

초청강연 I

농산물 중 잔류농약 허용기준 설정 및 분석법 체계

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최근 안전한 농산물을 섭취하는 것은 국민들 사이에서 건강의 필수불가결한 요소로 인식되고 있으며 이에 따라 농림부 및 식약처 등 정부기관에서도 안전한 먹거리 공급을 주요 국정목표 중 하나로 설정하고 있다. 농산물 재배과정에서 작물 보호를 위하여 불가피하게 사용되는 농약은 정도에 따라 다르기는 하나 필연적으로 수확물 중에 일부 잔류하게 된다. 이러한 잔류농약은 농산물의 안전성 확보에 가장 큰 영향을 미치는 인자 중의 하나로 인식되고 있으며 이에 따라 잔류농약에 대한 관리 및 규제가 계속적으로 강화되고 있다. 현재 농산물 및 식품 중 잔류농약의 안전성 확보에 대한 가장 중요하면서도 실용적인 관리 체계는 잔류허용기준 (MRL)의 설정 및 운용이다.

먼저 작물 재배과정에서 농약을 사용을 하였을 경우, 수확물 중에 잔존하는 잔류농약에 의한 만성독성학적 위해성 (risk)은 농약 자체의 만성독성 (chronic toxicity)과 노출량 (exposure)의 곱으로 표시된다. 만성독성은 실험동물에 대한 최대무작용량을 안전계수로 보정, 인간에 대하여 적용한 일일섭취허용량 (ADI)의 역수로 표시된다. 노출량은 식품 중 잔류농도에 식품 섭취량의 곱으로 표시되며 이 수치가 ADI를 초과하지 않도록 관리하는 것이 안전성 확보의 관건이다. ADI는 농약 고유의 독성이므로 그 수치는 일정하다. 또한 노출량 항목 중 식품 섭취량도 거의 일정한 수준이므로 관리가 가능한 부분은 식품 중 잔류 수준에 한정된다.

농산물 중 잔류 수준의 실질적 관리는 농약 및 농산물별로 MRL을 합리적으로 설정하는 것으로부터 출발한다. 즉, 정상적 경작조건에서 해충 방제를 위한 농약 사용은 허용하되 오남용을 방지하도록 수확물 중 잔류 수준의 상한선을 설정하는 것이다. 이를 근거로 농약 사용이 허가된 작물의 각 MRL에 농산물별 섭취량을 곱하여 합산한 이론적 최대섭취허용량 (TMDI)이 잔류농약의 섭취경로를 감안, ADI의 80%를 초과하지 않도록 농약 사용을 제한함으로써 위해에 대한 안전성이 확보된다.

MRL 설정은 정상적 경작 형태 (GAP)에서 최대 농약 살포 조건으로 수행한 잔류성 시험 (supervised trial)의 실험적 결과와 통계학적 평가에 근거한다. 즉, 각 잔류성 시험으로부터 수확물 중 평균 잔류량을 실험적으로 얻고, 다수 잔류성 시험결과와 변이성을 통계학적으로 평가하여 상위 95% 수준에서 설정한다. 통계학적 처리 방법 및 그에 요구되는 작물잔류성 시험 수는 국가 및 국제기관별로 상이하나 최근 OECD에서 제안한 MRL 설정법으로 통합되는 추세이다.

작물잔류성 시험에는 GAP에 근거한 표준적 포장시험과 더불어 신뢰성이 확보된 잔류분석 체계가 요구된다. 잔류농약 분석법은 그 분석 목적 및 1회당 분석성분 수에 따라 다성분 동시분석법 (multiresidue method, MRM)과 개별 분석법 (individual method)으로 대별된다. MRM의 경우 분석조각 1회당 검사가 가능한 농약수가 수십 가지 이상으로 분석효율은 매우 높으나 각각의 성분들에 대한 분석의 정밀도나 신뢰성은 다소 열등한 단점이 있다. 즉 다성분 동시분석법에서는 대상성분들을 물리화학적 특성범위별로 그룹핑하고 그룹별로 시료 추출, 정제 및 기기분석과정에 의하여 시료분석을 수행하므로 1개 분석 대상성분마다 최적의 분석법을 적용하는 것이 불가능하다. 따라서 각 대상성분에 대하여 최적화된 시료 조제 및 기기분석조건이 적용되는 개별 분석법에 비하여

분석 감도, 정밀성 및 신뢰도가 떨어질 수밖에 없다. 이에 따라 MRM은 적절한 분석기준을 만족하면서 분석효율에 중점을 두어 개발되며 주로 잔류농약검색용 (screening) 목적에 적합하다.

개별 분석법은 개별 농약성분별로 최적화시킨 분석법으로 MRM에 비하여 상대적으로 분석의 효율은 낮으나 최고의 분석 감도, 정밀성 및 신뢰도가 보장된다. 따라서 어떠한 시료의 다성분 검색 결과 검출된 의심성분을 보다 정확히 정성 및 정량하며, 특히 법적 및 행정적 목적으로 분석의 신뢰성이 요구될 때 이용된다. 또한 MRM에 포함시킬 수 없는 성분들에 대하여는 각각의 개별 분석법 확립이 잔류관리를 위하여 필수적이다.

작물잔류성 시험에는 정확한 정량 및 재현성을 요구하므로 MRM 보다는 개별분석법을 적용하여야 한다. 개별분석법을 적용하기 위해서는 분석법의 체계적 확립 및 실제 시료에 대한 분석법 검증이 필수적으로 선행되어야하며 실제 포장 시료군의 분석 시마다 분석의 항상성을 검증하기 위하여 추가의 회수율 시험을 요구하고 있다.

본 발표에서는 농산물 중 잔류 농약에 대한 안전성 확보를 위한 MRL 설정 체계를 설명하고자 한다. MRL의 기본적 개념, 설정 이론과 평가체계를 중심으로 국가/국제기관 별 평가법과 현재 국제적으로 통합되고 있는 설정 체계를 소개함과 동시에 국내 설정 체계와의 비교 및 개선 사항을 제시하고자 한다. 또한 MRL의 설정에 필수적으로 요구되는 작물잔류성 시험의 기본 요건 및 수행과 잔류분석법의 확립, 검증 및 재현성 확보에 필요한 사항을 국제 기준과의 부합성과 연관하여 제시하고자 한다.

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Use of Pesticides in Agriculture

- **Benefit** : Sustainable production of agricultural commodities.
- **Risk** : Side effects from acute and/or chronic toxicity.
- **Justification** : Economic poison (benefit >> risk).



Occurrence of Pesticide Residues

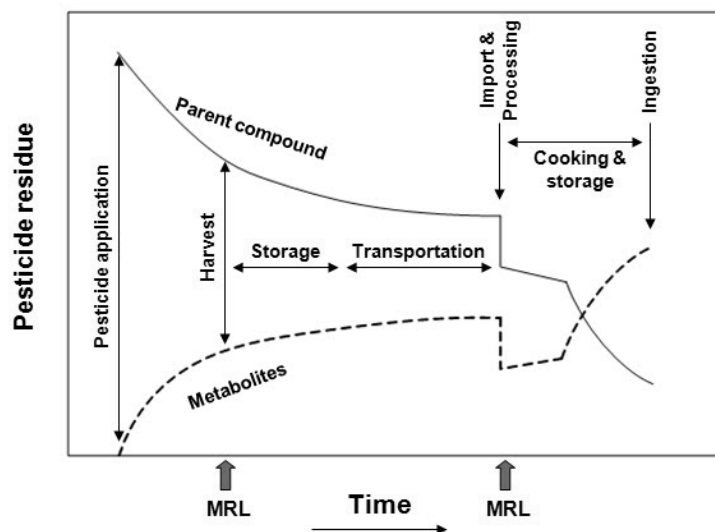
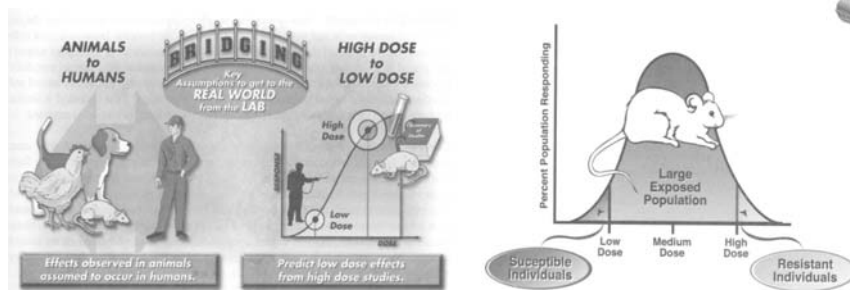


Fig. Dissipation and intake of the pesticide residue.

Chronic Risk by Pesticide Residues

- **Chronic risk = Hazard × Exposure**
 - **Hazard : 1/ADI (acceptable daily intake).**
 - **ADI = NOAEL / safety factor.**
 - **Exposure : Residue × food consumption.**
 - **Upper limit of exposure : ADI.**



Exposure Management of Pesticide Residues

- **Risk-cup for exposure of pesticide residues.**
 - Food intake 80%.
 - Drinking water 10%.
 - Residential exposure 10%.
- **MRLs (maximum residue limits) or tolerances for foodstuffs.**
 - Registration of crop for use.
 - Estimation of residue level in the harvest.
 - Establishment for upper limit of residue occurrence.

- **TMDI (theoretical maximum daily intake).**

- ? (MRL × Food consumption) = 80% of ADI.

- TMDI estimation for imidacloprid

Crop registered	Food consumed (kg)	MRL (mg/kg)	Pesticide intake (mg)
Mandarin	0.0832	0.5	0.04160
Kimchi cabbage	0.0118	3.5	0.04130
Lettuce	0.0034	5.0	0.01700
Cabbage	0.0048	3.5	0.01680
Apple	0.0318	0.5	0.01590
Pear	0.0244	0.5	0.01220
Rice	0.2211	0.05	0.01106
Others (16 crops)	0.0419	0.1 ~ 5.0	0.03363
TMDI			0.22312 (6.1%)

* Acceptable daily intake for human : ADI 0.06 mg/kg × 55 kg = 3.3 mg

Establishment of MRLs

- **Maximum residue limits (MRLs) or tolerances as legal limits for pesticide residues on/in foods and feeds with balancing.**
 - Should be set high enough so that most residues produced by the legal use of a pesticide are below the MRL value.
 - Can not be set so high that it is impossible to detect misuse.
- **Proposal of a MRL value.**
 - Using dataset from supervised field trials under GAP legally permitted.

Methods for Estimating MRLs

- **Round-up (eyeball) method.**
 - Rounding up of the largest residue value in a subjective manner.
 - Degree of round-up based on the reviewer professional judgment with no statistical theory and little guidance.
 - Production of different MRL values using the same or similar datasets.
- **Necessity of SOPs to reduce reviewer bias and to enhance the reproducibility of MRL determination in a objective manner.**

- **EU method I and II (1997).**
 - EU method I based on normal distribution with elimination of outlier by Dixon's *Q*-test and setting at the 95th percentile.
 - EU method II based on non-distributional empirical estimates setting at the 2 x 75th percentile using the Weibull procedure.
 - Right-skewed lognormal distribution provides better approximation for most residue dataset.
 - Real residue values may erroneously be eliminated.
 - No statistical support for the 2 x 75th percentile to ensure MRL high enough so as not to be exceeded.

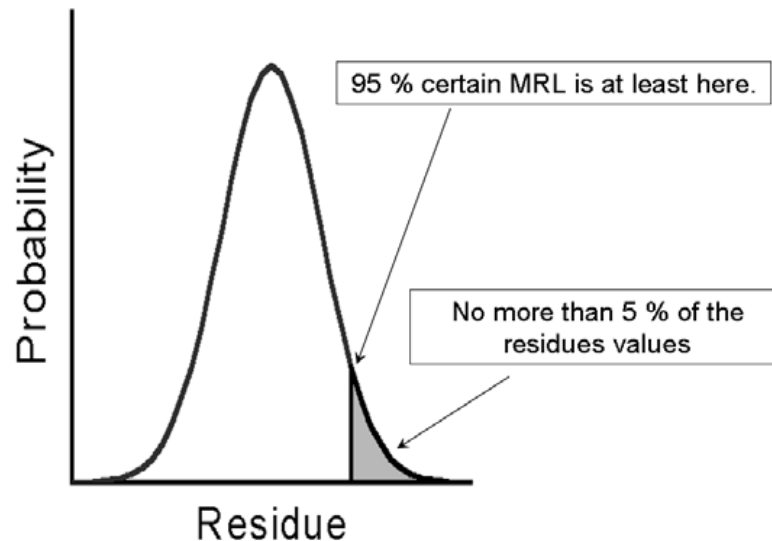


Fig. Assumption of normal distribution in EU Method I.

● **NAFTA method (2005).**

- **Dataset showing lognormal distribution.**
Minimum of 95% upper confidence limit on the 95th percentile, 99th percentile estimate (95/99 rule) for large dataset ($n = 15$), and UCLMedian95 ($3.9 \times$ upper prediction limit of the median) for small dataset ($n < 15$).
- **Dataset showing non-lognormal distribution.**
Mean + $3 \times$ SD (upper bound estimate of the 89% percentile for any distribution).
- **MLE (maximum likelihood estimation) technique for censoring (10 ~ 15%) to supplement a dataset.**
- **Assessment of lognormality assumption by Shapiro-Francia test.**
- **Large size of dataset is required for lognormal estimation.**
- **The unusually high MRL proposal in relation to the highest residue when residue data do not fit to lognormal distribution well at the upper end (Tailing effect).**

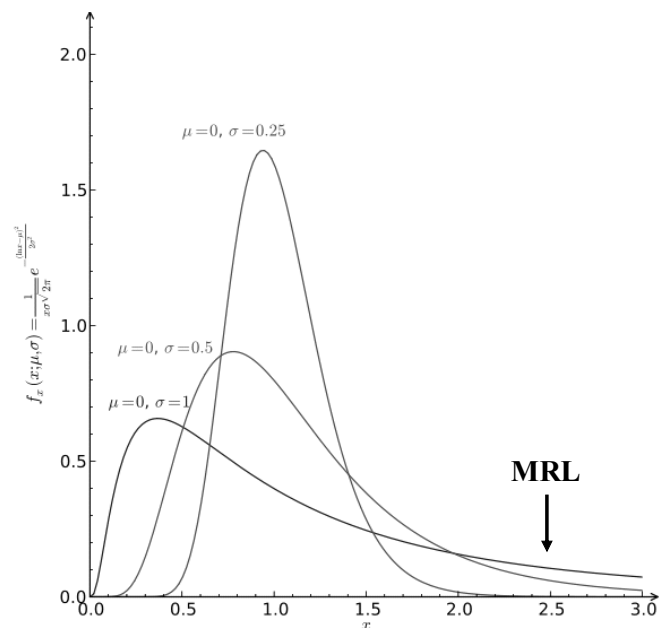
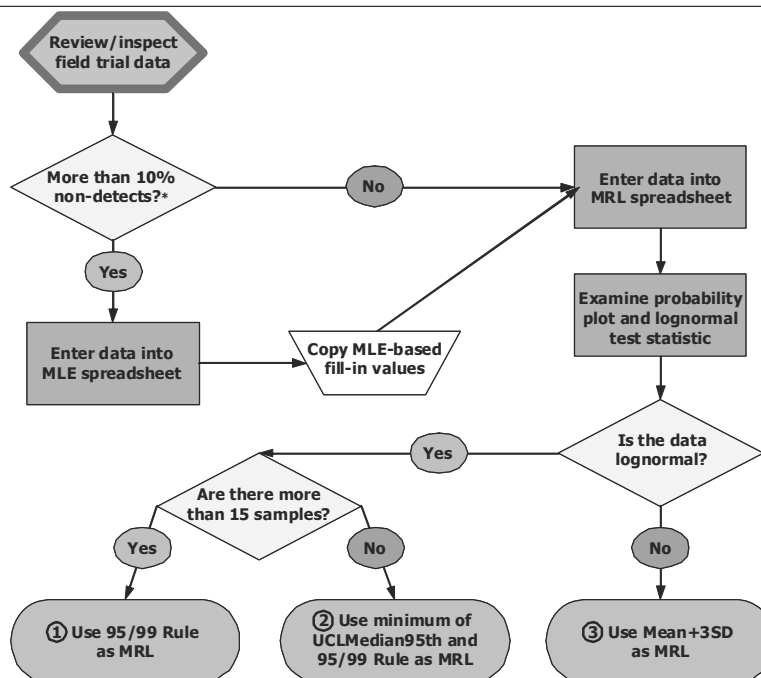


Fig. Assumption of lognormal distribution in NAFTA Method.



*If more than 60% of the data are non-detects, the fill-in values from the MLE spreadsheet should be used with caution.

Fig. Algorithm of NAFTA MRL calculator.

Development of OECD MRL Calculator

- Earlier version was based on NAFTA calculator and modified to harmonize EU methods and for the small dataset.
- Statistical fits using lognormal, normal and Weibull distributions have all shown the tailing effect.
- Earlier regulatory ceiling for MRL proposal ($2 \times$ Highest Residue and $3 \times$ Mean) eliminated real residue value as outlier, if unusually high, in EU method I.
- Non-distributional approach for the regulatory ceiling may be used to decrease the tailing effect.

Guiding Principles of OECD MRL Calculator

- Practical implementation of sound statistical methods.
- Simplicity for use without requiring extensive statistical knowledge.
- Clear and unambiguous MRL proposal for most residue dataset of field trials.
- Harmonization with EU and NAFTA procedures as much as possible.

Statistical goal

- **MRL proposal in the region of the 95th percentile (p95) of the underlying residue distribution.**
- **Conservative sense to make errors by overestimating p95 than by underestimating it for most datasets.**
- **Non-distributional approach to decrease the tailing effect.**
- **No restriction in relation to the earlier regulatory ceiling preventing MRL proposals greater than $2 \times \text{HR}$ or $3 \times \text{Mean}$.**

OECD MRL Calculating System (2011)

- **For not fully censored datasets, selects the maximum of**
 - **Highest residue value**
 - **Mean + $4 \times$ standard deviation (SD)**
 - **$3 \times \text{Mean} \times$ correction factor (CF)**

* **CF = $1 - 2/3 \times$ fraction censored data in the dataset.**
- **For fully censored datasets, selects the highest LOQ.**
- **Proposal on a case-by-case basis (reviewing by user).**
 - **Almost fully censored datasets but with several LOQ values.**
 - **Datasets with quantified values below the largest LOQ value.**

- **Highest residue value.**

- Lower-end floor to guarantee that MRL proposal is always greater than or equal to the highest residue.

- **Mean + 4 × standard deviation (SD).**

- The base of MRL proposal.

- **3 × Mean × correction factor (CF).**

- Another floor to guarantee that sample CV used in the calculation is at least 0.5, a condition verified by most residue datasets.
- CF to correct overestimation of the mean of a dataset for censored datasets.
- $CF = 1 - 2/3 \times \text{fraction censored data in the dataset}$.

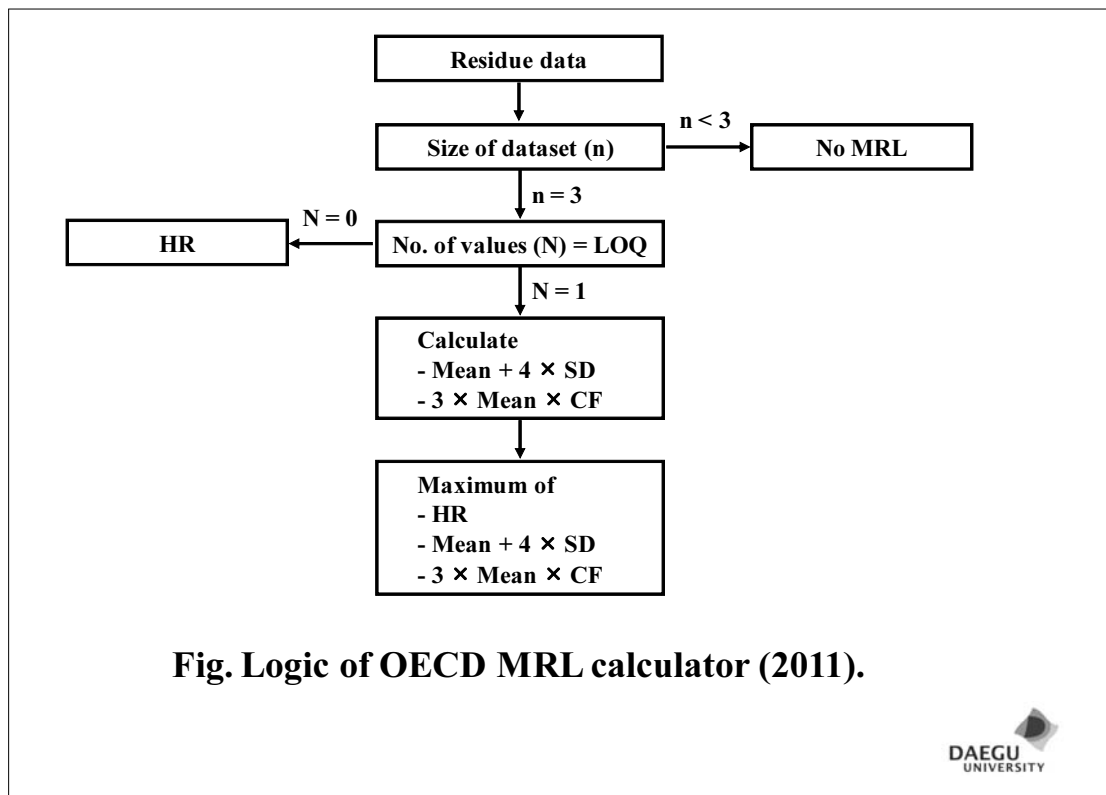
Censoring (%)	CF	3 × Mean × CF
0	1.00	3 Mean
50	0.67	2 Mean
100	0.33	1 Mean

- **Requires number of residue trials for**

- MRL calculation not possible if less than 3.
- High uncertainty of MRL estimate [Small dataset] for 3 ~ 7.
- 25% probability of MRL estimate below the 95th percentile for 8.
- Statistically significant MRL estimate for more than 8.

- **Data censoring**

- High uncertainty of MRL estimate [High level of censoring] if more than 50% of the dataset is censored.

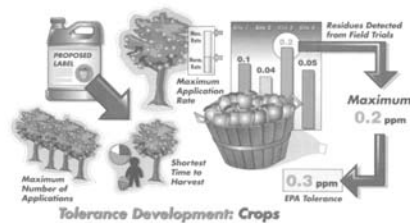


Supervised Residue Trial for MRL Proposal

- A independent residue trial is required to produce a residue value.
- Good field trial and reliable residue analysis are both prerequisite.

Supervised Field Trial

- **Critical good agricultural practices (cGAP) should be followed.**
 - Legally permitted GAP
 - Highest application rate
 - Greatest number of applications
 - Final application at minimum PHI (pre-harvest interval)
 - Deviations allowed : $\pm 25\%$ of the maximum application rate and $\pm 25\%$ of the minimum PHI. The only one of these parameters be allowed to deviate cGAP.



Residue data

- **One residue value per one supervised trial.**
- **The average or mean of the replicate values for residue trials with replicate field samples.**
- **The average or mean value of several analytical measurements for the same sample.**
- **Censored data (residue values < LOQ) by listing the LOQ value along with an asterisk .**



Captan residues in and between apple orchards

Captan	In orchard	Between orchards
Average (mg/kg)	2.00	2.27
Standard deviation	0.58	1.81
CV (%)	29.0	79.7

* *ca.* 50 apple samples were collected from each orchard.

Rounding

- MRL proposals are rounded as a last step in the calculation for globally harmonized MRLs.
 - Numbers between 1~10 are round to a single digit.
 - Numbers between 10~100 and 100~1000 are rounded to multiples of 10 and 100 and so on.
 - Intermediate values of 0.015, 0.15, 1.5, 15, etc, are introduced to avoid doubling MRLs on rounding.

0.001	0.0015	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009
0.01	0.015	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.1	0.15	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	1.5	2	3	4	5	6	7	8	9
10	15	20	30	40	50	60	70	80	90
100	150	200	300	400	500	600	700	800	900
1000									

- **Rounding down if the MRL proposal exceeds the lower MRL rounding possibility by less than 10% of the difference between the upper and lower MRL rounding possibilities.**

MRL class	10% Difference	Cut off point for rounding down
0.02	0.001	0.021
0.03	0.001	0.031
...
0.09	0.001	0.091
0.1	0.005	0.105
0.15	0.005	0.155
0.2	0.01	0.21
0.3	0.01	0.31
...
0.9	0.01	0.91
1	0.05	1.05
1.5	0.05	1.55
2	0.1	2.1
3	0.1	3.1



- **Some rounding examples :**

Unrounded proposal (mg/kg)	Rounded MRL proposal (mg/kg)
1.04	1
1.12	1.5
1.53	1.5
1.58	2
2.07	2
2.12	3
21.0	30

Performance of the OECD Calculator

● Performance against synthetic data.

- 100,000 datasets sampled from the lognormal distribution with the CV = 1.0.
- For smallest dataset with at least 3 data points, calculated MRL/p95 = 0.37 ~ 4.5, and calculated MRL/HR = 2.0 ~ 2.7.
- Failure rate (the chance to get a MRL below the p95)

Data point (n)	Failure rate (%)
3	42.5
8	25
29	5

- For smallest dataset with at least 3 data points, greater probability that the proposed MRL will be above the 95th percentile instead of below it (*ca.* 60% confidence).



● Performance against real data.

- The HR of 63 full datasets (at least 20 or 30 residue values) from EFSA* and JMPR data as reference point expected to be above 95th percentile.
- MRL proposals by OECD calculator from 10 subsets of 5, 8, 16 and 20 data points extracted from each of 63 full datasets.

Data point (n)	MRL proposal/HR of the full dataset
5 ~ 8	0.5 ~ 2.5 (much more frequently = 1)
16 ~ 20	Mostly = 1

* European Food Safety Authority.

● Comparison with historical MRLs.

- On average the MRL estimates yielded by the OECD Calculator exceed the MRLs proposed by EFSA and JMPR experts by 12% and 5%, respectively.
- On average the MRL estimates yielded by the OECD Calculator exceed the MRLs yielded by NAFTA Calculator by 11~15%.

Parameter	EFSA 215 datasets		JMPR 201 datasets	
	Rounded MRL/EFSA MRL	Rounded MRL/NAFTA MRL	Rounded MRL/JMPR MRL	Rounded MRL/NAFTA MRL
Min (overall)	0.30	0.43	0.40	0.50
Max (overall)	2.00	2.67	3.00	3.00
Mean (overall)	1.12	1.11	1.05	1.15
Mean (n = 4)	1.08	1.16	1.28	1.37
Mean (4 < n = 8)	1.12	1.09	1.08	1.12
Mean (8 < n = 16)	1.21	1.08	1.00	1.11
Mean (n > 16)	0.92	1.08	0.95	1.20
Mean (0% censored)	1.16	1.12	1.05	1.16
Mean (< 100% censored)	1.14	1.11	1.05	1.17
Mean (100% censored)	0.91	0.98	1.04	1.00



Harmonization of MRL Estimation between Korea and OECD

Field trial

- Number of field trials.
- Adoption of cGAP *in lieu of* GAP combinations.
- Collection and handling of samples.
- Input of the mean value of field replicates.

Residue analysis

- Employment of LOQ concept for HR or censoring.
- Validation of the analytical method.
- Analytical consistency (*e.g.* concurrent recovery).
- Input of the mean value of analytical measurements.



Requirements of Supervised Field Trials for the Establishment of MRL

Nation/ organization	No. of field trial	No. of treatment	No. of sampling	Replication of sampling	Replication of analysis	Total No. of measurement ²⁾
Korea	1	6~8	1~5	3	1	21 ~ 33
Codex ¹⁾	6	1	1	1 ~ 2	1 ~ 2	24
EU major crop ¹⁾	8	1	1	1	2	32
EU minor crop ¹⁾	4	1	1	1	2	16
USA ¹⁾	4 ~ 20	1	1	2	1	16 ~ 80

¹⁾ Critical GAP is followed for pesticide application in Codex, EU (North or South regions) and USA.

²⁾ Including control and treated samples.



Supervised Field Trials in Combination with GAP Parameters for both MRL and Safe Use Standard in Korea

● Supervised trial of etofenprox + methoxyfenozide SE in pear.

No. of application	No. of application timing	No. of sampling	Replication of sampling	Replication of analysis	Total No. of measurement ²⁾
2	2	1	3	1	21
3	2	1	3	1	
4	2	1	3	1	

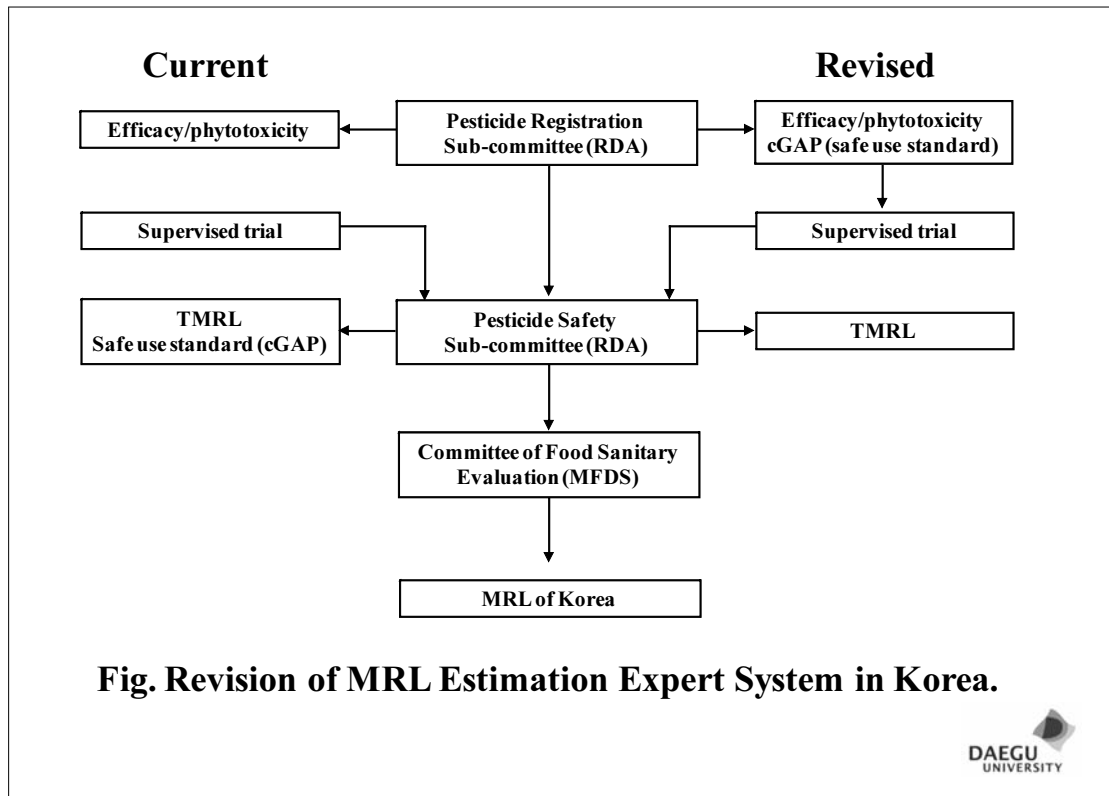
¹⁾ Including control and treated samples.

● Supervised trial of boscalid + metrafenone WG in green pepper.

No. of application	No. of application timing	No. of sampling	Replication of sampling	Replication of analysis	Total No. of measurement ²⁾
2	1	5	3	1	33
3	1	5	3	1	

¹⁾ Including control and treated samples.





Analytical System for Pesticide Residues

Residue Definition

- **Analytes : Parent compound (active ingredient), impurities and degradation products/metabolites of toxicological significance**

Pesticide	Residue definition*
DDT	Sum of <i>p,p'</i> -DDT, <i>o,p'</i> -DDT, <i>p,p'</i> -DDE and <i>p,p'</i> -DDD
Carbofuran	Sum of carbofuran, 3-hydroxycarbofuran and conjugated 3-hydroxycarbofuran, expressed as carbofuran
Phorate	Sum of phorate, its oxygen analogue, and their sulfoxides and sulfones, expressed as phorate

* CCPR

National System of Official Analysis for Pesticide Residues in Foods

► Multiple residue methods (MRMs)

- Monitoring and inspection methods for samples with no definitely known history.
- Optimized for coverage of maximum number of analytes, rapidity, and analytical efficiency.

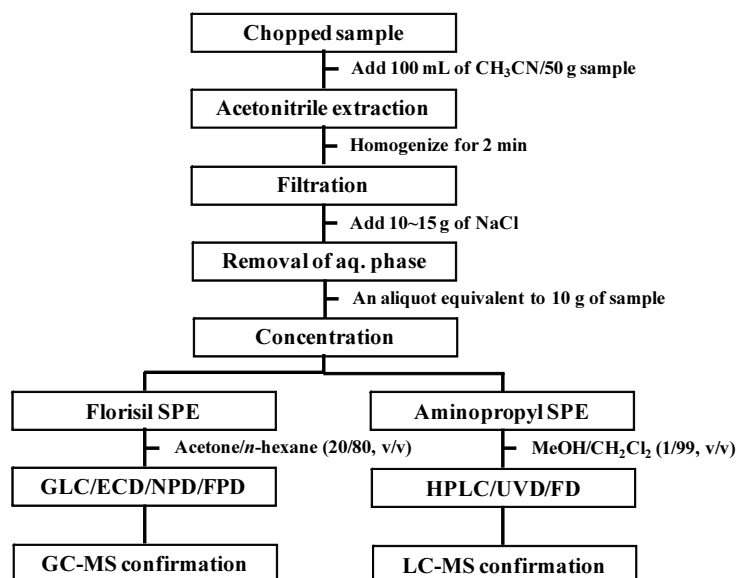
► Individual methods for group-based or single pesticides

- Legal or research-oriented methods for limited number of analytes.
- Complementary methods to verify MRM data.
- Optimized for precise quantitation of residues.
- Consisted of step by step procedures separately applicable to diverse analytes with similar characteristics.

Criteria of the Analytical Method for Pesticide Residues

Parameter	Multiple	Individual
Purpose	Screening	Quantitation
Analytical priority	Resolution/efficiency	Precision
Sample preparation	Group-based	Analyte-specific
Limit of quantitation (LOQ)	≤ 0.05 mg/kg or $\frac{1}{2}$ of MRL	≤ 0.05 mg/kg or $\frac{1}{2}$ of MRL
Recovery	70 ~ 130%	70 ~ 110%
Standard error (RSD)	= 30%	= 10%

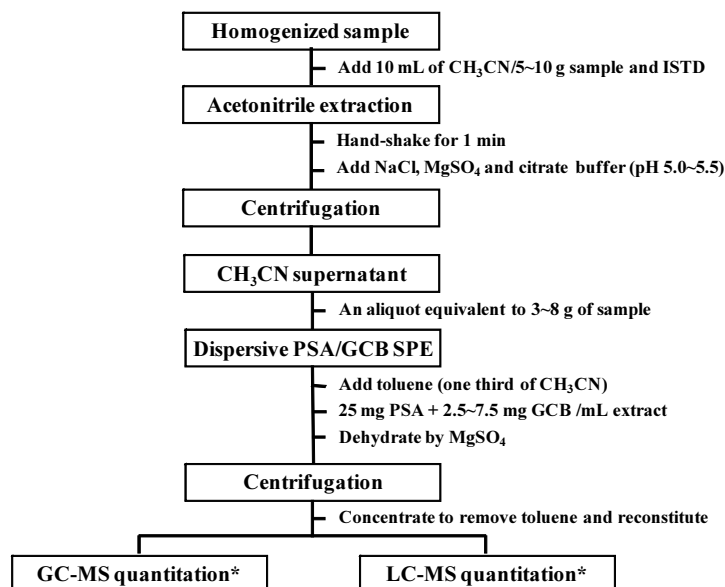
MFDS 4.1.2.2 (No. 83) Multi-Residue Analytical Method



MFDS PLS Method Using GC-LC/MS/MS (2013~2015)



QuEChERS Multi-Residue Analytical Method



*Confirmation by monitoring alternative fragment or daughter ions.



Development of Individual Analytical Methods



Year	Pesticide group	No. of pesticides	Instrumental analysis	
			Quantitation	Confirmation
2004	Oxime carbamates	2	GLC-NPD	GC-MS
	Pyrethroids	11	GLC-ECD	GC-MS
2005	Polar organophosphates	5	GLC-FPD	GC-MS
	Acaricides	8	HPLC-UVD	LC-MS
	Organotins	6	HPLC-UVD	-
2006	Organophosphates (sulfoxides)	3	GLC-FPD	GC-MS
	EBI fungicides/PGR	7	GLC-NPD	GC-MS
	Avermectines	2	HPLC-UVD/LC-MS	LC-MS
	Neonicotinoids	6	HPLC-UVD	LC-MS
2007	Benzimidazoles	4	HPLC-UVD/FLD	LC-MS
	Sulfonylureas	15	HPLC-UVD	LC-MS
2008	Graminicides	6	HPLC-UVD	LC-MS
	Strobilurin fungicides	6	HPLC-UVD	LC-MS
	Etofenprox	1	HPLC-UVD	LC-MS
	Indoxacarb	1	HPLC-UVD/LC-MS/MS	LC-MS
2009	Carbofuran/pro-carbofurans	4	LC-MS	LC-MS
	MBI fungicides	2	HPLC-UVD/LC-MS	LC-MS
2010	Synthetic pyrethroids	2	HPLC-UVD	LC-GC-MS
	Synthetic pyrethroids	5	GLC-ECD	GC-MS
2011	EBI fungicides	5	GLC-NPD (ISTD)	GC-MS
	Cyclohexanedione oximes	3	HPLC-UVD	LC-MS
2012	Phenylamide fungicides	4	GLC-NPD (ISTD)	GC-MS
	Urea herbicides	4	HPLC-UVD	LC-MS
Total		112		

Sample Preparation Procedures for Neutral Pesticides

Pesticide group	Log P _{ow}	Sample preparation procedure				
		Extraction	Liq.-liq. washing ¹	Liq.-liq. partition	HAP ²	CC sorbent
Pyrethroids	4.3~7.0	Acetone	N/A ³	Hexane	Yes.	SPE-Florisil
Polar OPs	-0.89~0.12	Acetone	Hexane	CH ₂ Cl ₂ /acetone (50/50)	No.	SPE-silica
Acaricides	2.4~6.4	MeOH	N/A	Hexane	N/A	SPE-NH ₂ /SPE-Florisil
EBI fungicides	2.9~4.4	Acetone	N/A	CH ₂ Cl ₂	Yes.	Florisil
Avermectines	4.4~5.9	MeOH	N/A	CH ₂ Cl ₂	Yes.	Florisil/SPE-NH ₂
Neonicotinoids	-0.64~1.6	CH ₃ CN	Hexane	CH ₂ Cl ₂	No.	Florisil
Strobilurins	2.4~4.7	CH ₃ CN	N/A	CH ₂ Cl ₂ /hexane (20/80)	Yes.	Florisil
Etofenprox	6.9	Acetone	N/A	Hexane	Yes.	Florisil
Indoxacarb	4.7	Acetone	N/A	CH ₂ Cl ₂ /hexane (20/80)	Yes.	Florisil
MBI fungicides	1.4~1.6	MeOH	Hexane	CH ₂ Cl ₂	No.	Silica gel

¹ Liquid-liquid washing or partition was followed to dilution of the sample extract with saline except pre-concentration of extracts for polar organophosphates and neonicotinoids.

² Hexane-CH₃CN partition for removal of fat.

³ Not applicable or applied.



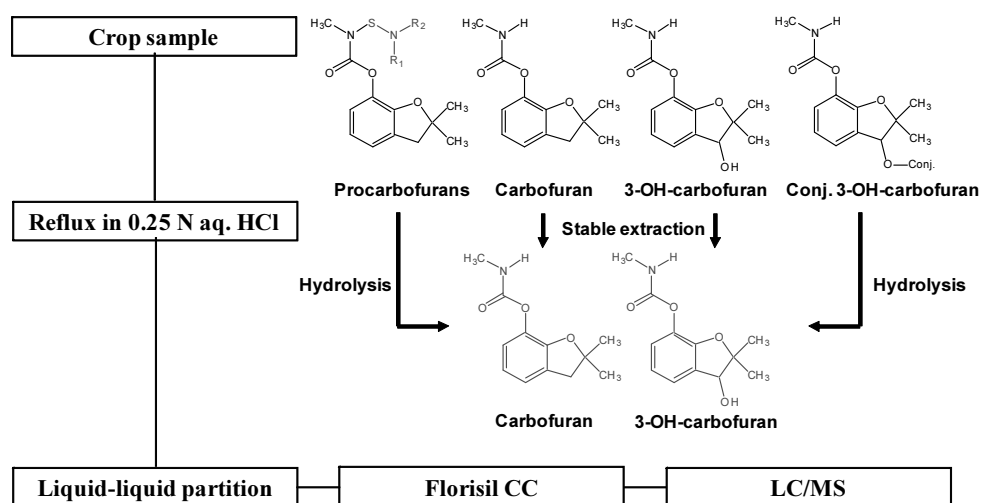
Sample Preparation Procedures for Ionizable Pesticides

Pesticide group	pKa ¹	Sample preparation procedure			
		Extraction	Liquid-liquid partition	HAP ²	CC sorbent
Benzimidazoles	4.2~4.7	MeOH	Ion-associated	No.	None.
Sulfonylureas	3.2~5.3	CH ₃ CN/H ₃ O ⁺	Ion-associated	No.	Florisil/SPE-NH ₂
Graminicides	2.3~3.8	CH ₃ CN/H ₃ O ⁺	Ion-associated	No.	SPE-NH ₂

¹pKa of conjugate acids for benzimidazoles.

² Hexane-CH₃CN partition for removal of fat.

Sample Preparation Procedure for Carbofuran and Its Congeners



Validation Parameters for Residue Analytical Methods

- **Sensitivity** **Limit of detection (LOD)**
 Limit of quantitation (LOQ)
- **Selectivity** **Degree of interference**
 (specificity)
- **Accuracy** **Calibration, recovery**
- **Precision** **Repeatability, reproducibility**

Sensitivity

- **Limit of quantitation (LOQ) : $S/N = 10$**
- * **Limit of detection (LOD) : $S/N = 3$**
- **Sensitivity Criteria for Analysis of Pesticide Residues¹**

MRL (mg/kg)	LOQ (mg/kg)
> 0.1	= 0.1
0.1	= 0.05
0.05	= 0.02
< 0.05	= MRL 0.5

¹Guidance Document on Residue Analytical Methods, European Commission, SANCO/825/00 rev.7-2004.

Selectivity (matrix interference)

- **Blank values in the area of analytical of interest from the matrices should not be higher than 30% of the LOQ.**

Accuracy

Calibration

- Matrix-matched standards where appropriate
- Bracketing calibration

Recovery experiment

- Validation recovery
- Concurrent recovery

Data set	CODEX		OECD/EC	
	Fortification level (mg/kg)	Replication	Fortification level (mg/kg)	Replication
Full	Control	5	Control	2
	Proposed LOQ	5	Proposed LOQ	5
	5 ~ 8 LOQ	4	10 LOQ or MRL	5
	90 ~ 100 LOQ	4	-	-
Reduced	Control	1	Control	1
	Proposed LOQ	3	Proposed LOQ	3
	10 ~ 12 LOQ	3	10 LOQ or MRL	3
	90 ~ 100 LOQ	3	-	-



Precision

Repeatability

Concentration level	Repeatability (RSD)	Range of mean recovery (%)
< 1 µg/kg	35	50 ~ 120
> 1 µg/kg = 0.01 mg/kg	30	60 ~ 120
> 0.01 mg/kg = 0.1 mg/kg	20	70 ~ 120
> 0.1 mg/kg = 1.0 mg/kg	15	70 ~ 110
> 1 mg/kg	10	70 ~ 110

¹Guidelines on Good Laboratory Practice in Residue Analysis, CAC/GL 40-1993, Rev.1-2003.

Reproducibility

- Intra-laboratory reproducibility (run effect)
- Inter-laboratory reproducibility (laboratory effect)



Matrix effect in QuEChERS method

- No. of pesticides : 138
- Analysis : Quechers preparation coupled with UPLC/TOF-MS

Infant foods	% of pesticides			
	Ion suppression = 40%	Ion suppression 30 ~ 39%	Ion suppression < 30% or ion enhancement = 10%	Ion enhancement > 10%
Apples	19	20	56	6
Apples & bananas	17	17	59	7
Apple juice	1	5	68	21
Bananas	12	13	65	9
Pears	20	12	59	7
Carrots	19	13	60	8
Creamed corn	28	19	49	3
Peas	14	12	68	4
Squash	23	13	58	5
Sweet potatoes	17	12	64	6

*J. Agric Food Chem., 57(9), 2162~2173, 2009.



Interlaboratory validation of QuEChERS method

- No of pesticides : 50, Quantitation : LC/MS/MS

Sample	Fortified at 0.01 mg/kg		Fortified at 0.1 mg/kg	
	Mean rec. (%)	SD (%)	Mean rec. (%)	SD (%)
Cucumber	63~108	3~30	59~105	2~45
Lemon	44~105	1~41	44~106	2~36
Wheat flour	35~109	3~85	41~103	1~71
Raisin	40~112	2~68	44~109	2~68

- No of pesticides : 29, Quantitation : GC/MSD and/or LC/MS/MS

Sample	Fortified at 0.025 mg/kg		Fortified at 0.25 mg/kg	
	Mean rec. (%)	SD (%)	Mean rec. (%)	SD (%)
Apple	78~124	1~17	79~106	1~13
Orange	75~119	1~15	78~108	1~21
Salad	70~115	1~20	75~109	3~15

*European Committee for Standardization in Germany (www.quechers.com)



Recovery and LOQ of avermectins using LC-MS ESI(-) SIM and LC/UVD

- Citrus with no matrix-matched calibration but highly specific purification.

Compound	Fortification (mg/kg)	Recovery (%) [*]		LOQ (mg/kg)	
		LC-MS	LC/UVD	LC-MS	LC/UVD
Abamectin B1a	0.047	85.1±4.9	87.0±6.5	0.002	0.003
	0.235	87.1±7.0	84.8±3.9		
Abamectin B1b	0.003	-	-	0.002	0.003
	0.015	82.5±8.5	82.1±4.6		
Milbemectin A ₃	0.021	88.8±6.3	94.1±6.8	0.002	0.003
	0.105	91.5±4.8	89.6±4.6		
Milbemectin A ₄	0.049	84.9±2.6	86.2±3.6	0.002	0.003
	0.245	88.4±4.7	86.9±3.8		

^{*} Mean values of triplicate samples with standard deviations.



Validation of the proposed method for indoxacarb (LC-MS/MS vs. HPLC/UVD)

- No matrix-matched calibration with ordinary purification.

Crop	Fortification (mg/kg)	Recovery±SD (%) [*]		LOQ (mg/kg)	
		LC-MS/MS	LC/UVD	LC-MS/MS	LC/UVD
Hulled rice	0.02	94.2 ± 9.0	95.1 ± 1.0		
	0.2	90.9 ± 13.6	96.6 ± 0.3	0.002	0.02
	2.0	123.8 ± 17.3	97.1 ± 1.6		
Apple	0.02	61.0 ± 10.8	93.9 ± 5.6		
	0.2	123.9 ± 9.4	95.9 ± 1.7	0.002	0.02
	2.0	123.5 ± 3.0	99.3 ± 3.2		
Mandarin	0.02	105.0 ± 21.0	103.7 ± 7.2		
	0.2	100.2 ± 8.5	94.7 ± 1.3	0.002	0.02
	2.0	83.5 ± 10.8	94.8 ± 1.0		
Chinese cabbage	0.02	83.4 ± 17.5	95.7 ± 0.5		
	0.2	101.2 ± 30.5	93.9 ± 0.6	0.002	0.02
	2.0	97.3 ± 24.9	92.5 ± 0.7		
Green pepper	0.02	108.8 ± 14.8	99.2 ± 1.3		
	0.2	102.1 ± 33.1	91.7 ± 0.2	0.002	0.02
	2.0	88.0 ± 13.7	92.8 ± 2.1		

^{*}Mean values of triplicate samples with standard deviations.



Validation of the proposed method for pyroquilon (HPLC/UVD vs. LC-MS SIM)

- Matrix-matched calibration with specific purification.

Crop	Fortification (mg/kg)	Recovery \pm SD (%) [*]		RSD (%)		LOQ (mg/kg)	
		LC/UVD	LC-MS	LC/UVD	LC-MS	LC/UVD	LC-MS
Hulled rice	0.02	85.2 \pm 1.7	80.8 \pm 1.1				
	0.2	81.4 \pm 2.6	92.5 \pm 3.6	2.7	7.8	0.02	0.002
	2.0	82.9 \pm 0.5	95.1 \pm 1.9				
Apple	0.02	95.7 \pm 3.7	93.6 \pm 9.7				
	0.2	86.4 \pm 1.8	92.1 \pm 2.5	6.9	6.4	0.02	0.002
	2.0	84.1 \pm 1.9	91.3 \pm 6.0				
Green pepper	0.02	89.3 \pm 5.6	93.8 \pm 5.3				
	0.2	86.0 \pm 1.0	97.7 \pm 3.0	4.8	4.0	0.02	0.002
	2.0	82.8 \pm 1.9	96.8 \pm 3.2				
Chinese cabbage	0.02	91.0 \pm 2.1	78.5 \pm 5.0				
	0.2	87.3 \pm 0.9	93.9 \pm 2.4	3.0	8.9	0.02	0.002
	2.0	86.4 \pm 2.2	90.7 \pm 4.0				

^{*}Mean values of triplicate samples with standard deviations.



Analytical efficiency for routine quantitation of pyroquilon and tricyclazole residues in crop samples

Quantitation	No. of analyte	No. of sample type ¹	No. of samples per type ²	No. of calibration std required		Run time per sample (min) ⁴	Total run time (h)
				Neat	MM ³		
HPLC/UVD	2	4	12	2	None.	17	34.0
LC-MS SIM	2	4	12	2	18	20	58.7

¹Hulled rice, apple, green pepper and Chinese cabbage.

²Triplicate samples of control and fortified at 0.02, 0.2 and 2.0 mg/kg each.

³Matrix-matched standard solution.

⁴Actual running time for analysis of a sample.