Proposed Regulatory Framework for Pesticide Residues in Food

Workshop on Pesticide Residues Testing

Single-Residue Methods in Pesticide Residues Analysis

9 June 2011

Pesticide Residues Analysis

- Single-Residue Method (SRM): analyse the target(s) with its specific method
- Method for analysis of pesticide ingredients in formulation and field studies
- Thus, single-residue methods usually target individual pesticide and/or its related metabolites

SRM vs MRM

- For routine pesticide residues analysis, multiple residues may be targeted in food
- Not desirable (practicable) to analyse target pesticide one by one
- Separated Extraction, clean-up, instrumentation

➔ Increased cost and time

- Combine individual pesticide residues methods into fewer methods

➔ Need of Multi-Residues Method (MRM) for residues analysis

Examples of MRM

- Multi-Residues Method (MRM) for common pesticides
- MRM methods are readily available
- PAM (I), DFG S19, AOAC, GB, EN, Japan MHLW, Netherlands, etc…
- Refer to our first workshop for more information

Grouping up the GC analysis

- MRM → GC-amenable?
- More-volatile, less polar → design sample prep

- Detector for traditional GC-based MRM:

- Halogen containing: ECD
- N & P containing: NPD
- P & S containing: FPD
- Mass selective: MSD, ITD

- Recent GC-based MRM: QQQ, TOF, Q-TOF, HR-TOF

Grouping up the LC analysis

- MRM → LC amenable?
- Less-volatile, more-polar, thermally labile
  → design sample prep suited for LC

- Detector for traditional LC-based MRM:

- DAD/UV
- FLD
- MSD

- Recent LC-based MRM: QQQ, TOF, Q-TOF, HR-TOF

MRM applicable ?

- Various GC-/LC- columns available
- Various GC-/LC- detectors available
- Various labs developed MRM extraction protocols: Liquid Extraction, QuEChERs, etc

- Still some analytes are not amenable to common GC- / LC- based MRM

- Require modifications to existing MRM protocols

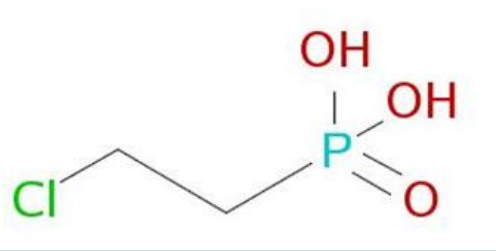
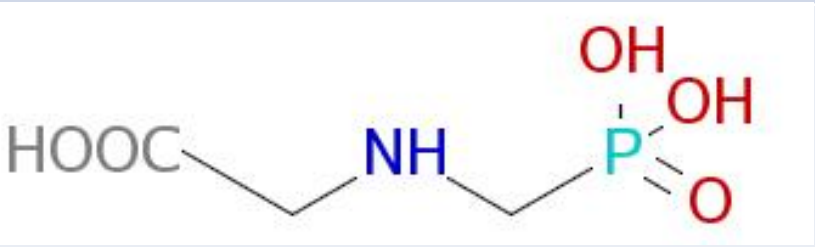
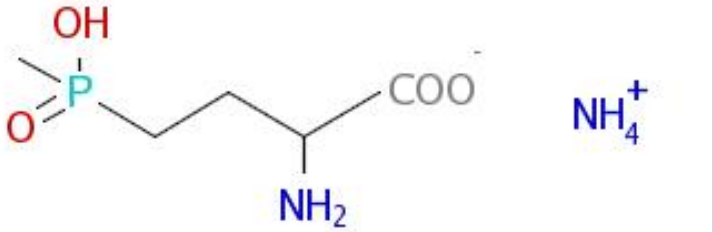
MRM vs SRM

- Development of MS detectors for GC- and LC-analysis, lower DL is achievable
- Same mass spectrometric instrument can now share the detection requirements of MRM and SRM
- Needs for traditional detectors are diminishing in modern single-residue analysis

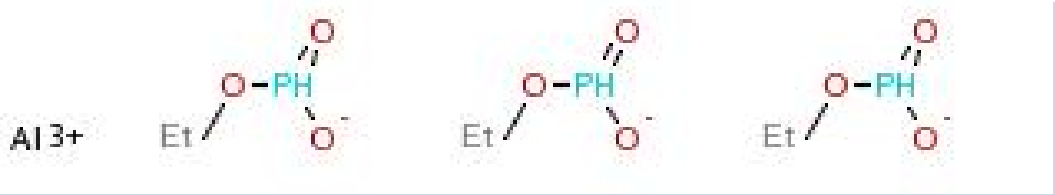
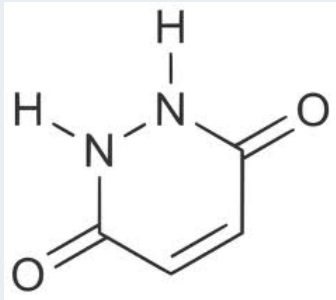
Single-residue Analysis

- Polar pesticides
- Fumigants
- Acidic pesticides
- Definition involving detection of moieties or conjugates

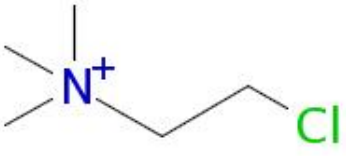
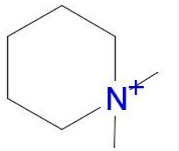
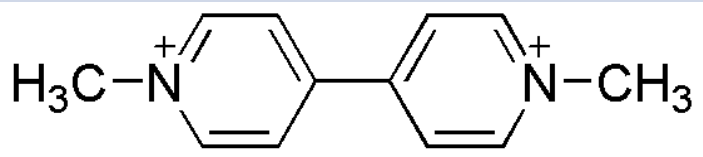
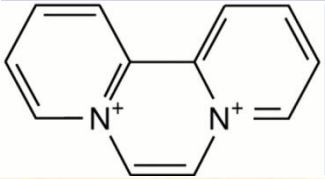
Polar Pesticides – Example 1

Name	Structure (with P-O-H)
Ethephon	
Glyphosate	
Glufosinate-Ammonium	

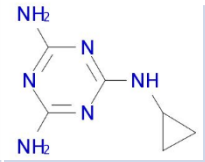
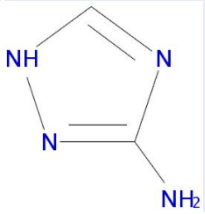
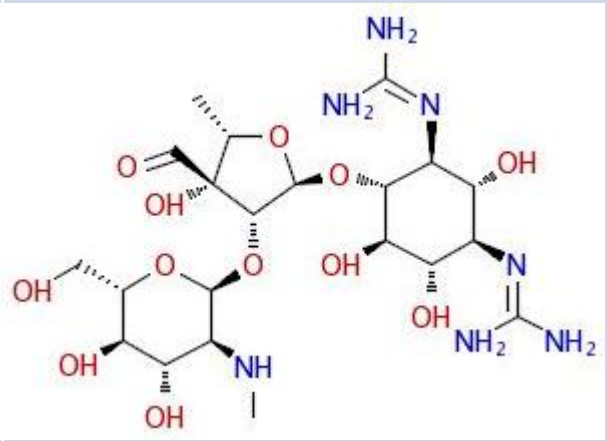
Polar Pesticides – Example 2

Name	Structure (with P-H, N-H)
Fosetyl-Al	
Maleic hydrazide	

Polar Pesticides – Example 3

Name	Structure (with quaternary ammonium)
Chlormequat	
Mepiquat	
Paraquat	
Diquat	

Polar Pesticides – Example 4

Name	Structure (with multiple -NH/-OH)
Cyromazine	
Amitrole	
Streptomycin	

SRM – Conventional Methods

Name	Single Residue Method in brief
Ethephon	Soxhlet extract with MeOH, derivatization for GC-FPD
Glyphosate	Extract with water, clean up by ion exchange column, derivatization for GC-FPD
Glufosinate-Ammonium	Homogenize with water, clean up by ion exchange column, derivatization for GC-FPD
Fosetyl-Al	Extract with dilute acid, *methylation* for GC-FPD
Maleic hydrazide	Alkaline digestion, distillation with *Zn* to release hydrazine, detection by UV
Chlormequat	Methanol extraction, clean up by ion exchange column, alkaline digest to release *acetylene*, HS-GC-FID analysis

SRM – Conventional Methods

Name	Single Residue Method in brief
Mepiquat	HCl-Methanol extraction, *demethylation*, GLC analysis
Paraquat	Extract with 5N sulfuric acid, clean-up by anion exchange column, reduction by sodium *dithionite*, determined by UV-VIS
Diquat	Reflux with sulfuric acid, clean-up by anion exchange column, reduction by sodium *dithionite*, determined by UV-VIS
Cyromazine	Extract with ACN-water (9:1), clean up by C18 Sep-Pak & anion exchange column, analysis by HPLC-UV
Amitrole	Extract with aqueous ethanol, *acetylation*, GC-NPD analysis
Streptomycin	Homogenized with aqueous solution, determined by microbiological method

SRM – Conventional Methods

- Long sample preparation
- Detection: FID, NPD, FPD, UV-VIS, microbiological, etc…
- Usually requires derivatization of analyte (methylation, demethylation, reduction, etc…)
- Single residues methods available, for example from PAM II, BfR, AOAC, etc…

SRM – Recent Developments

- One sample preparation to extract multiple number of polar pesticides
- Analysis by LC-MS/MS with optimized chromatographic conditions
- New SRM are available freely from EURL and other institutes

Polar Pesticides – Quick Sample Prep

1. Weigh sample into centrifuge tube
2. Add Internal standard solution
3. Add extraction solvent (acidified methanol)
4. Shake vigorously for 1 min
5. Centrifuge
6. Filter into vial with syringe filter
7. LC-MS/MS analysis

Polar Pesticides – LC-MS/MS

	Analyte	LC Column Example
1	Glyphosate, Ethephon, Glufosinate	Anion Exchange column
2	Fosetyl-Al, Maleic hydrazide, Chlormequat, Mepiquat, Paraquat, Diquat, Cyromazine, Amitrole, Streptomycin	Column with Zwitterionic sites

Fumigants

Fumigants Class	Residue definition
Carbon disulfide (CS_2)	Carbon disulfide
Methyl bromide	Methyl bromide
Propylene oxide	Propylene oxide (Cacao beans); Either as propylene oxide or propylene chlorohydrin (Fig)

Carbon Disulfide

- Boiling point: 46.3°C

- Extraction with aqueous acetone 5:1

or

- Extraction with aqueous acetone 9:1 followed by GC-FPD

or

- Purge and Trap GC with Hall electrolytic conductivity detector

Carbon Disulfide

1. Weigh sample
2. Add acetone:water (9:1)
3. Allow to stand for 24 hours
4. Isolate the extract
5. Direct inject into GC-FPD

Methyl Bromide

- Boiling point: 4°C

- Extraction with aqueous acetone 5:1

or

- Extraction with aqueous acetone 9:1 followed by HS-GC-ECD

or

- Blend the sample and analyse the headspace gas inside the blender by GC-ECD

Methyl Bromide

1. Weigh sample
2. Add acetone:water (9:1)
3. Allow to stand for 24 hours
4. Isolate the organic extract
5. Transfer extract to headspace bottle
6. Headspace GC-ECD analysis

Propylene Oxide

- Boiling point: 34°C
- Blend the sample with liquid nitrogen and analyse the sample by HS-GC-FID

Propylene Oxide

1. Cool the sample
2. Cryogenic mill the sample
3. Weight into headspace vial
4. Seal the vial immediately
5. Headspace GC-FID analysis

Propylene Chlorohydrins

- Similar sample preparation for propylene oxide can apply to 1-chloro-2-propylene and 2-chloro-1-propylene.

- Detection of by propylene chlorohydrins by HS-GC-ELCD (electrolytic conductivity detector)

Fumigants

Fumigants Class	Residue Definition
Hydrogen phosphide (PH_3)	All phosphides, **expressed as hydrogen phosphide**.
Dithiocarbamates (NR_2-CS_2^-)	Sum of all dithiocarbamates, **determined as CS_2 evolved during acid digestion**, expressed as mg CS_2/kg
Bromide ion	Bromide ion from all sources but **not including covalently bound bromine**.

Hydrogen Phosphide

- Can be in the form of zinc phosphide or aluminium phosphide
- Convert to hydrogen phosphide under acidic condition
- Headspace GC-FID analysis

Hydrogen Phosphide

1. Weigh the sample into headspace vial
2. Seal the vial
3. Add via syringe conc. HCl
4. Shake well and turn upside down the vial
5. Allow the vial to stand 30min at room temp
6. Inject the headspace into the chromatograph

Dithiocarbamates

- "Determined as CS_2 evolved during acid digestion"

- In the presence of Tin(II) chloride and Hydrochloric acid, formation of CS_2

Then,

→GC analysis

or

→ Formation of Cu:CS_2 complex, analyse by spectrophotometry

Dithiocarbamates

1. Weigh sample
2. Add isooctane
3. Add tin(II) chloride in HCl
4. Shaking water bath, 80°C, 2h
5. Cool the extract
6. Fill the extract into vial
7. GC-ECD analysis

Bromide ion

- Methyl bromide may degrade to inorganic bromide
- Ring opening of epoxide by bromide ion under acidic condition

→ suitable for LC / GC analysis

Epoxide = ethylene oxide or propylene oxide

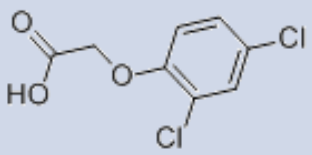
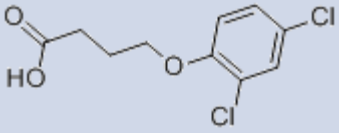
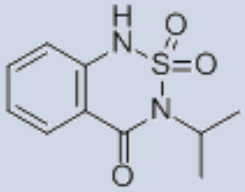
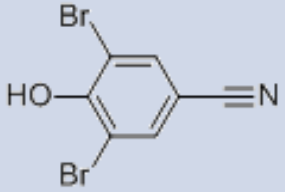
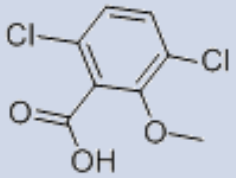
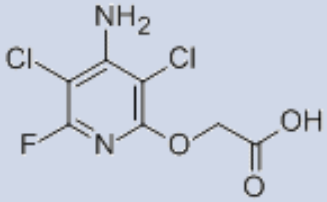
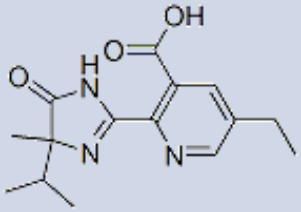
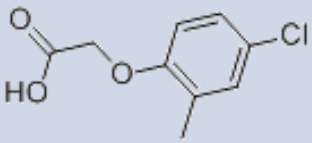
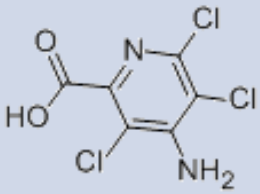
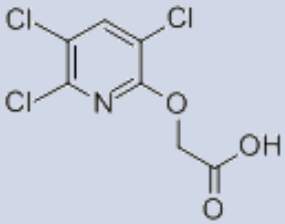
Bromide ion

1. Cut the sample into small size
2. Put into freezer overnight (-18 °C)
3. Mill the sample with coolant
4. Weigh 5 grams sample and 3 mL water
5. Add 5mL propylene oxide and 1mL H_2SO_4
6. Add 4g NH_4SO_4, extract by EtOAc
7. Organic layer for GC-ECD analysis

Single Analyte Test Methods

- Polar pesticides
- Fumigants
- Acidic pesticides
- Definition involving detection of moieties or conjugates

Acidic Pesticides

2,4-D	2,4-DB	Bentazone	Bromoxynil	Dicamba
				
Fluroxypyr	**Imazethapyr**	**MCPA**	**Picloram**	**Triclopyr**
				

Pesticide Name	Residue Definition
2,4-D	2,4-D
2,4-DB	Combined residues of 4- (2,4-dichlorophenoxy) butyric acid and its metabolite 2,4- dichlorophenoxyacetic acid
Bentazone	Bentazone
Bromoxynil	Bromoxynil
Dicamba	Combined residues of dicamba and its metabolite 3,6-dichloro-2-hydroxybenzoic acid
Fluroxypyr	Fluroxypyr
Imazethapyr	Imazethapyr
MCPA	2-methyl-4-chlorophenoxyacetic acid (MCPA)
Picloram	Picloram
Triclopyr	Triclopyr

Acidic pesticides – Cleanup issue

- Acidic compounds interact with PSA sorbent
- Losses of acidic compounds after cleanup

→ Skip PSA cleanup

Acidic pesticides – pH issue

- Ionization of pesticides at low or high pH-values

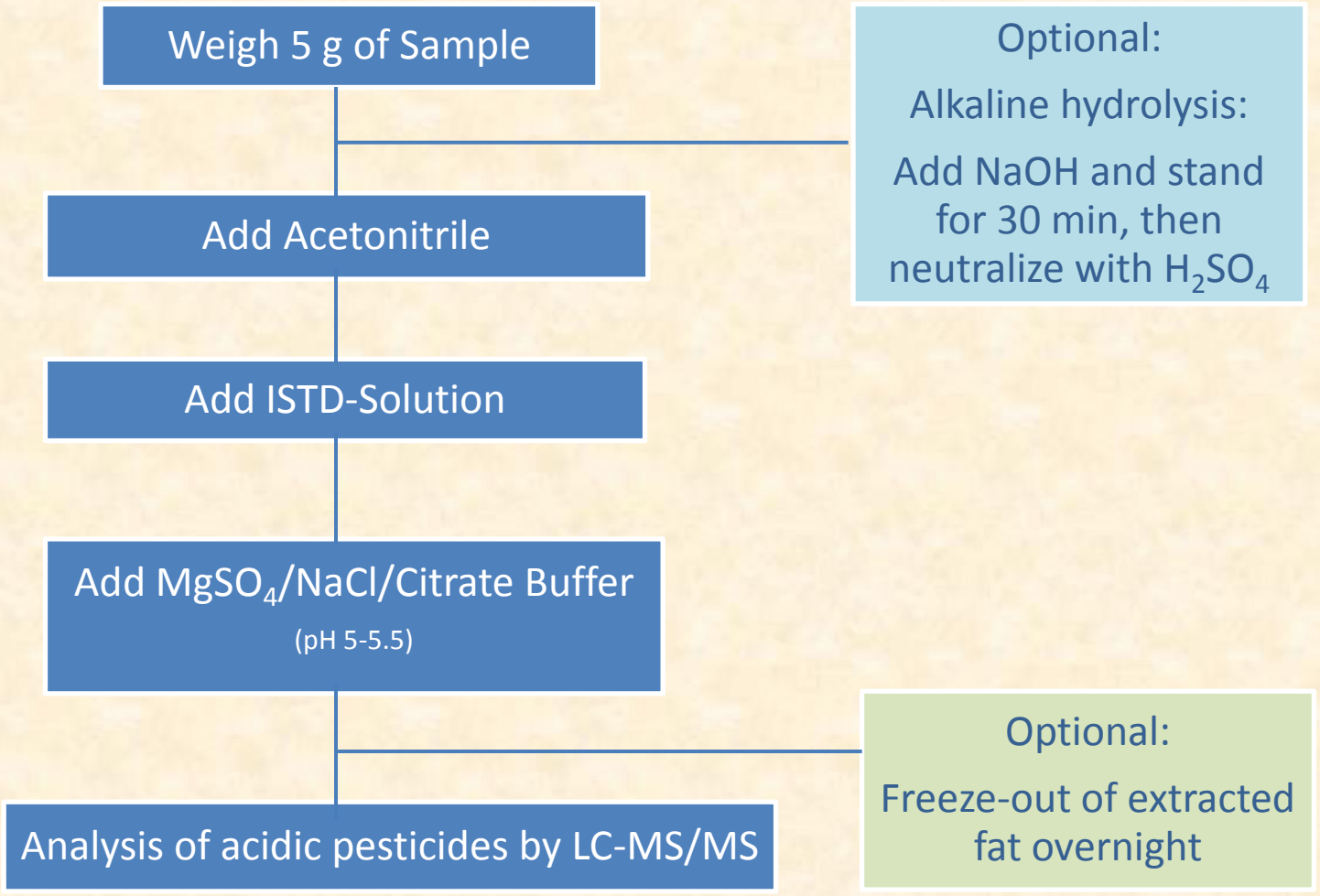
  Acids: $HX \rightleftharpoons H^+ + X^-$

  Bases: $B + H^+ \rightleftharpoons BH^+$

  → Ionic form prefers to stay in the water phase

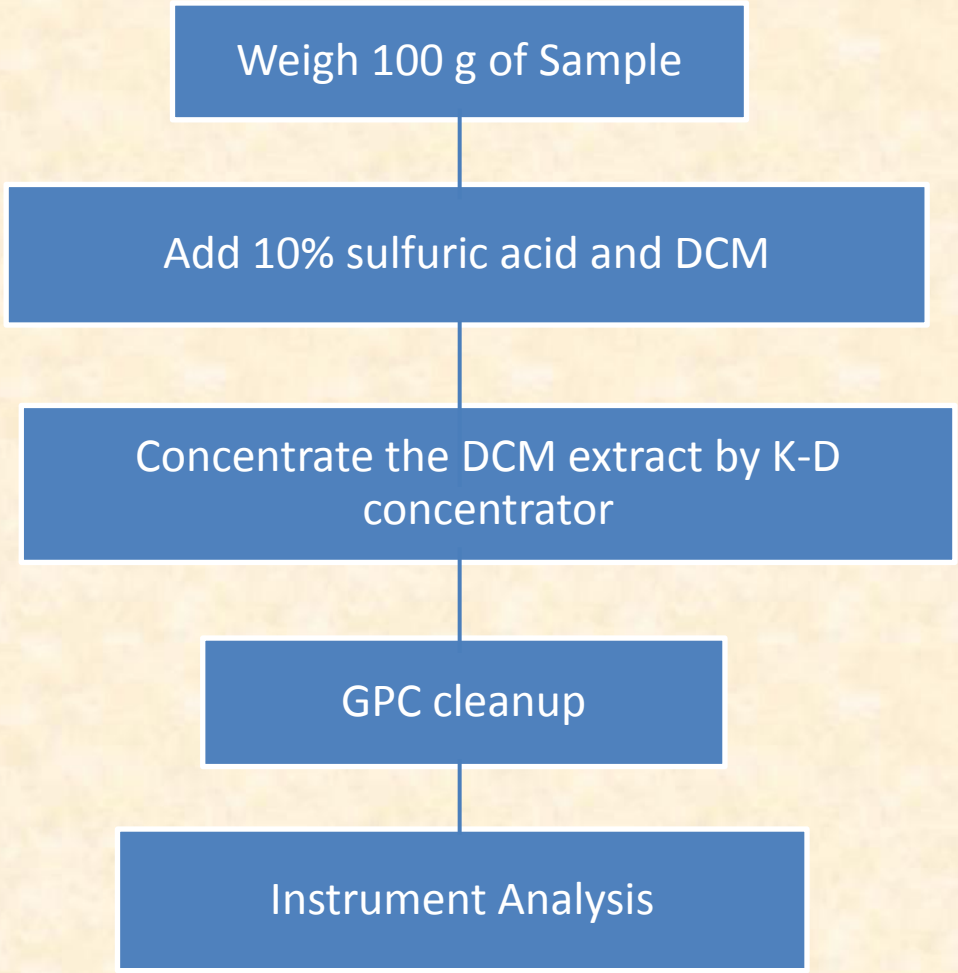
- pH-range of agriculture samples is $\sim 2.5 - 7$

QuEChERS modification for acidic pesticides

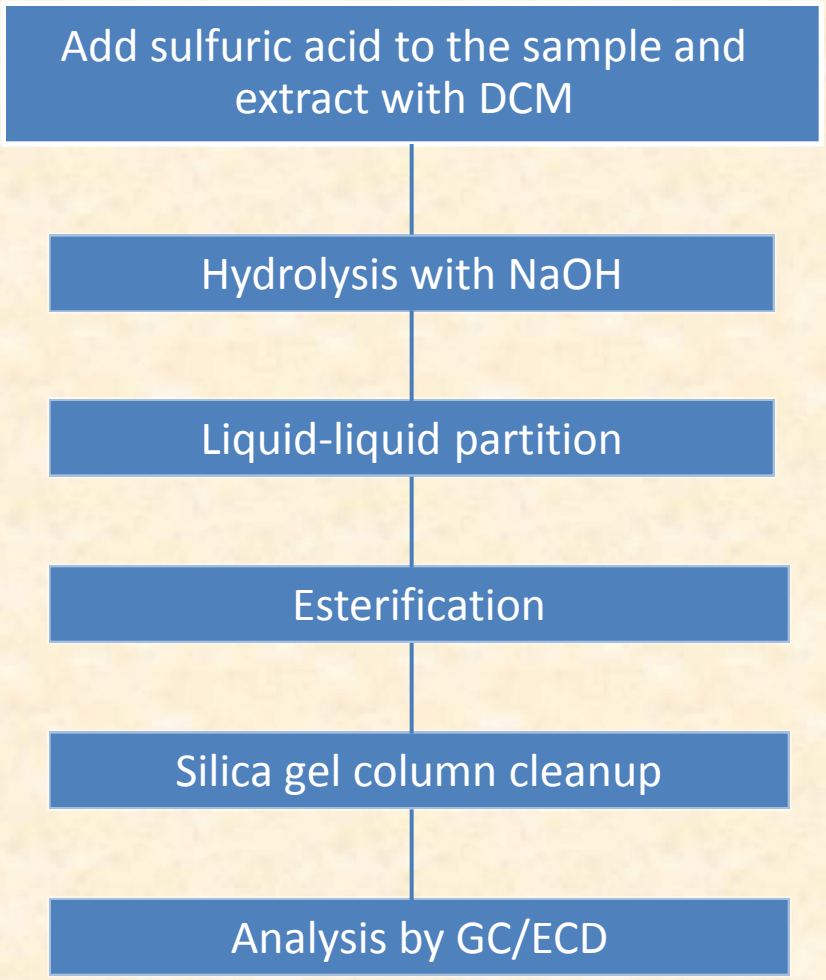


Alternative methods

- Residues are extracted from fruits and vegetables with DCM after acidification

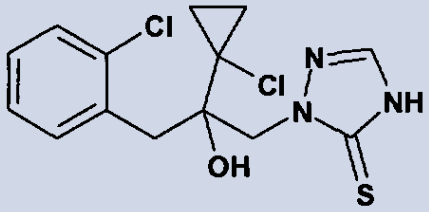
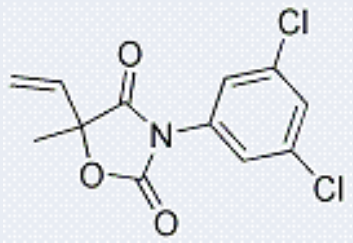


❖ Traditional method involve derivatization to trichloroethyl esters



Pesticides with Definition involving Moiety

- Examples of pesticides involving moiety:

Pesticide Name	MRL Definition
Prothioconazole 	Combined residue of prothioconazole and prothioconazole-desthio, and conjugates that can be converted to these two compounds by acid hydrolysis, calculated as prothioconazole
Vinclozolin 	Sum of vinclozolin and all metabolites containing the 3,5-dichloroaniline moiety, expressed as vinclozolin

Determination of Vinclozolin

- Parent and metabolite compounds are converted to dichloroaniline by alkaline hydrolysis and then derivatized to N-3,5-dichlorophenyl chloroacetamide

Weigh ~10-20 g of sample

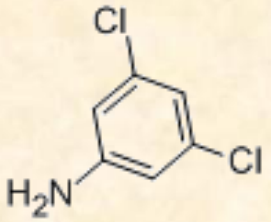
Hydrolysis/distillation with KOH

Partition aqueous distillation with DCM

Derivatize DCM phase with chloroacetyl chloride

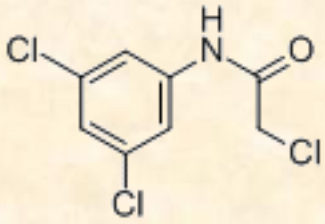
Concentrate and dissolved in ethyl acetate

Analysis by GC/ECD



dichloroaniline

derivatization



N-3,5-dichlorophenyl chloroacetamide (DCAD)

Calculation

- Vinclozolin concentration = C x Cf x Df/Sw

where C = Concentration of DCAD in the sample

Cf = Molecular weight (MW) conversion factor

= MW of Vinclozolin (286)/ MW of DCAD (238)

= 1.20

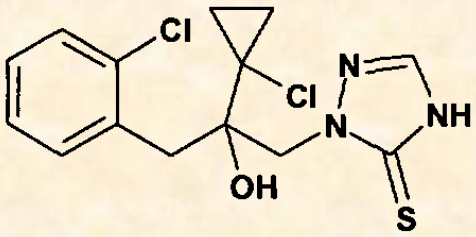
Df = Dilution factor

Sw = Sample weight

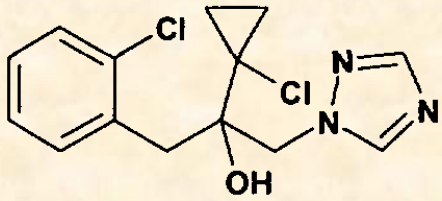
Determination of Prothioconazole and Desthio-Prothioconazole

- Definition: Combined residue of prothioconazole and prothioconazole-desthio, and conjugates that can be converted to these two compounds by acid hydrolysis, calculated as prothioconazole

- Extraction procedure involves acid hydrolysis

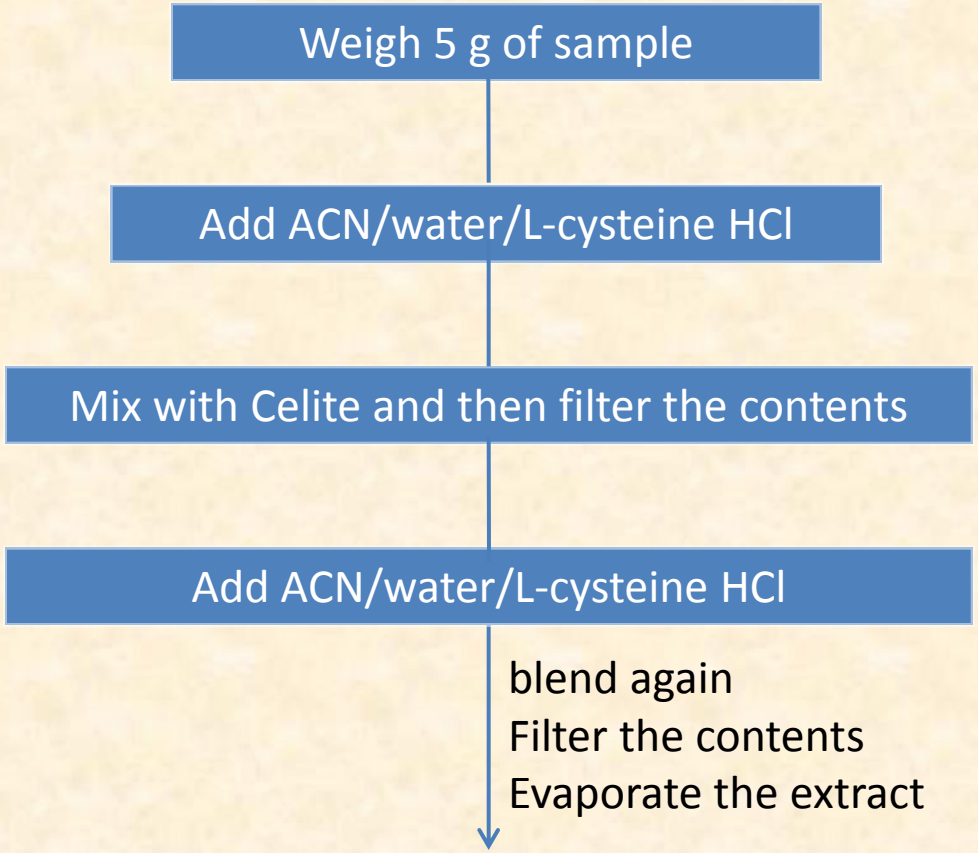


Prothioconazole

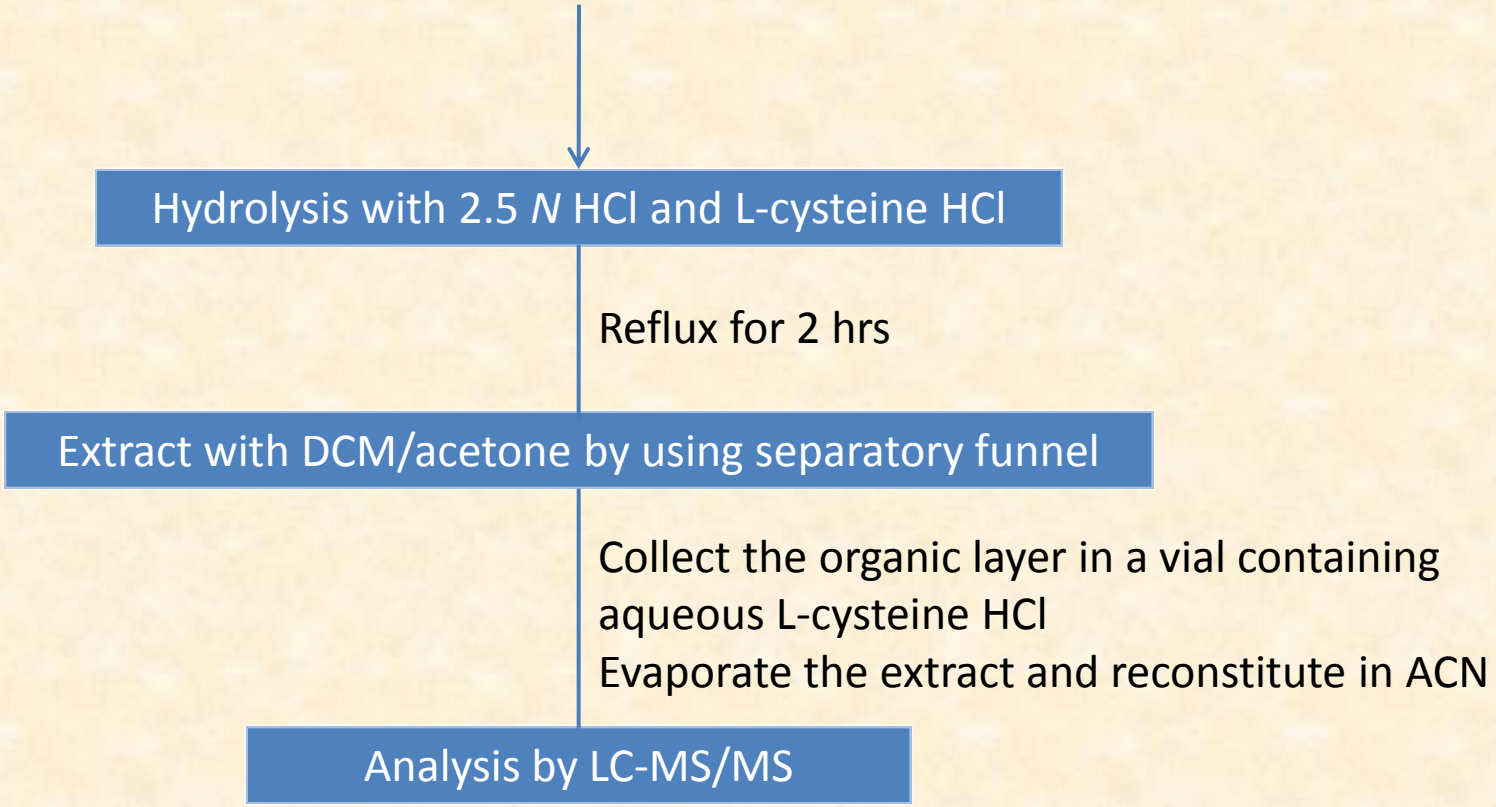


Desthio-prothioconazole

Determination of Prothioconazole and Desthio-Prothioconazole



Determination of Prothioconazole and Desthio-Prothioconazole (cont'd)



Method References

- German - Federal Institute for Risk assessment (BfR)

➔§ 35 LMBG official analytical methods: L 00.00 15 / L 00.00 16

– available freely from BfR website

➔"Manual of Pesticide Residue Analysis Volume I and II" – available as hardcopy

- USFDA / EPA :

➔"Pesticide Analytical Manual (PAM)" Volume I and II

➔"Residue Analytical Methods (RAM)"

Method References

- Joint FAO/IAEA:
  - ➔Food Contamination and Residue Information System (INFOCRIS)
- EURL (EU Reference Laboratories):
  - ➔EURL-SRM: single residue methods provided by EURL
- AOAC – “Official Methods of Analysis”
- Standard methods ISO, EN, GB etc…

Thank You!