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Bundesverband
Naturkost Naturwaren e. V.

Laboratory Performance Assessment

Analysis of Pesticides in Flaxseed

(Module 3 of BNN lab approval system)

Report

December 2023

Bundesverband
Naturkost Naturwaren e. V.

Summary

The laboratory performance assessment related to pesticides in flaxseed was designed and organised by Lach & Bruns in October 2023 on behalf of BNN e.V. (Bundesverband Naturkost Naturwaren, Berlin, Germany).

The test material consists of (whole) Flaxseeds, which was spiked with 6 analytes (5 pesticides and 1 synergist):

Procymidone, MCPA-glucoside, Piperonyl butoxide (PBO), Imazamox, Pirimiphos-methyl, and tau-Fluvalinate.

As Imazamox, Pirimiphos-methyl, and tau-Fluvalinate showed significantly deviating results of the spiked concentration levels during homogeneity and stability testing, these three substances were assessed by using the **z-score** (**comparability**) criterion according to Horwitz.
The other three substances (Procymidone, MCPA-glucoside calculated as MCPA free acid (sum), and PBO) were assessed by application of the **trueness criterion** (70% -120% of the spiking level).

The entire batch of the flaxseeds was thoroughly but carefully mixed avoiding any crushing or chopping of the seeds. Subsamples of the test material were distributed to sixteen (16) participants across four (4) European countries (Germany, Italy, The Netherlands, and Spain). Information with respect to the scope of analyses to be applied was provided to the laboratories in an instruction letter attached to the test sample.

All participants but one handed in their results before the deadline on 30th of October 2023. One participant submitted a quantitative result for one pesticide (Imazamox) after the deadline. The result was accepted, too, as it was possible to prove, that the result was determined before the official deadline. The result was below the current reporting limit and as a consequence missed to be sent out in time. Consequently, all results were considered for the assessment of results.

The performance assessment considers the following test criteria:

- No *false positive results*.
- Correct *identification* of the analytes with levels above 0,01 mg/kg (in total 6 analytes). Thus, no false negative results are accepted, independent whether an analyte is within the analytical scope of the laboratory or not.
- Correct *quantification* of at least 5 pesticides related to their residue definitions and the applied assessment criteria (trueness / comparability).

Based on these criteria, the laboratories were marked with one out of 3 marks, ranging from "*excellent*" to "*good*" to "*insufficient*". Laboratories with an excellent (12 out of 16 participants) or good result (2 participants) satisfy the quality standards of BNN.

2 participants do not meet the expected requirements of BNN as they both did not report tau-Fluvalinate.

Assessment of quantification

Analytical results within 70% - 120% of the spiked values are considered satisfying for the assessment of Procymidone, MCPA free acid (sum), Piperonyl butoxide (PBO).

Analytical results with z-scores ≤ |2| are considered satisfying for the assessment of Imazamox, Pirimiphos-methyl, and tau-Fluvalinate.

The overall performance is summarised as:

Substance	Spiked value [mg/kg]	Assigned Value [mg/kg]	Number of results	Correct quantification
Procymidone	0,023	-	16	16 (100%)
MCPA free acid (sum)	0.025	-	16	13 (81%)
PBO	0,027	-	16	16 (100%)
Imazamox	-	0.0234	16	16 (100%)
Pirimiphos-methyl	-	0.0397	16	16 (100%)
tau-Fluvalinate	-	0.0222	14	14 (88%)
TOTAL			94 of 96 (98%)	91 of 96 (95%)

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1. Test material preparation and design

The laboratory performance assessment was designed to verify the analytical competence related to BNN module M3 (oilseeds and vegetable oils and fats), area pesticides.

Organic flaxseeds were used for preparation of the test material. A sub-sample was taken and analysed (multi-method, polar pesticides, and acidic herbicides) to ensure that no incurred residues of pesticides (> 0,01 mg/kg) are present. The analysis of the sub-sample identified no levels of pesticides.

The organic flaxseeds were carefully mixed to avoid any crushing or chopping of the seeds. Subsamples of the non-spiked flaxseeds (ca. 100 g each) were also bottled as blank material (for internal analyses like homogeneity and stability testing only). Thereafter, the flax seeds were spiked on the surface with *MCPA-Glucoside, Piperonyl butoxide (PBO), Procymidone*, *Imazamox*, *Pirimiphos-methyl,* and *tau-Fluvalinate*. For that purpose, a mix-solution of the analytes was added gradually while the flaxseeds were carefully stirred to avoid crushing the seeds. Careful stirring was continued after spiking to ensure a homogeneous distribution of the analytes in the test material. After spiking, subsamples of ca. 200 g were transferred into plastic bottles. The test materials were stored at -18°C in the dark until distribution.

The test samples were stored in Styrofoam boxes and cooled with dry ice during the transport to the participants of the ring test.

The homogeneity and stability of the prepared test material was tested by an independent laboratory (homogeneity tests were performed one week before sample delivery date). Stability testing was performed after the last participant reported the results. The results showed sufficient stability across the entire test period. The results for homogeneity testing also confirm the homogeneity of the test material.

The samples were sent to the participating labs on October 23rd, 2023, thus arriving 24th/25th October at the participating laboratories (unannounced test). Reporting of results was scheduled for 30th October 2023.

Design of BNN flaxseed test material and homogeneity/stability testing

Analyte	**Spiked level (mg/kg)**	homogeneity / stability average results (mg/kg)	spike level recovery rate homogeneity (in %)	spike level recovery rate stability (in %)
Procymidone	0,023	0.018 / 0.0178	78	77
MCPA free acid (sum)	0.025	0.019 / 0.018	76	72
PBO	0,027	0.023 / 0.023	85	85
Imazamox	*0,038*	*0.023 / 0.025*	***61***	***66***
Pirimiphos-methyl	*0,025*	*0.037 / 0.036*	***148***	***144***
tau-Fluvalinate	*0,033*	*0.015 / 0.015*	***45***	***45***

Due to the deviating recoveries between the spiked levels and the results reported for homogeneity and stability testing, the reported results of Imazamox, Pirimiphos-methyl, and tau-Fluvalinate were assessed according to the assigned values (z-score).

2. Statistical evaluation of results

Accuracy (trueness) criterion

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

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

(x = reported result; sl = spiked level)

Accepted range:
Results, which correspond to a recovery of 70 to 120 % of the spiked level, are considered satisfying in this laboratory performance assessment.

z-score (comparability) criterion

The comparability of results is evaluated according to the z-score model based on an assigned value and the target standard deviation (acc. to Horwitz).

Assigned value

The assigned value x_{pt} is the robust mean, which is derived from the results of the participants according to ISO13528, Algorithm A [1] [1]. The Winsorisation algorithm is applied to minimise the influence of outliers.

The assigned values are subject to commercial rounding and are presented with an accuracy of three significant figures.

z-score

The z-score is derived of the result x_i of each participant, the assigned value x_{pt} and the target standard deviation according to Horwitz σ_H[2], [2] [1]:

$$z - score\ = \frac{x_i - x_{pt}}{\sigma_H}$$

[1] [1] Statistical methods for use in proficiency testing by interlaboratory comparison. ISO 13528:2015. Corrected version 2016-10-15.
[2] Horwitz W. Evaluation of Analytical Methods Used for Regulation of Foods and Drugs. Anal Chem. 1982;54(1):67A–76A.

The results of pesticides are assessed with YES resp. NO, depending on the value of the z-score:

z-scores **≤ |2|** are rated satisfactory (“YES”),
z-scores **> |2|** are rated dissatisfactory (“NO”).

3. Results

The laboratories received the test samples without prior announcement. Upon receipt of the test sample, the laboratories were informed about the test, the type of test material and the scope of the test by an enclosed instruction letter. The laboratories were requested to analyse for “pesticides multi method” and “phenoxycarboxylic acids (incl. alkaline hydrolysis)”.

Sixteen (16) participants across four European countries (Germany, Italy, The Netherlands, and Spain) took part in the laboratory performance assessment.

All participants but one handed in their results before the deadline. One participant submitted a quantitative result for one pesticide (Imazamox) after the deadline. The result was accepted, too, as it was possible to prove, that the result was determined before the official deadline. The result was below the current reporting limit and as a consequence missed to be send out in time. Consequently, all results were considered for the assessment of results. Each laboratory was given a randomly selected identifier, hereinafter referred to as laboratory code.

The laboratories were asked to report all sought and found pesticides, the reporting limits (RL) as well as the scope of the applied analytical methods.

A summary of the overall performance of the labs is provided in table 2. A detailed evaluation of the results of the participants is presented in tables 3 to 8 and in figures 1 to 6.

Additionally, the performance of the participants has been evaluated based on the BNN requirements using the following criteria:

- No *false positive results*.
- Correct *identification* of the analytes with a spiked value above 0,01 mg/kg (in total 6 analytes). Thus, no false negative results.
- Correct *quantification* in terms of accuracy (trueness).

The performance is evaluated as:

excellent: all criteria fulfilled (6 out of 6 results in conformity with BNN criteria).

good: single slight error (at least 5 out of 6 results in conformity with BNN criteria) AND **no** false positive AND **no** false negative result.

insufficient: more than 2 quantification deviations OR false negative result(s) OR false positive result(s).

Results in detail

Accuracy (trueness) criterion:

- **Procymidone and Piperonyl butoxide (PBO)** are reported within the accepted range related to the trueness criterion (70 - 120% recovery of spike) by 16 out of 16 participants, which is an **excellent overall performance**.
- **MCPA free acid (sum)** causes some challenges in the correct quantification. However, 13 participants meet the target area of 70% to 120% recovery of the spiking level (81%), which still is considered as a good overall performance. No lab missed the identification of MCPA.

z-score (comparability) criterion:

- **Pirimiphos-methyl** is reported within the accepted range related to the comparability criterion (z-score of $|z| \leq 2$) by 16 out of 16 participants, which is an **excellent overall performance**.
- **Imazamox** also showed in general excellent performances for the participants (16 participants out of 16).
- **Tau-Fluvalinate** also showed in general good performances. The laboratories which identified tau-Fluvalinate also quantified it correctly (z-score of $|z| \leq 2$; 14 out of 16 laboratories). However, 2 participants (lab codes no. 10 and no. 16) did not report results for this pesticide. Consequently, 14 out of 16 participants reported successful results (88%).
- **14 out of 16 participants (87,5%) are rated excellent resp. good!** (no or just one deviation in quantification)
- **2 laboratories failed** in this test (which corresponds to 12,5% of the participants) as they did not identify tau-Fluvalinate. One of these 2 laboratories (no. 16) additionally quantified MCPA outside the target range of 70 – 120% recovery of spike.

Excellent results (12 labs, sorted by lab code):

1 / 3 / 4 / 6 / 7 / 8 / 9 / 11 / 12 / 13 / 14 / 15

Good results (2 labs, sorted by lab code):

2 / 5

Insufficient results (2 labs, sorted by lab code):

10 / 16

4. Overview of laboratory evaluation

Table 1: laboratory evaluation

lab code	false negative	false positive	Quantification (recovery / z-score) achieved	Final evaluation based on reported results*
1	0	0	6 / 6	excellent
2	0	0	5 / 6	good
3	0	0	6 / 6	excellent
4	0	0	6 / 6	excellent
5	0	0	5 / 6	good
6	0	0	6 / 6	excellent
7	0	0	6 / 6	excellent
8	0	0	6 / 6	excellent
9	0	0	6 / 6	excellent
10	1	0	5/ 5	insufficient
11	0	0	6 / 6	excellent
12	0	0	6 / 6	excellent
13	0	0	6 / 6	excellent
14	0	0	6 / 6	excellent
15	0	0	6 / 6	excellent
16	1	0	4/ 5	insufficient

*__excellent__: all criteria fulfilled (6 out of 6 results in conformity with BNN criteria);

*__good__: single slight error (at least 5 out of 6 results in conformity with BNN criteria)
AND **no** false positive AND **no** false negative result;

*__insufficient__: more than 2 quantification deviations OR false negative result(s) OR false positive result(s).

5. Tables and figures

*Table 2: Summary of the overall performance - **recovery of spike** (1 of 2)*

	Procymidone		MCPA free acid (sum)		Piperonyl butoxide (PBO)	
	result relative to spike [%]	criterion passed	result relative to spike [%]	criterion passed	result relative to spike [%]	criterion passed
1	87	Yes	84	Yes	96	Yes
2	91	Yes	56	No	89	Yes
3	78	Yes	68*	Yes*	104	Yes
4	104	Yes	80	Yes	119	Yes
5	70	Yes	48	No	85	Yes
6	87	Yes	84	Yes	104	Yes
7	109	Yes	108	Yes	119	Yes
8	100	Yes	68*	Yes*	107	Yes
9	109	Yes	96	Yes	107	Yes
10	87	Yes	96	Yes	96	Yes
11	104	Yes	92	Yes	104	Yes
12	100	Yes	112	Yes	111	Yes
13	109	Yes	84	Yes	107	Yes
14	83	Yes	68*	Yes*	100	Yes
15	83	Yes	68*	Yes*	104	Yes
16	100	Yes	133	No	111	Yes

*As 70 % of the spiked level (0.025 mg/kg) corresponds to 0.0175 mg/kg, reported results of 0.017 mg/kg (= recovery of 68%) are considered satisfying.

*Table 2 (continued): Summary of the overall performance – **z-score** (2 of 2)*

	Imazamox		Pirimiphos-methyl		tau-Fluvalinate	
	z-score	criterion passed	z-score	criterion passed	z-score	criterion passed
1	0.3	Yes	-0.1	Yes	-0.2	Yes
2	-0.3	Yes	-0.5	Yes	0.0	Yes
3	-0.7	Yes	-0.9	Yes	-0.2	Yes
4	0.9	Yes	0.0	Yes	-0.9	Yes
5	-0.1	Yes	-0.7	Yes	-0.4	Yes
6	0.1	Yes	-0.9	Yes	-0.2	Yes
7	1.5	Yes	1.6	Yes	0.4	Yes
8	-0.3	Yes	0.3	Yes	-0.2	Yes
9	-0.1	Yes	0.5	Yes	0.4	Yes
10	0.1	Yes	0.2	Yes	n.d.	No**
11	0.5	Yes	0.4	Yes	0.8	Yes
12	-0.1	Yes	0.0	Yes	1.6	Yes
13	-0.1	Yes	0.6	Yes	-1.7	Yes
14	-0.5	Yes	-0.4	Yes	0.6	Yes
15	-0.7	Yes	-0.1	Yes	0.2	Yes
16	0.1	Yes	0.6	Yes	n.d.	No***

** Reporting limit: 0.01 mg/kg tau-Fluvalinate *** tau-Fluvalinate: out of scope

Table 3: Results of Procymidone

	Procymidone		
	spiked value [mg/kg]: 0.023		
	Accepted range [%]: 70 - 120	Accepted range [mg/kg]: 0.0161 - 0.0276	
Lab code	**result [mg/kg]**	**relative to spike [%]**	**satisfactory**
1	0.020	87	Yes
2	0.021	91	Yes
3	0.018	78	Yes
4	0.024	104	Yes
5	0.016	70	Yes
6	0.020	87	Yes
7	0.025	109	Yes
8	0.023	100	Yes
9	0.025	109	Yes
10	0.020	87	Yes
11	0.024	104	Yes
12	0.023	100	Yes
13	0.025	109	Yes
14	0.019	83	Yes
15	0.019	83	Yes
16	0.023	100	Yes

Table 4: Results of MCPA free acid (sum)

	MCPA free acid (sum)		
	spiked value [mg/kg]: 0.025		
	Accepted range [%]: 70 - 120	Accepted range [mg/kg]: 0.017(0) - 0.03	
Lab code	**result [mg/kg]**	**relative to spike [%]**	**satisfactory**
1	0.021	84	Yes
2	0.014	56	No
3	0.017	68*	Yes
4	0.020	80	Yes
5	0.012	48	No
6	0.021	84	Yes
7	0.027	108	Yes
8	0.017	68*	Yes
9	0.024	96	Yes
10	0.024	96	Yes
11	0.023	92	Yes
12	0.028	112	Yes
13	0.021	84	Yes
14	0.017	68*	Yes
15	0.017	68*	Yes
16	0.033	133	No

*As 70 % of the spiked level (0.025 mg/kg) corresponds to 0.0175 mg/kg, reported results of 0.017 mg/kg (= recovery of 68%) are considered satisfying.

Table 5: Results of Piperonyl butoxide (PBO)

	Piperonyl butoxide (PBO)		
	spiked value [mg/kg]: 0.027		
	Accepted range [%]: 70 - 120	Accepted range [mg/kg]: 0.0189 - 0.0324	
Lab code	**result [mg/kg]**	**relative to spike [%]**	**satisfactory**
1	0.026	96	Yes
2	0.024	89	Yes
3	0.028	104	Yes
4	0.032	119	Yes
5	0.023	85	Yes
6	0.028	104	Yes
7	0.032	119	Yes
8	0.029	107	Yes
9	0.029	107	Yes
10	0.026	96	Yes
11	0.028	104	Yes
12	0.030	111	Yes
13	0.029	107	Yes
14	0.027	100	Yes
15	0.028	104	Yes
16	0.030	111	Yes

Table 6: Results of Imazamox

	Imazamox		
	assigned value (AV) [mg/kg]: 0.023		
		Accepted range: z-scores ≤ |2|	
Lab code	**result [mg/kg]**	**z-score**	**satisfactory**
1	0.025	0.3	Yes
2	0.022	-0.3	Yes
3	0.020	-0.7	Yes
4	0.028	0.9	Yes
5	0.023	-0.1	Yes
6	0.024	0.1	Yes
7	0.031	1.5	Yes
8	0.022	-0.3	Yes
9	0.023	-0.1	Yes
10	0.024	0.1	Yes
11	0.026	0.5	Yes
12	0.023	-0.1	Yes
13	0.023	-0.1	Yes
14	0.021	-0.5	Yes
15	0.020	-0.7	Yes
16	0.024	0.1	Yes

Table 7: Results of Pirimiphos-methyl

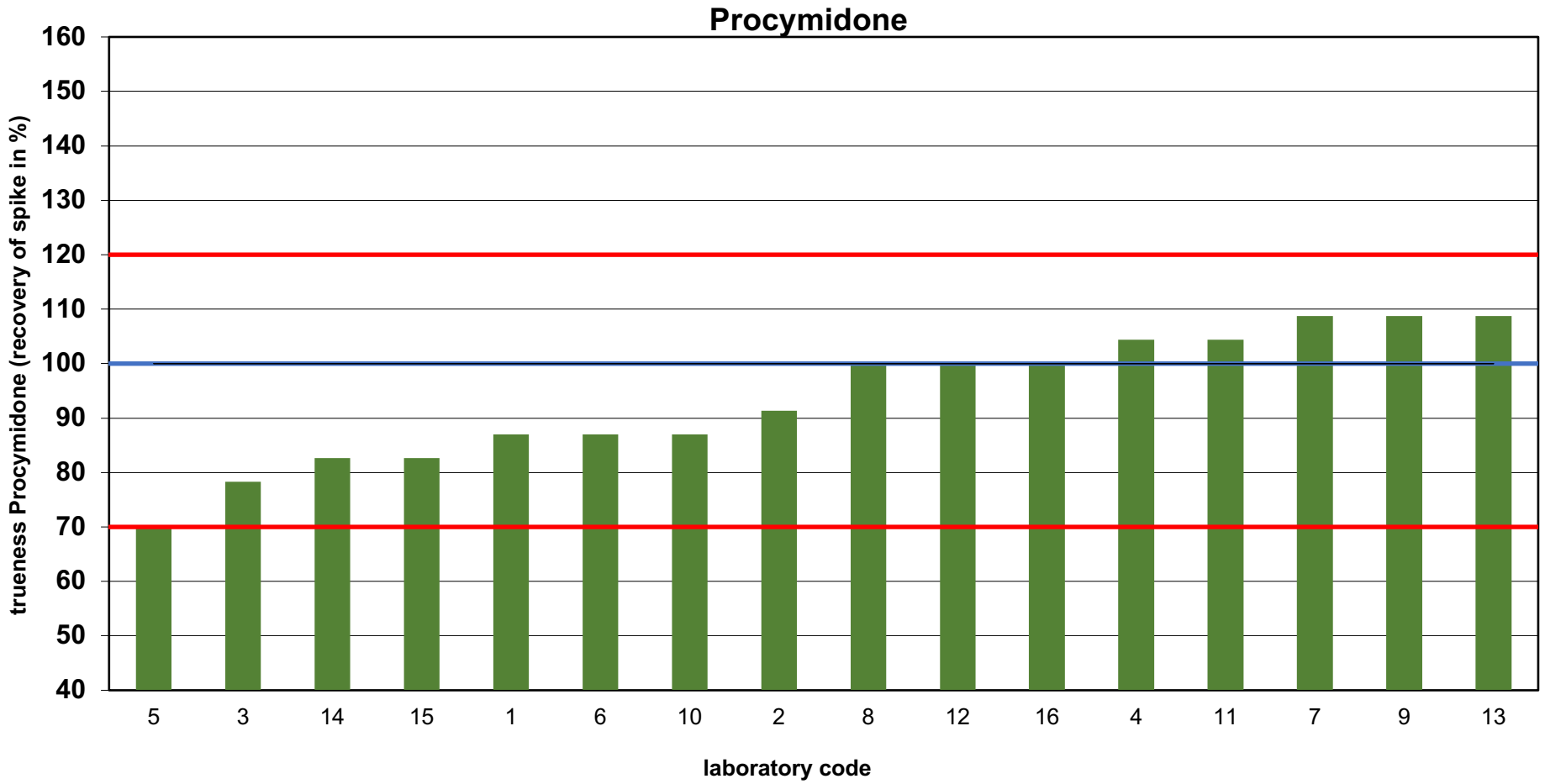
	Pirimiphos-methyl		
	assigned value (AV) [mg/kg]: 0.0397		
		Accepted range: z-scores ≤ |2|	
Lab code	**result [mg/kg]**	**z-score**	**satisfactory**
1	0.039	-0.1	Yes
2	0.035	-0.5	Yes
3	0.032	-0.9	Yes
4	0.040	0.0	Yes
5	0.034	-0.7	Yes
6	0.032	-0.9	Yes
7	0.054	1.6	Yes
8	0.042	0.3	Yes
9	0.044	0.5	Yes
10	0.041	0.2	Yes
11	0.043	0.4	Yes
12	0.040	0.0	Yes
13	0.045	0.6	Yes
14	0.036	-0.4	Yes
15	0.039	-0.1	Yes
16	0.045	0.6	Yes

Table 8: Results of tau-Fluvalinate

	tau-Fluvalinate		
	assigned value (AV) [mg/kg]: 0.0222		
		Accepted range: z-scores ≤ |2|	
Lab code	**result [mg/kg]**	**z-score**	**satisfactory**
1	0.021	-0.2	Yes
2	0.022	0.0	Yes
3	0.021	-0.2	Yes
4	0.018	-0.9	Yes
5	0.020	-0.4	Yes
6	0.021	-0.2	Yes
7	0.024	0.4	Yes
8	0.021	-0.2	Yes
9	0.024	0.4	Yes
10	n.r.	n.r.	No
11	0.026	0.8	Yes
12	0.030	1.6	Yes
13	0.014	-1.7	Yes
14	0.025	0.6	Yes
15	0.023	0.2	Yes
16	n.r.	n.r.	No

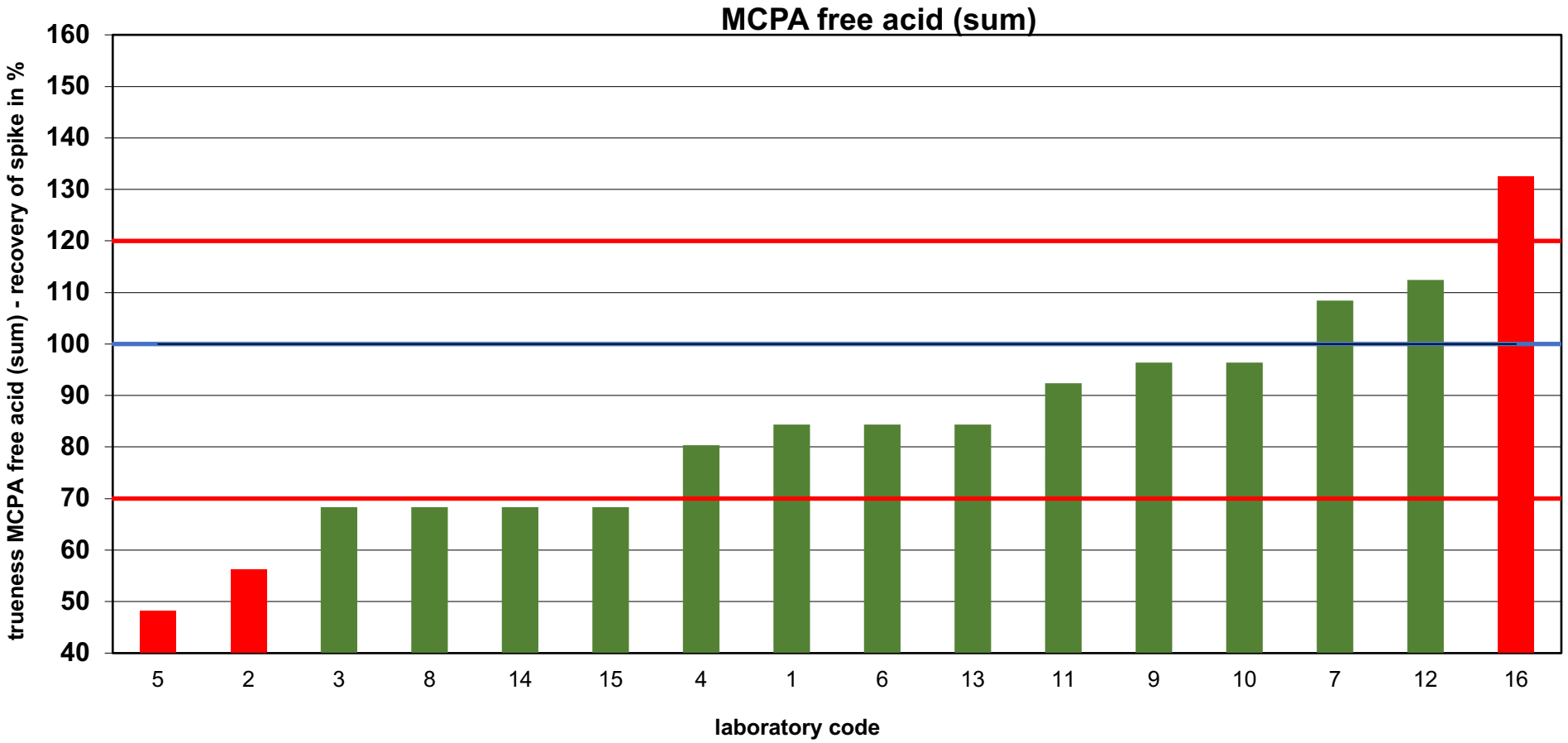
n.r.: not reported

Figure 1: Assessment of Procymidone - recovery of spike



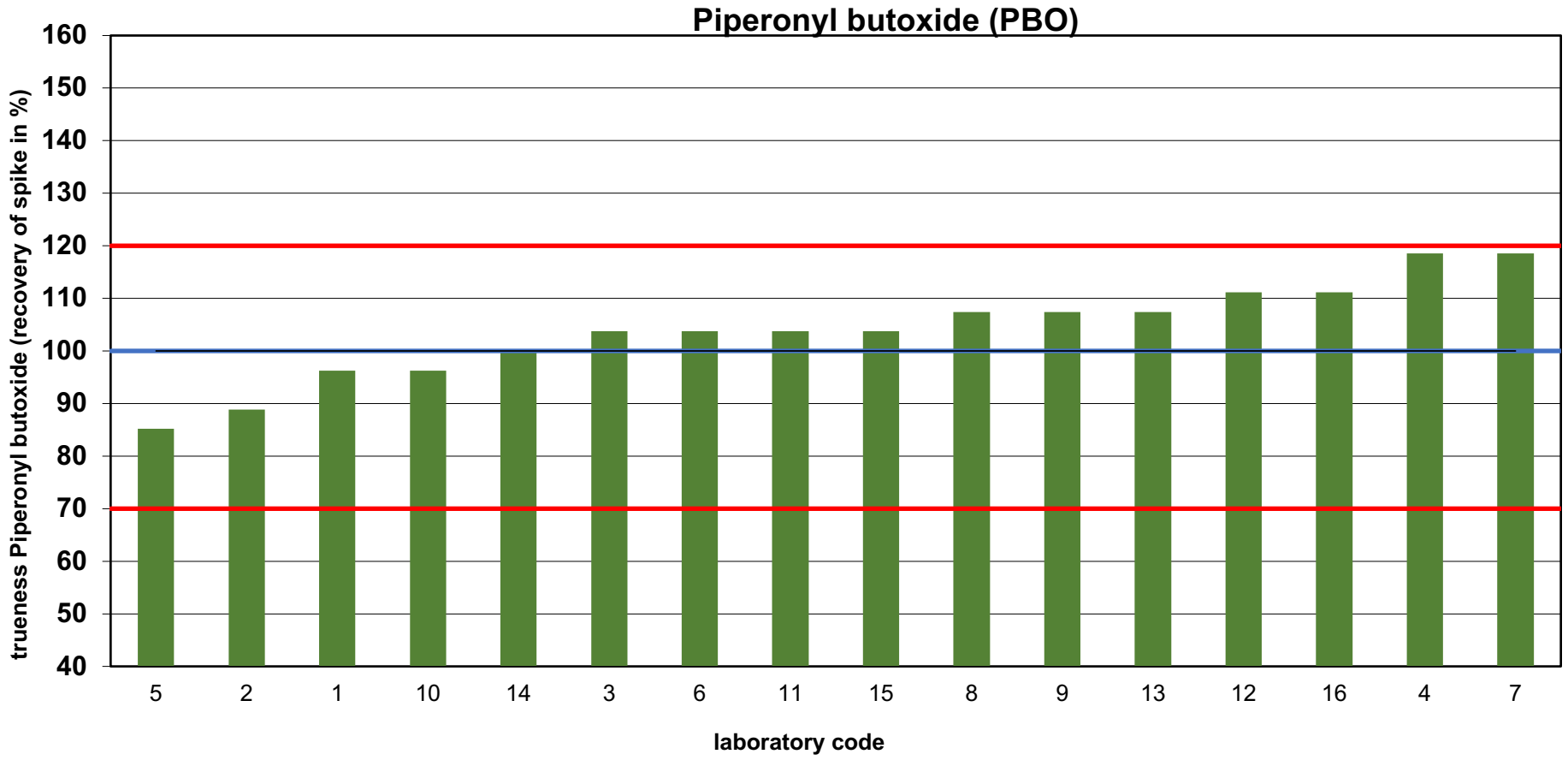
Green: satisfactory results, red: dissatisfactory results

Figure 2: Assessment of MCPA free acid (sum) - recovery of spike



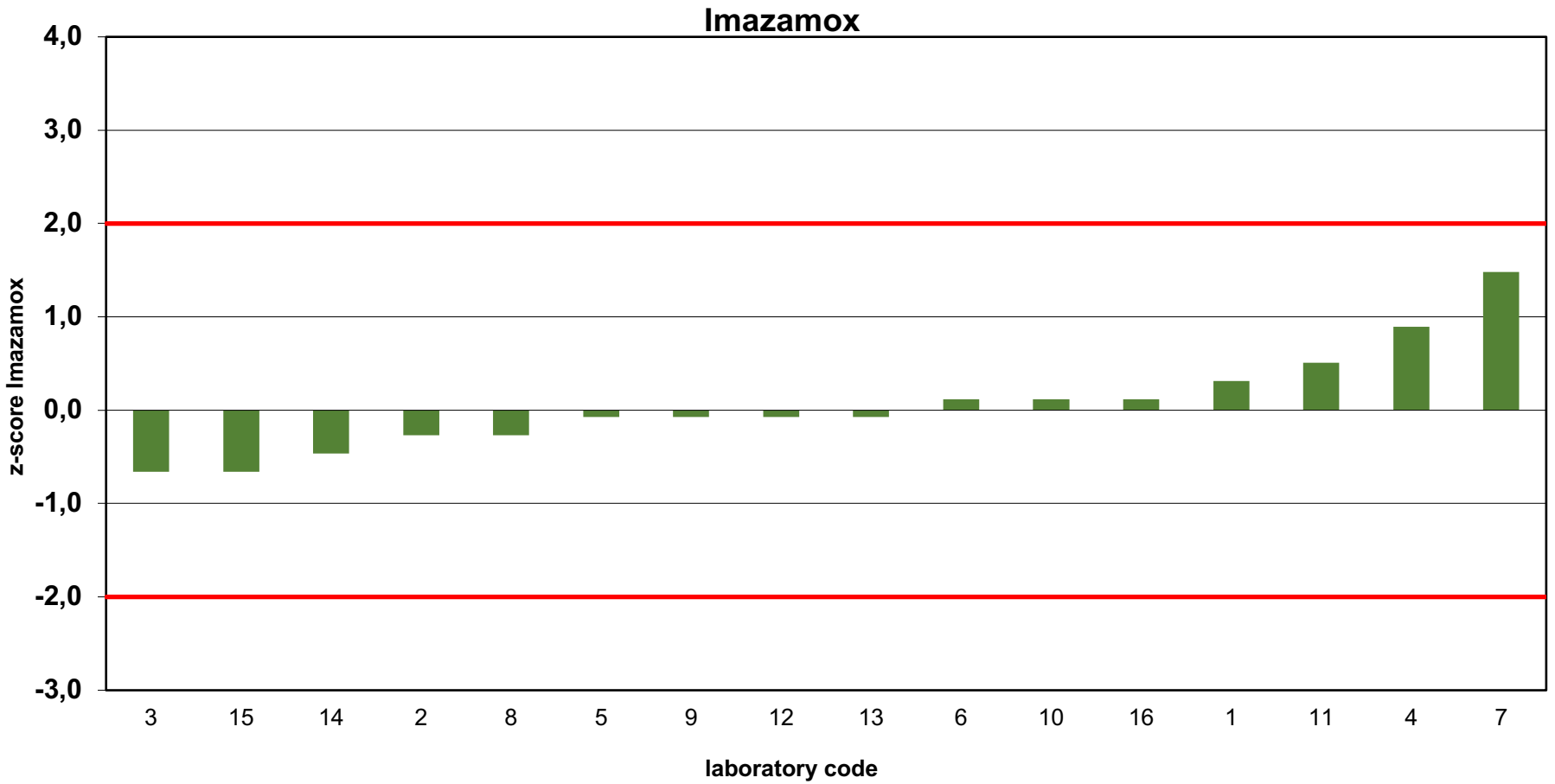
Green: satisfactory results, red: dissatisfactory results

Figure 3: Piperonyl butoxide (PBO) - recovery of spike



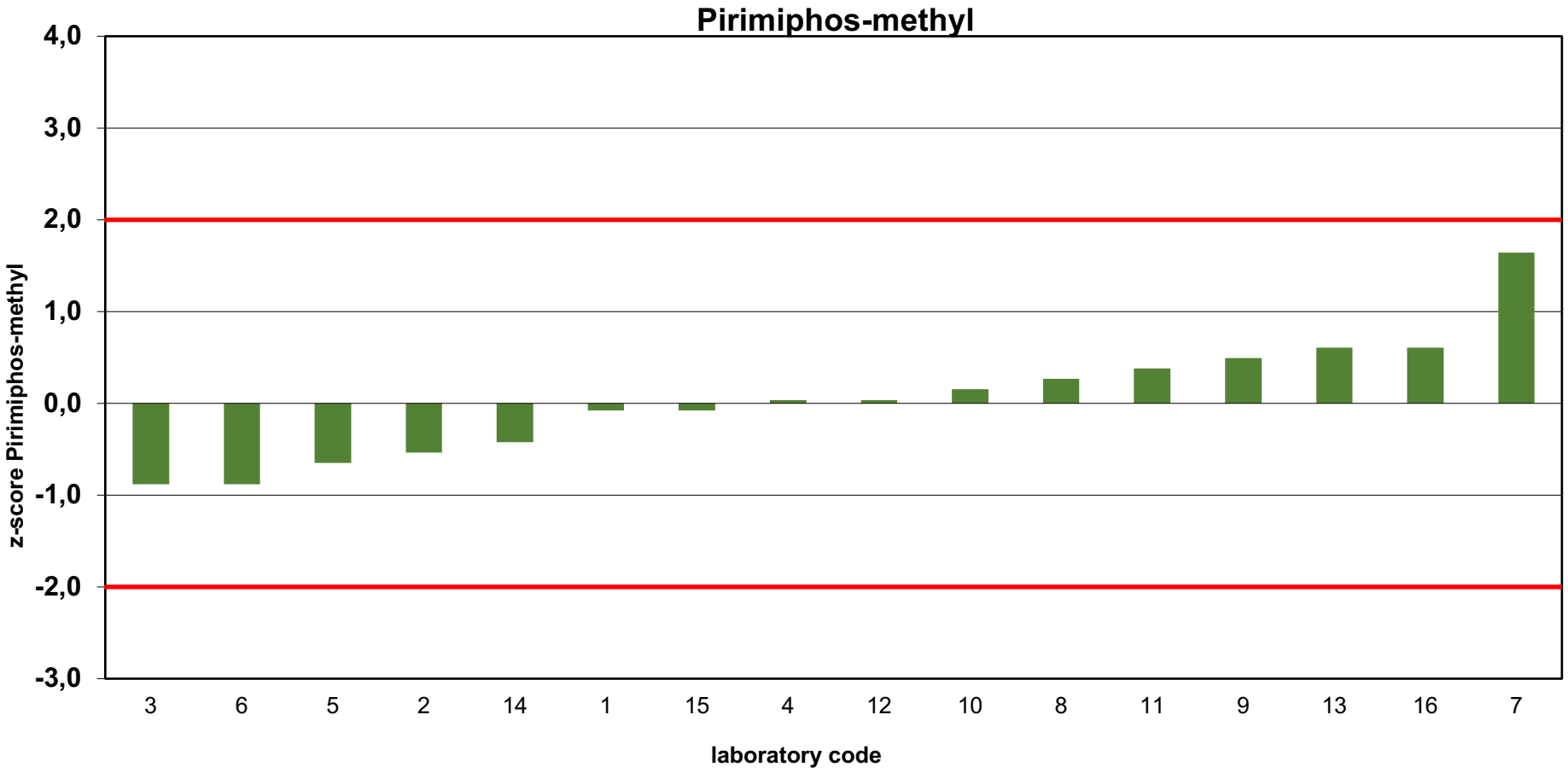
Green: satisfactory results, red: dissatisfactory results

Figure 4: Assessment of Imazamox – z-score



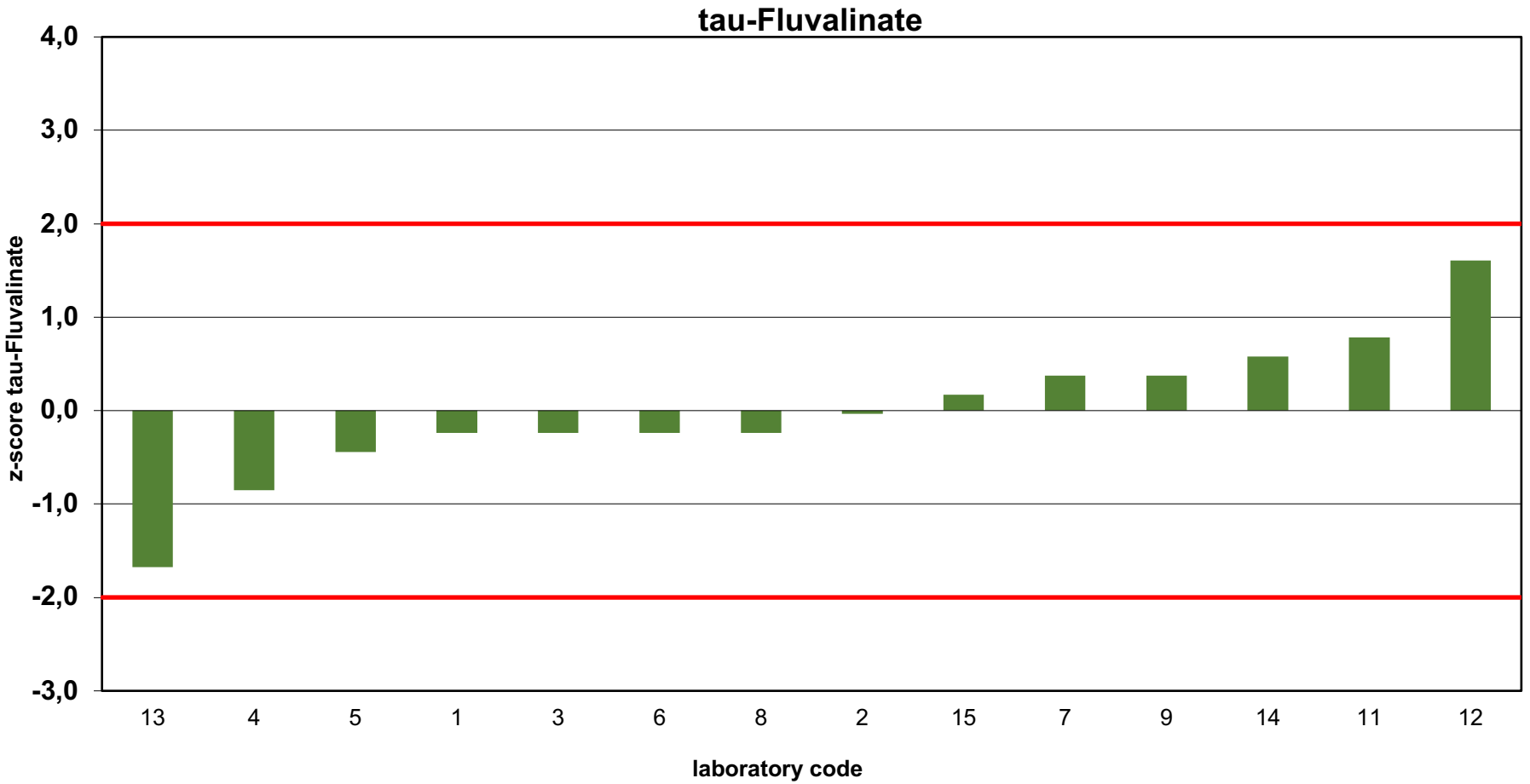
Green: satisfactory results, red: dissatisfactory results; n.r.: not reported

Figure 5: Assessment of Pirimiphos-methyl – z-score



Green: satisfactory results, red: dissatisfactory results

Figure 6: Assessment tau-Fluvalinate – z-score



Green: satisfactory results, red: dissatisfactory results