Bundesverband
Naturkost Naturwaren

Performance Test
with

“Undercover Samples”
within the BNN laboratory approval system

Analysis of
pesticide residues
in organic Kumquats at a
day-to-day (routine) level

January 2014

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Summary and Conclusion

The results of the undercover samples "Kumquat" were evaluated according to:

- False-positive findings,
- False-negative findings and
- The trueness criterion of the quantification:
  Difference between reported result and spiked level.

No participant reported false-positive results.

The overall performance is summarised in the following table:

Performance criterion	**Number of satisfactory participants**	**Total number of participants**	**Satisfactory (%)**
Correctly identified **Ethephon, Dicofol, Imazalil and Methidathion**	12	19	**63**
Correctly identified **AND** reported satisfactory results for **Ethephon, Dicofol, Imazalil and Methidathion**	1	19	**5**
Correctly identified **Fenbutatinoxid**	12	13	**92**
Correctly identified **AND** reported satisfactory results for **Fenbutatinoxid**	3	13	**23**

Assessment of quantification

The quantification performances were assed according to the trueness criterion of the quantification: Difference between reported result and spiked level (70-120% of the spiked levels are considered satisfying):

Analyte	Number of satisfactory results (70-120% of the spiked level)	Total number of scores	Satisfactory (%)
Ethephon	10	19	53
Dicofol	3	19	16
Imazalil	9	19	47
Methidathion	5	19	26
***Fenbutatinoxid* ***	*3*	*13*	*23*

* As Fenbutatinoxid is not covered by a multi-residue method (MRM) in every participating laboratory, the results are displayed for information purposes only. Nevertheless, the labs are highly requested to include this analyte into their MRM scope, as this is common practice in the majority of the labs.

The individual laboratory performances are discussed in detail in the individual evaluation reports for every single laboratory. The interpretation skills of the labs are part of the individual reports, too.

Contents

1 Introduction

Analysing so-called “undercover” samples is one important tool for laboratories to get knowledge about the daily performance under routine conditions. Undercover samples help to identify possible shortcomings, deficiencies and thus areas of improvement. For that reason, BNN decided to make use of this tool within the BNN lab performance assessment.

All in all nineteen (19) BNN approved laboratories across six (6) countries (Belgium, France, Germany, Italy, Netherlands, and Spain) took – unknowingly - part in this undercover performance test.

In order to compare the reported results the following conditions had to be ensured:

- Making use of inconspicuous Test Material.
- Similar conditions for the participants in terms of:
    - Limited time frame for analysis (as required for routine samples),
    - Guaranteed stability of analytes in the Test Material during the test period,
    - Asking for the provision of a test report (as required for routine samples).
- The Test Material had to look like a routine sample in order to pretend being a day-to-day routine sample which is analysed without “special care”:
    - Therefore, the samples were labelled as origin “Spain”, and
    - The official senders of the samples were organic fruit and vegetable agencies dealing which such kinds of foodstuff.
- Appropriate design and production of the Test Material in order to ensure that all participants had to deal with similar analytical challenges.

2 Test design and challenges

The design of the Kumquat Test Material is linked to the following principles:

- Pesticides commonly found in routine samples of this commodity,
- All pesticides are commonly covered by the scope of multi-methods (except Ethephon, which was ordered specifically, and Fenbutatinoxid, which was not covered by every participating lab)
- Reported pesticides for Kumquats in different publications (databases, monitoring programmes, rapid alert system etc.),
- Pesticides with concentration levels exceeding the related Maximum Residue Levels (MRL).

Several researches were performed in order to ensure a realistic but challenging Test Material design:

- 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

were considered for the determination of the final pesticide design.

The final design of the Test Material is listed in the table below:

Matrix: Kumquats 10 Kumquats per laboratory		
Pesticide	**Spiking level (mg/kg)**	**Maximum Residue Level (mg/kg)**
Ethephon *	0.180	0.05
Dicofol *	0.060	0.02
Imazalil	0.035	0.05
Methidathion *	0.045	0.02
Fenbutatinoxid	*0.075*	*0.05*

* MRL violation considering 50% expanded measurement uncertainty

The following challenges are considered:

1. The analytical routine performances of the BNN approved laboratories should be tested.
2. The sample must not be identified as Test Material of a performance test. Therefore, it was made up as routine sample.
3. All in all 5 analytes had to be identified and quantified.
4. The spiked levels of three of the pesticides in the Test Material violate the MRLs (without consideration of the expanded measurement uncertainty). Therefore, only *true* results will identify these violations.

The laboratories were faced with the following challenges:

1. The Kumquats were analysed according to routine samples. Therefore, no "special care analysis" was applied (as opposed to announced ring test samples).
2. All in all five analytes had to be identified and quantified by each laboratory.
3. The spiked levels covered a concentration range between 0,035 mg/kg (Imazalil) up to 0,18 mg/kg (Ethephon).
4. Dicofol requires experienced analytical skills for the analytical laboratories as it easily breaks down during sample preparation and injection into the GC system.

3 Test Material

An external institute was instructed to prepare the Test Material. Long-term experience with this institute already exists showing satisfying performance in the preparation of Test Material. The challenge to overcome was the preparation of non-homogenised test material in order to ensure that the laboratories do not realise the test character of the sample.

In order to achieve this objective, the Test Material was prepared as described below:

Ca. 5 kg of organic Kumquats were used for the Test Material preparation. A sub-sample of the 5 kg was taken for analysis of pesticides. This was done to ensure that no incurred pesticides are present in the Test Material. The analysis of the sub-sample did not show any positive pesticide levels above the reporting limit of the external institute.

All in all, ca. 270 Kumquats (including the Kumquat samples of the verification of the spiking and the stability testing) were individually prepared in the described way. A defined volume of a solution of a pesticide-mix was injected into each single Kumquat. Conclusively, it was known which pesticides in which quantity were present in the individual Kumquat.

10 Kumquats represented one laboratory sample. Having injected the defined pesticide mix, the total weight of the 10 pieces of Kumquats was measured. Each laboratory sample was

allocated a unique sample code and packed into a labelled plastic bag. All in all, 20 laboratory samples were prepared for the 19 participating laboratories (one backup sample).

Each laboratory received one laboratory sample of Kumquats (= all in all 10 Kumquats). The laboratory was instructed by the official client to homogenise the COMPLETE sample (all 10 pieces) for analysis.

Therefore, it was possible to determine the injected pesticide concentration in mg/kg related to each laboratory sample (= 10 Kumquats).

The weight of the single Kumquats varies (naturally), while the volume of the pesticide solution spiked to each Kumquat remains constant (see chapter 6.1, "Individual weight of Kumquat sample (g)"). Therefore, the exact spiking levels of each laboratory sample slightly deviate from each other. The individual spiked levels of each laboratory sample (= 10 Kumquats) in mg/kg were calculated from the concentration of the pesticide-mix spiking solution, the injected volume of spiking solution and the individual weight of the Kumquat laboratory sample (see chapter 6.1 "spike (mg/kg)").

The spiking was verified as described in detail in chapter 7. The stability testing was performed as described in detail in chapter 8.

4 Performance in practice

The undercover Test Samples were sent to the participating laboratories with help of "official laboratory clients" within week 3 (January 2014).

The laboratories were asked to analyse the WHOLE sample (all 10 Kumquats) according to multi residue testing with GC/MS resp. LC/MSMS plus Ethephon as a single residue analysis.

As the samples pretended being routine samples, no specific instructions were provided for the way of reporting results. As a conclusion, the way of reporting results depends on the laboratories themselves. Routine conditions therefore are represented in an authentic way.

5 Evaluation of Results

The results of the undercover samples were evaluated according to false-positive findings, false-negative findings and according to the trueness criterion of the quantification: Difference between reported result and spiked level. The recoveries of the spiked levels are calculated in order to work out if the laboratory actually found 70-120% of the spiked levels. This way of assessment regards the analytical result of each laboratory in isolation (and not in comparison with a statistical mean).

An **overview of all analytical results** is provided in **chapter 6.1**. The graphical representations of the results related to each laboratory and each analytical result are provided as a **bar chart** in **chapter 6.2**. Here, the individual recovery rates of the spiked levels are presented against the lab codes.

5.1 Results – related to individual pesticides

Ethephon, range of spiked level: 0.180 - 0.182 mg/kg

Ethephon was ordered specifically to be included into the scope of analyses. All laboratories - with the exception of one lab (lab code 18) - were able to identify Ethephon.

Eight (8) laboratories reported results corresponding to recoveries < 70%. Five (5) out of them reported recoveries close to 70%. Laboratory 18 reported Ethephon as "not detected", thus as a false negative result. Labs 6, 15 and 19 reported levels with significantly low recoveries compared to the spiked level.

Dicofol, range of spiked level: 0.060 – 0.061 mg/kg

Dicofol was the most challenging of all pesticides examined in this test:
5 laboratories failed to identify Dicofol at all (labs 14, 15, 16, 18, 19). Lab 17 reported Dicofol as below RL (reporting limit 0.01 mg/kg).

Thirteen (13) labs identified and quantified Dicofol, but just three (3) labs reported results within 70–120 % of the spiking level. One lab (10) quantified a much too high level (142%) while nine (9) labs reported results below 70% of the spiked level.

This situation is unsatisfying. As it is well know since a long time, that Dicofol is one of the most challenging analytes in pesticide analyses because of its easy degradation especially during sample injection into the GC system, this result is not really surprising.

Imazalil, range of spiked level: 0.035 – 0.036 mg/kg

All laboratories identified Imazalil correctly. Just one lab (18) delivered a much too high result (142%), while 9 laboratories showed too low recoveries of the spiking levels (minimum 31% of lab 15). Therefore, Imazalil is still challenging in terms of correct quantification.

Methidathion, range of spiked level: 0.045 – 0.046 mg/kg

This pesticide shows dissatisfying results as well: 3 laboratories failed to identify Methidathion at all (labs 3, 13, 15).
Sixteen (16) labs identified and quantified Methidathion, but just five (5) labs reported a result within 70–120 % of the spiked level. Five (5) labs (out of eleven (11) labs with results below 70%) reported levels below 50%, which is much too low compared to the spiking level.

Fenbutatinoxid, range of spiked level: 0.075 – 0.077 mg/kg

As Fenbutatinoxid is not included in the scope of the applied MRMs of every participant, the results are displayed for information purposes only. It is known, that there are possibilities to include Fenbutatinoxid into the scope of the QuEChERS MRM-method without significant modifications of the method. On the other hand it is known as well, that this may be not possible using different MRMs like the Dutch MRM, the Luke method or others. Therefore, it depends on the kind of the applied MRM, whether a lab may identify and quantify Fenbutatinoxid within the MRM-scope (thus without a special instruction of the lab-client to analyse the sample in particular for Fenbutatinoxid). If a laboratory applies a MRM different from the QuEChERS method it is recommended to inform the clients about that fact. This is of high relevance if there is a request to analyse f. ex. citrus fruits, where Fenbutatinoxid is frequently detected.

5 laboratories (labs 6, 13, 14, 16, 19) did not report Fenbutatinoxid, as this analyte was not part of the analytical scope of their applied multi-methods. Laboratory 12 did not report Fenbutatinoxid. However, Fenbutatinoxid was part of the laboratory's generally offered analytical scope. But as the "official client" did not order that parameter explicitly, the laboratory did not analyse for Fenbutatinoxid in the Kumquat sample.

9 laboratories reported results below 70% recovery of the spiked level, while one (1) laboratory (lab 9) reported a result slightly above 120% of the spiked level (121%).

6 Results

6.1 Overall Results

Lab Code	Individual weight of Kumquat sample (g)	*Ethephon*			*Dicofol*			*Imazalil*			*Methidathion*			*Fenbutatinoxid*		
		Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike
1	118.0	0.182	0.16	88	0.061	0.032	53	0.035	0.027	76	0.045	0.039	86	0.076	0.011	15
2	117.4	0.182	0.118	65	0.061	0.034	56	0.036	0.021	59	0.046	0.024	53	0.076	0.059	78
3	118.0	0.182	0.205	113	0.061	0.034	56	0.035	0.021	59	0.045	n.r.	-	0.076	0.037	49
4	118.6	0.181	0.12	66	0.060	0.023	38	0.035	0.015	43	0.045	0.012	27	0.075	0.039	52
5	116.9	0.183	0.138	75	0.061	0.009	15	0.036	0.025	70	0.046	0.013	28	0.076	0.037	48
6	116.1	0.184	0.062	34	0.061	0.036	59	0.036	0.018	50	0.046	0.016	35	0.077	n.r.*	-
7	118.0	0.182	0.16	88	0.061	0.03	50	0.035	0.03	85	0.045	0.04	88	0.076	0.06	79
8	118.2	0.181	0.12	66	0.060	0.01	17	0.035	0.03	85	0.045	0.04	88	0.076	traces	-
9	117.6	0.182	0.2	110	0.061	0.051	84	0.035	0.030	85	0.046	0.033	73	0.076	0.092	121
10	119.1	0.180	0.16	89	0.060	0.085	142	0.035	0.035	100	0.045	0.046	102	0.075	0.089	119
11	117.6	0.182	0.12	66	0.061	0.04	66	0.035	0.03	85	0.045	0.03	66	0.076	0.04	53
12	118.4	0.181	0.19	105	0.060	0.05	83	0.035	0.032	91	0.045	0.028	62	0.075	n.r.*	-
13	117.4	0.182	0.14	77	0.061	0.044	72	0.036	0.021	59	0.046	n.r.	-	0.076	n.r.*	-

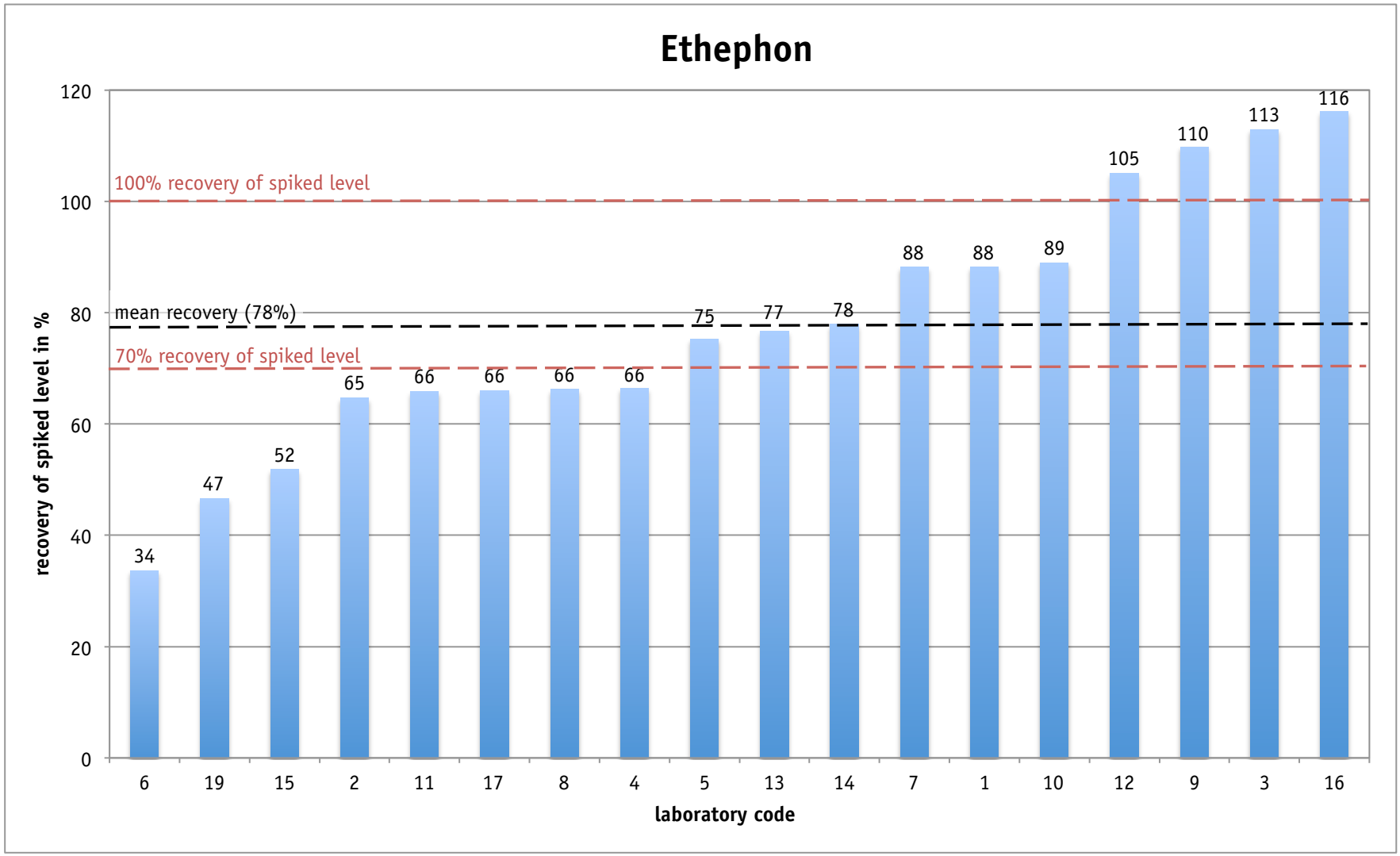
Lab Code	Individual weight of Kumquat sample (g)	***Ethephon***			***Dicofol***			***Imazalil***			***Methidathion***			***Fenbutatinoxid***		
		Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike	Spike (mg/kg)	reported result (mg/kg)	% of spike
14	117.5	0.182	0.142	78	0.061	n.r.	-	0.035	0.021	59	0.046	0.015	33	0.076	n.r.*	-
15	118.0	0.181	0.094	52	0.060	n.r.	-	0.035	0.011	31	0.045	n.r.	-	0.076	0.012	16
16	118.5	0.181	0.21	116	0.060	n.r.	-	0.035	0.03	85	0.045	0.03	66	0.075	n.r.*	-
17	117.9	0.182	0.12	66	0.061	< 0.01	-	0.035	0.02	57	0.045	0.03	66	0.076	0.05	66
18	118.1	0.181	n.r. (<0.050)	-	0.060	n.r. (<0.003)	-	0.035	0.050	142	0.045	0.016	35	0.076	0.042	56
19	117.5	0.182	0.085	47	0.061	n.r.	-	0.035	0.024	68	0.046	0.027	59	0.076	n.r.*	-

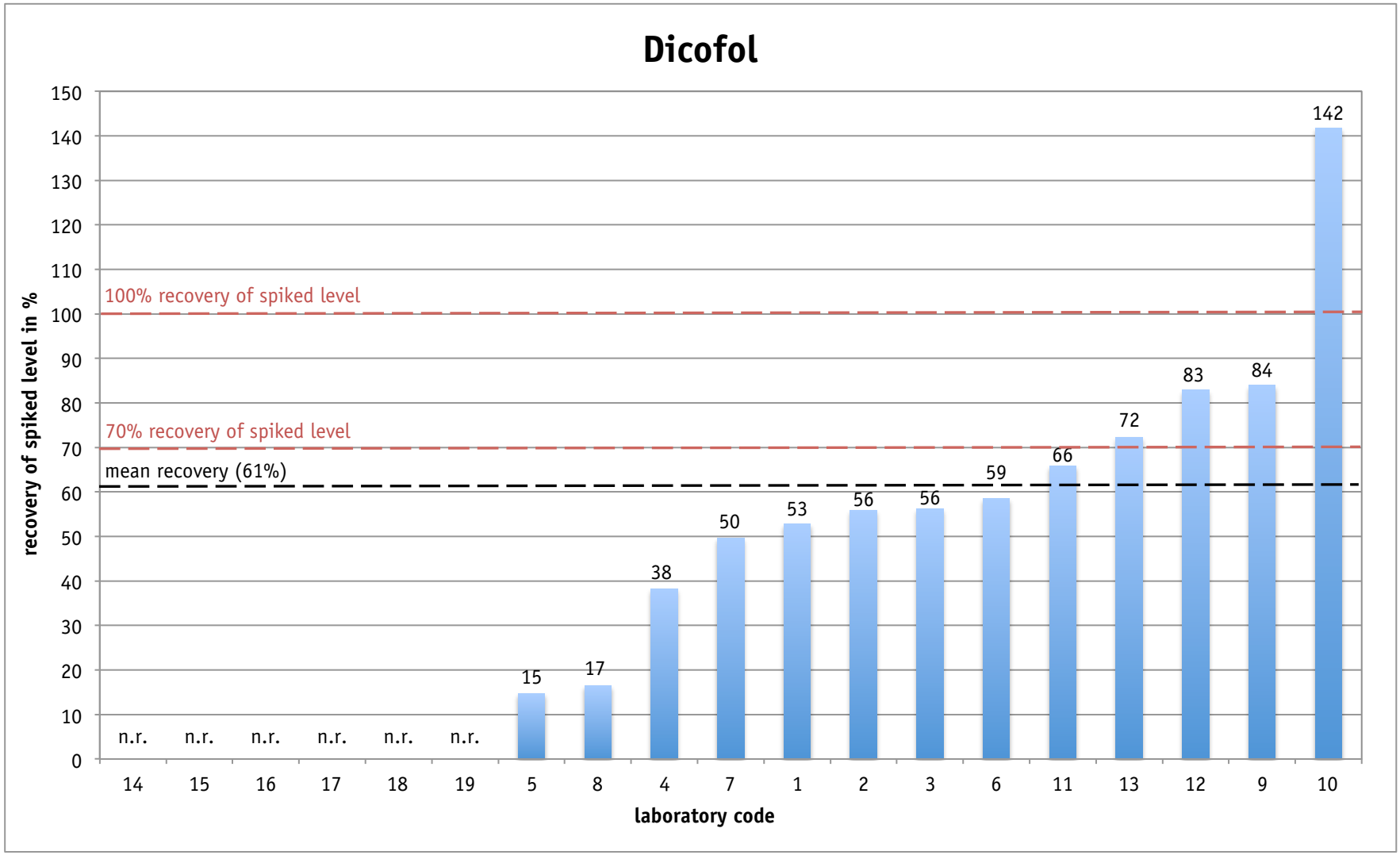
mean recovery of spike (%):	78	61	73	60	63

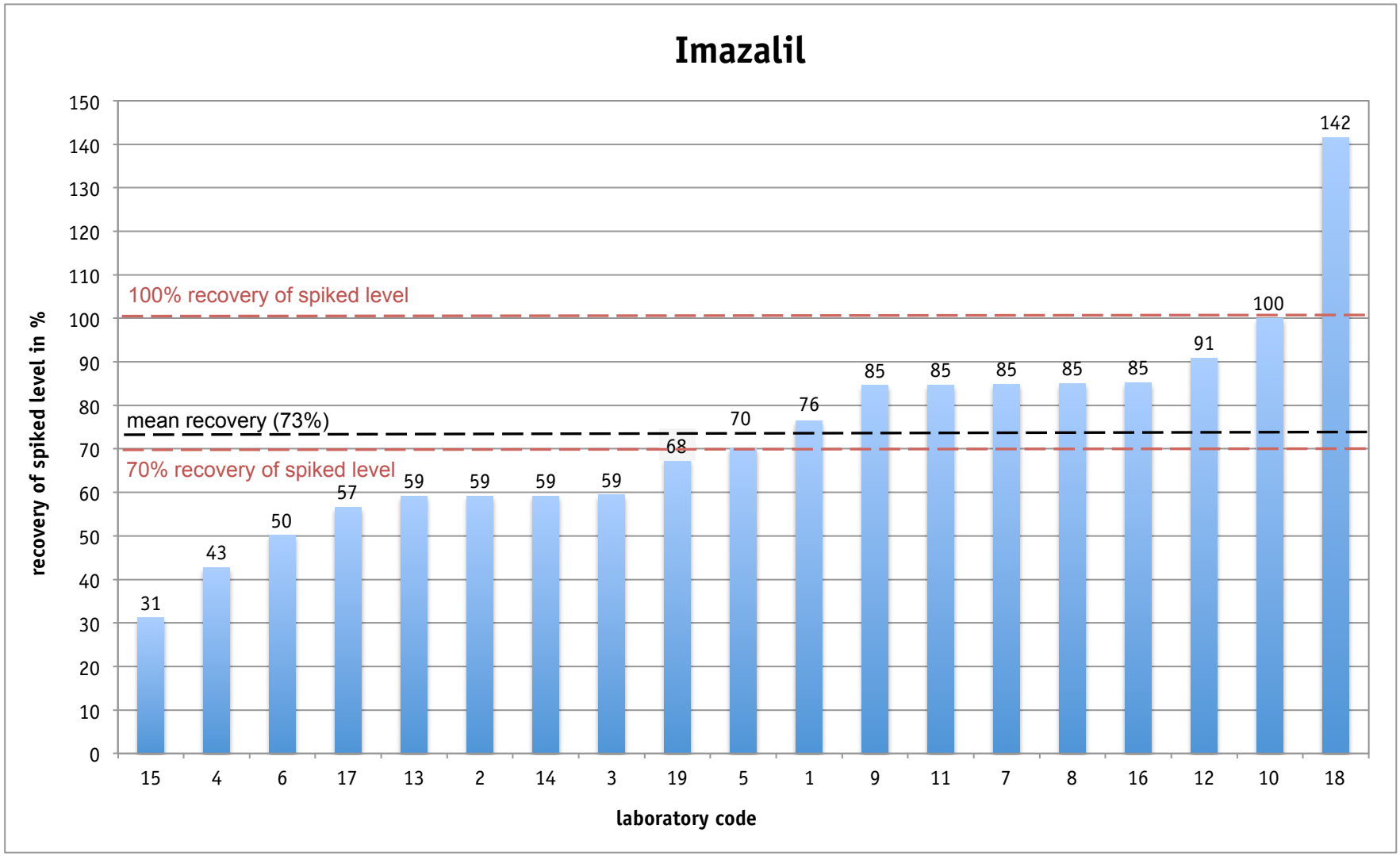
n.r.: not reported

*: not within the scope of the laboratory

6.2 Detailed Results




Dicofol
recovery of spiked level in %
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100% recovery of spiked level
70% recovery of spiked level
mean recovery (61%)
n.r.
n.r.
n.r.
n.r.
n.r.
n.r.
15
17
38
50
53
56
56
59
66
72
83
84
142
14
15
16
17
18
19
5
8
4
7
1
2
3
6
11
13
12
9
10
laboratory code


Imazalil
recovery of spiked level in %
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100% recovery of spiked level
mean recovery (73%)
70% recovery of spiked level
31
43
50
57
59
59
59
59
68
70
76
85
85
85
85
85
91
100
142
15
4
6
17
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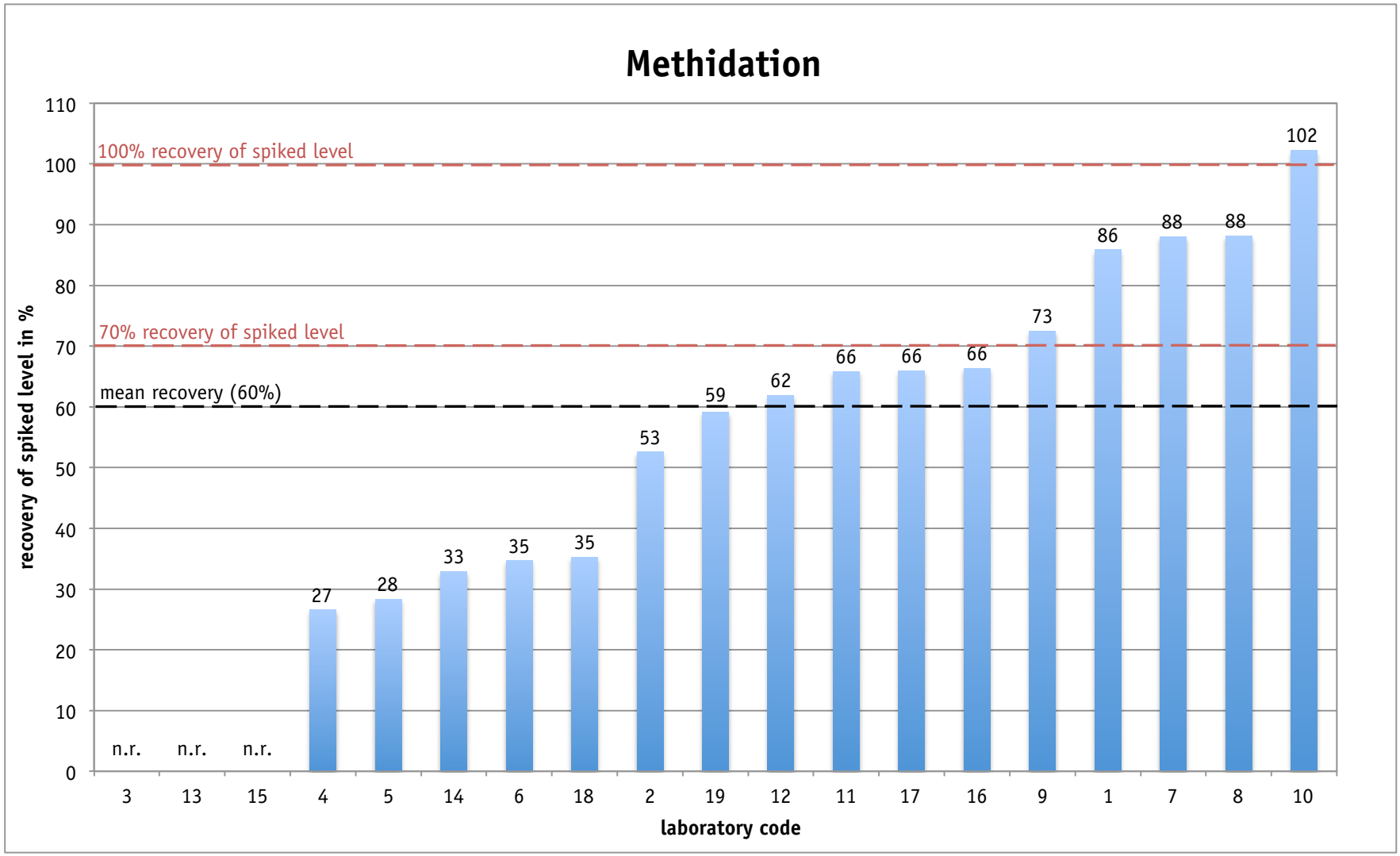
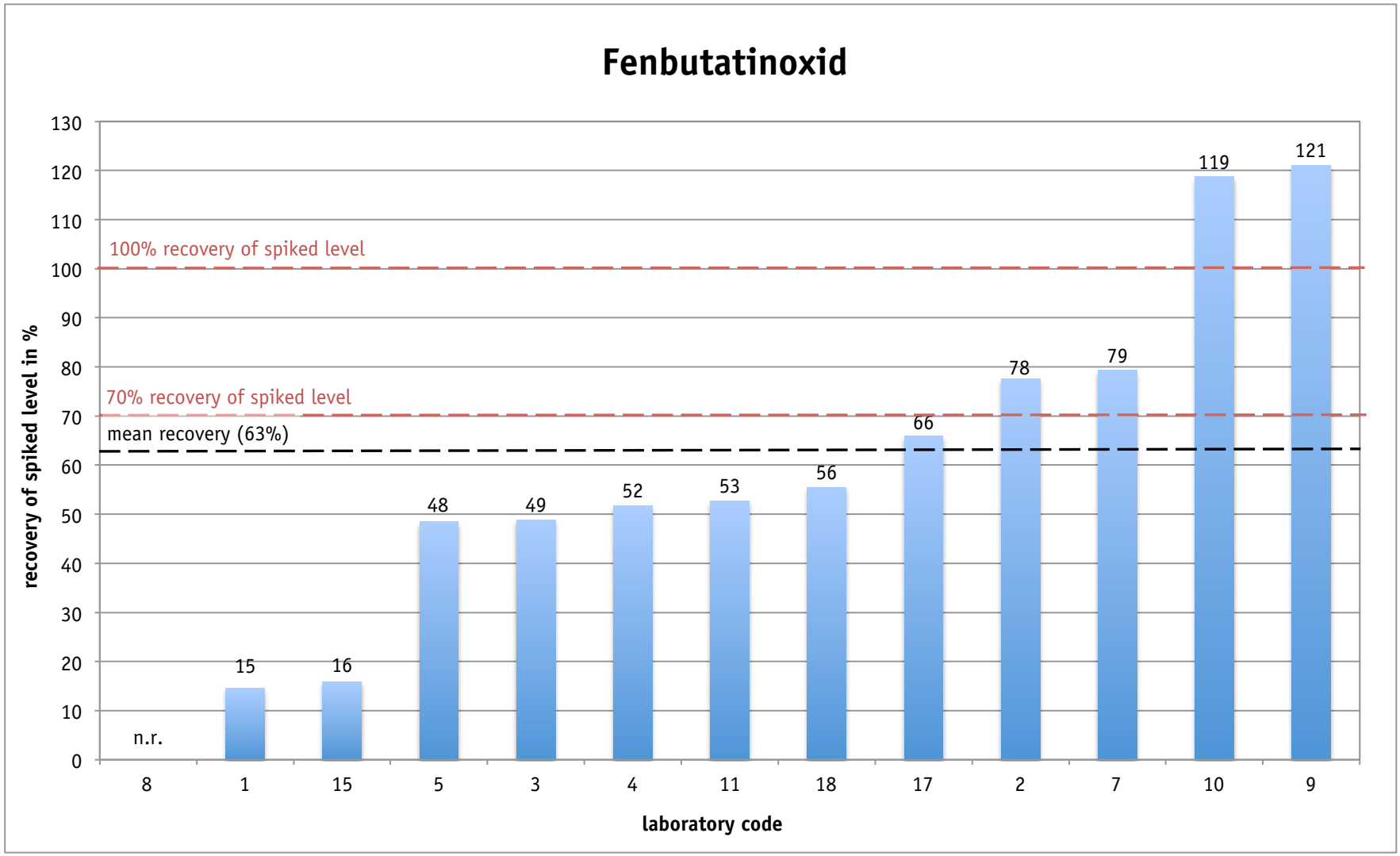
3
19
5
1
9
11
7
8
16
12
10
18
laboratory code


Methidation
recovery of spiked level in %
laboratory code
100% recovery of spiked level
70% recovery of spiked level
mean recovery (60%)
110
100
90
80
70
60
50
40
30
20
10
0
n.r.
n.r.
n.r.
27
28
33
35
35
53
59
62
66
66
66
73
86
88
88
102
3
13
15
4
5
14
6
18
2
19
12
11
17
16
9
1
7
8
10


Fenbutatinoxid
recovery of spiked level in %
130
120
110
100
90
80
70
60
50
40
30
20
10
0
100% recovery of spiked level
70% recovery of spiked level
mean recovery (63%)
n.r.
15
16
48
49
52
53
56
66
78
79
119
121
8
1
15
5
3
4
11
18
17
2
7
10
9
laboratory code

7 Verification of the correct spiking

The correct spiking (injection of the pesticide-mix) was verified by five additional Kumquat samples (consisting of 10 Kumquat per sample). Those samples were prepared like described above for the laboratory samples (see chapter 3). Ethephon, Imazalil and Methidathion were analysed in the five Kumquat samples (representing the full set of pesticides spiked to the Kumquats) by the external institute. The analytical results were compared with the actual quantities of pesticides, which were spiked into the Kumquat samples. The analytical results, presented below, confirm the correct spiking of the Kumquats.

	Ethephon				
Sample No.	Spike [mg/kg]	Result (extraction 1) [mg/kg]	Result (extraction 2) [mg/kg]	Mean result (Extr. 1/2) [mg/kg]	Recovery of spike [%]
1	0.182	0.182	0.189	0.186	102
2	0.180	0.193	0.160	0.177	98
3	0.182	0.196	0.173	0.185	101
4	0.182	0.171	0.173	0.172	95
5	0.180	0.198	0.169	0.184	102

	Imazalil				
Sample No.	Spike [mg/kg]	Result (extraction 1) [mg/kg]	Result (extraction 2) [mg/kg]	Mean result (Extr. 1/2) [mg/kg]	Recovery of spike [%]
1	0.035	0.038	0.037	0.038	107
2	0.035	0.036	0.036	0.036	103
3	0.036	0.035	0.037	0.036	100
4	0.036	0.036	0.035	0.036	99
5	0.035	0.033	0.033	0.033	94

	Methidation				
Sample No.	Spike [mg/kg]	Result (extraction 1) [mg/kg]	Result (extraction 2) [mg/kg]	Mean result (Extr. 1/2) [mg/kg]	Recovery of spike [%]
1	0.045	0.049	0.051	0.050	111
2	0.045	0.049	0.049	0.049	109
3	0.045	0.050	0.046	0.048	107
4	0.045	0.046	0.046	0.046	102
5	0.045	0.047	0.046	0.047	103

8 Stability testing

Two additional Kumquat samples were prepared for stability testing in the same batch and in the same way as described above for the laboratory samples (see chapter 3). Those two samples were stored in a fridge for stability testing later on. After all laboratories had reported the results to their "clients", those two Kumquat samples (stored in a fridge) were analysed. Again, Ethephon, Imazalil and Methidathion were analysed in the Kumquat samples, representing the full set of pesticides spiked to the Kumquats.

The analytical results of the pesticides, presented below, confirmed the stability of the pesticides in the Test Material.

	Ethephon		
Sample No.	Spike [mg/kg]	Result [mg/kg]	Recovery of spike [%]
1	0.183	0.186	102
2	0.183	0.181	99

	Imazalil		
Sample No.	Spike [mg/kg]	Result [mg/kg]	Recovery of spike [%]
1	0.036	0.034	94
2	0.036	0.034	94

	Methidathion		
Sample No.	Spike [mg/kg]	Result [mg/kg]	Recovery of spike [%]
1	0.046	0.040	87
2	0.046	0.040	87