BENAKI
PHYTOPATHOLOGICAL
INSTITUTE

Method validation: A pivotal tool for regulatory purposes in the control of pesticides formulations

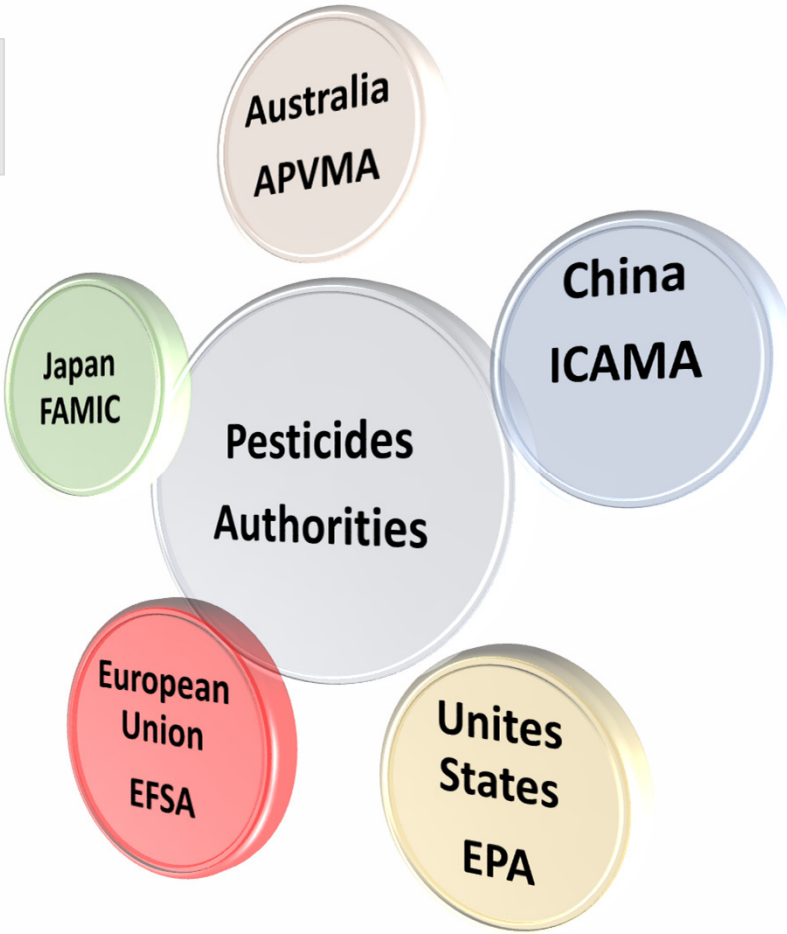
CIPAC SYMPOSIUM 2019
Braunschweig, Germany
18 June

Tzanetou Evangelia
Email: ev.tzanetou@bpi.gr

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Pesticides Authorities

Pesticides are some of the most tested and regulated products in the world.



BENAKI PHYTOPATHOLOGICAL INSTITUTE

Regulation at EU level

❖ **Plant Protection Product:** Regulation (EC) no. 1107/2009 (repealing Council Directives 79/117/EEC and 91/414/EEC)

❑ **Data requirements – Active Substance**:

1) Commission Regulation (EU) no. 283/2013

2) Commission Communications 2013/C 95/01

❑ **Data requirements – Plant Protection Product:**

1)Commission Regulation (EU) no. 284/2013

2)Commission Communications 2013/C 95/02

- *There are also national requirements which are specific to each Member State.*

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Data Requirements

Applicants

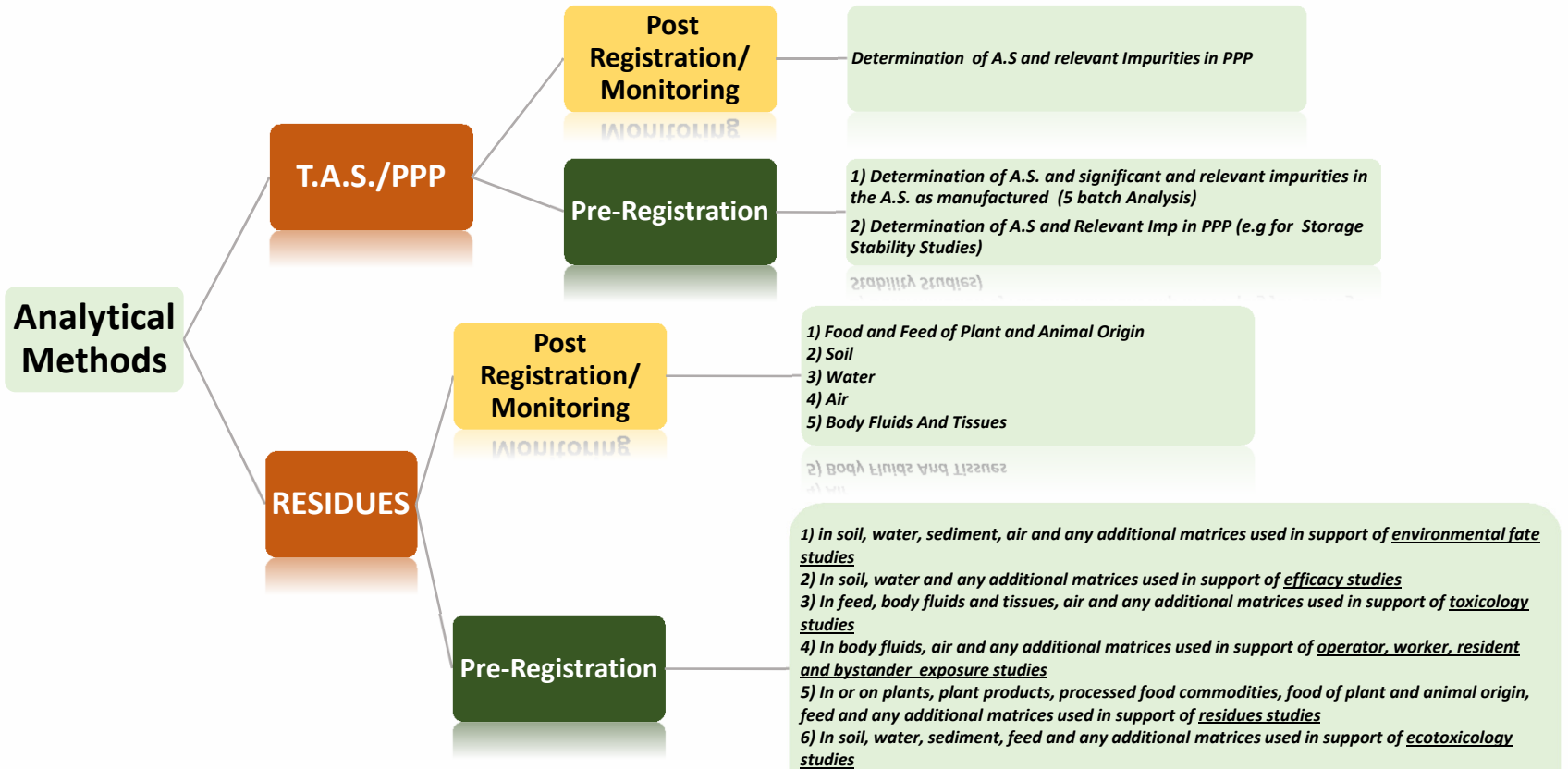
Introduce in the market

Active substance or PPP

- Data Requirements
  - Physico-Chemistry
    - Identity
    - Physical and Chemical properties
    - Analytical methods
      - Technical Active Substance (T.A.S)/Plant Protection Products (PPP)
      - Residues
  - Toxicology
  - Residues
  - Behavior in the environment
  - Ecotoxicology
  - Efficacy

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Analytical Methods



BENAKI PHYTOPATHOLOGICAL INSTITUTE

Regulation at EU level for T. A.S. and PPP

❖ **SANCO/3030/99 rev. 4** (European Commission, 2000a)
"***Technical Material and Preparations***: *Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A section 5) of directive 91/414.*"

❑ Same data requirements for both purposes (pre- as for post-registration methods).

❖ **SANCO/3030/99 rev. 5** (European Commission, 2019c)

- "***Technical Active Substance and Plant protection product***s: *Guidance for generating and reporting methods of analysis in support of pre- and post- registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013.*"

The main reasons for this update:

❑ Be in line with the Regulations (EU) No. 283/2013 and 284/2013
❑ Improvement of a common understanding on the required validation data

❖ The applicability date is for dossiers submitted on or after 01 October 2019.

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Regulation at EU level for residues

❖**SANCO/3029/99** (European Commission, 2000)

*"**Residues**: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, section 4) and Annex III (part A, Section 5) of directive 91/414".*

Pre-Registration Purposes

❖ **SANCO/825/00 rev.8.1** (European Commission, 2010)

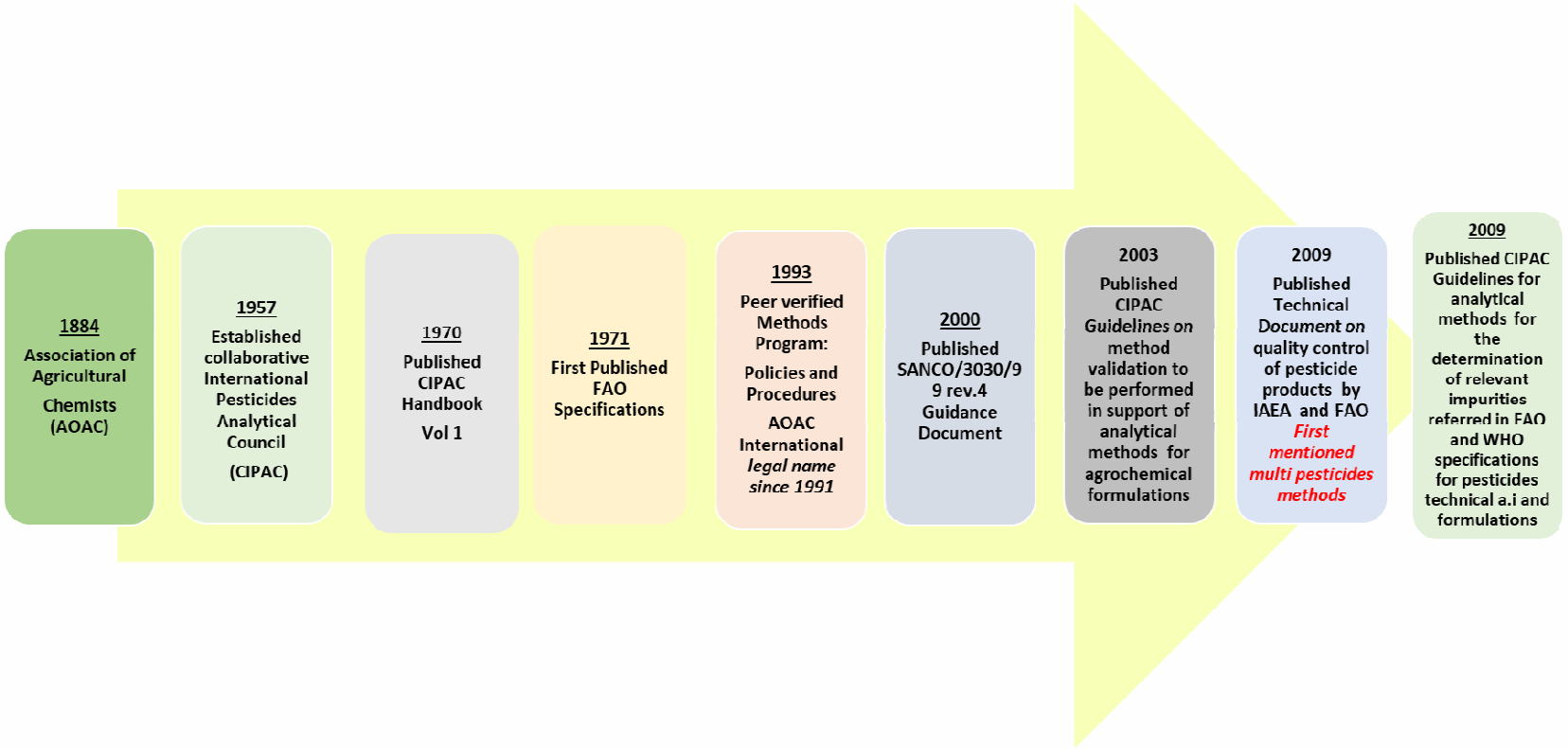
"Guidance document on pesticides residue analytical methods"

Post-Registration Purposes

- ❑ The Revisions of the two Guidance Documents under Regulation (EC) No 1107/2009 are on going (prepared by DE)
- ❑ The first drafts of the revised documents will be presented soon.

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Recognized guidance documents on quality control of plant protection products



BENAKI PHYTOPATHOLOGICAL INSTITUTE

Method Validation

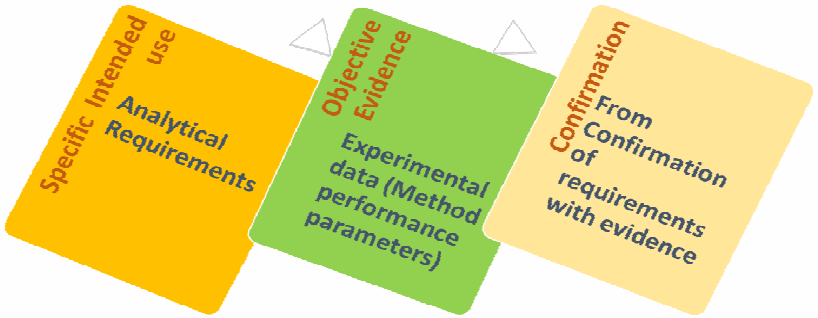
➢ ***Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled***

ISO 8402:1994

✓ **Method validation provides the necessary proof that a method is *"fit for purpose".***

✓ Method validation is required for the following reasons:

1. Robust Science
2. Regulatory Requirements
3. High quality and International Competition of PPPs



BENAKI PHYTOPATHOLOGICAL INSTITUTE

Method validation in Guidelines and related topics

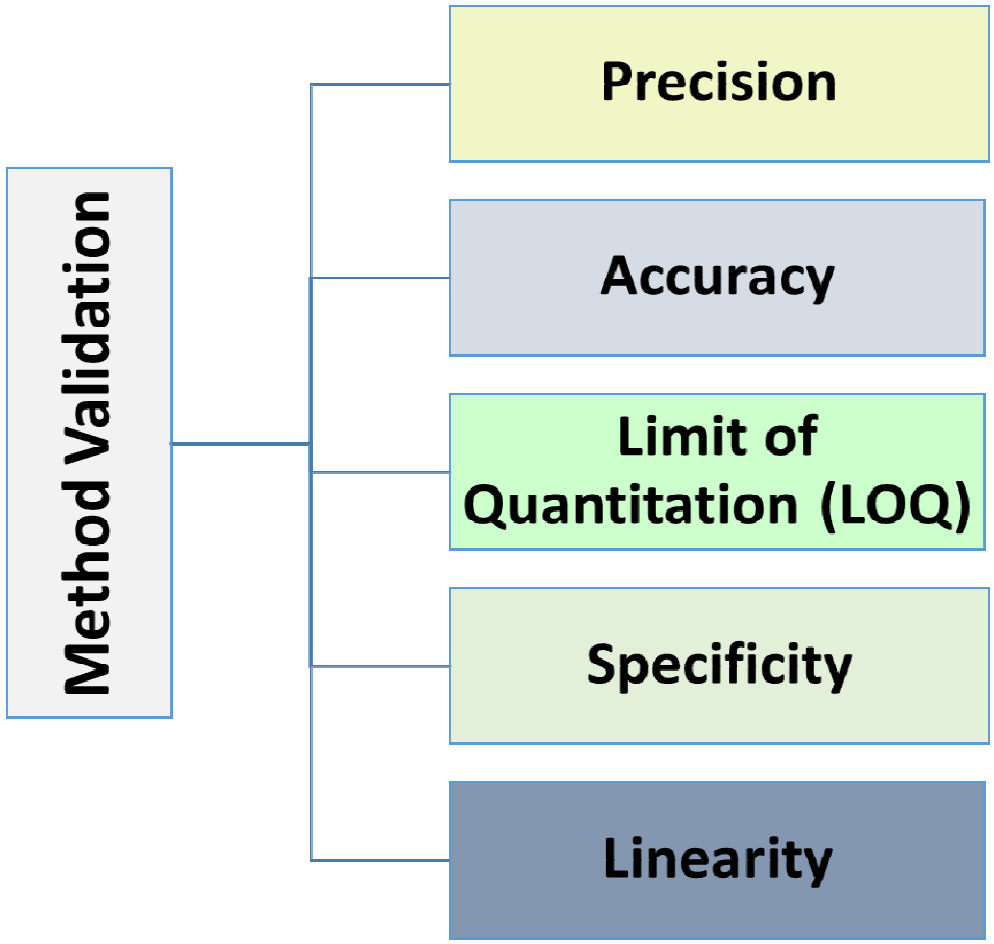
1) APVMA GL26 (2004). Australian Pesticides & Veterinary Medicines Authority. Guidelines for the validation of analytical methods for active constituent, agricultural and veterinary chemical products
2) ISO/IEC 17025 Second edition (2005). General Requirements for the competence of testing and calibration laboratories.
3) ISO/TS 21748:2004 Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation
4) OECD (2007), ENV/JM/MONO(2007)17, Guidance Document on Pesticide Residue Analytical Methods
5) OECD(2014) ENV/JM/MONO(2014)20, Guidance Document for Single Laboratory Validation of Quantitative Analytical Method
6) FAO third revision (2016), Manual on Development and Use of FAO Specifications for Plant Protection Products
7) ECHA (2014). Guidance on the Biocidal Products Regulation. Vol I: Identity/physico-chemical properties/analytical methodology
8) CAC/GL 90-2017. Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed
9) ESYD G-FYTOPROST (2016). Hellenic Accreditation System. Guidance document on method Validation and Quality Control Procedures for Pesticides residues
10) Albert, R & Hurwitz, W 1997, 'A heuristic derivation of the Hurwitz curve', Analytical Chemistry, vol. 69, pp. 789–790.
11) IAEA (2009). Quality Control of Pesticides Products.
12) Validation guidelines for pesticide residue analysis in food and feed by SANCO/12571/2013
13) AOAC official methods of analysis(2012). Appendix F: Guidelines for standard method performance requirements
14) ENFSI Standing Committee (QCC-VAL-001, 2006)
15) CEN, (EN 15662:2008)
16) The Cooperation on International Traceability in Analytical Chemistry (CITAC, 2002)
17) EURACHEM (Magnusson & O¨ rnemark, 2014. Eurachem Guide: The Fitness for Purpose of Analytical Methods; A Laboratory Guide to Method Validation and Related Topics
18) EURACHEM/CITAC (2017) Guide to Quality in Analytical Chemistry
19) IUPAC.,Thompson, Ellison, & Wood, (2002). Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis.
20) ICH (1995). Guideline on Validation of Analytical Procedures: Definitions and Terminology.
21) The International Laboratory Accreditation Cooperation (ILAC)
22) The World Health Organization (WHO)
23) ISO/IEC 17025 Second edition (2005). General Requirements for the competence of testing and calibration laboratories.
24) Green, JM 1996, 'A practical guide to analytical method validation', Analytical Chemistry, vol. 68, pp. 305A–1309A.
25) Grubs, FE & Beck, G 1972, 'Extension of sample sizes and percentage points for significance tests of outlying observations', Technometries, vol. 14, pp. 847–854.

Differences Between the various Guidelines:

- In the requirements
- In the established limits

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation Parameters for Regulatory purposes



BENAKI PHYTOPATHOLOGICAL INSTITUTE

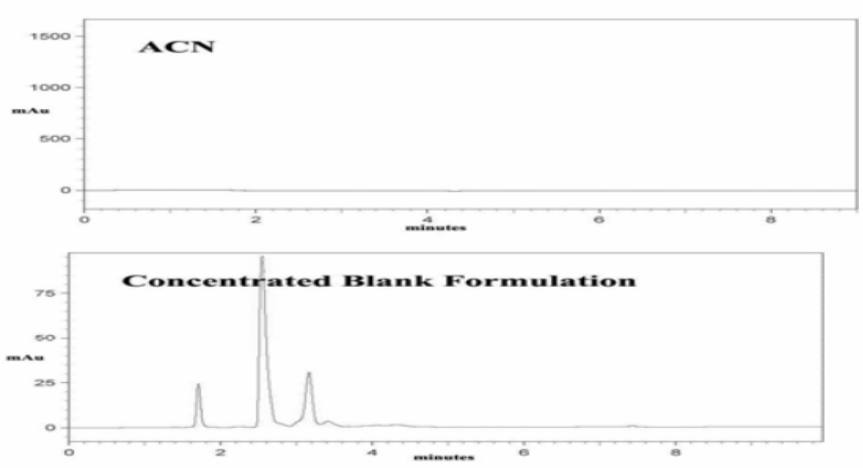
Specificity

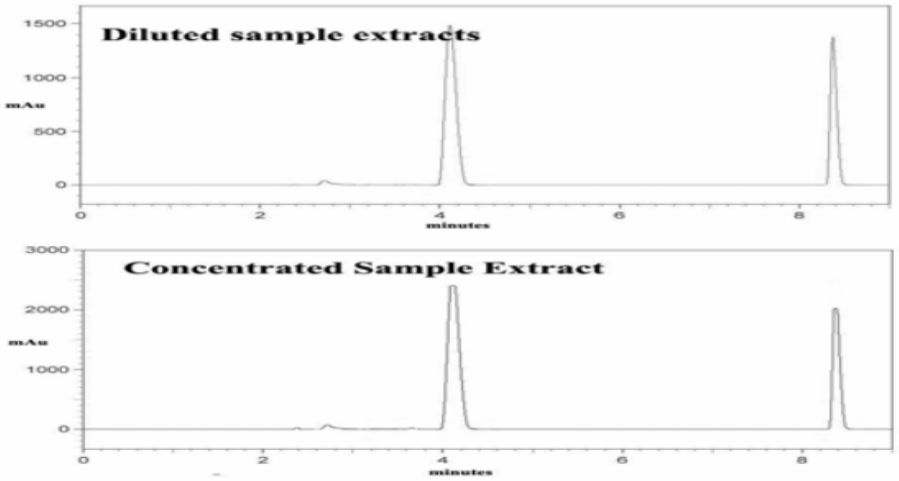
Specificity of the separation :

- The ability of the analytical method to distinguish the analyte to be determined from degradation products, metabolites or known additives was investigated.
- **The degree of interference should** be demonstrated by providing chromatograms of of blank formulations and sample extracts.

Example: Validation of a 'multi-pesticide' (MP) liquid chromatographic method with UV detection (HPLC-DAD), for the quantitative determination of two active ingredients (famaxadone and cymoxanil).

Source: IAEA (2009). Quality Control of Pesticides Products.





BENAKI PHYTOPATHOLOGICAL INSTITUTE

Linearity -LOQ

- **The linearity is evaluated** by inspecting a typical **calibration plot of signal (response factor) versus analyte concentration**

- Calibration Range
- Equation of the calibration curve
- Correlation coefficient (r)
- Representative chromatograms
- Standard Calibration Solutions
- Number of determinations and concentration levels

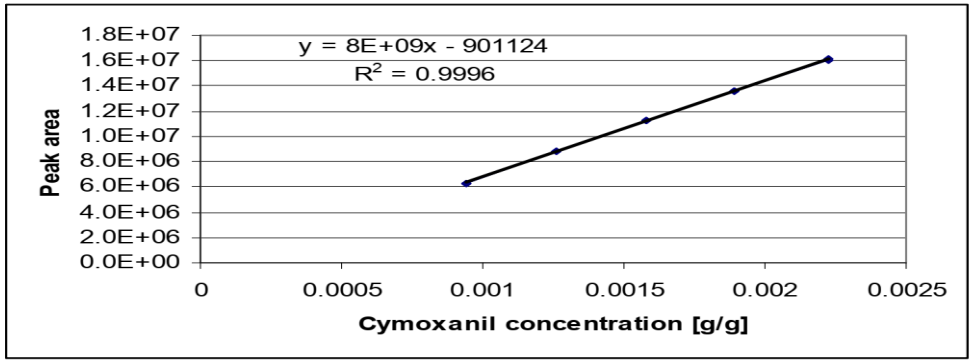
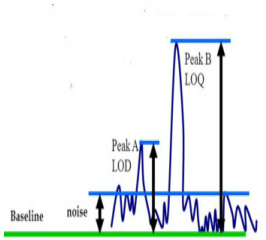


FIG. 1. Regression line and regression equation for cymoxanil using Chromolith RP-18e column.

Validation of a 'multi-pesticide' (MP) liquid chromatographic method with UV detection (HPLC-DAD), for the quantitative determination of two active ingredients (famaxadone and cymoxanil). ***Source: IAEA (2009). Quality Control of Pesticides Products.***

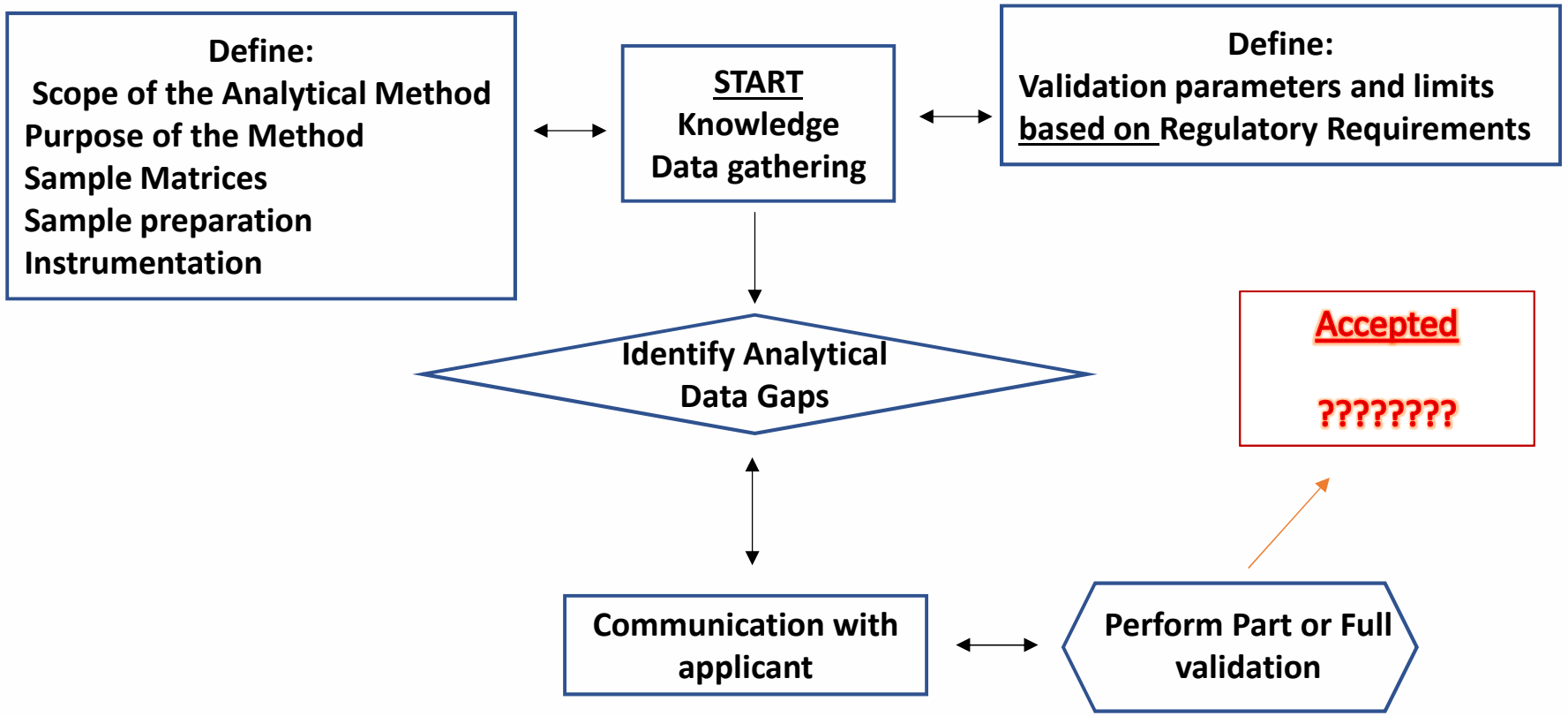
- **The LOQ is expressed as: the lowest validated concentration tested (in terms of accuracy and precision).**

- Only tested Concentrations can be defined as LOQ.



BENAKI PHYTOPATHOLOGICAL INSTITUTE

The Work Flow Example for Evaluation of A.M Validation



BENAKI PHYTOPATHOLOGICAL INSTITUTE

Evaluating the Analytical Method on a case by case basis

Considering

✓ the validation extent

✓ the purpose of the analytical method (e.g. LOQ and concentration ranges tested in (eco)toxicological studies)

Key

✓ **Collaboration** with the experts from the respective sections:

To identify which is the level of the endpoint, to inform for the validated levels

➢ **Expert judgment** to conclude whether a method can be considered *fit-for-purpose*

BENAKI
PHYTOPATHOLOGICAL
INSTITUTE

Regulatory requirements on the Validation of Analytical methods

Part A: For the determination of Active substance and impurities in Technical Active substance and Plant Protection Products

(Source: SANCO/3030/99 rev. 4 and rev.5)

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Analytical Methods

MATRIX	RELEVANT SUBSTANCE
In Technical Active Substance (T.A.S)	Active substance
	Significant Impurities (≥0.1% w/w)
	Relevant impurities (impurities with toxicological/ecotoxicological/ environmental concern)
	Additives
In Plant Protection Product (PPP)	Active substance
	Relevant impurities
	relevant co-formulants *(on going definition at EU level)*

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Standard Collaboratively tested Methods

Collaboratively tested Standard methods are regarded as validated and recommended for use at the **stage of pesticide registration**.

- **Do not need to be evaluated** (not full validation data are required)
- **Applicability of the method must be reported:**
  - ✓ ***Specificity data*** (e.g lack of interference in chromatograms).

Standardized Analytical Methods	Technical	Formulations
	Active Substance/ Impurities	
Handbooks of Collaborative International Pesticide Analytical Council (CIPAC)	☺	☺
The Association of Official Analytical Chemists' (AOAC International)	☺	☺
The European Committee for Normalization (CEN, 2008)	☺	
The International Organization for Standardization (ISO)	☺	

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

Specificity

- **For the A.S in T.A.S/PPP:**
  - The degree of interferences should be reported.
  - The interferences from other substances should not contribute more than 3% to the total peak area measured for the target analyte.
- **For impurities (significant and/or relevant impurities) in T.A.S/PPP:**
  - Confirmatory techniques are required to support impurities identification when the primary method is not considered **as *High specific method.***

Analytical methods used for regulatory purposes must be reported in detail and should be highly specific or specific.

- ***Highly specific methods are*:**
  - GC-MS or LC-MS with 3 ions and an m/z ratio >100
  - HPLC-MS/MS or GC-MS/MS with 2 transitions
- ***Specific method*:** HPLC or GC method with a retention match with a reference standard of the analyte.
- *Non-specific method*: Any analytical method in which quantification is based on a functional group (moiety) within the analyte rather than for the specific analyte.

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

Linearity

•Calibration range must extend over the highest and lowest nominal content of the analyte (with an appropriate range $\pm 20\%$) .

• Duplicate determinations (different weights) at three concentration levels or single determinations at five concentrations levels should be made.

•The correlation coefficient
- r > 0.99 Acceptable
- r <0.99 Explanation on how accurate linearity is to be maintained

- Concentration of the solutions should be reported and expressed as mg/L

- Concentration range A.S /Impurity in the T.A.S/PPP should be expressed as (m)g/Kg

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

A.S. in T.A.S

- Experimental determination of the LOQ is not required ← **(EU) no. 283/2013**

Impurities (significant and relevant) in T.A.S:

- Experimental determination of the LOQ is not required ← **(EU) no. 283/2013**

✓ The method should be validated in terms of accuracy and precision:

1) at specification levels for significant impurities

2) at least at 20% less for relevant impurities

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

- **Relevant impurities in PPP:**

✓ ***A data requirement*** **independently on whether the relevant impurity is formed or not during manufacture or from degradation during storage** ⟸ ***EFSA Technical Report (May 2017)***

✓ If the relevant impurity is formed during storage, then its content should be determined before and after storage (accelerated and shelf-life studies).

✓ The **LOQ** of the method should be below the anticipated concentration, *taking into consideration:*

- 1) The max limit of the relevant impurities in the A.S. as specified in the approval regulation of the active substance
- 2) Content of the T.A.S in PPP

Source: SANCO 3030/99(EU, 2019c):

✓ **If the content of the A.S is too low in PPP** and can be demonstrated that the desired LOQ **cannot be reached.**

Validation at the possible lowest concentration level is acceptable

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

Accuracy:

- Recovery Not Required for A.S in the T.A.S.
- At least 2 independent recovery determinations should be made for impurities (same or different fortification levels)

% (w/w) active substance	Mean recovery %	% (w/w) impurities	Mean recovery %
>10	98-102	>1	90-110
1-10	97-103	0.1-1	80-120
<1	95-105	<0.1	75-125
0.01-0.1	90-110		
<0.01	80-120		

Source: SANCO 3030/99(EU, 2000a)

% (w/w) substance (active substance or impurities)	Mean recovery %
≥ 10	97- 103
≥1 - < 10	90- 110
≥0.1 - < 1	80- 120
≥ 0.01 - < 0.1	75- 125
< 0.01	70- 130

Revised Guidance

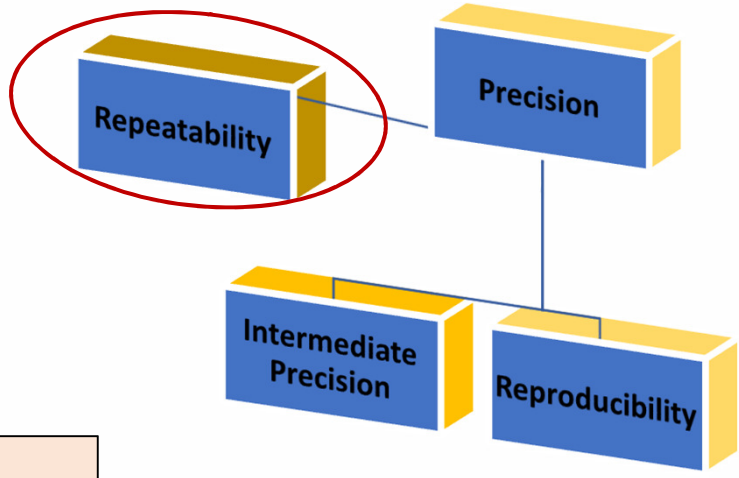
Source: SANCO 3030/99(EU, 2019c)

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for T.A.S/PPP

For Precision

- Min. 5 determinations (at each fortification level).
- Reported RSD
- Acceptability Should be based on the **Horrat (Horwitz ratio) value, Hr:**



%RSD/%RSDr

Revised Guidance

Criteria:
Hr ≤1, acceptable
1 < Hr ≤ 2, acceptable in case of a suggested explanation
Hr > 2, not acceptable

Source: SANCO 3030/99(EU, 2019c)

BENAKI
PHYTOPATHOLOGICAL
INSTITUTE

Regulatory requirements on the Validation of Analytical methods
PART B: For pesticides Residues

Source: SANCO/825/00 rev.8.1

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Analytical Methods

MATRIX GROUP	
Plants, Plant products, foodstuff of Plant Origin	• Barley, rice, wheat, etc. **(Dry Commodities)** • Apples, bananas, cherries, lettuce, etc. **(High water)** • Avocados, nuts, olives, etc. **(High oil content)** • Grapefruits, grapes, lemons, oranges (**High acid content)** • No group
Foodstuff of Animal Origin	• Milk • Eggs • Meat • Fat • Liver/Kidney
Enviromental matrices	• Soil • Water: Drinking/groundwater & Surface water • Air
Body fluids and tissues	• Blood, serum, plasma or urine • Meat, liver or kidney

The compounds as included in the residue definition for the particular matrix (A.S. and metabolites)

(EU) no. 283/2013

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for Residues

Selectivity

- Representative chromatograms of standard(s) and fortified samples at the lowest fortification level, matrix blank solutions
- Blank values should not contribute more than 30% LOQ or detailed justification

- Confirmatory methods to demonstrate the selectivity of the primary method.

1) simultaneous to the primary detection using: GC-MS or HPLC-MS by monitoring 2 additional ions or HPLC-MS/MS or GC-MS^n by monitoring 1 additional transition.
2) by an independent analytical technique: different chromatographic principle, or detector or stationery or mobile phase

- Validation data are required

Calibration

- **Concentration range shall be covered from 30% of the LOQ to 20% of the highest level**
- **Standards solutions** prepared in blank matrix extracts

or

In solvent solutions if matrix effects are not significant

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for Residues

- **A Validation of the primary method in an Independent Laboratory (ILV) should be submitted:**

1) Plants, plant product, food stuff of plant origin:

- samples of representative commodities of all matrix groups or at least 2 matrices for identical method (one of high water content)
- No ILV for multiresidue method published by standardization body (e.g AOAC, CEN)

2) Foodstuff of animal origin:

- samples of representative commodities of all matrix groups or at least 2 matrices for identical method

3) Drinking Water

(EU) no. 283/2013

<u>In order to ensure independence:</u>

- Not laboratory must not involved in the method development
- Should not be in the same location (May be in the applicant's organization)
- Any additions or modification to the primary method must be justified/reported.
- Same numbers of samples and fortification levels
- Confirm LOQ

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for Residues

MATRIX	LOQs
Plants, foodstuff of Plant Origin	• 0.01 mg/kg or lowest MRL *For Difficult to analyze 50% MRL
Foodstuff of Animal Origin	• 0.01 mg/kg or lowest MRL
Soil	• 0.05 mg/kg or LC_{50} (toxic concentration) of the most sensitive non-target organism *For phytotoxic herbicides with EC_{50} of the most sensitive crop
Drinking water	• 0.1 µg/L
Surface water	• Lowest effect concentration (e.g NOEC for either fish or Daphnia or EC50 for algae)
Air	• concentration from $AOEL_{inhalative}$ (or $AOEL_{systemic}$ or established limits
Body fluids	• 0.05 mg/L
Body tissues	• 0.1 mg/kg

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of Methods for Residues

Accuracy and Precision

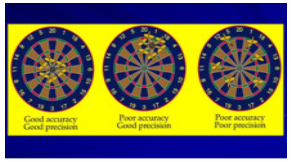
FORTIFICATION LEVELS

- **LOQ** 5 Samples
- **10 x LOQ** 5 Samples (except body fluids and tissues)
- **Control** 2 Samples

Mean recovery and precision criteria for plant matrices and animal matrices

Concentration level	Range of mean recovery (%)	Precision, RSD (%)
> 1 µg/kg ≤ 0.01 mg/kg	60- 120	30
> 0.01 mg/kg ≤ 0.1 mg/kg	70- 120	20
> 0.1 mg/kg ≤ 1 mg/kg	70- 110	15
> 1 mg/kg	70- 110	10

Source: SANCO 825/00 (EU, 2010)/ OECD 2007, ENV/JM/MONO(2007)17



In General:

- Mean Recovery Range

70% - 120% (per level)

- RSD % at each fortification level and overall RSD%

< **20% per level**

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Thinks to consider

- There are formulations containing more than one active substance.
- **Need** for the development of multi-pesticide/analyte (MP) methods.

APPLICATION OF MULTI-ANALYTE METHODS FOR PESTICIDE FORMULATIONS

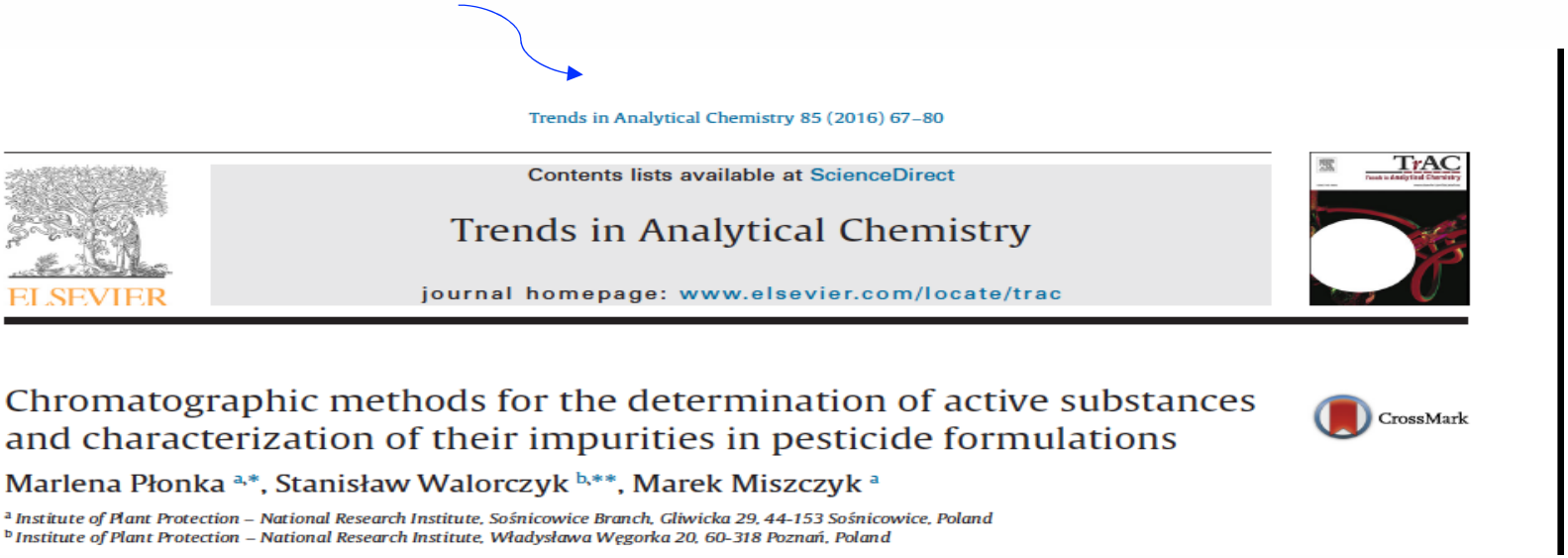
The applicability of gas chromatographic multi-analyte methods has been tested for **44 different pesticides containing 31 active substances.**
J. Lantos

compiled by Á. Ambrus (2009) in

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Thinks to Consider

- **Review article** published on 2016 concerning techniques and methods used for the determination of active substances and their impurities in formulated plant protection products up to now.
- 4 articles described methods which allow for the analysis of multiple active substances



Trends in Analytical Chemistry 85 (2016) 67–80

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Chromatographic methods for the determination of active substances and characterization of their impurities in pesticide formulations

Marlena Płonka [a,*], Stanisław Walorczyk [b,**], Marek Miszczyk [a]

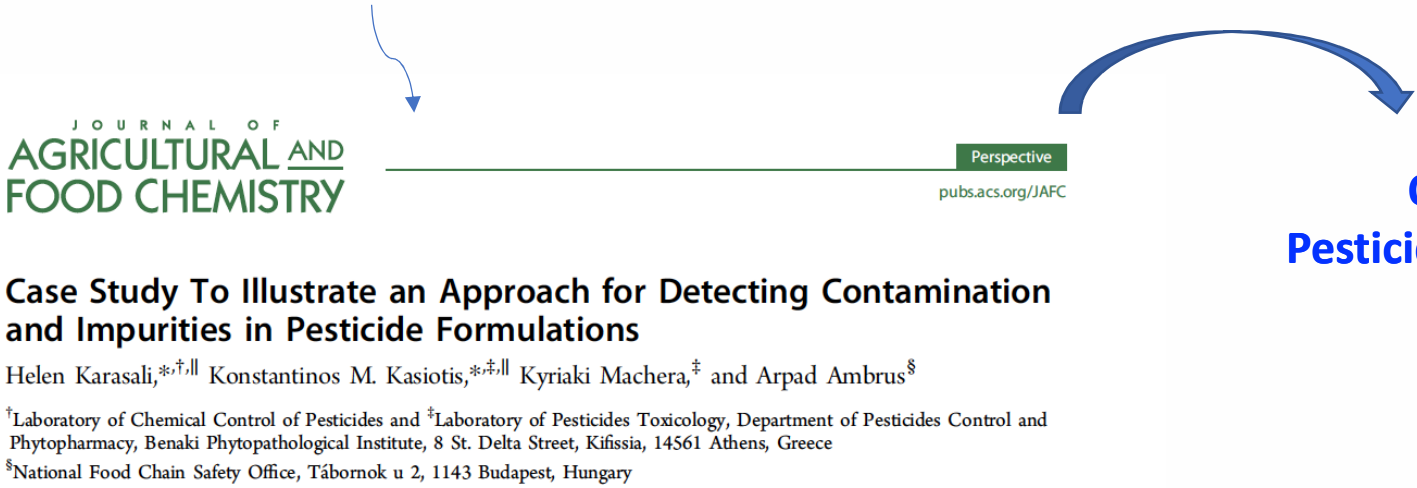
[a] Institute of Plant Protection – National Research Institute, Sośnicowice Branch, Gliwicka 29, 44-153 Sośnicowice, Poland
[b] Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland

BENAKI PHYTOPATHOLOGICAL INSTITUTE

Thinks to Consider

- Monitoring of relevant impurities in pesticide formulations is needed to ensure proper quality of PPPs

- Initial screening of PPPs revealed the presence of carbaryl in copper oxychloride formulation



Quality of Pesticide Formulations

BENAKI
PHYTOPATHOLOGICAL
INSTITUTE

Thank You Very Much!