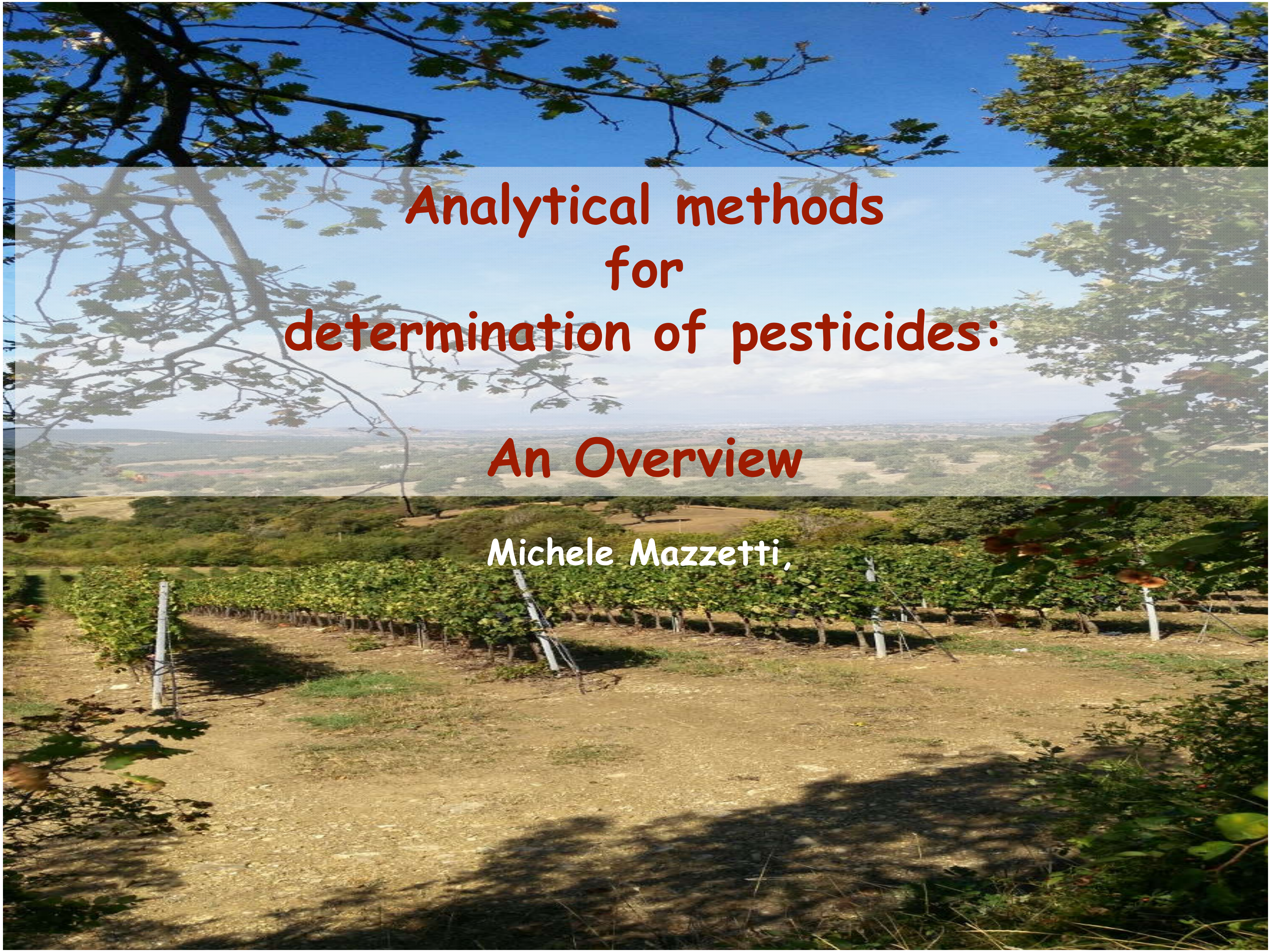


Analytical methods for determination of pesticides:

An Overview

Michele Mazzetti,

What is a Pesticide?

A 'pesticide' is something that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport.
The term includes, amongst others: herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, rodenticides, growth regulators, repellents, rodenticides, and biocides.

European
Commission

http://ec.europa.eu/food/plant/pesticides_en

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide,and various other substances used to control pests

(EPA, 2009)

What is a Pesticide?

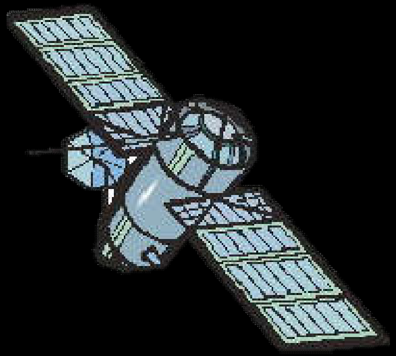
Definition of pesticide varied with times and countries. However, the essence of pesticide remains basically constant, i.e., it is a (mixed) substance that is poisonous and efficient to target organisms and is safe to non-target organisms and environments (hopefully n.d.r.)

Proceedings of the International Academy of Ecology and Environmental Sciences, 2011, 1(2):125-144

History of pesticides

in the first phase (the period before 1870s) natural pesticides, for instance sulfur in ancient Greece, were used to control pests;

the second phase was the era of inorganic synthetic pesticides (the period 1870s-1945). Natural materials and inorganic compounds were mainly used during this period

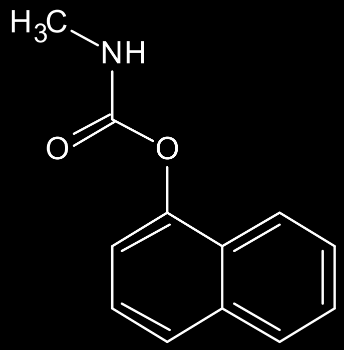


the third phase (since 1945) is the era of organic synthetic pesticides. Since 1945, the man-made organic pesticides, e.g., DDT, 2,4-D, and later HCH, dieldrin, have terminated the era of inorganic and natural pesticides.

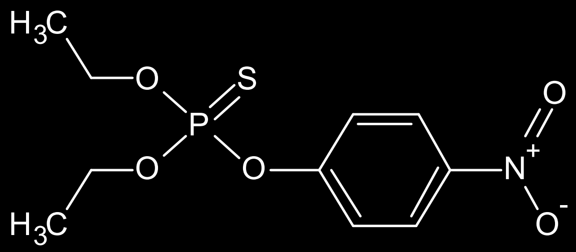
Proceedings of the International Academy of Ecology and Environmental Sciences, 2011, 1(2):125-144

History of pesticides

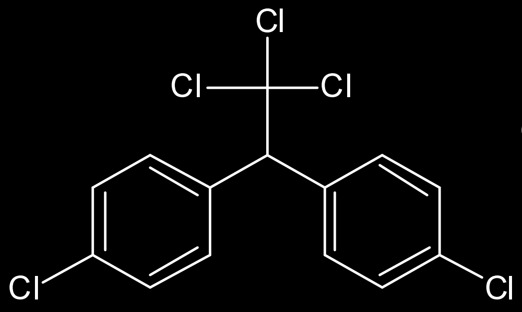
In the earlier period of organic synthesized pesticides, there were mainly three kinds of insecticides,



carbamated insecticides:



organophosphorus insecticides



organochlorined insecticides.

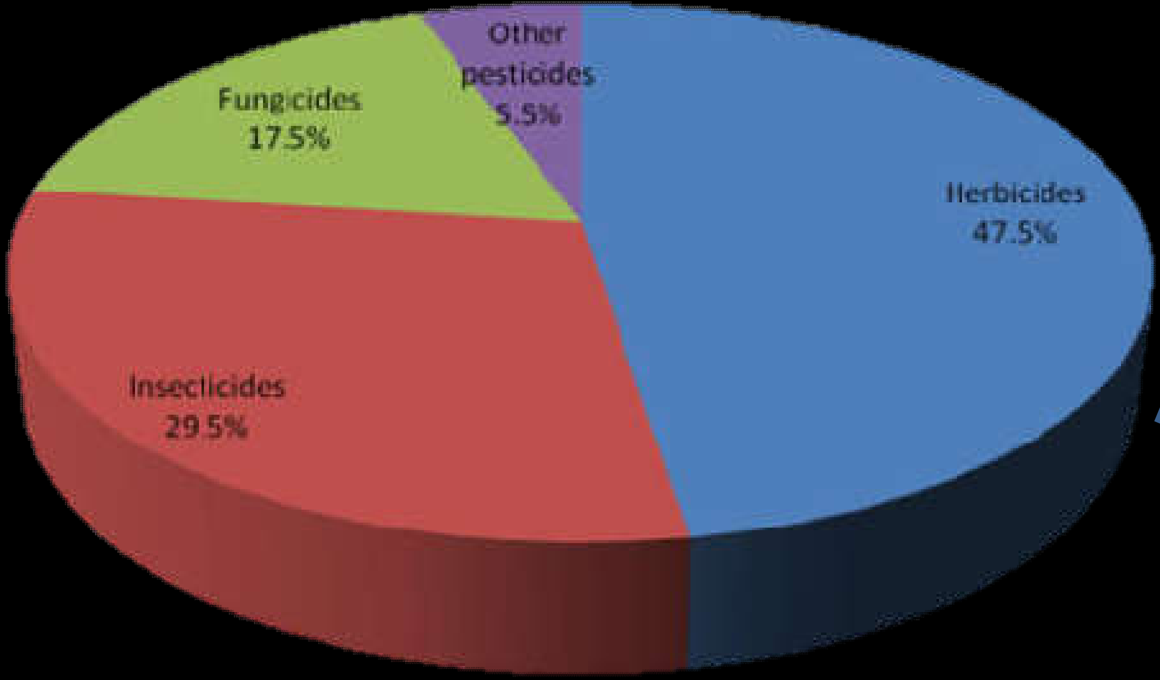
Sooner after that herbicides and fungicides achieved a considerable development as well

Proceedings of the International Academy of Ecology and Environmental Sciences, 2011, 1(2):125-144

Consumption of pesticides

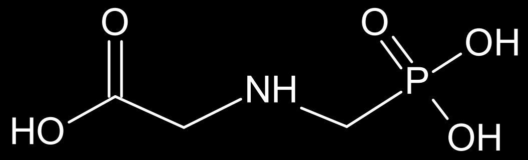
The consumption of insecticides is estimated to decline gradually and the use of herbicides would be popular in the future.

This trend may be found from the changes of the structure of pesticide consumption worldwide



Worldwide consumption of pesticides (2013)

…for example Glyphosate



A. De et al.,Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles, Springer Briefs in Molecular Science, DOI: 10.1007/978-81-322-1689-6_2,

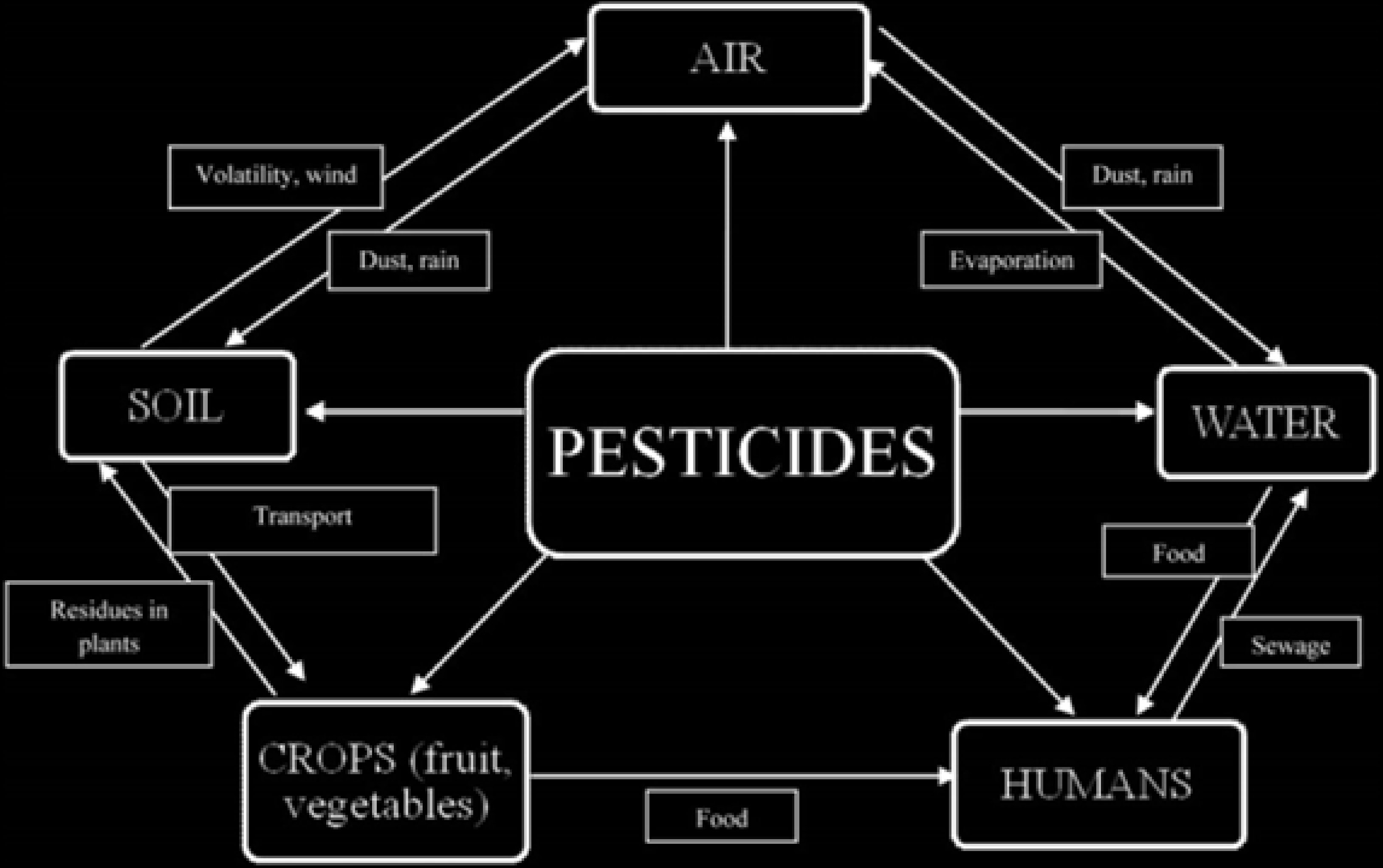
Proceedings of the International Academy of Ecology and Environmental Sciences, 2011, 1(2):125-144

Consumption of pesticides

Note that pesticide sales in North America haven't grown very much — and usage actually seems to be declining in the United States (more on that below). The growth in Europe, meanwhile, is largely driven by a big uptick in sales in Eastern Europe. Meanwhile, sales are more or less stagnant in the Middle East and Africa.

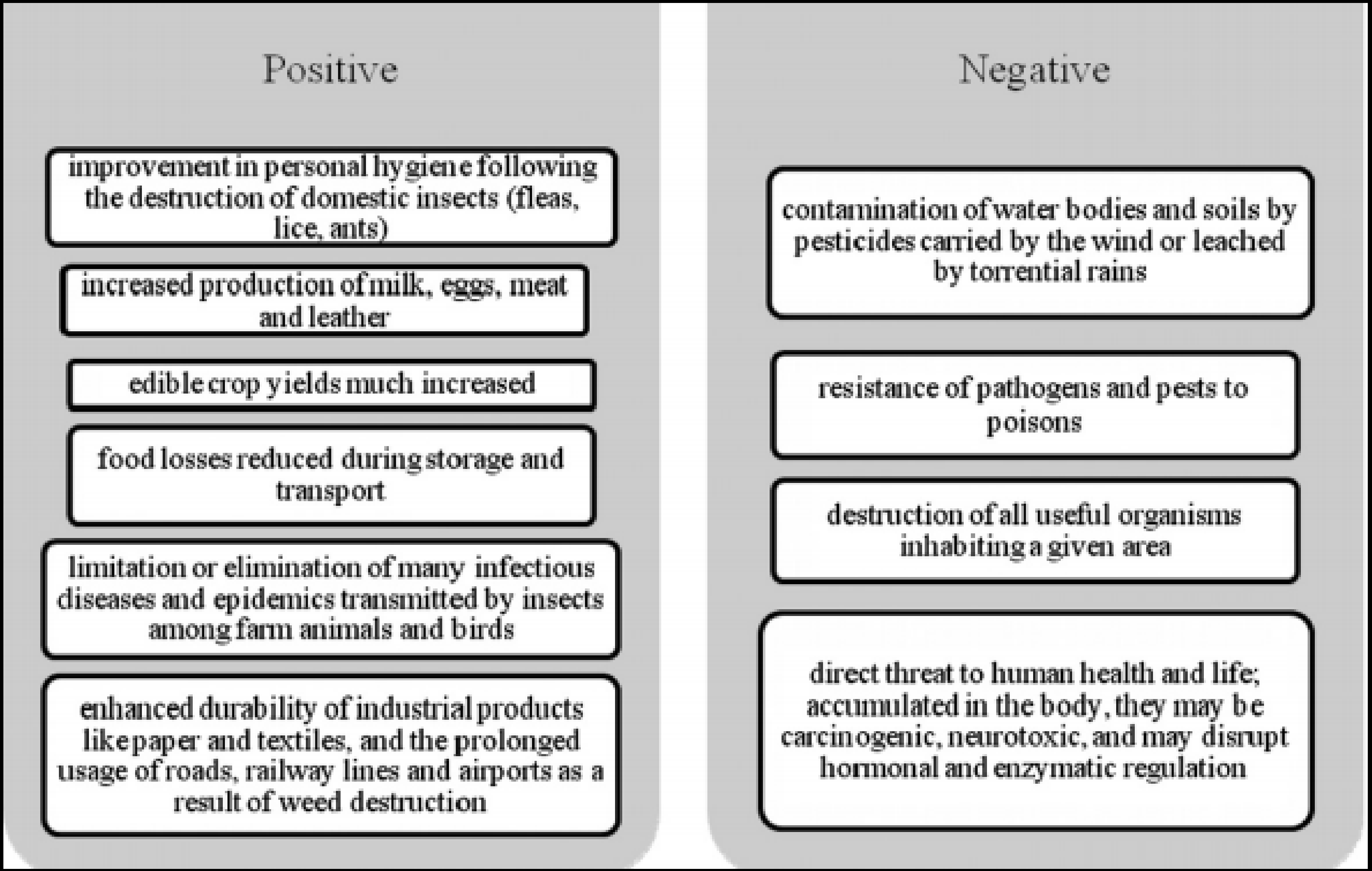
FAO: FAO Statistical Yearbook 2013: World Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. 2015, p. 65. Available at: http://www.fao.org/docrep/018/i3107e/i3107e00.htm

The circulation of pesticides in nature *(including crops)*



Trend in Analytical Chemistry, Vol. 30, No. 6, 2011

The effects of using pesticides



Positive

- improvement in personal hygiene following the destruction of domestic insects (fleas, lice, ants)
- increased production of milk, eggs, meat and leather
- edible crop yields much increased
- food losses reduced during storage and transport
- limitation or elimination of many infectious diseases and epidemics transmitted by insects among farm animals and birds
- enhanced durability of industrial products like paper and textiles, and the prolonged usage of roads, railway lines and airports as a result of weed destruction

Negative

- contamination of water bodies and soils by pesticides carried by the wind or leached by torrential rains
- resistance of pathogens and pests to poisons
- destruction of all useful organisms inhabiting a given area
- direct threat to human health and life; accumulated in the body, they may be carcinogenic, neurotoxic, and may disrupt hormonal and enzymatic regulation

Trend in Analytical Chemistry, Vol. 30, No. 6, 2011

PESTICIDES IN FOOD/FEED: REGULATORY FRAMEWORK
MAXIMUM RESIDUE LEVELS (MRL)

REGULATION (EC) No 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 February 2005

Article 3

Definitions

(d) **‘maximum residue level’ (MRL)** means the upper legal level of a concentration for a pesticide residue in or on food or feed set in accordance with this Regulation, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers;

efsa

European Food Safety Authority

26 October 2016

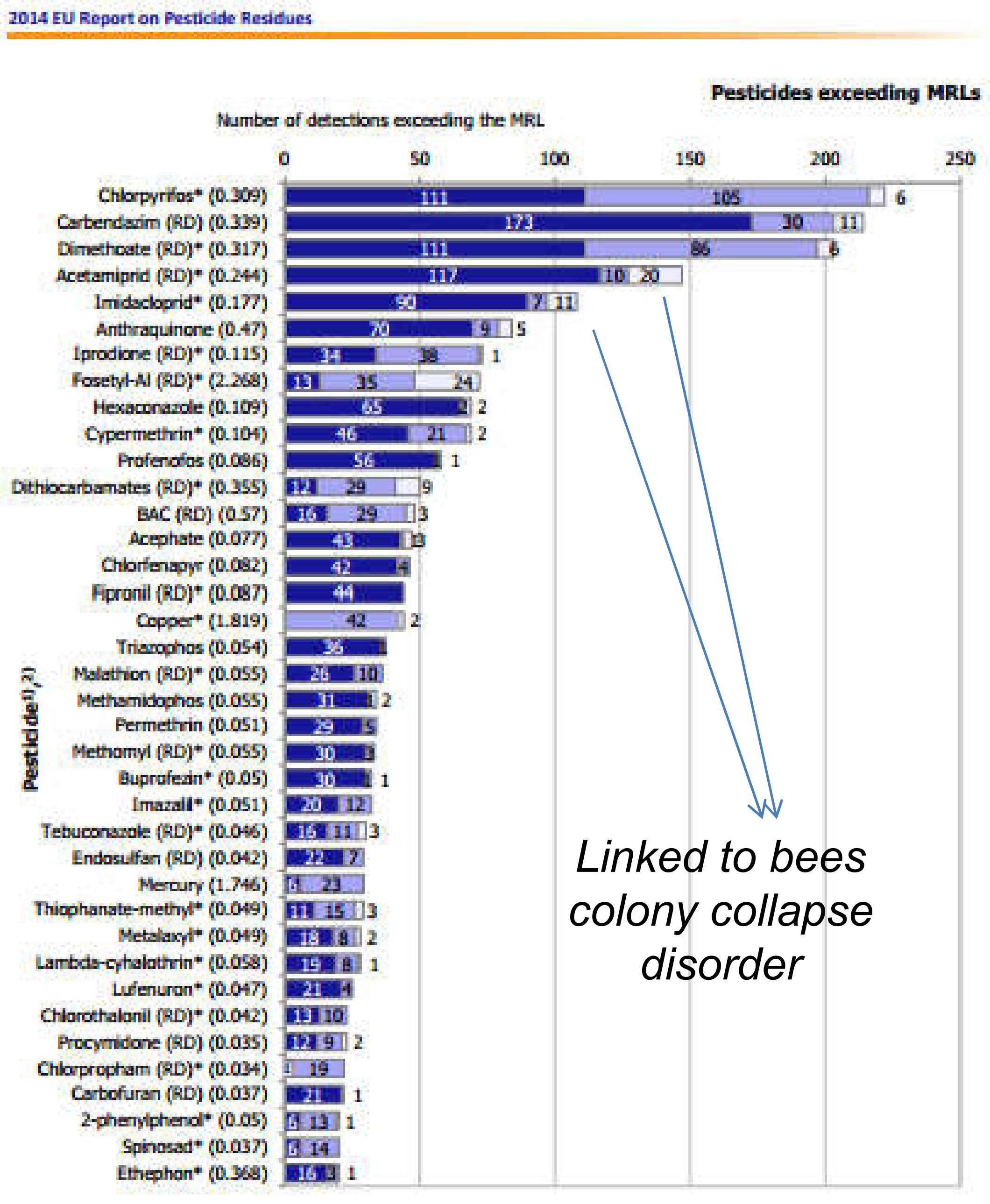
Pesticide residues in food: risk to consumers remains low

- 97% of samples analysed were within legal limits.
- Of these, 53.6% were free of quantifiable residues and 43.4% contained residues that were within permitted concentrations.
- Of the samples originating from EU/EEA countries, 1.6% contained residues exceeding legal limits; the corresponding figure for samples from third countries was 6.5%.
- No quantifiable residues were found in 91.8% of baby food samples.
- 98.8% of organic products were either free of residues or contained residues within legal limits.

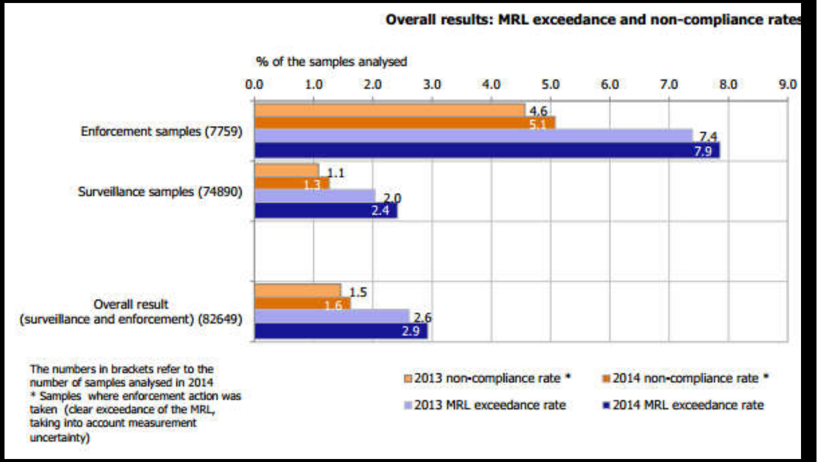
EFSA concluded that exposure is unlikely to pose a threat to human health.

EFSA Journal

The 2014 European Union Report on Pesticide Residues in Food European Food Safety Authority



Linked to bees colony collapse disorder



The numbers in brackets refer to the number of samples analysed in 2014
* Samples where enforcement action was taken (clear exceedance of the MRL, taking into account measurement uncertainty)

The main stages in analytical procedures for determining pesticides in samples of fruit and vegetables

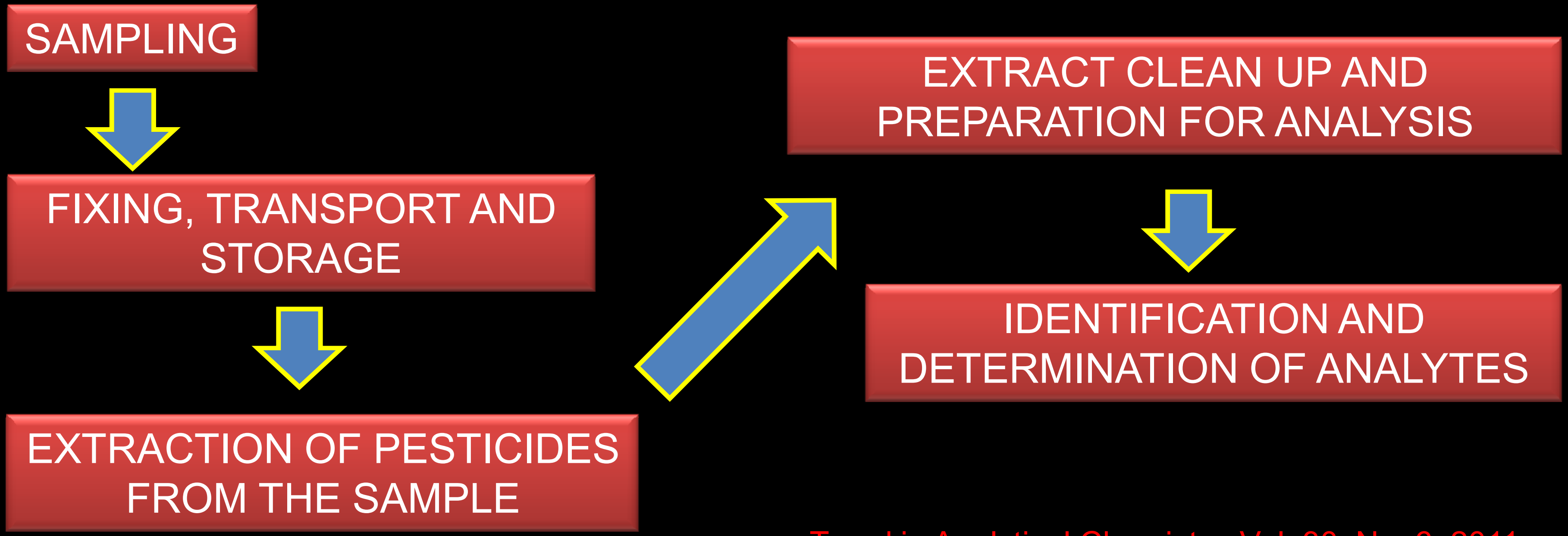
The analysis of pesticides in biological samples continues to present challenges to analysts..

A number of problems crop up in the analysis of pesticide residues:

(1) the complexity and the diversity of matrices in biological materials;,

(2) the low concentrations of pesticides in samples of fruit and vegetables.

Target analytes must, therefore, be isolated from matrices and then be enriched before the final determination can be undertaken



SAMPLING

FIXING, TRANSPORT AND STORAGE

EXTRACTION OF PESTICIDES FROM THE SAMPLE

EXTRACT CLEAN UP AND PREPARATION FOR ANALYSIS

IDENTIFICATION AND DETERMINATION OF ANALYTES

Trend in Analytical Chemistry, Vol. 30, No. 6, 2011

Multiresidue Methods (MRMs):

Aim of MRMs:

Cover as many pesticides as possible from a single sample portion employing a single sample preparation procedure

But, still more than one determinative analysis run is required to cover all analytes of interest with sufficient selectivity and sensitivity…

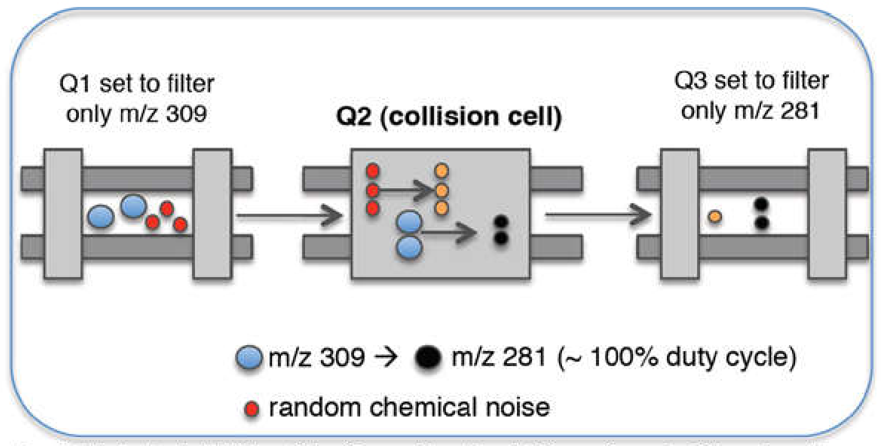


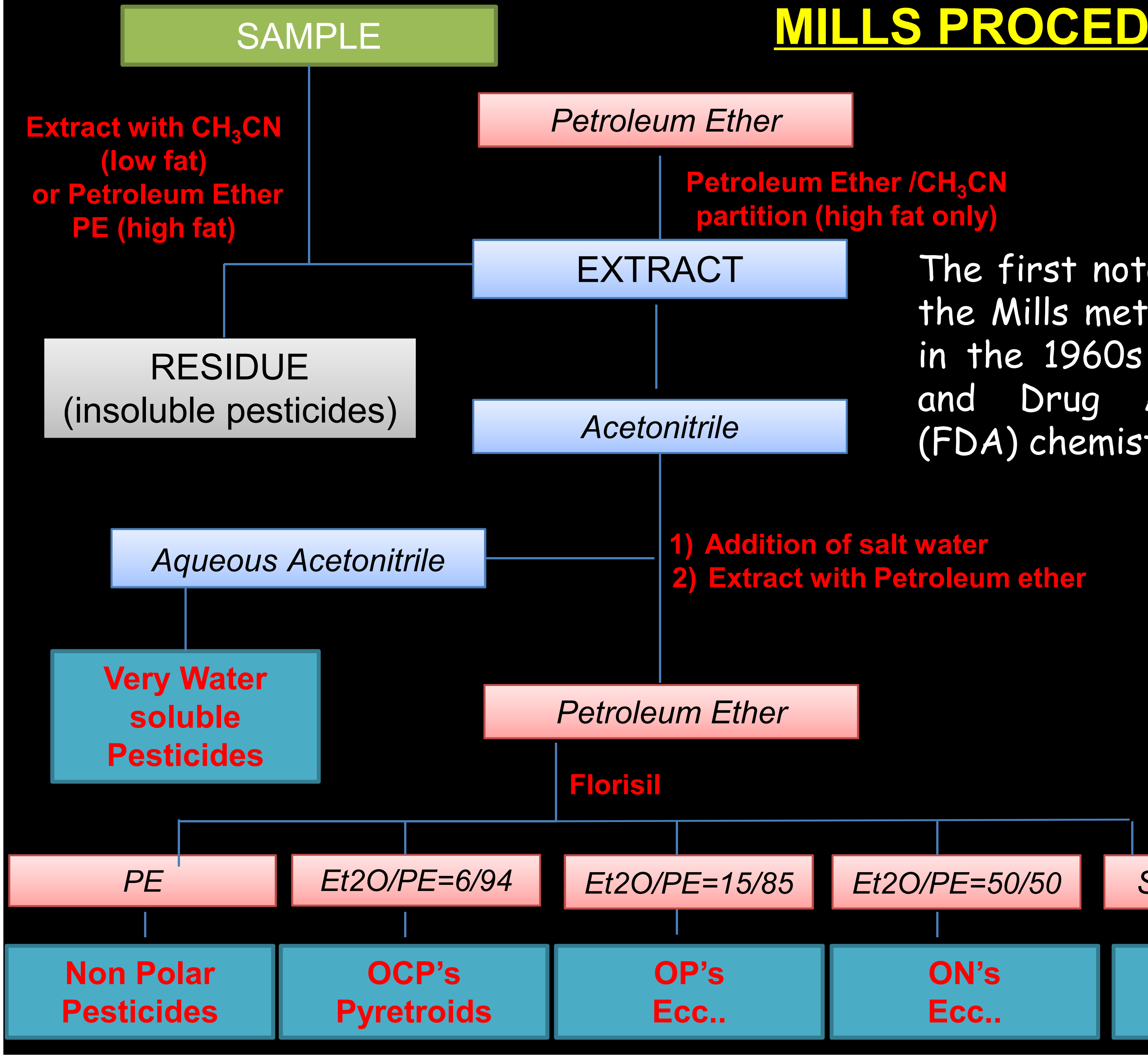
Figure 1: Triple Quadrupole MS MRM monitoring offers complete analyte selectivity, even for samples within complex matrices.

The broader the spectrum of analytes covered by the MRM,

- The less additional methods are required to cover all analytes
- The more efficient and economical the analysis
- Less time, personnel, materials...

Michelangelo Anastassiades, Stuttgart, 2006

MILLS PROCEDURE (PAM)



SAMPLE

Extract with CH_3CN (low fat) or Petroleum Ether PE (high fat)

Petroleum Ether

Petroleum Ether /CH_3CN partition (high fat only)

EXTRACT

RESIDUE (insoluble pesticides)

Acetonitrile

1) Addition of salt water
2) Extract with Petroleum ether

Aqueous Acetonitrile

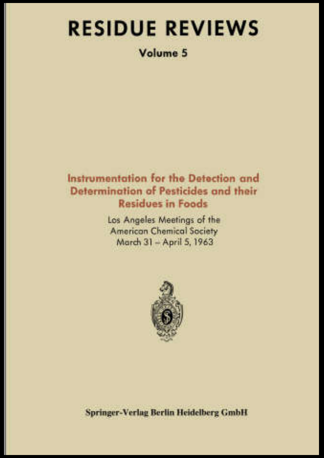
Very Water soluble Pesticides

Petroleum Ether

Florisil

PE	*Et2O/PE=6/94*	*Et2O/PE=15/85*	*Et2O/PE=50/50*	*Spent Florisil*
Non Polar Pesticides	**OCP's Pyretroids**	**OP's Ecc..**	**ON's Ecc..**	**Very Polar Pesticides**

The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills

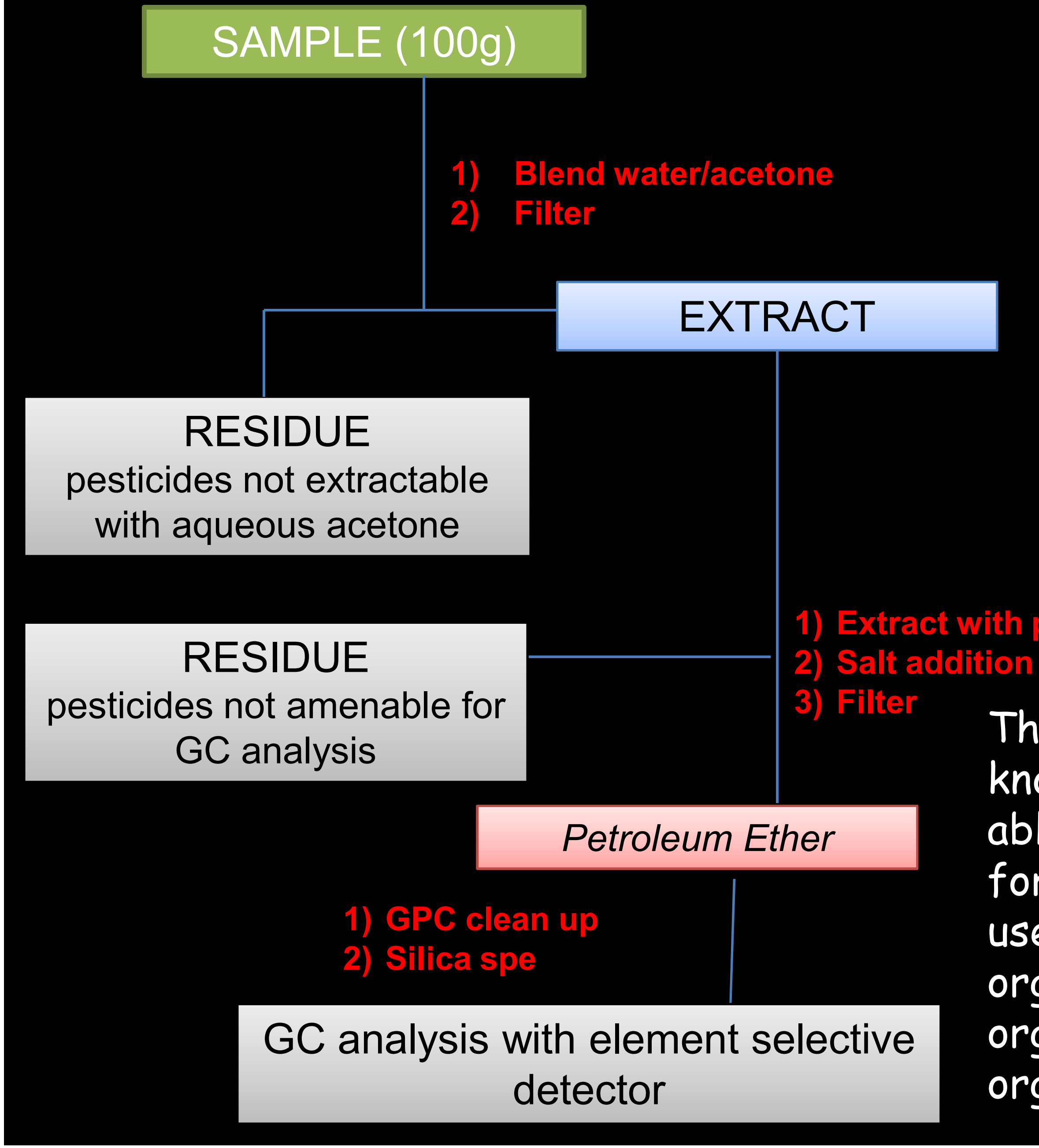


RESIDUE REVIEWS
Volume 5

Instrumentation for the Detection and Determination of Pesticides and their Residues in Foods
Los Angeles Meetings of the American Chemical Society
March 31 – April 5, 1963

Springer-Verlag Berlin Heidelberg GmbH

ACETONE EXTRACTION METHOD



SAMPLE (100g)

1) Blend water/acetone
2) Filter

EXTRACT

RESIDUE
pesticides not extractable with aqueous acetone

1) Extract with petroleum ether and dichloromethane
2) Salt addition
3) Filter

RESIDUE
pesticides not amenable for GC analysis

Petroleum Ether

1) GPC clean up
2) Silica spe

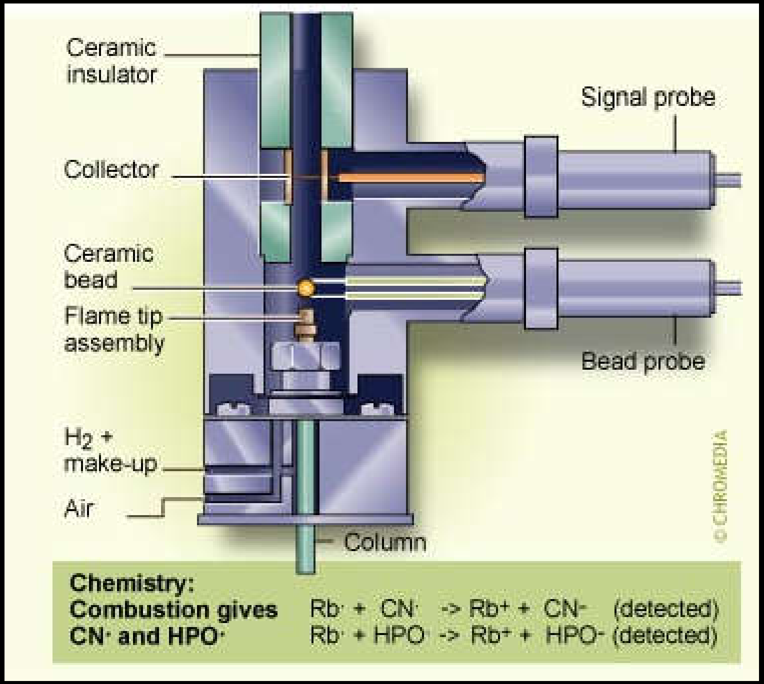
GC analysis with element selective detector

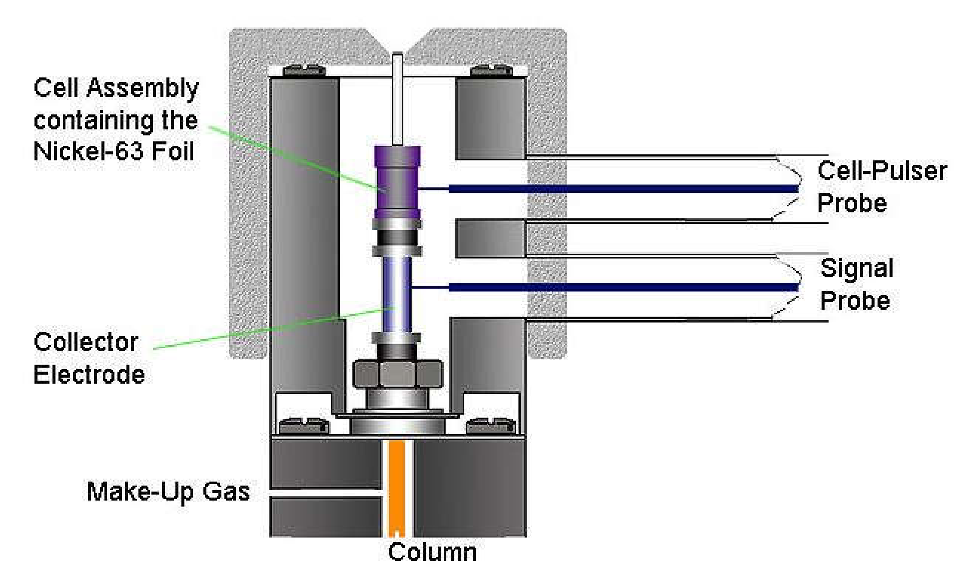
In 1975, Milton Luke and colleagues at the U.S. Food and Drug Administration (FDA) introduced a new method for multiclass, multiresidue pesticide analysis of fruit, vegetable, grains, and other food samples

This method, which became widely known as the "Luke method," was able to achieve high recoveries for the major types of pesticides used at that time (e.g. organochlorines, organophosphates, organonitrogens)

ACETONE EXTRACTION METHOD

The use of multiple selective detectors in gas chromatography (GC), such as electron-capture detection (ECD), flame photometric detection (FPD), electrolytic conductivity detection (ELCD or Hall detector), and nitrogen phosphorus detection (NPD), allowed an expanded scope over common previous methods, which generally were effective only for single class of pesticides, such as organochlorines using GC-ECD





Double column confirmation

ACETONE EXTRACTION METHOD

The Luke method achieved AOAC Official Method status (Method 985.22) in 1985 based on a study involving FDA labs.

For many years to follow, analytical technologies continued to improve and agrochemical companies registered many more pesticides from different classes. Although the registration process often required companies to first test the ability of the Luke method to recover the newly registered pesticides, fewer modern pesticides could be included in the FDA multiclass monitoring method, and this required the companies to develop single analyte methods in the registration process to be used for enforcement.

However, monitoring labs had too few resources to use the typically very complicated methods for so many different pesticides, and little or no monitoring was done of those types of pesticides.

ACETONE EXTRACTION METHOD

In terms of analytical technology, mass spectrometry (MS) was coupled to GC in the commercial bench-top instruments during the 1980s, and they were initially used for qualitative confirmation purposes in pesticide analyses. In the 1990s, the performance features of the instruments improved to the point that detection limits were acceptably low enough that GC-MS could be used to replace selective GC detectors for quantitative as well as qualitative analysis and reduce the need for multiple injections in GC. By the late 1990s, GC-MS had become commonplace in monitoring labs

Mighty 90s

HP 5890 (GC) *coupled with*
HP 5972 (*single quadrupole*)

ACETONE EXTRACTION METHOD

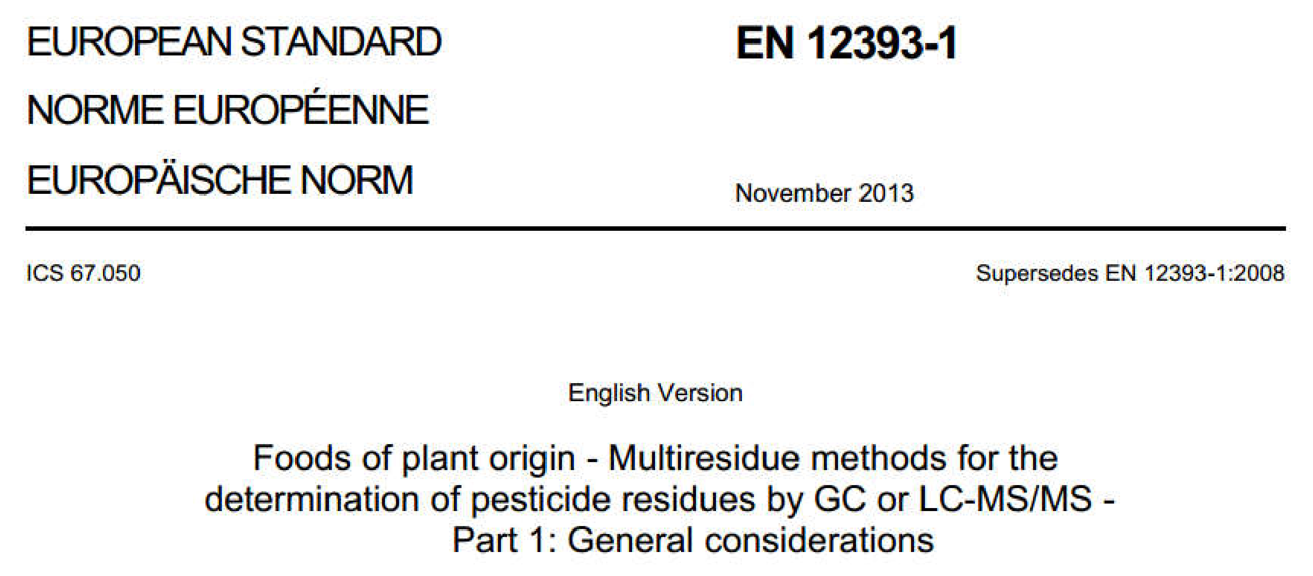
Additionally, the price reduced and performance improved for high quality commercial bench-top LC-MS/MS (tandem mass spectrometry) instruments. This allowed multiclass, multiresidue analysis of many LC-type pesticides that could previously be detected only by single-analyte methods.

EARLY 2000

Applied Bioscience API 2000
Triple Quadrupole

Thermo DECA XP
Ion Trap

ACETONE EXTRACTION METHOD



EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 12393-1

November 2013

ICS 67.050

Supersedes EN 12393-1:2008

English Version

Foods of plant origin - Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS - Part 1: General considerations

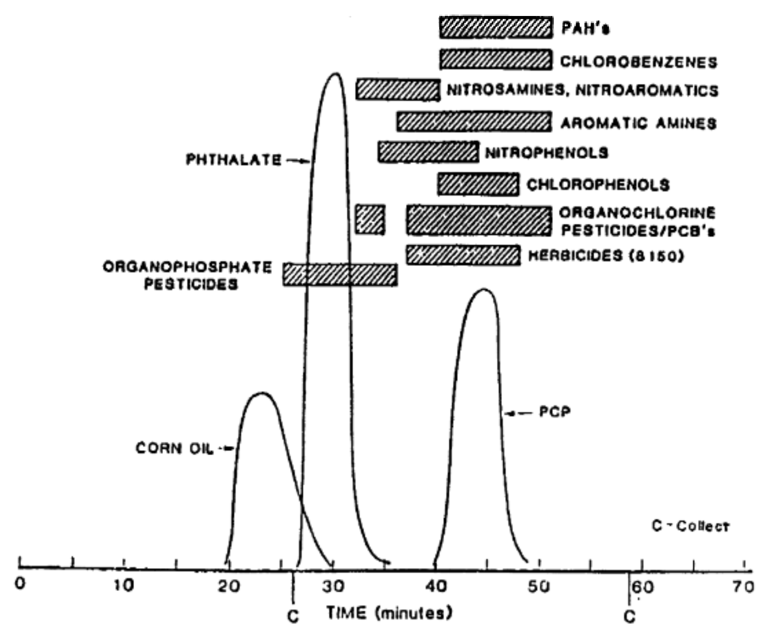
This European Standard contains the following methods that have been subjected to interlaboratory studies and/or are adopted throughout Europe

method M: Extraction with acetone and liquid-liquid partition with dichloromethane/light petroleum if necessary clean-up on Florisil®
method N: Extraction with acetone, liquid-liquid partition with dichloromethane or cyclohexane/ethyl acetate and clean-up with gel permeation and silica gel chromatography;
method P: Extraction with ethyl acetate and, if necessary, clean-up with gel permeation chromatography

ACETONE EXTRACTION METHOD

However, the Luke method, which used acetone for extraction and partitioning from water with a combination of methylene chloride and petroleum ether (and addition of salt for more polar pesticides), was not sufficiently effective, environmentally-friendly, safe, and efficient enough for “twenty-first century” standards

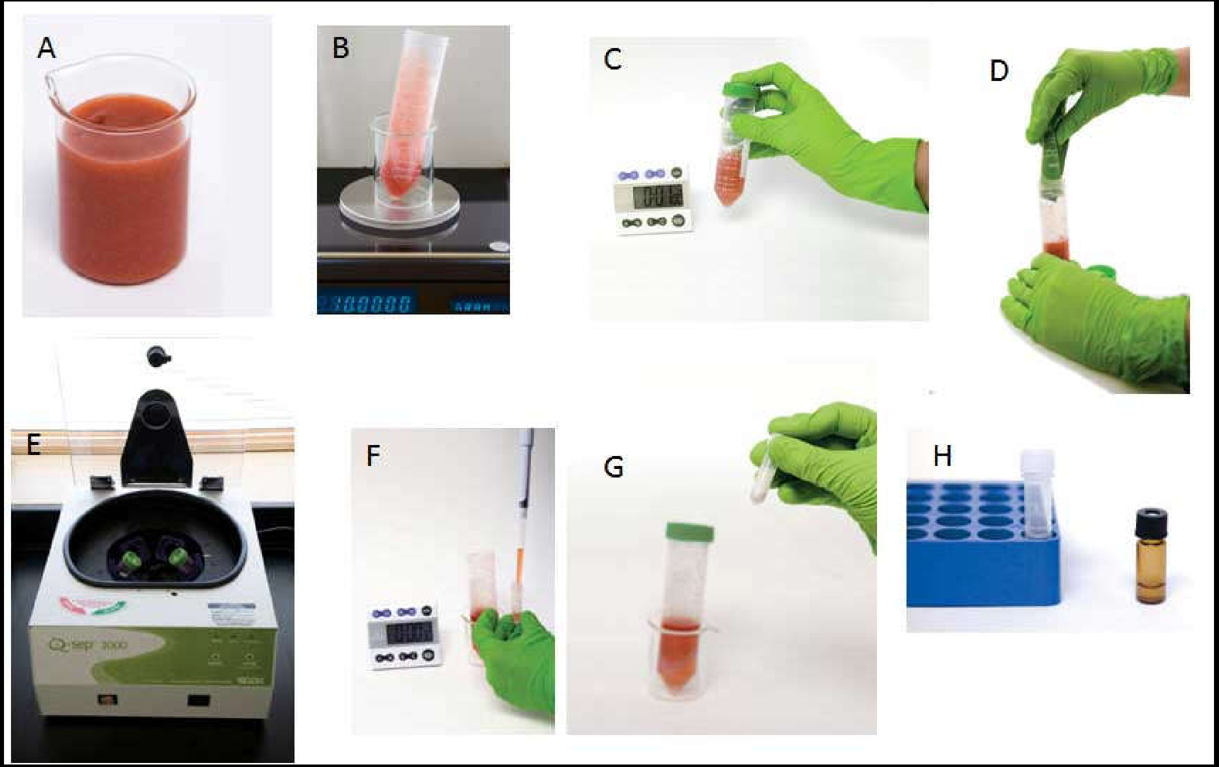
Even 12393-1 is really complicated and the consumption of solvent is very high.

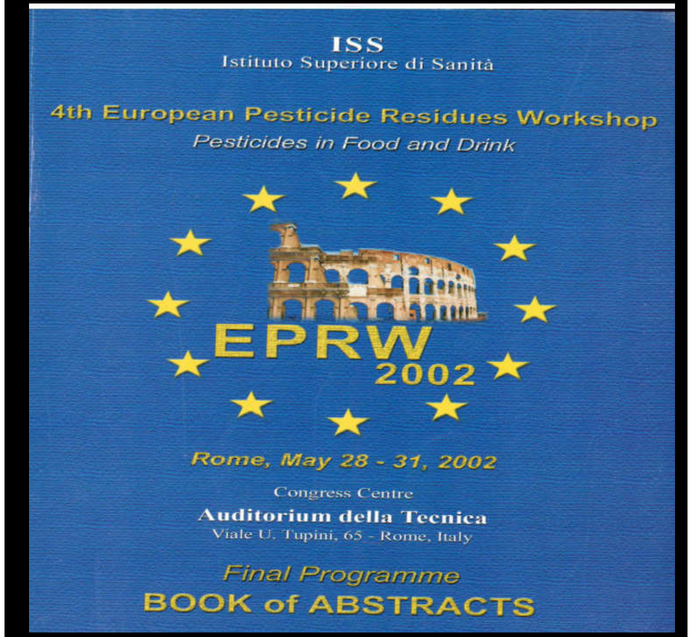


GPC profile from EPA 3640

QuEChERS METHOD

Generic steps of the QuEChERS technique




ISS
Istituto Superiore di Sanità

4th European Pesticide Residues Workshop
Pesticides in Food and Drink

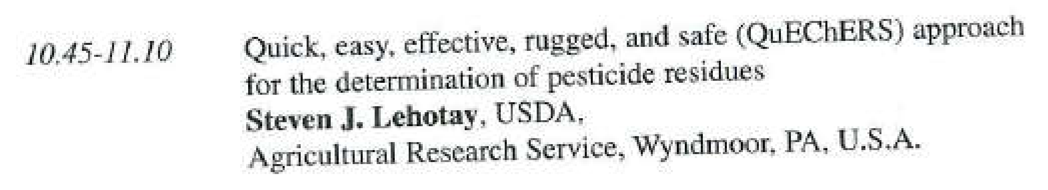
EPRW
2002

Rome, May 28 - 31, 2002

Congress Centre
Auditorium della Tecnica
Viale U. Tupini, 65 - Rome, Italy

Final Programme
BOOK of ABSTRACTS


Friday 31st May - Morning

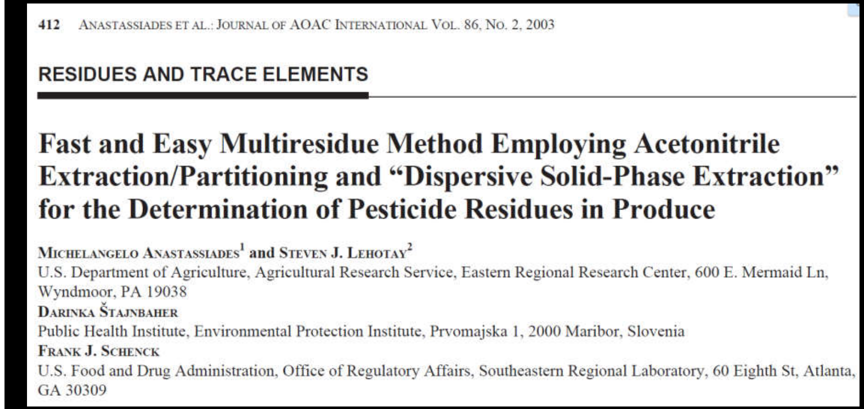

10.45-11.10 Quick, easy, effective, rugged, and safe (QuEChERS) approach for the determination of pesticide residues
Steven J. Lehotay, USDA,
Agricultural Research Service, Wyndmoor, PA, U.S.A.

- A) homogenized sample
- B) weight 10 grams of sample
- C) 10 mL of CH_3CN and shake (1 min.)
- D) add extraction salts and shake
- E) centrifuge
- F) dSPE cleanup of an aliquot of extract
- G) shake the dSPE tube and centrifuge
- H) the sample is ready for analysis

QuEChERS METHOD

Streamlined aspects of QuEChERS

1. reduced subsample size from a thoroughly homogenized sample
2. extraction by shaking of sample with solvent in a centrifuge tube
3. partitioning of water from the sample using MgSO4 in combination with other salt(s)
4. centrifugation to separate the extract from the water and non- soluble material rather than filtration
5. taking an aliquot of the extract rather than trying to collect the entire portion
6. use of internal standard(s) to improve accuracy and precision of the results rather than having to make calculations of extract volume depending on water content of the sample
7. injection of the same extract, preferably without solvent exchange or concentration steps, in both GC-MS and LC-MS/MS analyses.



412 ANASTASSIADES ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 86, NO. 2, 2003

RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

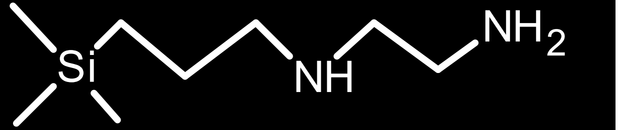
MICHELANGELO ANASTASSIADES[1] and STEVEN J. LEHOTAY[2]
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Ln, Wyndmoor, PA 19038
DARINKA ŠTAJNBAHER
Public Health Institute, Environmental Protection Institute, Prvomajska 1, 2000 Maribor, Slovenia
FRANK J. SCHENCK
U.S. Food and Drug Administration, Office of Regulatory Affairs, Southeastern Regional Laboratory, 60 Eighth St, Atlanta, GA 30309

QuEChERS METHOD

Streamlined aspects of QuEChERS

QuEChERS over previous sample preparation techniques is the use of dispersive solid-phase extraction dSPE) for cleanup

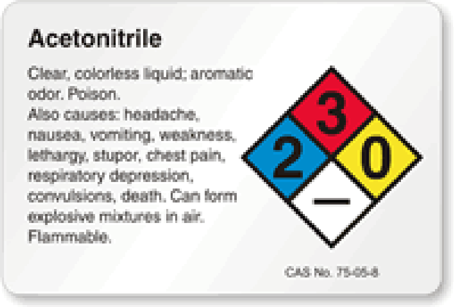
Lehotay and Anastassiades realized that the previous work of Fillion (an effective column/cartridge-based cleanup for MeCN pesticide extracts, which had been salted out from water, with a combination of primary secondary amine (PSA), octadecylsilyl (ODS or C18), and graphitized carbon black) was a "chemical filtration" approach in which certain common matrix components in foods (e.g. fatty acids, chlorophyll, sterols, anthocyanins) remained on the sorbents and the MeCN served as the elution solvent for the pesticide analytes



Anastassiades had the idea to dispense an aliquot of the extract into a centrifuge tube containing loose sorbent(s), and then to take a second aliquot after shaking and centrifugation for analysis

QuEChERS METHOD

Choice of Acetonitrile as Solvent



Acetonitrile

Clear, colorless liquid; aromatic odor. Poison.
Also causes: headache, nausea, vomiting, weakness, lethargy, stupor, chest pain, respiratory depression, convulsions, death. Can form explosive mixtures in air. Flammable.

CAS No. 75-05-8

PROs

- Selective (Few Co-Extractives but still broad pesticide Spectrum covered)
- Compatible with LC-and SPE-Applications
- Not Chlorinated
- Miscible with Water (Good for Initial Extraction)
- Separ. from Water-Phase by Salt-Add. (No Non-Polar Solv. Needed)
- Easier to Remove Water (with $MgSO_4$) than from Acetone

CONs

- Difficult to Evaporate
- High Expansion Volume (advisable the use of solvent vent injection mode)
- Not Compatible With NPD (advisable the use of solvent vent injection mode)
- Not Compatible with GPC (But, Lipid-Co-Extraction is Low)
- Rel. Toxic (But, Method Performed in a Closed Vessel, thus minimal exposure)
- Low Lipid Solubility

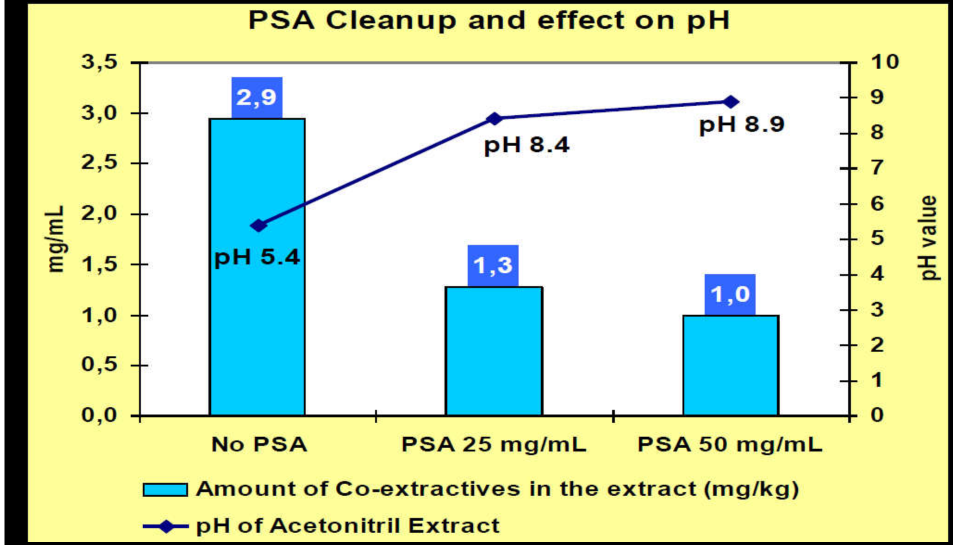
Losses of non-polar pesticides (Recov. consistent at same Lipid/solvent ratio)
Accessibility problems of pesticides enclosed in Lipid particles (Ultra Turrax))

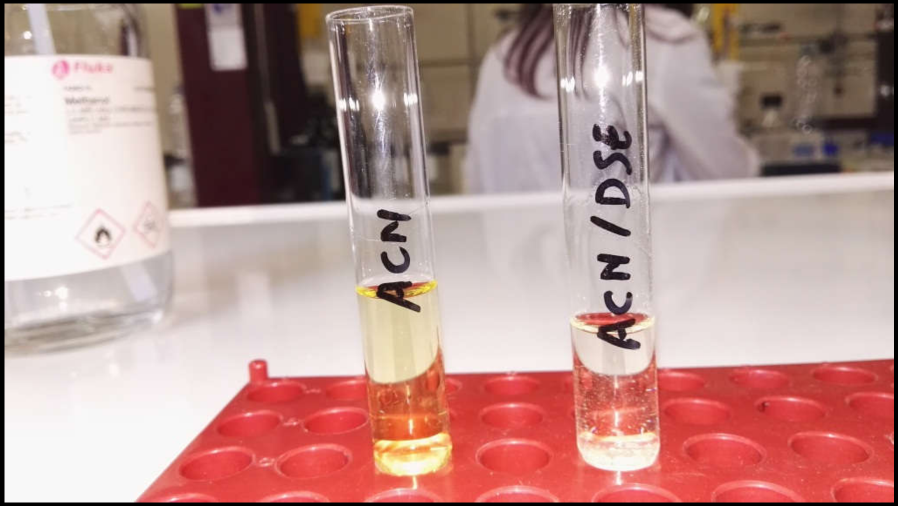
Michelangelo Anastassiades, Stuttgart, 2006

QuEChERS METHOD

Dispersive SPE (dSPE)

Anastassiades had the idea to dispense an aliquot of the extract into a centrifuge tube containing loose sorbent(s), and then to take a second aliquot after shaking and centrifugation for analysis

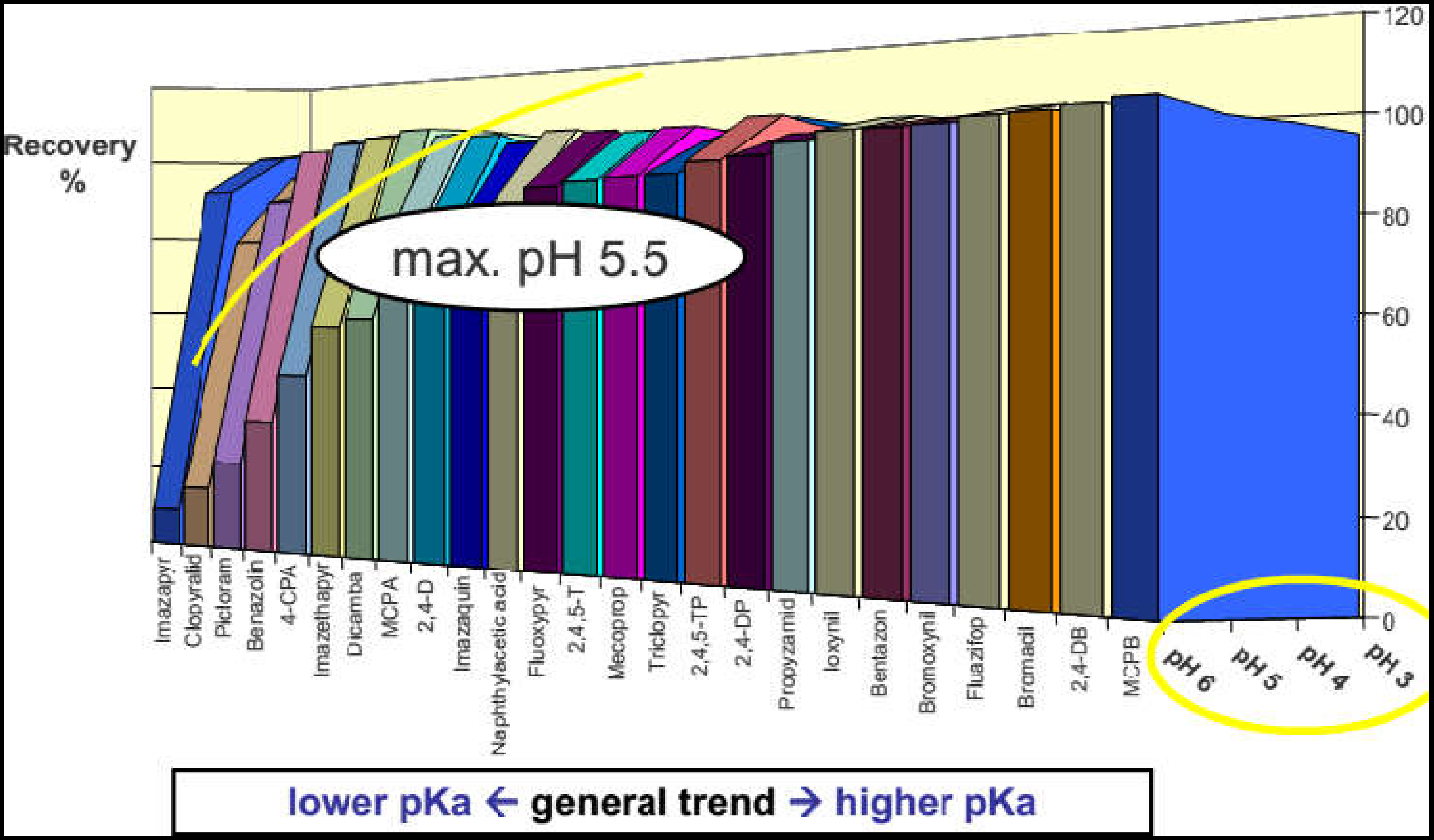




By using dSPE, the trappings of traditional cartridge based SPE disappeared, such as needing a manifold, vacuum system, collection tubes, elution solvents, solvent evaporation apparatus, and reliance on limited commercial products

QuEChERS METHOD

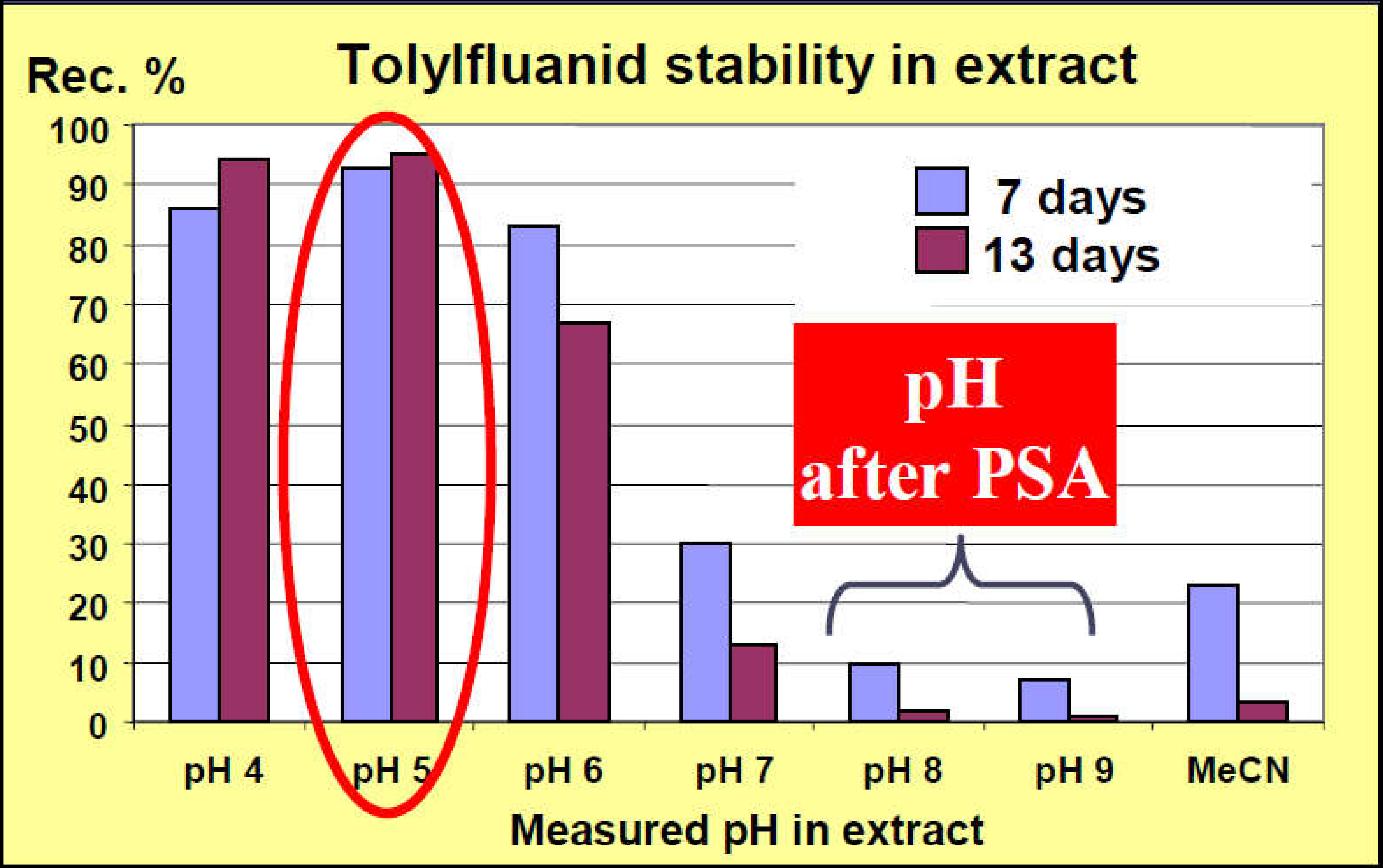
NO-PSA Clean up



For Acidic pesticides recovery drop at pH 6

Michelangelo Anastassiades, Stuttgart, 2006
Open Chem., 2015; 13: 980–1010

QuEChERS METHOD



Addition of formic acid (5% in ACN):

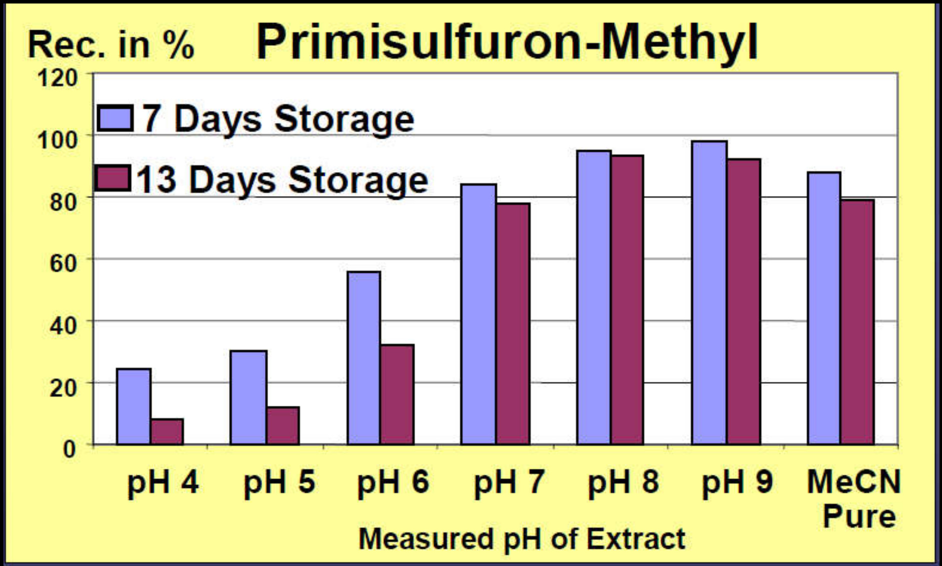
Michelangelo Anastassiades, Stuttgart, 2006
Open Chem., 2015; 13: 980–1010

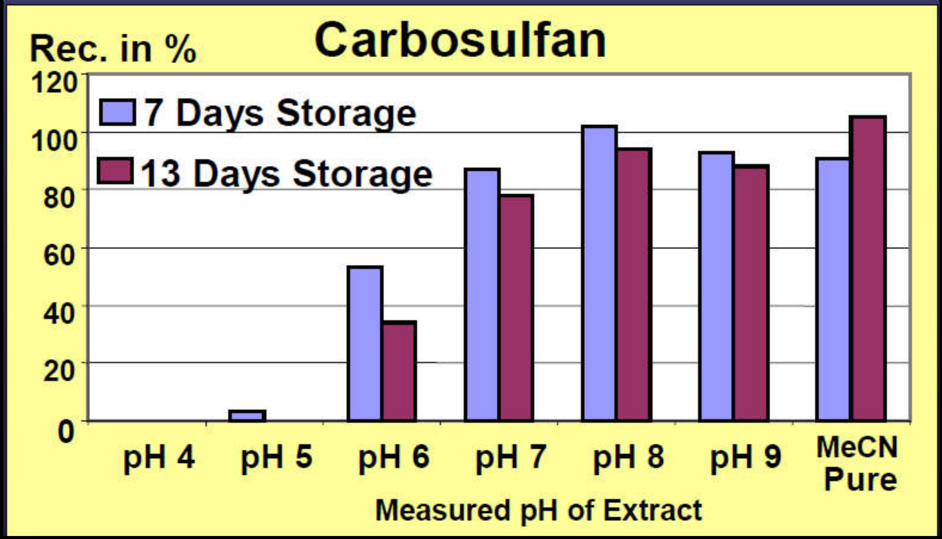
QuEChERS METHOD

Addition of formic acid (5% in ACN):

Some pesticides are acid labile
Sulfonylureas, Carbosulfan

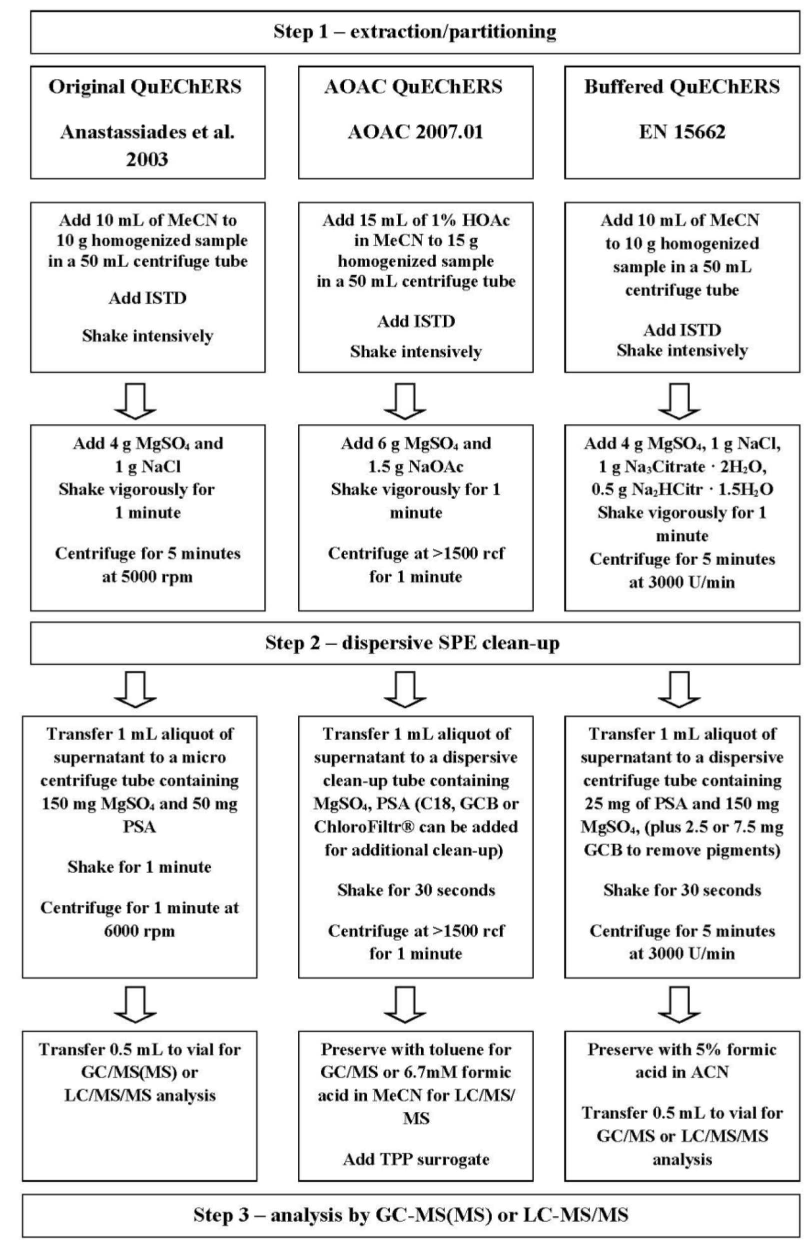
If these compounds are included in the target spectrum use an aliquot of the final extract before acidifying





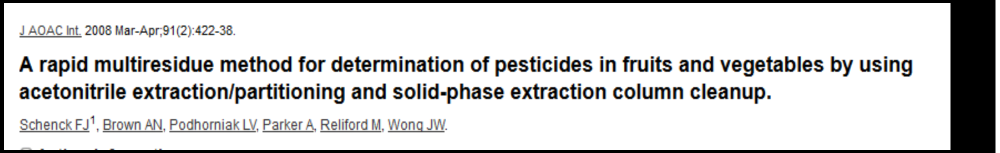
Michelangelo Anastassiades, Stuttgart, 2006
Open Chem., 2015; 13: 980–1010

QuEChERS METHOD



Step 1 – extraction/partitioning		
Original QuEChERS Anastassiades et al. 2003	**AOAC QuEChERS** AOAC 2007.01	**Buffered QuEChERS** EN 15662
Add 10 mL of MeCN to 10 g homogenized sample in a 50 mL centrifuge tube Add ISTD Shake intensively	Add 15 mL of 1% HOAc in MeCN to 15 g homogenized sample in a 50 mL centrifuge tube Add ISTD Shake intensively	Add 10 mL of MeCN to 10 g homogenized sample in a 50 mL centrifuge tube Add ISTD Shake intensively
Add 4 g $MgSO_4$ and 1 g NaCl Shake vigorously for 1 minute Centrifuge for 5 minutes at 5000 rpm	Add 6 g $MgSO_4$ and 1.5 g NaOAc Shake vigorously for 1 minute Centrifuge at >1500 rcf for 1 minute	Add 4 g $MgSO_4$, 1 g NaCl, 1 g $Na_3Citrate \cdot 2H_2O$, 0.5 g $Na_2HCitr \cdot 1.5H_2O$ Shake vigorously for 1 minute Centrifuge for 5 minutes at 3000 U/min
Step 2 – dispersive SPE clean-up		
Transfer 1 mL aliquot of supernatant to a micro centrifuge tube containing 150 mg $MgSO_4$ and 50 mg PSA Shake for 1 minute Centrifuge for 1 minute at 6000 rpm	Transfer 1 mL aliquot of supernatant to a dispersive clean-up tube containing $MgSO_4$, PSA (C18, GCB or ChloroFiltr® can be added for additional clean-up) Shake for 30 seconds Centrifuge at >1500 rcf for 1 minute	Transfer 1 mL aliquot of supernatant to a dispersive centrifuge tube containing 25 mg of PSA and 150 mg $MgSO_4$, (plus 2.5 or 7.5 mg GCB to remove pigments) Shake for 30 seconds Centrifuge for 5 minutes at 3000 U/min
Transfer 0.5 mL to vial for GC/MS(MS) or LC/MS/MS analysis	Preserve with toluene for GC/MS or 6.7mM formic acid in MeCN for LC/MS/MS Add TPP surrogate	Preserve with 5% formic acid in ACN Transfer 0.5 mL to vial for GC/MS or LC/MS/MS analysis
Step 3 – analysis by GC-MS(MS) or LC-MS/MS		

Various versions of
QuEChERS Method



J AOAC Int. 2008 Mar-Apr;91(2):422-38.

A rapid multiresidue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction/partitioning and solid-phase extraction column cleanup.

Schenck FJ[1], Brown AN, Podhorniak LV, Parker A, Reliford M, Wong JW.

INTERESTING

Michelangelo Anastassiades, Stuttgart, 2006
Open Chem., 2015; 13: 980–1010

QuEChERS METHOD

Internal Standard

Internal Standard	Suitable for
$^{13}C_{12}$-Aldrin	GC
d_5-Atrazine	LC and GC
d_4-Carbendazim	LC
d_3-Carbofuran	LC (and GC)
d_{10}-Diazinon	LC and GC
d_6-a-HCH	GC
d_6-Malathion	LC and GC
d_6-Methoxychlor	GC
d_{10}-Parathion	GC
d_6-Parathion-methyl	GC (and LC)
d_3-Propoxur	LC and GC
Triphenylphosphate	LC and GC
Triethylphosphate	LC and GC

An INTERNAL STANDARD MUST

1. *not occur in the sample to begin with;*
2. *be stable;*
3. *give consistently high recoveries;*
4. *be readily available and inexpensive;*
5. *not interfere with any analytes;*
6. *ideally be readily detected in GC-MS and LC-MS/MS without being affected by matrix effects in either case*

Suggested internal standards for LC and GC

Mass Spectrometry in Food Safety, Humana Press, 2011, S. Lehotay, Chapter 4

QuEChERS METHOD

Dispersive SPE (dSPE)

PSA not satisfying when high contents of carotenoids or chlorophyll

Carbon Sorbent is more Effective

GCB(Graphitized Carbon Black) was best in handling-Used in combination with PSA at small amounts Cleanup time (shaking) extended from 30 s to 2 min

Problem with GCB

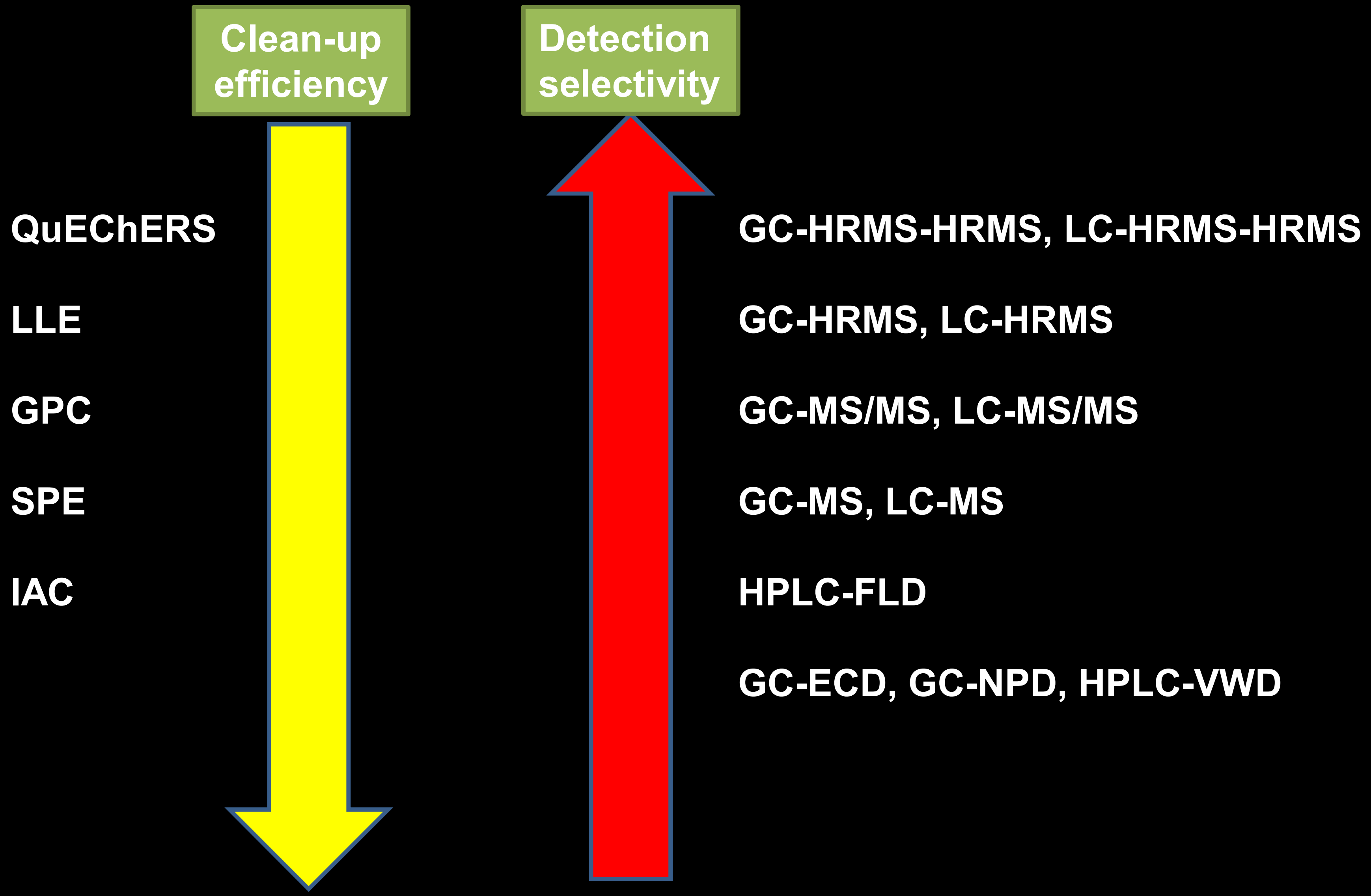
Planar pesticides have a high affinity towards GCB e.g. hexachlorobenzene, chlorothalonil, thiabendazole.

Anthracene may be used as surrogate QC standard. Recoveries > 70% will indicate that no unacceptable losses of pesticides have occurred.

Michelangelo Anastassiades, Stuttgart, 2006

QuEChERS METHOD

Simplified sample preparation challenges



Clean-up efficiency

Detection selectivity

QuEChERS

LLE

GPC

SPE

IAC

GC-HRMS-HRMS, LC-HRMS-HRMS

GC-HRMS, LC-HRMS

GC-MS/MS, LC-MS/MS

GC-MS, LC-MS

HPLC-FLD

GC-ECD, GC-NPD, HPLC-VWD

QuEChERS METHOD

Simplified sample preparation challenges

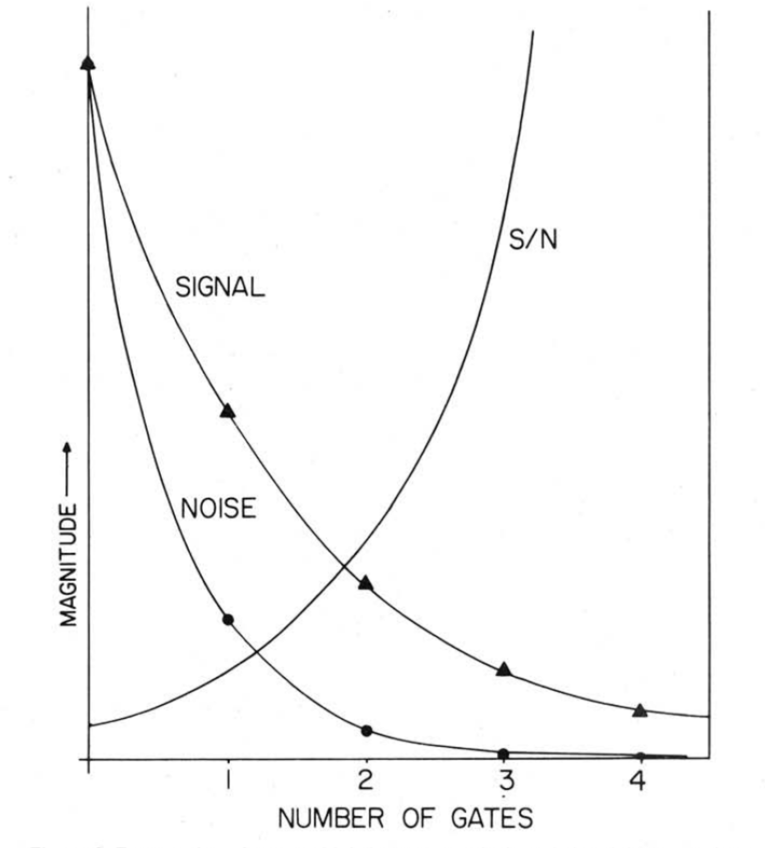
SAMPLE

QuEChERS

GC-MS/MS

LC-MS/MS

EN 15662 defines as GC tool a simple GC-MS in SIM mode but…



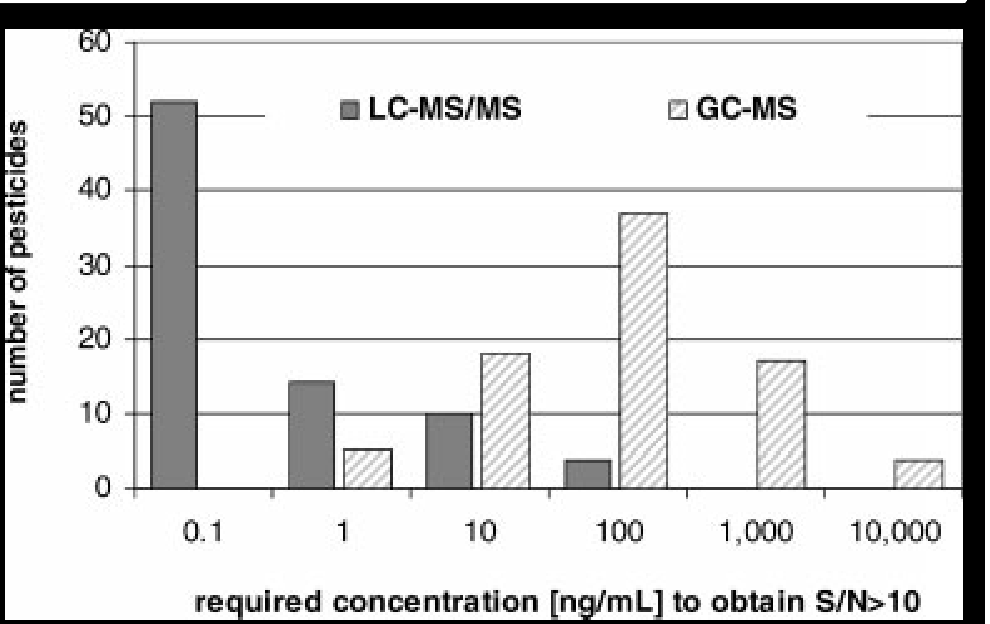
For a series of gates which favor trasmission of signal due to analyte over that due to chemical noise, the signal-to-noise ratio for detection of the analyte increases in spite of an attenuation in analyte signal

Increase of Sensitivity,
by the
increase of Selectivity

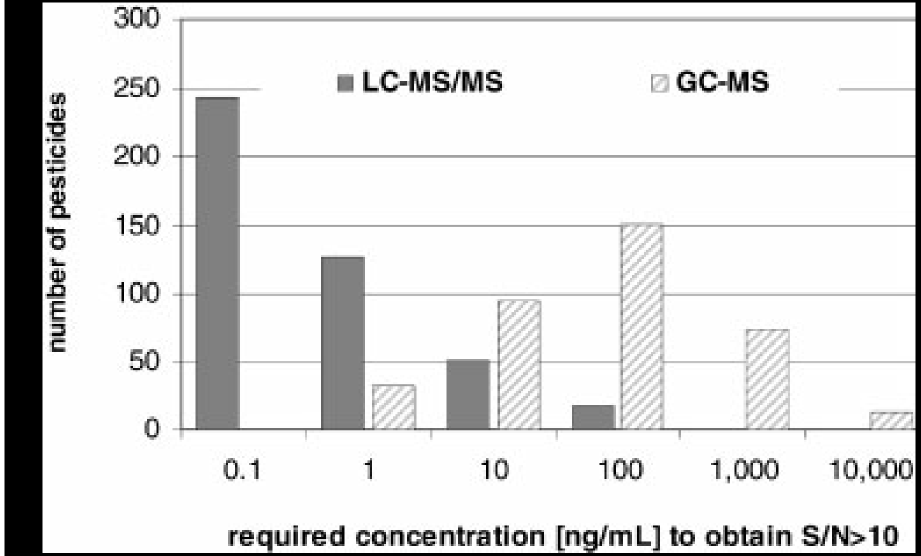
R.G. Cooks and K.L. Busch, *J. Chem. Educ.* **59**(11), 926–933 (1982).

QuEChERS METHOD

RESIDUE ANALYSIS :
BETTER BY *GC-MS* OR LC-MS/MS?



Distribution of limit of quantification (LOQ) data of all organophosphorus pesticides

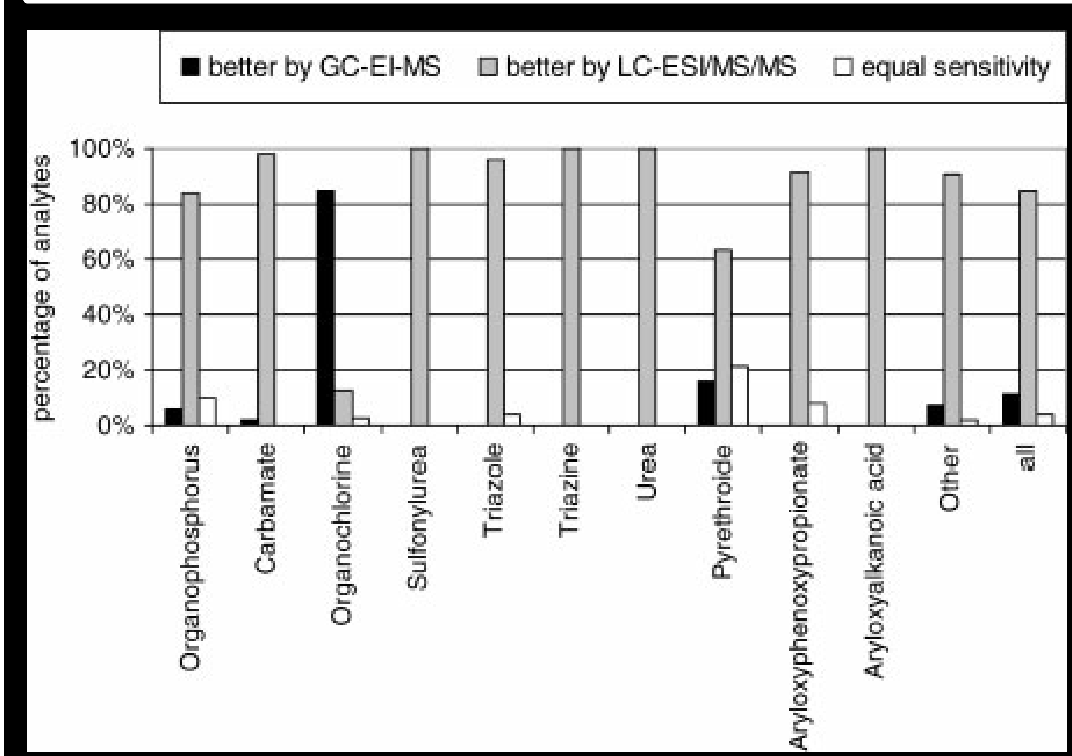


Distribution of limit of quantification (LOQ) data of all pesticides/metabolites.

Mass Spectrometry Reviews, 2006, 25, 838– 865

QuEChERS METHOD

RESIDUE ANALYSIS :
BETTER BY GC-MS OR LC-MS/MS?



Comparison of GC-MS sensitivity versus LC-MS/MS sensitivity of individual pesticides summarized for different pesticide classes

The better performance of LC-MS/MS is probably determined by several reasons. Among them the higher injection volume used in LC-MS/MS (20 μL vs. 1 μL) and the lower amount of fragmentation during ionization (ESI vs. EI) may explain some of these differences.

The use of GC-MS/MS introduces some little variations in the framework.

BUT

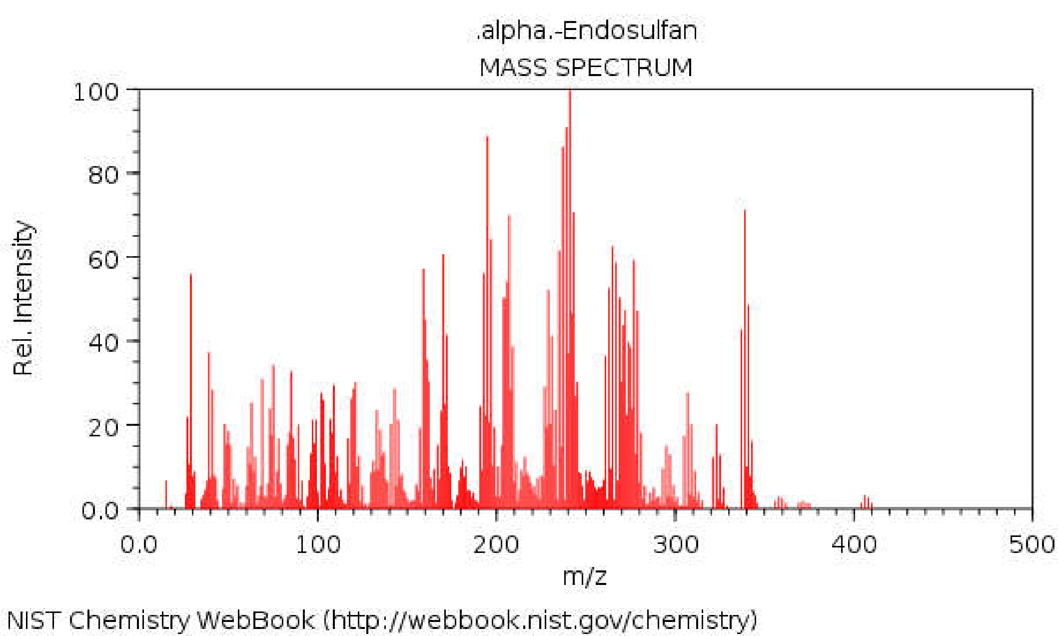
The high extent of the fragmentation still remain as a unfavorable factor.

Mass Spectrometry Reviews, 2006, 25, 838– 865

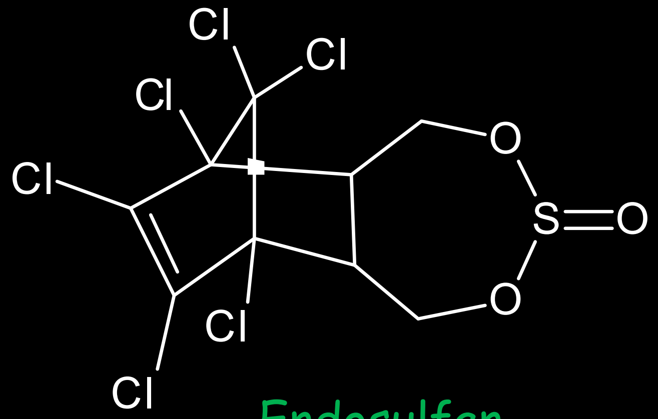
QuEChERS METHOD

RESIDUE ANALYSIS :
BETTER BY GC-MS OR LC-MS/MS?

Infact, there is another unique feature of pesticide analysis with mass spectrometry. Relative to other contaminants, many pesticides including OCs, OPs, pyrethroids, and chloroacetanilides exhibit low intensity for the molecular ion regardless of whether EI or CI is used. Consequently in SIM mode the quantitative or qualifier ion is rarely selected as the molecular ion. In general >90% of pesticides do not monitor the molecular ion by EI or CI methods as at the working concentration ranges of trace analysis generally the molecular ion is too low in abundance to be observe .



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)



Endosulfan

406.925 Da

R. Raina,, Pesticides - Strategies for Pesticides Analysis

QuEChERS METHOD

RESIDUE ANALYSIS :
BETTER BY GC-MS OR LC-MS/MS?

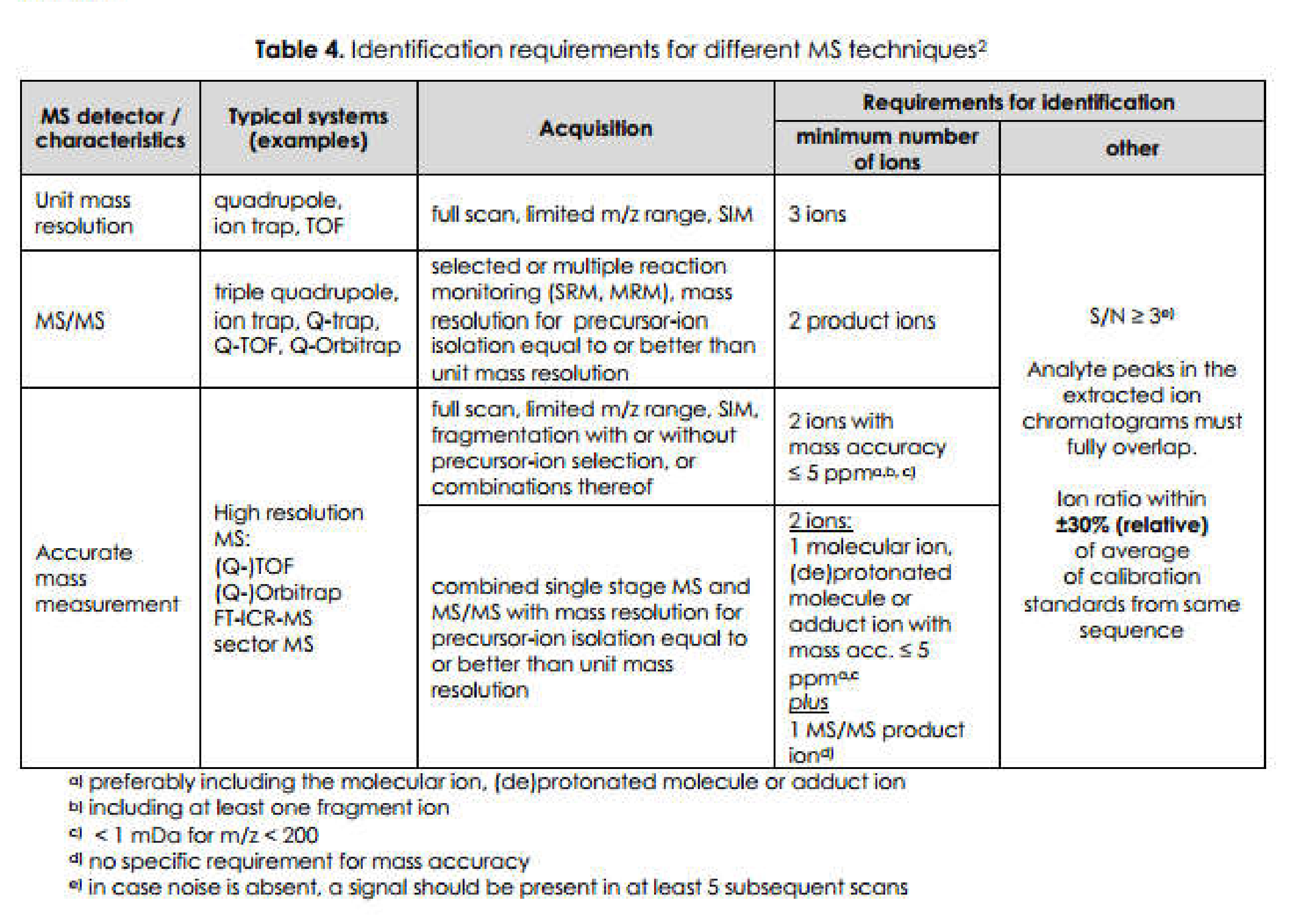


Table 4. Identification requirements for different MS techniques[2]

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[e] Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within **±30% (relative)** of average of calibration standards from same sequence
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm[a,c] plus 1 MS/MS product ion[d]	

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion

[b] including at least one fragment ion

[c] < 1 mDa for m/z < 200

[d] no specific requirement for mass accuracy

[e] in case noise is absent, a signal should be present in at least 5 subsequent scans

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Safety of the Food Chain
Pesticides and biocides

SANTE/11945/2015
30 November -1 December 2015 rev. 0

Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

SANTE/11945/2015

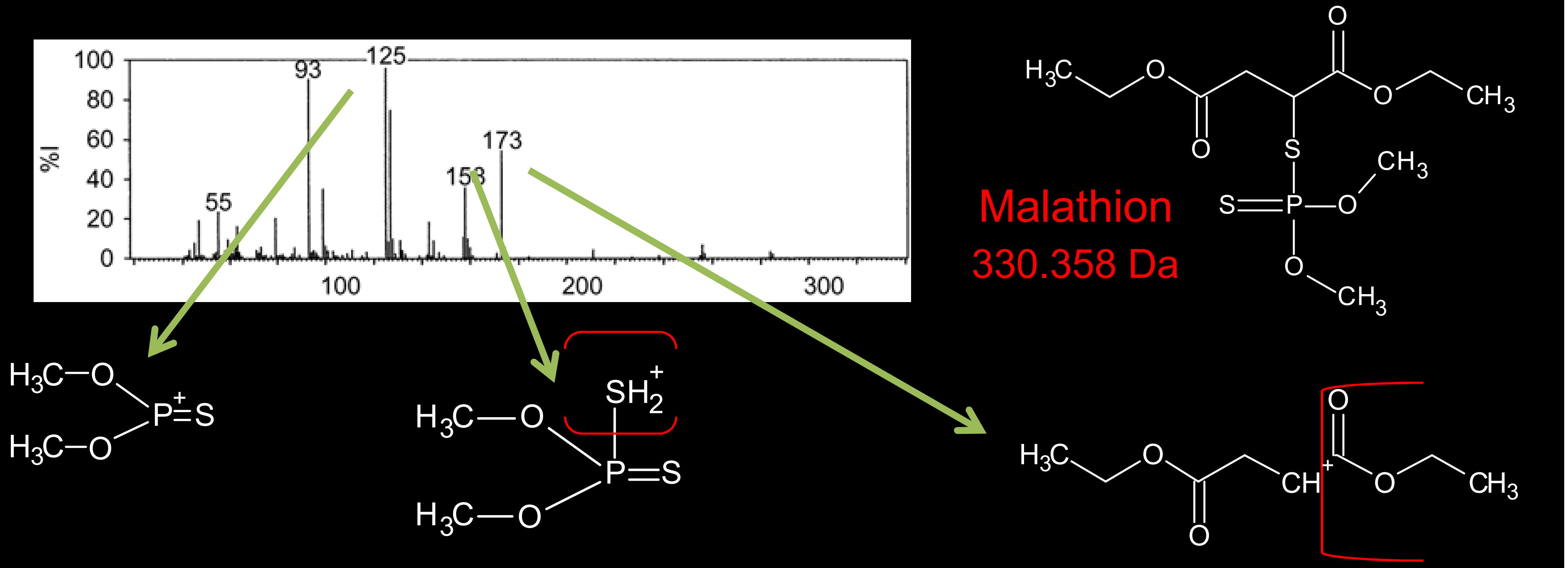
Supersedes

SANCO/12571/2013

Implemented by 01/01/2016

QuEChERS METHOD

RESIDUE ANALYSIS : BETTER BY GC-MS OR LC-MS/MS?



Malathion

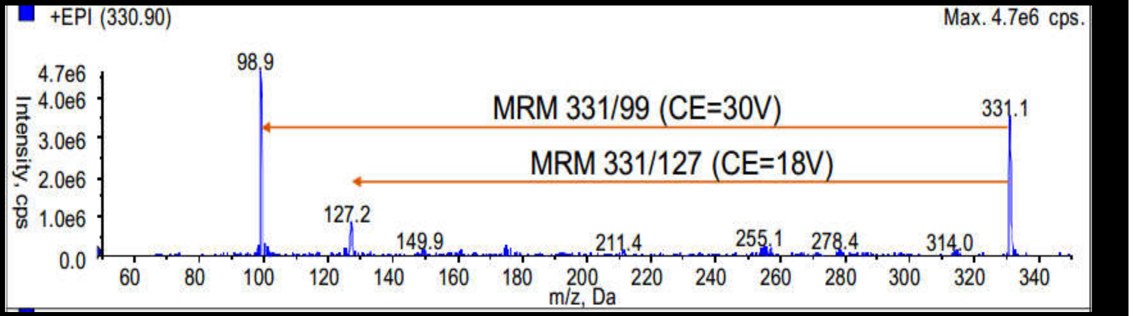
330.358 Da

GC-MS/MS MRM transition

158 → 125

173 → 99

LC-MS/MS MRM transition



QuEChERS METHOD

RESIDUE ANALYSIS :
CHOICE OF ANALYSIS TECNIQUE

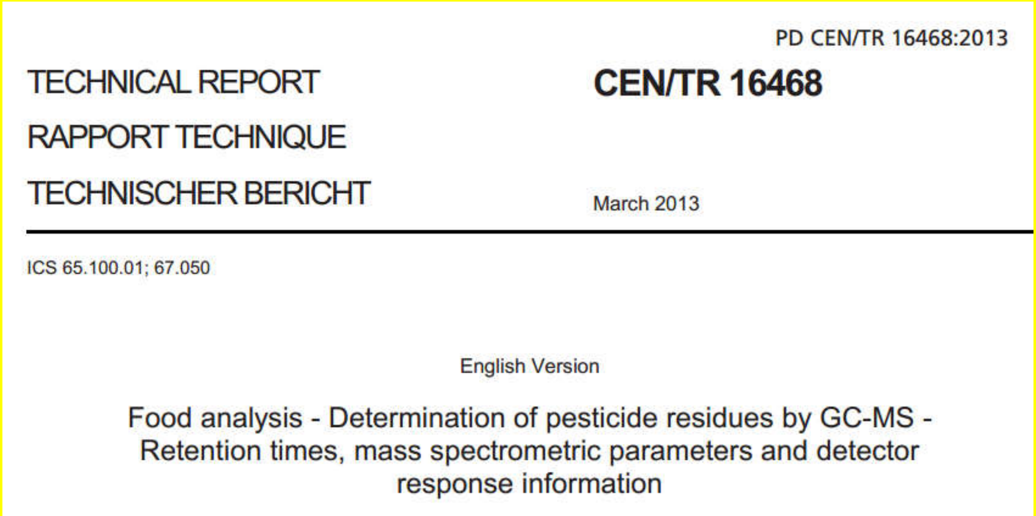
My personal advice (and experience) is to use GC-MS (better GC-MS/MS) for anlaysis of non API/ESI-ionizable pesticides (mainly Organochlorine pesticides) and use LC-MS/MS for the other class .

Agilent
GC7890-QQQ 7000 B
Example of GC-MSMS instrument

Thermo
Orbitrap Exactive HCD
Example of LC-HRMS instrument

QuEChERS METHOD

RESIDUE ANALYSIS :
CHOICE OF ANALYSIS TECNIQUE



PD CEN/TR 16468:2013

TECHNICAL REPORT
RAPPORT TECHNIQUE
TECHNISCHER BERICHT

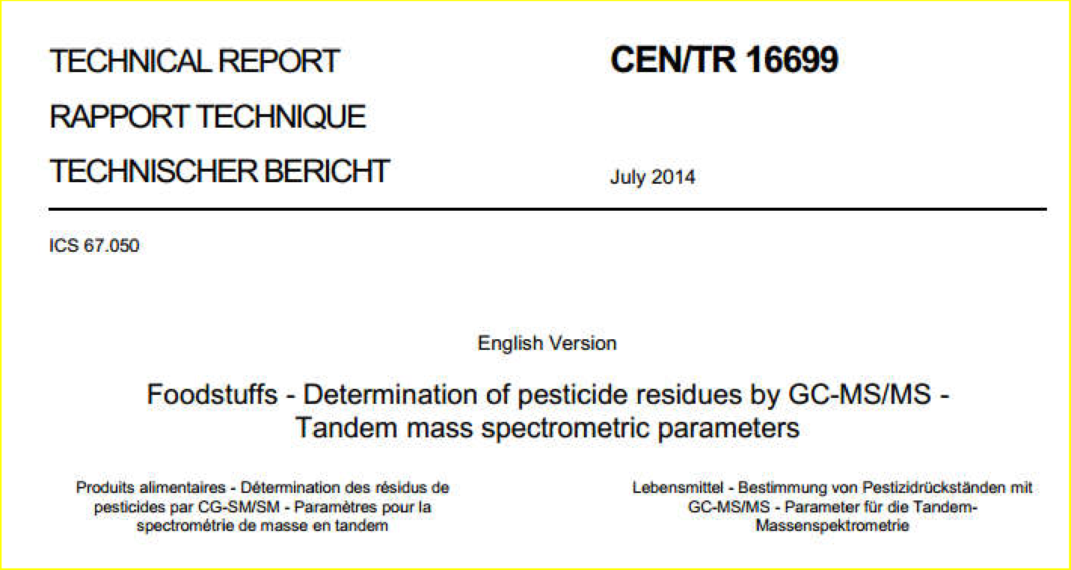
CEN/TR 16468

March 2013

ICS 65.100.01; 67.050

English Version

Food analysis - Determination of pesticide residues by GC-MS - Retention times, mass spectrometric parameters and detector response information



TECHNICAL REPORT
RAPPORT TECHNIQUE
TECHNISCHER BERICHT

CEN/TR 16699

July 2014

ICS 67.050

English Version

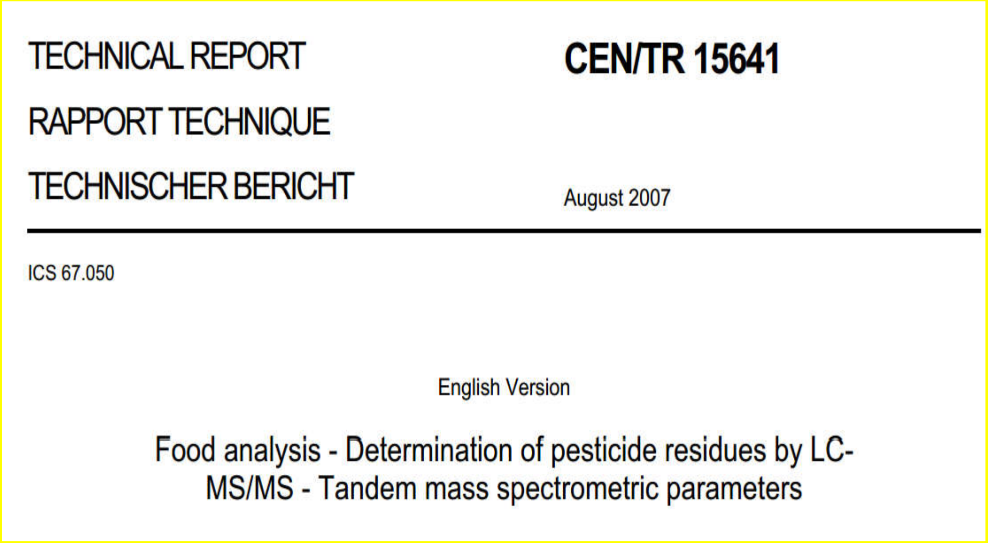
Foodstuffs - Determination of pesticide residues by GC-MS/MS - Tandem mass spectrometric parameters

Produits alimentaires - Détermination des résidus de pesticides par CG-SM/SM - Paramètres pour la spectrométrie de masse en tandem

Lebensmittel - Bestimmung von Pestizidrückständen mit GC-MS/MS - Parameter für die Tandem-Massenspektrometrie

QuEChERS METHOD

RESIDUE ANALYSIS :
CHOICE OF ANALYSIS TECNIQUE



TECHNICAL REPORT
RAPPORT TECHNIQUE
TECHNISCHER BERICHT

CEN/TR 15641

August 2007

ICS 67.050

English Version

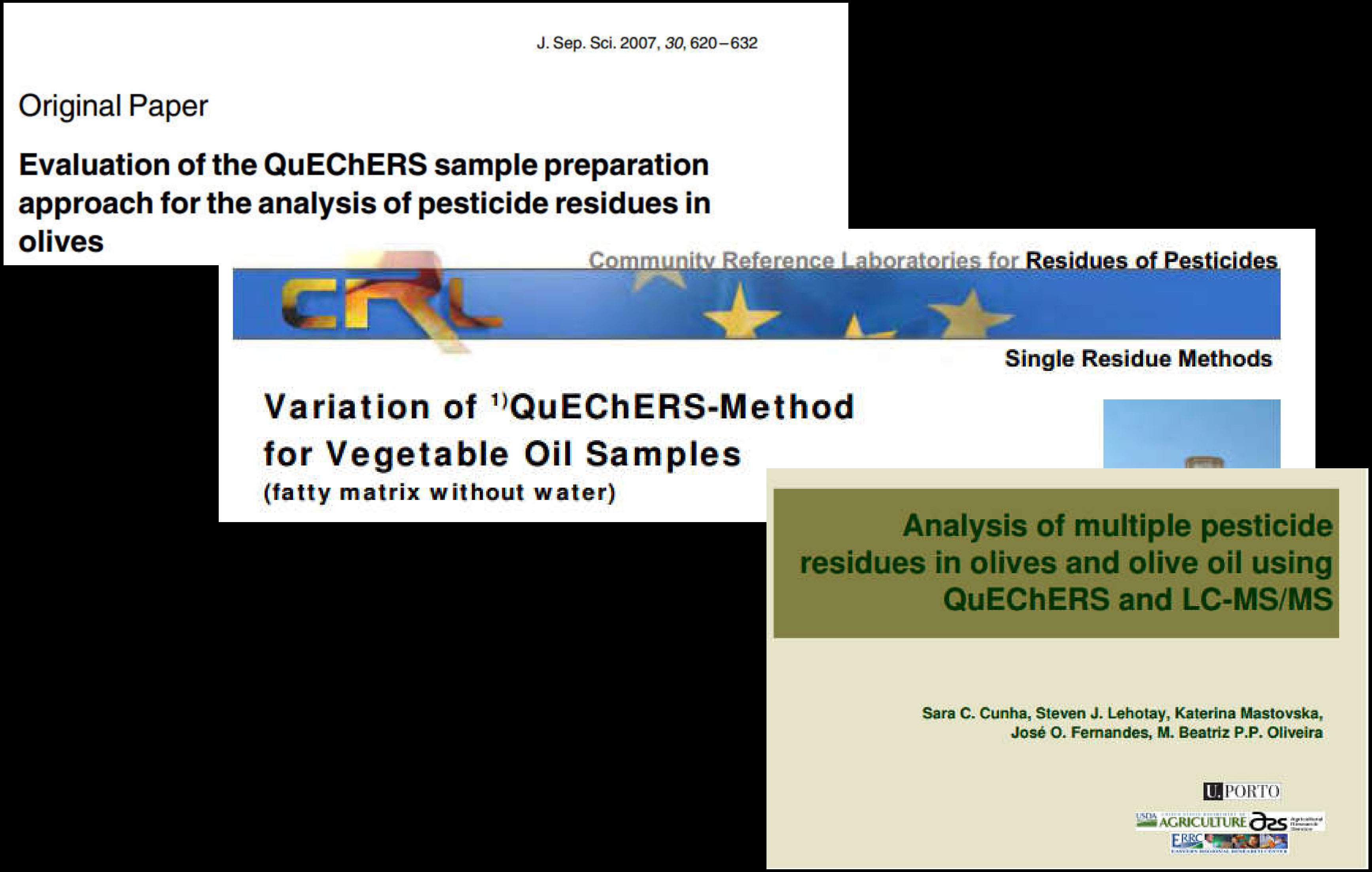
Food analysis - Determination of pesticide residues by LC-MS/MS - Tandem mass spectrometric parameters

Contains for approx. 500 pesticides:

- ***CAS-Number***
- ***Ionization method***
- ***Structure of quasimolecular ion***
- ***Mass of parent ion***
- ***Declustering potential***
- ***Mass of two main fragments***
- ***Appropriate collision energies***
- ***Relative retention times***
- ***Classification of response***

QuEChERS METHOD

Case Study: Olive Oil



J. Sep. Sci. 2007, *30*, 620–632

Original Paper

Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives

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Community Reference Laboratories for **Residues of Pesticides**

CRL

Single Residue Methods

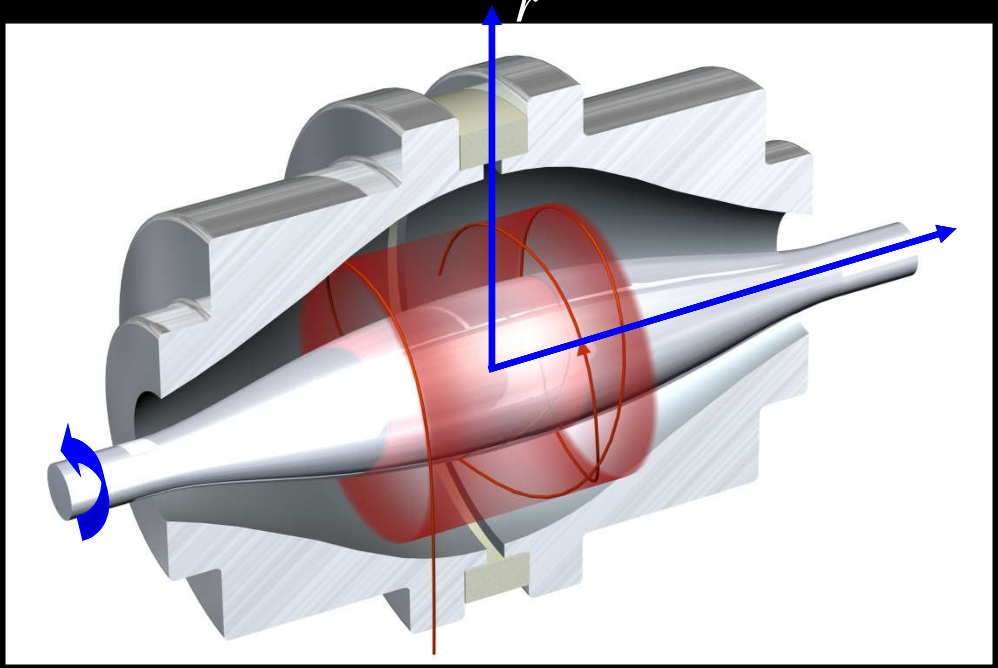
Variation of [1)]QuEChERS-Method for Vegetable Oil Samples

(fatty matrix without water)

Analysis of multiple pesticide residues in olives and olive oil using QuEChERS and LC-MS/MS

Sara C. Cunha, Steven J. Lehotay, Katerina Mastovska, José O. Fernandes, M. Beatriz P.P. Oliveira

New Development
LC-HRMS: ORBITRAP



Hyper-logarithmic potential distribution in the Orbitrap: "ideal Kingdon trap"

$$U(r,z)=\frac{k}{2}\cdot\left\{z^2-r^2/2+R_m^2\cdot\ln(r/R_m)\right\}$$

Characteristic frequencies:

Frequency of rotation ω_φ
Frequency of radial oscillations ω_r
Frequency of axial oscillations ω_z

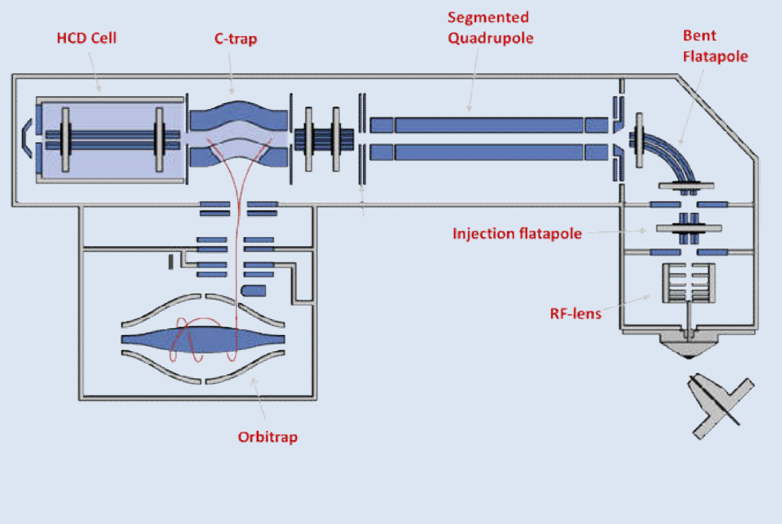
$$\omega_\varphi=\frac{\omega_z}{\sqrt{2}}\sqrt{\left(\frac{R_m}{R}\right)^2-1}$$

$$\omega_z=\sqrt{\frac{k}{m/q}}$$

Only this frequency does not depend on energy, angle, etc. and is used for mass analysis

- Korsunskii M.I., Basakutsa V.A. *Sov. Physics-Tech. Phys.* 1958; **3**: 1396. Knight R.D. *Appl.Phys.Lett*. 1981, **38**: 221.
- Gall L.N.,Golikov Y.K.,Aleksandrov M.L.,Pechalina Y.E.,Holin N.A. *SU Pat. 1247973*, 1986.

New Development
LC-HRMS

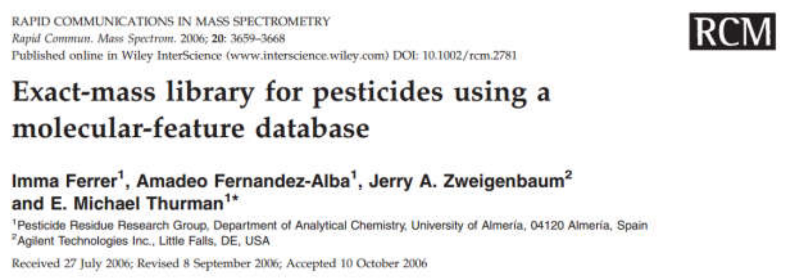


In this configuration, the apparatus is capable to operate in full scan mode with a resolution up to 100000 (1Hz) and an accuracy up to 2 ppm (positive).

- ***No MRM timetable***
- ***Identification of the analytes on the base of molecular ions***

IF NEEDED

Total fragmentation in HCD cell allows the record of an MS/MS spectra (with some limitations)



RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2006; **20**: 3659–3668

RCM

Exact-mass library for pesticides using a molecular-feature database

Imma Ferrer[1], Amadeo Fernandez-Alba[1], Jerry A. Zweigenbaum[2] and E. Michael Thurman[1]*
[1]Pesticide Residue Research Group, Department of Analytical Chemistry, University of Almería, 04120 Almería, Spain
[2]Agilent Technologies Inc., Little Falls, DE, USA

Received 27 July 2006; Revised 8 September 2006; Accepted 10 October 2006

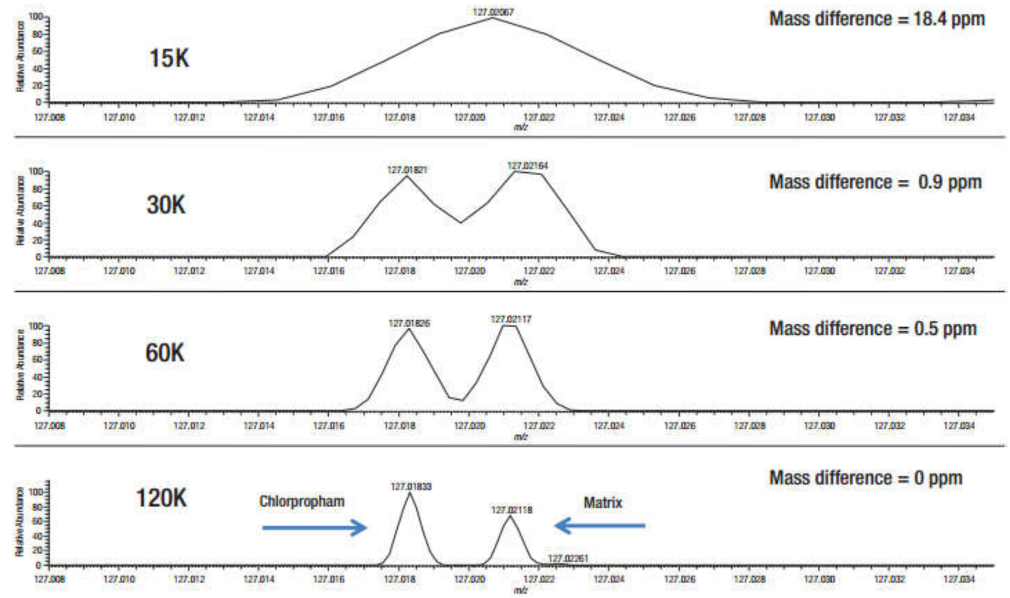
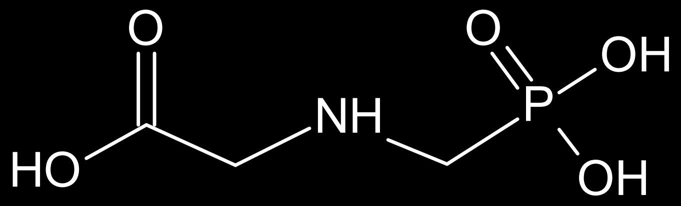


Figure 6. Effect of resolving power on mass accuracy of an analyte in matrix. Mass profiles of a diagnostic ion of chlorpropham at 10 ng/mL in leek, acquired at different resolutions of 15K, 30K, 60K, and 120K. At 15K and 30K the chlorpropham ion is not resolved from matrix interference resulting in poorer mass accuracy. At 15K, under screening criteria applied in this study, this pesticide would have been missed (false negative).

Glyphosate analysis

Glyphosate, is a broad-spectrum herbicide and, without doubts, is the world's biggest-selling chemical used for weed control in agricultural, silvicultural and urban environments

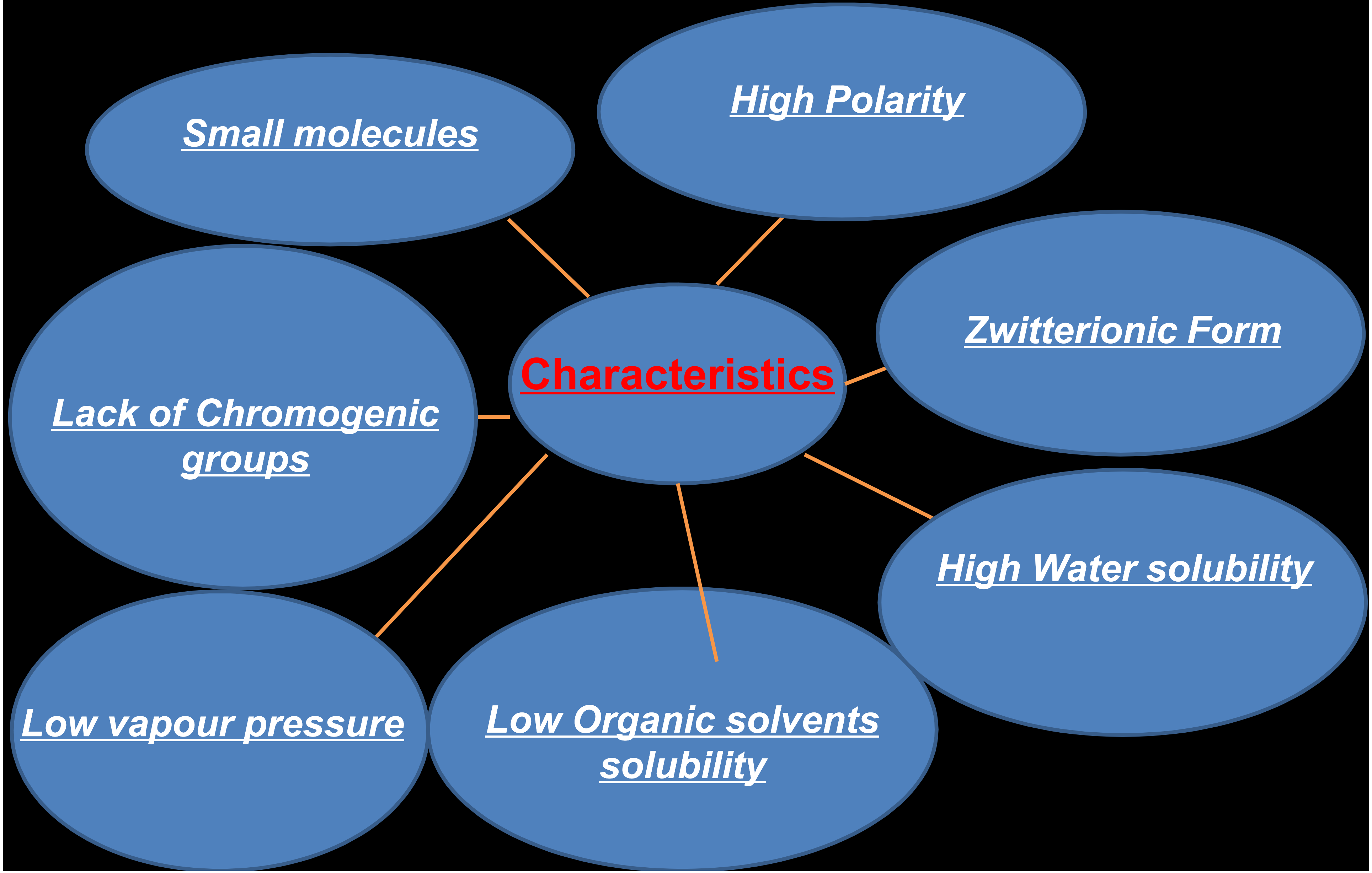


Common Name ISO: GLYPHOSATE

Chemical name IUPAC: N-(phosphonomethyl)-glycin

Solvent	Solubility	Solvent	Solubility
Water	**pH 2: 10.5 ± 0.2 g/l** **20 °C, 995 g/kg**	Methanol	0.231 g/l
Acetone	0.078 g/l	n-Octanol	0.020 g/l
Dichloromethane	0.233 g/l	Propan-2-ol	0.020 g/l
Ethylacetate	0.012 g/l	Toluene	0.036 g/l
Hexane	0.026 g/l		

Analysis of Glyphosate and AMPA



Small molecules

High Polarity

Lack of Chromogenic groups

Characteristics

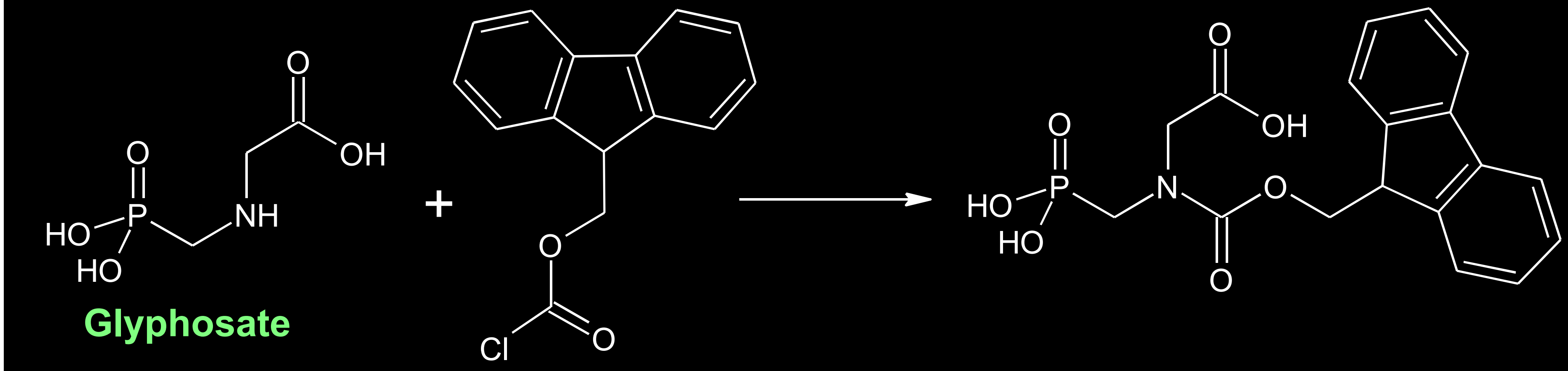
Zwitterionic Form

High Water solubility

Low vapour pressure

Low Organic solvents solubility

Derivatization of Glyphosate and AMPA with FMOC-Cl

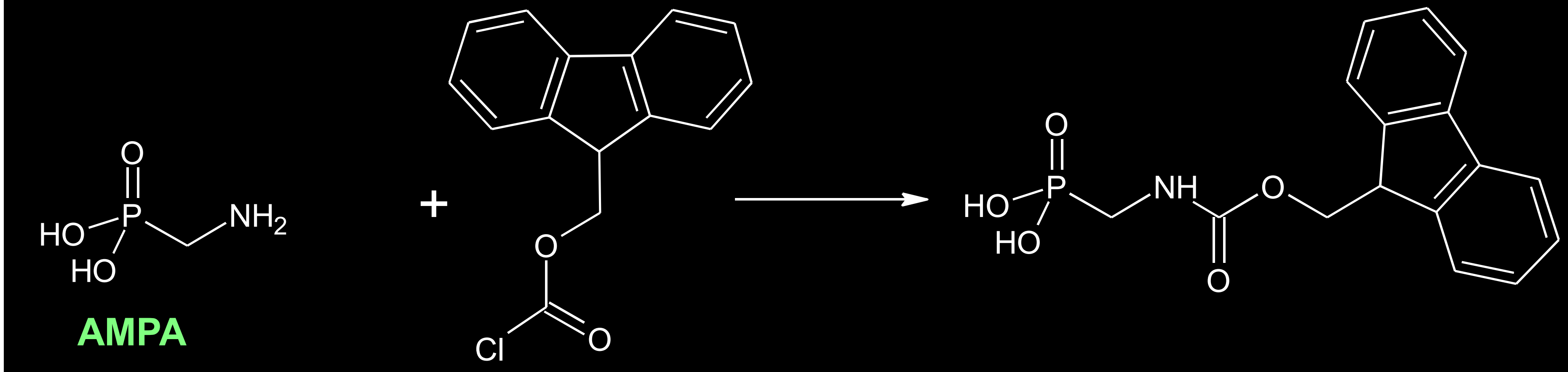


Glyphosate

FMOC-Cl

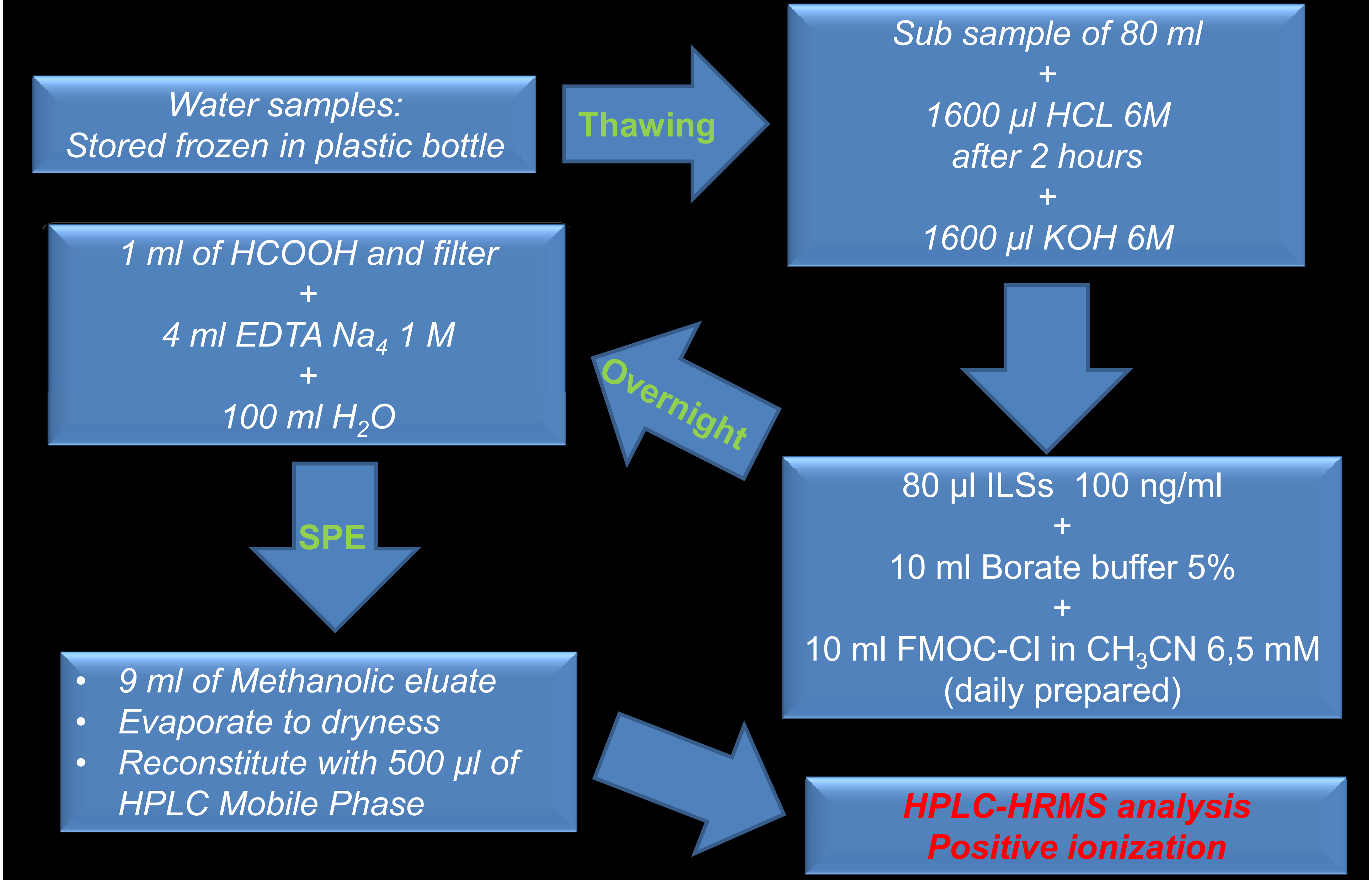
IUPAC name: Chloroformic acid 9H-fluoren-9-ylmethyl ester

Other names:9-Fluorenylmethyl chloroformate; 9-Fluorenylmethoxycarbonyl chloride;



AMPA

Analysis of Glyphosate and AMPA



Water samples:
Stored frozen in plastic bottle

Thawing

Sub sample of 80 ml
+
1600 µl HCL 6M
after 2 hours
+
1600 µl KOH 6M

80 µl ILSs 100 ng/ml
+
10 ml Borate buffer 5%
+
10 ml FMOC-Cl in CH_3CN 6,5 mM
(daily prepared)

Overnight

1 ml of HCOOH and filter
+
4 ml EDTA Na_4 1 M
+
100 ml H_2O

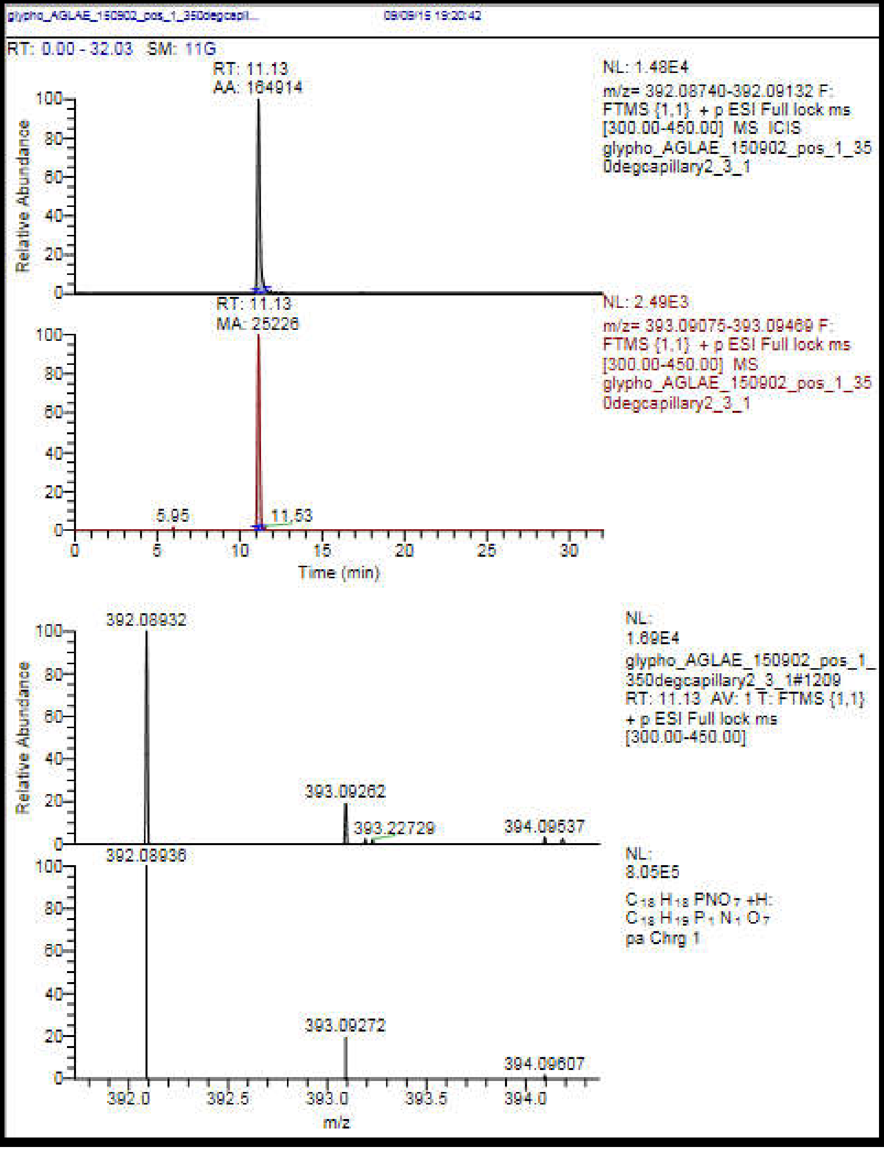
SPE

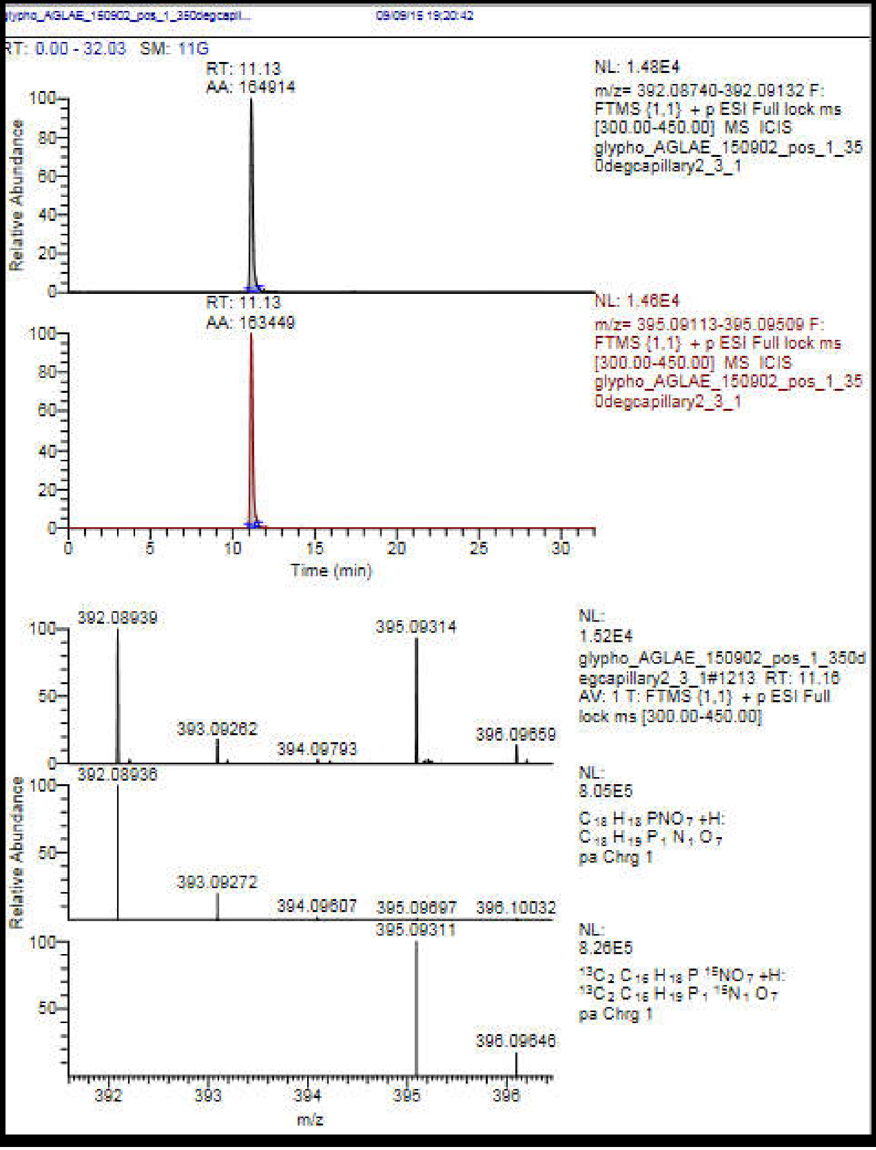
- *9 ml of Methanolic eluate*
- *Evaporate to dryness*
- *Reconstitute with 500 µl of HPLC Mobile Phase*

HPLC-HRMS analysis
Positive ionization

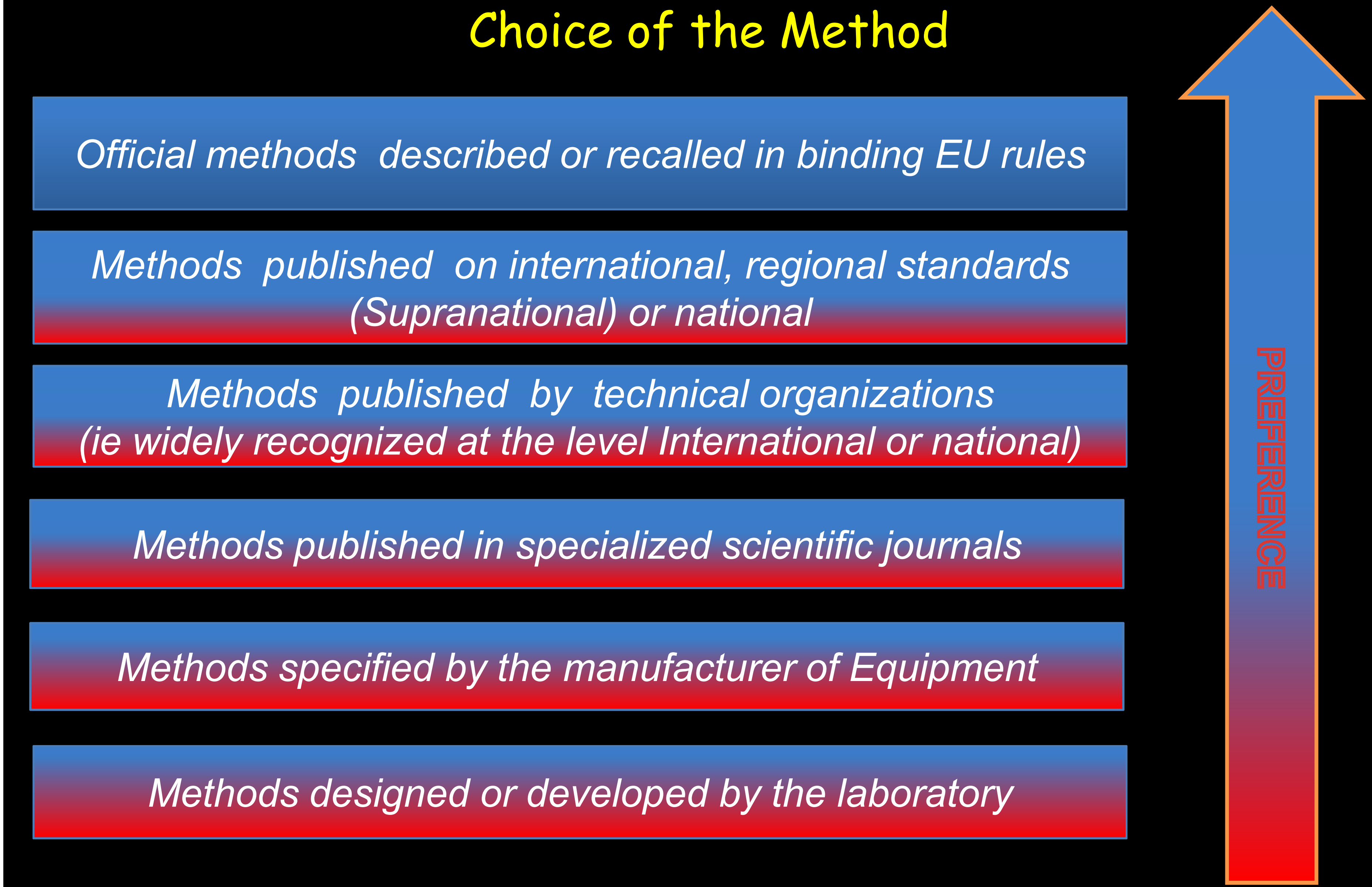
LC-HRMS: Full scan

Mineral water spiked with Glyphosate /Glyphosate ILS
0,2 µg/l e.a.





PESTICIDES IN FOOD/FEED:
Choice of the Method



Official methods described or recalled in binding EU rules

Methods published on international, regional standards (Supranational) or national

Methods published by technical organizations (ie widely recognized at the level International or national)

Methods published in specialized scientific journals

Methods specified by the manufacturer of Equipment

Methods designed or developed by the laboratory

Legal basis, Directive 85/591, Preamble and Article 2

Why and when we should use standardized methods

IT'S LOGIC

WHY?

- *Methods are based on widely accepted methods with sufficient validation data.*
- *Standards are available in three languages (EN, DE and FR).*
- *Clear description with all details including calibration and calculation.*
- *Checked by experts from many member states.*
- *More easy to convince accreditation bodies*

WHEN?

- *If analytical results cause international trade barriers.*
- *As starting point for new laboratories*

Main problems in standardization of methods

IT’S LOGIC

- ***Validation requirements not easily to fulfill.***
- ***Editorial process very laborious, because many comments have to be considered.***
- ***Official character of “old” methods may hinder analytical progress***

Whenever possible, standardized methods should offer the flexibility to apply methods in a changing “analytical world”, e.g.

PESTICIDES IN FOOD/FEED:
METHOD VALIDATION – TECHNICAL FRAMEWORK

In accordance with Article 12 of Regulation 882/2004, laboratories designated for official control of pesticide residues must be accredited to ISO/IEC 17025

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Safety of the Food Chain
Pesticides and biocides

SANTE/11945/2015
30 November -1 December 2015 rev. 0

Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

SANTE/11945/2015

Supersedes

SANCO/12571/2013

Implemented by 01/01/2016

1. **The key objectives are:**
(i) to provide a harmonized cost-effective quality assurance system in the EU
(ii) to ensure the quality and comparability of analytical results
(iii) to ensure that acceptable accuracy is achieved
(iv) to ensure that false positives or false negatives are not reported
(v) to support compliance with ISO/IEC 17025 (accreditation standard)

Validation Model

Fulfilment of legal requirements

Pesticide concept

LOD‘s /LOQ in different types of matrix reproducibiity

List of all pesticides analysed in routine

Recoveries, 70 – 120 %

Repeatability

Which pesticide is detected how?

Reproducibility

Thanks