

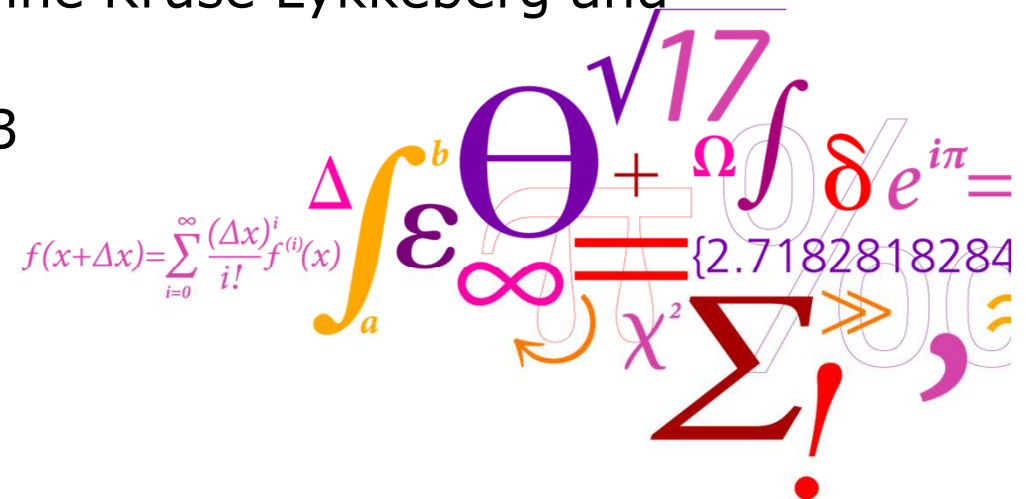


EUPT-CF7


Incurred and spiked pesticides in feed for laying hens

Mette Erecius Poulsen, Anne Kruse Lykkeberg and
Gitte Andersen

Almeria, 24 October 2013



Overall conclusions

- A difficult PT
 - High number of pesticide residues
 - 23 > 0.04 mg/kg
 - 8 < 0.02 mg/kg (not evaluated)
 -  High number of false negative and positive results
- An easy PT
 - Matrix was easy to analyse.
 - Average Qn was 20%
 - Could be analysed by standard multimethod used for cereals

PTs on cereals/feed – overview

		2007 EUPT-C1 /SRM2	2008 EUPT-C2	2009 EUPT-C3 /SRM4	2010 EUPT-C4	2011 EUPT-C5 /SRM6	2012 EUPT-C6	2013 EUPT-CF7
	Test material	Wheat flour	Wheat flour	Oat flour	Rye flour	Rice flour	Barley	Feed
	no. of participants	64	74	111	118	155 (133)	149 (127)	120 (106)
	No. of target pesticides	37	55	60	72	104	107	116
MRM	no. of incurred pesticides	3	9	14	13	10	8	19
	no. of spiked pesticides	4	4		3	7	10	4
SRM	no. of incurred pesticides	2	2	2	2			
	no. of spiked pesticides	1		2	1	7		
	Total no. of pesticides in test item	10	15	18	19	24	18	23

← **Additional 8 incurred pesticides at low levels <0.02 mg/kg**

Organising team at EURL

- **Mette Erecius Poulsen, Senior Chemist**
- **Anne Kruse Lykkeberg, Senior Chemist**
- **Gitte Andersen, Chemist**
- **Merete B. Ludwigsen, Chemical Technician**
- **Inge Schröder, Chemical Technician**
- **Lisbet Pilhøj, Chemical Technician**
- **Arne Bent Jensen, System Developer**

Scientific Group

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Stewart Reynolds

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Quality Group

Antonio Valverde

Stewart Reynolds

Participation

Country	# labs	Country	# labs	Country	# labs
Argentina	1	Germany	16	Poland	12
Austria	1	Ghana	1	Portugal	2
Belgium	3	Greece	3	Romania	5
Brazil	2	Hungary	5	Serbia	1
Bulgaria	2	Ireland	1	Singapore	1
Chile	1	Italy	16	Slovakia	2
Costa Rica	2	Kenya	2	Slovenia	4
Cyprus	1	Latvia	1	Spain	10
Czech Republic	2	Lithuania	1	Sweden	2
Denmark	1	Luxembourg	0	Switzerland	1
Egypt	1	Malta		Turkey	1
Estonia	1	Netherlands	4	United Kingdom	2
Finland	1	Norway	1		
France	6	Peru	1	Total	120

Participation

Country	# labs	Country	# labs	Country	# labs
Argentina	1	Germany	16	Poland	12
Austria	1	Ghana	1	Portugal	2
Belgium	3	Greece	3	Romania	5
Brazil	2	Hungary	5	Serbia	1
Bulgaria - No NRL	2	Ireland	1	Singapore	1
Chile	1	Italy	16	Slovakia	2
Costa Rica	2	Kenya	2	Slovenia	4
Cyprus	1	Latvia	1	Spain	10
Czech Republic	2	Lithuania	1	Sweden	2
Denmark	1	Luxembourg	0	Switzerland	1
Egypt	1	Malta		Turkey	1
Estonia	1	Netherlands	4	United Kingdom	2
Finland	1	Norway	1		
France	6	Peru	1	Total	120

Target Pesticide List

- 116 pesticides
- 9 new
 - Aldrin
 - Chlordane, cis-
 - Chlordane, oxy
 - Chlordane, trans-
 - Dieldrin
 - Endrin
 - Heptachlor
 - Heptachlorepoxyd-cis
 - Heptachlorepoxyd-trans

(Directive 2002/32 on
undesirable substance in
animal feed)



TARGET PESTICIDE LIST

for the EUPT – CF7 2013

(last updated: 19.12.2012)

Nine new pesticides added to the Target Pesticide List from EUPT-C6 (2012) are marked in bold.

MRM-Compounds

Pestide no.	Pesticides	MRRL (mg/kg)
1	2-phenyl phenol	0.01
2	3-hydroxy-carbofuran	0.01
3	Acephate	0.01
4	Aldrin	0.01
5	Azinphos-methyl	0.01
6	Azoxystrobin	0.01
7	Bifenthrin	0.01
8	Boscalid	0.01
9	Captan	0.01
10	Carbaryl	0.01
11	Carbendazim	0.01



BAPF-C2
Wood

Rye

Barley

Maize

Soybean cake

Soybean meal

Vitacarb
Lime

Mineral and
Vitamin premix

SOJAOLIE

K11-1263
Bjor-334
BAST
VELEGNET TIL
SALATER
DRESSINGER
OG STEGNING
10 L

Feed for laying hens

Ingredients	Test sample	Test blank
Wheat	25%	25%
Barley	13%	12%
Rye	13%	12%
Maize	13%	12%
Sum Cereals	63%	62%
Soybean meal	25%	
Soybean cake		27%
Lime	9%	9%
soya oil	3%	1%
Mineral and vitamin premix	1%	1%
Total amount	100%	100%

- Ingredients

- Wheat, rye and barley are grown in Denmark in 2006, 2007, 2009, 2011

- Maize, soybean meal, soybean cake, lime, mineral and vitamin premix was delivered from DLG – Danish feed producer

- Production

- Raw materials were shipped to Forschungsinstitut Futtermitteltechnik der IFF, Braunschweig-Thune in Germany

- Internationale Forschungsgemeinschaft Futtermitteltechnik

Pesticide	Application in the field	Spike in laboratory	EUPT-C1 wheat	EUPT-C2 wheat	EUPT-C4 rye	EUPT-C6 barley
Aldrin		x				
Azoxystrobin	x		x	x	x	
Boscalid	x					x
Carbendazim	x			x	x	
Chlorpyrifos-methyl	x	x		x	x	
Cypermethrin	x	x		x		
Endosulfan-alpha		x				
Endosulfan-sulfate		x				
Epoxiconazole	x			x		x
Fenpropidin	x					x
Fenpropimorph	x				x	
Fluquinconazole	x				x	
Flutriafol	x				x	
Iprodione	x	x		x		
Kresoxim-methyl	x				x	
Lindane		x				
Malathion	x	x			x	
Propiconazole	x	x				x
Pyraclostrobin	x					x
Spiroxamine	x			x	x	
Tebuconazole	x					x
Triadimenol	x			x		
Trifloxystrobin	x			x		

Tietjen hammer mill type VHM 15



Amandus Kahl laboratory
flat die pelleting press type 14-175



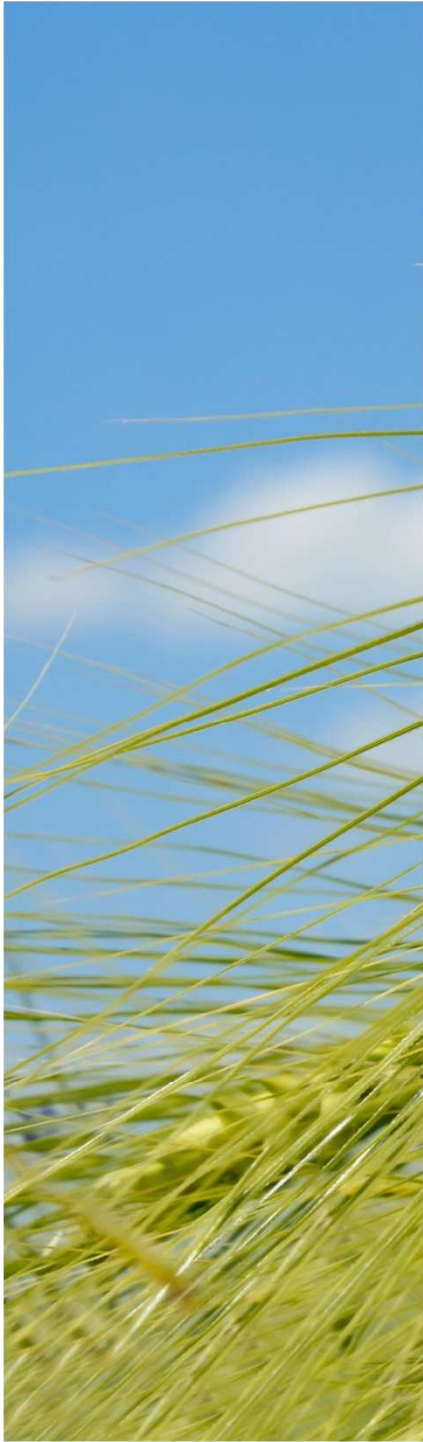
Dinnissen twin-shaft paddle mixer type 300 L





Ho







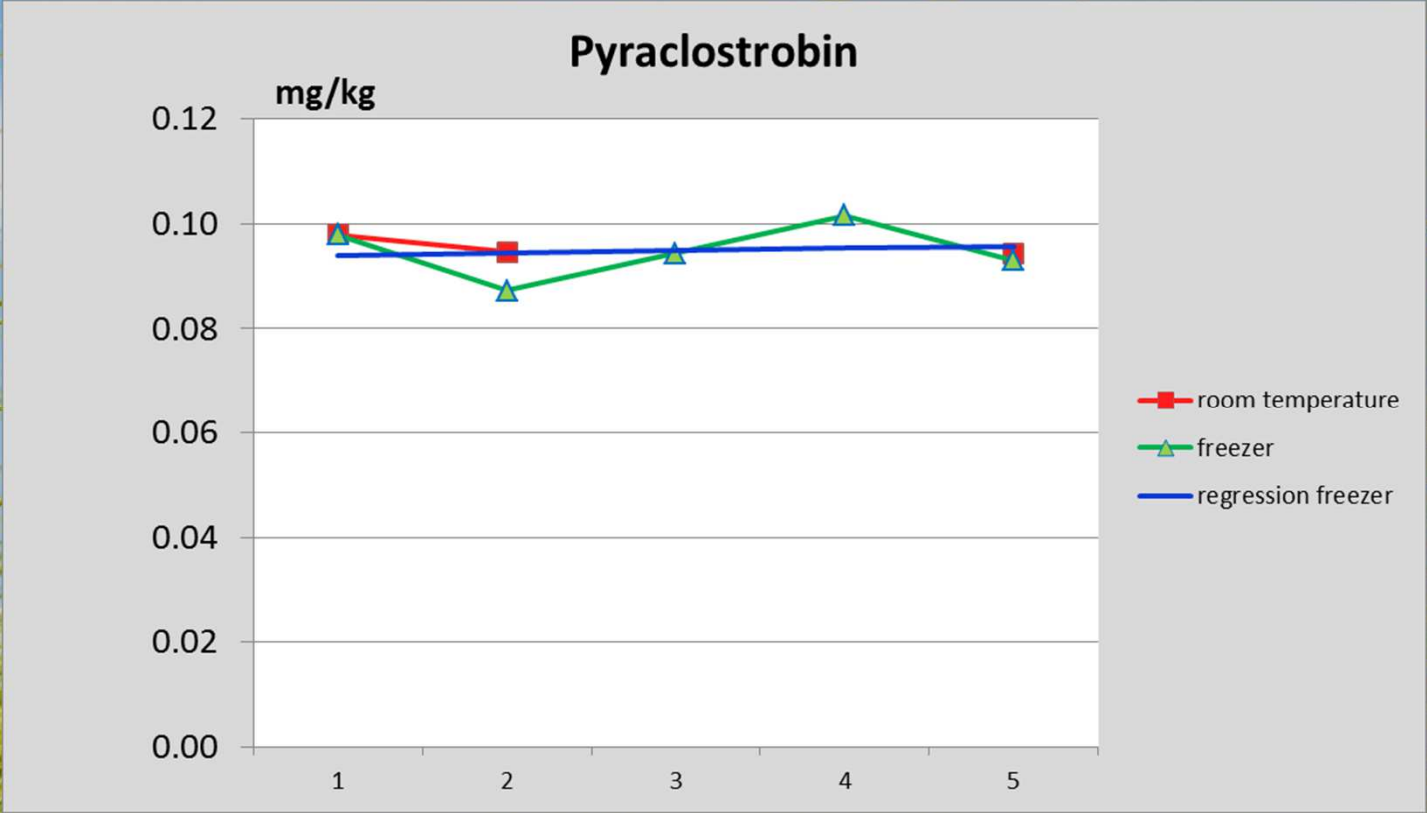


Sample shipment

- Samples were distributed on Monday 13 May 2013 (6 May to Third Countries)
- Most samples were delivered to EU laboratories on 14 or 15 May



Stability test

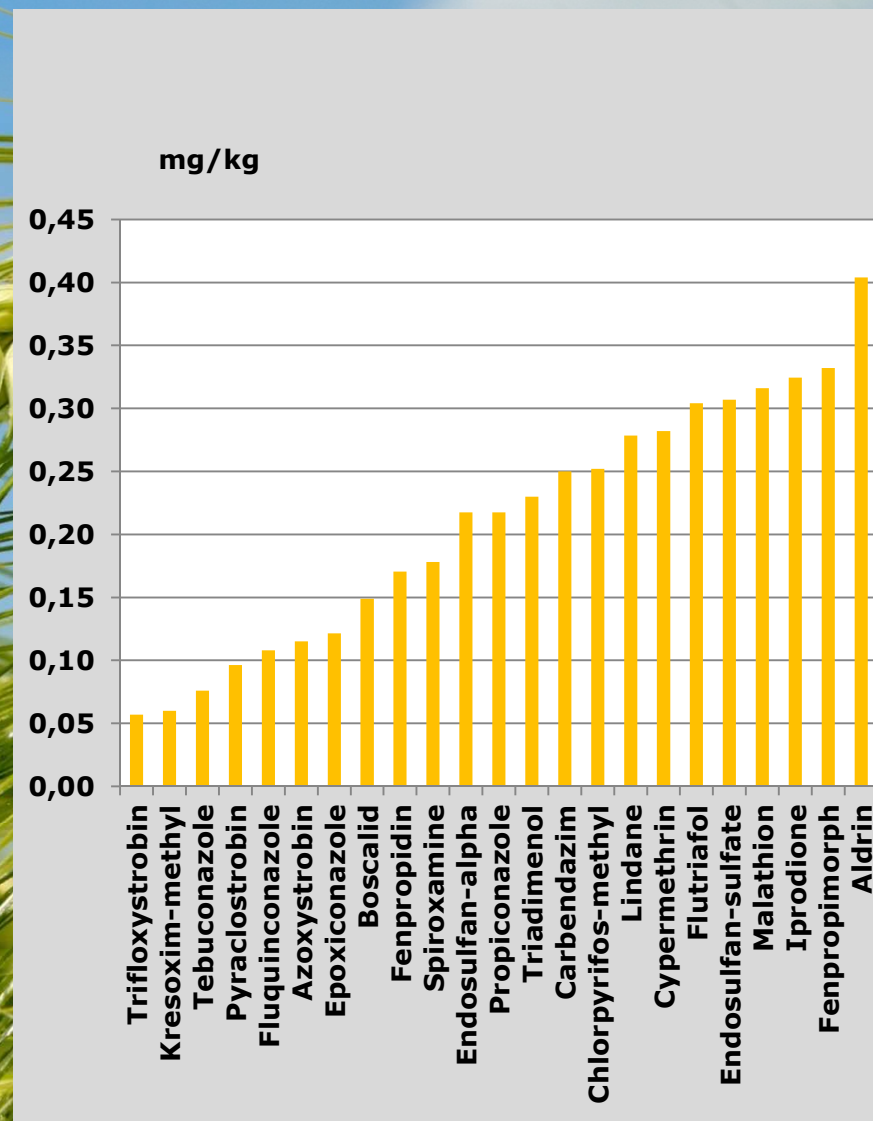


Calculation of assigned values and uncertainty of assigned values

- Median values of the results from EU laboratories (and EFTA)
 - Only result from participant that
 - Reported to add water to the samples before extraction or
 - used mixture of solvent and water or
 - used ASE
- Outliers
 - results with z-scores >5
 - Result from one lab submitting results from recovery samples
- Uncertainty
 - $u = 1.25 * (s^* / \sqrt{n})$
 - s^* is robust standard deviation
 - N is the number of participants

Pesticides	Assigned values, mg/kg
Aldrin	0.405
Azoxystrobin	0.115
Boscalid	0.149
Carbendazim	0.252
Chlorpyrifos-methyl	0.252
Cypermethrin	0.282
Endosulfan-alpha	0.220
Endosulfan-sulfate	0.307
Epoxiconazole	0.121
Fenpropidin	0.171
Fenpropimorph	0.333
Fluquinconazole	0.107
Flutriafol	0.308
Iprodione	0.325
Kresoxim-methyl	0.060
Lindane	0.279
Malathion	0.316
Propiconazole	0.217
Pyraclostrobin	0.096
Spiroxamine	0.176
Tebuconazole	0.076
Triadimenol	0.230
Trifloxystrobin	0.057

Assigned values



Additional pesticides

PESTICIDES	Calculated content mg/kg	Median all results mg/kg	Submitted results
Bifentrin	0.010	0.013	57
Deltametrin	0.007	0.012	20
Diazinon	0.009	0.009	23
Difenoconazole	0.001	0.006	11
Lambda-cyhalothrin	0.008	0.011	30
Pirimicarb	0.005	0.006	7
Pirimiphos-methyl	0.009	0.012	48
Prothioconazole-desthio	0.011	0.015	33

Re

Pesticide	No. of reported results	No. of NA	False negatives	% results
Aldrin	95	8	3	92
Azoxystrobin	87	16	3	84
Boscalid	86	17		83
Carbendazim	69	34		67
Chlorpyrifos-methyl	94	9		91
Cypermethrin	92	11	2	89
Endosulfan-alpha	100	3	5	97
Endosulfan-sulfate	95	8	2	92
Epoxiconazole	81	22	1	79
Fenpropidin	62	41	4	60
Fenpropimorph	78	25		76
Fluquinconazole	78	25	1	76
Flutriafol	79	24	5	77
Iprodione	82	21		80
Kresoxim-methyl	88	15	4	85
Lindane	97	6	3	94
Malathion	94	9	1	91
Propiconazole	88	15		85
Pyraclostrobin	69	34	5	67
Spiroxamine	69	34	2	67
Tebuconazole	87	16	2	84
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Trifloxystrobin	84	19	3	82

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False positive results

Lab code	Pesticide	Concentration mg/kg	Determination technique
6	Trifluralin	0.072	LC-MS/MS QQQ
26	Imazalil	0.018	LC-MS/MS QQQ
31	p'p'-DDT	0.063	GC- (μ) ECD
31	Quinoxifen	0.046	GC-Ion Trap
34	HCH-beta	0.239	GC-MS/MS (QQQ)
35	Hexaconazole	0.018	LC-MS/MS QQQ
68	HCH-beta	0.270	GC-MS/MS (QQQ)
91	Chlorpyrifos	0.021	GC-MS/MS (QQQ)
91	Pendimethalin	0.017	LC-MS
122	p'p'-DDT	0.015	GC-Ion Trap
125	Permethrin	0.020	?
126	HCH-beta	0.092	GC-MSD
129	HCH-beta	0.150	GC-MSD

Results <0.01 mg/kg

Lab code	Pesticide	Concentration mg/kg	Determination technique	RL, mg/kg
27	Pirimicarb-desmethyl	0.006	LC-MS/MS QQQ	0.01
61	Chlorpyrifos	0.0011	GC-MS/MS (QQQ)	0.005
61	Pirimicarb-desmethyl	0.0041	LC-MS/MS QQQ	0.005
61	Prochloraz	0.0014	LC-MS/MS QQQ	0.005
61	Triadimefon	0.0014	LC-MS/MS QQQ	0.005
67	HCH-alpha	0.0024	GC-MS/MS (QQQ)	0.01
67	Pirimicarb-desmethyl	0.0056	LC-MS/MS QQQ	0.01
113	Malaoxon	0.0008	?	0.01
113	Pirimicarb-desmethyl	0.0075	LC-MS/MS QQQ	0.01
125	Chlorpyrifos	0.001	?	?
125	Pendimethalin	0.001	?	?

Calculations of z-scores

- For each laboratory/pesticide combination :

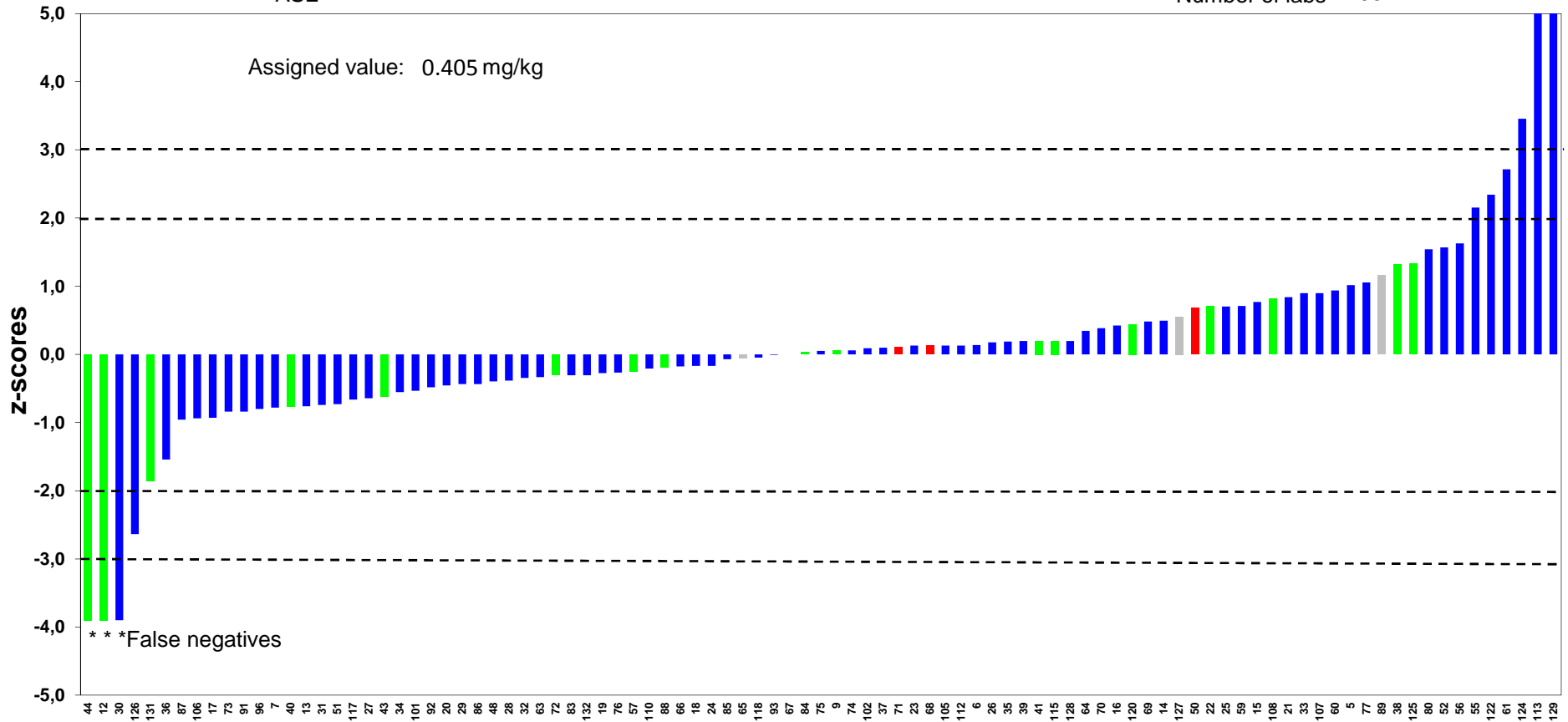
$$z = (x - X) / \sigma$$

- x is the result reported by the participant or the specific reporting limit of the lab for those labs not having detected the pesticide present in the test material
 - X is the assigned value, mean without outliers
 - σ is the target standard deviation obtained by multiplying the median by the FFP RSD of 25%
-
- $|z| \leq 2$ Acceptable
 - $2 < |z| \leq 3$ Questionable
 - $|z| > 3$ Unacceptable

- Water added
- No water added
- Not specified
- ASE

Aldrin

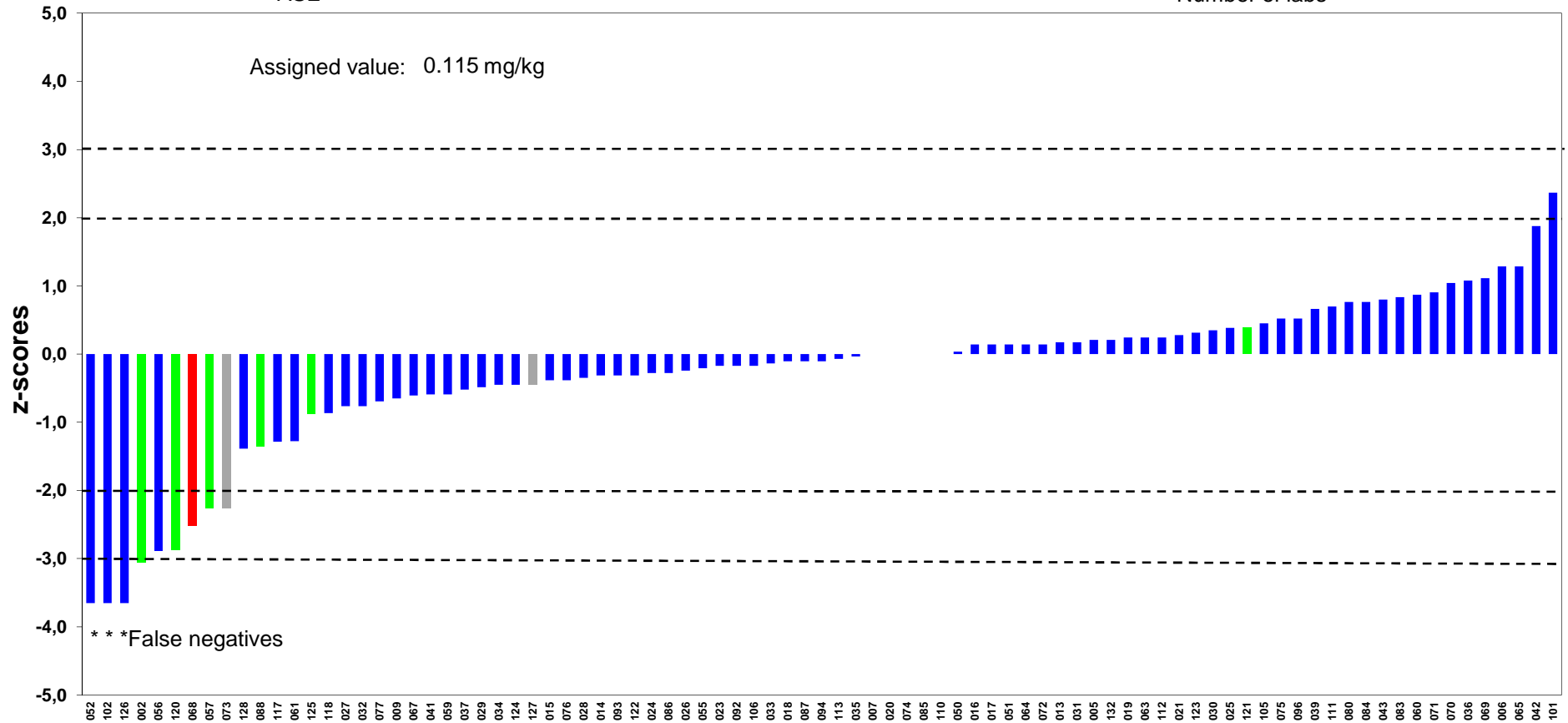
Acceptable 85
 Questionable 4
 Unacceptable 6
 Number of labs 95



- Water added
- No water added
- Not specified
- ASE

Azoxystrobin

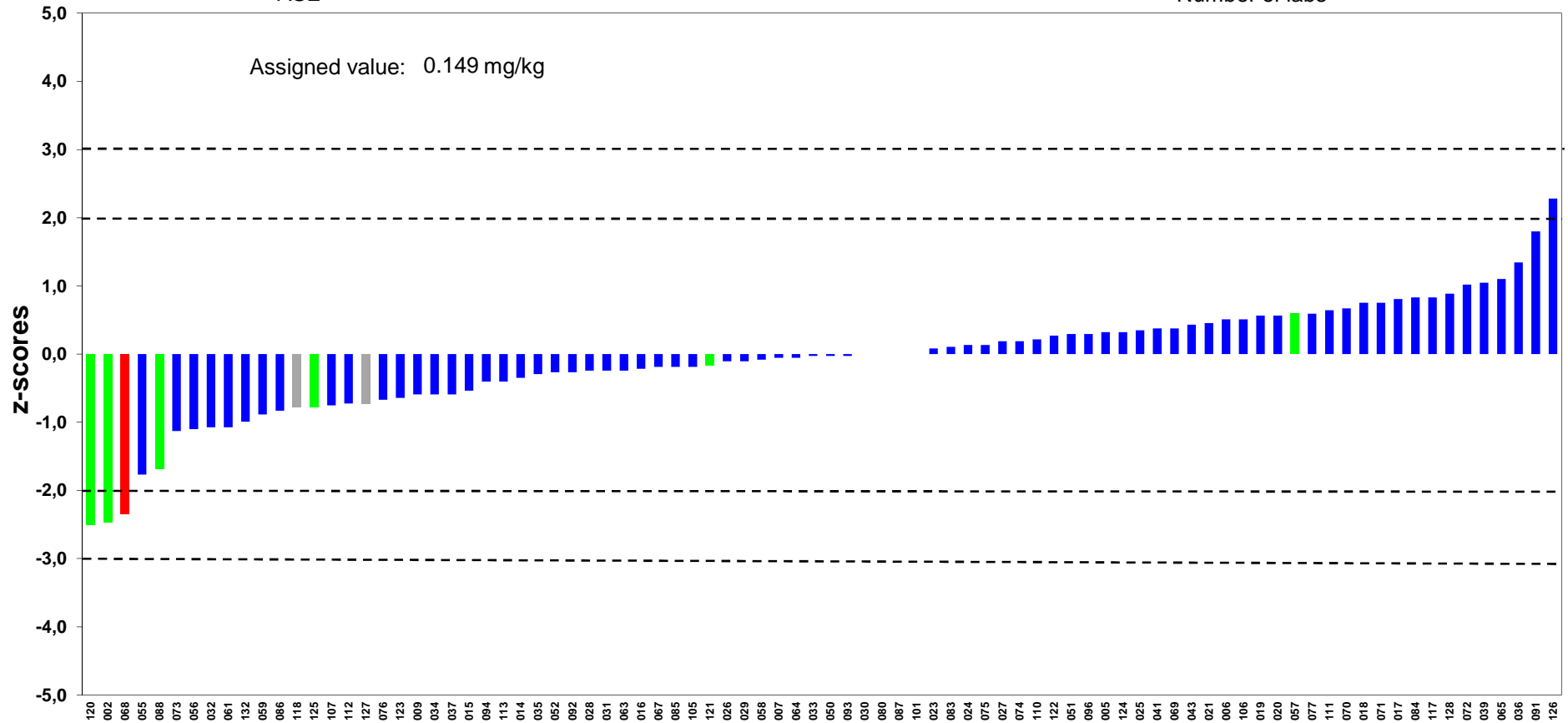
Acceptable	77
Questionable	6
Unacceptable	4
Number of labs	87



- Water added
- No water added
- Not specified
- ASE

Boscalid

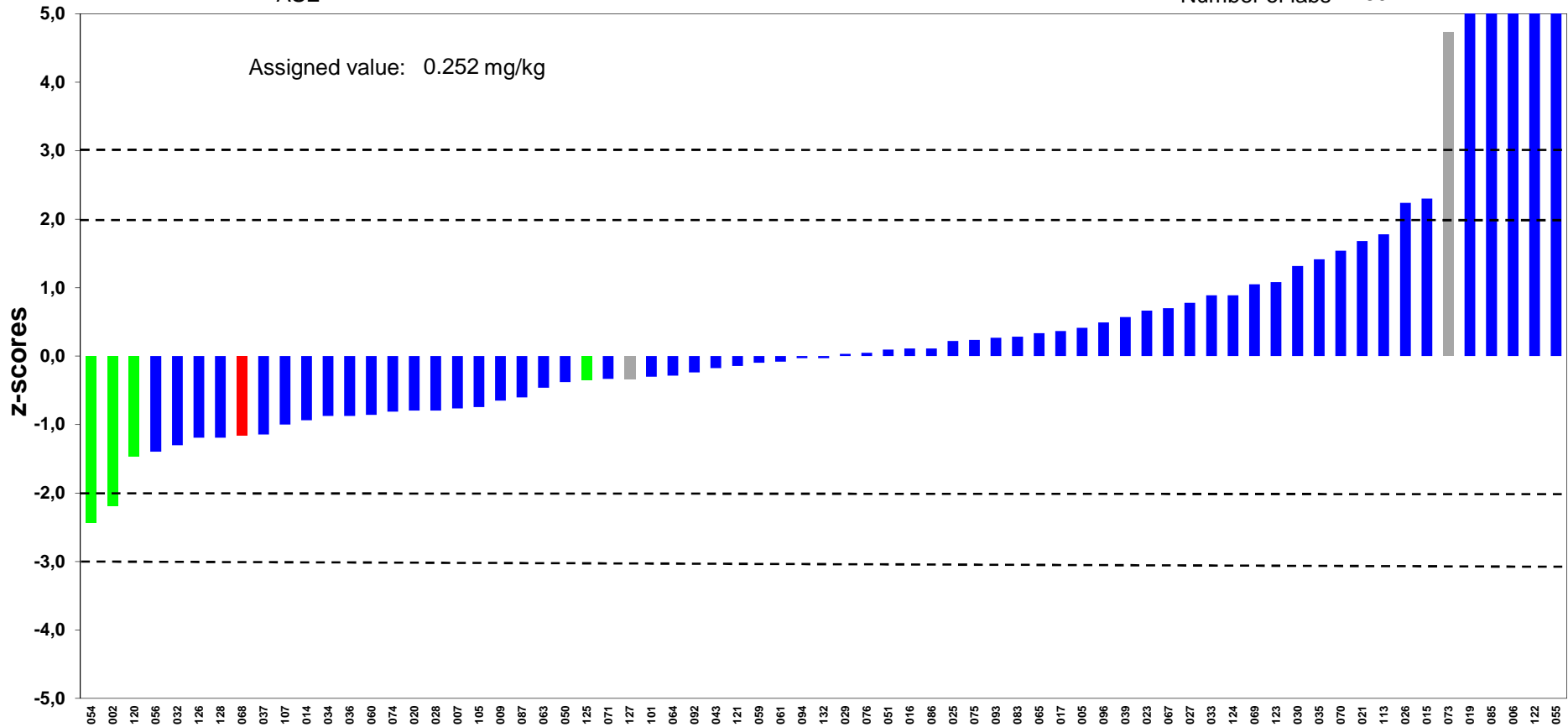
Acceptable 82
 Questionable 4
 Unacceptable 0
 Number of labs 86



- Water added
- No water added
- Not specified
- ASE

Carbendazim

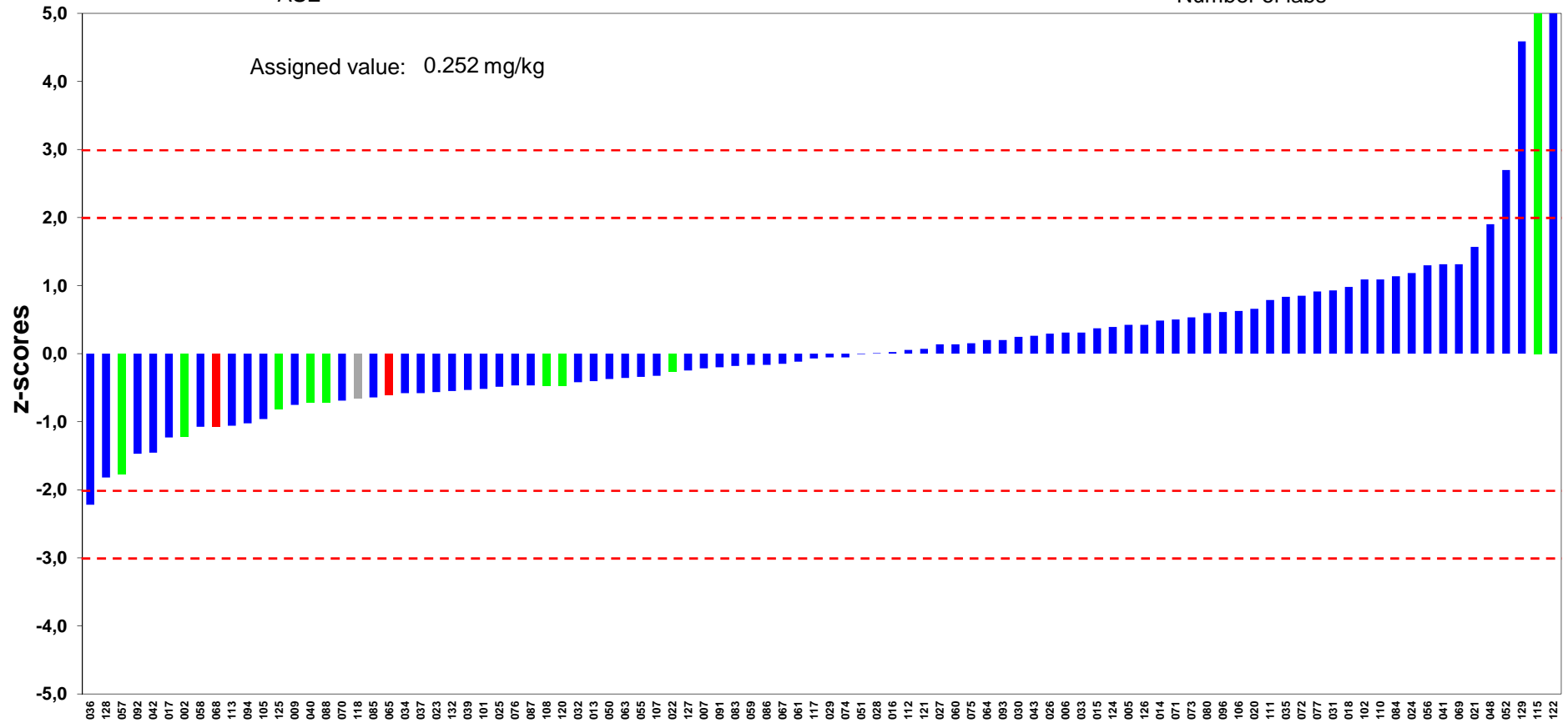
Acceptable 59
 Questionable 4
 Unacceptable 6
 Number of labs 69



- Water added
- No water added
- Not specified
- ASE

Chlorpyrifos-methyl

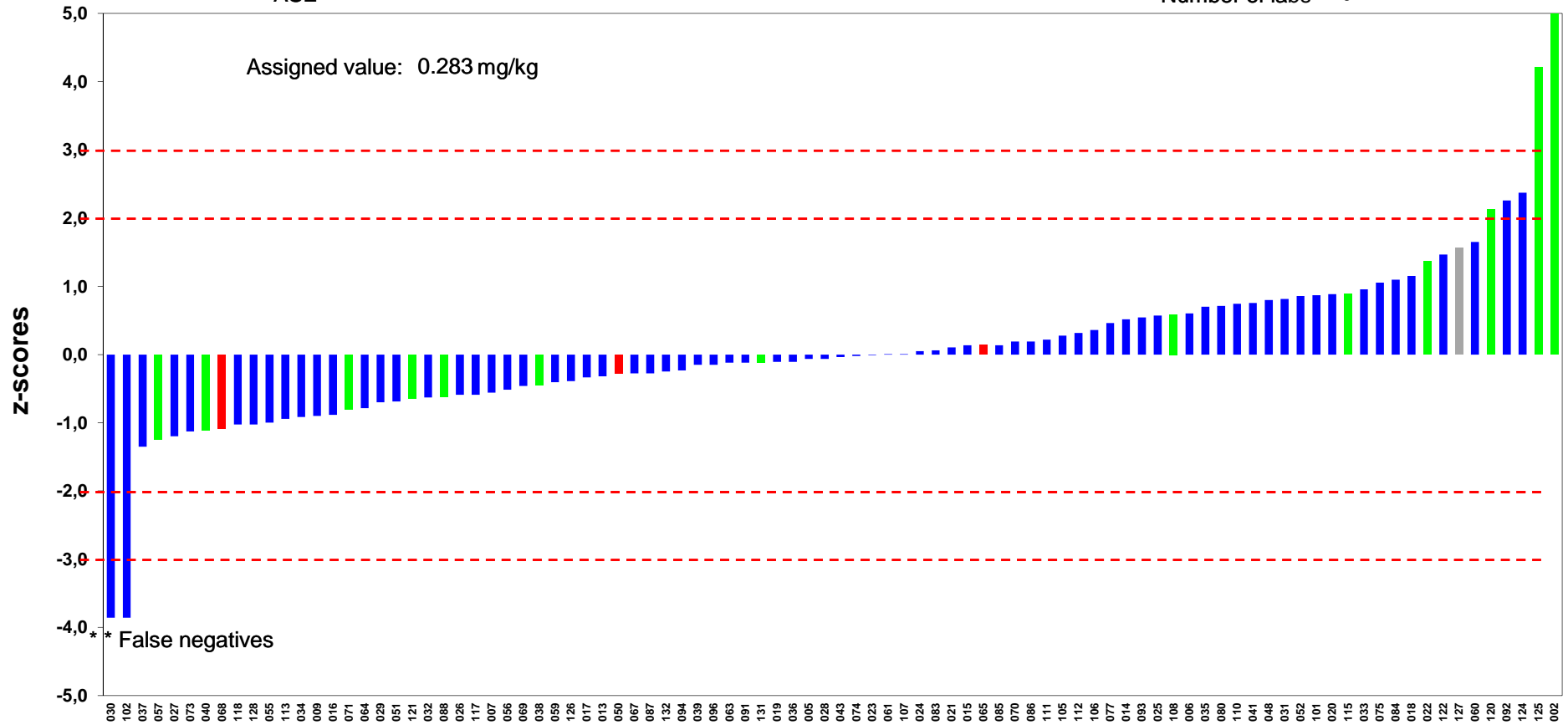
Acceptable 89
 Questionable 2
 Unacceptable 3
 Number of labs 94



Cypermethrin

Acceptable	85
Questionable	3
Unacceptable	4
Number of labs	92

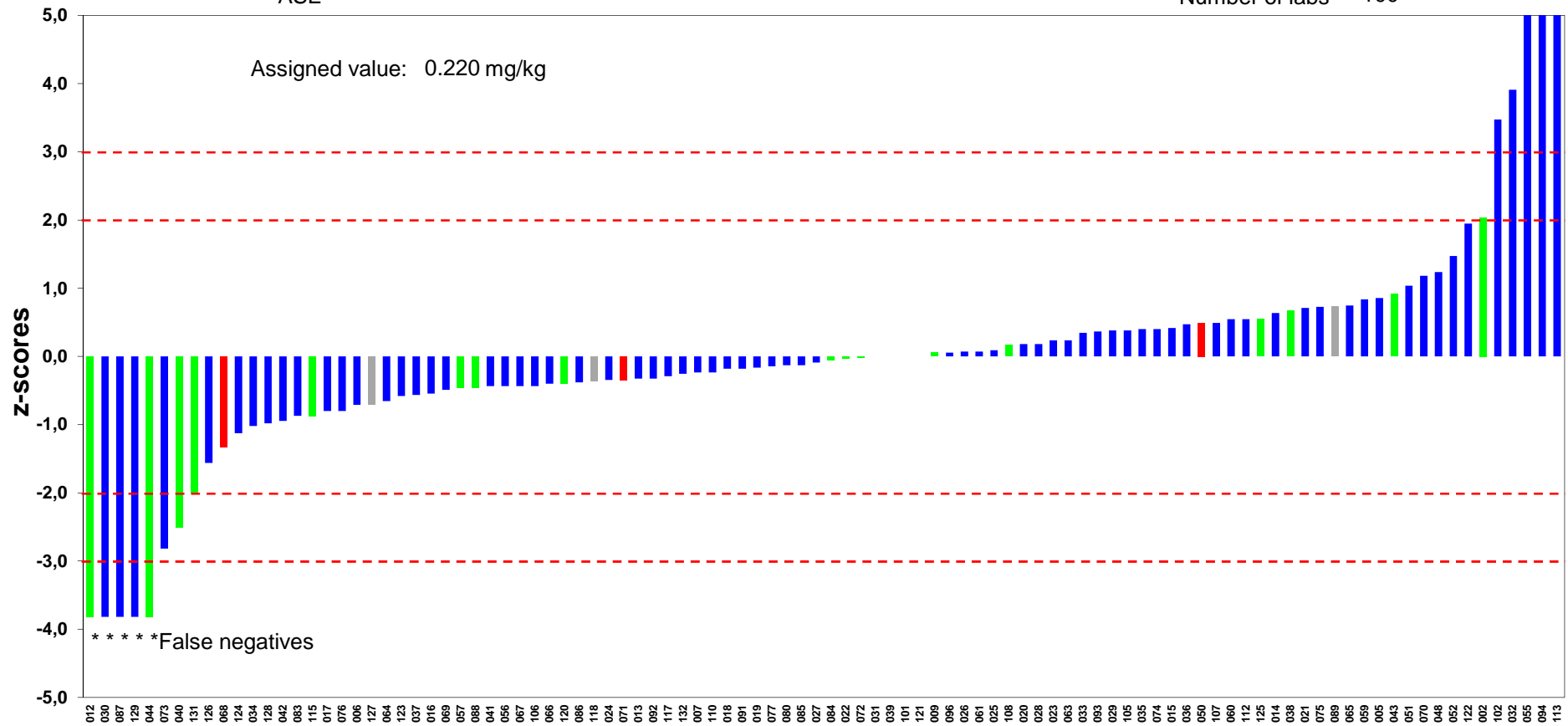
- Water added
- No water added
- Not specified
- ASE



- Water added
- No water added
- Not specified
- ASE

Endosulfan-alpha

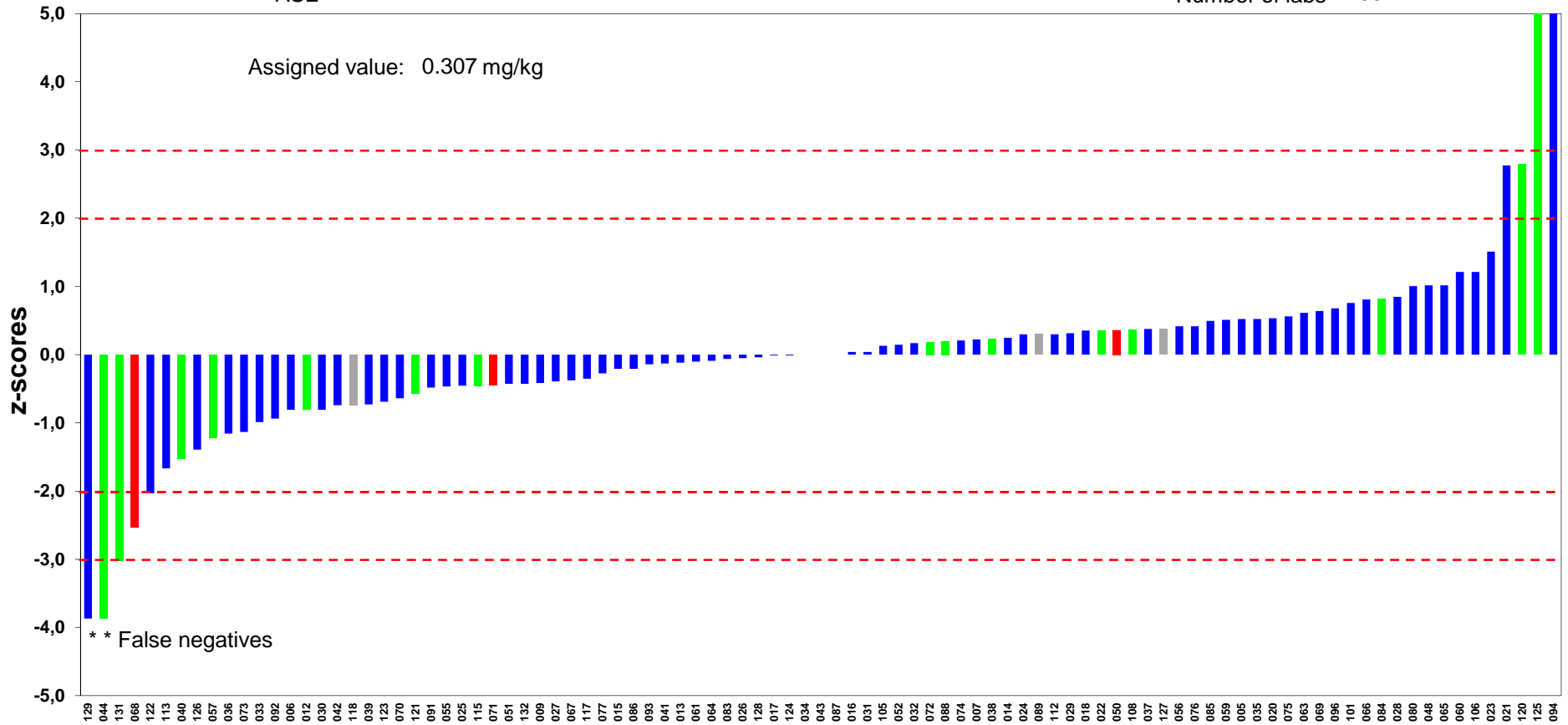
Acceptable 88
 Questionable 2
 Unacceptable 10
 Number of labs 100



- Water added
- No water added
- Not specified
- ASE

Endosulfan-sulfate

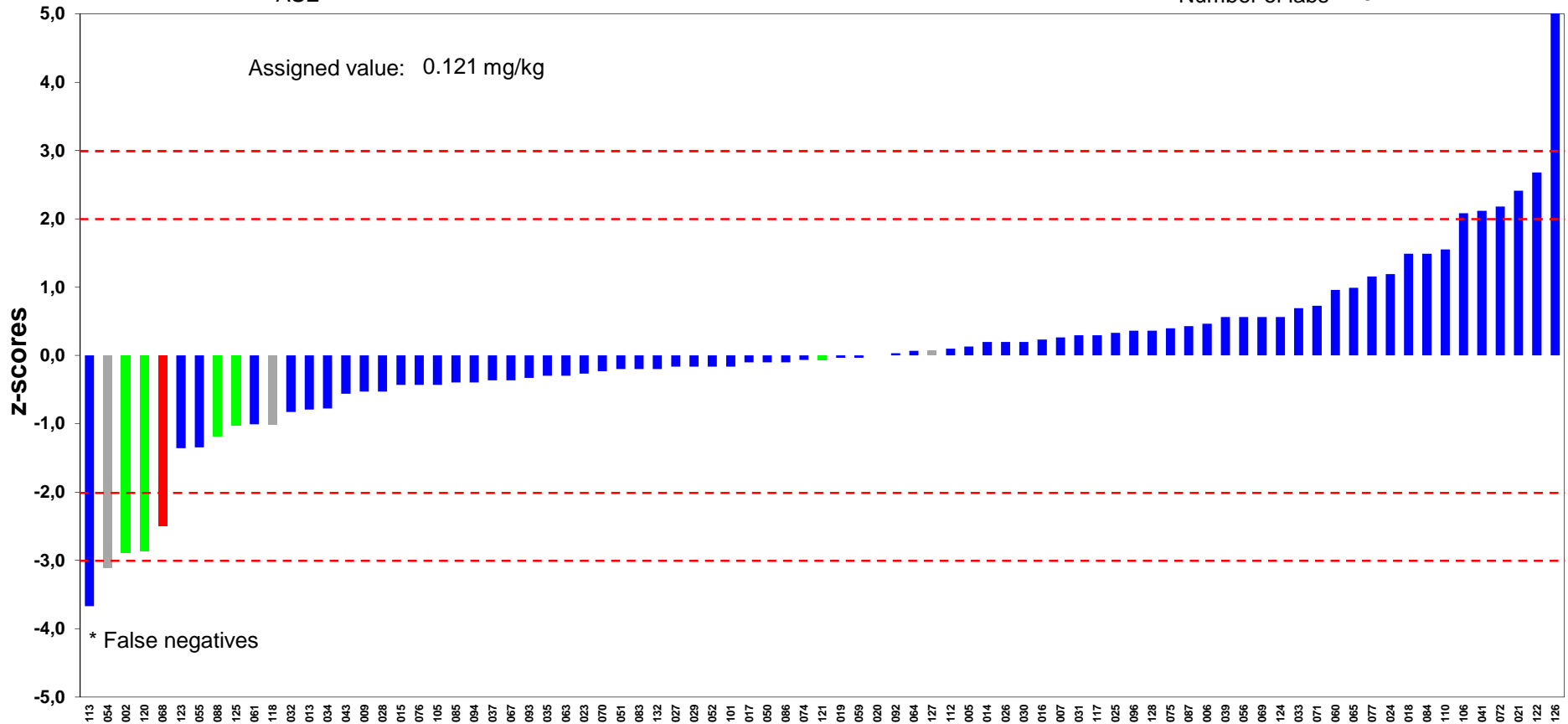
Acceptable	87
Questionable	4
Unacceptable	4
Number of labs	95



- Water added
- No water added
- Not specified
- ASE

Epoxiconazole

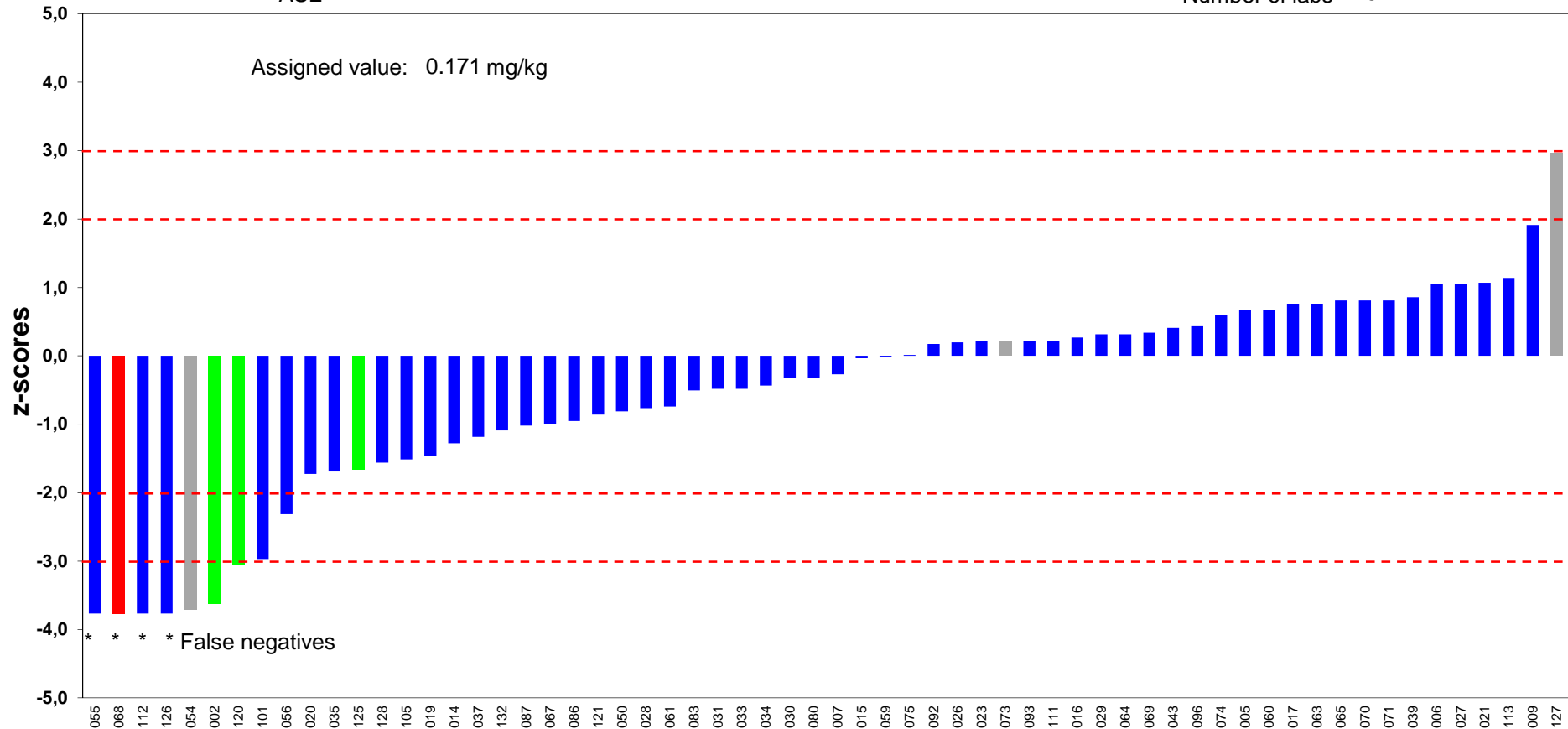
Acceptable 70
 Questionable 8
 Unacceptable 3
 Number of labs 81



- Water added
- No water added
- Not specified
- ASE

Fenpropidin

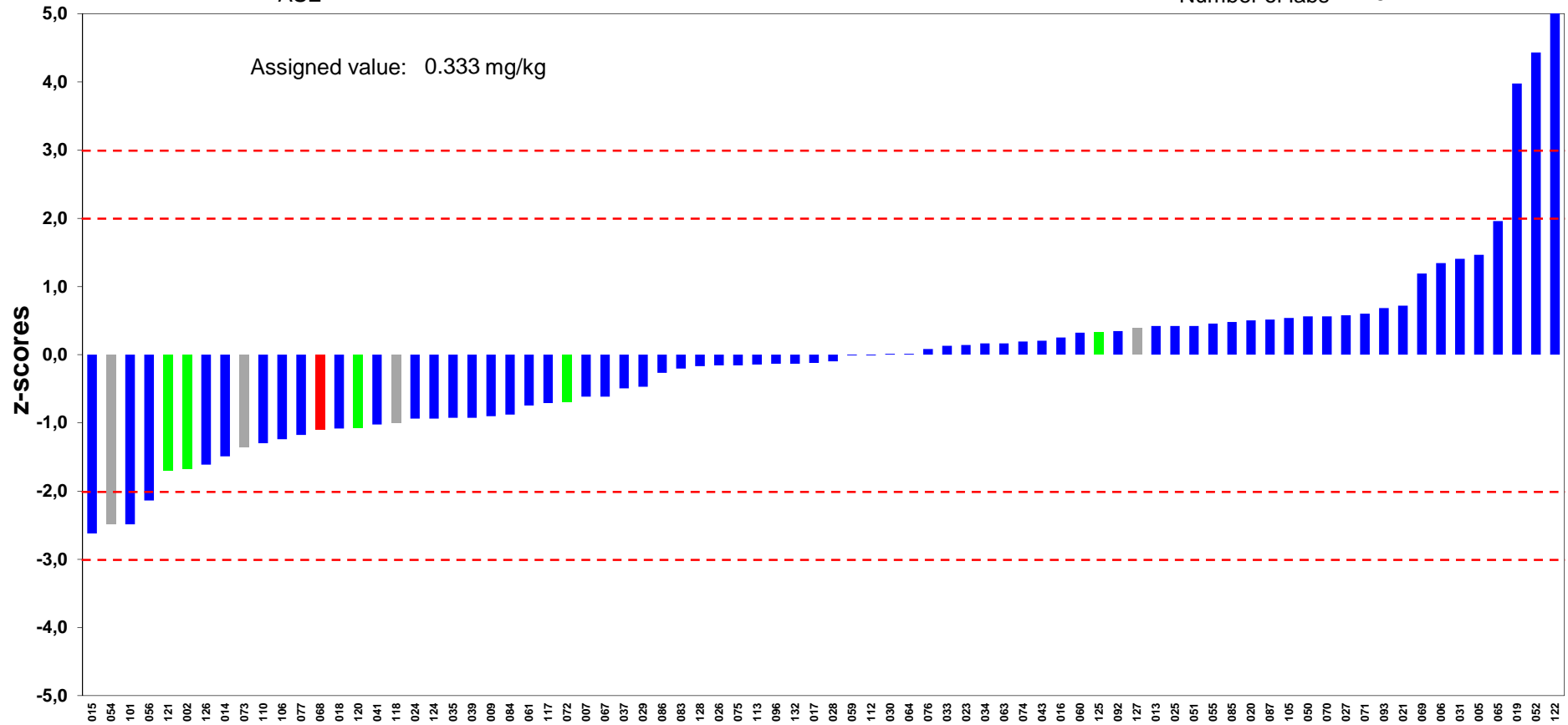
Acceptable 52
 Questionable 4
 Unacceptable 6
 Number of labs 62



- Water added
- No water added
- Not specified
- ASE

Fenpropimorph

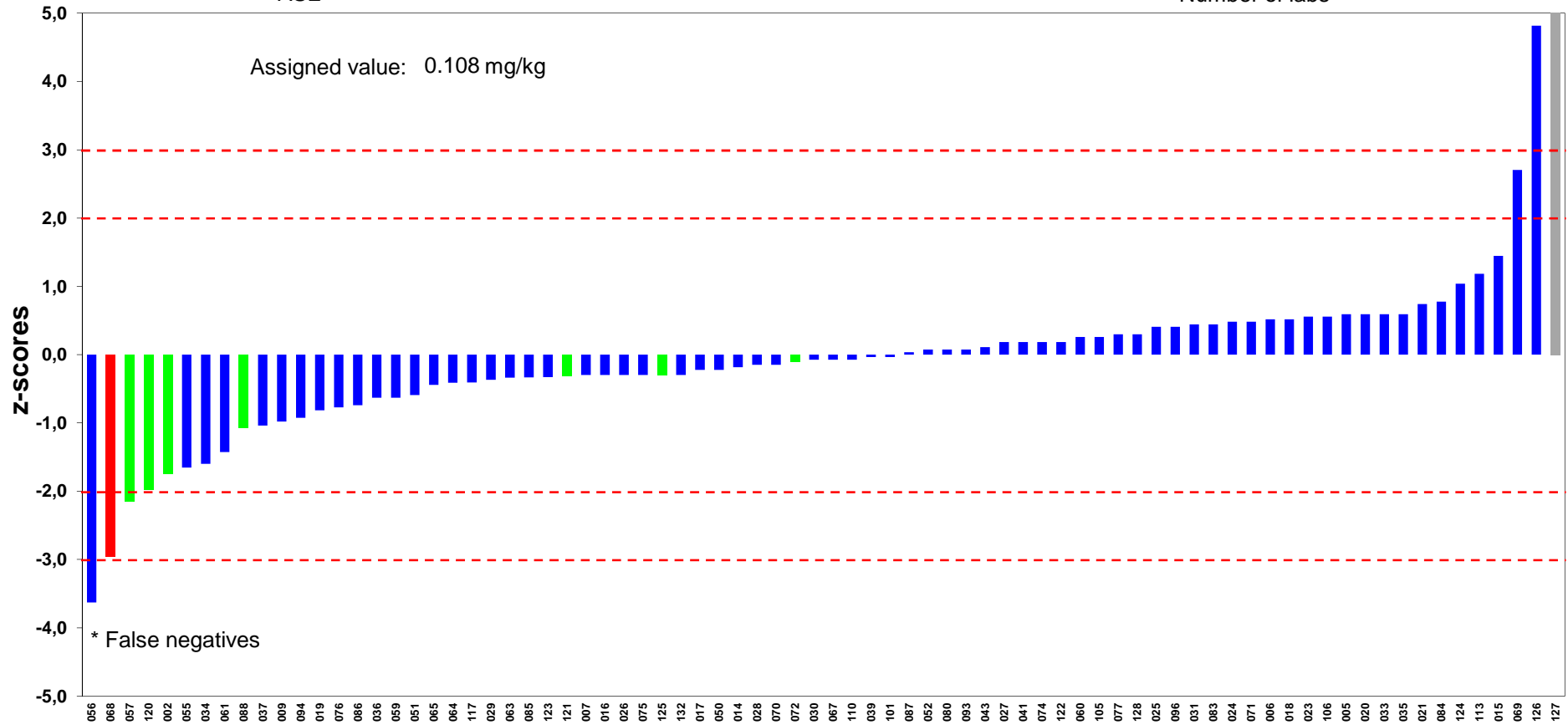
Acceptable 71
 Questionable 4
 Unacceptable 3
 Number of labs 78



- Water added
- No water added
- Not specified
- ASE

Fluquinconazole

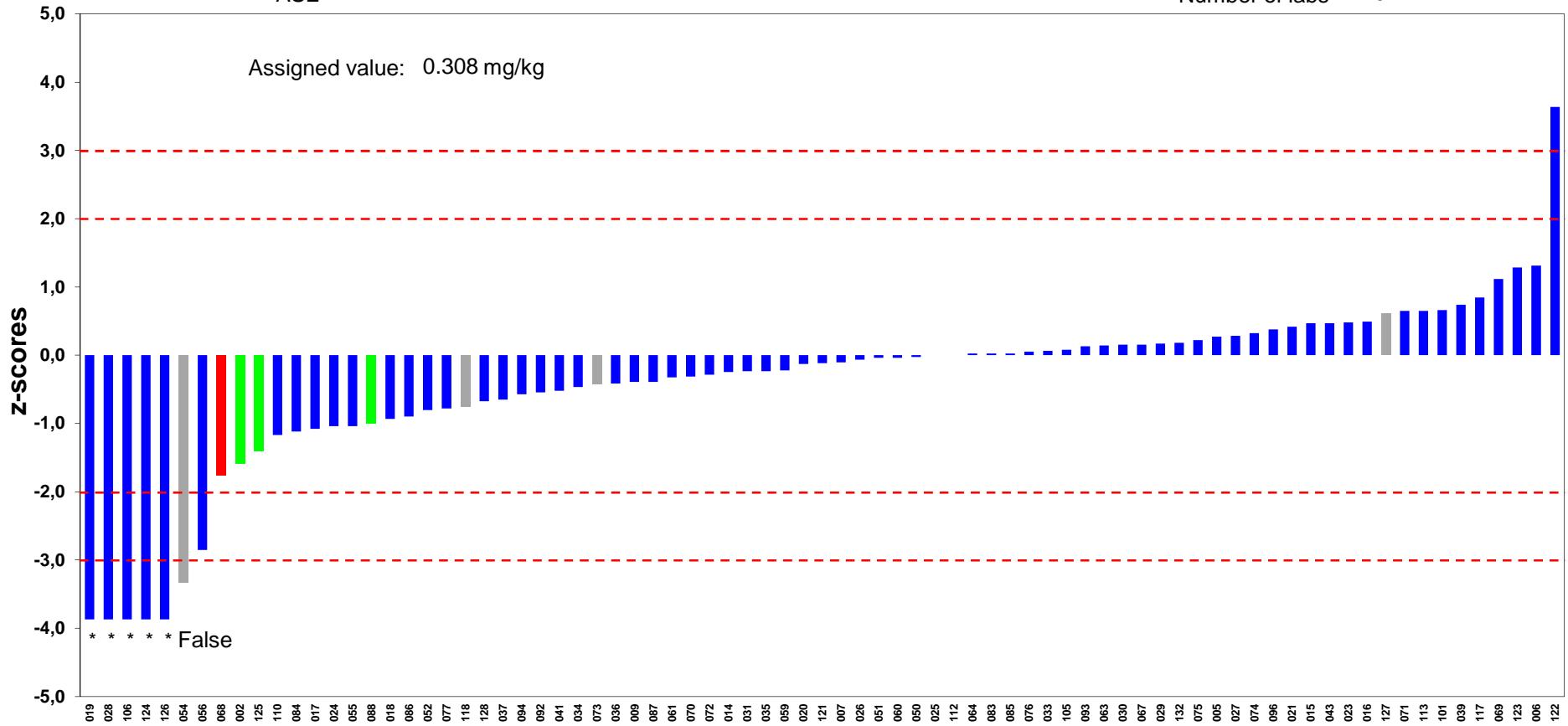
Acceptable 72
 Questionable 3
 Unacceptable 3
 Number of labs 78



- Water added
- No water added
- Not specified
- ASE

Flutriafol

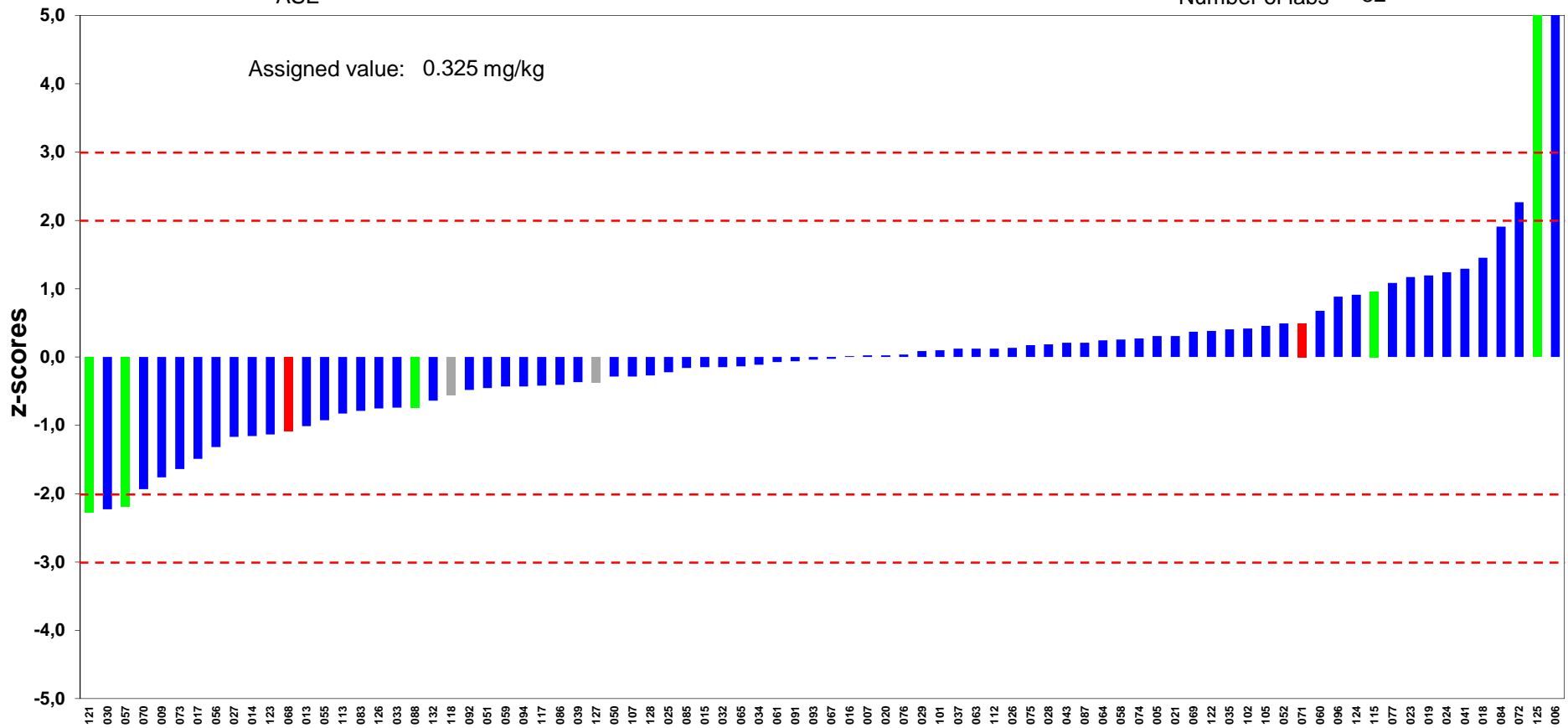
Acceptable 71
 Questionable 1
 Unacceptable 7
 Number of labs 79



- Water added
- No water added
- Not specified
- ASE

Iprodione

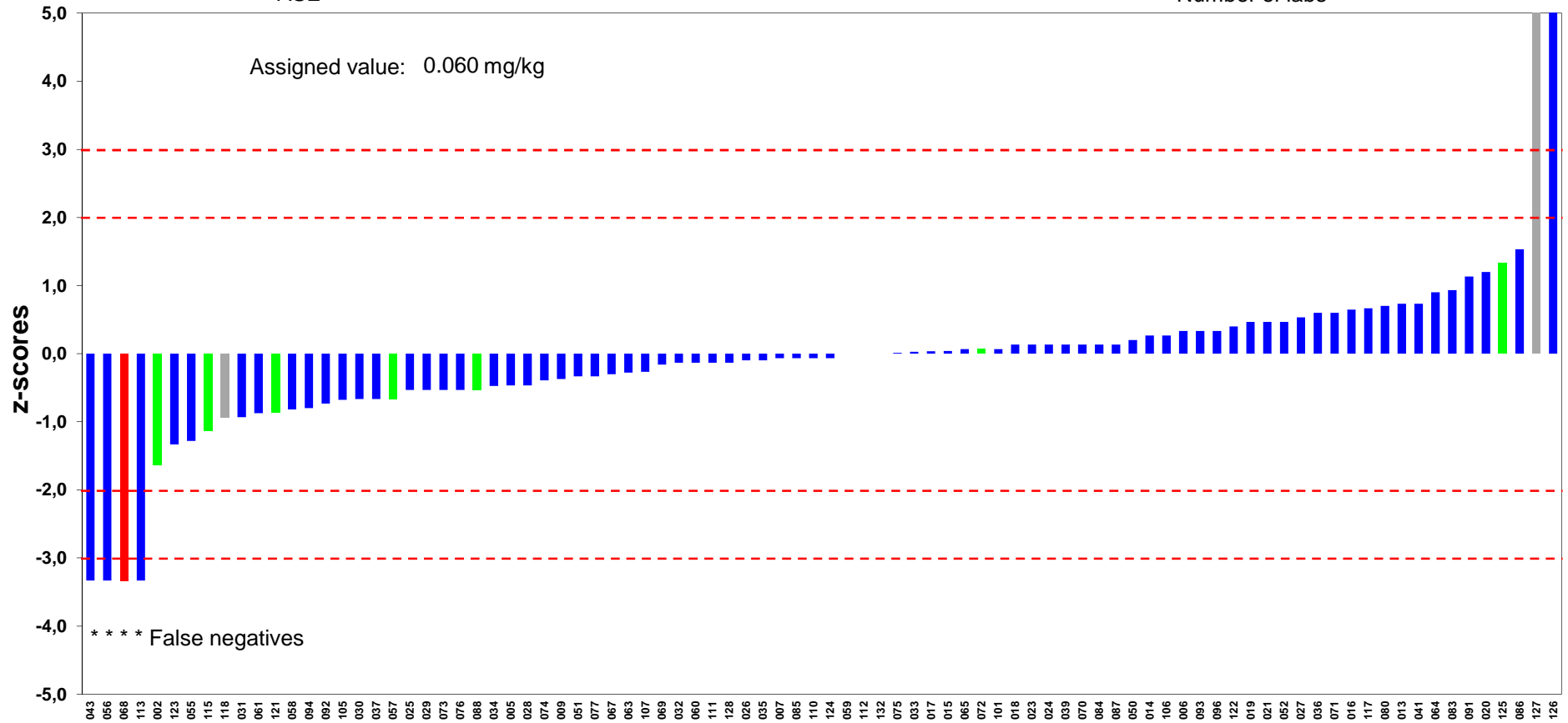
Acceptable 76
 Questionable 4
 Unacceptable 2
 Number of labs 82



- Water added
- No water added
- Not specified
- ASE

Kresoxim-methyl

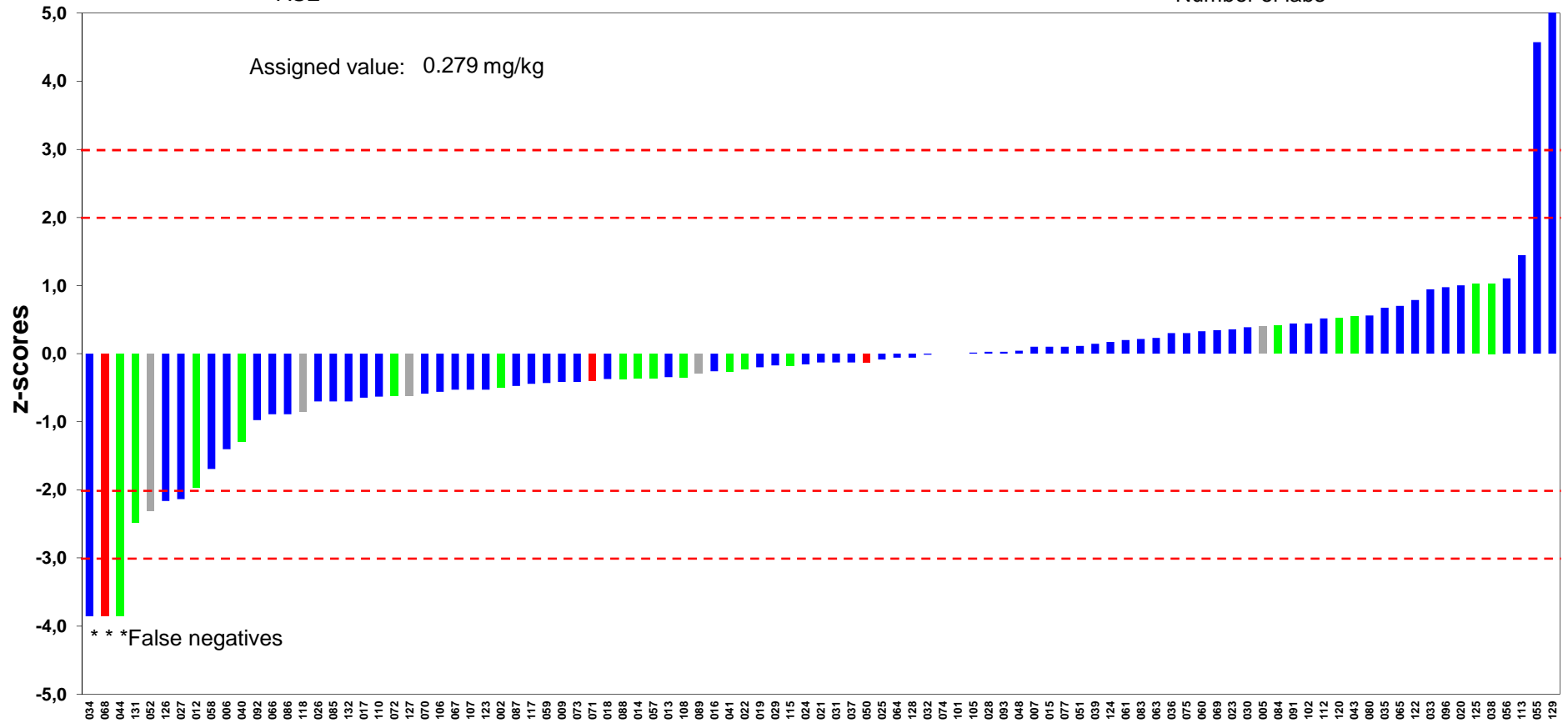
Acceptable 82
 Questionable 0
 Unacceptable 6
 Number of labs 88



- Water added
- No water added
- Not specified
- ASE

Lindane

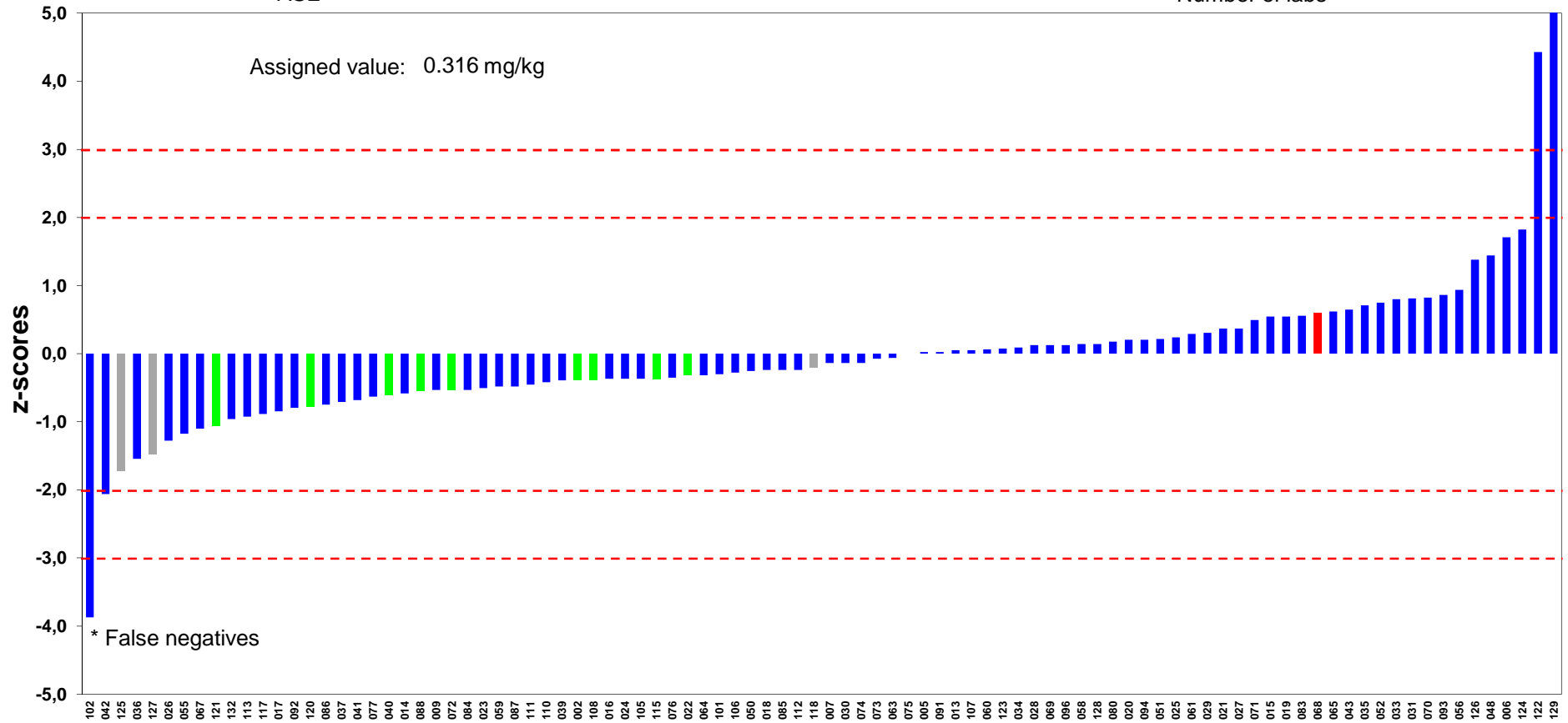
Acceptable 88
 Questionable 4
 Unacceptable 5
 Number of labs 97



- Water added
- No water added
- Not specified
- ASE

Malathion

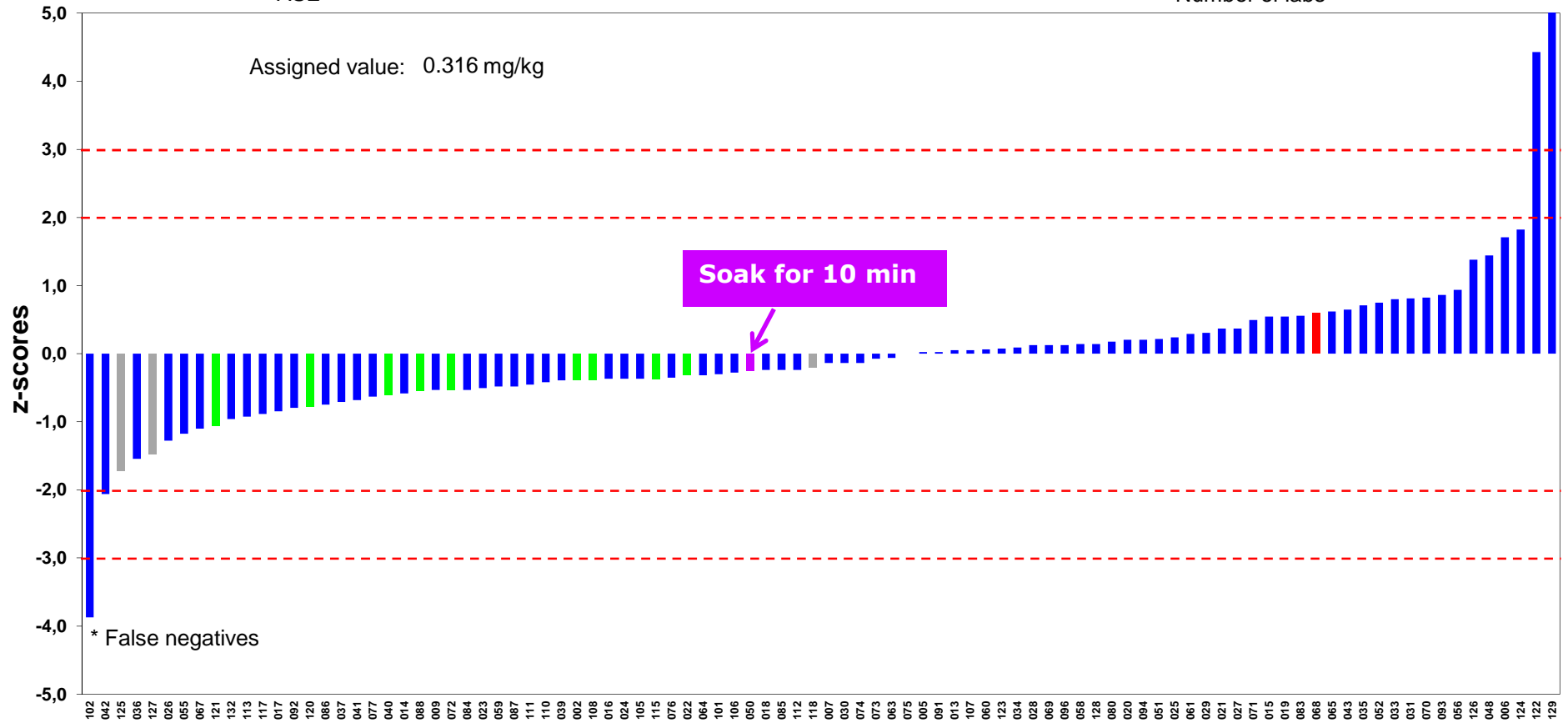
Acceptable 90
 Questionable 1
 Unacceptable 3
 Number of labs 94



- Water added
- No water added
- Not specified
- ASE

Malathion

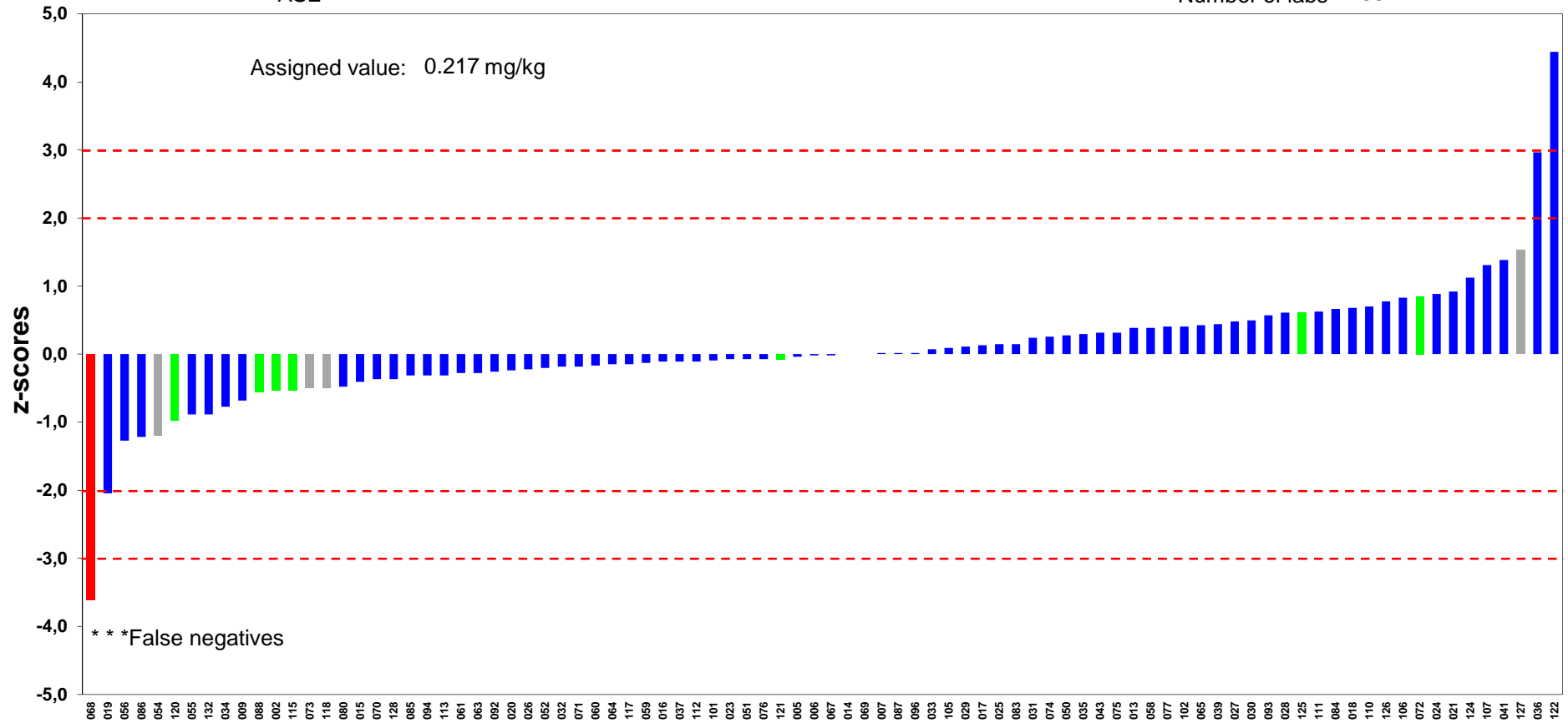
Acceptable	90
Questionable	1
Unacceptable	3
Number of labs	94



- Water added
- No water added
- Not specified
- ASE

Propiconazole

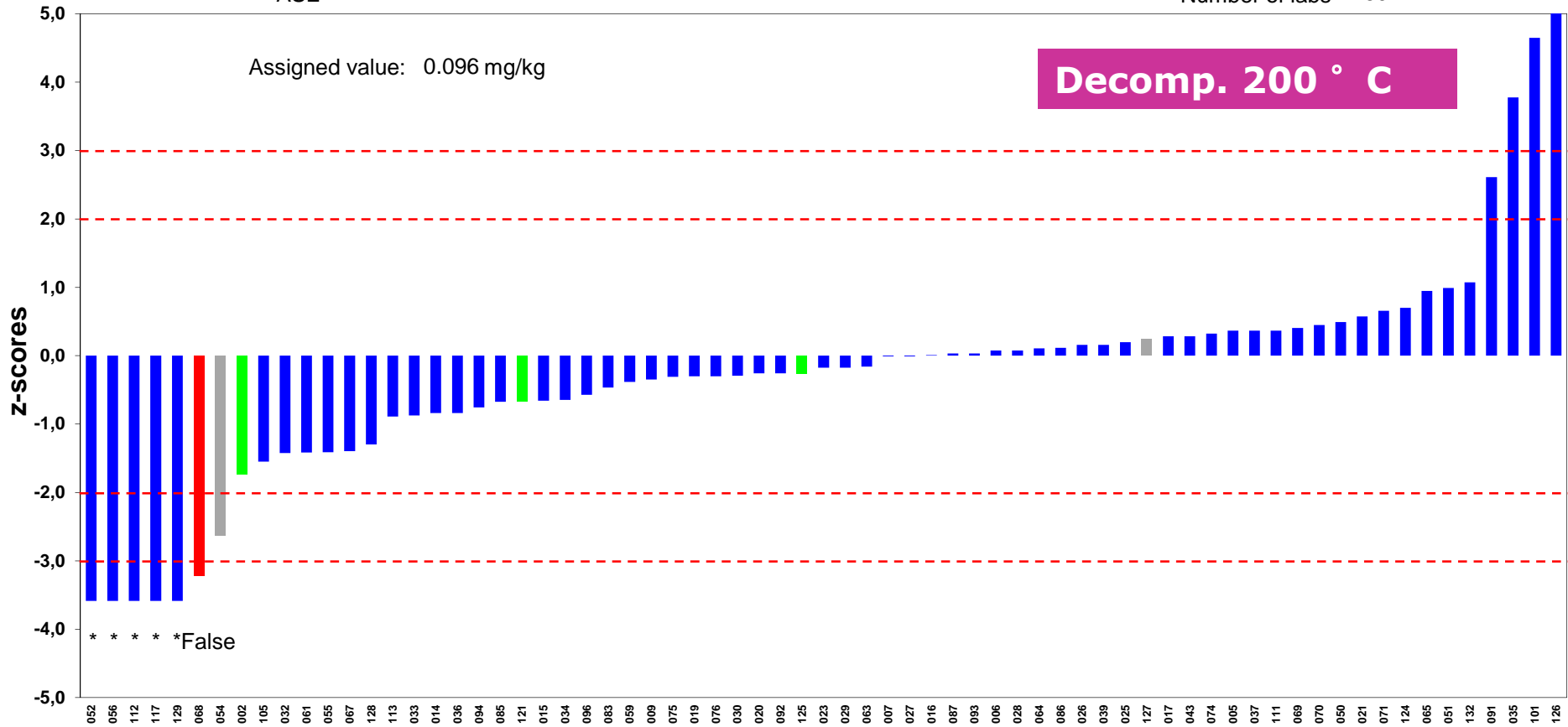
Acceptable 85
 Questionable 1
 Unacceptable 2
 Number of labs 88



- Water added
- No water added
- Not specified
- ASE

Pyraclostrobin

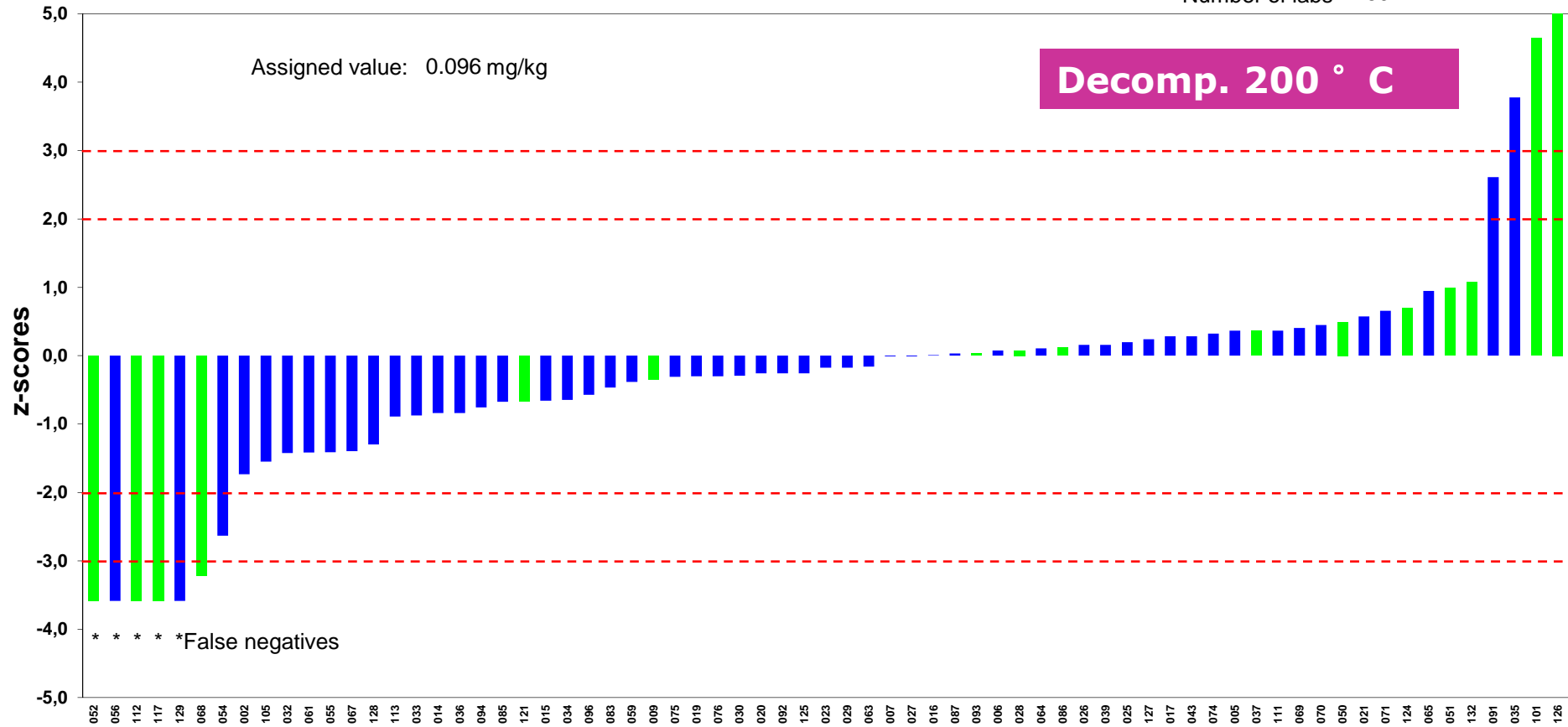
Acceptable 58
 Questionable 2
 Unacceptable 9
 Number of labs 69



■ LC
■ GC

Pyraclostrobin

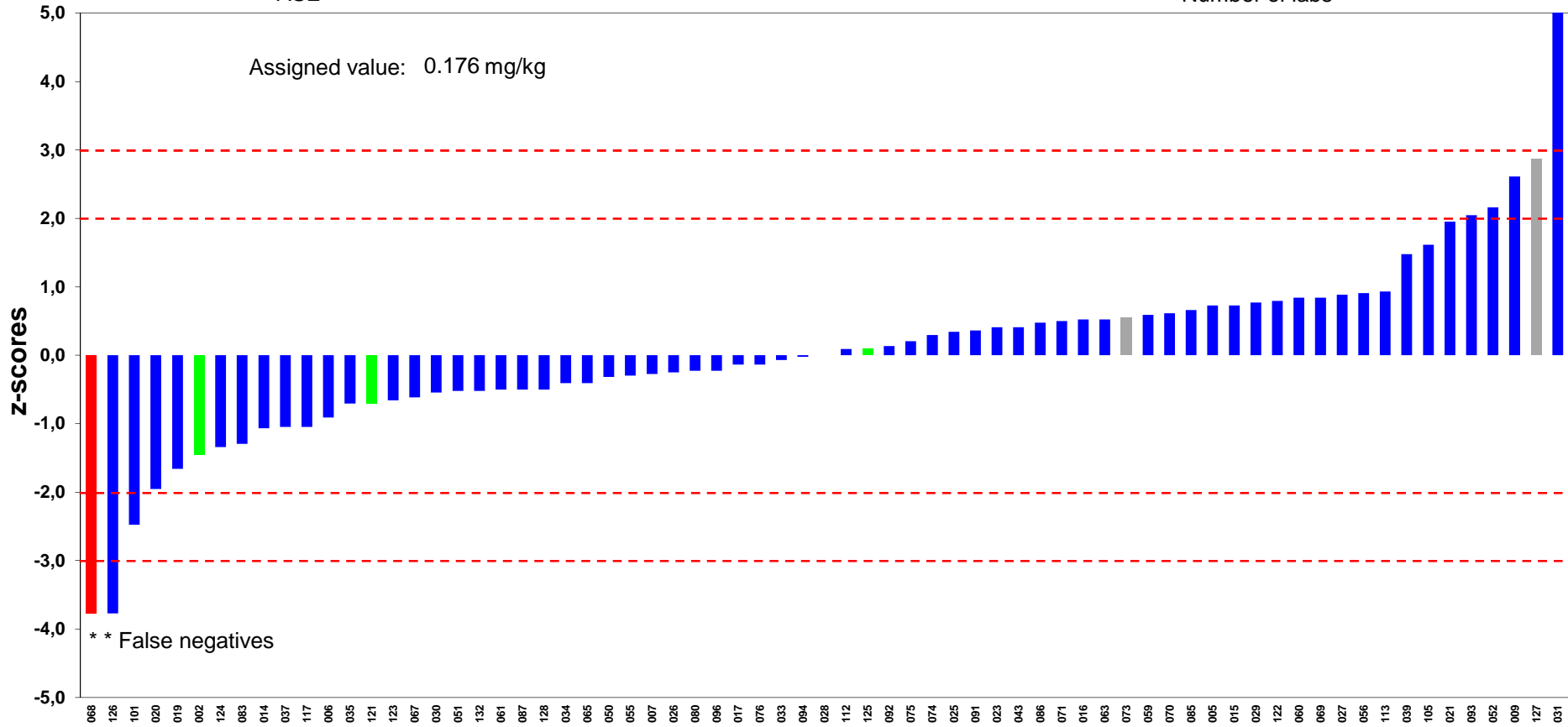
Acceptable 58
Questionable 2
Unacceptable 9
Number of labs 69



- Water added
- No water added
- Not specified
- ASE

Spiroxamine

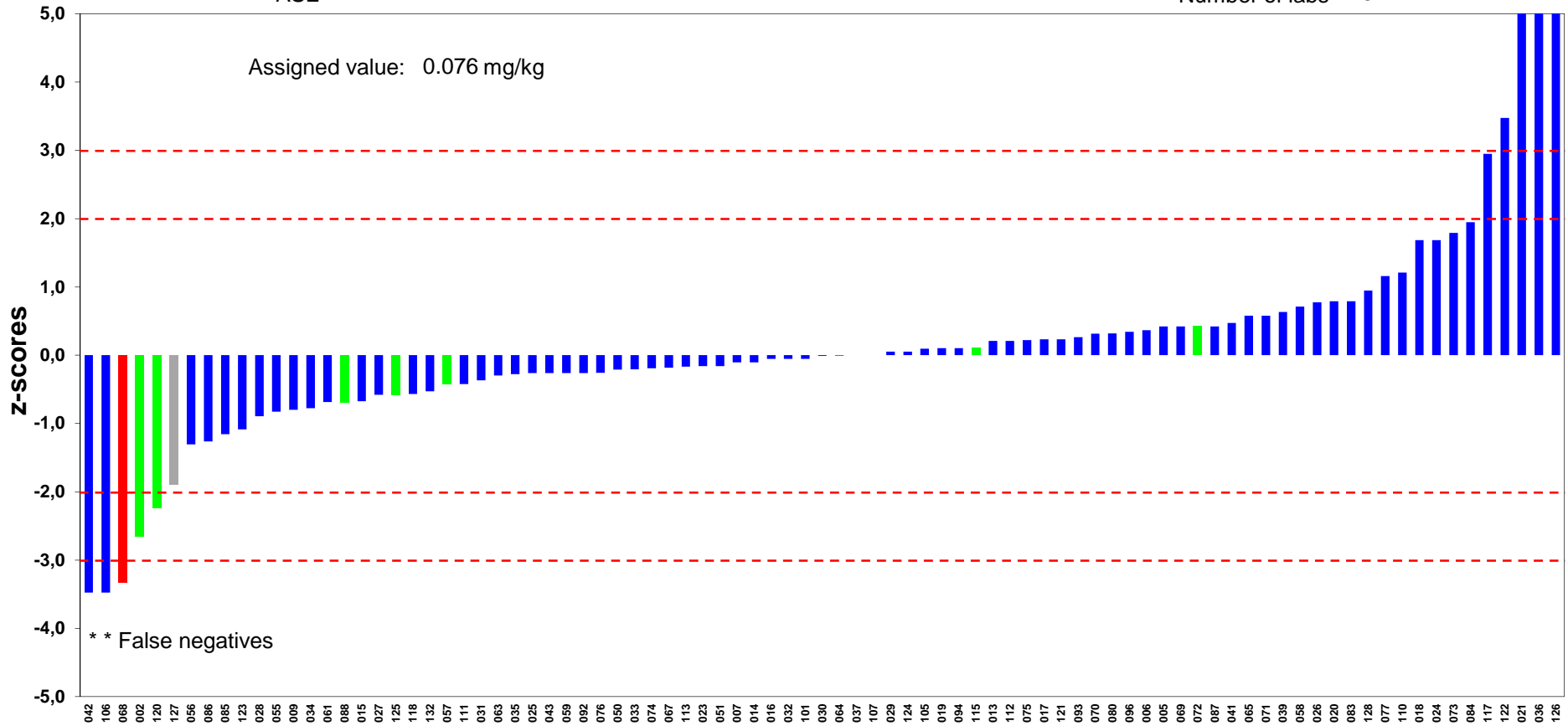
Acceptable 62
 Questionable 4
 Unacceptable 3
 Number of labs 69



- Water added
- No water added
- Not specified
- ASE

Tebuconazole

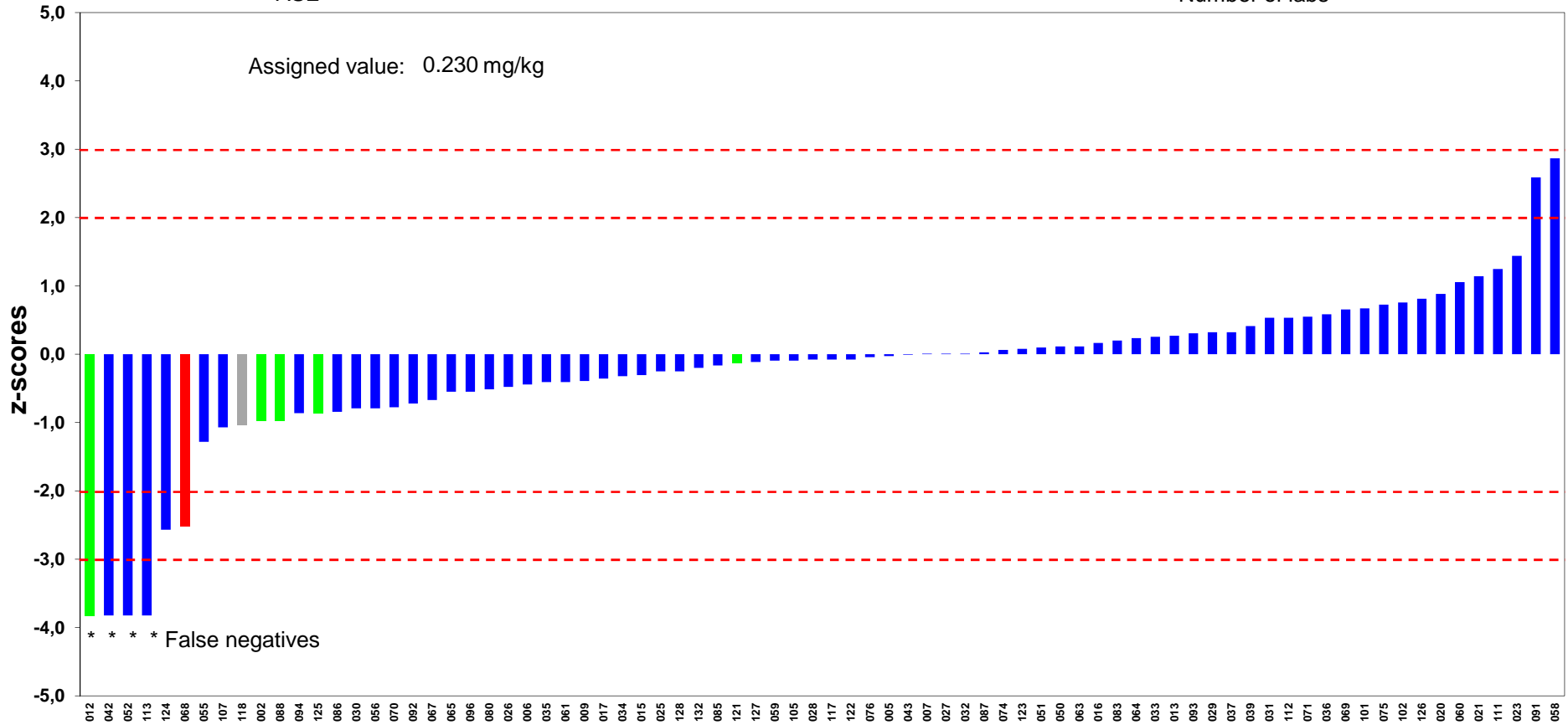
Acceptable	77
Questionable	3
Unacceptable	7
Number of labs	87



- Water added
- No water added
- Not specified
- ASE

Triadimenol

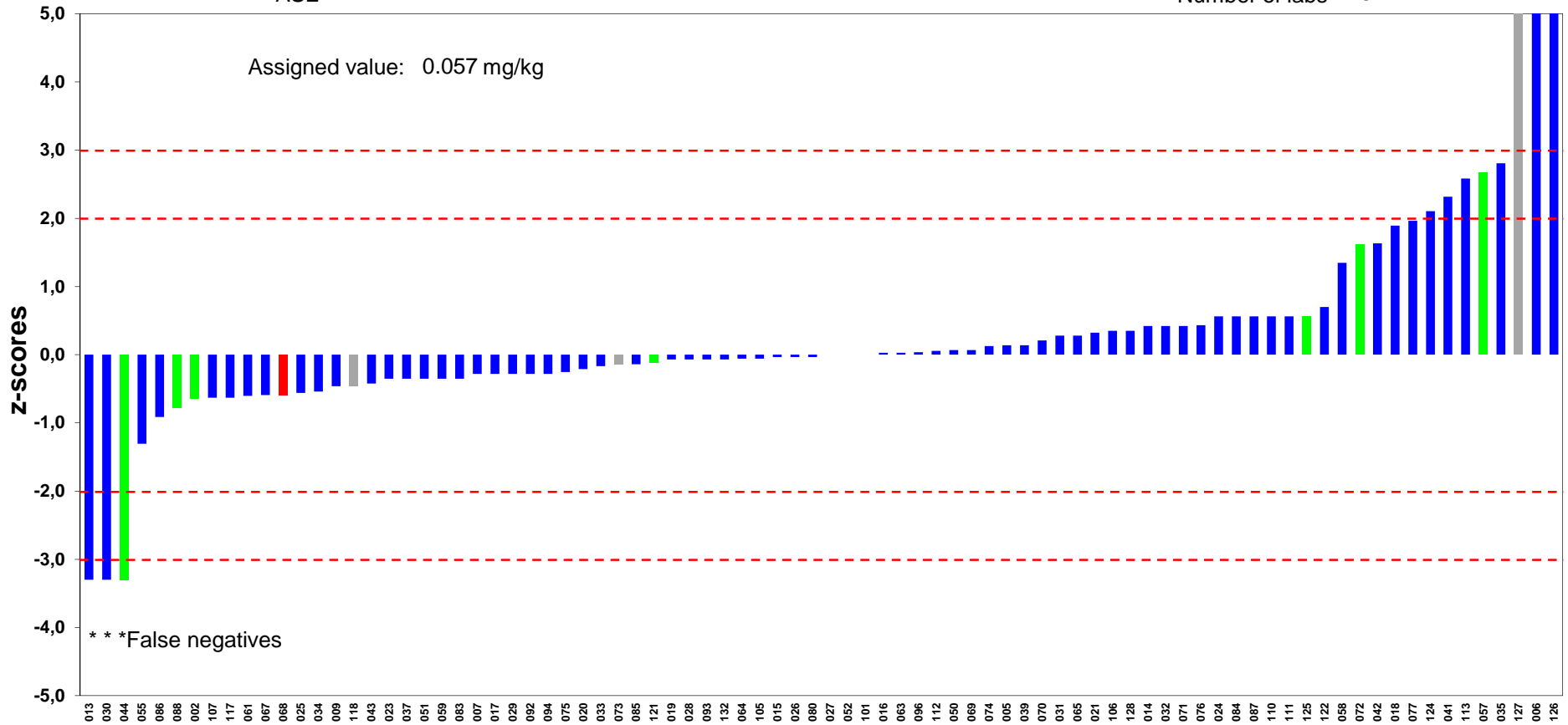
Acceptable 70
 Questionable 4
 Unacceptable 4
 Number of labs 78



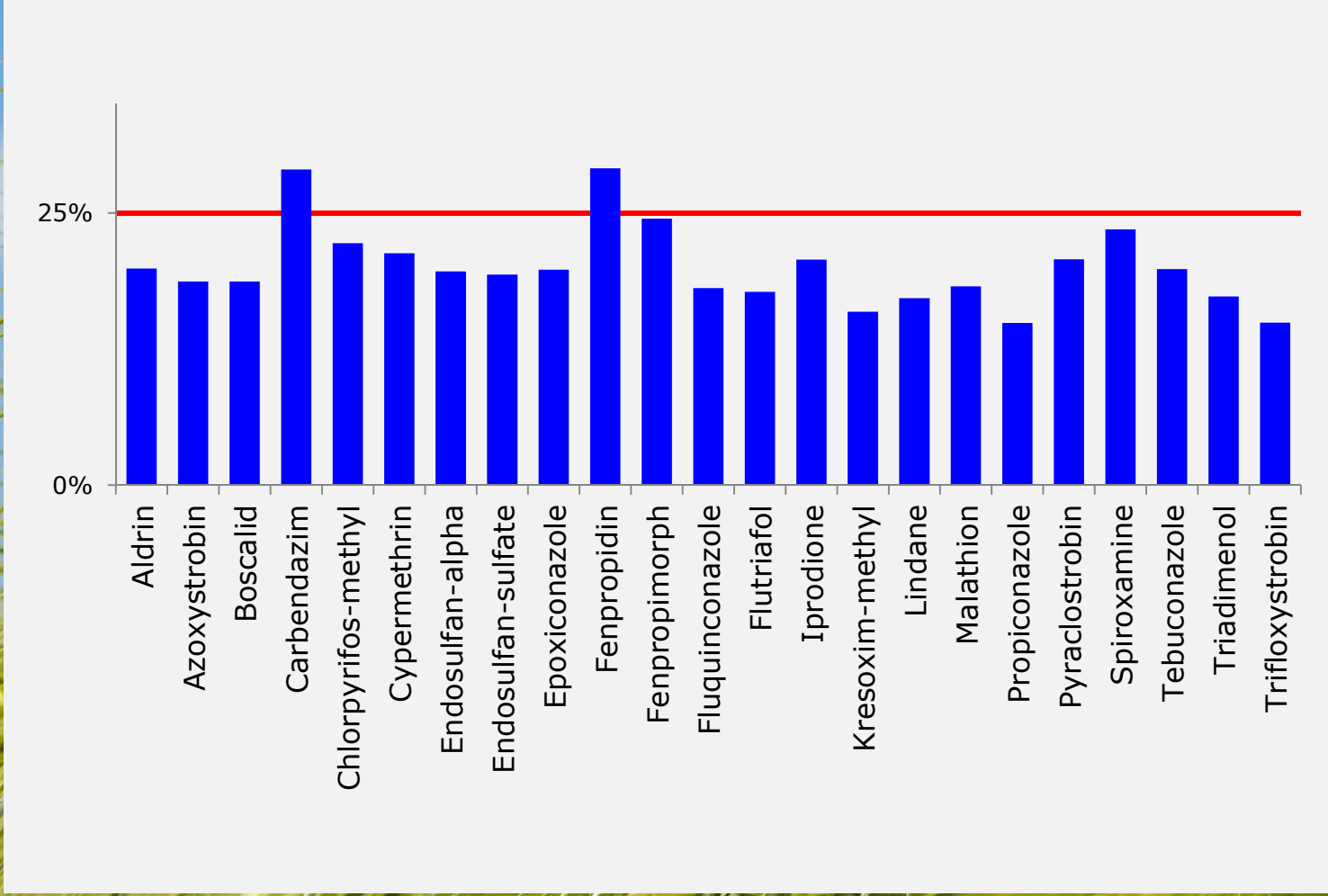
- Water added
- No water added
- Not specified
- ASE

Trifloxystrobin

Acceptable 73
 Questionable 5
 Unacceptable 6
 Number of labs 84



Qn



	No. of reported results	Acceptable %	Questionable %	Unacceptable %	False negatives %
Aldrin	95	89	4	6	3
Azoxystrobin	87	89	7	5	3
Boscalid	86	95	5	0	
Carbendazim	69	86	6	9	
Chlorpyrifos-methyl	94	95	2	3	
Cypermethrin	92	92	3	4	2
Endosulfan-alpha	100	87	3	10	5
Endosulfan-sulfate	95	92	4	4	2
Epoxiconazole	81	86	10	4	1
Fenpropidin	62	84	6	10	6
Fenpropimorph	78	91	5	4	
Fluquinconazole	78	92	4	4	1
Flutriafol	79	90	1	9	6
Iprodione	82	93	5	2	
Kresoxim-methyl	88	93	0	7	5
Lindane	97	91	4	5	3
Malathion	94	96	1	3	1
Propiconazole	88	95	2	2	
Pyraclostrobin	69	84	3	13	7
Spiroxamine	69	90	6	4	3
Tebuconazole	87	89	3	8	2
Triadimenol	78	90	5	5	5
Trifloxystrobin	84	87	6	7	4

	No. of reported results	Acceptable %	Questionable %	Unacceptable %	False negatives %
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Carbendazim	69	86	6	9	
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Cypermethrin	92	92	3	4	2
Endosulfan-alpha	100	87	3	10	5
Endosulfan-sulfate	95	92	4	4	2
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Fenpropidin	62	84	6	10	6
Fenpropimorph	78	91	5	4	
Fluquinconazole	78	92	4	4	1
Flutriafol	79	90	1	9	6
Iprodione	82	93	5	2	
Kresoxim-methyl	88	93	0	7	5
Lindane	97	91	4	5	3
Malathion	94	96	1	3	1
Propiconazole	88	95	2	2	
Pyraclostrobin	69	84	3	13	7
Spiroxamine	69	90	6	4	3
Tebuconazole	87	89	3	8	2
Triadimenol	78	90	5	5	5
Trifloxystrobin	84	87	6	7	4

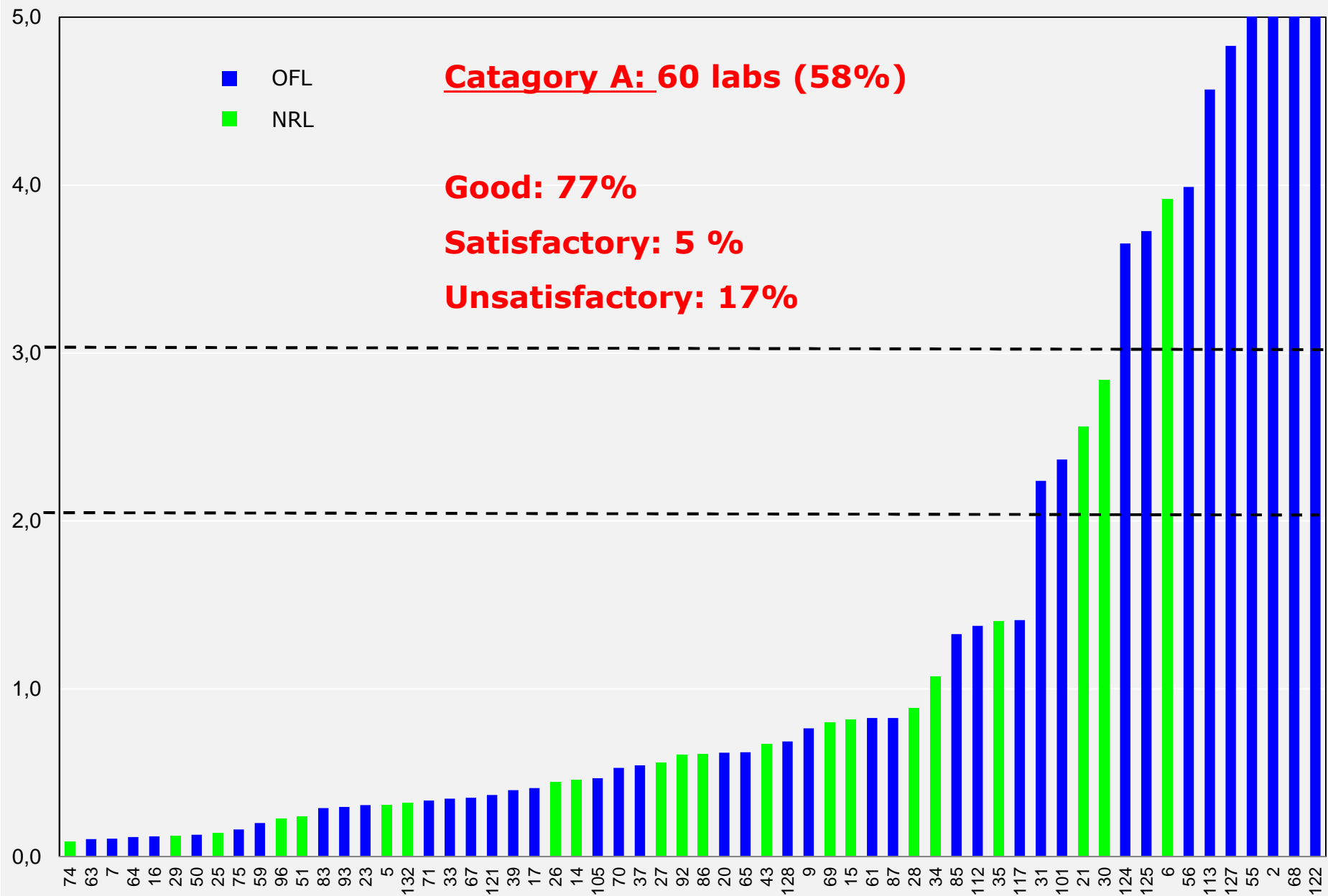
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Triadimenol	78	90	5	5	5
Trifloxystrobin	84	87	6	7	4

Category A laboratories and combined z-scores

- To be classified as Category A laboratory the labs had to report at least 90% of the pesticide present in the test material (≥ 16 pesticide residues) and report no false positive.
- Evaluation of the overall performance, the Average of the Squared z-Score (AZ²) are calculated:

$$AZ^2 = \frac{\sum_{i=1}^n |z_i| |z_i|}{n}$$

- $z \leq 2$ Good
- $2 < z \leq 3$ Satisfactory
- $z > 3$ Unsatisfactory



Conclusions

- EUPT-CF7 was the first EUPT focusing on cereal-based feed Test Items.
- The cereal used to prepare the feed was sprayed in the field with commercially available pesticide formulations, and the soya oil used was spiked in the laboratory.
- The treated Test Item contained 14 incurred pesticides, 5 pesticides that were incurred and spiked and 4 that were spiked only.
- 106 laboratories, representing 29 EU and EFTA countries participated
- 14 laboratories from Third Countries representing 10 countries participated

Conclusions

- Both the number of false positives and false negatives increased considerable in comparison to the last EUPT on cereals EUPT-C6.
 - The 13 false positive results were reported by 11 laboratories
 - The 50 false negative results were reported by 22 laboratories
- For boscalid, chlorpyrifos-methyl, cypermethrin, endosulfan-sulfate, fenpropimorph, fluquinconazole, flutriafol, iprodione, kresoxim-methyl, lindane, malathion, propiconazole, spiroxamine and triadimenol acceptable results were obtained for **90-96%** of the laboratories.
- For aldrin, azoxystrobin, carbendazim, endosulfan-alpha, epoxiconazole, fenpropidin, pyraclostrobin, tebuconazole and trifloxystrobin acceptable results were obtained by **84-89%** of the laboratories.

EUPT-CF8

- What
 - Wheat with incurred pesticides
- When
 - April/May 2014

**Thank you to the Advisory Group
Thank you to the Quality Group**

**Thank you very much for your
attention**

