes	0.6
11	0.026	84	0.01	100	yes	0.7	0.21	83	0.1	84	yes	-0.5
12	0.023	100	0.010	88	yes	0.1	0.201	95	0.10	80	yes	-0.7
13	0.024	92	0.01	92	yes	0.3	0.21	82	n.r.	84	yes	-0.5
14	0.027	104	0.01	104	yes	0.9	0.18	75	0.10	72	yes	-1.1
15	0.036	96	0.01	**138**	**no**	2.8	0.218	95	0.2	87	yes	-0.3
16	0.021	94	0.01	81	yes	-0.3	0.23	94	0.1	92	yes	-0.1
17	0.025	-	0.005	96	yes	0.5	0.24	-	0.1	96	yes	0.2
18	0.022	110	0.01	85	yes	-0.1	0.21	98	0.1	84	yes	-0.5
19	0.020	110	0.01	77	yes	-0.5	n.a.	-	-	-	**no**	-
20	0.027	79	0.01	104	yes	0.9	0.2	86	0.1	80	yes	-0.6

RL: reporting limit; ND: not detected; n.a.: not analysed, the analysis of phosphonic acid is subcontracted to another laboratory

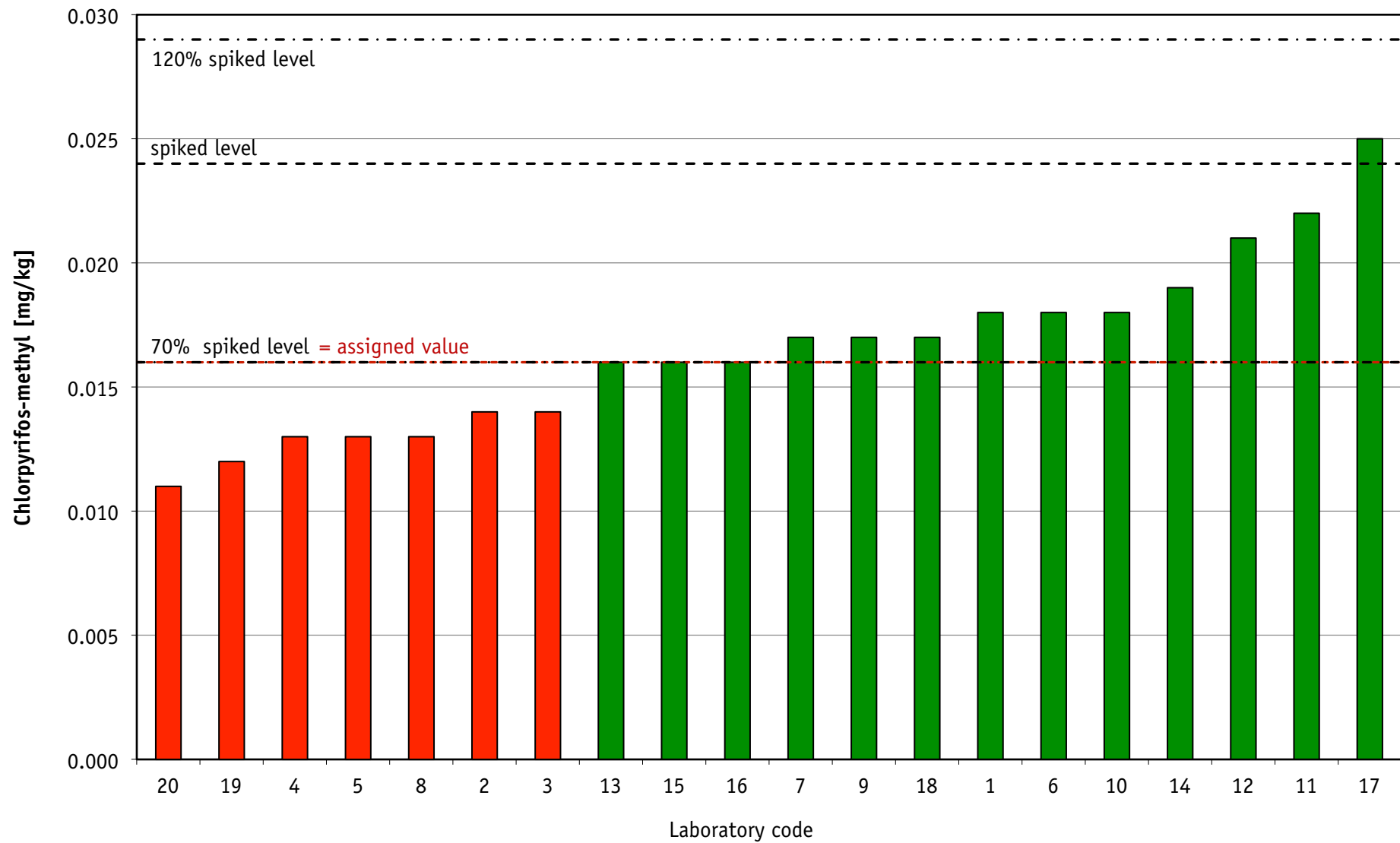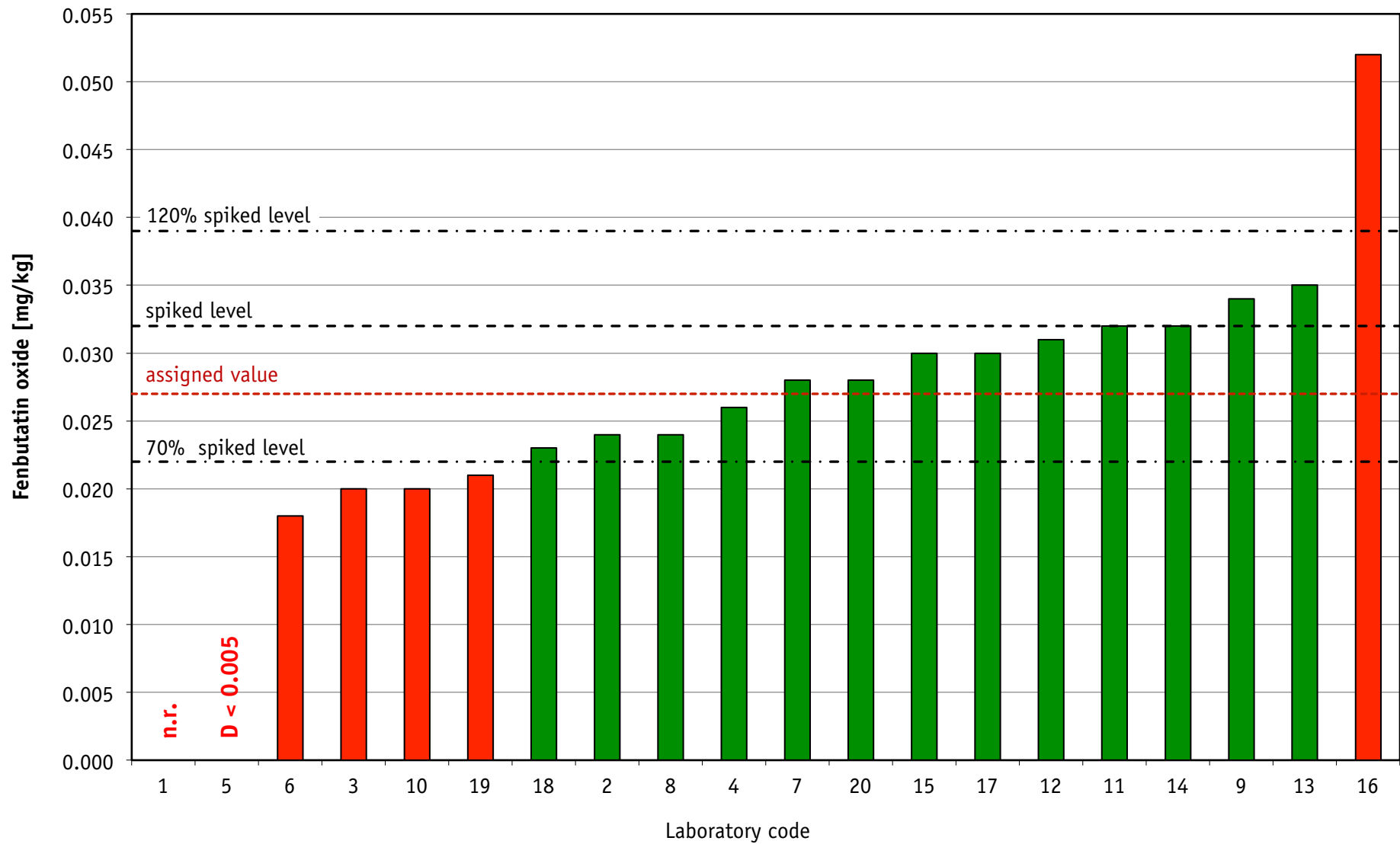Figure 1. Assessment of Chlorpyrifos-methyl (spiked level: 0.024 mg/kg)

Figure 2. Assessment of Fenbutatin oxide (spiked level: 0.032 mg/kg)

n.r.: not reported; D: detected

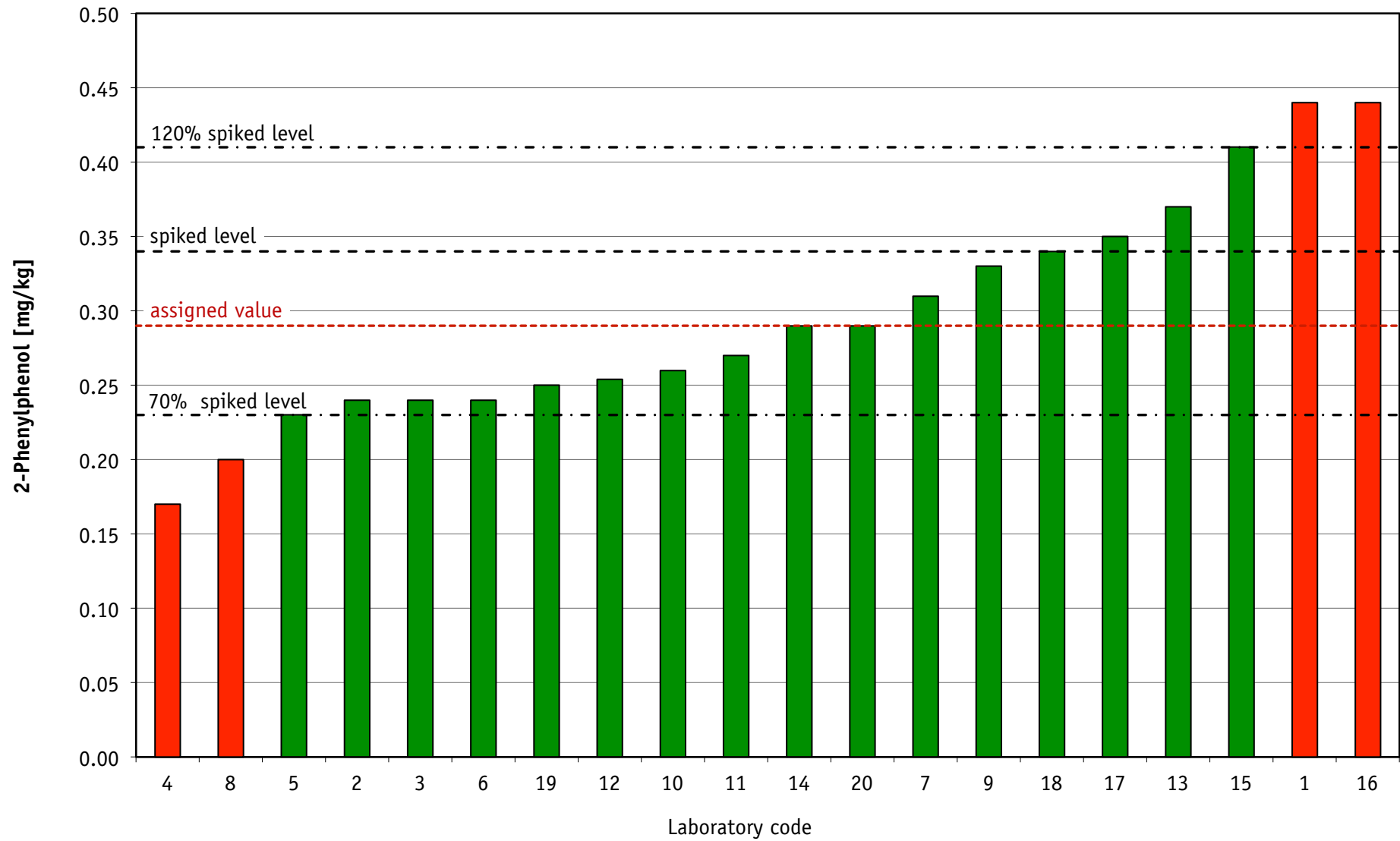*Figure 3. Assessment of 2-Phenylphenol (spiked level: 0.34 mg/kg)*

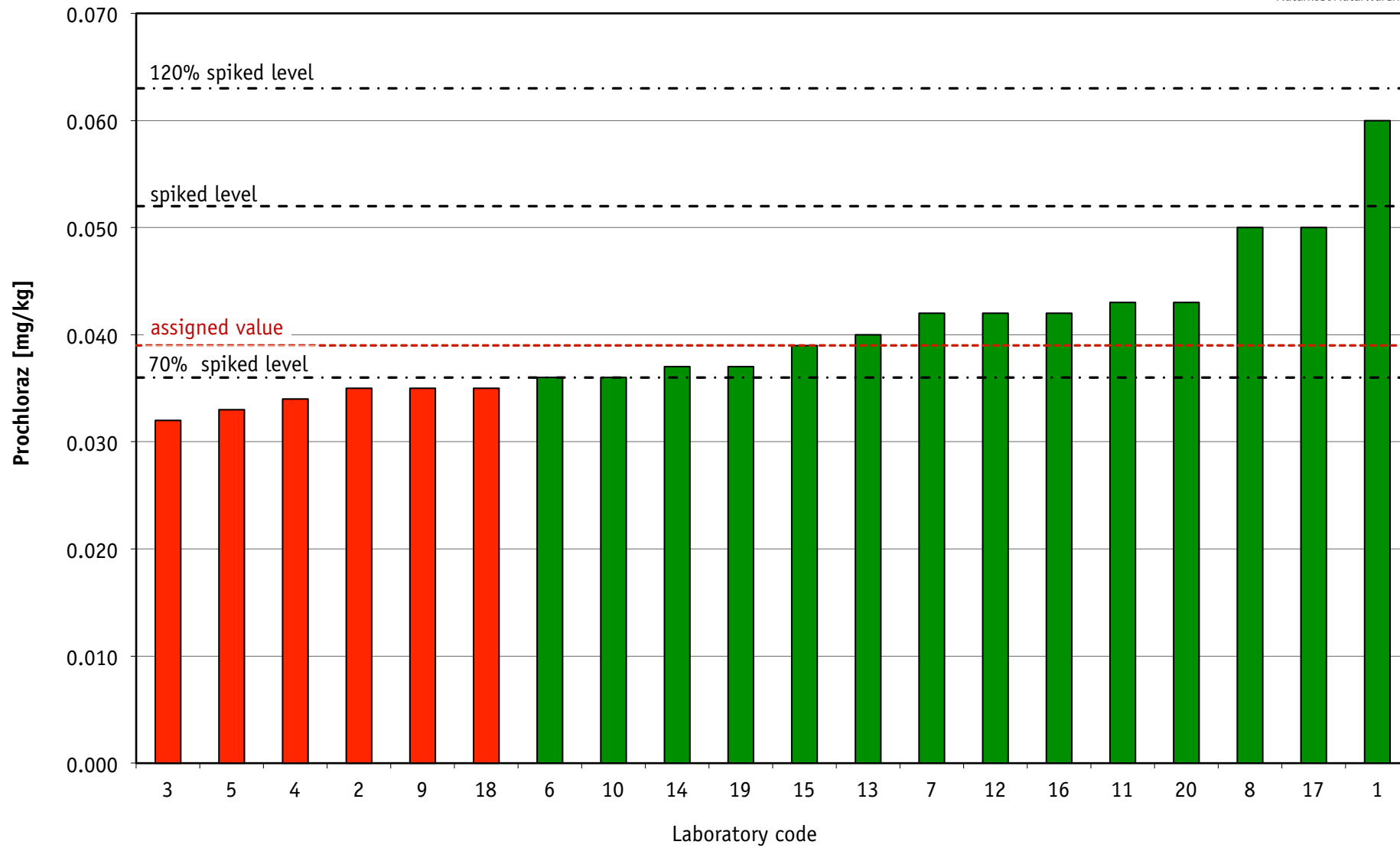Figure 4. Assessment of Prochloraz (spiked level: 0.052 mg/kg)

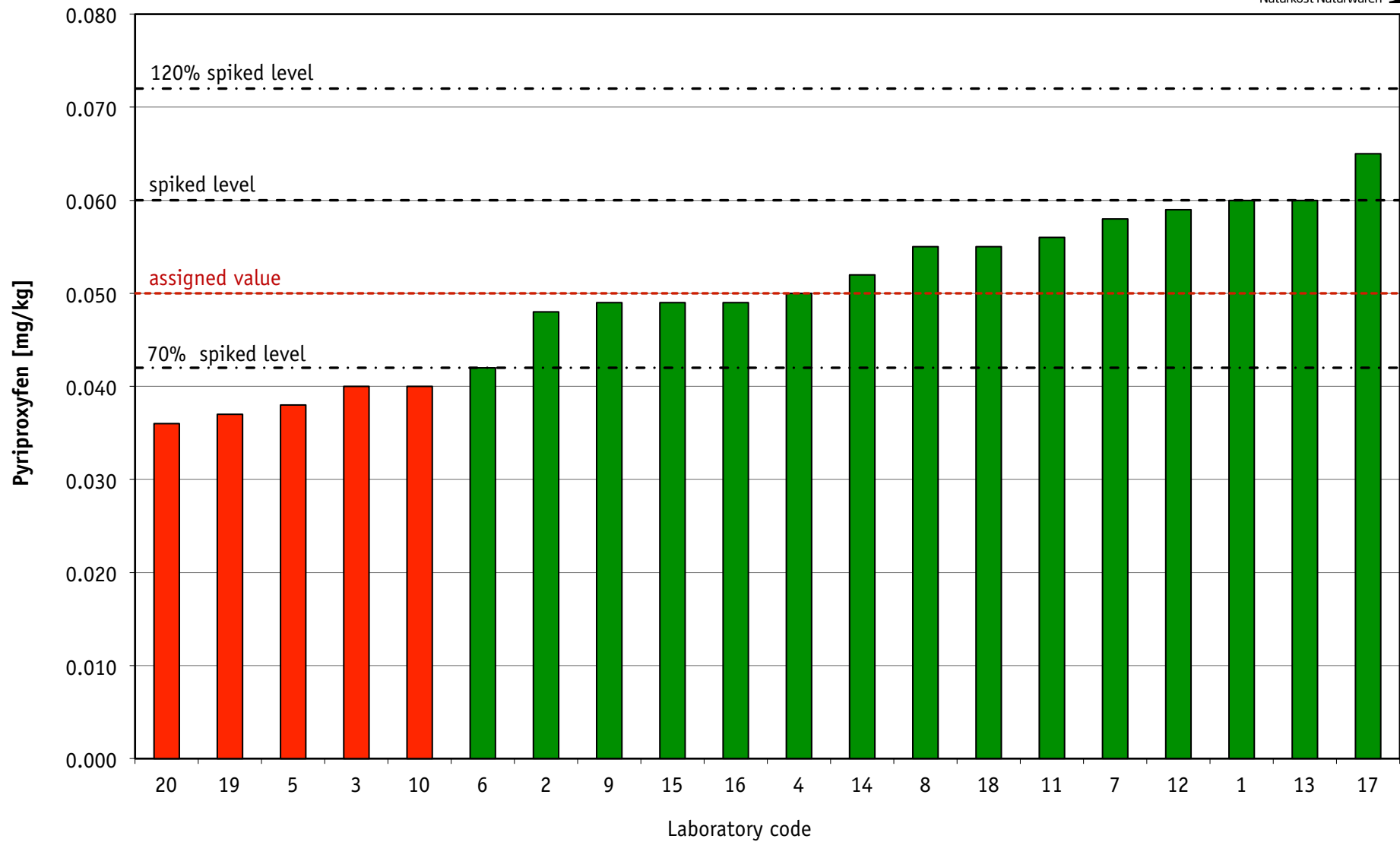*Figure 5. Assessment of Pyriproxyfen (spiked level: 0.060 mg/kg)*

Figure 6. Assessment of Tebufenpyrad (spiked level: 0.014 mg/kg)
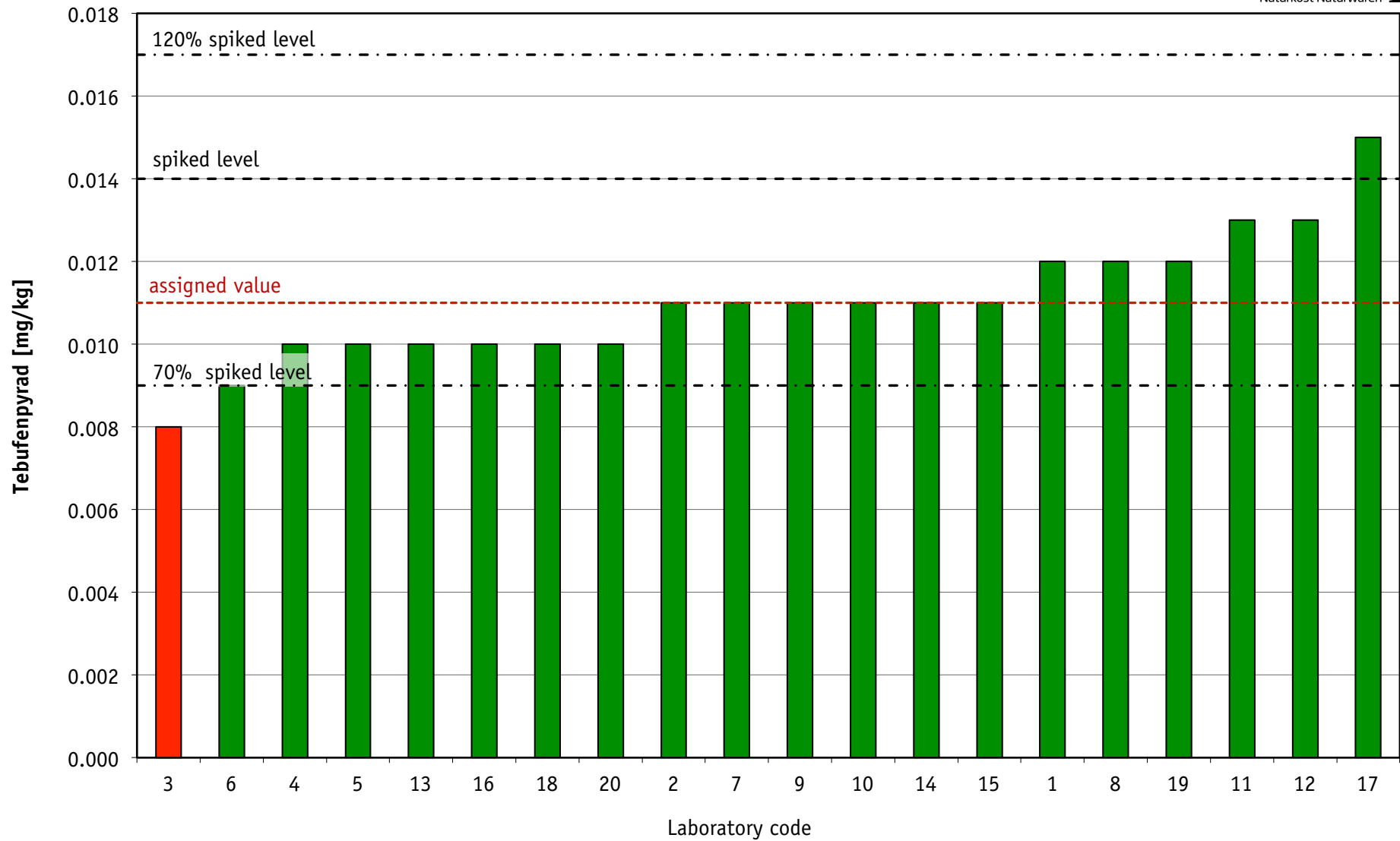
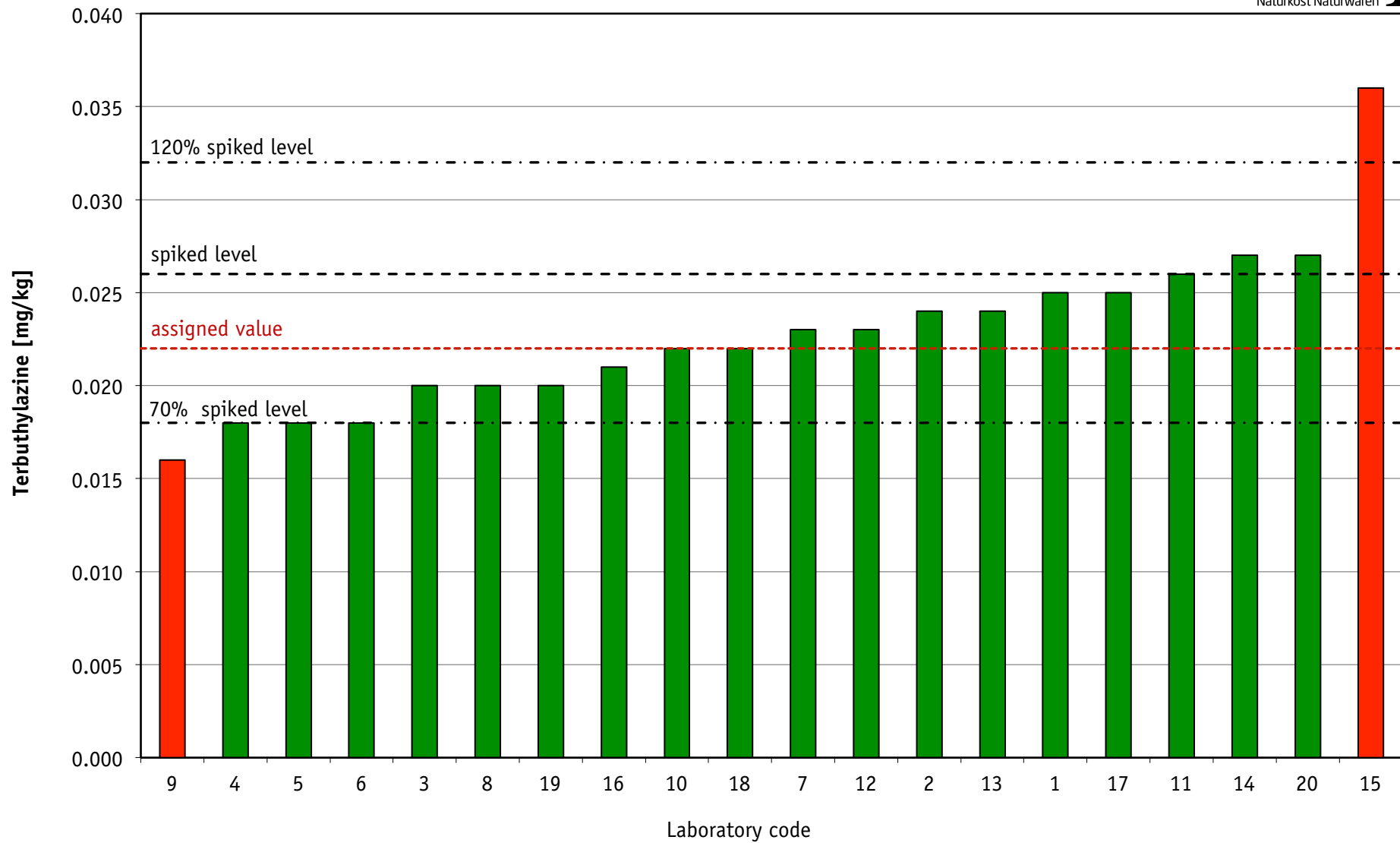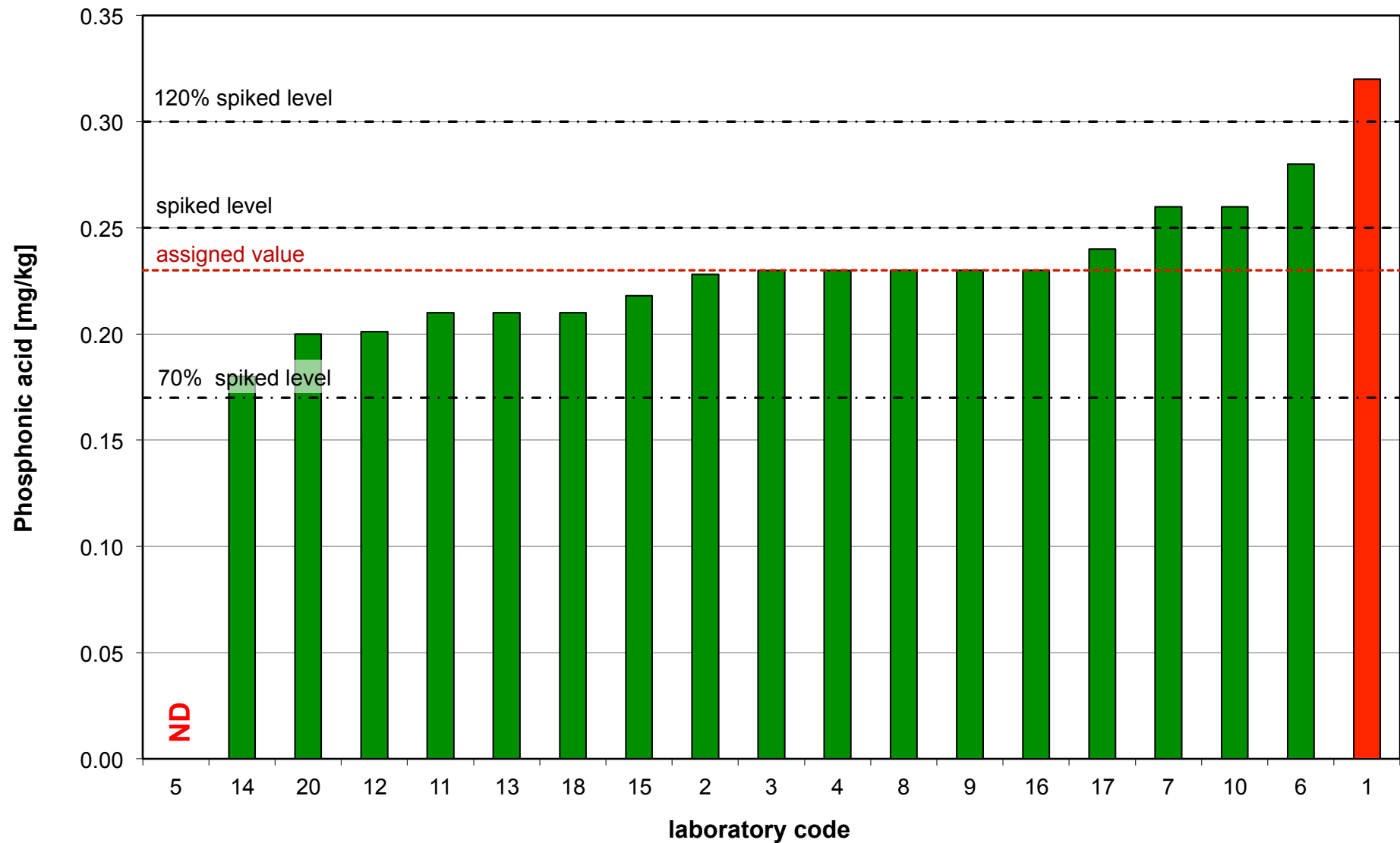*Figure 7. Assessment of Terbuthylazine (spiked level: 0.026 mg/kg)*

Figure 8. Assessment of Phosphonic acid (spiked level: 0.25 mg/kg)

ND: not detected

6. Proof of concept study

Prior to the laboratory performance assessment a proof of concept study was conducted to confirm the suitability of the applied method of sample treatment. For this purpose, a solution of pesticides was injected to a set of six samples of lemons (10 lemons per sample) as described in detail in section 3. Each sample (10 lemons each) was homogenised thereafter (all in all six different homogenates). Each of the six homogenates is analysed in duplicate. The results of the proof of concept study confirm the suitability of the spiking procedure by means of reproducibility and accuracy (table 6).

Table 6. Results of the proof of concept study

Subsample No.	Overall weight of the sample - 10 lemons (g)	Extraction No.	Chlorpyrifos-methyl [mg/kg]	Fenbutatin oxide [mg/kg]	2-Phenyl-phenol [mg/kg]	Prochloraz [mg/kg]	Pyriproxy-fen [mg/kg]	Tebufen-pyrad [mg/kg]	Terbuthyl-azine [mg/kg]	Phosphonic acid [mg/kg]
1	1627	1	0.030	0.036	0.38	0.053	0.077	0.018	0.030	0.258
		2	0.031	0.038	0.40	0.049	0.080	0.019	0.030	0.255
2	1655	1	0.024	0.039	0.37	0.060	0.076	0.018	0.034	0.270
		2	0.024	0.039	0.33	0.053	0.058	0.011	0.032	0.265
3	1616	1	0.033	0.036	0.31	0.058	0.075	0.017	0.037	0.307
		2	0.027	0.036	0.32	0.053	0.062	0.014	0.034	0.325
4	1638	1	0.027	0.036	0.31	0.047	0.075	0.013	0.031	0.302
		2	0.029	0.032	0.34	0.049	0.084	0.018	0.031	0.307
5	1642	1	0.025	0.034	0.34	0.052	0.076	0.016	0.033	0.240
		2	0.029	0.034	0.33	0.055	0.080	0.013	0.035	0.258
6	1650	1	0.025	0.034	0.31	0.052	0.070	0.018	0.035	0.290
		2	0.024	0.032	0.32	0.061	0.078	0.013	0.034	0.262
Mean [mg/kg]			0.027	0.036	0.34	0.054	0.074	0.016	0.033	0.28
Standard deviation [mg/kg]			0.0031	0.0024	0.029	0.0044	0.0075	0.0027	0.0022	0.027
Coefficient of variation [%]			**11.2**	**6.7**	**8.6**	**8.1**	**10.1**	**17.3**	**6.7**	**9.6**
Spiked level [mg/kg]			0.025	0.035	0.37	0.056	0.065	0.015	0.029	0.28
Recovery [%]			**109**	**99**	**92**	**96**	**114**	**104**	**114**	**100**

7. Confirmation of the spiked values

One sample of lemons (7 pieces) was randomly chosen of the prepared test samples (see section 3). The sample was homogenised and analysed in duplicate directly after the preparation to confirm the spiked values before shipment of the samples. The recoveries of the results (92 to 109 %) confirm the spiked levels (table 7).

Table 7. Confirmation of the spiked values

Subsample No.	Overall weight of the sample - 7 lemons (g)	Extraction No.	Chlorpyrifos-methyl [mg/kg]	Fenbutatin oxide [mg/kg]	2-Phenyl-phenol [mg/kg]	Prochloraz [mg/kg]	Pyriproxy-fen [mg/kg]	Tebufen-pyrad [mg/kg]	Terbuthyl-azine [mg/kg]	Phosphonic acid [mg/kg]
1	1834	1	0.020	0.034	0.32	0.047	0.064	0.012	0.027	0.29
		2	0.024	0.037	0.32	0.048	0.062	0.013	0.027	0.23
Mean [mg/kg]			0.022	0.035	0.32	0.048	0.063	0.013	0.027	0.26
Spiked level [mg/kg]			0.024	0.032	0.34	0.052	0.060	0.014	0.026	0.25
Recovery [%]			**92**	**109**	**94**	**92**	**105**	**93**	**104**	**104**

8. Stability testing

One sample of lemons (7 pieces) was randomly chosen of the prepared test samples (see section 3). The sample was stored at +7 °C for one week (test period) and analysed in duplicate thereafter. The results confirm the stability of all parameters throughout the whole testing period (table 8).

Table 8. Results of the stability testing

Subsample No.	Overall weight of the sample - 7 lemons (g)	Extraction No.	Chlorpyrifos-methyl [mg/kg]	Fenbutatin oxide [mg/kg]	2-Phenyl-phenol [mg/kg]	Prochloraz [mg/kg]	Pyriproxy-fen [mg/kg]	Tebufen-pyrad [mg/kg]	Terbuthyl-azine [mg/kg]	Phosphonic acid [mg/kg]
1	1833	1	0.026	0.035	0.25	0.037	0.078	0.016	0.020	0.27
		2	0.016	0.030	0.22	0.064	0.075	0.013	0.035	0.26
Mean [mg/kg]			0.021	0.033	0.24	0.051	0.077	0.014	0.028	0.27
Spiked level [mg/kg]			0.024	0.032	0.34	0.052	0.060	0.014	0.026	0.25
Recovery [%]			**88**	**103**	**71**	**98**	**128**	**100**	**108**	**108**

9. Annex

Table 9. Overall sample weight of the lemon samples in the test

Laboratory code	Sample weight [g]
1	1848
2	1831
3	1835
4	1835
5	1841
6	1844
7	1829
8	1824
9	1841
10	1849
11	1827
12	1842
13	1830
14	1843
15	1846
16	1827
17	1840
18	1833
19	1842
20	1814
Mean sample weight [g]	1836
Minimum sample weight [g]	1814
Maximum sample weight [g]	1849