

Proposed Regulatory Framework for Pesticide Residues in Food

Workshop on Pesticide Residue Testing

6 May 2011



Topics for Discussion

1st Workshop (6 May 2011)

- Overview on pesticide residue testing
- Multi-residues test methods
- Analytical performance criteria approach

2nd Workshop (Jun 2011)

- Single residue methods



Proposed Regulatory Framework for Pesticide Residues in Food

Preliminary List of Proposed MRLs and EMRLs for Pesticide Residues in Food in Hong Kong

http://www.cfs.gov.hk/english/whatsnew/whatsnew_fstr/files/Draft_MRL_f_or_technical_meeting_28032011.pdf

- Name of Pesticide
- Residue Definition
- Food
- Codex Food Code
- Proposed Limit (mg/kg)



	Pesticide	除害劑	Residue Definition	殘留物定義	MRL最高殘餘限量(M)/EMRL 再殘餘限量(E)	Codex Food Code 食物編號		Proposed limit 建議限量 (mg/kg)	
119	16	Emamectin	甲氨基阿維菌素	Combined residues of emamectin (MAB1a + MAB1b isomers) and the associated 8,9-Z isomers (8,9-ZB1a + 8,9-ZB1b)	甲氨基阿維菌素 (MAB1a + MAB1b 異構體) 及其相關 8,9-Z 異構體 (8,9-ZB1a + 8,9-ZB1b) 殘餘之和。	M	MO 1289	Sheep liver	0.05
119	17	Emamectin	甲氨基阿維菌素	Combined residues of emamectin (MAB1a + MAB1b isomers) and the associated 8,9-Z isomers (8,9-ZB1a + 8,9-ZB1b)	甲氨基阿維菌素 (MAB1a + MAB1b 異構體) 及其相關 8,9-Z 異構體 (8,9-ZB1a + 8,9-ZB1b) 殘餘之和。	M	MO 1293	Horse liver	0.05
120	1	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	DT 1114	Tea, Green, Black (black, fermented and dried)	20
120	2	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FB 0020	Blueberries	0.3
120	3	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FB 0209	Grapes	2
120	4	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FB 0275	Strawberry	2
120	5	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0326	Avocado	0.5
120	6	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0332	Custard apple	0.5
120	7	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0343	Litchi	2
120	8	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0345	Mango	0.5
120	9	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0350	Papaya	0.5
120	10	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0352	Persimmon, American	2
120	11	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FI 0353	Pineapple	1
120	12	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FM 0183	Milk	0.1
120	13	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FP 0009	Pork	1
120	14	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0014	Plum	2
120	15	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0240	Apricot	2
120	16	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sweet	2
120	17	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sour	2
120	18	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Bitter	2
120	19	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sweet	2
120	20	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sweet	2
120	21	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sweet	2
120	22	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	FS 0244	Cherry, Sweet	2
120	23	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	GC 0647	Oats	0.3
120	24	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	GC 0650	Rye	0.3
120	25	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	GC 0654	Wheat	0.3
120	26	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	GS 0659	Sugar cane	0.5
120	27	Endosulfan	硫丹	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate	alpha-硫丹、beta-硫丹和硫酸硫丹之和。	M	HS 0190	Spices, Seeds	1

Food Blueberries

Pesticide Endosulfan

Codex Food Code FB 0020

Proposed limit 0.3 mg/kg

Blueberries

Residue Definition Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate

Group : Berries and other small fruits

Proposed Regulatory Framework for Pesticide Residues in Food

- Number of pesticides to be included:
 - More than 300
- Number of Maximum Residue Level (MRL) /Extraneous Maximum Residue Limit (EMRL)
 - About 6000
- Food types:
 - Include different foods from plant and animal origins
 - Include foods of high fiber, high protein, high fat, high carbohydrate, high water content, etc.



Testing Objectives

- The Testing Method(s) shall be able to analyse the pesticide residues according to the stated **residue definition**.
- The **Reporting Limit** of an appropriate testing method shall be at least equal to or lower than the proposed limit.

Codex recommendation : LOQ should be $1/5$ or $2/5$ of specified level (ML) depending on concentration



Pesticide Residue Definition

Residue definition means the combination of the pesticide and its metabolites, derivatives and related compounds to which an MRL or EMRL applies.

Examples of different types of residue definitions

1) Residue Definition : Parent Pesticide

Pesticide	Residue Definition
2,4-D	2,4-D
Acephate	Acephate

Most of pesticide definitions belong to this type



Pesticide Residue Definition

2) Residue Definition : Sum of compounds

Pesticide	Residue Definition
DDT	Sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE (DDD)
Endosulfan	Sum of alpha-endosulfan, beta-endosulfan and endosulfan sulphate

3) Residue Definition : Expression of parent compounds

Pesticide	Residue Definition
Methiocarb	Sum of methiocarb, its sulphoxide and its sulphone, expressed as methiocarb
Methomyl	Sum of methomyl and thiodicarb, expressed as methomyl



Pesticide Residue Definition

4) Residue Definition : Conversion of compounds

Pesticide	Residue Definition
Dithiocarbamates	Sum of all dithiocarbamates, determined as CS ₂ evolved during acid digestion, expressed as mg CS ₂ /kg
Prothioconazole	Combined residue of prothioconazole and prothioconazole-desthio, and conjugates that can be converted to these two compounds by acid hydrolysis, calculated as prothioconazole

5) Residue Definition : Definition involving moiety

Pesticide	Residue Definition
Vinclozolin	Sum of vinclozolin and all metabolites containing the 3,5-dichloroaniline moiety, expressed as vinclozolin
Propachlor	Sum of propachlor and its metabolites containing the N-isopropylaniline moiety, expressed as 2-chloro-N-isopropylacetanilide



Type of Testing Methods for Pesticide Residues

Test methods for pesticides can be generally divided into two categories:

➤ Multi-residues test methods

➤ Pesticide residues are suitable for analysis by multi-residues test methods which can analyze more than a hundred kinds of pesticide residues under the same testing procedure.

➤ In general, multi-residues test methods are suitable for following types of residue definitions:

- Parent pesticide
- Sum of compounds
- Expression of parent compounds



Type of Testing Methods for Pesticide Residues

➤ Single residue test methods

➤ Based on the characteristics of pesticide residue or the definition of the pesticide, the pesticide needs to be analyzed by individual test method.

➤ In general, single-residue test methods are suitable for following types of residue definitions:

➤ Conversion of compounds

➤ Definition involving moiety

➤ Other pesticides with special characteristics, such as highly polar analytes, volatile analytes, inorganic or organometallic analytes, etc. are also suitable.

Due to the rapid research and development of the multi-residues test methods, many pesticides, which are used to be analyzed by single residue test methods in the past, are now able to be analyzed by multi-residues test methods.



Trend of Method Development

The use of the number of pesticides is continuously increasing internationally. In order to fulfill the needs of speeding up analytical time and improving the quality, the direction of method development has also been changed :

- Traditionally, international organizations and different national / regional institutions would publish standards or reference test methods for specific pesticides.
- Regarding the test method recognition and quality control, the international trend has gradually changed from developing prescribed test methods to the establishment of **analytical performance criteria** so that satisfactory comparability of results can be achieved by employing different analytical methods but with performance meeting the same requirements under internationally recognized protocols.



Trend of Method Development

For the above reasons:

Different laboratories can consider their testing needs and intended purposes, facilities and resources, and use relevant criteria protocols or reference methods compiled by international or national organizations, or adopt appropriate test methods and fulfill the quality requirements being specified.

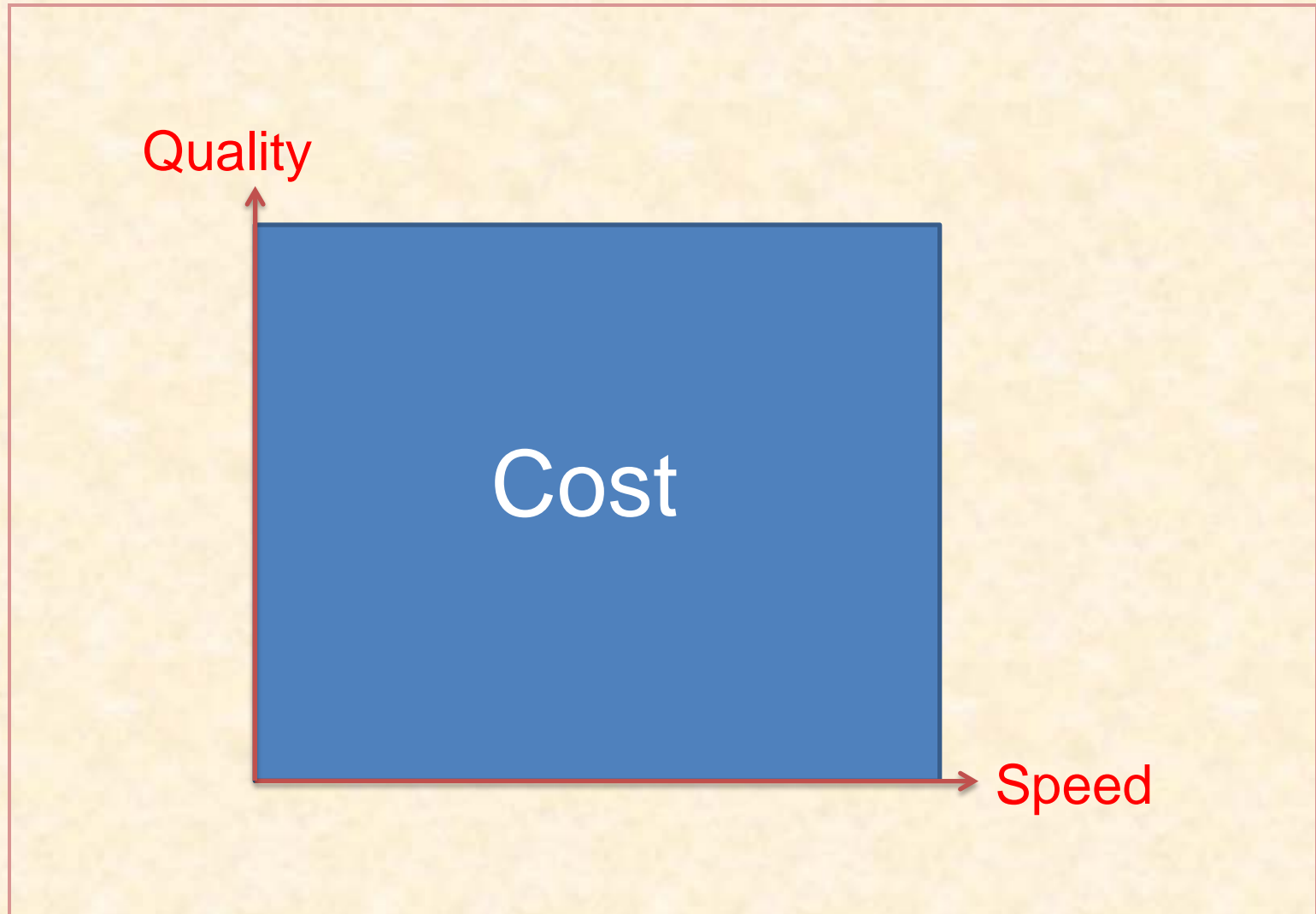


Preparation for Pesticide Residues Testing

- Selection or development of appropriate test methods based on the needs and intended purposes, facilities and resources
- Procurement of pesticide standard reference materials and other related chemicals
- Instrument acquisition
- Laboratory staff training



Considerations on Selection of Test Method



Consideration on Selection of Test Method

Instrumentation Specificity

ECD,FPD → MSD → MSMS, HRMS, TOF → Quality & Speed

GPC → SPE → Dispersive SPE (QuEChERS) → Cleanup Speed



Test Method References

Reference test methods from national institutions or international organizations related to the proposed regulation framework

- Codex Alimentarius Commission (Codex) Standard – Analysis of Pesticide Residues : Recommended Methods
- National Standards of the People’s Republic of China “Compilation of Official Methods of Analysis for Pesticide Residues” (Vol. 1 & II), 2009.
- United States Environmental Protection Agency (USEPA) - Residue Analytical Method (RAM)



QuEChERS

Currently, the sample preparation procedure for pesticide multi-residues analysis is mainly based on QuEChERS method or its modifications.

QuEChERS is the abbreviation of Quick, Easy, Cheap, Effective, Rugged, Safe.



Test Method References

Multi-residues reference test methods, such as:

- AOAC Official Methods of Analysis 2007.01
- GB/T 19648-2006 & 20769-2008
- Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food, Department of Food Safety, Ministry of Health, Labour and Welfare, Japan
- British Standards BS EN 15662:2008 & BS EN 15637:2008
- European Union Reference Laboratories (EURL)
- United States Food and Drug Administration (FDA) - Pesticide Analytical Manual (PAM) Volume I, 3rd Edition



Multi-residues Test Method

Main steps for multi-residues analysis

- ✓ Sampling
- ✓ Homogenization
- ✓ Extraction
- ✓ Cleanup
- ✓ Instrumental Analysis



Sampling - Portion of Commodity for Analysis

Codex Alimentarius Commission (Codex) Standard

- Portion of Commodities to which is analysed
CAC/GL 41-1993 (Amendment 2010)
- Codex Classification of Foods and Animal Feeds
Codex Alimentarius Volume 2, Pesticides
Residues in Food, Second Edition.



Sampling - Portion of Commodity for Analysis

Codex Alimentarius Commission (Codex) Standard

Water spinach (Leafy Vegetables)
– Whole commodity



Mao melon (Fruiting Vegetables – Inedible Peel)
– Whole commodity



Orange (Citrus Fruits)
– Whole commodity



Peach (Stone fruits)– Whole commodity after removal of stems and stone, calculated on the whole commodity without stem



Sampling - Portion of Commodity for Analysis

Peach (Stone fruits) – Whole commodity after removal of stems and stone, calculated on the whole commodity without stem



Calculations:

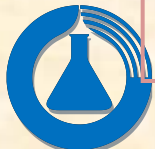
For example, Peach

- Weight of the whole commodity **without stem (with stone)** = 100 g
- Weight of the whole commodity after **removal of stems and stone** = 80 g

If the **concentration** of pesticide for the portion of commodity for analysis (the whole commodity after removal of stems and stone) = **1.0 mg/kg**

then the concentration of the pesticide for the peach

$$= 1.0 \text{ mg/kg} * 80\text{g} / 100\text{g} = 0.8 \text{ mg/kg}$$



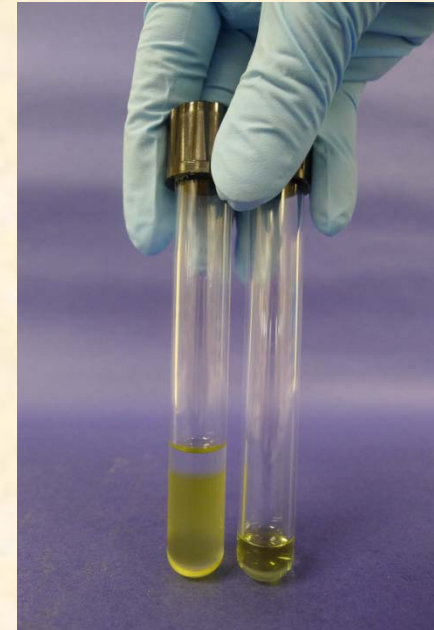
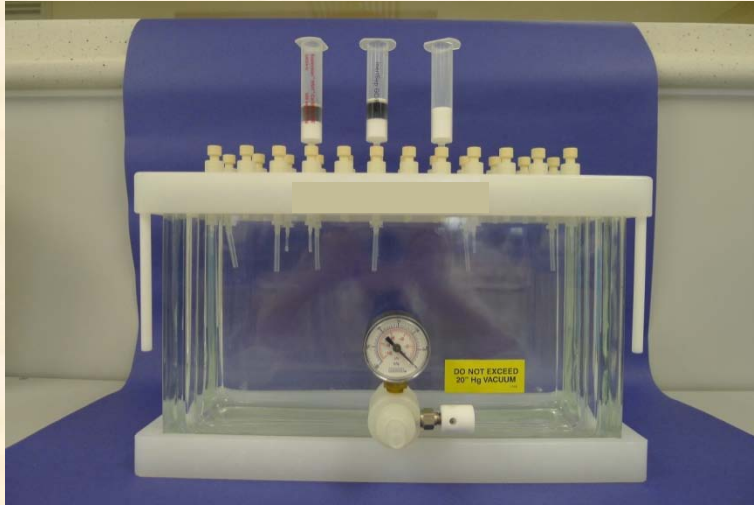
Homogenization



Extraction



Cleanup



Instrumental Analysis

Instrumental analysis for multi-residues analysis is mainly divided into gas chromatography (GC) and liquid chromatography (LC) analyses.

For GC amenable pesticides, GC with ECD, FPD, MSD and GC-MS/MS are mainly employed.

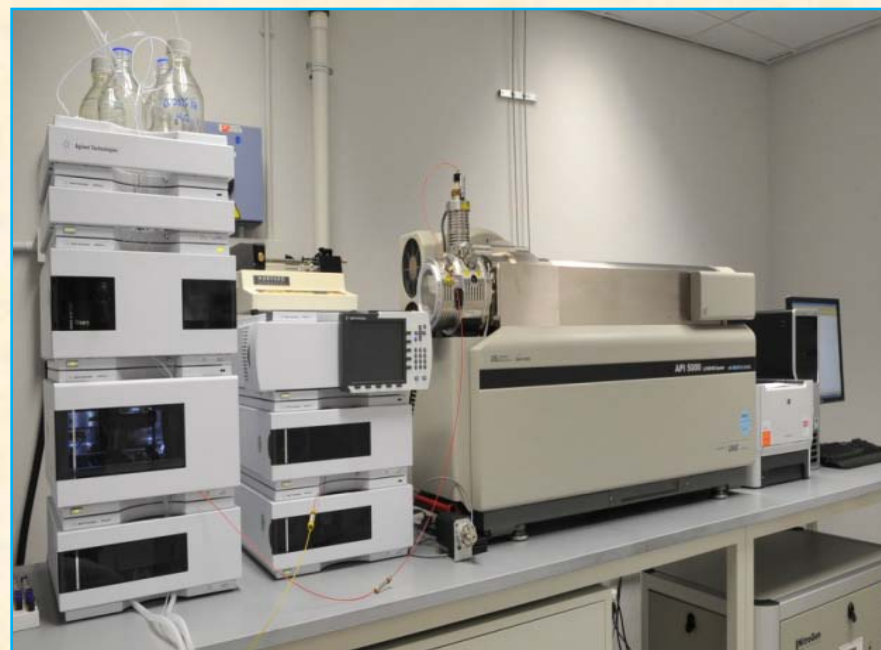
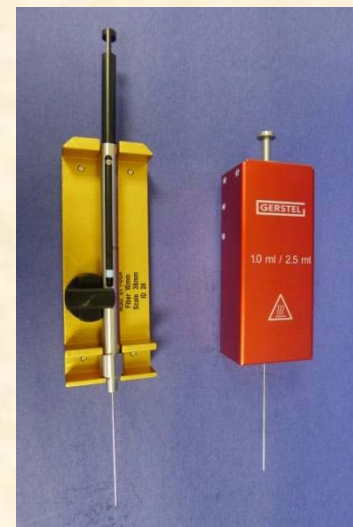
For LC amenable pesticides, LC with DAD, FLD and LC-MS/MS are mainly used.



Instrumental Analysis



Instrumental Analysis



Comparison of Multi-residues Methods - Sample Extraction

Fruit and vegetables	AOAC official method 2007.01	GB/T 19648-2006 & 20769-2008	BS EN 15662:2008	BS EN 15637:2008	Japan MHLW
Approach	QuEChERS		QuEChERS		
Sample size (g)	15	20	10	10	20
Solvent	1% acetic acid in ACN	ACN	ACN	methanol	ACN
Solvent volume (mL)	15	40	10	20	50
Salts added	1.5g NaAc, 6g anh. MgSO ₄	5g NaCl	4g anh. MgSO ₄ , 1g NaCl, 1g trisodium citrate dihydrate, 0.5g disodium hydrogencitrate sesquihydrate	NaCl (in 2.5mL 20%w/w solution)	10g NaCl , 20mL 0.5M phosphate buffer pH 7.0
Internal standard	Triphenyl phosphate (TPP)	Heptachlor epoxide	TPP, PCBs and others	TPP	
Extraction by	Shake & centrifuge	Comminute with homogenizer & centrifuge	Shake & centrifuge	Shake & centrifuge	Comminute with homogenizer, filter & centrifuge

Comparison of Multi-residues Methods - Sample Cleanup

Fruit and vegetables	AOAC official method 2007.01	GB/T 19648-2006 & 20769-2008	BS EN 15662:2008	BS EN 15637:2008	Japan MHLW
Technique	dSPE	SPE	freezing out fat/wax/sugar (opt.) & dSPE	SPE	SPE
Materials	PSA, MgSO ₄ , GCB (opt.), C ₁₈ (opt.)	C ₁₈ SPE column, N-propyl amine + GCB SPE column with Na ₂ SO ₄	PSA, MgSO ₄ , GCB (opt.)	diatomaceous earth column	N-propyl amine + GCB SPE column
Cleanup by	Shake & centrifuge	ACN + toluene elution	Shake & centrifuge, acidified by formic acid	DCM elution	ACN + toluene elution

Comparison of Multi-residues Methods – Instrumentation

Fruit and vegetables	AOAC official method 2007.01	GB/T 19648-2006 & 20769-2008	BS EN 15662:2008	BS EN 15637:2008	Japan MHLW
Instrumentation	1. GC-MS (SIM) mode 2. LC-MS/MS (SRM) mode	1. GC-MS (SIM) mode 2. LC-MS/MS (SRM) mode	1. GC-MS (SIM) mode 2. LC-MS/MS (SRM) mode	LC-MS/MS (SRM) mode	1. GC-MS (SIM) mode 2. LC-MS/MS (SRM) mode

Process of Pesticide Multi-residues Test Method

Traditionally, **direct use of quantitative method** was usually employed for determination of concentration of pesticides. With the increasing number of pesticides in testing, the multi-residues test method can firstly **screen out** the pesticides in the samples with **stronger signal** and subsequently confirmation and quantitation analysis are performed.

Analysis can be divided into three steps:

- 1) Screening
- 2) Confirmation
- 3) Quantitation

This method can significantly reduce work in data analysis.



Screening of Pesticide

Screening Specifications:

The accuracy of the screening is based on **false negative rate** to indicate the accuracy of detection.

According to the Codex Alimentarius Commission and the EU guidelines:

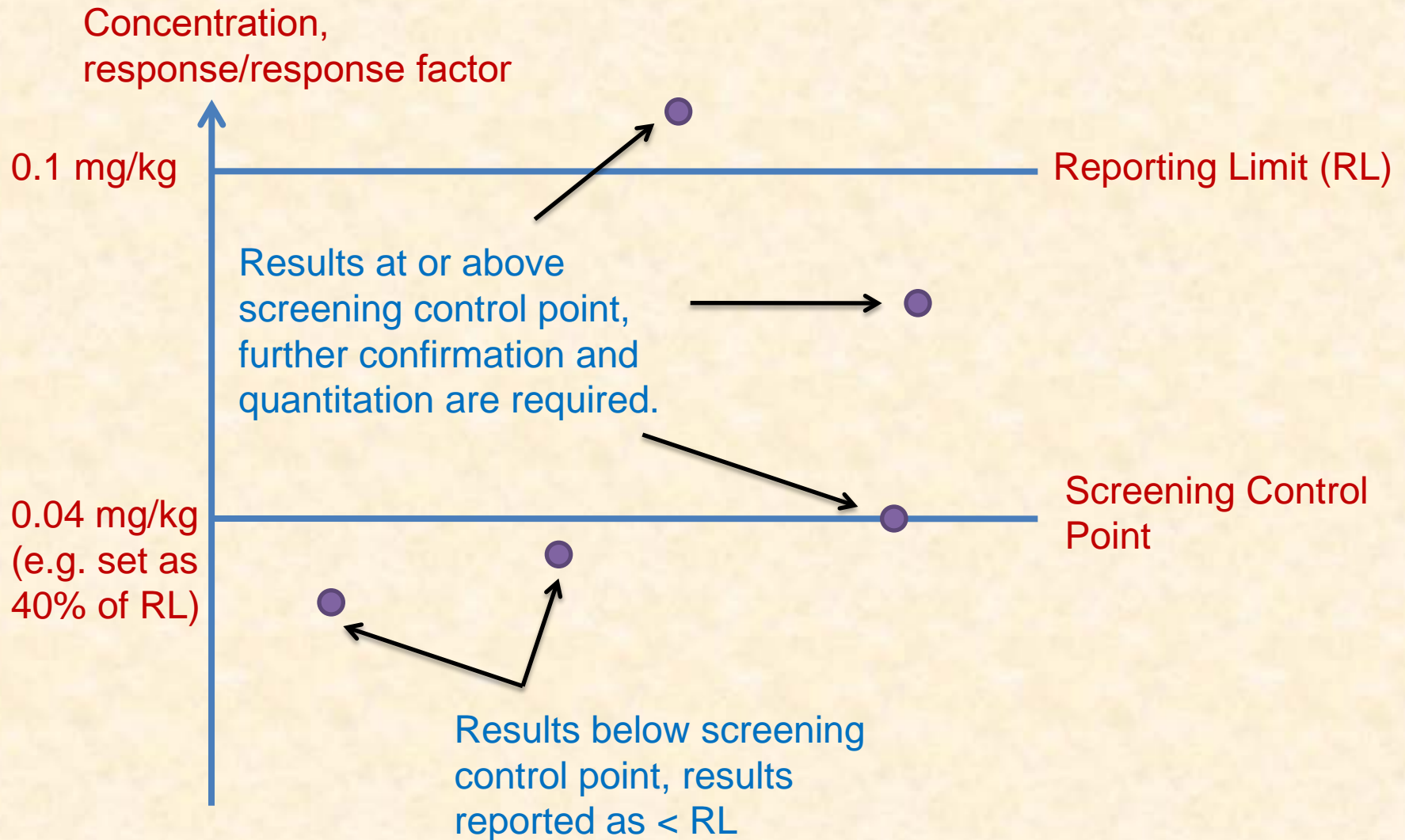
False negative rate : < 5%

A control point for screening is a level of concentration, response or response factor above which further confirmation and quantitation of the pesticide in food are required.

In general, level of control point should be set at or below 70% of the reporting limit.



Screening of Pesticide



Confirmation of Pesticide

Confirmation Requirements(SANCO/10684/2009):

- 1) Deviation of Relative retention time, GC: 0.5%,
LC: 2.5%
- 2) Mass Spectrometer (ion ratios)



Quantitation of Pesticide

Quantitation Requirements:

- Include all requirements in confirmation
- Calibration Curve – at least 3 points excluding blank, standard addition may be employed if encountering matrix interferences
- Method Blank
- Duplicates, replicates
- Spike Recovery
- Internal Standard or Surrogate Recovery
- Matrix reference sample if applicable



Thank you!

