


Pesticide Residues Monitoring :
Practical Aspects (e.g. sampling) and
Analytical Tools Available

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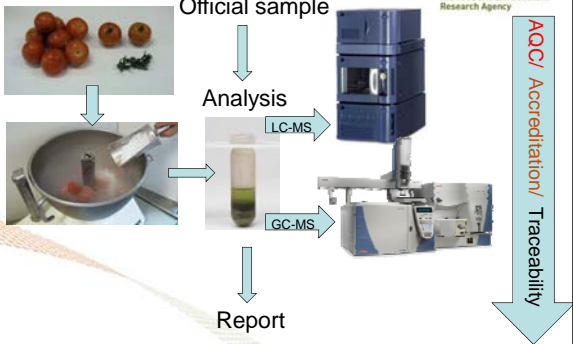


Topics to be discussed

- Sampling
 - Guidelines
 - Practice
- Analytical Methods
 - Scope of methods
 - Instrumentation
 - Analytical Quality Control
- Sources of information /training



Overview –pesticides in fruits and vegetables



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graph TD
    A[Official sample] --> B[Analysis]
    B --> C[Report]
    B --> D[LC-MS]
    B --> E[GC-MS]
    F[AQC/ Accreditation/ Traceability]
  
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Taking a representative sample



Official guidelines

- Commission Directive 2002/63 (mirrors Codex guidelines)
Codex Alimentarius Commission Vol 2, 1993 (ISBN 92-5-103271-8)
..... aimed at protecting consumers' health and ensuring fair practices in the food trade.....

Codex methods of sampling are designed to ensure that fair and valid sampling procedures are used

..... But it isn't always as easy as this sounds.....can be subjective **but the sample is the legal sample**

Traceability



- Documented Procedures (SOP, sampling plan)
- Trained and authorised samplers (e.g. government officials)
- Use appropriate tamper evident bottles/sealed bags
- Detailed sample forms
- Transport sample to laboratory using containers to maintain sample in good condition

Analysis of pesticide residues



- Multi-residue Methods (MRMs) based on Mass Spectrometry preferred (100s of analytes)
- Single Residue methods (SRMs) also required
- e.g. dithiocarbamates, glyphosate
- Methods not prescribed
- Laboratories can choose to use any method but must provide evidence to demonstrate that results are valid

Method (step 1)
Sample Preparation & Sample Processing



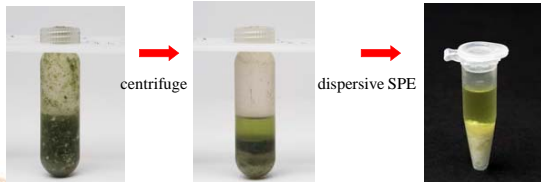
- Unavoidable steps in the analysis of pesticides
- Prerequisite to obtain representative sub-samples
- Possible losses of pesticide residues during homogenisation of sample



Method (step 2) - Extraction & Clean-up e.g. QuEChERS

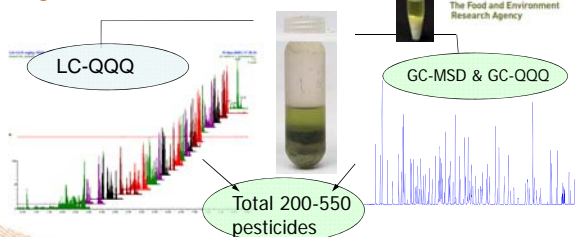


Qu (quick) E (easy) Ch (cheap) E (effective) R (rugged) S (safe)



- < sample (10 g) < solvent < reagents < equipment < space < cost
- Extracts easy to analyse by LC-MS *but not GC-MS* (high sensitivity GC-MS/MS may provide a solution)

Typical multi-residue approach e.g. QuEChERS



- Target analyte approach only detects pesticides in the 'predefined list'
- Other residues present will not be detected (TOFMS will provide more information)

Methods sources (1)

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

<http://www.cen.eu/CENORM/Sectors/TechnicalCommittees/Workshops/CENTechnicalCommittees/WP.asp?param=6256&title=CEN%2FTC+275>

- EN15055: 2006 chlormequat and mepiquat (LCMS/MS)
- EN15662: QuEChERS (GC-MS and LC-MS)
- EN15637: ChemElut method (LC-MS/MS only)
- TR15641: Collection of MS/MS parameters

CEN methods validated by collaborative trial

Method sources (2)

Community Reference Laboratories

CRL-Web-Portal: <http://www.crl-pesticides.eu/>

- Details of multi-residue and single residue methods

CRL Data Pool: <http://www.crl-pesticides-datapool.eu>

Method Validation data:

- > 650 compounds, > 100 commodities, > 50,000 results

See later slides for further information on CRL activities

Method sources (3)

www.quechers.com

Chemisches und Veterinäruntersuchungsamt Stuttgart CVUA

Pesticide	Commodity	Determination	Cleanup with PSA	No cleanup	Remarks
Atrazine	Apple	LC-MS/MS	x	x	
	Cucumber	LC-MS/MS	x	x	
Atrazine-desethyl	Apple	LC-MS/MS	x	x	
	Cucumber	LC-MS/MS	x	x	
Atrazine-desisopropyl	Apple	LC-MS/MS	x	x	
	Cucumber	LC-MS/MS	x	x	
Azinphos-methyl	Cucumber	GC-MSD	x	x	Sensitive towards decomposition in the injector
Azoxystrobin	Apple	GC-MSD	x	x	
	Cucumber	GC-MSD	x	x	
Beflubutamid	Apple	GC-MSD	x	x	
	Cucumber	GC-MSD	x	x	
Benalaxyl	Apple	GC-MSD	x	x	
	Cucumber	GC-MSD	x	x	
Benazolin	Apple	LC-MS/MS		x	pH up to 6 avoid PSA

Other methods for pesticide analysis (fruits and vegetables)



QuEChers acetonitrile - not the only method

- Ethyl acetate - H G. J. Mol *et al*, *Anal. Bioanal. Chem.* (2007) 389 1715-1754
- Acetone – M A Luke *et al*, *J. AOAC Int.*, 1975, **58**, 1020-1026
W Specht *et al*, *Fresenius' Journal analytical chemistry* 1995, **353**, 1432-1130
- Methanol - Klein J, Alder L. *J. AOAC Int.*, 2003, **86**, 1015-1037 (only for LC-MS/MS)

Analysis of fish and high fat samples



- Version of QuEChERS with a 'freezing step' to remove fat but not ideally suited to analysis of fish and high fat AP samples
- Suitable methods usually require
 - Extraction of fat
 - Separation of pesticides from fat (gel permeation chromatography)
 - Additional SPE clean-up of GPC extracts
 - Concentration
 - GC-MS/MS analysis (preferred to GC-MS)

Method Analytical Quality Control



Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed Document N° SANCO/2007/3131

http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf

Provide guidelines on best laboratory practice
- from sample receipt through to reporting of the result(s)

Define minimum method performance criteria that are required
- allows laboratories to use any method of their choice

The key objectives of the guidelines (SANCO/2007/3131)



- To ensure false positives or false negatives are not reported
- Define criteria for identification of residues
- Define acceptable accuracy and precision
- To harmonise cost-effective AQC in the EU
- To support compliance with the ISO/IEC 17025 accreditation standard

Proficiency Testing



- Good method performance can be demonstrated by analysis of proficiency test samples
e.g. EUPT Series, FAPAS
- Independent assessment of laboratory performance (shows any bias of results from the 'true' value)
- Requirement of accreditation bodies
- Requirement of European Commission for official control laboratories

EU Proficiency Tests



Article 28 of Regulation 396/2005/EC

'All laboratories analysing samples for the official controls on pesticide residues shall participate in the European Community Proficiency Tests for pesticide residues organised by the Commission'.

EU Proficiency tests 'Carried out on an annual basis in order to ensure the quality, accuracy and comparability of the residue data reported by EU Member States to the European Commission as well as to other Member States'.

EU PT Protocols



- General protocol
Assessment of results and definitions
MRRLs, false positives, false negatives, assigned value, fit-for-purpose standard deviation, z-score, combined z-scores, classification
- Specific protocol
All information necessary to participate in a particular PT

Assignment of MRRLs & ND results



- Minimum required reporting levels (MRRLs)
- encourage labs to achieve acceptable reporting levels (LOQs)
- to allow false positive results to be easily identified
- Not Detected (ND)
- any compound present in the test material at, or above, the MRRL and reported as a ND is considered to be a false negative result

EU Proficiency tests (fruits & vegetables)



- PT 1 Jan 1997 sweet peppers
 - PT 2 Mar 1999 apples
 - PT 3 May 1999 cucumbers
 - PT 4 May 2002 oranges
 - PT 5 May 2003 lettuce
 - PT 6 May 2004 tomatoes
 - PT 7 May 2005 grapes
 - PT 8 June 2006 aubergine (egg plant)
 - PT 9 May 2007 strawberries
 - PT 10 May 2008 carrot
 - PT 11 June 2009 cauliflower
- PTs for Animal products and cereals/products
 - First PT for **screening** pesticides spiked in extracts of oranges

Performance of Laboratories



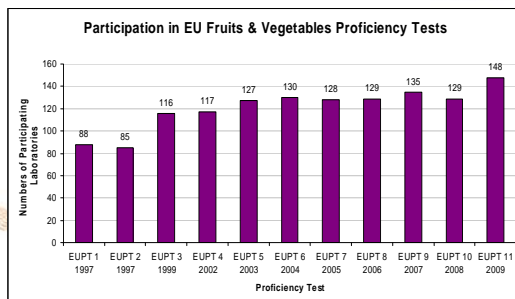
- No false positive results
- No false negative results
- Demonstrated sufficient scope
- Ranked according to:
 - Numbers of pesticides sought
 - Sum of Weighted z-Scores (SWZ)

Performance of Laboratories

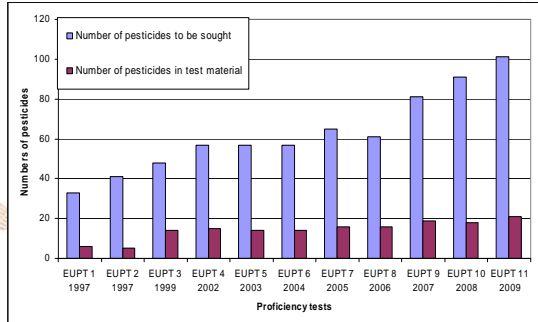


Lab Code	No of Pesticides Sought	SWZ	Classification
121	18	0.3	Good
067	18	0.5	Good
063	18	1.1	Good
097	18	2.2	Satisfactory
019	18	3.4	Unsatisfactory
110	17	0.3	Good
022	17	1.8	Good
080	17	4.0	Unsatisfactory
127	17	4.1	Unsatisfactory
010	16	0.6	Good
043	16	2.1	Satisfactory

Participation in EU PTs – pesticides in fruit and vegetables



Number of pesticides in each EU PT round (fruit and vegetables)



Legislation – Regulation 882/2004 (Official Controls)



Articles 32 & 33:

Describes a concept of a network of Community Reference Laboratories (CRLs) and National Reference Laboratories (NRLs) to improve the overall quality of official labs in the EC

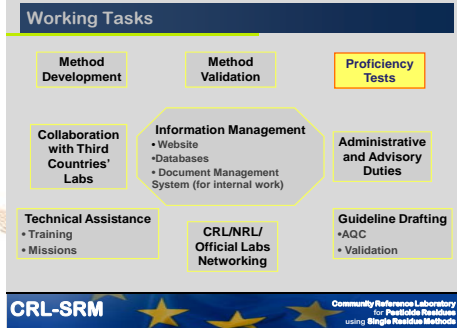
Community Reference Laboratories (CRLs) pesticides



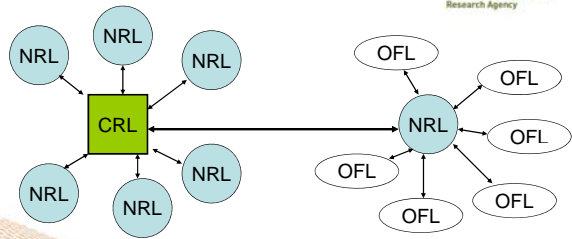
- Fruits & Vegetables Spain, Almeria/Valencia
- Cereals & Animal Feeds Denmark, Copenhagen
- Products of Animal Origin Germany, Frieburg
- Single Residue Methods Germany, Stuttgart

<http://www.crl-pesticides.eu>

CRLs Work Programmes



Networking



Conclusions



- Methods performance criteria approach preferred to the use of prescribed methods
- Multi-residue approaches based on MS are now capable of screening hundreds compounds in a single analysis
- Since the introduction of quality control procedures the harmonisation of performance of residues testing laboratories, and the reliability of data, has improved
- Ever increasing pressure from the European Commission for laboratories to continue to improve
