National Institute of Metrology, China

Report of the APMP.QM-S16

Fipronil-sulfone in Chicken Egg Powder

Final Report
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1 Introduction

Fipronil is a broad-spectrum insecticide attributed to the phenylpyrazole family. Due to its high toxicity to invertebrates and long persistence, it had been one of the most widely used insecticide to control household and agricultural pests from its appearance in market. Fipronil is a neurotoxic compound which could prevent the inhibition of invertebrate gamma-aminobutyric acid gated chloride channels in the central nervous system. Fipronil also showed genotoxicity and cytotoxicity to many vertebrate animals, like mammals, birds, fish, reptiles and so on. Moreover, fipronil could have negative effects on the liver, kidneys and thyroid glands of human. Its oxidation metabolite is fipronil-sulfone that could induce even higher toxicity than fipronil. In 2017, a fipronil in egg scandal occurred in the European Union (EU) that involved more than 15 states and astonished the world. The detected concentration of the sum of fipronil and fipronil-sulfone was many times higher than the EU maximum residue limit in bird egg (5 μg/kg). Besides European countries, fipronil contaminated eggs were also found in Asian areas like China's Hong Kong and South Korea. Systematic monitoring and control of pesticide residues are necessary to safeguard public health.

As part of its commitment to strengthen regional chemical metrology infrastructure, the Asia-Pacific Metrology Programme (APMP) has been organizing inter-comparisons for the purpose of establishing the technical basis for mutual recognition of measurement capabilities among national metrology institutes (NMIs)/designated institutes (DIs). At the CCQM Organic Analysis Working Group (OAWG) Meeting held in Chengdu, October 2018 and the APMP TCQM Meeting held in Singapore, November 2018, an APMP supplementary comparison (APMP.QM-S16) on the determination of fipronil-sulfone in chicken egg powder was proposed for participation by NMIs/DIs worldwide. The proposal was supported by the OAWG and APMP TCQM.

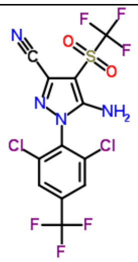
2 Objectives

The comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of incurred fipronil metabolite (fipronil-sulfone) in chicken egg powder. The mass fraction (μg/kg) should be reported on dry basis. Participants are advised that the results of the supplementary comparison may be used to support calibration & measurement capability (CMC) claims from participating laboratories under the CIPM MRA.

3 Measurand

The measurand of this study is fipronil-sulfone in chicken egg powder.

Table 1: Information of fipronil-sulfone

Measurand	Fipronil-sulfone
CAS Number	120068-36-2
Molecular formula	$C_{12}H_4Cl_2F_6N_4O_2S$
Molecular weight (g/mol)	453.147
pK_{ow}	-4.42
Structure	

4 Study material

4.1 Preparation

The level of fipronil-sulfone in the study material was adjusted by mixing natural contaminated chicken eggs with blank chicken eggs. After stirring and mixing, the liquid egg sample was freeze-dried, powdered and homogenized. The homogenized powder was separately dispensed into plastic double-layer vacuum packaging bags with content of about 5 g each.

4.2 Homogeneity study

The homogeneity of fipronil-sulfone in the study material was established by employing liquid chromatography-isotope dilution tandem mass spectrometry (LC-ID-MS/MS). 11 packages of samples were randomly selected and analyzed in duplicate as shown in Figure 1. The sample size of 0.5 g was used in the homogeneity study. A one-way analysis of variance (ANOVA) was used to evaluate homogeneity and the study material was concluded to be homogeneous by *F*-test at 95% confidence interval as shown in Table 2. The relative uncertainty of the between-bottle homogeneity was found to be 1.04%.

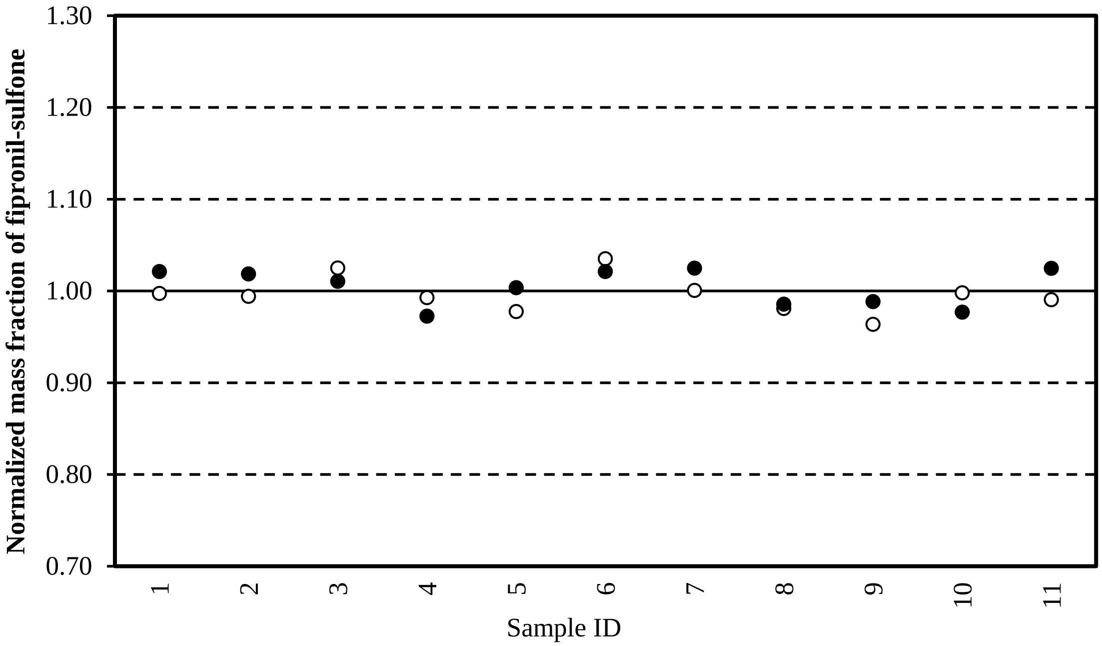


Figure 1: Graphical presentation of homogeneity study of fipronil-sulfone in the material

Table 2: Summary of ANOVA for homogeneity study of fipronil-sulfone in the material

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.005726	10	0.000573	2.3022	0.09375	2.8536
Within Groups	0.002736	11	0.000249			
Total	0.008462	21				

4.3 Stability study

The stability of fipronil-sulfone in the study material was established by LC-ID-MS/MS. A short-term stability study using isochronous design was carried out over a period of 28 days at a simulated transport temperature of 40 °C. Two randomly selected packages were transferred from the reference temperature of -20 °C to 40 °C on four occasions over the study period. Duplicate subsamples were then taken from each bottle and analyzed. The trend graph of stability was shown in Figure 2. The trend-analysis technique proposed by ISO Guide 35 was applied to assess the stability. The effect of time on the stability was evaluated using a linear approximation model by fitting linear regression lines to the data set. The statistical results for the significance test of the regression coefficient are given in Table 3. The results showed that the slope of the regression line was not significantly different from zero, using Student's *t*-test at 95 % confidence level, i.e. no significant instability was observed when the study sample was stored at about 40 °C.

The stability of the study material was also evaluated through one-way ANOVA test on the regression with results summarized in Table 4. The obtained *p*-value (greater than 0.05) indicated that the regression was insignificant at 95% confidence level.

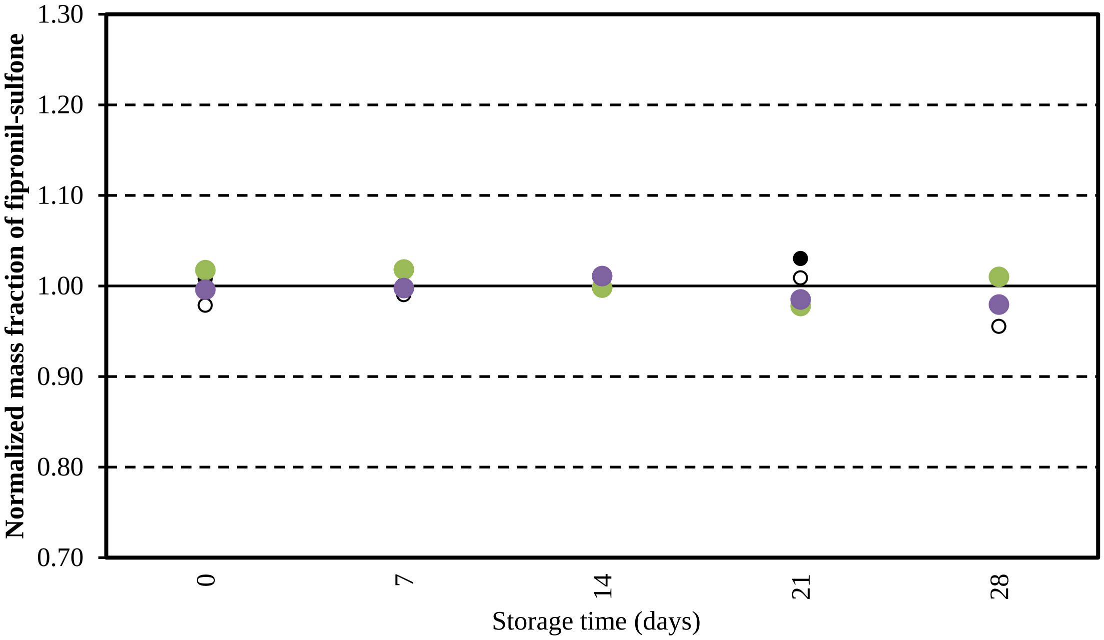


Figure 2: Graphical presentation of short-term stability study of fipronil-sulfone in the material

Table 3: Summary of Student's *t*-test for short-term stability study of fipronil-sulfone in the material

Descriptions	*Values*	*Descriptions*	*Values*
Slope of the regression line (b)	-0.0006	Uncertainty of slope [$s(b)$]	0.0004
Intercept of the regression line (b_0)	1.00535	Calculated t ($\frac{\|b\|}{s(b)}$)	1.4251
Standard deviation of the points (s)	0.0173	Critical t factor ($t_{0.95,n-2}$)	2.1009

Table 4: Summary of ANOVA for short-term stability study of fipronil-sulfone in the material

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.001375	4	0.000344	1.1180	0.3847	3.0556
Within Groups	0.004614	15	0.000308			
Total	0.005989	19				

The same approach was used to determine the stability of fipronil-sulfone in the study material under the storage temperature of -20 °C using classical design. The study was carried out on five occasions over a period of 18 months. No significant instability of the study material was observed at 95% level of confidence.

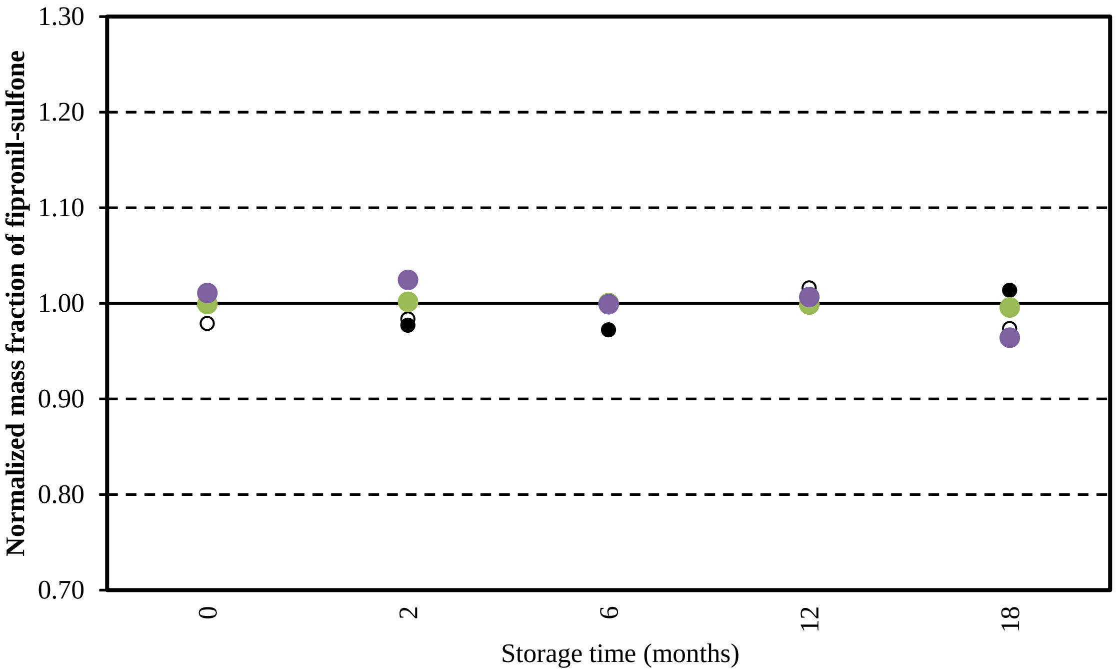


Figure 3: Graphical presentation of long-term stability study of fipronil-sulfone in the material

Table 5: Summary of Student's *t*-test for long-term stability study of fipronil-sulfone in the material

Descriptions	*Values*	*Descriptions*	*Values*
Slope of the regression line (*b*)	-0.0003	Uncertainty of slope [*s*(*b*)]	0.0006
Intercept of the regression line (b_0)	0.99953	Calculated *t* ($\frac{\lvert b \rvert}{s(b)}$)	0.5994
Standard deviation of the points (*s*)	0.0170	Critical *t* factor ($t_{0.95,n-2}$)	2.1009

Table 6: Summary of ANOVA for long-term stability study of fipronil-sulfone in the material

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	F_{crit}
Between Groups	0.000962	4	0.000241	0.836554	0.522947	3.055568
Within Groups	0.004313	15	0.000288			
Total	0.005275	19				

5 Instructions for study participants

Each participant will receive 4 packages, each containing about 5 g of chicken egg powder, shipped on dry ice. Two sample packages are intended for method development and the other two are to be used for determination of the final results. The samples will be transported at a temperature not exceeding 40 °C. A temperature strip will be attached to the sample, which records the highest temperature experienced during transport. Upon receipt of the sample pack, participants should check (i) the physical conditions of the sample packages; and (ii) inspect the temperature strip and record the highest temperature experienced during the transport; and (iii) complete the Sample Receipt Form and return it to the coordinator (E-mail: guozh@nim.ac.cn). Replacement will be arranged if the test samples are identified to be not suitable for analysis. The samples should be kept at -20 °C or below before analysis.

Participants are requested to determine the mass fraction (in μg/kg) of fipronil-sulfone (on a dry mass basis) in the test sample using the analytical method of their choice. The analysis should be conducted with a recommended sample size of at least 0.5 g. Participants are requested to report the mean value of replicate measurements of the test material. The expected mass fraction of fipronil-sulfone in the chicken egg powder sample is in the range of 4-400 μg/kg.

Participants are also requested to carry out dry mass correction. The determination of dry mass correction should be conducted with a recommended size of at least 0.5 g. The test sample portion taken for dry mass correction should be placed over anhydrous calcium sulphate (DRIERITE®) in a desiccator at room temperature for a minimum of 20 days until a constant mass is reached. Dry mass correction should be carried out at the same time as the test sample portion is to be analyzed in the same package of sample. Participants should report results based on mass change of three replicate sub-samples.

The test sample should be stored at -20 °C or below. The samples should be warmed up to room temperature (at 20 ± 5 °C and relative humidity <85%) before opening for analysis. The samples should be processed as soon as possible and carefully re-sealed after use and stored in a freezer at -20 °C or below.

Participants should investigate the particular local customs and quarantine requirements for the samples to be sent to their countries. If special permits are required, please inform the organizer. Besides, the organizer will not be responsible for any charges such as import taxes or related charges for the importation of the samples.

6 Programme schedule

The time schedule for the various stages of the programme is shown as follows:

Table 7: Time schedule for APMP.QM-S16

Stage	Period
Official call for participation	17 June 2019
Distribution of samples	July 2019
Deadline for submission of results	31 October 2019
Extended deadline	22 November 2019

The organizer announced on 17 October 2019 to extend the deadline for submission of results from 31 October to 22 November upon the requests of some participants with the approval of the APMP TCQM Chair.

7 Registration, sample distribution and report submission

A total of seven NMIs/DIs participated in the APMP.QM-S16 comparison. Each participant received 4 packages, each containing about 5 g of chicken egg powder, shipped on dry ice. A temperature strip was attached to the sample, which recorded the highest temperature experienced during the transport. The technical protocol, sample receipt form, result report form and the core competency table were sent to the participants by e-mail. All samples were well received in August 2019 or before except the sample sent to TISTR which received the sample in October 2019 due to the problem of the import license. All the participating NMIs/DIs submitted their results on or before the final deadline.

Information on participating NMIs/DIs, contacts, sample receipts and report submissions are summarized in Table 8.

Table 8: Information on participating NMIs/DIs, contacts, sample receipts and report submissions

No.	Participating Institutes, Department/Laboratory	Economy	Contact Person	Sample Receipt Date	Report Submission Date
1	GLHK Government Laboratory Hong Kong	Hong Kong SAR, China	Dr. Clare Ho	22 July 2019	12 Nov 2019
2	HSA Health Sciences Authority, Chemical Metrology Laboratory	Singapore	Ms Cheow Pui Sze	14 Aug 2019	15 Nov 2019
3	EC-JRC-Geel Joint Research Centre, Reference Material Production Laboratory	European Commission	Dr. Marta Dabrio	24 July 2019	22 Oct 2019
4	NIMT National Institute of Metrology, Thailand, Chemical metrology and Biometry Department	Thailand	Dr. Kittiya Shearman	15 July 2019	22 Nov 2019
5	TISTR Thailand Institute of Scientific and Technological Research, Analytical Chemistry Laboratory	Thailand	Ms. Thippaya Junvee Fortune	28 Oct 2019	22 Nov 2019
6	UME TUBITAK National Metrology Institute, Organic Chemistry Laboratory	Turkey	Dr. Mine Bilsel	7 Aug 2019	22 Nov 2019

8 Reporting of results

A Report of Results Form was provided to the participating NMIs/DIs for completion. The participating NMIs/DIs were requested to report the mean value of replicate measurements, each with a recommended size of not less than 0.5 g. The results were reported in the unit of μg/kg, which included standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. A complete description of the analytical procedure and the uncertainty estimation was also provided by the participating NMIs/DIs.

8.1 Reference materials used by the participating laboratories

The summary of the reference standards and internal standards used in the programmes by the participating laboratories was showed in Table 9. NIM used its own fipronil-sulfone certified reference material (CRM) GBW(E) 062661 as calibrant. GLHK, NIMT also used CRM from NIM, while HSA and TISTR used fipronil-sulfone CRM from NMIA (P1731) as calibrant. For UME, purity analysis on commercial fipronil-sulfone standard was performed by qNMR and used as calibrant. JRC used a commercial fipronil-sulfone standard but the metrological traceability was not substantiated.

All participants used Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$ as internal standard from Cambridge Isotopes Laboratories (100 μg/mL in methanol) except for JRC which didn’t use internal standard.

Table 9: Summary of reference standards and internal standards used by the participating laboratories

NMI/DI	Calibrant				Internal standard
	Source	Purities and Uncertainties (95% CI)	Identity verification	Purity assessment	
GLHK	CRM: NIM GBW(E) 062661	99.6%±0.3% (*k*=2)			Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$
HSA	CRM: NMIA P1731	99.5%±0.4% (*k*=2)	Compare its mass spectrum with GBW(E)062661 using GC-MS/MS and LC-MS/MS.	The purity value of the calibrant was verified with GBW(E)062661.	Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$
EC-JRC-Geel	Sigma-Aldrich 32333	99.0%			
NIM	CRM: NIM GBW(E) 062661	99.6%±0.3% (*k*=2)	1H NMR and LC-MS/MS.	1) HPLC based Mass Balance method by subtracting the impurities including structure related impurities, water, VOCs and inorganic residues; 2) Quantitative nuclear magnetic resonance (qNMR) method traceable to high purity CRM of ethyl hydroxybenzoate (GBW(E)100064) from NIM, China with a purity value of 99.7 ± 0.2%	Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$
NIMT	CRM: NIM GBW(E) 062661	99.6%±0.3% (*k*=2)	LC-MS/MS		Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$
TISTR	CRM: NMIA P1731	99.5%±0.4% (*k*=2)			Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$

UME	Toronto Research Chemicals F342215	98.89%±0.27% (*k*=2)	UHPLC-HR-MS/MS	qNMR (Traceabile to UME-CRM 1301)	Fipronil Sulfone-$^{13}C_4$,$^{15}N_2$

8.2 Methods used by the participating laboratories

Participants are encouraged to determine the mass fraction (in μg/kg) of fipronil-sulfone in the test sample on a dry mass basis using the analytical method of their choice. The details of method for sample reconstitution, extraction, clean-up, instrumental analysis and quantitation were summarized in Table 10 ~ 12.

Most participating laboratories applied isotope dilution mass spectrometry (IDMS) technique with fipronil-sulfone-$^{13}C_4$,$^{15}N_2$ as internal standard spiked before sample extraction for quantitation in this programme, except EC-JRC-Geel which used matrix matched external calibration method. LC-ID-MS/MS was chosen by four participating laboratories (GLHK, HSA, NIM and NIMT), LC-ID-HRMS was chosen by two participating laboratories (GLHK and UME), and GC-ID-MS/MS was chosen by two participating laboratories (HSA and TISTR). It should be noted that GLHK chose both LC-ID-MS/MS and LC-ID-HRMS, while HSA chose both LC-ID-MS/MS and GC-ID-MS/MS. However, various extraction techniques and clean-up methods were used. Except TISTR which didn’t provide the reconstitution method, the other 6 participating laboratories reconstituted egg powder with different amount of water. Acetonitrile was selected as extraction solvent for all participating laboratories and GLHK also added 1% formic acid in it. Moreover, typical QuEChERS salts ($MgSO_4$ and NaCl) were added by UME and DisQuE extraction salts (NaSesquihydrate, NaCitrate, NaCl and MgSO4) by EC-JRC-Geel, NIM and NIMT. After extraction, n-hexane was used to defat by HSA and NIMT. For clean-up method, PRiME HLB was used by GLHK, EC-JRC-Geel, NIM, NIMT and dSPE was selected by NIMT, TISTR and UME.

For LC-MS measurement, 5 participating laboratories (GLHK, HSA, EC-JRC-Geel, NIM, and NIMT) applied triple quadruple mass spectrometry operated under the multiple reaction monitoring mode (MRM) using the electrospray ionization technique. Most participating laboratories used the MRM transition 451 > 415 for fipronil-sulfone and 457> 421 for isotopic internal standard for quantitation, whereas NIM selected 451 > 282 and 457> 288 as quantitation ion pairs respectively. In addition, GLHK and UME used orbitrap based LC-HRMS. Ions 450.9263 for fipronil-sulfone and 456.9338 for isotopic internal standard were monitored and used for quantitation. For GC-MS measurement, both HSA and TISTR applied triple quadruple mass spectrometry. The MRM transition 383 > 255 for fipronil-sulfone and 389> 257 for isotopic internal standard were monitored and used for quantitation. GLHK, HSA, NIM and UME used IDMS quantitation method with single-point and bracketing calibration. NMIT and TISTR applied matrix-matched IDMS quantitation method with single-point and bracketing calibration. EC-JRC-Geel is the only laboratory applied five-point matrix matched external calibration method for quantitation.

All participants applied the suggested protocol for moisture determination and results were reported in dry mass basis.

Table 10: Summary of sample pre-treatment and internal standard spiking

NMI/DI	Sample intake (g)	Sample reconstituted with water	Amount of water (g or mL)	IS Spikes Before or After reconstitution	IS Equilibration time, temperature
GLHK	0.5	Y	6 mL	Before	30 min, Rt
HSA	0.5	Y	1.5 g	After	Overnight, 4°C
EC-JRC-Geel	1	Y	4 mL	No[b]	No
NIM	0.5	Y	1.5 mL	After	30 min, Rt
NIMT	0.5	Y	2 mL	After	Overnight (16 hours)
TISTR	1	/	/	/	/
UME	0.5	Y	1.92 g	Meanwhile[c]	1 h, Rt

a) No information was provided.

b) No internal standard was spiked.

c) Water and internal standard were added at the same time.

Table 11: Summary of sample extraction, clean-up methods

NMI/DI	Extraction method	Clean-up method
GLHK	Extracted with 10mL of 1% formic acid in acetonitrile. Vertical shaking at 1000 strokes per minute for 15 minutes.	2mL of extract cleaned by passing through Oasis® PRiME HLB Cartridge Plus Light syringe filter (100mg sorbent per cartridge). No dilution or concentration step was performed.
HSA	Extracted with 5 mL acetonitrile. Shaken on a multitube shaker for 1 min and sonicated for 5 min. 1 g of sodium chloride was added and vortexed to mix well. Centrifuged at 4,200 rpm for 10 min, the supernatant solution was collected. The extraction with acetonitrile was repeated two more times.	The combined supernatant was re-extracted with 10 mL of n-hexane. After vortexing, the mixture was centrifuged at 3,000 rpm for 5 min, and the supernatant was decanted. The bottom acetonitrile layer was evaporated to dryness under nitrogen and reconstituted with 1 mL of acetonitrile. The reconstituted solution was transferred into an amicon Ultra-0.5 centrifugal filter (consisting of regenerated cellulose membrane), and was centrifuged at 13,000 rpm for 10 min
EC-JRC-Geel	Modified QuEChERS method: to the reconstituted egg sample 10 mL water, 10 mL Acetonitrile and DisQuE extraction salts were added, 30 min shaker at maximum speed and then centrifugation.	After extraction and centrifugation supernatant was filtered over Oasis PRiME HLB syringe filter and injected directly into the analytical system.
NIM	The sample was added with 2 mL of water and 10 mL of acetonitrile, vortexed for 1 min. Then, 2.6 g of DisQuE extraction salts (DisQuE Pouch for 50 mL CEN 0.5g NaSesquihydrate 1g NaCitrate/1g NaCl/4g MgSO4) was added and the tube was shaked by hand for one minute vigorously and sonicated for 10 min. After the sample was centrifuged at 8,000 rpm for 5 min, the supernatant solution was collected.	5mL of extract was cleaned by passing through Oasis PRiME HLB SPE column and filtered through a 0.22 µm GHP filter for LC-MS/MS analysis.
NIMT	Solid-liquid extraction (also for protein precipitation) was carried out using 10 mL of acetonitrile (centrifuge at 12000 rpm for 5 min). The solution was extracted using DisQuE Pouch 50 mLCEN 0.5g Na Sesquihydrate 1g Na Citrate/1g NaCl/4g MgSO4.	Liquid-liquid extraction was performed using 10 mL of hexane for defattening. Further clean-up was performed on Oasis PRiME HLB Plus light cartridges (100 mg). The reconstitute solution (2 mL) was applied through the cartridges. The solution collected was then dried at 45 °C under N_2. The dried residue was reconstituted with 1.5 mL of acetonitrile and clean-up using dSPE (150 mg MgSO4, 50 mg PSA, 50 mg C18, 50 mg GCB), Then the solution was filtered through 0.2 um filter disk and analyed using LC-MS/MS

TISTR	Sample was extracted with acetonitrile (10ml) and centrifuge at 3500 rpm for 5 minutes.	Clean up with C18, $MgSO_4$ and PSA.
UME	20 mL of HPLC grade acetonitrile was added and vortex was applied for 1 minute. The mixture was sonicated for 30 minutes. Then 3 g of $MgSO_4$ and 2 g NaCl was added to centrifuge tube and tube was shaked by hand for two minutes vigorously and centrifuged at room temperature for 5 min at 5000 g.	Then the supernatant was transferred to 15 mL centrifuge tube and was kept at -20 ˚C for 1 h. After this step 10 mL of supernatant was transferred into the 15 mL centrifuge tube which contains 400 mg of primary secondary amine (PSA), 400 mg octadecyl-modified silica (C18) and 45 mg graphitized carbon black (GCB) and vortex was applied for 1 min and then sample was centrifuged at room temperature for 5 min at 5000 g. Then supernatant was filtered through a 0.2 µm PTFE filter for LC/MS analysis

Table 12: Summary of instrumental analysis and quantitation methods

NMI/DI	Analytical instrument(s) used	Chromatographic column	Method of quantitation	Type of calibration	Ions/MRM transitions
GLHK	1) LC-MS/MS. Thermo Vanquish horizon UPLC system with Thermo Altis triple quadrupole mass spectrometer. 2) LC-HRMS. Thermo 3400RS UPLC system with Thermo QExactive quadruople orbitrap mass spectrometer.	LC-MS/MS and LC-HRMS: Waters CORTECS UPLC T3 column (150 mm x 2.1 mm x 1.6 µm) with in-line filter (Waters stainless steel in-line filter kit, 2.1mm x 0.2 µm).	IDMS	Single-point, bracketing	1) LC-MS/MS Calibrant 451 > 415* 451 > 282 451 > 244 IS 457 > 421 457 > 288 457 > 247 2) LC-HRMS Calibrant 450.92634* 414.94967 281.99256 243.98948 IS 456.93383

HSA	1) GC-MS/MS: Thermo Scientific TSQ$^{\text{TM}}$ 9000 triple quadrupole GC-MS/MS system; 2) LC-MS/MS: AB Sciex Qtrap® 5500 MS/MS instrument coupled with Shimadzu Prominence UFLC XR LC system	1) GC-MS/MS: DB-5MS (30 m x 0.25 mm, 0.25 µm) 2) LC-MS/MS: Hypersil BDS C18 (50 × 2.1 mm, 5 µm)	Exact-matching IDMS	Single-point calibration	1) GC-MS/MS: Calibrant 383.0> 255.0* 383.0> 241.0 383.0> 335.0 IS 389.0> 257.0* 389.0> 241.0 389.0> 341.0 2) LC-MS/MS: Calibrant 450.9> 415.0* 450.9> 282.0 IS 456.7> 420.6* 456.7> 287.8
EC-JRC-Geel	LC-MS/MS: Waters XEVO TQ-S	Acquity UPLC BEH C18 1.7µm, 2.1x100mm	External calibration	Five-point matrix matched calibration	Calibrant 451 > 415* 451 > 282 451 > 244
NIM	LC-MS/MS: Waters UPLC Xevo TQ-S	Waters BEH C18 (50 mm × 2.1 mm, 1.7 µm)	IDMS	Single-point calibration	Calibrant 451 > 282* 451 > 415 IS 457 > 288* 457 > 421
NIMT	LC-MS/MS: Shimadzu LC system equipped with API 4000 MS/MS from AB Sciex.	Clipeus C18 chromatography column (100 x 3.0 mm i.d., 5 mm packing, Higgins Analytical, Inc.)with Phenomenex C18 SecurityGuard column (4.0 × 3.0 mm)	Exact-matching double IDMS	Single-point and bracketing	Calibrant 450.8 > 414.9* 450.8 > 218.9 IS 456.8 > 420.9* 456.8 > 287.9

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TISTR	GC-MS/MS	DB5-MS	Matric matching IDMS.	Bracketing IDMS.	Calibrant 383> 255* IS 389> 257*
UME	LC-MS/MS: Thermo Scientific Dionex Ultimate 3000 UHPLC connected to Thermo Scientific Qexactive HR-MS/MS	ACQUITY UPLC BEH C18 1.7 µm 2.1x100 mm	IDMS	Single-point	Calibrant 450.92639* IS 456.93420*

8.3 Results reported by the participating laboratories

All participating laboratories submitted their results by the extended deadline. The results were summarized in Table 13 and plotted in Figure 4.

Table 13: Summary of results reported by the participating laboratories

NMI/DI	Moisture content (g/g)	Standard deviation (g/g)	Mass fraction on dry mass basis (μg/kg)	Combined standard uncertainty *u* (μg/kg)	Coverage factor (*k*)	Expanded uncertainty *U* (μg/kg)
GLHK	0.0219	0.0034	122.4	5.6	2	11.2
HSA	0.0157	0.002096	123.5	4.6	2	9.2
EC-JRC-Geel	0.02	0.0014	121.9	4.7	2	9.3
NIM	0.0152	0.0008	122.8	2.9	2	5.9
NIMT	0.021969	0.0011713	120.8	4.2	2.16	9.1
TISTR	0.01534	0.00021	116.96	4.26	2	8.52
UME	0.015	0.002	123.5	3.6	2	7.2

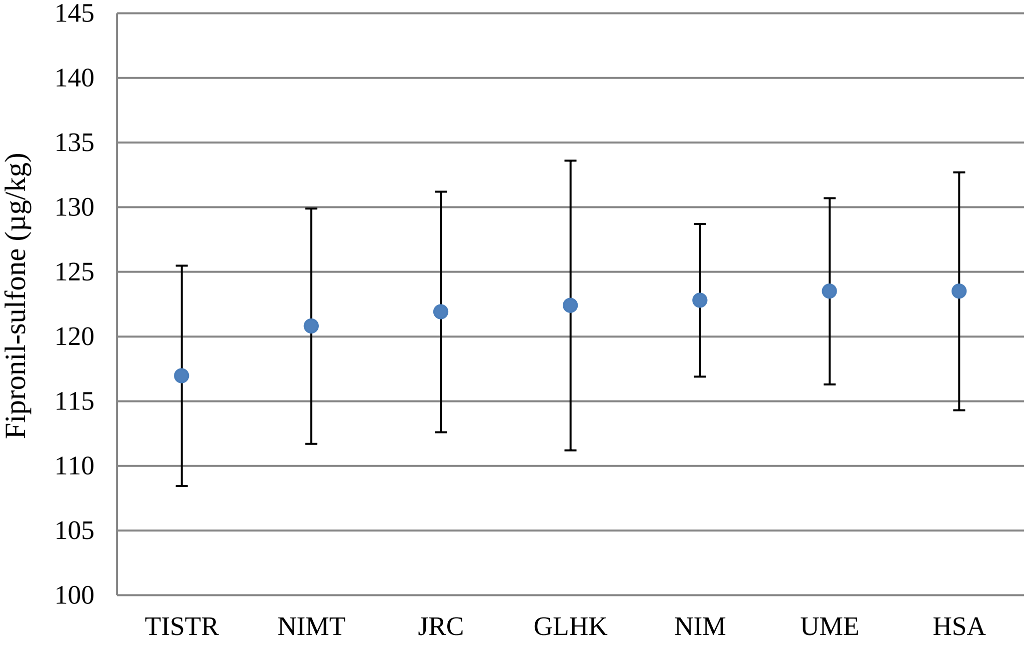


Figure 4: Reported results from all participants for fipronil-sulfone

Dots represent the reported mean values, *x*; bars represent their 95 % expanded uncertainties, *U*(*x*). The thin horizontal gridlines are provided for visual guidance.

9 Measurement equation and estimation of measurement uncertainty

Full reports by all the participants on their measurement equations and uncertainty estimates are provided in Appendix I, and any additional information is provided in Appendix II.

10 Provisional Supplementary Comparison Reference Value (SCRV)

A preliminary report was sent to participants in November 2019 and discussed in December 2019 in the APMP meetings in Sydney, Australia. During the meeting in Sydney, tentative SCRV was discussed such as calculation method, treat of the results from NMIT and TISTR. After this meeting, Dr. Lindsey Mackay confirmed to CCQM that both NIMT and TISTR can be included in the calculation of SCRV, and they can use their DoEs to underpin their CMCs. In that case, the CMCs must be completely different claims. As the metrological traceability for the calibrant from JRC was not substantiated, the measurement result reported by JRC was excluded in the calculation of SCRV. The Dixon and the Grubbs statistical tests indicated that there are no location outliers amongst the remaining dataset. As a result, six sets of valid results were used for the estimation of the Supplementary Comparison Reference Value (SCRV) for fipronil-sulfone.

Potential candidate SCRV values, X, and standard uncertainties, $u(X)$, for fipronil-sulfone mass fraction (µg/kg), calculated from the 6 results, are listed in Table 14. Neither the arithmetic mean and standard deviation of the mean, nor the robust median and median absolute deviation from the median (MADe) account for the participants' reported uncertainties, $u(x_i)$ [1]. It was preferred to utilise the reported $u(x_i)$ in SCRV calculation because they were considered to be generally reliable and account for the main sources of variance in the data set. For consistent data sets with 4~10 results, the Hierarchical Bayes is considered the appropriate estimator and includes the reported $u(x_i)$, all of which are larger than the estimate of dark uncertainty calculated with this approach (1.1 µg/kg). The SCRV was estimated using a Hierarchical Bayesian procedure via the NIST Consensus Builder (NICOB)[2].

Table 14: Candidate SCRV estimators for fipronil-sulfone with their standard uncertainties

Estimator	X (µg/kg)	$u(X)$ (µg/kg)
Arithmetic Mean	121.66	1.02
Median	122.60	0.68
Hierarchical Bayes*	121.82	1.77

* Estimated using NICOB [2]

As the data are mutually consistent, normally distributed and contain believable participant uncertainties, the Hierarchical Bayesian procedure implemented in the NIST Consensus Builder (NICOB) [2] was considered the most appropriate approach and implemented to estimate the SCRV and associated uncertainty. This method is based on a Gaussian random effects model:

$$X_i = \mu + \lambda_i + E_i$$

Where i indexes the participating laboratories, X_i are the lab-reported means, μ is the consensus value, λ_i are the laboratory effects distributed as Gaussian with mean 0 and variance σ_λ^2, and E_i are the lab-specific measurement errors distributed as Gaussian with mean 0 and variance $u(X_i)^2$. The parameter σ_λ^2 directly estimates the excess variance and the estimate of μ is close to the weighted mean.

The model is estimated via Markov Chain Monte Carlo (MCMC) resampling, which produces large numbers of realisations (draws) of the parameters of the random effects model. This allows the value, standard uncertainty, and 95% credible interval of a parameter to be estimated, respectively, as the arithmetic mean, standard deviation, and interval between the 2.5th percentile and 97.5th percentile of a sufficiently large number (typically several tens of thousands) of draws.

Within the NICOB option for the Hierarchical Bayesian procedure, the SCRV was calculated using default values for prior distribution scales and for MCMC settings. The participants' results, the provisional SCRV of the Bayes approach and their associated standard uncertainties are plotted in Figure 5.

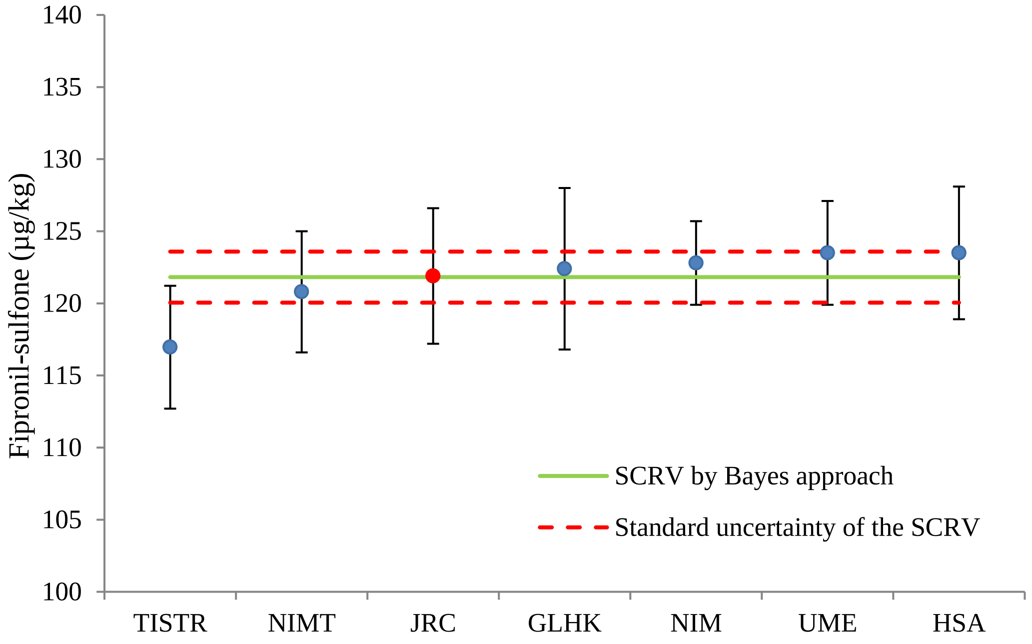


Figure 5: Plots of participants' results relative to the Bayes SCRV for APMP.QM-S16

The solid blue dots represent values included for SCRV calculation. The solid red dot represents the value excluded from SCRV calculation. Bars represent their standard uncertainties. The solid green horizontal line denotes the candidate SCRV. The dotted red horizontal lines denote the standard uncertainty interval of the candidate SCRV.

11 Degrees of equivalence (DoE) calculation

The degree of equivalence (DoE) for each participating NMI/DI's result was calculated using the same Hierarchical Bayes model employed to estimate the SCRV and associated uncertainty [2].

The absolute degree of equivalence (D_i) was calculated as follows:

$$D_i = X_i - X_{\mathrm{SCRV}}$$

whereby X_i is the result reported by participant *i* and X_{SCRV} is the KCRV estimate. Using a Monte Carlo (MC) technique to estimate the SCRV, the respective D_i and their expanded uncertainties, $U(D_i)$, that provide intervals corresponding to a confidence level of approximately 95 %, are readily estimated along with the SCRV. The distributions of the D_i were assessed as essentially symmetric, thus the $U(D_i)$ were estimated as the half-width of the interval between the 2.5th and 97.5th percentiles of the MC draws.

To enable comparison with the degrees of equivalence estimates from other studies, it is convenient to express the D_i and $U(D_i)$ as percentages relative to the SCRV:

$$\%D_i = 100 \times \frac{D_i}{X_{\mathrm{SCRV}}}$$

$$U(\%D_i) = 100 \times \frac{U(D_i)}{X_{\mathrm{SCRV}}}$$

Tables 15 and Figures 6 summarise the participants' D_i (µg/kg) and $\%D_i$ for fipronil-sulfone.

Table 15: Degrees of equivalence [*Di*] and their uncertainties at 95% level of confidence for fipronil-sulfone in chicken egg powder

NMI/DI	D_i (µg/kg)	$U(D_i)$ (µg/kg)	$\%D_i$	$U(\%D_i)$
GLHK	0.58	11.99	0.48	9.84
HSA	1.68	10.06	1.38	8.26
EC-JRC-Geel	0.08	10.29	0.07	8.45
NIM	0.98	7.37	0.80	6.05
NIMT	-1.02	9.67	-0.84	7.94
TISTR	-4.86	9.55	-3.99	7.84
UME	1.68	8.43	1.38	6.92

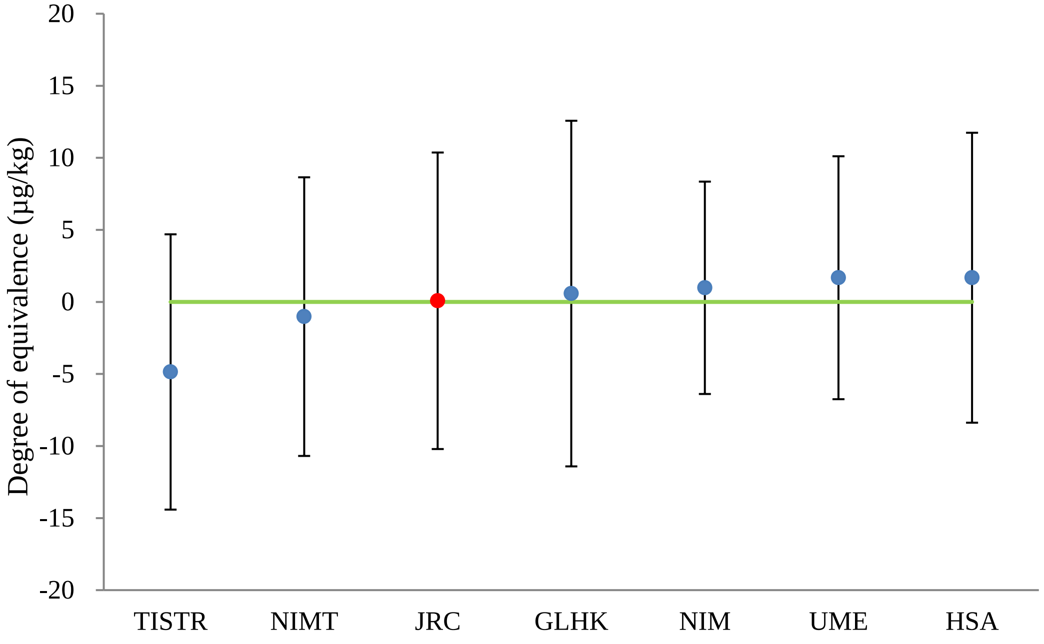


Figure 6a: Degrees of equivalence estimates and 95% coverage intervals for APMP.QM-S16

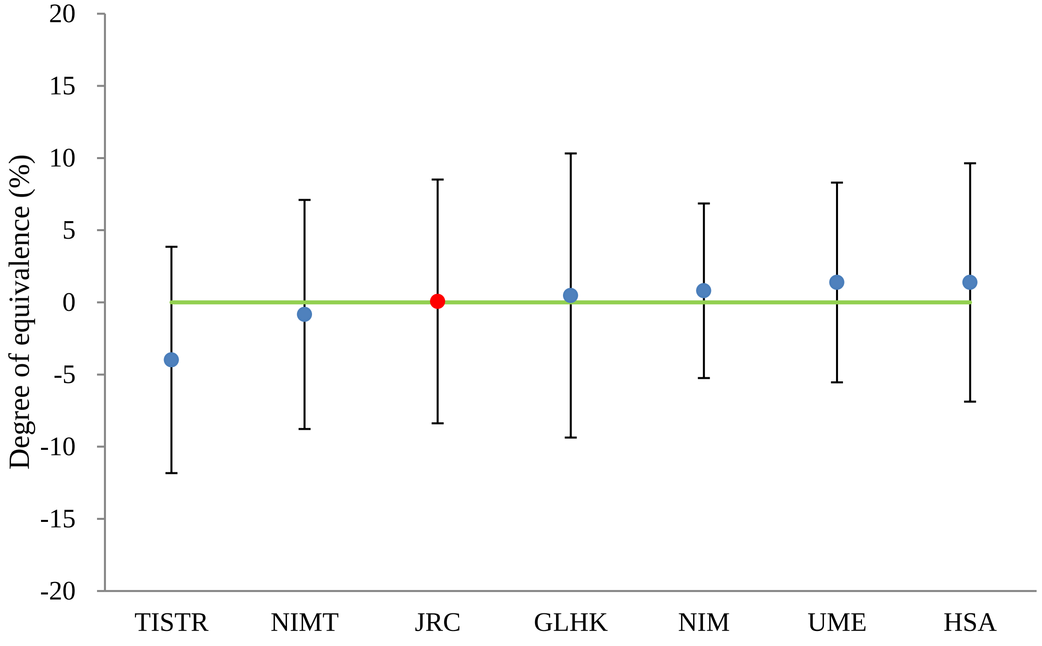


Figure 6b: Relative degrees of equivalence estimates and 95% coverage intervals for APMP.QM-S16

12 Core competency and How Far Does the Light Shine?

This study provides the means for assessing measurement capabilities for determination of low-polarity measurands in a procedure that requires extraction, clean-up, analytical separation, and selective detection in a food matrix. Generally, it provides demonstration of a laboratory's capabilities in determining the medium molecular weight analytes (mass range 300-500) with low polarity (pKow < -2) at mass fraction levels of 4 to 400 µg/kg in a high fat, high protein and low carbohydrate food matrix that falls within Sector 4 of the AOAC Food-matrix Triangle. Core competency tables for each participant can be found in Appendix III.

13 Use of report

This report is intended to be used to demonstrate capabilities and underpin CMC submissions in line with the How Far Does the Light Shine statement.

Acknowledgement

The Coordinators of this comparison would like to express their sincere thanks to all the participating institutes for their contributions, as well as for the support of Dr Kazumi Inagaki, Chair, APMP TCQM and Dr Lindsey Mackay, Chair, CCQM OAWG.

References

1) CCQM/13-22 Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence, 11-Apr-2013

2) Calculated using the NIST Consensus Builder online application available at https://consensus.nist.gov

Appendix I: Measurement equation and full uncertainty evaluation reported by the participating laboratories

GLHK

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

1.1 Calculation of the moisture content in sample

The % moisture content, W, in the sample is calculated as:

$$\%M = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

Where W_3 = weight of (glass vial + glass stopper) with sample after drying (g)

W_2 = weight of (glass vial + glass stopper) sample before drying (g)

W_1 = weight of (glass vial + glass stopper) (g)

1.2 Dry mass correction factor (F_{Dry})

The dry mass correction factor (F_{Dry}) is calculated as follows:

$$F_{Dry} = 1 - \frac{\%M}{100}$$

1.3 Calculate the peak area ratio (AR) between Fipronil sulfone and labelled Fipronil sulfone as follows:

$$AR = \frac{A_x}{A_{IS}}$$

Where Ax = Peak area of Fipronil sulfone (Quantitation MRM or HRMS ion)

A_{IS} = Peak area of Fipronil sulfone-$^{13}C_4$, $^{15}N_2$ (Quantitation MRM or HRMS ion)

1.4 Calculate the mass fraction of Fipronil Sulfone in sample (ng/g) by single-level exact matching calibration with continuous bracketing, according to the following equation:

$$M_X = \frac{\left(\frac{(AR_S)}{(AR_{cal,n} + AR_{cal,n+1})/2} \times MR_{cal} \times M_{IS}\right)}{M_S}$$

Where M_X = Mass fraction of Fipronil Sulfone in sample (ng/g)

AR_S = area ratio of Fipronil sulfone : labelled Fipronil sulfone $^{13}C_4,^{15}N_2$ in sample

$AR_{cal,n}$ area ratio of Fipronil sulfone : labelled Fipronil sulfone $^{13}C_4,^{15}N_2$ in calibration standard analyzed before the sample

$AR_{cal,n+1}$ area ratio of Fipronil sulfone : labelled Fipronil sulfone $^{13}C_4,^{15}N_2$ in calibration standard analyzed after the sample

MR_{cal} = mass ratio of Fipronil sulfone : labelled Fipronil sulfone $^{13}C_4,^{15}N_2$ in calibration standard (unitless mass fraction ratio)

M_{IS} = mass of labelled Fipronil sulfone-$^{13}C_4,^{15}N_2$ added into the sample (ng)

M_S = mass of sample (g)

1.5 Moisture corrected mass fraction (C_{xspl})

$$C_{Xspl} = \frac{M_X}{F_{Dry}}$$

Where C_{xspl} = Moisture corrected mass fraction of Fipronil Sulfone in sample (ng/g)

M_X = Mass fraction of Fipronil Sulfone in sample (ng/g)

F_{Dry} = Dry mass correction factor

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Uncertainties were estimated based on contribution from four factors: 1) purity of reference material, 2) method precision, 3) method bias, 4) uncertainty from moisture content determination. Detailed breakdowns are given as follows:

Description	*Value x*	*Std. Unc.*	*Rel. Std. Unc. u(x)*
RM [u(std)]	1	0.006299	0.006299
Precision [u(pres)]	1	0.028190	0.028190
Method Bias [u(bias)]	1	0.035167	0.035167
Moisture [u(water)]	1	0.002692	0.002692
Combined Rel. Std. Unc.		0.045589	

Combined standard uncertainty, (u(Fipronil sulfone)), ug/kg

$$= \mathit{Dried\ mass\ fraction\ of\ fipronil\ sulfone} \times \sqrt{\mathrm{u(std)}^2 + \mathrm{u(pres)}^2 + \mathrm{u(bias)}^2 + \mathrm{u(water)}^2}$$

$$= 122.4 \times 0.045589$$

$$= 5.6$$

Expanded uncertainty, (U(Fipronil sulfone), ug/kg

$$= \mathrm{u(Fipronil\ sulfone)} \times \mathrm{k(where\ k = coverage\ factor\ of\ 2}$$

$$= 5.6 \times 2$$

$$= 11.2$$

HSA

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

$$C_X = C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}} \cdot \frac{1}{(1 - F_{mois})}$$

where

Factors	Details of the factors	How the values were determined
C_Z =	Mass fraction of fipronil sulfone in the calibration standard solution used to prepare the calibration blend	Gravimetric values of serial dilution of the calibration solution and the purity value of fipronil sulfone calibrant
F_{mois} =	Dry mass correction factor based on moisture determination of the study sample $Cx = Cx' \cdot 1/(1-F_{mois})$, where F_{mois} represents the moisture content and Cx' represents the mass fraction of fipronil sulfone in chicken egg powder based on wet mass of study sample	Gravimetric values of study sample before and after drying. The moisture content of individual packet was used for dry mass correction for each reporting package, i.e. 0.0175 g/g moisture content for package S16-042 and 0.0139 g/g moisture content for package S16-059.
m_Y =	Mass of internal standard solution added to the sample blend	Weighing
m_{Yc} =	Mass of internal standard solution added to the calibration blend	Weighing
m_{Zc} =	Mass of standard solution added to the calibration blend	Weighing
m_X =	Mass of study material in the sample blend	Weighing
R_X =	Observed isotope abundance ratio in the study material	Peak area ratio of [(383.0/255.0)/(389.0/257.0)] for GC-MS/MS and [(450.9/415.0)/(456.7/420.6)] for LC-MS/MS in the study material
R_Y =	Observed isotope abundance ratio in the internal standard	Peak area ratio of [(383.0/255.0)/(389.0/257.0)] for GC-MS/MS and [(450.9/415.0)/(456.7/420.6)] for LC-MS/MS in the internal standard solution
R_Z =	Observed isotope abundance ratio in the calibration standard	Peak area ratio of [(383.0/255.0)/(389.0/257.0)] for GC-MS/MS and [(450.9/415.0)/(456.7/420.6)] for LC-MS/MS in the calibration standard solution
R_B =	Observed isotope abundance ratio in the sample blend	Peak area ratio of [(383.0/255.0)/(389.0/257.0)] for GC-MS/MS and [(450.9/415.0)/(456.7/420.6)] for LC-MS/MS in the sample blend
R_{Bc} =	Observed isotope abundance ratio in the calibration blend	Peak area ratio of [(383.0/255.0)/(389.0/257.0)] for GC-MS/MS and [(450.9/415.0)/(456.7/420.6)] for LC-MS/MS in the calibration blend

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

$$C_X = F_{prec} \cdot F_{ip} \cdot F_{rec} \cdot F_{mtd} \cdot C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}} \cdot \frac{1}{(1 - F_{mois})}$$

where

additional factors (F) contributing to biases in the result value of fipronil sulfone were included, with an uncertainty associated to each factor.

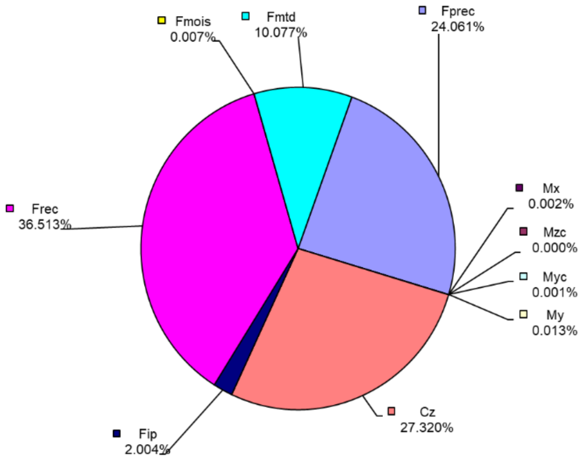
F_{prec} = Factor representing method precision

F_{ip} = Factor representing any bias in the result due to choice of different ion pairs

F_{rec} = Factor representing method recovery

F_{mtd} = Factor representing any bias in the result due to the choice of calibration method, i.e. exact matching IDMS vs linear regression IDMS

The full uncertainty budget for the determination of fipronil sulfone is given in the Table below:



Parameter	x_i	u_{xi}	u_{xi}/x_i	Contribution	Sources of uncertainty
F_{prec}	1	0.01816	1.8164%	24.061%	Standard deviation of seven independent determinations on the study material using GC-MS/MS and LC-MS/MS.
F_{ip}	1	0.00524	0.5242%	2.004%	Comparison of results obtained using different ion pairs on the same subsamples.
F_{rec}	1	0.02238	2.2376%	36.513%	Method recovery using in-house prepared quality control by spiking fipronil sulfone into the study material.
F_{mois}	0.0139	0.000314	2.2613%	0.007%	• Uncertainty in weighing before and after drying based on balance calibration certificate. • The larger relative standard deviation of the mean of moisture content determined using two subsamples from the two reporting packages. • Uncertainty due to possible re-absorption of moisture content in the study sample.
F_{mtd}	1	0.01176	1.1755%	10.077%	Comparison of results obtained using exact matching IDMS and linear regression IDMS on the same subsamples.
C_Z	0.1187	0.00230	1.9355%	27.320%	• Uncertainty in the purity value of fipronil sulfone certified reference material (NMIA P1731). • Uncertainty in weighing based on balance calibration certificate. • Bias in the preparation of calibration blends. • Bias in results determined using the solution calibration blend (in acetonitrile) vs the matrix-matched calibration blend.
m_Y	0.2026	0.0000849	0.0419%	0.013%	Uncertainty in weighing based on balance calibration certificate.
m_{Yc}	0.6127	0.0000849	0.0138%	0.001%	
m_{Zc}	1.5600	0.0000849	0.0054%	0.0002%	
m_X	0.5155	0.0000849	0.0165%	0.002%	
R_X, R_Y, R_Z	Negligible				
R_B, R_{Bc}	Uncertainty included in method precision				

JRC

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

Quantification of fipronil sulfone is done by external calibration by using the peak area. The calibration curve are obtained using the peak area. The concentration obtained from the application is the concentration in the extractant of the sample. An extra calculation is needed to finally get the concentration of the sample. C sample=(C extract * m extract)/m sample remark: result are dry mass and recovery corrected (recovery 95.3%)

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Uncertainty budget is estimated during the full method validation following a top down approach. Contributions are the uncertainty of repeatability, the uncertainty of the intermediate precision, the uncertainty of the trueness and the calibration. The expanded uncertainty is calculated taking into account the contributions specified in the following equation. RSDrep=4.2%, RSDip=3.4%, u trueness=0.5%, u cal=0.3% remark: n1: number replicates, n2: number of days

$$U = k * \sqrt{\frac{RSD_{rep}^2}{n_1} + \frac{RSD_{ip}^2}{n_2} + u_{true}^2 + u_{cal}^2}$$

NIM

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

1) Calculate the moisture content in egg powder sample:

$F_{mois} = (w_1 - w_2) / (w_1 - w_0)$

F_{mois}: Content of moisture in egg powder sample (g/g);
w_1: Total mass of weighing bottle and egg powder sample before drying (g);
w_2: Total mass of weighing bottle and egg powder sample after drying (g);
w_0: Mass of weighing bottle (g);

2) Calculate the mass fraction of fipronil-sulfone in egg powder sample:

$X = (A_s/A_i)_s \times (A_i/A_s)_r \times (M_s/M_i)_r \times (m_i)_s / w / (1 - F_{mois})$

X: Mass fraction of fipronil-sulfone in egg powder sample (µg/kg);
$(A_s/A_i)_s$: Peak area ratio of measurand and internal standard in sample solution;
$(A_i/A_s)_r$: Peak area ratio of internal standard and measurand in standard solution;
$(M_s/M_i)_r$: Mass ratio of measurand and internal standard in standard solution;
$(m_i)_s$: Mass of internal standard added in egg powder sample (ng);
w: Mass of egg powder sample (g);
F_{mois}: Content of moisture in egg powder sample (g/g).

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

Uncertainty budget					
Parameter	Source of uncertainty	Source of data	x_i	$u_{rel}(x_i)$	*Contribution*
Method precision	Precision of the method	Standard deviation of 12 independent determinations on the study material	121.0 μg/kg	1.77%	41.42%
Standard solution	Purity of calibration standard	CRM certificate	996 mg/g	0.15%	3.51%
	Weight of calibration standard	Balance calibration certificate	0.003 g	0.64%	14.98%
	Weight of solvent		30 g	0.00%	0.01%
	Weight of standard solution		1 g	0.02%	0.44%
	Weight of internal standard solution		1 g	0.02%	0.44%
	Weight of solvent		10 g	0.00%	0.04%
Sample pretreatment	Weight of sample		0.5 g	0.04%	0.89%
	Weight of internal standard solution		0.2 g	0.10%	2.22%
Method recovery	Recovery of the method	Method recovery of spiked standard into the study material	100%	1.45%	33.93%
Moisture content	Content of moisture	Standard deviation of 4 independent determinations on the study material	0.9848 g/g	0.09%	2.11%
Combined standard uncertainty			2.39%		
Relative expanded uncertainty (k=2)			4.78%		
Uncertainty Analysis Results					
Cx=			122.8 mg/kg		
u(x) =			2.94 mg/kg		
k=			2		
U(x) =			5.87 mg/kg		

NIMT

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

$$w_x = F_P.F_E.F_I.w_{zc} \cdot \frac{m_y \cdot m_{zc}}{F_{drymass}.m_x \cdot m_{yc}} \cdot \frac{R'_b}{R'_{bc}}$$

Where:

w_x = mass fraction of fipronil sulfone in chicken egg powder

w_{zc} = mass fraction of fipronil sulfone in the calibration solution used to prepare the calibration blend

m_y = mass of spike solution (internal standard) added to sample blend

m_{yc} = mass of spike solution (internal standard) added to calibration blend

m_{zc} = mass of standard solution added to calibration blend

m_x = mass of sample added to sample blend

F_E = extraction efficiency factor, given a value of 1

F_I = interference effect, given a value of 1

F_P = method precision factor, given a value of 1

$F_{drymass}$ = dry mass correction factor obtained from moisture content analysis

R'_b and R'_{bc} = observed isotope amount ratios in the sample blend and the calibration blend, respectively

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

$$\frac{u(w_x)}{w_x} = \sqrt{\left(\frac{u(w_{ZC})}{w_{ZC}}\right)^2 + \left(\frac{u(m_y)}{m_y}\right)^2 + \left(\frac{u(m_{yc})}{m_{yc}}\right)^2 + \left(\frac{u(m_{zc})}{m_{zc}}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(F_{drymass})}{F_{drymass}}\right)^2 + \left(\frac{u(F_I)}{F_I}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2}$$

Where;

u(wz,c) is the standard uncertainty of the mass fraction of analyte in the calibration solution used to prepare the calibration blend. The value was estimated from the purity of fipronil sulfone standard, masses weighed for preparation of stock solutions and uncertainty using different standards (standard comparison).

$u(m_y)$, u(my,c), u(mx) and u(mz,c) are standard uncertainties of the masses. These values were estimated from the bias and precision effect of the balance.

u (F_P) is the standard uncertainty of the precision factor. This value was estimated from standard deviation of the multiple IDMS results.

$u(F_I)$ is the standard uncertainty of the interference effect. This value was estimated from potential bias between primary ion pair and secondary ion pair of the MRM program.

$u(F_E)$ is the standard uncertainty of the extraction efficiency factor which was estimated from the liquid-solid extraction and solid-phase –extraction.

$u(F_{drymass})$ is the standard uncertainty of the dry mass correction factor which was estimated from the moisture content analysis.

Note: For the uncertainty contributing to the R'b and R'b,c ,the precision in measuring the isotope amount ratios of the analyte and the internal standard in the sample and calibration blends was assumed to be incorporated in the overall method precision. The effect of any biases on these ratios was assumed to be negligible as any systematic biases should cancel out since the calibration blends and sample blends were exact-matched for analyte concentration and isotope ratio. Other biases that may arise from interferences, extractions are captured in other factors.

Combination of Uncertainties

Factor	**Values**	**Uncertainties**	
	x	**u(x)**	**u(x)/(x)**
Measurement equation factors			
Method Precision, F_P	1.0000	0.02918	2.918%
mzc	0.29314	0.000028	0.0096%
my	0.29413	0.000028	0.0096%
myc	0.29578	0.000028	0.0095%
Fdrymass	0.97803	0.000575	0.0588%
mx	0.50161	0.000028	0.0056%
wz	199.1682	3.032840	1.5228%
Additional Factors		Enter u(x) = 0 and veff = 1 for unused factors.	
Extraction effects, F_E	1.000	0.0100	1.000%
Interference from two different ion pairs, F_I	1.000	0.0050	0.503%

Uncertainty Analysis Results

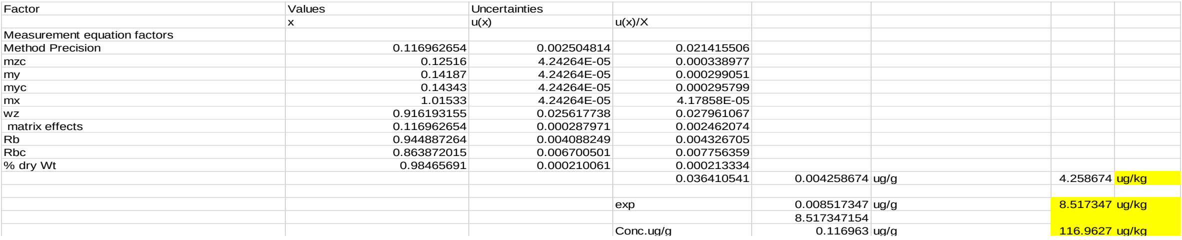
Cx=	**120.8**	ng/g
u(x) =	4.200	ng/g
u(x)/x =	3.48%	
Veff(total) =	13.621	
k=	2.16	(@ 95% level)
U(x) =	**9.074**	
%U(x) =	**7.51%**	

TISTR

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

$$w_x = F_P . F_E . F_I . w_z \cdot \frac{m_y \cdot m_{zc}}{F_{drymass} . m_x \cdot m_{yc}} \cdot \frac{R'_b}{R'_{bc}}$$

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.



Factor	Values	Uncertainties					
	x	u(x)	u(x)/X				
Measurement equation factors							
Method Precision	0.116962654	0.002504814	0.021415506				
mzc	0.12516	4.24264E-05	0.000338977				
my	0.14187	4.24264E-05	0.000299051				
myc	0.14343	4.24264E-05	0.000295799				
mx	1.01533	4.24264E-05	4.17858E-05				
wz	0.916193155	0.025617738	0.027961067				
matrix effects	0.116962654	0.000287971	0.002462074				
Rb	0.944887264	0.004088249	0.004326705				
Rbc	0.863872015	0.006700501	0.007756359				
% dry Wt	0.98465691	0.000210061	0.000213334				
			0.036410541	0.004258674	ug/g	4.258674	ug/kg
			exp	0.008517347	ug/g	8.517347	ug/kg
				8.517347154			
			Conc.ug/g	0.116963	ug/g	116.9627	ug/kg

UME

1) The measurement equations used to calculate the mass fraction of each analyte. Please provide details of all the factors listed in the equations and indicate how these values were determined.

$$Cx = \frac{A_x \times n_{\imath sx}}{A_{\imath sx} \times RF \times M_{sample}} \qquad RF = \frac{A_{xstock} \times C_{\imath sstock}}{A_{\imath sstock} \times C_{xstock} \times P}$$

C_x	Mass fraction of unknown analyte in sample (µg/kg)		
A_x	Area of unknown native analyte in sample	A_{xstock}	Area of native analyte in stock solution used for calibration
$A_{\imath sx}$	Area of labelled analyte in sample	$A_{\imath sstock}$	Area of labelled analyte in stock solution used for calibration
$n_{\imath sx}$	total amount of added labelled compound (ng)	$C_{\imath sstock}$	Mass fraction of labelled analyte in stock solution used for calibration
RF	Response factor	C_{xstock}	Mass fraction of native analyte in stock solution used for calibration
M_{sample}	Sample intake (g)	P	purity

2) Estimation of uncertainties for each factor. Give a complete description of how the estimates were obtained and combined to calculate the overall uncertainty. Please provide a table detailing the full uncertainty budget.

$$Cx = \frac{A_x \times n_{\imath sx}}{A_{\imath sx} \times RF \times M_{drysample}} \qquad RF = \frac{A_{xstock} \times C_{\imath sstock}}{A_{\imath sstock} \times C_{xstock} \times P} \qquad n_{ISX} = M_{IS} \times C_{ISstock}$$

$$Cx = \frac{A_x \times A_{ISX} \times C_{xstock} \times P \times M_{\imath sx} \times C_{\imath sstock}}{A_{\imath sx} \times A_x \times C_{\imath sstock} \times M_{drysample}} \qquad Cx = \frac{M_{\imath sx} \times C_{xstock} \times P}{M_{drysample}}$$

Bottom up approach was used

Sources :

1 Mass of drysample intake ($M_{drysample}$)
2 Spiking of labelled stock solution (M_{ISX})
3 Native stock solution (C_{xstock})
4 Repeatability
5 Recovery

1-2/Mass of dry sample intake and spiking of labelled stock solution

		Value	Standard Measurement Uncertainty
Mass of Tare	Calibration	mtare (g)	umcalibration 0.000233
Mass of egg powder intial	Calibration	meggpowder (g) 0.5	umcalibration (g) 0.000233
Mass of egg powder final	Calibration	meggpowder (g) 0.485	umcalibration (g) 0.000233
Mass of IS stock solution	Calibration	mIS (g) 0.12	umcalibration (g) 0.000233

$$u(M_{DSI}) = \sqrt{(u_{mcalibtare})^2 + (u_{mcalibsample\,int})^2 + (u_{mcalibsample\,final})^2 + (u_{mcalibIS})^2}$$

mdrysample (g)	uMDSI
0.485	0.00047

$$u(M_{IS}) = \sqrt{(u_{mcalibtare})^2 + (u_{mcalibsample\,int})^2 + (u_{mcalibIS})^2}$$

mIS (g)	uMIS
0.12	0.00040

3-Native Stock Solution

1st stock solution

Purity of Fipronil sulfone	upurity
0.9889	0.00135

	Value	Standard Measurement Uncertainty		
Mass of Tare				
Calibration	mtare (g)	umcalibration		
		0.000233		
Mass of fipronil sulfone				
Calibration	mfipronil sulfone(g)	umcalibration (g)		
	0.02	0.000233		
Mass of solvent				
Calibration	mSolvent (g)	umcalibration (g)	msample	umsample
		0.000233	0.02	0.00040

$$u(C_{1ststocksol}) = \sqrt{(u_{purity})^2 + (u_m)^2}$$

C1ststocksol (mg/kg)	uC1stock sol
1068.33	0.0014

$$u(C_{2ndstocksol}) = \sqrt{(u_{1ststcsol})^2 + (u_m)^2}$$

2nd stock solution

C2ndstocksol	uC2ndstock sol
56.90	0.0015

$$u(C_{3rdstocksol}) = \sqrt{(u_{2ndstcsol})^2 + (u_m)^2}$$

3rd stock solution

C3rdstocksol (mg/kg)	uC3rdstock sol
0.599	0.00152

4- Repeatability

$$u(r) = \frac{SD}{\sqrt{n}}$$

Results	
123.33	
123.99	
122.98	
123.75	
123.66	
124.14	
Average	123.47
STD	0.458
u_r=SD/SQRT (n)	0.187
n (number of replicate)	6

5-Uncertainty of Recovery

Preparation of spiked sample

	Value	Standard Measurement Uncertainty			
Mass of chicken egg sample	$m_{eggpowder}$(g)	umcalibrationsample (g)	umSI	0.000466	g
Calibration	0.5	0.000233	mSI	0.5	g
			umSI/mSI	0.000932	
Mass of Tare	m_{tare} (g)	umcalibrationtare			
Calibration	5	0.000233			
			$u_{C3rd\ stock\ solution}$ (mg/kg)	0.00152	(mg/kg)
Mass of Native standard addition	m_{NS} (g)	umcalibration	$C_{3rdstock\ solution}$ (mg/kg)	0.599	(mg/kg)
Calibration	0.1	0.000233	u3rdSS/C3rdSS	0.00254	
Mass of Labelled standard addition	m_{LS} (g)	umcalibration	ucert	0.326	
Calibration	0.1	0.000233	Ccert	120.74	
			uobs	3.470	
			Cobs	121.38	

$$u(R_m) = R_m\sqrt{\left(\frac{u(\overline{C_{obs}})}{\overline{C_{obs}}}\right)^2 + \left(\frac{u(C_{cert})}{C_{cert}}\right)^2} \qquad R_m = \frac{\overline{C_{obs}}}{C_{cert}}$$

uCobs	standard measurement uncertainty of observed concentration of analyte
Cobs	observed concentration of analyte
uCcert	standard measurement uncertainty of certified concentration of analyte
Ccert	certified concentration of analyte
Rm	

$$\frac{u_c(Analyte)}{c_{Analyte}} = \sqrt{(\frac{u(M_{DSI})}{M_{DSI}})^2 + (\frac{u(M_{IS}\ )}{M_{IS}})^2 + +(\frac{u(C_{NSS})}{C_{NSS}})^2 + (\frac{u(r)}{r})^2 + (\frac{u(R_m)}{R_m})^2}$$

Uncertainty Budget of Fipronil Sulfone				
Parameters	Unit	Value (X)	u(x)	u(x)/X
Sources :				
Mass of drysample intake (Mdrysample)	g	0.49	0.00047	0.000961
Spiking of labelled stock solution (MISX)	g	0.12	0.0004	0.00336
Native stock solution (CNSS)	mg/kg	0.60	0.002	0.00254
Repeatability		123.5	0.187	0.00151
Recovery		1.01	0.0289	0.0287
Relative Standard Measurement Uncertainty				0.029
Result (µg/kg)		123.5		
Combined Standard Measurement Uncertainty			3.6	
Expanded Uncertainty (k=2)			7.2	
Relative Measurement Uncertainty (%)			5.8	

Appendix II: Other information provided in report of results

GLHK

(i) Suggested protocol for moisture determination was used.

(ii) For reference, the moisture-content-uncorrected analyte contents are given as below:

Fipronil sulfone : 119.7 µg/kg.

(iii) Mass fraction is average measurement value from LC-MS/MS and LC-HRMS went through identical sample preparation.

(iv) 3 sub-samplings were taken from the packet. From each sub-sampling, the cleaned extract was analyzed 5 times on LC-MS/MS to obtain a mean measurement value and another 5 times on LC-HRMS for a mean measurement value, calibrated using same CRM and calibration function.

(v) Mass of electron was included in the calculation of monoisotopic experimental mass ion.

HSA

The comparison study material was spiked gravimetrically with fipronil sulfone solution prepared from GBW(E)062661 (NIM). The spiked material was analysed in parallel with the SBs for quality control (QC). Each QC was subjected to the same extraction and clean-up as the study sample. The recovery results ranged between 97.9% to 100.7% (for GC-MS/MS) and 95.5% to 99.3% (for LC-MS/MS) were used to estimate the uncertainty of method recovery (Frec).

A matrix-matched calibration blend prepared from in-house prepared chicken egg powder (pre-screened to contain negligible amount of fipronil sulfone) was also used to analyse the SBs. No significant difference in the results was found using Student's t-test. The results were used to estimate the uncertainty in the use of matrix-matched calibration vs solution calibration which was incorporated in the uncertainty of Cz.

Subsamples of the study samples were analysed using different ion pairs (quantifying vs qualifying) from GC-MS/MS and LC-MS/MS. The larger uncertainty from the GC-MS/MS and LC-MS/MS results was used to estimate the uncertainty in the use of different ion pairs (Fip).

The equilibration time of the SB at about 4 °C after spiking with the internal standard solution was studied. No significant difference in the results obtained from the SB equilibrated overnight vs equilibrated for two nights was found. Hence, the SB was equlibrated overnight at 4 °C.

Extraction using acidified solvent was studied. No significant difference in the results obtained from the SB extracted using acetonitrile vs using acidified acetonitrile was found.

NIM

1) The effect of IS spiked before or after reconstitution was studied, no significant difference of results was found.

2) The determination results were also verified by GC-IDMS method.

In detail, 1 g of test sample was weighed into a 50 mL centrifuge tube, and 3 mL of water was added for reconstitution. Then, an appropriate amount of isotopically-labeled internal standard solution was added and weighted into the same centrifuge tube. The sample was vortexed and equilibrated for 5 min at room temperature. Following the equilibration, the sample was added with 20 mL of acetonitrile, vortexed for 1 min and sonicated for 5 min. Then, 1 g of NaCl was added and the tube was vortexed for 1 min and centrifugated for 5 min at 9000 rpm. The supernatant solution was collected then mixed with 20 mL of hexane. After 1 min vortex and 2 min centrifugation at 3000 rpm, the supernatant hexane was decanted. Then, 10 mL acetonitrile extract was purified by HLB column. After nitrogen evaporation to dry at 40 °C, 1 mL acetonitrile was added to reconstitute the residual. After filteration through a 0.22 µm GHP filter, the sample was injected for GC-MS analysis. Injection volume of sample was 1 µL in splitless mode and injection port temperature was 100 °C. Oven temperature ramping was: 100 °C (1 min), 40 °C/min to 170 °C, 10 °C/min to 265 °C, 50 °C/min to 300 °C (1 min). Helium worked as carrier gas at 1 mL/min though column. The mass spectrometer was operated in electron ionization (EI) mode with ionization energy of 70 eV. The source temperature was kept on 230 °C. The quantitation ions for fipronil sulfone and fipronil sulfone-13C4, 15N2 were 382.8 and 389.0, and the qualification ions were 384.8 and 391.0, respectively.

NIMT

Sample packaging is not convenient to use. It is hard to find blank sample.

UME

In order to verify the moisture content of the sample, determination of moisture was also carried out by Karl Fischer and TGA measurements.

However, the result of moisture content obtained by Karl Fischer is 0.029 g/g, by TGA is 0.031

Calibration curve obtained by 6 multiple points was used for verification of the value-assignment

Appendix III: Core competency tables reported by the participating laboratories

GLHK

APMP.QM-S16	***GLHK***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*pK*ow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 µg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		NIM China Fipronil Sulfone CRM : GBW(E)062661
Identity verification of analyte in calibration material. #	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	Retention time, LC-MS/MS with 3 MRM transitions, LC-HRMS monoisotopic molecular ions
Extraction of analyte(s) of interest from matrix	√	Mechanical vertical shaking
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	Syringe-SPE cartridge pass-through clean-up
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	√	LC-MS/MS and LC-HRMS
Calibration approach for value-assignment of analyte(s) in matrix	√	IDMS - bracketing
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

HSA

APMP.QM-S16	***HSA***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*pK*ow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 µg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	High purity certified reference material (CRM) of fipronil sulfone (P1731) from the National Measurement Institute, Australia (NMIA) with a purity value of 99.5 ± 0.4 % was used as the calibrant. The purity value of the calibrant was verified with fipronil sulfone CRM GBW(E)062661 (99.6 ± 0.3%) from the National Institute of Metrology (NIM), China.
Identity verification of analyte in calibration material. #	✓	The identity of fipronil sulfone was verified by comparing its mass spectrum with fipronil sulfone CRM GBW(E)062661 from NIM, based on retention time and m/z ratio on the GC-MS/MS and LC-MS/MS.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and m/z ratio of the parent and daughter ions on the LC-MS/MS and GC-MS/MS.
Extraction of analyte(s) of interest from matrix	✓	The study material was weighed into a 50-mL amber centrifuge tube, and about 1.5 g of water was added. The mixture [sample blend (SB)] was vortexed and equilibrated overnight at about 4 °C after gravimetrically spiking with an appropriate amount of isotope-labelled fipronil sulfone (Cambridge Isotopes Laboratories) internal standard solution. Following the equilibration, the SB was added with 5 mL of acetonitrile, shaken on a multitube shaker for 1 min and sonicated for 5 min. Then, 1 g of sodium chloride was added and vortexed to mix well. After the sample was centrifuged at 4,200 rpm for 10 min, the supernatant solution was collected. The extraction with acetonitrile was repeated two more times. The combined supernatant was re-extracted with 10 mL of n-hexane. After vortexing, the mixture was centrifuged at 3,000 rpm for 5 min, and the supernatant was decanted. The bottom acetonitrile layer was

		evaporated to dryness under nitrogen and reconstituted with 1 mL of acetonitrile.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	The reconstituted solution was transferred into an amicon Ultra-0.5 centrifugal filter (consisting of regenerated cellulose membrane), and was centrifuged at 13,000 rpm for 10 min before injecting the solution for GC-MS/MS and LC-MS/MS analysis.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	a) GC-MS/MS: Thermo Scientific TSQ^{TM} 9000 triple quadrupole GC-MS/MS system; and b) LC-MS/MS: AB Sciex Qtrap® 5500 MS/MS instrument coupled with Shimadzu Prominence UFLC XR LC system
Calibration approach for value-assignment of analyte(s) in matrix	✓	A single-point calibration, using exact-matching IDMS method.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	The comparison study material was spiked gravimetrically with fipronil sulfone solution prepared from GBW(E)062661 (NIM). The spiked material was analysed in parallel with the SBs for quality control (QC). Each QC was subjected to the same extraction and clean-up as the study sample. The recovery results ranged between 97.9% to 100.7% (for GC-MS/MS) and 95.5% to 99.3% (for LC-MS/MS) were used to estimate the uncertainty of method recovery (F_{rec}).
Other		

NIM

APMP.QM-S16	***NIM***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*pK*ow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 μg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	√	High purity CRM of fipronil sulfone (GBW(E) 062661) from NIM, China was used.
Identity verification of analyte in calibration material. #	√	The identity of fipronil sulfone was confirmed by 1H NMR and LC-MS/MS.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	√	1) HPLC based Mass Balance method by subtracting the impurities including structure related impurities, water, VOCs and inorganic residues; 2) Quantitative nuclear magnetic resonance (qNMR) method traceable to high purity CRM of ethyl hydroxybenzoate (GBW(E)100064) from NIM, China with a purity value of 99.7 ± 0.2%.
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	Retention time and MRM transitions with two ion pairs
Extraction of analyte(s) of interest from matrix	√	Liquid – liquid extraction by acetonitrile, vortex, shake, and ultrasonic extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	Oasis PRiME HLB SPE cleanup
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	√	LC-MS/MS: Waters UPLC Xevo TQ-S
Calibration approach for value-assignment of analyte(s) in matrix	√	IDMS, Single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	√	Method recovery of spiked standard into the study material was 96.33% ~ 101.12% (mean = 98.55%, RSD = 1.77%, n = 6).
Other	√	The determination results were also verified by GC-IDMS method.

NIMT

APMP.QM-S16	***NIMT***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*p*Kow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 µg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Yes, high pure standard fipronil-sulfone (CRM) (GBW(E)062661 from NIM China
Identity verification of analyte in calibration material. #	✓	LC-MS/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	-	
For calibrants which are a calibration solution: Value-assignment method(s).#	-	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Chromatographic retention time (LC-MS/MS), MRM mode with two ion pairs for identification
Extraction of analyte(s) of interest from matrix	✓	Liquid-solid extraction, Liquid-liquid extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE cleanup
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	-	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) Exact-matching double IDMS (matrix-matched calibration blends) b) single-point, bracketing calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	-	
Other	-	

TISTR

APMP.QM-S16	***TISTR***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*pK*ow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 μg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?		<CRM NMIA P1731:Fipronil Sulfone>
Identity verification of analyte in calibration material. #		<not confirm> from Certificate the identity was confirmed by NMR, IR, MS. The certified purity was obtained by mass balance .,
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#		<No>
For calibrants which are a calibration solution: Value-assignment method(s).#		<No>
Sample Analysis Competencies		
Identification of analyte(s) in sample		<GC-MSMethods used to identify the analyte>
Extraction of analyte(s) of interest from matrix		<Acetonitrile>
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)		<C18, PSA,MgSO4>
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)		<No>
Analytical system		<GC-MS/MS>
Calibration approach for value-assignment of analyte(s) in matrix		<Matrix matching IDMS>
Verification method(s) for value-assignment of analyte(s) in sample (if used)		<No>
Other		

UME

APMP.QM-S16	***UME***	**Fipronil-sulfone in Chicken Egg Powder**
Scope of Measurement: Successful participation in this APMP supplementary comparison will demonstrate participant's capabilities in determining the low-polarity analytes (*p*Kow < -2) with molecular mass range from 300 to 500 g/mol at mass fraction levels of 4 to 400 µg/kg in high fat, high protein, low carbohydrate food matrices. This may include demonstration of measurement capabilities such as: (1) value assignment of primary reference standards; (2) value assignment of calibration solutions; (3) extraction of analyte of interest from the matrix; (4) cleanup and separation of analyte of interest from other interfering matrix or extract components; (5) separation and quantification using techniques such as LC-MS, LC-HRMS or GC-MS.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	√	*Highly pure substance was used* *Research Chemicals, Cat No: F342215, CAS: 120068-36-2, 100 mg neat*
Identity verification of analyte in calibration material. #	√	*UHPLC-HR-MS/MS*
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	√	*The purity determination of Fipronil Sulfone was performed by qNMR in traceability chain of UME-CRM-1301. The purity is 98.89%, uncertainty is 0.27% at k=2 and 95% confidence level*.
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	-
Sample Analysis Competencies		
Identification of analyte(s) in sample	√	*UHPLC-HR-MS/MS, retention time, m/z*
Extraction of analyte(s) of interest from matrix	√	*Solid/liquid extraction by acetonitrile, ultrasonication and centrifugation*
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	√	*primary secondary amine (PSA), octadecyl-modified silica (C18), graphitized carbon black (GCB) centrifugation*
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	-
Analytical system	√	*UHPLC-HR-MS/MS*
Calibration approach for value-assignment of analyte(s) in matrix	√	*IDMS, Single point*
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	-
Other	√	*Calibration curve obtained by 6 multiple points was used for verification of value-assignment*