RIKILT
WAGENINGEN UR

Proficiency test for parasiticides in salmon muscle

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Distribution list:

- 24 participating laboratories

Summary

The proficiency test for parasiticides in salmon muscle was organized by RIKILT Wageningen UR.

For this proficiency test, four test materials were dispatched:

- salmon muscle containing ivermectin and emamectin with assigned values of 65.1 µg/kg and 133 µg/kg respectively;
- salmon muscle containing cypermethrin, deltamethrin and emamectin with assigned values of 33.6 µg/kg, 34.0 µg/kg and 82.2 µg/kg respectively;
- salmon muscle containing cypermethrin and deltamethrin with assigned values of 25.5 µg/kg and 8.77 µg/kg respectively;
- salmon muscle containing cypermethrin with an assigned value of 45.1 µg/kg.

The first three materials were prepared by spiking blank salmon muscle materials followed by cryogenic homogenization. The fourth material was not cryogenically homogenized, because this material had to be analysed in its entirety. During homogeneity testing, the three materials proved to be sufficiently homogenous for proficiency testing. The stability test demonstrated statistically significant (small) losses of some compounds, which was accounted for in the calculation of the z-scores.

Twenty-four laboratories subscribed for participation in this test. Within the time frame of the study 23 laboratories submitted results and one lab showed optimal performance by detecting all compounds, the absence of false positives and false negatives and a correct quantification. Within the participant's scope (not all participants included emamectin, ivermectin, cypermethrin and deltamethrin in its analysis), nine extra labs showed optimal performance.

In the avermectins analysis three false negative results and nine false positive result were reported. Nine out of 19 labs that analysed avermectins reported no false negative or false positive results and satisfactory z-scores. In the pyrethroids analysis 13 false negative results and seven false positive results were reported. Eight out of 16 labs that analysed pyrethroids reported no false negative or false positive results and satisfactory z-scores.

Contents

1 Introduction

Proficiency testing is conducted to provide laboratories with a powerful tool to evaluate and demonstrate the reliability of the data that is produced. Next to validation and accreditation, proficiency testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [1] and is demanded by ISO 17025:2005 [2].

The aim of this proficiency study was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of parasiticides in salmon muscle. This study also provided an evaluation of the methods applied for the quantitative analysis of parasiticides in salmon muscle.

The preparation of the materials, including the suitability testing of the materials and the evaluation of the quantitative results were carried out by RIKILT Wageningen UR.

2 Material en methods

This proficiency test focused on α-cypermethrin (CYP, a pyrethroid), deltamethrin (DEL, a pyrethroid), emamectin (EMA, an avermectin) and and ivermectin (IVM, an avermectin). The European and Japanese maximum residue limits (MRLs) for these compounds, except for ivermectin, in salmon muscle are presented in Table 2.

Table 1. European and Japanese MRLs in salmon muscle of the compounds included in the proficiency test [10].

Marker residue	Compound	EU-MRL in salmon muscle (µg/kg)	Japanese MRL in salmon muscle (µg/kg)
CYP	Cypermethrin (sum of isomers)	50	30
DEL	Deltamethrin	10	30
EMA	Emamectin B1a	100	100

2.1 Sample preparation

One material (A) containing EMA and IVM, one material (B) containing CYP, DEL and EMA, one material (C) containing CYP and DELTA and one material (D) containing CYP were prepared. Materials A-C were prepared by adding methanolic solutions of the selected compounds to blank salmon muscle aiming at the levels as presented in Table 2. Each of the materials was homogenized under cryogenic conditions according to in-house standard operating procedures [3]. Material D was prepared by adding a methanolic solution of CYP to 5 grams of blank salmon muscle. This material had to be analysed in its entirety by the participants.

Table 2. Target amount of parasiticides in the proficiency test materials.

Material code	Target amount (µg/kg)			
	CYP	DEL	EMA	IVM
A	-	-	200	100
B	35	35	100	-
C	55	12	-	-
D	60	-	-	-

2.2 Sample identification

After homogenization, the sample materials were divided into sub-portions and stored in polypropylene containers. Each contained at least 50 gram of sample. The samples for materials A-C for the participants were randomly selected and coded from 001 through 102. For each laboratory a sample set was prepared consisting of one randomly selected sample of material A, B and C. The codes of the samples belonging to each sample set are presented in Annex I. In addition, every participant received material D, which was a 50 ml tube containing 5 grams of salmon muscle. The remaining samples were used for homogeneity and stability testing.

2.3 Participants

Twenty-four laboratories subscribed for participation in the proficiency study of which 15 are situated within Europe, seven in South-America, one in North-America and one in Asia. On the invitation each participant was asked to indicate which compounds were included in their scope.

2.4 Homogeneity study

The homogeneity of the materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [5] and ISO 13528 [6], taking into account the insights discussed by Thompson [7] regarding the Horwitz equation. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) are compared with the target standard deviation derived from the Horwitz equation (σ_H, §4.3). The method applied for homogeneity testing is considered suitable if $s_w < 0.5*\sigma_H$ and a material is considered adequately homogeneous if $s_s < 0.3* \sigma_H$.

Ten containers of material A were analyzed in duplicate for EMA, ten containers of material B were analyzed in duplicate for EMA and ten containers of materials C were analyzed in duplicate for CYP to determine the homogeneity of the materials. The homogeneity of material D was not tested, because the material had to be analysed in its entirety by the participants. The homogeneity of other compounds in materials A, B and C were not tested, because the homogeneity test of EMA and CYP was considered sufficient to prove the homogeneity of the material. The results of the homogeneity study and their statistical evaluation are presented in Annex II-IV. All materials demonstrated to be sufficiently homogeneous for use in the proficiency test.

2.5 Sample distribution and instructions

Each of the participating laboratories received a randomly assigned laboratory code (1 through 24). The sample sets with the corresponding number, consisting of three coded samples (Annex I) were sent to the participating laboratories on June 11th, 2012. The sample sets were packed in an insulating box containing dry ice or cool packs and were dispatched to the participants immediately by courier. Finally all but one laboratory confirmed the receipt of the samples in good condition. This laboratory did not get the samples through customs.

The samples were accompanied by a letter (Annex V) describing the requested analyses, an acknowledgement of receipt form and a results form.

The laboratories were asked to store the samples until analysis according to their own laboratory's procedure. A single analysis of each sample was requested. The deadline for sending in the results was July 5th 2012.

2.6 Stability

On June 11th, the day the materials were distributed to the participants, 6 randomly selected samples of each material were stored at <-70 °C. It is assumed that the compounds included in this proficiency test are stable at these storage conditions. The remaining samples were stored at -20 °C.

In the morning of July 10th 2012 a set of six randomly selected samples of each material was selected from the samples stored at -20 °C. These samples were stored at room temperature for one day to verify if a possible delay in the transport does not affect the stability of the samples.

On August 8th 2012, 58 days after distribution of the samples, six samples that had been stored at -20°C, six samples that were stored at room temperature and six samples that had been stored at <-70°C were analysed for CYP and DEL. This was performed for material B and C. On September 17th, 98 days after distribution of the samples, a similar procedure was applied for EMA and IVM for materials A and B. For each set of samples, the average of the results and the standard deviation was calculated.

First it was determined if a 'consequential instability' occurred [5,6]. A consequential instability occurs when the average value of the samples stored at -20°C or the samples stored at room temperature is more than 0.3σH below the average value of the samples stored at <-70 °C. If so, the instability has a significant influence on the calculated z-scores. Second, it was determined if a statistically significant instability occurred using a Students t-test [6]. The results and statistical evaluation of the stability test are presented in Annex VI.

For EMA in material A a consequential difference was observed between the samples stored at <-70°C, at -20°C and at room temperature for one day. The average result was lower than the average of the samples that were stored at <-70°C. The concentration of EMA showed a decrease of 15.0% (from 172.4 µg/kg to 146.6 µg/kg) for the samples stored at room temperature for one day and of 7.0% (from 172.4 µg/kg to 160.3 µg/kg) for the samples stored at -20°C. Therefore, for EMA in material A the instability is incorporated in the calculation of the z'_{ai}-scores (§4.4).

For IVM in material A a consequential difference was observed between the samples stored at <-70°C and at room temperature for one day. The average result of the samples stored at room temperature was lower than the average of the samples that were stored at <-70°C. The concentration of IVM showed a decrease of 7.1% (from 131.5 µg/kg to 122.1 µg/kg). Therefore, for IVM in material A the instability is incorporated in the calculation of the z'_{ai}-scores (§4.4).

For EMA in material B no stability data are available due to derivatization problems. However, since the instability of EMA in A showed a decrease for both conditions, it can be assumed that EMA in B also shows instability. Therefore, for EMA in material B the instability is incorporated in the calculation of the z'_{ai}-scores (§4.4).

For CYP in material B a consequential and a statistical significant difference were observed between the samples stored at <-70°C, at -20°C and at room temperature for one day. The average result was lower than the average of the samples that were stored at <-70°C. The concentration of CYP in material B showed a decrease of 12.8% (from 44.6 µg/kg to 38.9 µg/kg) for the samples subjected to storage at room temperature for one day. The concentration of CYP showed a decrease of 9.5% (from 44.6 µg/kg to 40.4 µg/kg) for the samples stored at -20°C. Therefore, for CYP in material B the instability is incorporated in the calculation of the z_{ai}-scores (§4.4).

For DEL in material B a consequential and a statistical significant difference were observed between the samples stored at <-70°C and the samples stored at room temperature for one day. The average result was lower than the average of the samples that were stored at <-70°C. The concentration of DEL in material B showed a decrease of 7.4% (from 38.2 µg/kg to 35.4 µg/kg).

Therefore, for DEL in material B the instability is incorporated in the calculation of the z'_{ai}-scores (§4.4).

For CYP in material C a consequential and a statistical significant difference were observed between the samples stored at <-70°C and the samples stored at room temperature for one day. The average result was lower than the average of the samples that were stored at <-70°C. The concentration of CYP in material C showed a decrease of 6.7% (from 36.0 µg/kg to 33.6 µg/kg) for the samples stored at -20°C. Therefore, for CYP in material C the instability is incorporated in the calculation of the z'_{ai}-scores (§4.4).

For DEL in material C no consequential significant difference was observed between the samples stored at <-70°C, at -20°C and at room temperature for one day. However, a statistical significant difference was observed between the samples stored at <-70°C and the samples stored at -20°C.

The stability tests were performed 58 (pyrethroids) and 98 days (avermectins) after the shipment of the samples. The time between the shipment of the samples and the deadline was 24 days. The decrease in instability was likely to be smaller after 24 days than after 58 or 98 days, but the instabilities do not change the overall results of this proficiency test much.

3 Applied method of analysis

Twenty-three laboratories carried out one or more quantitative analyses. An overview of the quantitative methods applied and the compounds included in the methods is presented in Annexes VII and VIII.

3.1 Avermectins

Nineteen laboratories applied a method for the quantitative analysis of avermectins in materials A and B (Annex VII). The compounds were extracted with ACN, ethyl acetate, (acidified) MeOH or ACN with McIlvaine buffer. For sample purification eleven labs applied SPE of which two used an ASPEC™ system and one dispersive SPE. One lab applied a QuEChERS based method with ethyl acetate. Remaining labs applied ultrasonification, dilution or LLE to purify the samples. The applied detection techniques for the quantitative analysis of avermectins in salmon muscle were LC-MS/MS (six labs), LC-FLD (eleven labs) and two labs applied both (UP)LC-MS/MS and LC-FLD. Three labs used an internal standard:

- Selamectin (twice)
- Isoproturon-d_6

Sixteen labs did not use an internal standard.

3.2 Pyrethroids

Sixteen labs applied a quantitative method for pyrethroids in materials B, C and D (Annex VIII). The compounds were extracted with ethyl acetate, ACN, hexane/aceton, hexane or ACN/water/ formic acid. Sample purification showed a variety of procedures, ranging from centrifugation, dilution, (HP)GPC, liquid-liquid-extraction to the use of QuEChERS, magnesia-loaded silica gel, SPE florisil or dispersive SPE. The applied detection techniques for the quantitative analysis of parasiticides in salmon muscle were GC-ECD (six labs), LC-MS/MS (two labs), GC-MS(/MS) (five labs) and two labs applied both LC-MS/MS and GC-MS/MS. The internal standards used were:

- Cypermethrin-d_6
- Octachlorostyrene
- Tetrachloronaphthalin
- HCH gamma-d_6
- Trans permethrin-d_6

Nine labs did not use an internal standard.

4 Statistical evaluation

The statistical evaluation of the quantitative part of the study was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [5], elaborated by ISO, IUPAC and AOAC and ISO 13528 [6] in combination with the insights published by the Analytical Methods Committee [6,8,9] regarding robust statistics.

For the evaluation of the quantitative results the assigned value, the uncertainty of the assigned value, a target standard deviation and z-scores were calculated.

4.1 Calculation of the assigned value (X)

The assigned value (X) was determined using robust statistics [6,8,9]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data does not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the assigned value [6,8,9]. The assigned value is therefore a consensus value.

4.2 Calculation of the uncertainty of the assigned value (u)

The uncertainty of the assigned value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. A high uncertainty of the assigned value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the assigned value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory. In other words, illegitimate conclusions could be drawn regarding the performance of the participating laboratories from the calculated z_a-scores if the uncertainty of the assigned value is not taken into account.

The uncertainty of the assigned value (the robust mean) is calculated from the estimation of the standard deviation of the assigned value and the number of values used for the calculation of the assigned value [7]:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:

u = uncertainty of the assigned value;

n = number of values used to calculate the assigned value;

$\hat{\sigma}$ = the estimate of the standard deviation of the assigned value resulting from robust statistics.

According to ISO 13528 [6] the uncertainty of the assigned value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_H$

where:

u = the uncertainty of the assigned value;

σ_H = target standard deviation (§4.2.3).

In case the uncertainty of the assigned value does not comply with this criterion, the uncertainty of the assigned value should be taken into account when evaluating the performance of the participants regarding the accuracy (§4.4).

4.3 Calculation of the target standard deviation (σ_H)

According to Commission Decision 2002/657/EC [4], the coefficient of variation for the repeated analysis of a reference or fortified material under reproducibility conditions, shall not exceed the level calculated by the Horwitz equation. The Horwitz equation, $\sigma_H = 0.02c^{0.8495}$, presents a useful and widespread applied relation between the expected relative standard deviation of a singular analysis result under reproducibility conditions, and the concentration, c (g/g). It expresses inter-laboratory precision expected in inter-laboratory trials. Therefore, this relation is suitable for calculating the target standard deviation in proficiency tests.

Thompson [5] demonstrated that the Horwitz equation is not applicable to the lower concentration range (<120 µg/kg) as well as to the higher concentration range (>138 g/kg). Therefore a complementary model is suggested:

For analyte concentrations <120 µg/kg:

$\sigma_H = 0.22c$

For analyte concentrations >138 g/kg:

$\sigma_H = 0.01c^{0.5}$

where:

σ_H = expected standard deviation in inter-laboratory trials;
c = concentration of the analyte (g/g).

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [6] is applied. According to these guidelines z_a-scores are classified as presented in Table 3.

Table 3. Classification of z_a-scores.

$\|z_a\| \leq 2$	Satisfactory
$2 < \|z_a\| < 3$	Questionable
$\|z_a\| \geq 3$	Unsatisfactory

If the calculated uncertainty of the assigned value complies with the criterion mentioned in §4.2.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from:

$$z_a = \frac{\bar{x} - X}{\sigma_H}$$ Equation I

where:

z_a = accuracy z-score;

$\bar{x}$ = the average result of the laboratory;

X = assigned value;

σ_H = target standard deviation.

However, if the uncertainty of the assigned value does not comply with the criterion mentioned in § 4.2.2, it could influence the evaluation of the laboratories. Although, according to ISO 13528 in this case no z-scores can be calculated if a consensus value is used as the assigned value, we feel that evaluation of the participating laboratories is of main importance justifying the participating laboratories' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [6]:

$$z'_a = \frac{\bar{x} - X}{\sqrt{\sigma_H^2 + u^2}}$$ Equation II

where:

z'_a = accuracy z-score taking into account the uncertainty of the assigned value;

$\bar{x}$ = the average result of the laboratory;

X = assigned value;

σ_H = target standard deviation;

u = uncertainty of the assigned value.

If a consequential instability of the proficiency test materials is observed, this can influence the evaluation of the laboratory performance. Therefore, in that case the consequential instability is taken into account when calculating z-scores. Because instability only regards one side of the confidence interval (a decrease of the concentration) this correction only applies to the lower 2s limit and results in an asymmetrical confidence interval.

In the case of a consequential instability the accuracy z-score for the laboratories that reported an amount below the assigned value is corrected for this instability by:

$$z_{ai} = \frac{\overline{x} - X}{\sqrt{\sigma_H^2 + \Delta^2}}$$

Equation III

where:

z_{ai} = accuracy z-score taking into account the instability of the assigned value;

$\overline{x}$ = the average result of the laboratory;

X = assigned value;

σ_H = target standard deviation;

Δ = difference between average concentration of compound stored at -70°C and average concentration after thaw-freeze cycle.

In some cases the uncertainty of the assigned value does not comply with the criterion in §4.2.2 and a consequential instability is observed. In this case the z'_a score for the laboratories that reported an amount below the assigned value is corrected for this instability by:

$$z'_{ai} = \frac{\overline{x} - X}{\sqrt{\sigma_H^2 + \Delta^2 + u^2}}$$

Equation IV

where:

z'_{ai} = accuracy z-score taking into account the uncertainty and instability of the assigned value;

$\overline{x}$ = the average result of the laboratory;

X = assigned value;

σ_H = target standard deviation;

Δ = difference between average concentration of compound stored at -70°C and average concentration after thaw-freeze cycle;

u = uncertainty of the assigned value.

5 Results and discussion

Twenty-four laboratories subscribed for the participation and 23 reported results for the proficiency test for parasiticides in salmon muscle. Ten labs included all compounds in their analysis. The performance of individual labs is summarized in Annex IX.

An overview of the compounds found in the samples is presented in Annex IX. Annex X gives an overview of false positive and false negative results. Sixteen false positive and 16 false negative results were reported. Lab 5 reported three false positive results (eprinomectin in materials A, B and C), lab 8 reported three false positive results (diflubenzuron in A, B and C), lab 9 reported two false positive results (EMA in C and DEL in D), lab 14 reported two false positive results (bifenthrin in A and DEL in D), lab 16 reported five false positive results (moxidectin in A, B and C, EMA and abamectin in C) and lab 23 reported one false positive results (DEL in D).

5.1 EMA in material A

Seventeen laboratories carried out a quantitative analysis for EMA in material A (Annex XI). The lowest value reported is 2.0 µg/kg and the highest value is 276 µg/kg. The assigned value of EMA in material A is 133 µg/kg with a robust standard deviation of 50.6 µg/kg. This is nearly two times higher than the value suggested by Thompson: 28.8 µg/kg. The uncertainty of the assigned value is 15.4 µg/kg which does exceed $0.3\sigma_H$ (§4.2). A consequential and statistic instability during storage of 98 days was observed, so z'_{ai}-scores were calculated. A decrease of 172 µg/kg to 146 µg/kg (15.0%) was observed. At the assigned level of 133 µg/kg this means a decrease of 19.8 µg/kg. With correction for the consequential instability, the accuracy of three results (labs 3, 5 and 14) was unsatisfactory. When no consequential instability was observed and equation II (§4.4) was used for calculating the z'_a-scores, the three results would still be unsatisfactory and one extra result of lab 6 would be questionable.

5.2 IVM in material A

Sixteen laboratories carried out a quantitative analysis for IVM in material A (Annex XII). Labs 10 and 19 reported a false negative result. The lowest value reported is 35.2 µg/kg and the highest value is 98 µg/kg. The assigned value of IVM in material A is 65.1 µg/kg with a robust standard deviation of 19.7 µg/kg. This is higher than the value suggested by Thompson: 14.3 µg/kg. The uncertainty of the assigned value is 6.59 µg/kg which does exceed $0.3\sigma_H$ (§4.2). A consequential instability during storage of 98 days was observed, so z'_{ai}-scores were calculated. A decrease of 131.5 µg/kg to 122.1 µg/kg (7.1%) was observed. At the assigned level of 65.1 µg/kg this means a decrease of 4.91 µg/kg. With correction for the consequential instability, the accuracy of one result (lab 3) was questionable. When no consequential instability was observed and equation II (§4.4) was used for calculating the z'_a-scores, the result would still be questionable.

5.3 EMA in material B

Seventeen laboratories carried out a quantitative analysis for EMA in material B (Annex XII). Lab 14 reported a false negative result. The lowest value reported is 29.56 µg/kg and the highest value is 176 µg/kg. The assigned value of EMA in material B is 82.2 µg/kg with a robust standard

deviation of 17.8 µg/kg. This is comparable to the value suggested by Thompson: 18.1 µg/kg. The uncertainty of the assigned value is 5.57 µg/kg which does exceed $0.3\sigma_H$ (§4.2). In material A a consequential and statistic instability for EMA during storage of 98 days was observed, so also for material B z'_{ai}-scores were calculated. At the assigned level of 82.2 µg/kg a decrease of 15.0% (§5.1) means a decrease of 12.2 µg/kg. With correction for the consequential instability, the accuracy of two results (labs 2 and 6) was questionable and one result was unsatisfactory (lab 3). When no consequential instability was observed and equation II (§4.4) was used for calculating the z_a-scores, the results would still be questionable and unsatisfactory.

5.4 CYP in material B

Sixteen laboratories carried out a quantitative analysis for CYP in material B (Annex XIII). Labs 17, 19 and 23 reported a false negative result. Lab 19 intentionally did not report the presence of CYP due to an unexpected profile (only one peak present instead of the expected four isomer peaks). The lowest value reported is 8.16 µg/kg and the highest value is 41 µg/kg. The assigned value of CYP in material B is 33.6 µg/kg with a robust standard deviation of 4.12 µg/kg. This is much lower than the value suggested by Thompson: 7.40 µg/kg. The uncertainty of the assigned value is 1.43 µg/kg which does not exceed $0.3\sigma_H$ (§4.2). A consequential and statistic instability during storage of 58 days was observed, so z_{ai}-scores were calculated. A decrease of 44.6 µg/kg to 38.9 µg/kg (12.8%) was observed. With correction for the consequential instability, the accuracy of one result (lab 6) was questionable. When no consequential instability was observed and equation I (§4.4) was used for calculating the z_a-scores, the result would be unsatisfactory.

5.5 DEL in material B

Sixteen laboratories carried out a quantitative analysis for DEL in material B (Annex XIV). Labs 17 and 23 reported a false negative result. The lowest value reported is 16.53 µg/kg and the highest value is 56 µg/kg. The assigned value of DEL in material B is 34.0 µg/kg with a robust standard deviation of 12.5 µg/kg. This is more than 1.5 times higher than the value suggested by Thompson: 7.47 µg/kg. The uncertainty of the assigned value is 4.16 µg/kg which does exceed $0.3\sigma_H$ (§4.2). A consequential and statistic instability during storage of 58 days was observed, so z'_{ai}-scores were calculated. A decrease of 38.2 µg/kg to 35.4 µg/kg (7.4%) was observed. With correction for the consequential instability, the accuracy of two results (labs 4 and 14) was questionable. When no consequential instability was observed and equation II (§4.4) was used for calculating the z'_a-scores, two extra results would be questionable (labs 6 and 20).

5.6 CYP in material C

Sixteen laboratories carried out a quantitative analysis for CYP in material C (Annex XV). Labs 17, 19 and 23 reported a false negative result. Lab 19 intentionally did not report the presence of CYP due to an unexpected profile. The lowest value reported is 8.20 µg/kg and the highest value is 34 µg/kg. The assigned value of CYP in material C is 25.5 µg/kg with a robust standard deviation of 6.23 µg/kg. This is comparable to the value suggested by Thompson: 5.60 µg/kg. The uncertainty of the assigned value is 2.16 µg/kg which does exceed $0.3\sigma_H$ (§4.2). A consequential and statistic instability during storage of 58 days was observed, so z'_{ai}-scores were calculated. A decrease of 36.0 µg/kg to 33.6 µg/kg (6.7%) was observed. With correction for the consequential instability, the accuracy of one result (lab 6) was questionable. When no consequential instability was

observed and equation II (§4.4) was used for calculating the z'_a-scores, the result would still be questionable.

5.7 DEL in material C

Sixteen laboratories carried out a quantitative analysis for DEL in material C (Annex XVI). Labs 10, 17 and 23 reported a false negative result. Labs 7 and 22 did not report DEL, because the concentration was lower than their LOQ. The lowest value reported is 6 µg/kg and the highest value is 14.87 µg/kg. The assigned value of DEL in material C is 8.77 µg/kg with a robust standard deviation of 2.26 µg/kg. This is comparable to the value suggested by Thompson: 1.93 µg/kg. The uncertainty of the assigned value is 0.85 µg/kg which does exceed $0.3\sigma_H$ (§4.2). No consequential and statistic instability during storage of 58 days was observed. With regard to the accuracy, two results (labs 4 and 6) were questionable.

5.8 CYP in material D

Fifteen laboratories carried out a quantitative analysis for CYP in material D (Annex XVII). Labs 17 and 19 reported a false negative result. Lab 19 intentionally did not report the presence of CYP due to an unexpected profile. The lowest value reported is 7.49 µg/kg and the highest value is 100 µg/kg. The assigned value of CYP in material D is 45.1 µg/kg with a robust standard deviation of 17.8 µg/kg. This is almost two times higher than the value suggested by Thompson: 9.93 µg/kg. The uncertainty of the assigned value is 6.17 µg/kg which does exceed $0.3\sigma_H$ (§4.2). No stability test was performed for this material. With respect to the accuracy two results (labs 4 and 6) were unsatisfactory.

6 Conclusions

Twenty-three laboratories reported results for the proficiency study of parasiticides in salmon muscle. Lab 15 showed optimal performance by detecting all compounds, the absence of false positives and false negatives and a correct quantification. Within the participant's scope (not all participants included all compounds), nine other labs showed optimal performance: labs 1, 7, 11, 12, 18, 20, 21, 22 and 24. An overview of each participant's performance is shown in Annex XIX.

In the avermectins analysis three false negative results and nine false positive result were reported. Eight out of nine false positive results were reported by labs that applied LC-FLD, one by a lab that combined LC-FLD and LC-MS/MS. The false negative results were caused by the failure to detect IVM (twice) and EMA (once). False negative, questionable or unsatisfactory results were not reported by mainly one application. Nine out of 19 labs that analysed avermectins reported no false negative or false positive results and satisfactory z-scores (labs 7, 11, 12, 15, 17, 18, 22, 23 and 24). One lab used an internal standard and six labs applied LC-FLD, two applied LC-MS/MS and one applied a combination of LC-FLD and LC-MS/MS.

In the pyrethroids analysis 13 false negative results and seven false positive results were reported. The false positive results were all caused due to reporting DEL in material D. The false negative results were caused by the failure to detect CYP (eight times) and DEL (five times). The unexpected profile (two isomer peaks instead of four) of the CYP in this test, resulted in three false negative results by lab 19. False negative, questionable or unsatisfactory results were not reported by mainly one analysis method. Eight out of 16 labs that analysed pyrethroids reported no false negative or false positive results and satisfactory z-scores (labs 1, 5, 7, 12. 15. 20. 21 and 22). Three of these labs used an internal standard, five applied GC-ECD, two applied GC-MS/MS and one applied a combination of GC-MS/MS and LC-MS/MS.

Table 4 presents the overall performance of this proficiency test.

Table 4. Overview of the results of the proficiency test.

Material	Compound	Satisfactory results (%)	Assigned value (µg/kg)
A	EMA	82	133
A	IVM	80	65.1
B	EMA	76	82.2
B	CYP	75	33.6
B	DEL	75	34.0
C	CYP	75	25.5
C	DEL	69	8.77
D	CYP	73	45.1

References

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2 ISO/IEC 17025:2005(E). 2005. General Requirements for the Competence of Calibration and Testing Laboratories.

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5 Thompson M, Ellison SL,Wood R. 2006. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem. 78(1):145-196.

6 ISO 13528:2005(E). 2005. Statistical methods for use in proficiency testing by inter-laboratory comparison, 1st edition.

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8 Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 1. Basic concepts. Analyst 114:1693-1697.

9 Analytical Methods Committee. 1989. Robust statistics - How not to reject outliers Part 2. Inter-laboratory trials. Analyst. 114:1699-1702.

10 Council Regulation (ECC) No 2377/90. 26 June 1990. Laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Off. J. Eur. Commun. L224: 1.

Annex I
Codification of the samples

Lab number	Material A*	Material B*	Material C*	Material D
1	013	091	029	MATERIAL D
2	063	045	092	MATERIAL D
3	060	003	076	MATERIAL D
4	068	062	069	MATERIAL D
5	088	093	095	MATERIAL D
6	032	044	038	MATERIAL D
7	050	022	048	MATERIAL D
8	053	065	055	MATERIAL D
9	094	099	079	MATERIAL D
10	037	084	057	MATERIAL D
11	077	064	061	MATERIAL D
12	075	024	080	MATERIAL D
13	049	100	052	MATERIAL D
14	074	047	005	MATERIAL D
15	012	071	028	MATERIAL D
16	007	046	027	MATERIAL D
17	011	067	073	MATERIAL D
18	010	096	031	MATERIAL D
19	051	019	035	MATERIAL D
20	043	026	009	MATERIAL D
21	097	087	033	MATERIAL D
22	030	020	090	MATERIAL D
23	002	085	066	MATERIAL D
24	016	001	004	MATERIAL D

* *All sample codes start with PAR/2012/SALMON.*

Annex II
Statistical evaluation of homogeneity data of material A for emamectin

Emamectin (µg/kg)		
Sample number	Replicate 1	Replicate 2
Hom/A001	187.42	228.56
Hom/A002	247.34	240.81
Hom/A003	241.38	194.88
Hom/A004	264.71	238.71
Hom/A005	236.29	235.77
Hom/A006	252.33	223.60
Hom/A007	238.41	254.84
Hom/A008	272.07	229.51
Hom/A009	233.88	258.75
Hom/A010	241.96	215.48
Grand mean	236.83	
Cochran's test		
C	0.246	
Ccrit	0.602	
C<Ccrit?	NO OUTLIERS	
Target s = σ_H	Horwitz: 47.067	
S_x	14.55	
S_w	20.98	
S_s	0.00	
Critial = $0.3\sigma_H$	14.12	
S_s<critical?	ACCEPTED	
S_w<$0.5\sigma_H$?	ACCEPTED	

S_x = standard deviation of the sample averages.
S_w = within-sample standard deviation.
S_s = between-sample standard deviation.

Annex III
Statistical evaluation of homogeneity data of material B for emamectin

	Emamectin (µg/kg)	
Sample number	Replicate 1	Replicate 2
Hom/B001	86.91	95.53
Hom/B002	119.97	103.06
Hom/B003	111.07	125.40
Hom/B004	103.92	112.46
Hom/B005	102.85	117.77
Hom/B006	115.11	109.47
Hom/B007	118.63	98.68
Hom/B008	98.07	109.08
Hom/B009	101.49	96.92
Hom/B010	98.45	84.85
Grand mean	105.48	
Cochran's test		
C	0.246	
Ccrit	0.602	
C<Ccrit?	NO OUTLIERS	
Target s = σ_H	Horwitz: 23.21	
S_x	8.97	
S_w	8.99	
S_s	6.32	
Critial = $0.3\sigma_H$	6.96	
S_s<critical?	ACCEPTED	
S_w<$0.5\sigma_H$?	ACCEPTED	

S_x = standard deviation of the sample averages.
S_w = within-sample standard deviation.
S_s = between-sample standard deviation.

Annex IV
Statistical evaluation of homogeneity data of material C for cypermethrin

	Cypermethrin (µg/kg)	
Sample number	Replicate 1	Replicate 2
Hom/C001	31.77	30.98
Hom/C002	28.24	31.90
Hom/C003	29.21	30.29
Hom/C004	27.65	29.51
Hom/C005	28.19	31.26
Hom/C006	30.36	33.64
Hom/C007	29.41	30.39
Hom/C008	29.28	31.21
Hom/C009	28.68	33.16
Hom/C010	29.77	32.75
Grand mean	30.38	
Cochran's test		
C	0.277	
Ccrit	0.602	
C<Ccrit?	NO OUTLIERS	
Target s = σ_H	Horwitz: 6.68	
S_x	1.00	
S_w	1.90	
S_s	0.00	
Critial = $0.3\sigma_H$	2.01	
S_s<critical?	ACCEPTED	
S_w<$0.5\sigma_H$?	ACCEPTED	

S_x = standard deviation of the sample averages.
S_w = within-sample standard deviation.
S_s = between-sample standard deviation.

Annex V
Instruction letter

RIKILT WAGENINGEN UR

For quality of life

P.O. Box 230 | 6700 AE Wageningen | The Netherlands

DATE
May 31, 2012

SUBJECT
Proficiency study parasiticides
in salmon muscle
Pr. Nr 122.72.734.01

OUR REFERENCE
12/RIK0369
Esc

POSTAL ADDRESS
P.O. Box 230
6700 AE Wageningen
The Netherlands

VISITORS ADDRESS
Wageningen Campus
Building 123
Akkermaalsbos 2
6708 WB Wageningen

INTERNET
www.rikilt.wur.nl

C of C NUMBER
09098104

HANDLED BY
J.J.W. Elbers

TELEPHONE
0317-480314

FAX
0317-417717

EMAIL
ingrid.elbers@wur.nl

Dear participant,

Thank you very much for your interest in the proficiency study for the analysis of parasiticides in salmon muscle.
Hereby I send you a parcel containing three randomly coded samples. Each sample consists of at least 50 g salmon muscle. The samples may contain one or more analytes (in alphabetical order):

Abamectin	Deltamethrin	Fenvalerate
Azamethiphos	Diflubenzuron	Ivermectin
Bifenthrin	Dichlorvos	Moxidectin
Cyfluthrin	Doramectin	Resmethrin
Cyhalothrin	Emamectin	Teflubenzuron
Cypermethrin	Eprinomectin	Trichlorfon

Please fill out the accompanied 'acknowledgement of receipt form' and return it immediately upon receipt of the samples, preferably by e-mail.

Your laboratory code is:

Instructions:

- After arrival store the samples according to your laboratory's procedures.
- Defrost the samples before analysis and homogenize them according to your laboratory's procedures.
- Please analyze the samples according to your routine methods and make use of your own reference standards.
- Carry out a **single analysis** for each sample. Please report the results before **July 5th 2012** using the results form.

Please contact me if you have any questions or need any assistance.

Kind regards,

Ingrid Elbers

Wageningen UR (Wageningen University, Van Hall Larenstein University of Applied Sciences and various research institutes) is specialised in the domain of healthy food and living environment.

RIKILT, part of Wageningen UR, carries out research into the safety, quality and health of food and feed and provides consultancy services to (inter)national government authorities. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described at www.rva.nl (no. L014 and R013)).

Annex VI
Statistical evaluation of stability data

Statistical evaluation for EMA in material A			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0	98	
Calculated amounts (µg/kg)	151.98	170,64	159.17
	161.77	134,61	137.75
	170.49	182,92	160.78
	188.62	*	138.32
	159.89	167,54	*
	201.74	145,81	137.11
Average amount (µg/kg)	172.42	160.3	146.63
n	6	5	5
st. dev (µg/kg)	19.048	19.627	12.207
Difference		12.11	25.79
$0.3\sigma_H$	10.783		
Consequential difference? Diff < 0.3 σ_H		YES	YES
t		1.04	2.60
t_{crit}		2.26	2.26
Statistical diference? T < t_{crit}		NO	YES

Statistical evaluation for IVM in material A			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0		
Calculated amounts (µg/kg)	124.24	134.84	134.16
	129.63	115.04	116.12
	136.18	146.02	127.35
	143.32		119.18
	126.40	139.72	
	129.19	135.15	113.71
Average amount (µg/kg)	131.5	134.2	122.1
n	6	5	5
st. dev (µg/kg)	7.059	11.604	8.481
Difference		-2.66	9.39
$0.3\sigma_H$	8.556		
Consequential difference? Diff < 0.3 σ_H		NO	YES
t		0.47	2.01
t_{crit}		2.26	2.26
Statistical diference? T < t_{crit}		NO	NO

Continued Statistical evaluation of stability data.

Statistical evaluation for CYP in material B			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0	58	58
Calculated amounts (µg/kg)	44.36	39.44	39.86
	42.71	42.92	39.81
	42.24	40.41	38.35
	46.58	39.07	40.35
	45.85	40.76	38.30
	45.87	39.72	36.80
Average amount (µg/kg)	44.6	40.4	38.9
n	6	6	6
st. dev (µg/kg)	1.805	1.388	1.336
Difference		4.22	5.69
0.3σH	2.944		
Consequential difference? Diff < 0.3 σH		YES	YES
t		4.54	6.21
tcrit		2.23	2.23
Statistical diference? T < tcrit		YES	YES

Statistical evaluation for DEL in material B			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0	58	58
Calculated amounts (µg/kg)	37.79	36.01	35.21
	38.14	37.09	35.80
	37.12	36.76	35.07
	38.27	35.76	36.34
	38.82	35.71	35.19
	39.35	35.68	34.93
Average amount (µg/kg)	38.2	36.2	35.4
n	6	6	6
st. dev (µg/kg)	0.781	0.607	0.538
Difference		2.08	2.83
$0.3\sigma_H$	2.524		
Consequential difference? Diff < 0.3 σ_H		NO	YES
t		5.15	7.30
t_{crit}		2.23	2.23
Statistical diference? T < t_{crit}		YES	YES

Continued Statistical evaluation of stability data.

Statistical evaluation for CYP in material C			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0	58	58
Calculated amounts (µg/kg)	33.89	34.51	33.62
	39.76	32.90	32.05
	34.60	34.49	35.08
	36.52	32.34	34.90
	36.89	35.76	32.94
	34.34	33.59	32.93
Average amount (µg/kg)	36.0	33.9	33.6
N	6	6	6
st. dev (µg/kg)	2.207	1.240	1.196
Difference		2.07	2.41
$0.3\sigma_H$	2.376		
Consequential difference? Diff < $0.3\ \sigma_H$		NO	YES
t		2.00	2.36
t_{crit}		2.23	2.23
Statistical diference? $T < t_{crit}$		NO	YES

Statistical evaluation for DEL in material C			
Storage temp	-70°C	-20°C	1 day room temp
Time at -20°C (days)	0	58	58
Calculated amounts (µg/kg)	8.11	7.40	7.99
	8.84	7.64	7.70
	7.95	8.01	8.29
	8.45	7.99	8.31
	8.28	8.19	7.90
	7.95	7.69	7.82
Average amount (µg/kg)	8.27	7.82	8.00
N	6	6	6
st. dev (µg/kg)	0.344	0.292	0.252
Difference		0.44	0.26
$0.3\sigma_H$	0.545		
Consequential difference? Diff < $0.3\ \sigma_H$		NO	NO
t		2.41	1.51
t_{crit}		2.23	2.23
Statistical diference? $T < t_{crit}$		YES	NO

Annex VII
Overview of the applied quantitative methods for avermectins

Lab	Extraction	Sample purification	Internal standard	Detection method	Compounds included
2	ACN	shaking, ultrasonic bath, addition of water and heptane, removal of heptane, ASPEC, evaporation	-	LC-MS/MS	EMA IVM
3	ACN/McIlvaine buffer pH4	hexane and SPE Oasis HLB	selamectin	LC-MS/MS	EMA IVM
5				LC-FLD	EMA IVM
6	Ethyl acetate	QuEChERS, NaCl and MgSO4, shaking, centifugation, dispersive SPE PSA	-	LC-MS/MS	EMA
7	ACN	SPE Sep-Pack C18	-	LC-FLD	EMA IVM
8	ACN	evaporation, derivatization	-	LC-FLD	EMA
9	ACN	dilution, SPE C18, evaporation	-	LC-FLC UPLC-MS/MS	EMA IVM
10			isoproturon-d6	LC-MS/MS	EMA IVM
11	ACN	SPE C18, evaporation, filtration	-	LC-MS/MS	IVM
12			selamectin	LC-MS/MS	IVM
14			-	LC-FLD	EMA IVM
15	organic solvent	SPE C18, evaporation	-	LC-FLD LC-MS/MS	EMA IVM
16	ACN	Oasis HLB, evaporation, derivatization	-	LC-FLD	EMA IVM

Lab	Extraction	Sample purification	Internal standard	Detection method	Compounds included
17	ACN for IVM acid-MeOH for EMA	ultrasonification, centrifugation, derivatization ultrasonification, centrifigation, cleaning with ACN, derivatization	-	LC-FLD	EMA IVM
18	ACN	triethylamin, SPE C8, evaporation, derivatization	-	LC-FLD	EMA IVM
19	MeOH	LLE with water/hexane/dichloromethan, SPE aminopropyl, derivatization	-	LC-FLD	EMA IVM
22	ethyl acetate	LLE, derivatization	-	LC-FLD	EMA IVM
23			-	LC-FLD	EMA IVM
24	ACN	ultrasonic bath, SPE, ASPEC 4XL, C18 column, evaporation, derivatization	-	LC-FLD	EMA IVM

Annex VIII
Overview of the applied quantitative methods for pyrethroids

Lab	Extraction	Sample purification	Internal standard	Detection method	Compounds included
1	mixed with florisyl	packed in syringe with silica and Na2SO4, elution with ethyl acetate	-	GC-ECD	CYP DEL
4	ACN/water/formic acid	centrifugation	-	LC-MS/MS	CYP DEL
5			cypermethrin-d6	GC-MS/MS	CYP DEL
6	ethyl acetate	QuEChERS, NaCl and MgSO4, shaking, centifugation, SPE florisil	-	GC-MS/MS	CYP DEL
7	hexane		-	GC-ECD	CYP DEL
9	ACN	dilution, SPE C18, filtration	-	GC-MS	CYP DEL
10			octachlorostyrene	GC-MS	CYP DEL
12		fat extraction, GPC, silica gel	tetrachloronaphthalin	GC-ECD	CYP DEL
14			-	GC-MS/MS LC-MS/MS	CYP DEL
15	ethyl acetate	HPGPC	HCH gamma-d6	GC-MS/MS	CYP DEL
17	no information				CYP DEL
19	hexane/aceton	LLE with ACN, magnesia-loaded silica gel, MgO3Si, SPE florisil	fenvalerate	GC-ECD	CYP DEL

Lab	Extraction	Sample purification	Internal standard	Detection method	Compounds included
20	hexane/aceton	concentration, extraction with ACN, evaporation	-	GC-ECD	CYP DEL
21	ethyl acetate	alumina silica, evaporation	-	GC-ECD	CYP DEL
22		QuEChERS, dispersive SPE	-	GC-MS/MS LC-MS/MS	CYP DEL
23			trans permethrin-d6	LC-MS/MS	CYP DEL

Annex IX
Overview of quantitative results

Lab	Material A	Material B	Material C	Material D
1		CYP DEL	CYP DEL	CYP
2	EMA IVM (not quantified)	EMA		
3	EMA IVM	EMA		
4		CYP DEL	CYP DEL	CYP
5	EMA IVM eprinomectin (FP)	CYP DEL EMA eprinomectin (FP)	CYP DEL eprinomectin (FP)	
6	EMA	CYP DEL EMA	CYP DEL	CYP
7	EMA IVM	CYP DEL EMA	CYP*	CYP
8	EMA diflubenzuron (FP)	EMA diflubenzuron (FP)	diflubenzuron (FP)	
9	EMA IVM	CYP DEL EMA	CYP DEL EMA (FP)	CYP DEL (FP)
10	EMA FN for IVM	CYP DEL EMA	CYP FN for DEL	CYP
11	IVM			
12	IVM	**CYP** **DEL**	CYP DEL	CYP
14	EMA IVM bifenthrin (FP)	CYP DEL FN for EMA	CYP DEL	CYP DEL (FP)
15	EMA IVM	CYP DEL EMA	CYP DEL	CYP
16	EMA IVM moxidectin (FP)	EMA moxidectin (FP)	EMA (FP) moxidectin (FP) abamectin (FP)	
17	EMA IVM	EMA FN for CYP FN for DEL	FN for CYP FN for DEL	FN for CYP
18	EMA IVM	EMA		
19	EMA FN for IVM	DEL EMA FN for CYP	DEL FN for CYP	FN for CYP
20		CYP DEL	CYP DEL	CYP

Lab	Material A	Material B	Material C	Material D
21		CYP DEL	CYP DEL	CYP
22	EMA IVM	CYP DEL EMA	CYP*	CYP
23	EMA IVM	FN for CYP FN for DEL EMA	FN for CYP FN for DEL	CYP DEL (FP)
24	EMA IVM	EMA		

* *LOQ for DEL = 10 µg/kg.*

Annex X
False positive and false negative results

False positive results

Lab code	Sample code	Material	Compound confirmed
05	088	A	eprinomectin
05	093	B	eprinomectin
05	095	C	eprinomectin
08	053	A	diflubenzuron
08	065	B	diflubenzuron
08	055	C	diflubenzuron
09	079	C	EMA
09		D	DEL
14	074	A	bifenthrin
14	005	D	DEL
16	007	A	moxidectin
16	046	B	moxidectin
16	027	C	moxidectin
16	027	C	EMA
16	027	C	abamectin
23		D	DEL

False negative results

Lab code	Sample code	Material	Compound missed
10	037	A	IVM
10	057	C	DEL
14	047	B	EMA
17	067	B	CYP
17	067	B	DEL
17	073	C	CYP
17	073	C	DEL
17		D	CYP
19	051	A	IVM
19	019	B	CYP
19	035	C	CYP
19		D	CYP
23	085	B	CYP
23	085	B	DEL
23	066	C	CYP
23	066	C	DEL

Annex XI
Results for the analysis emamectin in material A

Emamectin Assigned value: 133 µg/kg Uncertainty of assigned value: 15.4 µg/kg Target standard deviation (Horwitz, Thompson): 28.8 µg/kg Robust standard deviation: 50.6 µg/kg		
Lab code	Result (µg/kg)	z'_{ai}-score
2	187	1.67
3	276	**4.40**
5	10.4	**-3.20**
6	67.05	-1.72
7	156.1	0.72
8	146.10	0.41
9	181.19	1.49
10	96	-0.96
14	2.0	**-3.42**
15	155	0.68
16	77.8	-1.44
17	154.2	0.66
18	130	-0.07
19	156.7	0.74
22	157.3	0.75
23	96.69	-0.94
24	144	0.35

Continued results for the analysis of emamectin in material A.

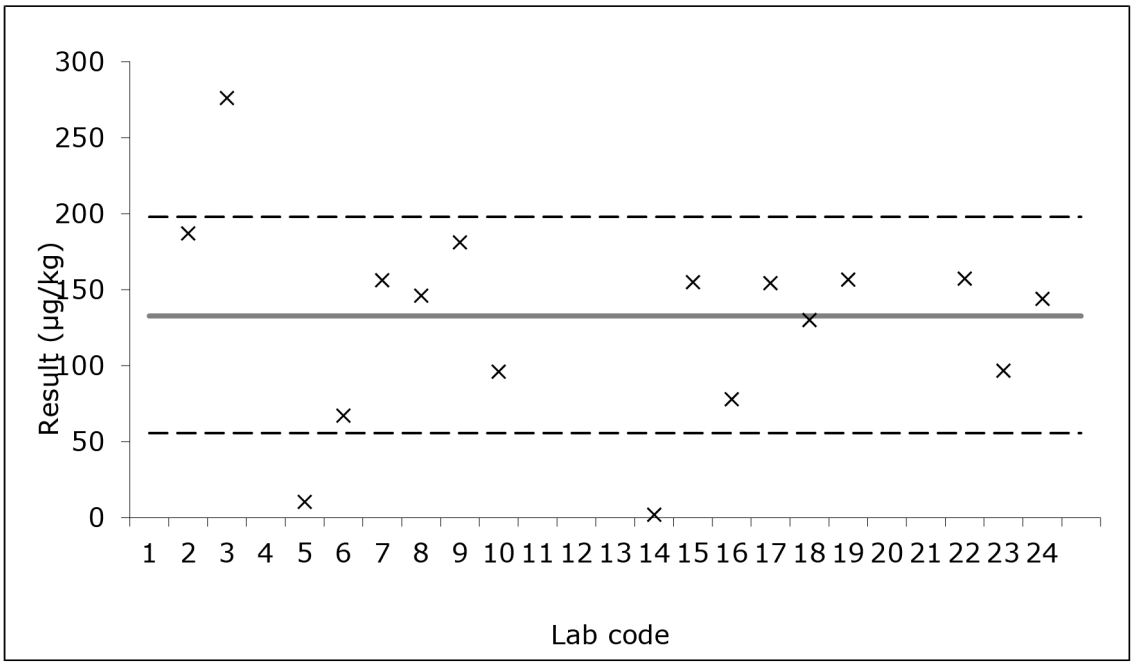

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation II in §4.4. The X - 2σ_H line (dotted) is calculated according to equation IV in §4.4.

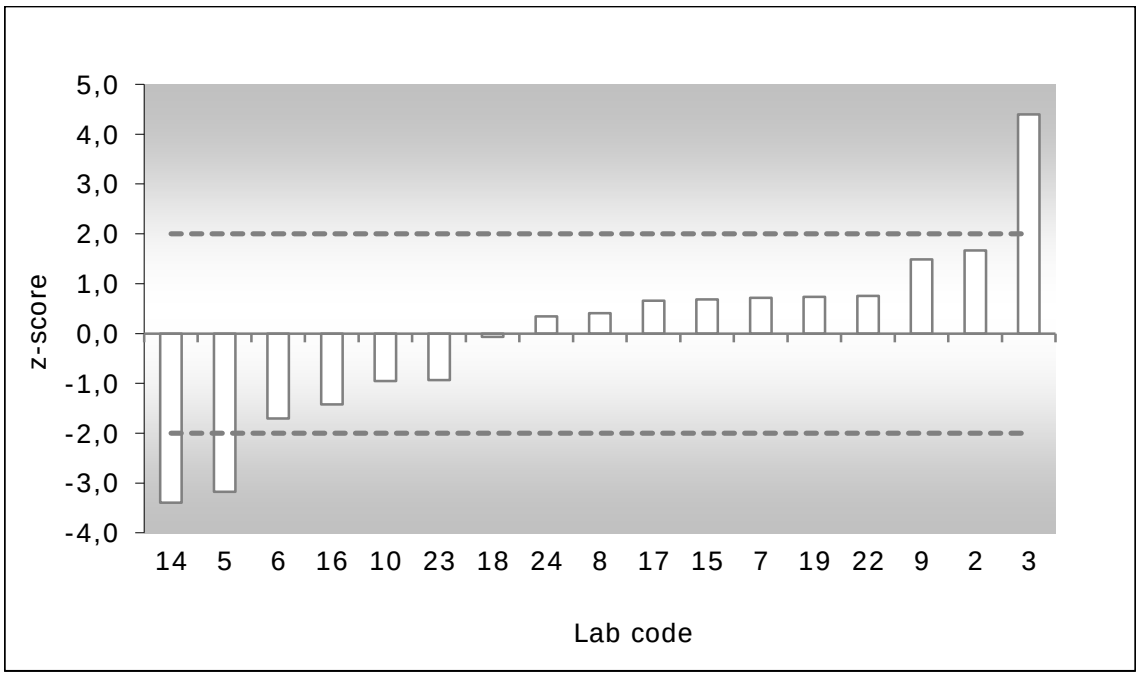

Figure b. Graphical representation of z'$_{ai}$-scores.

Annex XII
Results for the analysis of ivermectin in material A

Ivermectin Assigned value: 65.1 µg/kg Uncertainty of assigned value: 6.59 µg/kg Target standard deviation (Horwitz, Thompson): 14.3 µg/kg Robust standard deviation: 19.7 µg/kg		
Lab code	Result (µg/kg)	z'_{ai}-score
3	98	**2.09**
5	54.3	-0.66
7	81.1	1.02
9	85.76	1.31
11	61	-0.25
12	46.7	-1.12
14	45.0	-1.22
15	73	0.50
16	56.5	-0.52
17	53.4	-0.71
18	68	0.19
22	35.2	-1.82
23	81.13	1.02
24	75	0.63

Continued results for the analysis of ivermectin in material A.

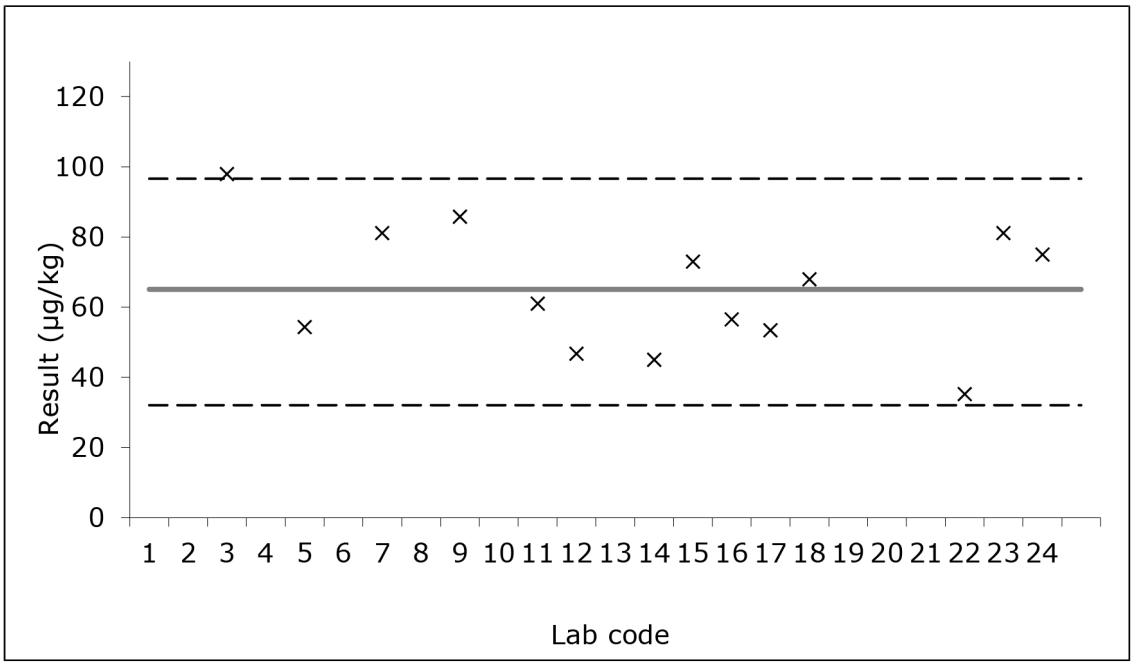

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation II in §4.4. The X - 2σ_H line (dotted) is calculated according to equation IV in §4.4.

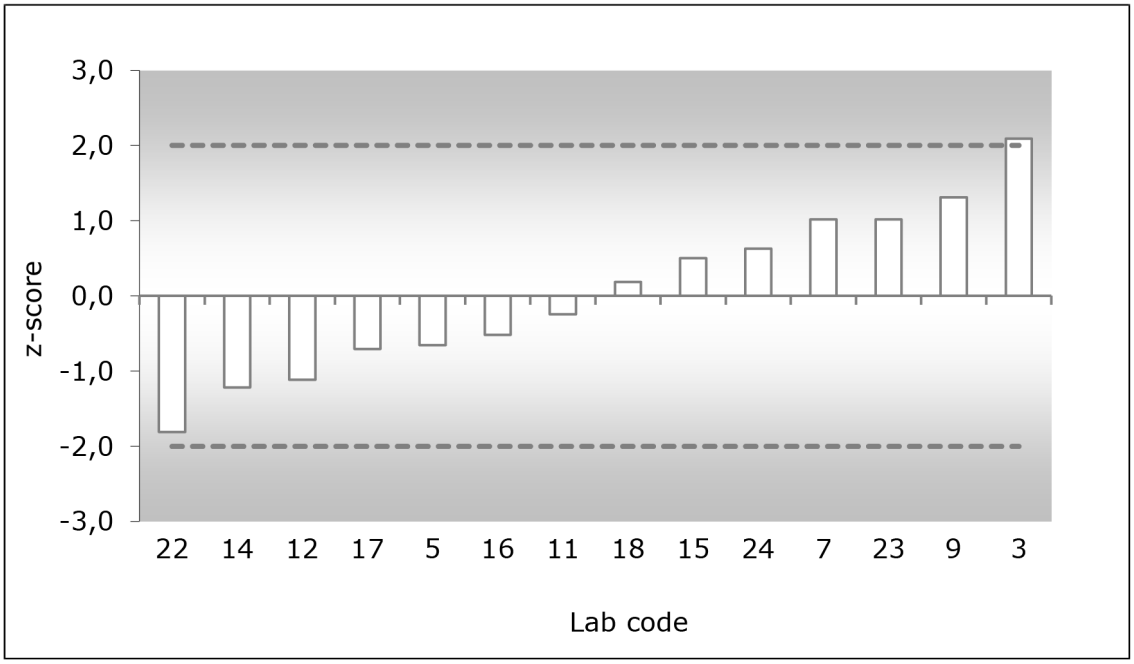

Figure b. Graphical representation of z'$_{ai}$-scores.

Annex XIII
Results for the analysis of emamectin in material B

Emamectin Assigned value: 82.2 µg/kg Uncertainty of assigned value: 5.57 µg/kg Target standard deviation (Horwitz, Thompson): 18.1 µg/kg Robust standard deviation: 17.8 µg/kg		
Lab code	Result (µg/kg)	z'_{ai}-score
2	123	**2.15**
3	176	**4.95**
5	49.7	-1.44
6	29.56	**-2.33**
7	82.8	0.03
8	85.0	0.15
9	85.76	0.19
10	61	-0.94
15	87	0.25
16	54.1	-1.25
17	93.5	0.60
18	75	-0.32
19	90.7	0.45
22	95.6	0.71
23	52.85	-1.30
24	89	0.36

Continued results for the analysis of emamectin in material B.

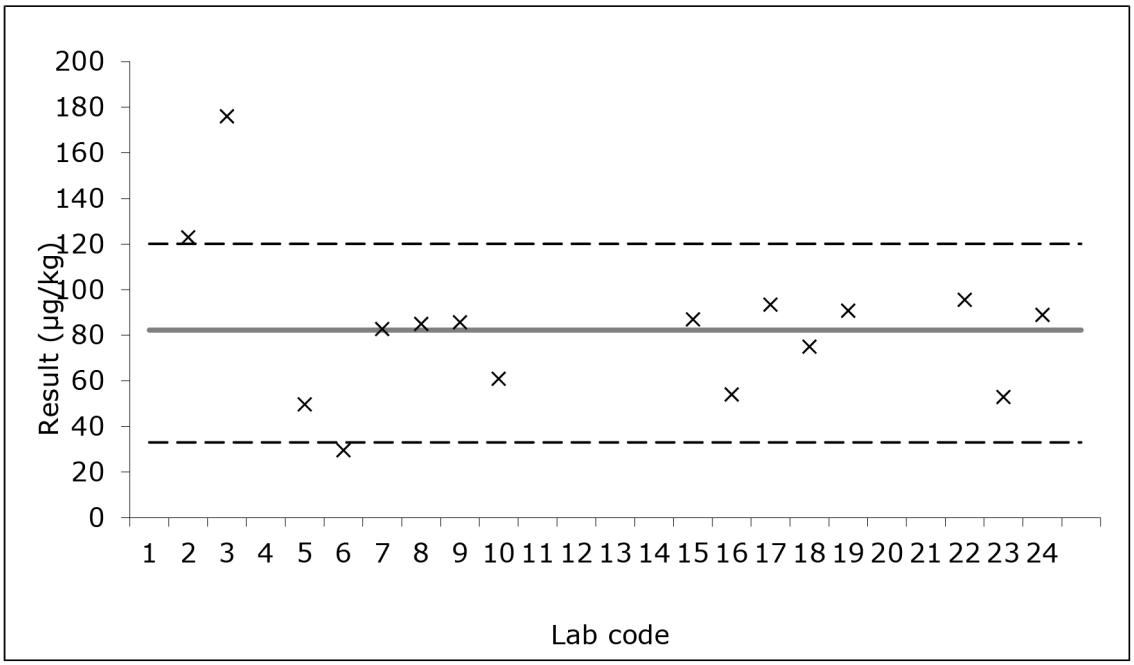

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation II in §4.4. The X - 2σ_H line (dotted) is calculated according to equation IV in §4.4.

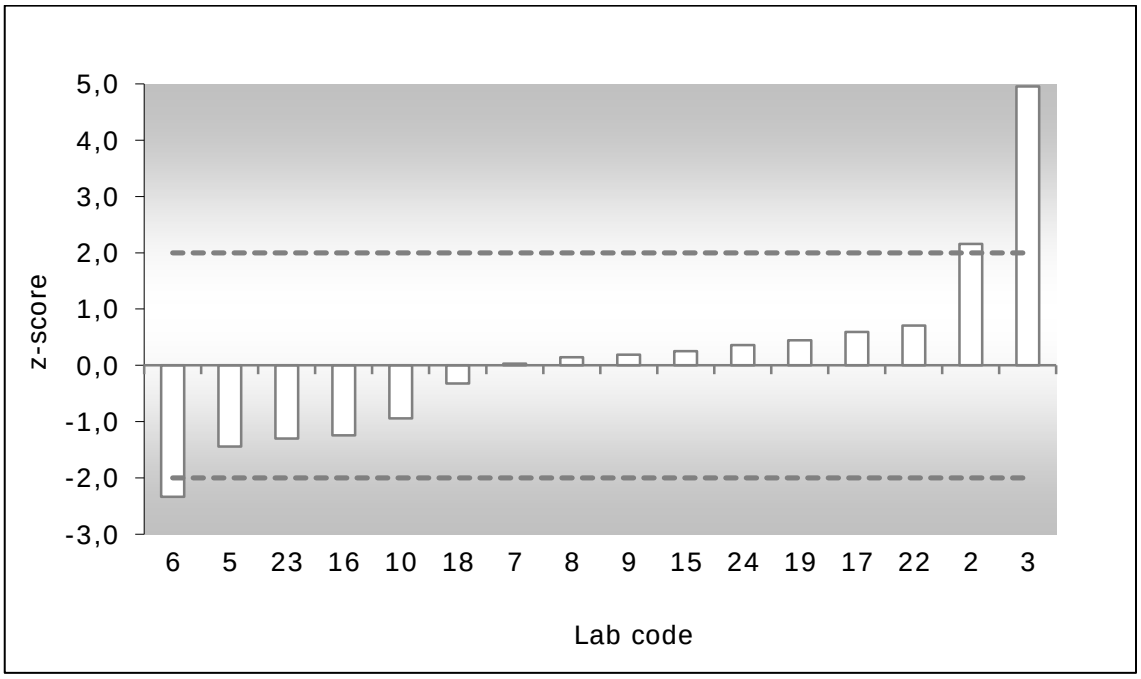

Figure b. Graphical representation of z'$_{ai}$-scores.

Annex XIV
Results for the analysis of cypermethrin in material B

Cypermethrin Assigned value: 33.6 µg/kg Uncertainty of assigned value: 1.43 µg/kg Target standard deviation (Horwitz, Thompson): 7.40 µg/kg Robust standard deviation: 4.12 µg/kg		
Lab code	Result (µg/kg)	z_{ai}-score
1	36.2	0.35
4	41	1.00
5	28.5	-0.60
6	8.16	**-2.98**
7	29.9	-0.44
9	34.75	0.15
10	37	0.46
12	35.0	0.19
14	25.0	-1.01
15	34	0.05
20	37.2	0.48
21	35.5	0.25
22	26	-0.89

Continued results for the analysis of cypermethrin in material B.

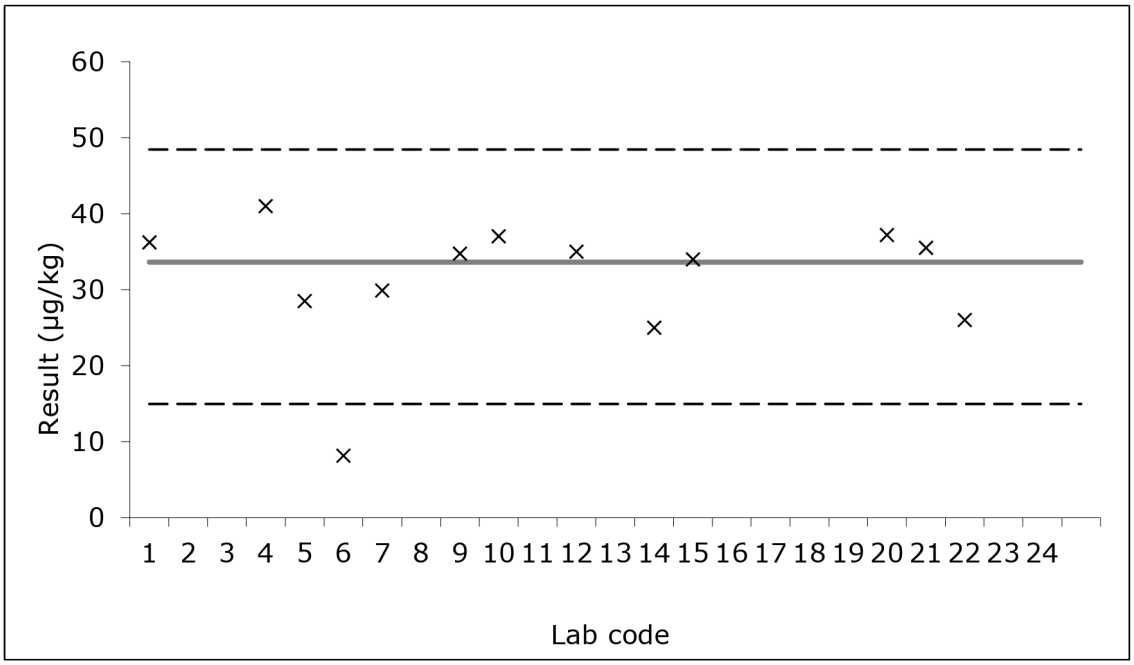

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation I in §4.4. The X - 2σ_H line (dotted) is calculated according to equation III in §4.4.

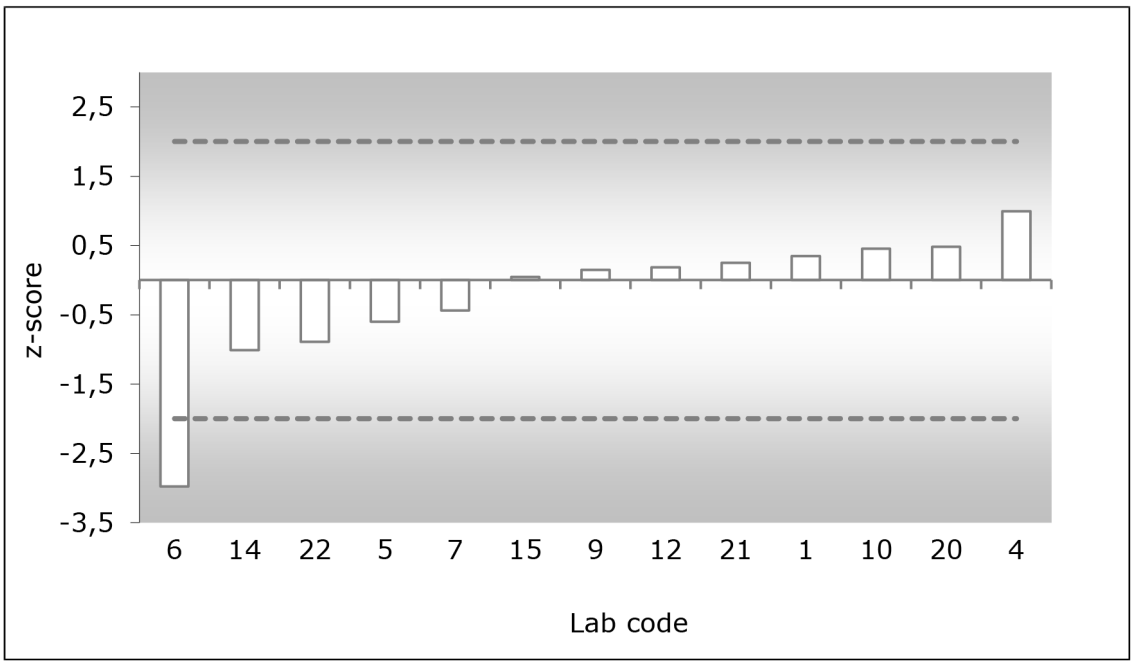

Figure b. Graphical representation of z_{ai}-scores.

Annex XV
Results for the analysis of deltamethrin in material B

Deltamethrin Assigned value: 34.0 µg/kg Uncertainty of assigned value: 4.16 µg/kg Target standard deviation (Horwitz, Thompson): 7.47 µg/kg Robust standard deviation: 12.5 µg/kg		
Lab code	Result (µg/kg)	z'_{ai}-score
1	41.2	0.84
4	56	**2.57**
5	42.0	0.94
6	16.53	-1.94
7	20.3	-1.52
9	37.77	0.44
10	27	-0.77
12	34.0	0.00
14	52.0	**2.11**
15	32	-0.22
19	36.3	0.27
20	16.5	-1.94
21	39.5	0.65
22	25	-1.00

Continued results for the analysis of deltamethrin in material B.

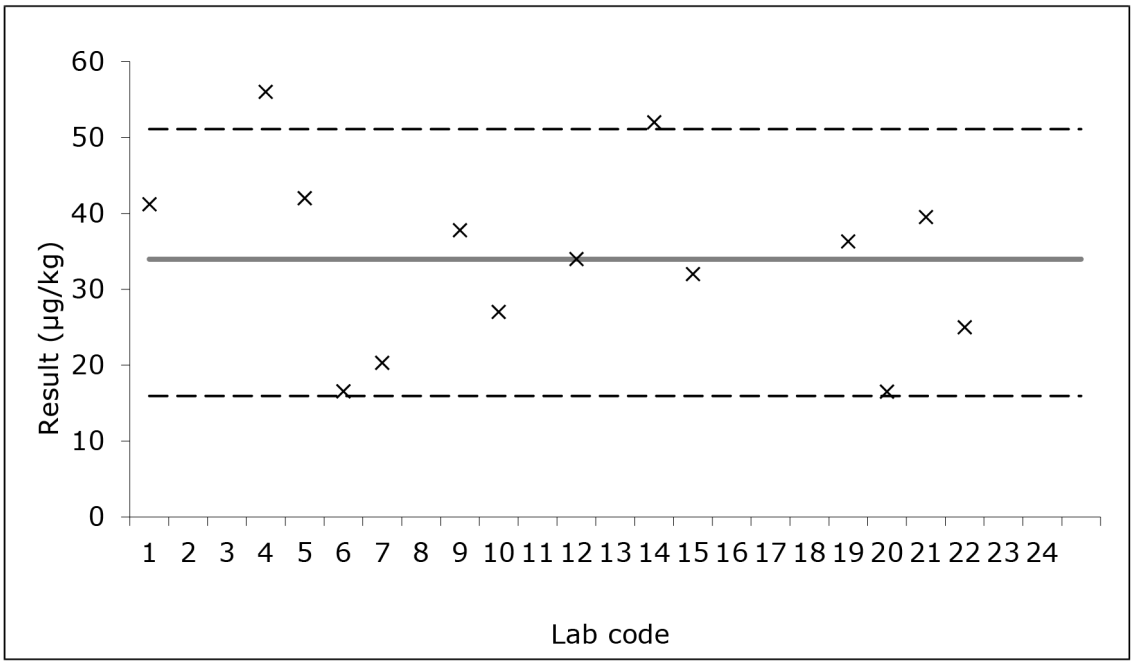

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation II in §4.4. The X - 2σ_H line (dotted) is calculated according to equation IV in §4.4.

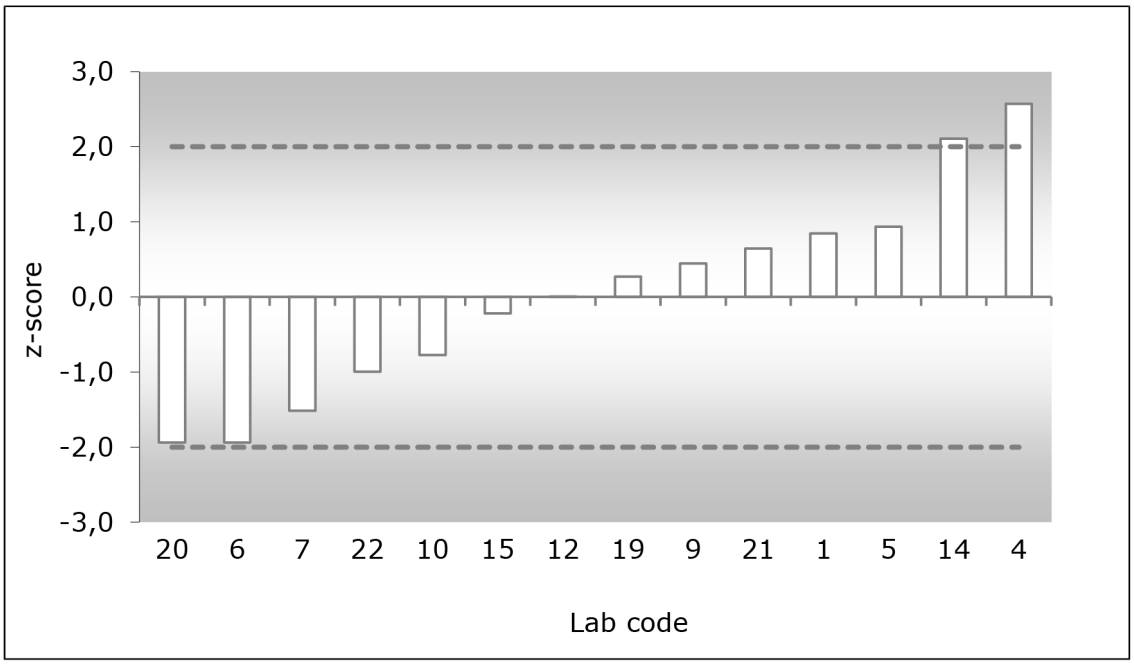

Figure b. Graphical representation of z'$_{ai}$-scores.

Annex XVI
Results for the analysis of cypermethrin in material C

Cypermethrin Assigned value: 25.5 µg/kg Uncertainty of assigned value: 2.16 µg/kg Target standard deviation (Horwitz, Thompson): 5.60 µg/kg Robust standard deviation: 6.23 µg/kg		
Lab code	Result (µg/kg)	z'_{ai}-score
1	31.2	0.96
4	34	1.42
5	27.0	0.26
6	8.20	**-2.77**
7	20.3	-0.83
9	21.32	-0.66
10	31	0.92
12	25.4	-0.01
14	18.0	-1.20
15	25	-0.07
20	30.9	0.91
21	26.4	0.16
22	24	-0.23

Continued results for the analysis of cypermethrin in material C.

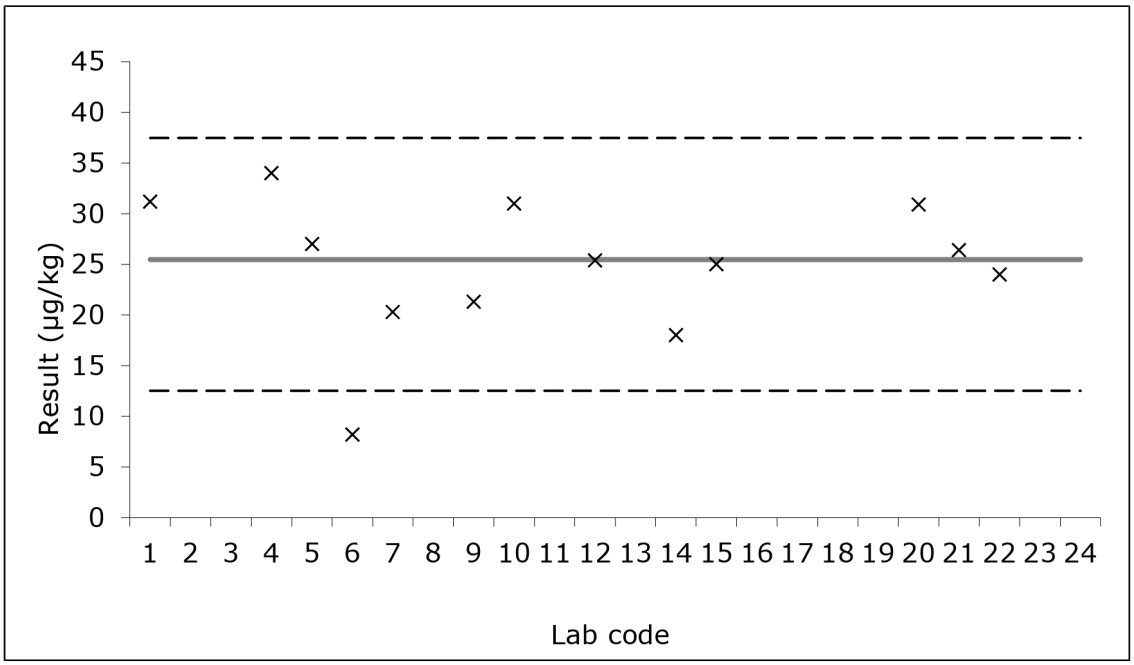

Figure a. Graphical representation of the reported results. The X + 2σ_H line (dotted) is calculated according to equation II in §4.4. The X - 2σ_H line (dotted) is calculated according to equation IV in §4.4.

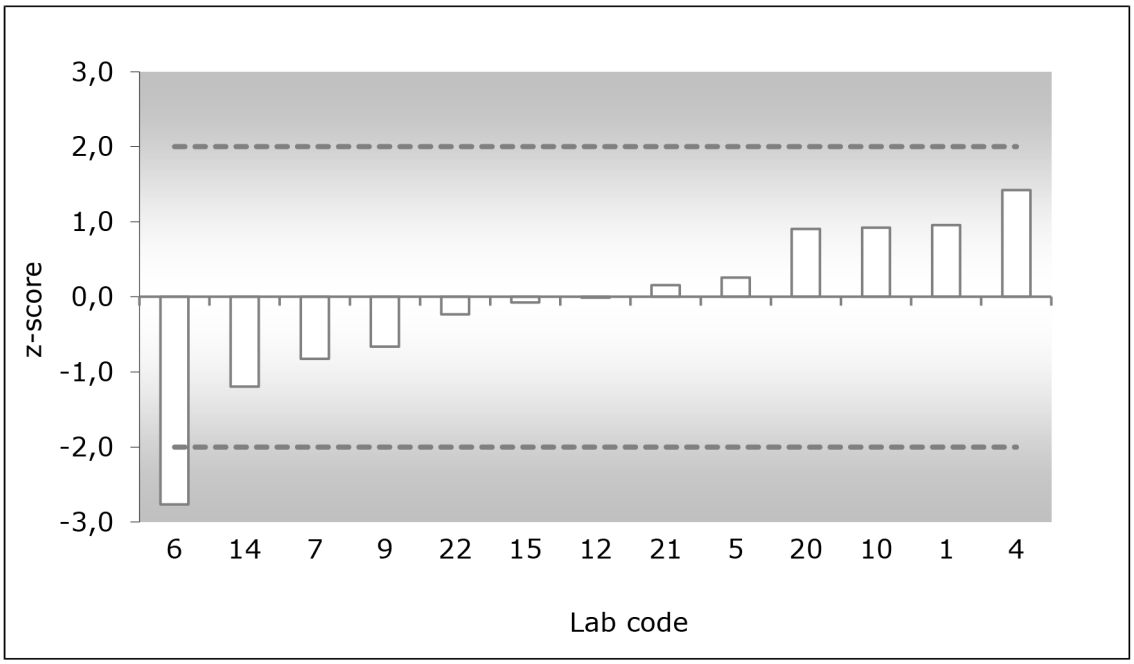

Figure b. Graphical representation of z'$_{al}$-scores.

Annex XVII
Results for the analysis of deltamethrin in material C

Deltamethrin Assigned value: 8.77 µg/kg Uncertainty of assigned value: 0.85 µg/kg Target standard deviation (Horwitz, Thompson): 1.93 µg/kg Robust standard deviation: 2.26 µg/kg		
Lab code	Result (µg/kg)	z'_a-score
1	9.5	0.35
4	13	**2.01**
5	11.5	1.30
6	14.87	**2.90**
9	7.17	-0.76
12	6.3	-1.17
14	8.5	-0.13
15	6	-1.31
19	7.4	-0.65
20	8.4	-0.17
21	9.2	0.21

Continued results for the analysis of deltamethrin in material C.

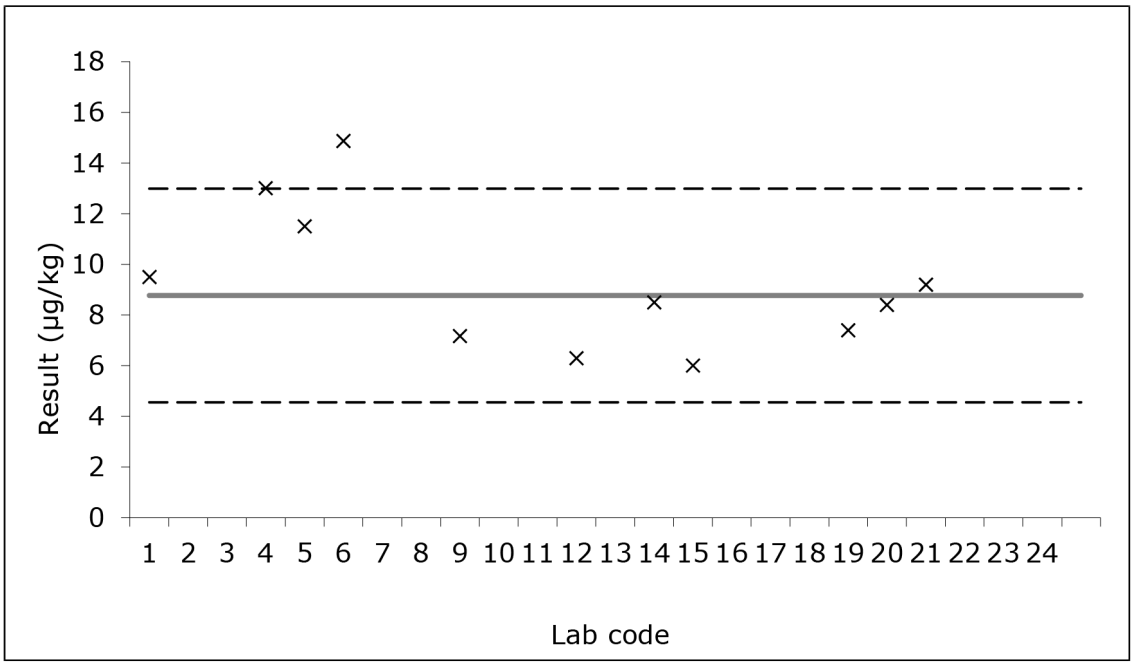

Figure a. Graphical representation of the reported results. The X ± 2σ_H lines(dotted) are calculated according to equation II in §4.4.

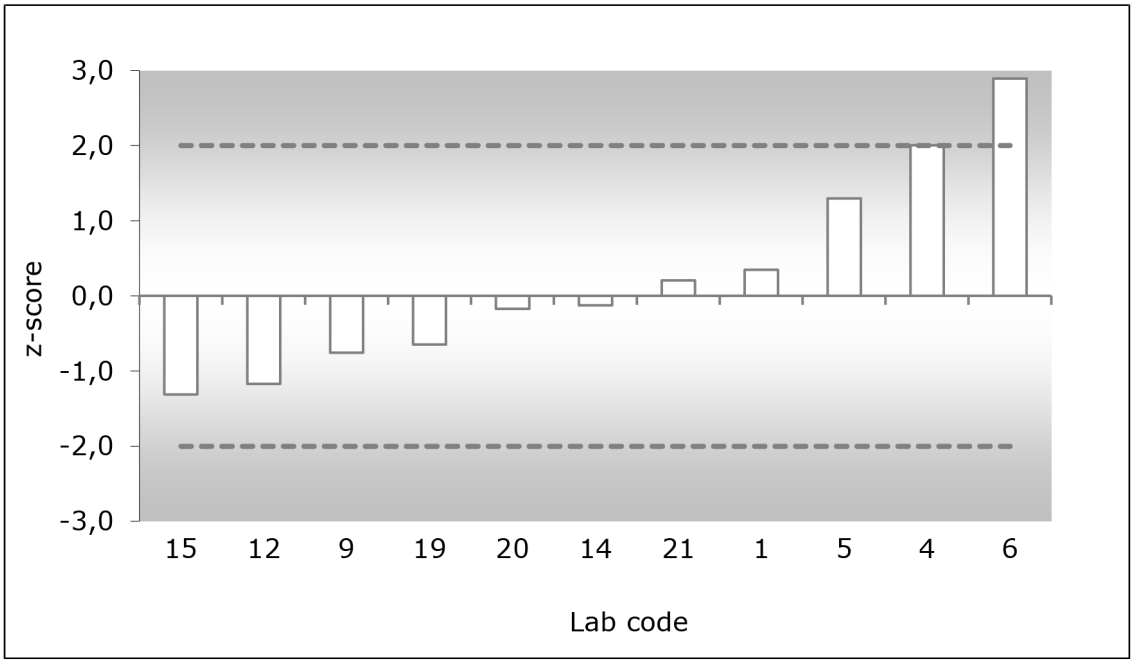

Figure b. Graphical representation of z'$_a$-scores.

Annex XVIII
Results for the analysis of cypermethrin in material D

Cypermethrin Assigned value: 45.1 µg/kg Uncertainty of assigned value: 6.17 µg/kg Target standard deviation (Horwitz, Thompson): 9.93 µg/kg Robust standard deviation: 17.8 µg/kg		
Lab code	Result (µg/kg)	z'_a-score
1	55.3	0.87
4	100	**4.70**
6	7.49	**-3.22**
7	61.1	1.37
9	42.52	-0.22
10	29	-1.38
12	40.1	-0.43
14	31	-1.21
15	51	0.50
20	60	1.27
21	60.3	1.30
22	33	-1.04
23	34	-0.95

Continued results for the analysis of cypermethrin in material D.

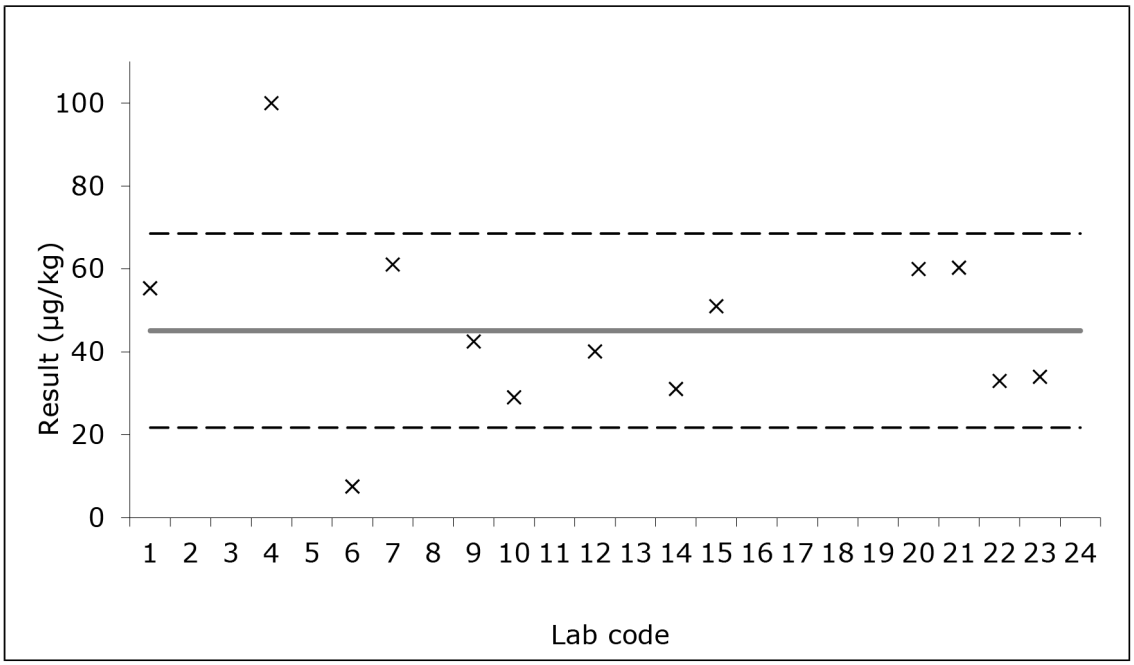

Figure a. Graphical representation of the reported results. The X ± 2σ_H lines(dotted) are calculated according to equation II in §4.4.

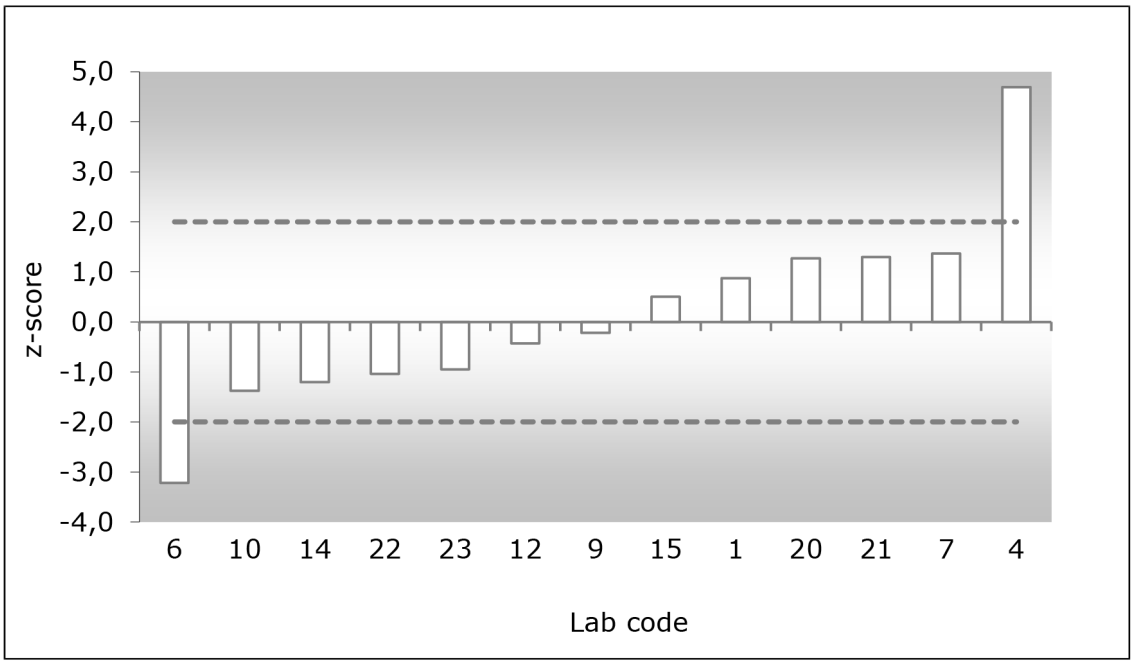

Figure b. Graphical representation of z'$_a$-scores.

Annex XIX
Overall score participants

Lab code	Satisfactory z-scores	Questionable z-scores	Unsatisfactory z-scores	False negatives	False positives	Compounds included	Remarks
1	5	0	0	0	0	CYP, DEL	
2	1	1	0	0	0	EMA, IVM	Could not quantify IVM in material A
3	0	1	2	0	0	EMA, IVM	
4	2	2	1	0	0	CYP, DEL	
5	6	0	1	0	3	EMA, IVM, CYP, DEL	Did not analyse CYP in material D
6	2	4	1	0	0	EMA, CYP, DEL	
7	7	0	0	0	0	EMA, IVM, CYP, DEL	DEL in material C under LOQ
8	2	0	0	0	3	EMA	
9	8	0	0	0	2	EMA, IVM, CYP, DEL	
10	6	0	0	2	0	EMA, IVM, CYP, DEL	
11	1	0	0	0	0	IVM	
12	6	0	0	0	0	IVM, CYP, DEL	
14	5	1	1	1	2	EMA, IVM, CYP, DEL	
15	8	0	0	0	0	EMA, IVM, CYP, DEL	
16	3	0	0	0	5	EMA, IVM	

Lab code	Satisfactory z-scores	Questionable z-scores	Unsatisfactory z-scores	False negatives	False positives	Compounds included	Remarks
17	3	0	0	5	0	EMA, IVM, CYP, DEL	
18	3	0	0	0	0	EMA, IVM	
19	4	0	0	4	0	EMA, IVM, CYP, DEL	
20	5	0	0	0	0	CYP, DEL	
21	5	0	0	0	0	CYP, DEL	
22	7	0	0	0	0	EMA, IVM, CYP, DEL	DEL in material C under LOQ
23	4	0	0	4	1	EMA, IVM, CYP, DEL	
24	3	0	0	0	0	EMA, IVM	

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

RIKILT advises national and international governments on establishing standards and methods of analysis. RIKILT is available 24 hours a day and seven days a week in cases of incidents and food crises.

The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs and the Netherlands Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

More information: www.wageningenUR.nl/en/rikilt