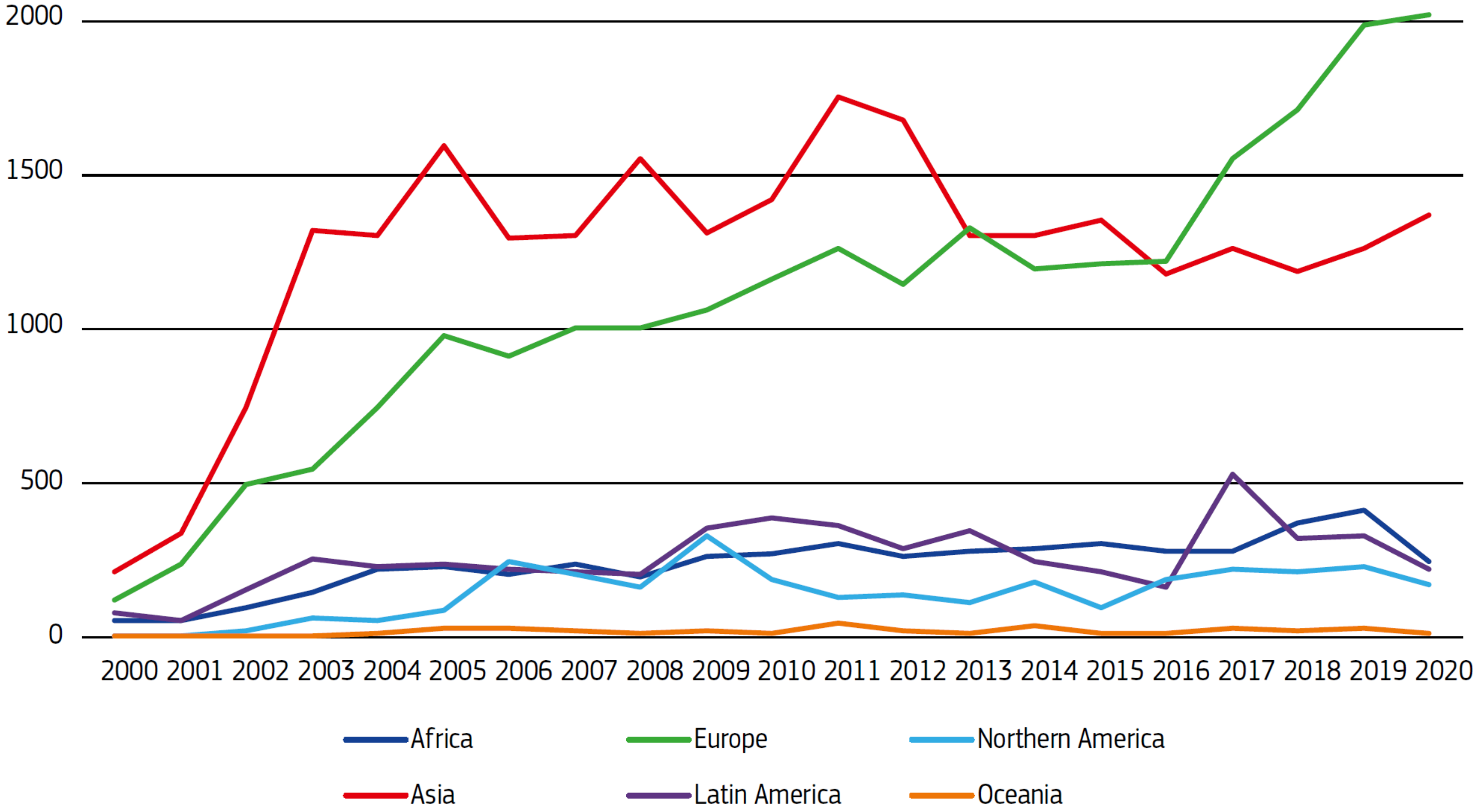
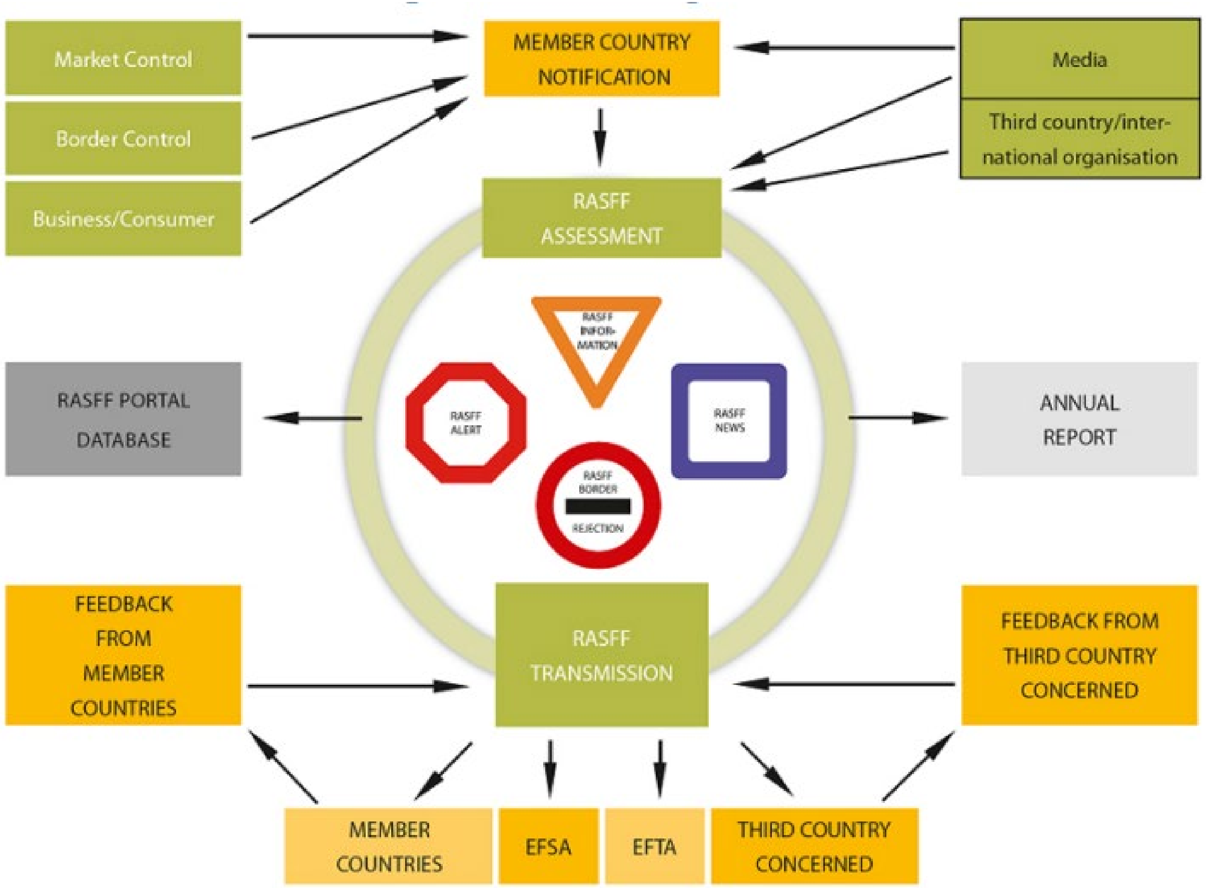
Current practices and challenges in pesticide residue analysis

Jana Hajslova, Leos Uttl, Dana Schusterova, Michal Stupak,
Monika Tomaniova. Aristeidis Tsagkaris

Hazard notifications by world regions

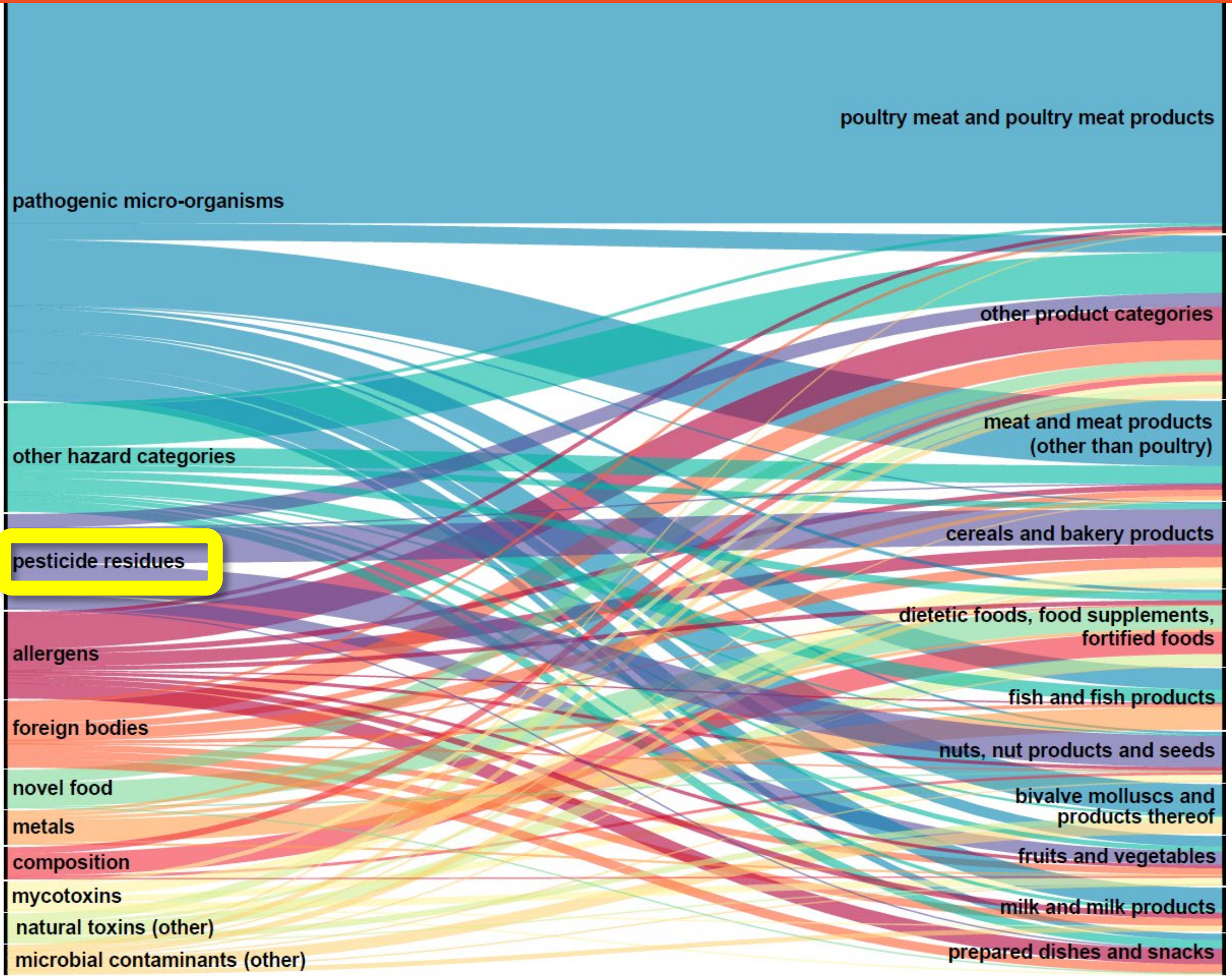


Rapid alert system for food and feed (RASFF)



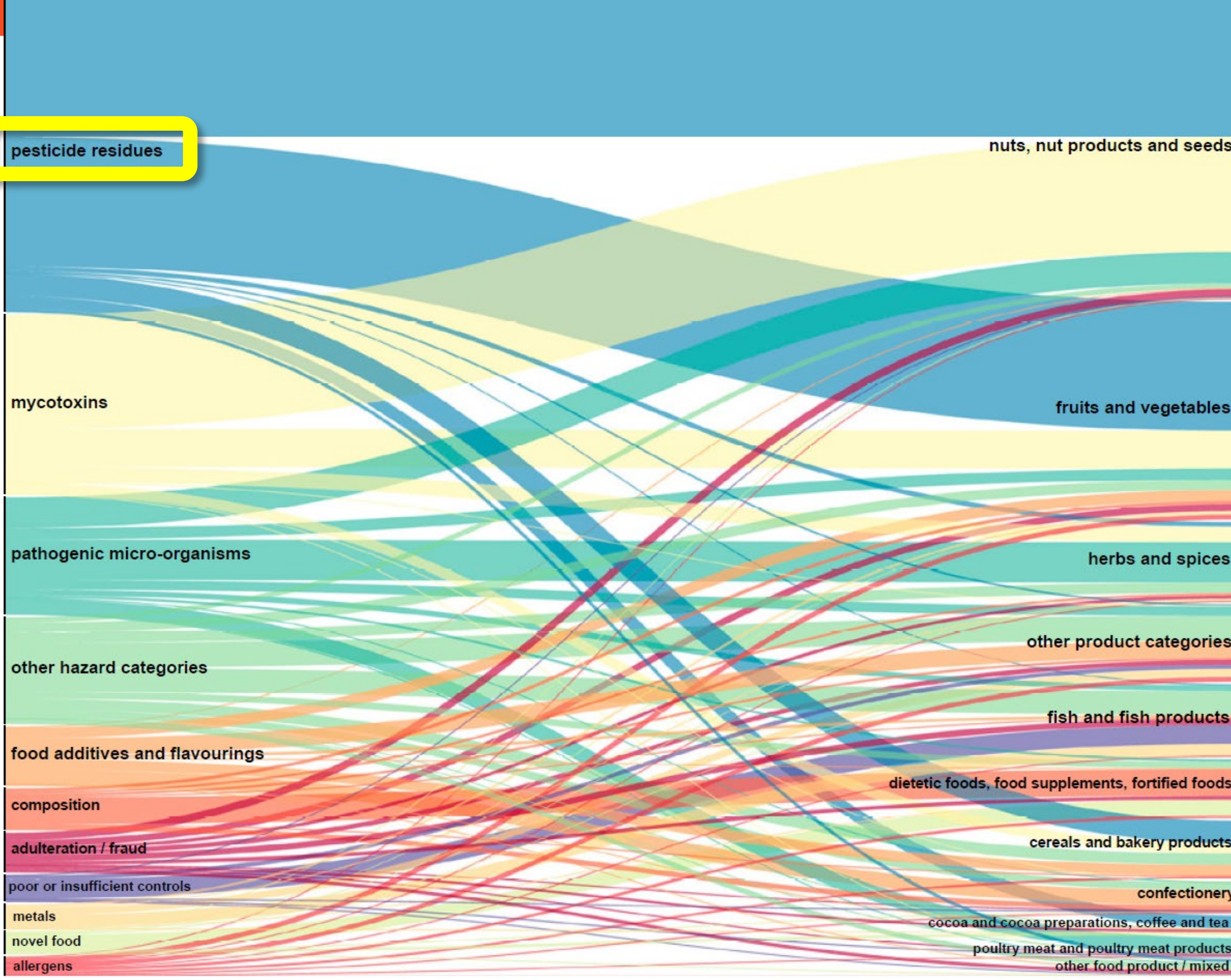
https://ec.europa.eu/food/animals/movement-pets/eu-legislation_en

2020 top 10 hazard and product categories on food products originating from member countries



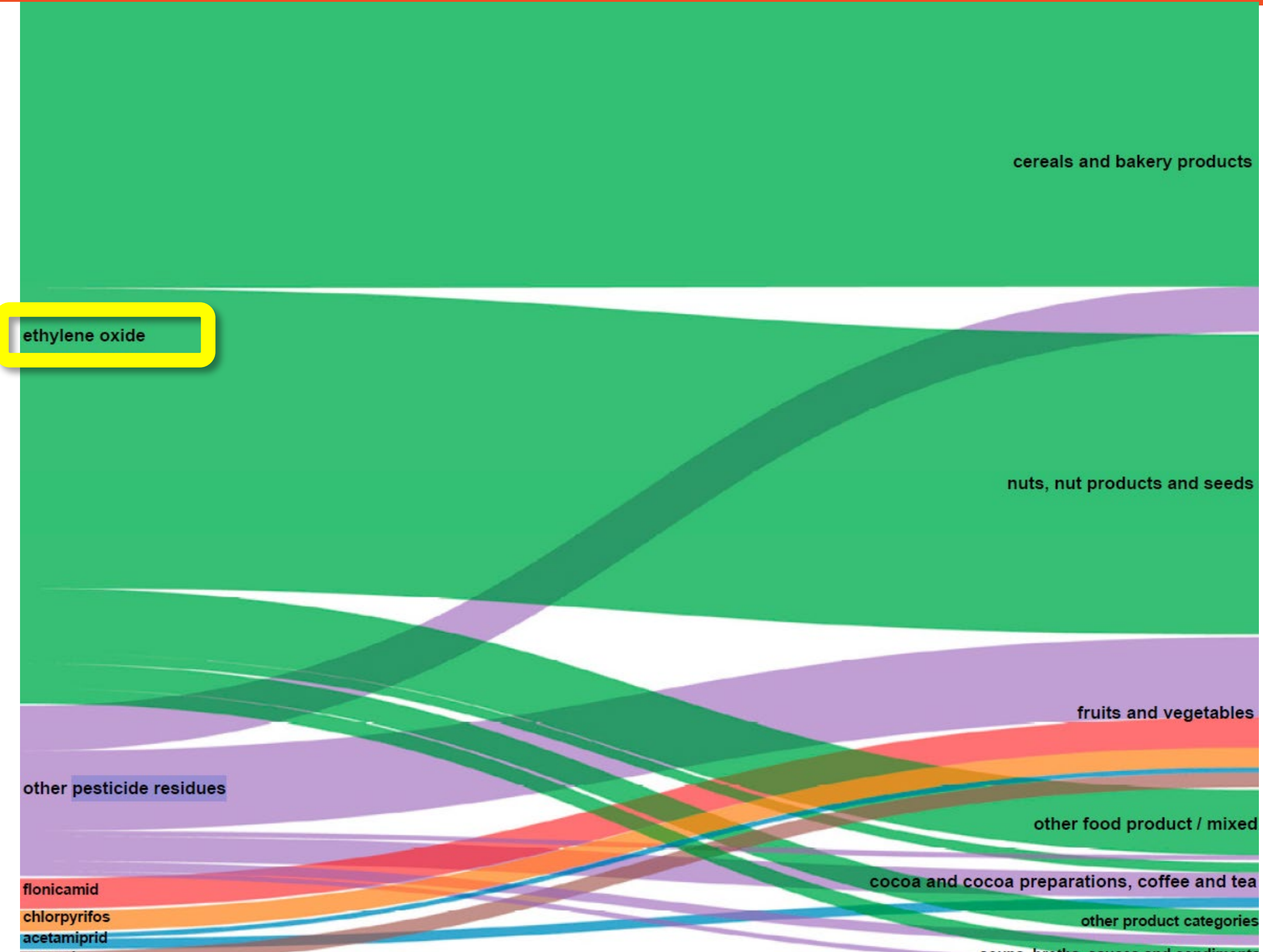
RASFF
Rapid Alert System for Food and Feed

2020 top 10 hazard and product categories on food products originating from non-member countries



UCT PRAGUE

Pesticide residues notified in 2020, set out against food product category on food products originating from member countries

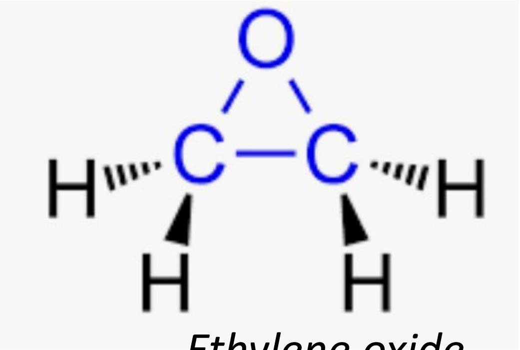


Ethylene oxide:
an emerged contaminant

Ethylene oxide

- Ethylene oxide is a gaseous disinfectant banned in EU since 1991
- It is classified as a category 1 carcinogen by the International Agency Research of Cancer (IARC)
- **Contamination of seeds, spices, food additives, milk products, breads…**
- During storage, ethylene oxide reacts with chloride ions yielding 2-chloroethanol.

MLR definition: Ethylene oxide (sum of ethylene oxide and 2-chloro-ethanol expressed as ethylene oxide)



Ethylene oxide

SEARCH CONSUMERS TRACES

Show search criteria

Ethylene oxide in food, food supplements, food additives….

782 NOTIFICATIONS

08/20 – 02/22

Ref.	Category	Type	Subject	Date	Country	Class.	Decision
2022.0868	Dietetic foods, food…	food	Ethylene oxide in supplements from France	14 FEB 2022	Spain	alert notification	serious
2022.0849	Dietetic foods, food…	food	Ethylene oxide in food supplements	14 FEB 2022	Belgium	alert notification	serious
2022.0819	Other food product / mixed	food	Emulsifying agent contaminated with ethylene oxide from Turkey	10 FEB 2022	France	information notification for attention	serious
2022.0802	Food additives and flavourings	food	Locust bean gum contaminated with ethylene oxide from Turkey	10 FEB 2022	France	information notification for attention	serious
2022.0791	Food additives and flavourings	food	Locust bean gum contaminated with ethylene oxide from Turkey	9 FEB 2022	France	information notification for attention	serious

Implementation of GC-MS/MS method


Sesame seeds


Spice


Rice

Samples

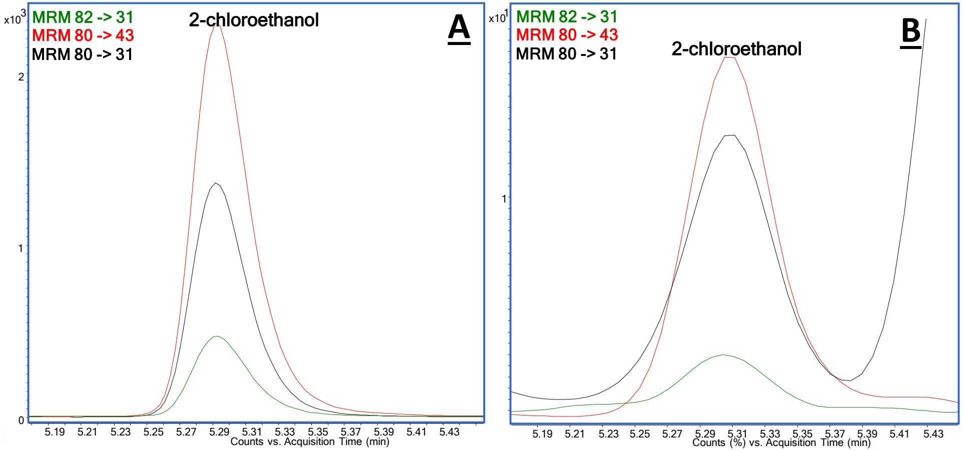
Analysis of total EtO – conversion of EtO to 2CE by acid hydrolysis + NaCl followed by ethylacetate extraction and dSPE clean-up

Screening 2-CE (contamination marker) – extraction by aqueous acetonitrile followed by dSPE clean-up


GC-MS/MS analysis

Analysis of ethylene oxide and 2-chloroethanol

- Performance characteristics of implemented analytical method
  - **Recovery** - 73-99% (**EtO**) and 86-119% (**2CE**)
  - **Repeatability** (RSD) – 2-20%
  - **LOQ** – 0.01 mg/kg (**2CE**) and 0.02 mg/kg (**EtO**)
  - **Accuracy** was proven through analysis of EUPT-SRM16 – sesame seeds

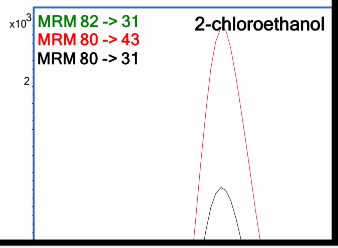


◀ ***Chromatographic records (GC-MS/MS)***

- ***A** - EUPT-SRM 16-2021 (sesame seeds, 2CE=5.07 mg/kg)*
- ***B** – ground pepper (2CE=0.02 mg/kg)*

Analysis

- Performance c
  - Recovery -
  - Repeatabili
  - LOQ – 0.01
  - Accurancy



STUPAK ET A

Gas Chromatography Tandem Mass Spectrometry Analysis of Ethylene Oxide: An Emerged Contaminant in Seeds and Spices

Michal Stupák, Filatova Maria, Vladimir Kocourek, and Jana Hajšlová, University of Chemistry and Technology Prague, Faculty of Food and Biochemical Technology, Department of Food Analysis and Nutrition, Prague, Czech Republic

The occurrence of the banned insecticide, ethylene oxide (EtO), and its transformation product, 2-chloroethanol (2-CE), has recently been reported in various food commodities. In this study, two alternative approaches based on gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) were developed to control maximum residue limit (defined as the sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide). The first approach offered a rapid screening of 2-CE (the contamination marker) in an aqueous acetonitrile extract purified by dispersive solid-phase extraction (dSPE). The total EtO was determined by the second approach, which involved conversion of EtO to 2-CE by acid hydrolysis in the presence of chloride; the ethyl acetate extract was purified prior to instrumental analysis by dSPE. The achieved limit of quantification for EtO (the sum of EtO and 2-CE expressed as EtO) was low enough to ensure compliance with regulatory requirements. The accuracy of the results was successfully verified by analysis of the EUPT test material (EUPT-SRM16 - 2021).

LC|GC europe
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September 2021 | Volume 34 Number 9
www.chromatographyonline.com

ame seeds

ic records (GC-MS/MS)

M 16-2021 (sesame

.07 mg/kg)

epper (2CE=0.02 mg/kg)

Stupak M., Filatova M., Kocourek V., Hajslova J.: *Gas Chromatography Tandem Mass Spectrometry Analysis of Ethylene Oxide: An Emerged Contaminant in Seeds and Spices*. LCGC (2021) Special Issues 34(s10): 5-10. (on-line)

References

- *Sesame seeds*. Photography. *Britannica ImageQuest*, Encyclopædia Britannica, 25 May 2016. quest.eb.com/search/132_1250313/1/132_1250313/cite. Accessed 25 Oct 2021.
- *Freshly ground coarse black pepper in white paper*. Photograph. *Britannica ImageQuest*, Encyclopædia Britannica, 25 May 2016. quest.eb.com/search/118_810398/1/118_810398/cite. Accessed 25 Oct 2021.
- *Spoonful Of Sesame Seeds*. Photography. *Britannica ImageQuest*, Encyclopædia Britannica, 25 May 2016. quest.eb.com/search/156_2425086/1/156_2425086/cite. Accessed 25 Oct 2021.
- www.Agilent.com
- *Spices for sale on market in the Rue Ste. Claire, Annecy, Haute Savoie, Rhone-Alpes, France, Europe*. Photography. *Britannica ImageQuest*, Encyclopædia Britannica, 25 May 2016. quest.eb.com/search/151_2482591/1/151_2482591/cite. Accessed 15 Feb 2022.

Current multi-residue methods (MRM)

Methods for analysis of multiple pesticide residues

LC–MS/MS: 360

Abamectin, Acephate, Acetamiprid, Acetochlor, Acrinathrin, Alachlor, Aldicarb, Aldicarb-sulfone, Aldicarb-sulfoxide, Ametryn, Atrazine, Azadirachtin, Azoxystrobin, Benalaxyl, Bendiocarb, Bitertanol, Boscalid, Bromoxynil, Carbaryl, Carbendazim, Carbofuran, Carbofuran-3-hydroxy, Chloroxuron, Chlorsulfuron, Cinerin, Clofentezine, Clomazone, Clothianidin, Cyanazine, Cyazofamid, Cymoxanil, Cyproconazole, Demeton-S-methyl, Demeton-S-methyl-sulfone, Desmedipham, Desmethylpirimicarb, Desmetryn, Dichlorvos, Dicrotophos, Diethofencarb, Diflubenzuron, Diflufenican, Dimethenamide, Dimethoate, Dimethomorph, Dimoxystrobin, Diniconazole, Disulfoton, Disulfotone-sulfone, Disulfotone-sulfoxid, Diuron, DMSA, DMST, Dodine, EPN, Epoxiconazole, Ethiofencarb, Ethofumesate, Etofenprox, Etrimfos, Fenamiphos, Fenamiphos-sulfon, Fenamiphos-sulfoxide, Fenazaquin, Fenbuconazole, Fenhexamid, Fenoxaprop-P, Fenpropathrin, Fenprophimorph, Fenpropidin, Fenpyroximate, Fensulfothion, Fenthion, Fipronil, Flonicamid, Fluazifop, Fluazifop-P-butyl, Fluazinam, Fludioxonyl, Flufacenate, Flufenacet, Flufenoxuron, Fluoxastrobin, Fluquinconazole, Fluroxypyr, Flusilazole, Formetanate, Formothion, Haloxyfop-acid, Hexaconazole, Hexazinon, Hexythiazox, Imazalil, Imazaquin, Imazethapyr, Imidacloprid, Indoxacarb, Iodosulfuron-methyl, Iprovalicarb, Isoproturon, Jasmolin, Lenacil, Linuron, Lufenuron, Mefenpyr-diethyl, Mepanipyrim, Neprobil, Metalaxyl, Metazachlor, Metconazole, Methamidophos, Methiocarbsulfone, Methiocarbsulfoxide, Methomyl, Methoxyfenozide, Metobromuron, Metolachlor, Metolcarb, Metoxuron, Mevinphos, Monocrotophos, Monolinuron, Monuron, Myclobutanil, Naled, Napropamide, Neuron, Norflurazone, Omethoate, Oxadixyl, Oxamyl, Oxydemeton-methyl, Paclobutrazol, Pencycuron, Phenmedipham, Phorate, Phorate-sulfon, Phorate-sulfoxide, Phosphamidon, Picoxystrobin, Piperonylbutoxide, Pirimicarb, Prochloraz, Prometon, Prometryn, Propachlor, Propamocarb, Propaquizafop, Propiconazole, Propoxur, Propyzamide, Proquinazid, Prosulfocarb, Pyraclostrobin, Pyrethrin, Pyridate, Pyrifenox, Pyrimethanil, Pyriproxyfen, Quinmerac, Quinoxyfen, Quizalofop-P-ethyl, Resmethrin, Simazine, Simetryn, SpinosynA, SpinosynD, Spiroxamin, Tau-Fluvalinate, Tebufenozide, Tebufenpyrad, Teflubenzuron, Terbufos-sulfoxide, Terbuthylazine, Terbutryn, Thiabendazole, Thiacloprid, Thiamethoxam, Thiodicarb, Thiometon, Thiophanate-methyl, Tolylfluanid, Triadimenol, Trichlorfon, Triflumuron, Triforine, Vamidothion……………………

GC–MS: 205

azinphos-ethyl , azinphos-methyl , bifenthrin , bupirimate , buprofezin , cadusafos , carbophenothion , hlorfenvinphos , chlorpropham , chlorpyrifos , chlorpyrifos-methyl , cyfluthrin , cyhalothrin , cypermethrin , cyprodinil , deltamethrin , diazinon , dichlofluanid , diclofop-methyl , difenoconazole , ethion , ethoprophos , fenarimol , fenoxycarb , fonofos , haloxyfop-ethoxyethyl , haloxyfop-methyl , heptenophos , isofenphos , isofenphos-methyl , kresoxim-methyl , malaoxon , malathion , mecarbam , methacrifos , methidathion , methiocarb , oxyfluorfen , penconazole , pendimethalin , permethrin , phenothrin , phenthoate , phosalone , phosmet , pirimiphos-ethyl , pirimiphos-methyl , profenofos , propargite , propham , pyrazophos , pyridaben , quinalphos , sulfotep , tebuconazole , terbufos , terbufos-sulfone , tetraconazole , tolclofos-methyl , triadimefon , triazophos , trifloxystrobin…………

bromophos-ethyl , bromophos-methyl , bromopropylate , chlorobenzilate , chlorothalonil , DDD (o , p') , DDD (p , p') , dicofol , dieldrin , diphenylamine , endosulfan (alpha) , endosulfan (beta) , endosulfan-sulfate , endrin , fenamidone , fenchlorphos , fenitrothion , fenthion , fenvalerate (1) , fenvalerate (2) , flucythrinate , fluvalinate , HCH (alpha) , HCH (beta) , HCH (delta) , HCH (gamma) , heptachlor-epoxide (endo) , heptachlor-epoxide (exo) , iprodione , methoxychlor , nitrofen , parathion-ethyl , parathion-methyl , phenylphenol (o) , procymidone , prothiofos , pyridaphenthion , quintozene , tecnazene , tefluthrin (cis) , tetradifon , trifluralin , vinclozolin ………

pesticides both LC and GC amenable

Selection of MRM analytical strategy

Cleaning effect (↑)	Cleaning effect (↓)
QuEChERS	GC-HRMS/HRMS, LC-HRMS/HRMS
LLE	GC-HRMS, LC-HRMS
GPC	GC-MS/MS, LC-MS/MS
SPE	GC-MS, LC-MS
IAC	HPLC-DAD
	GC-NPD, GC-ECD, HPLC-DAD

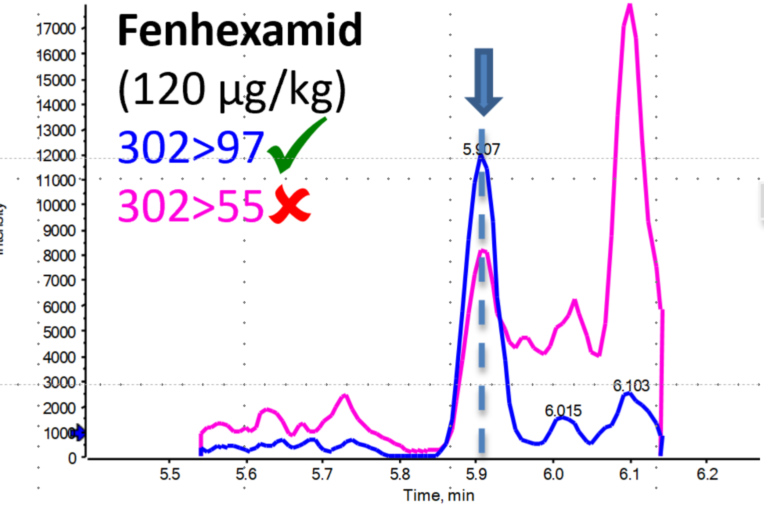
High resolution mass spectrometry (HRMS): challenges in pesticide residue analysis

HRMS enables : ▶ **to distinguish between compounds with the same nominal mass,** ▶ **to determine elemental compositions,** ▶ **identify unknowns; retrospective data mining**

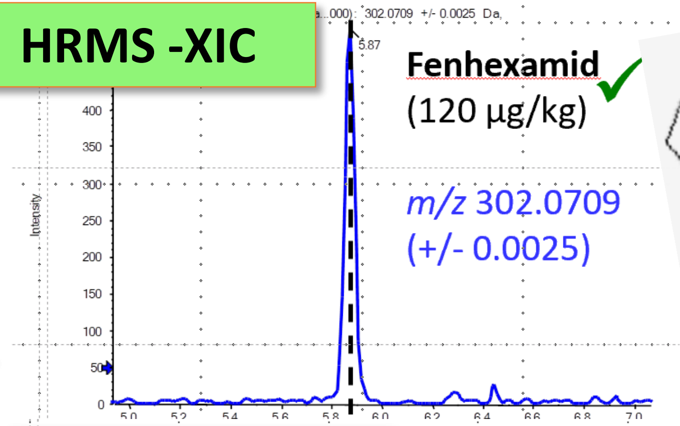
MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scale, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N $\geq$ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

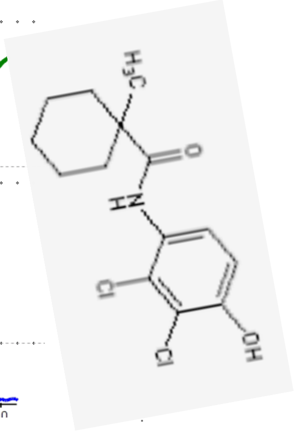
Example: orthogonal selectivity of HRMS and MS/MS

MS/MS

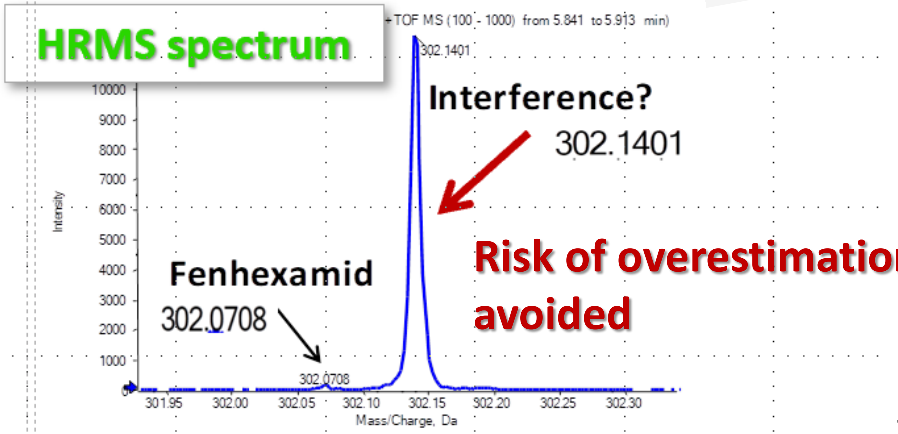


HRMS -XIC





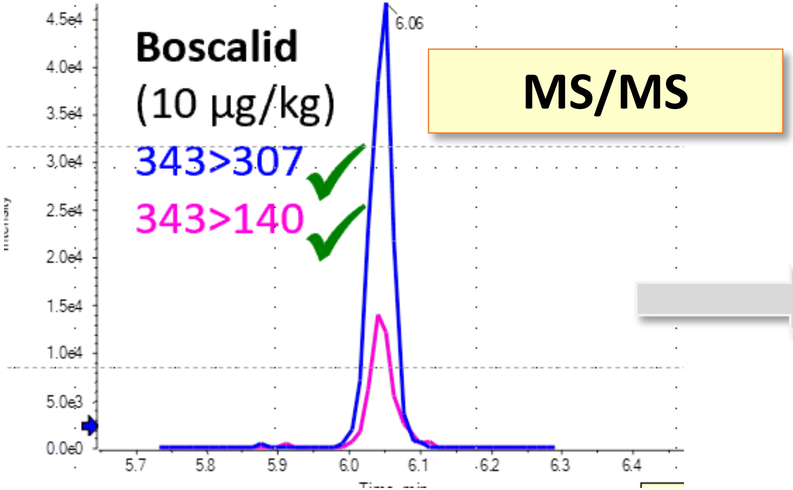
HRMS spectrum

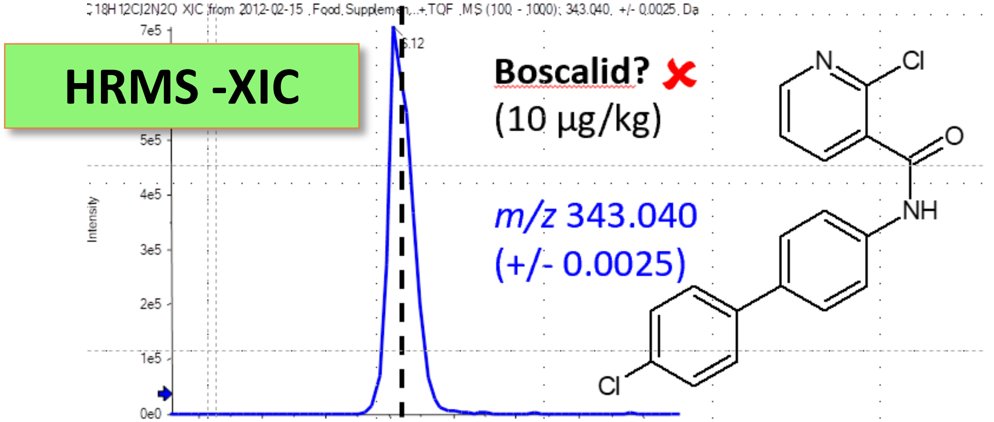


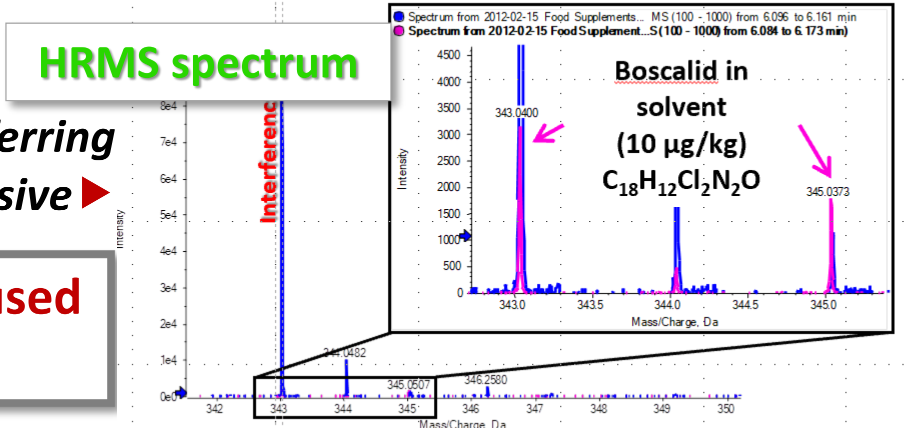
Risk of overestimation avoided

Mass difference of analyte and interferring ion ≈ 0.07 Da ▶

Example: orthogonal selectivity of HRMS and MS/MS







Mass difference between analyte and interferring ion ≈ 0.005 Da; Interference 25× more intensive ▶

Neither ^{35}Cl nor ^{37}Cl isotopes can be used for quantification.

Multiresidue – multimatrix method developed and transferred

EUCHINASAFE
中欧食品安全

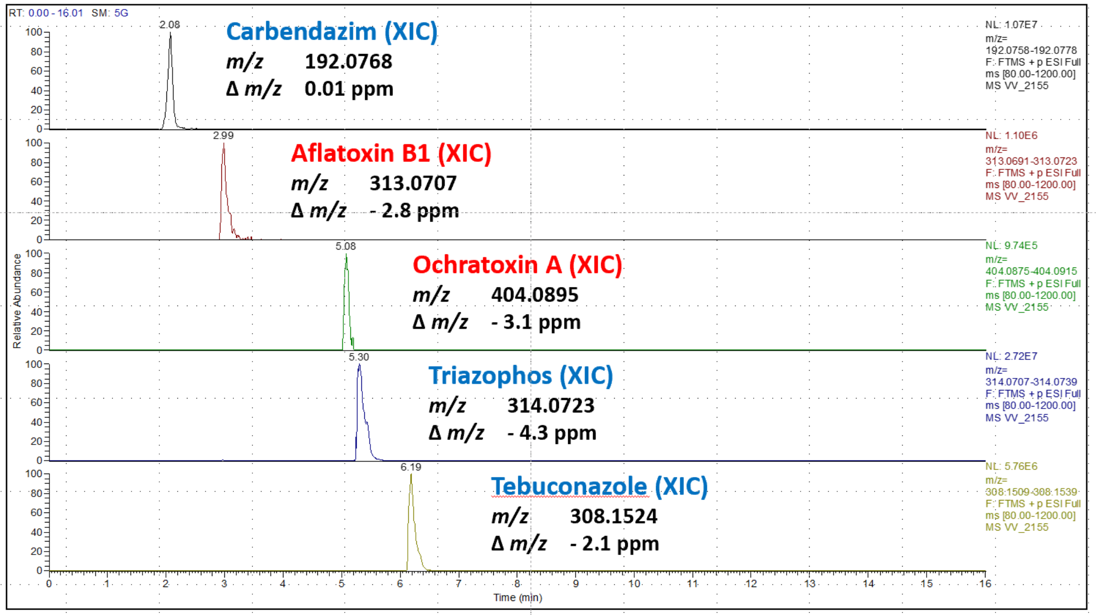
UHPLC-HRMS/MS method for multiple contaminants (mycotoxins + pesticides)

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

EUCHINASAFE 中欧食品安全

Simultaneous determination of pesticide residues and mycotoxins using multi-detection LC–MS method

SOP code	ILC-Multires-2021-SOP
Version / date of issue	2 / 07-07-2021
Institute / Laboratory	University of Chemistry and Technology / Testing Laboratory
Approved by:	J. Hajslova (UCT)
Signature:	
Institute:	University of Chemistry and Technology Prague Department of Food Analysis and Nutrition Technicka 3/1903; 166 28 Prague 6, Czech Republic jana.hajslova@vscht.cz; https://uapv.vscht.cz/?jazyk=en



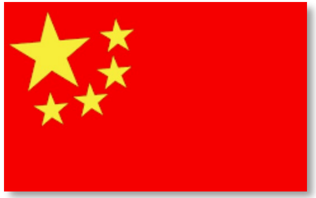
Inter-Laboratory Comparison Study on Pesticide Residues in Food (ILC)

EUCHINASAFE 中欧食品安全

➡ The aim of ILC: obtaining information regarding the **quality, accuracy and comparability of pesticide residue data in food** reported within the framework of EU and China laboratories implementing multidetection LC-MS method developed within the project.

➡ Task for participants: to use the UPLC-HRMS multiresidue method developed by UCT Prague and described in Standard Operation Procedure (SOP) provided to Chinese partners for analysis of **green tea sample contaminated by multiple pesticide residues**

Inter-laboratory Comparison Study on Pesticide Residues in Food (ILC)



Participating laboratories:

- **Academy of National Food and Strategic Reserves Administration**, Institute of Cereals and Oils Quality and Safety (China)
- **Beijing Center For Disease Control and Prevention**, Central Lab (China)
- **Chinese Academy of Inspection and Quarantine**, Institute of Food Safety (China)
- **China National Center for Food Safety Risk Assessment**, Food Chemistry (China)
- **Shanghai Customs**, China, Animal, Plant and Food Inspection and Quarantine Technical Center (China)
- **Shanghai Municipal Center For Disease Control and Prevention** (China)
- ***Metrological and Testing Laboratory, University of Chemistry and Technology Prague*** *(organizer, EU)*

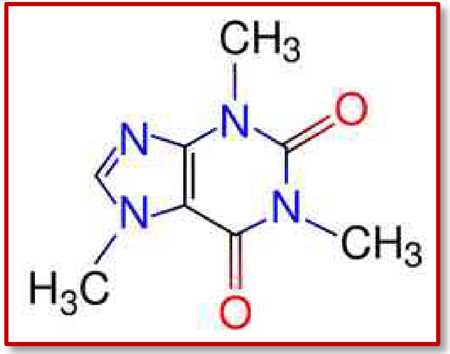
Results were submitted by all 7 laboratories (100 %) before the closing date: 10-09-2021

Tea is a difficult matrix to analyze…

Main constituents of tea leaves

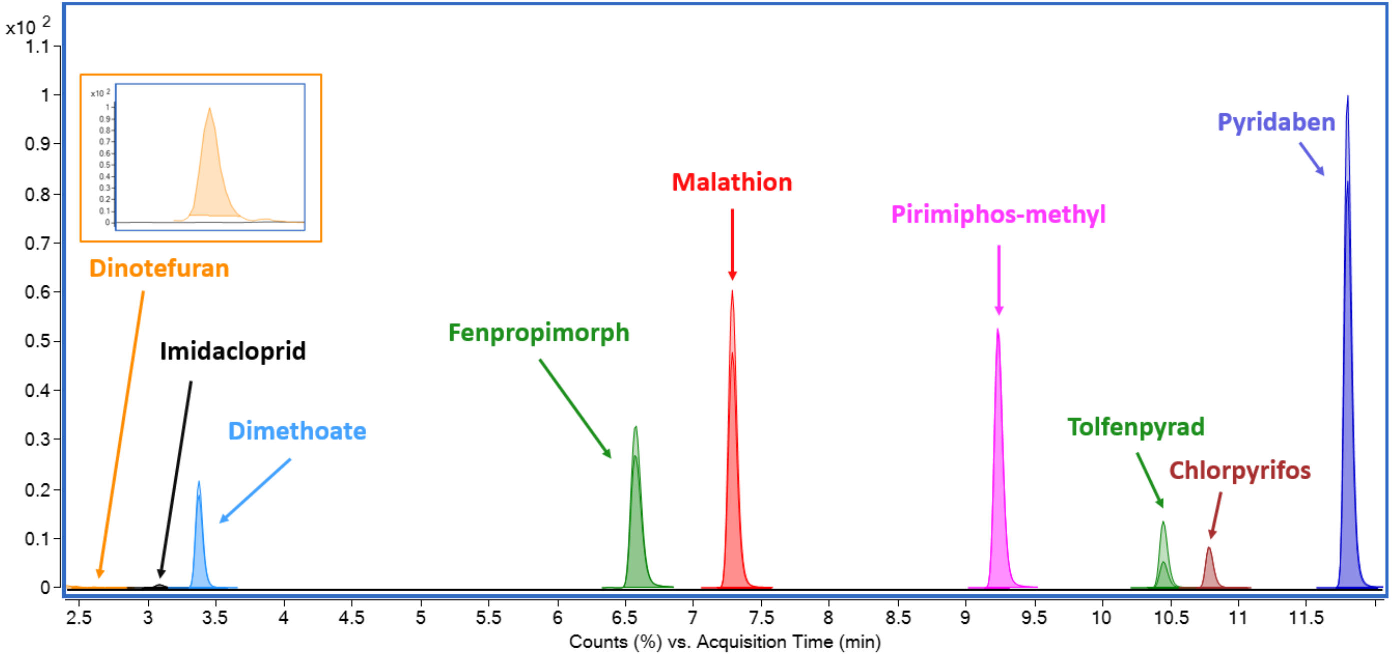
Component	Content (% dry weight)
Polyphenols	25–35
flavanols	80% of total polyphenols
Sacharides	25
polysacharides	14–22
Proteins	15
Minerals	5
Free aminoacids	4
Chlorophyll	0.5
CAFFEINE	**2.5–5.5**

CAFFEINE



40% dry matter soluble in water (fermented tea)

Pesticide residues in ILC green tea



Test material - prepared and tested according to the ISO/IEC 17043:2010 - was provided by FERA Science Ltd (the participant in the EU-China-Safe project).

ILC Study - some method details (as reported):

EA MLA Signatory
Český institut pro akreditaci, o.p.s.
Olšanská 54/3, 130 00 Praha 3

issues

according to section 16 of Act No. 22/1997 Coll., on technical requirements for products, as amended

CERTIFICATE OF ACCREDITATION

No. 192/2019

Vysoká škola chemicko-technologická v Praze
with registered office Technická 1905/5, 166 28 Praha 6, Dejvice, Company Registration No. 60461373

to the Testing Laboratory No. **1316.2**
Metrological and Testing laboratory

Scope of accreditation:

Chemical analysis of food including beverages and food supplements, food and pharmaceutical raw materials and products, feedstuffs, chemical preparations, biological materials of human, plant and animal origin, environmental components and forensic samples including addictive substances to the extent as specified in the appendix to this Certificate.

This Certificate of Accreditation is a proof of Accreditation issued on the basis of assessment of fulfillment of the accreditation criteria in accordance with

ČSN EN ISO/IEC 17025:2018

In its activities performed within the scope and for the period of validity of this Certificate, the Body is entitled to refer to this Certificate, provided that the accreditation is not suspended and the Body meets the specified accreditation requirements in accordance with the relevant regulations applicable to the activity of an accredited Conformity Assessment Body.

This Certificate of Accreditation replaces, to the full extent, Certificate No.: 202/2018 of 18. 4. 2018, or any administrative acts building upon it.

The Certificate of Accreditation is valid until: **29. 4. 2024**

Prague: 29. 4. 2019

Jiří Růžička
Director
Czech Accreditation Institute
Public Service Company

*ILC was organised by the ISO 17025 accredited testing laboratory of the **University of Chemistry and Technology Prague** (UCT Prague, CZ), in collaboration with Queens University Belfast (QUB, UK) and China National Center for Food Safety Risk Assessment,(CFSA, China) and supported by the FERA Science LtD (UK).*

References:

[1] ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing"

[2] EA-4/21 INF:2018 Guidelines for the assessment of the appropriateness of small interlaboratory comparisons

[3] General protocol for EU Proficiency Tests on Pesticide Residues in Food and Feed provided by European Reference Laboratories, 9th Ed., Nov 2019

[4] ISO 13528: Statistical methods for use in proficiency testing by interlaboratory comparisons

[5] Protocol for Proficiency testing Schemes (Part 1: Common Principles), version 7, Jan 2021, FERA Science Ltd, Sand Hutton, York, UK.

ILC Study on Pesticide Residues in Food

Analyte	assigned value (X_a) [mg/kg]	number of scores $\|z\| \leq 2.0$	total number of analytes	% $\|z\| \leq 2.0$	number of False Negative
Chlorpyrifos (ethyl)	0.119	5	7	71 %	1
Dimethoate	0.068	7	7	100 %	-
Dinotefuran	0.056	7	7	100 %	-
Fenpropimorph	0.079	6	7	86 %	1
Imidacloprid	0.047	6	7	86 %	1
Malathion	0.107	6	7	86 %	-
Pirimiphos-methyl	0.138	6	7	86 %	-
Pyridaben	0.081	6	7	86 %	-
Tolfenpyrad	0.077	6	7	86 %	-

Total number of False Positive results: 3
Total number of False Negative results: 3

Table 1: Concentration of the pesticides (mg/kg) reported by laboratories and z-scores using Fit-For-Purpose RSD (relative standard deviation for proficiency = 25 %). MRRL: Minimum Required Reporting Level

Laboratory code	chlorpyrifos	Z scores (FFP RSD (25%)	dimethoate	Z scores (FFP RSD (25%)	dinitefuran	Z scores (FFP RSD (25%)	fenpropimorph	Z scores (FFP RSD (25%)	imidacloprid	Z scores (FFP RSD (25%)	malathion	Z scores (FFP RSD (25%)	pirimiphos-methyl	Z scores (FFP RSD (25%)	pyridaben	Z scores (FFP RSD (25%)	tolfenpyrad	Z scores (FFP RSD (25%)
MRRL	**0,050**		**0,010**		**0,020**		**0,010**		**0,010**		**0,010**		**0,010**		**0,010**		**0,010**	
Assigned value	**0,119**		**0,068**		**0,057**		**0,079**		**0,047**		**0,107**		**0,138**		**0,081**		**0,077**	
LAB 10	0,131	0,41	0,072	0,24	*0,050*	-0,47	0,066	-0,63	0,045	-0,16	0,112	0,19	0,146	0,22	0,088	0,31	0,084	0,37
LAB 11	0,182	2,12	0,084	0,94	0,055	-0,11	0,106	1,40	0,049	0,21	0,141	1,28	0,191	1,54	0,107	1,27	0,115	1,97
LAB 12	0,140	0,71	0,068	-0,02	0,042	-1,05	0,080	0,07	0,042	-0,42	0,109	0,08	0,171	0,96	0,084	0,12	0,072	-0,25
LAB 13	0,134	0,49	0,071	0,19	0,050	-0,46	0,078	-0,02	0,040	-0,55	0,107	0,01	0,171	0,95	0,089	0,40	0,072	-0,22
LAB 14	0,141	0,74	0,073	0,31	0,065	0,61	0,089	0,52	0,045	-0,17	0,120	0,49	0,147	0,26	0,088	0,34	0,085	0,41
LAB 15	FN	FN	0,050	-1,05	0,060	0,23	FN	FN	FN	FN	0,050	-2,13	0,023	-3,33	0,020	-3,01	0,210	6,95
LAB 16	0,122	0,10	0,057	-0,64	0,044	-0,90	0,079	0,01	0,043	-0,31	0,108	0,02	0,141	0,09	0,086	0,24	0,084	0,35

False positive:
LAB 14 fenpropathrin
LAB 15 cyanazine, pirimicarb

False negative:

LAB 15 chlorpyrifos, fenpropimorph, imidacloprid

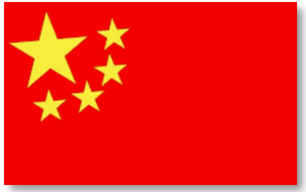
ILC Study – some method details (as reported):

Chromatographic column used

Lab 10:	Acclaim RSLC 120 C18 (150 x 2.1 mm), 2.2 µm
Lab 11:	not specified
Lab 12:	Accucore aQ (150 x 2.1 mm), 2.7 µm; Thermo Scientific, Phenomenex (USA)
Lab 13:	Poroshell 120 EC-C18 (150 x 3.0 mm), 2.7 µm
Labs 14, 15, 16:	Waters ACQUITY UPLC HSS T3 column (100 x 2.1 mm),1.7 µm

Mass spectrometry

Labs 13, 14, 15, 16:	MS/MS Triple Quadrupole
Labs 11, 12:	**High Resolution MS (Q-)Orbitrap**
Lab 10:	**High Resolution MS**



Calibration / quantitation:

Labs 10, 13, 15, 16:	matrix-matched calibration
Labs 11, 12:	standard addition

Authentication of organic crops based on UHPLC-HRMS

EUCHINASAFE
中欧食品安全

Challenge: bio-wines authentication

Bacgreound of study: Growing popularity of ´bio-wines´ has raise a demand for comntrol of compliance of organic farming practices usewd in grapes production (Commission Regulation 889/2008).

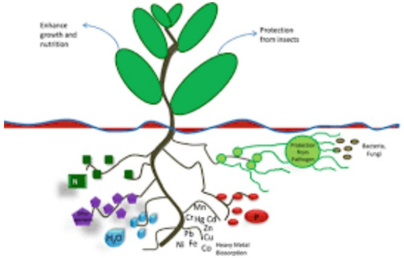
PROBLEM: **occurrence of unauthorized pesticide residues close to 0.01 mg/kg in ´organic´ grapes found by Control Authority, however, how to interpret it?**

- **Accidental contamination e.g. through atmospheric transport**

or

- **Illegal use of pesticide preparations**

Biotransformation of pesticides in plants



Pesticide

Phase 1
- oxidation
- hydrolysis
- reduction

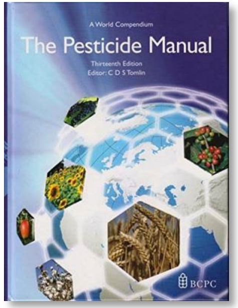
Phase 2
- conjugation
  - sugars
  - glutathione
  - aminoacids

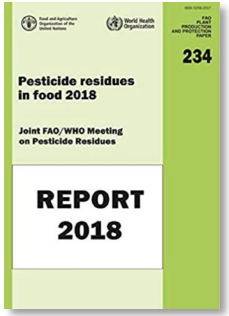
Phase 3
- conversion of Phase 2 metabolites into (nontoxic) 2º conjugates
- moving metabolites to vacuole for storage
- Incorporating metabolites into cell wall

Sources employed for Database of pesticide metabolites construction

JMPR documents, EFSa opinions, Pesticide manual, scientific literature..

European Food Safety Authority



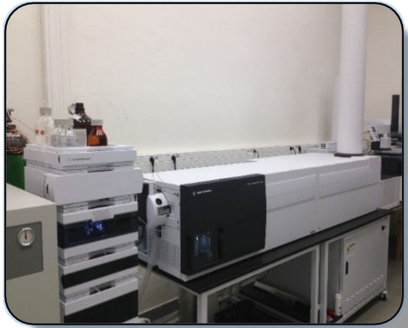


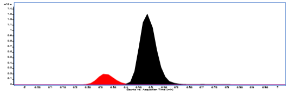
HRMS/MS screening and confirmation

Accurate mass (± 5 ppm), isotope profile

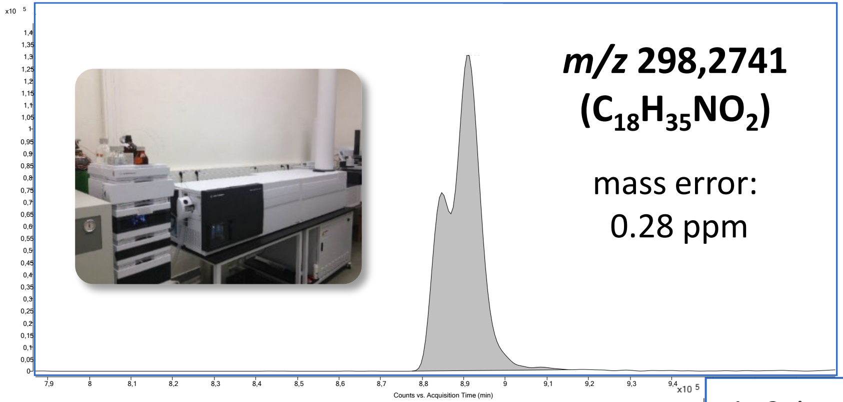
Interpretation of MS/MS fragments

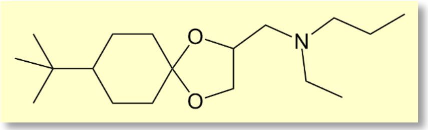
- Diagnostic fragments
- Fragments complying to parent molecule
- Fragments characterizing metabolic transformation
- Neutral losses





Example of difficult decision: is the wine organic? Pesticide metabolites as markers!



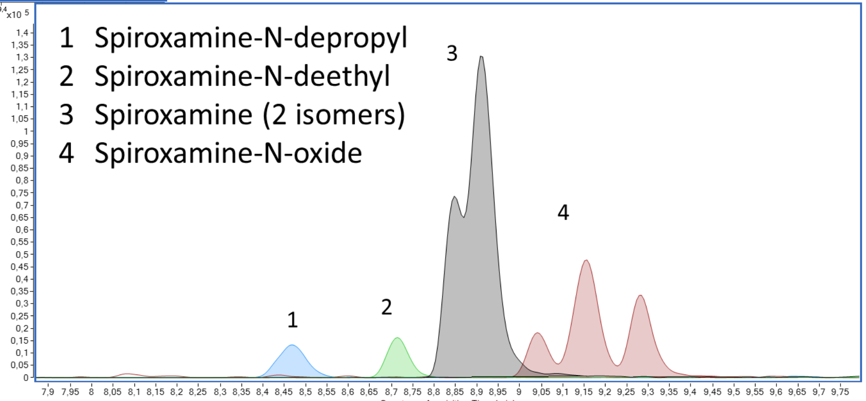


◀ **Spiroxamine – 0.01 mg/kg** → „BIO“ MLR

1st step ✓

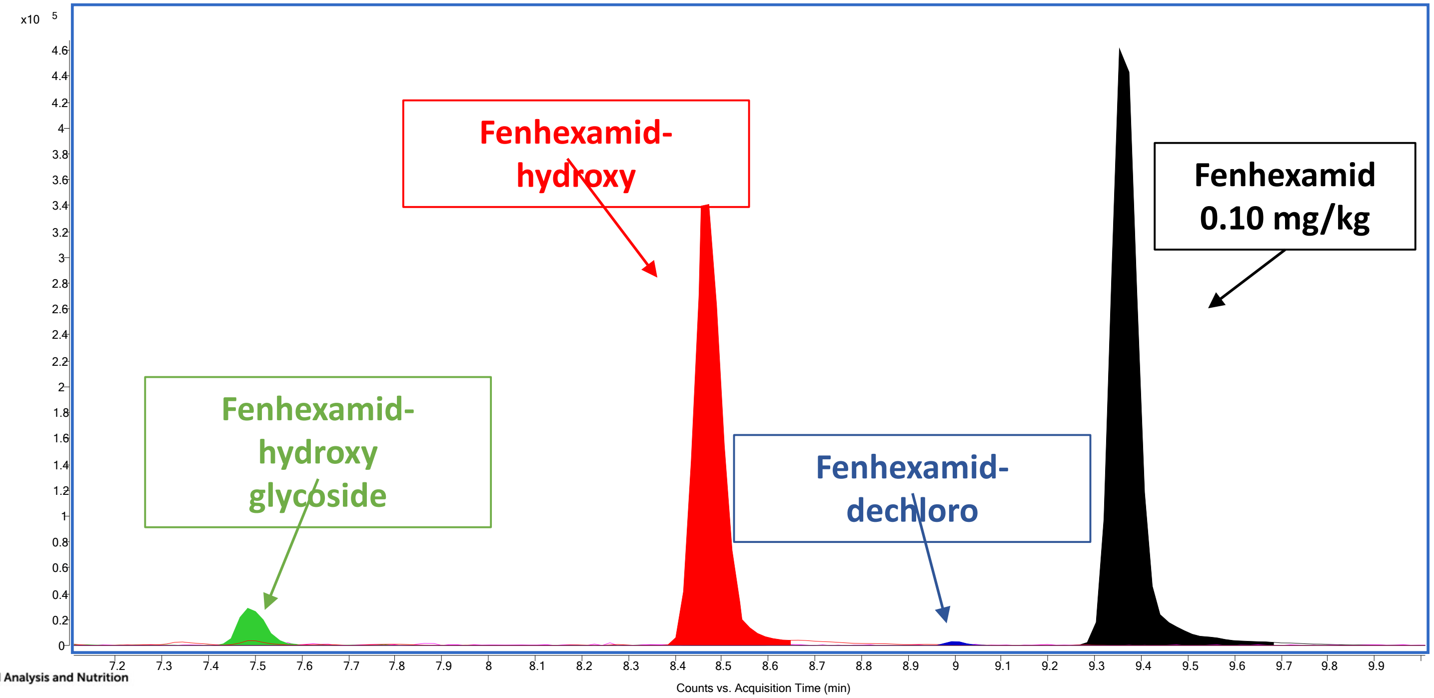
Spiroxamine + metabolites → this does not comply with „BIO“ MLR ▶

2nd step X



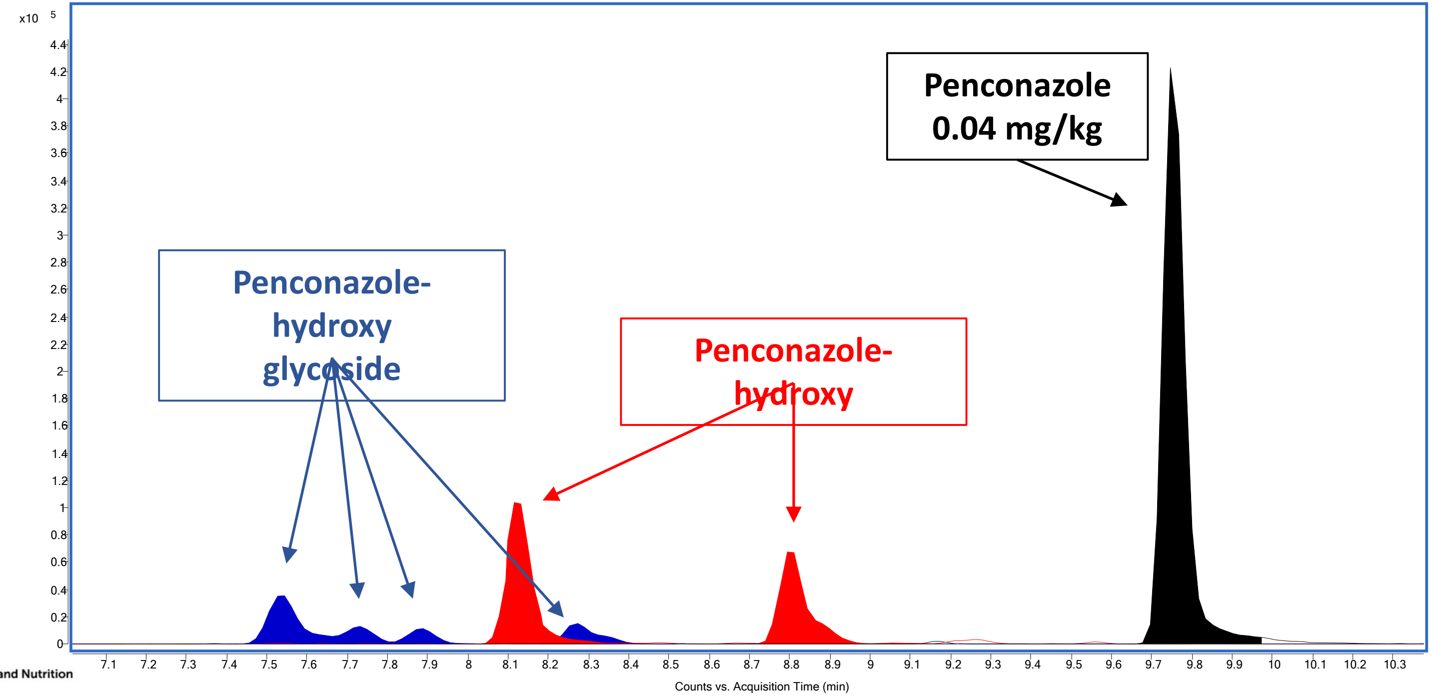
Metabolites of fenhexamid in grapes

- EIC Fenhexamid (*m/z* 302.0709) and metabolites Fen-OH (*m/z* 318.0658), Fen-OH-glycoside (*m/z* 480.1187) and Fen-dechloro (*m/z* 268.1099)



Metabolites of penconazole in grapes

- EIC Penconazole (*m/z* 284.0721), penconazole-hydroxy (*m/z* 300.0665) and penconazole-hydroxy glycoside (*m/z* 462.1176)



ACS award: article of the month

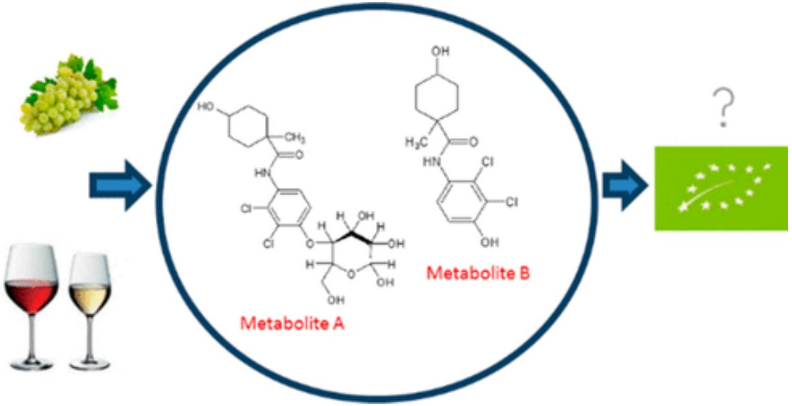
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

2019, 67, 22m
6102 - 6115

ACS Chemistry for Life®
AMERICAN CHEMICAL SOCIETY

Can Occurrence of Pesticide Metabolites Detected in Crops Provide the Evidence on Illegal Practices in Organic Farming?

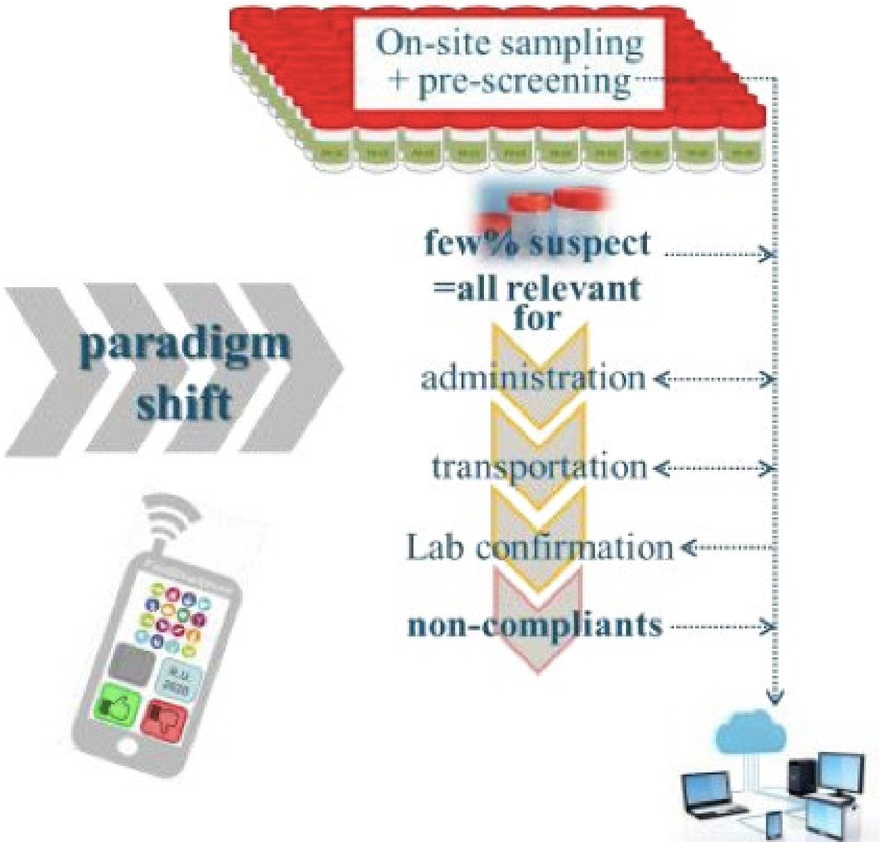
Schusterova D, Suchanova M, Pulkrabova J, Koourek V, Hajslova J



Curret challenge: screening tools bsed on smartphone

Dr. Tsagkaris Aristeidis <tsagkara@vscht.cz>

The FoodSmartphone vision



- Less paperwork
- Less transport
- Less storage
- More data

Can even citizens be involved??

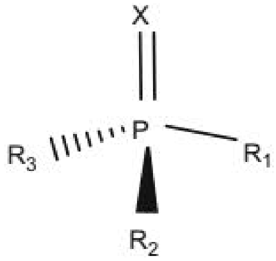
The Telegraph

World's cheapest smartphone, costing under £3, begins shipping next week

http://www.telegraph.co.uk/technology/2016/07/01/worlds-cheapest-smartphone-costing-under-3-

New approach based on an old principle

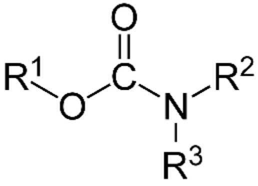
with smartphone readout



AChE strip assays

for pesticides detection

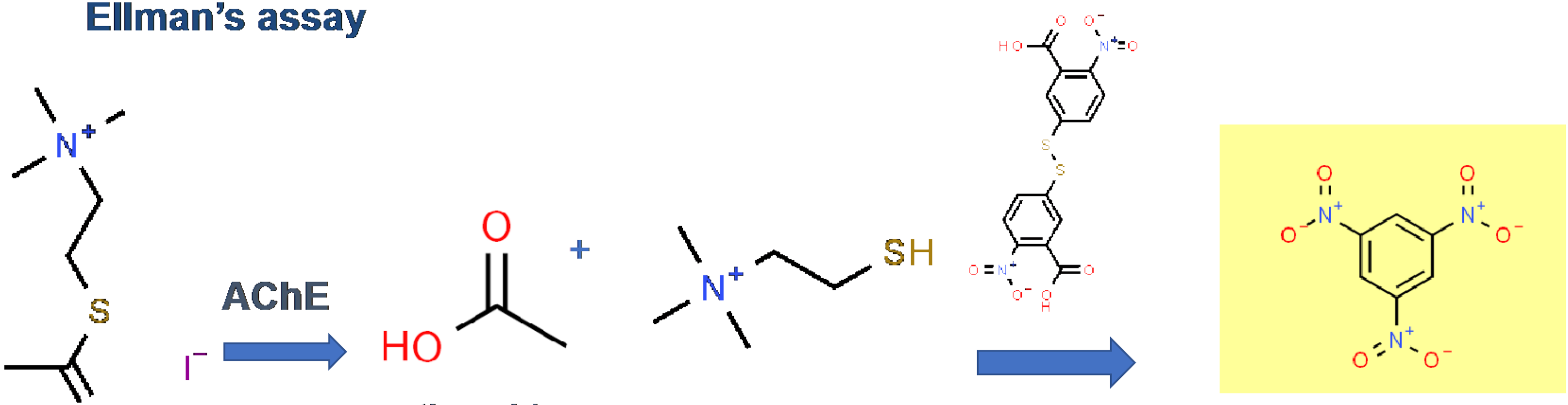
AChE



NEUROTOXIC

AChE assay principle

Ellman's assay



acetylthiocholine

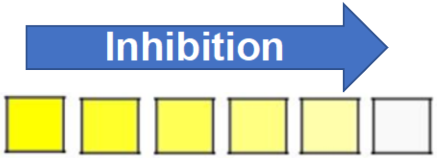
acetic acid

thiocholine

DTNB

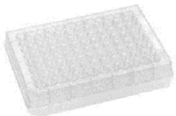
TNB

Active ChE No pesticide

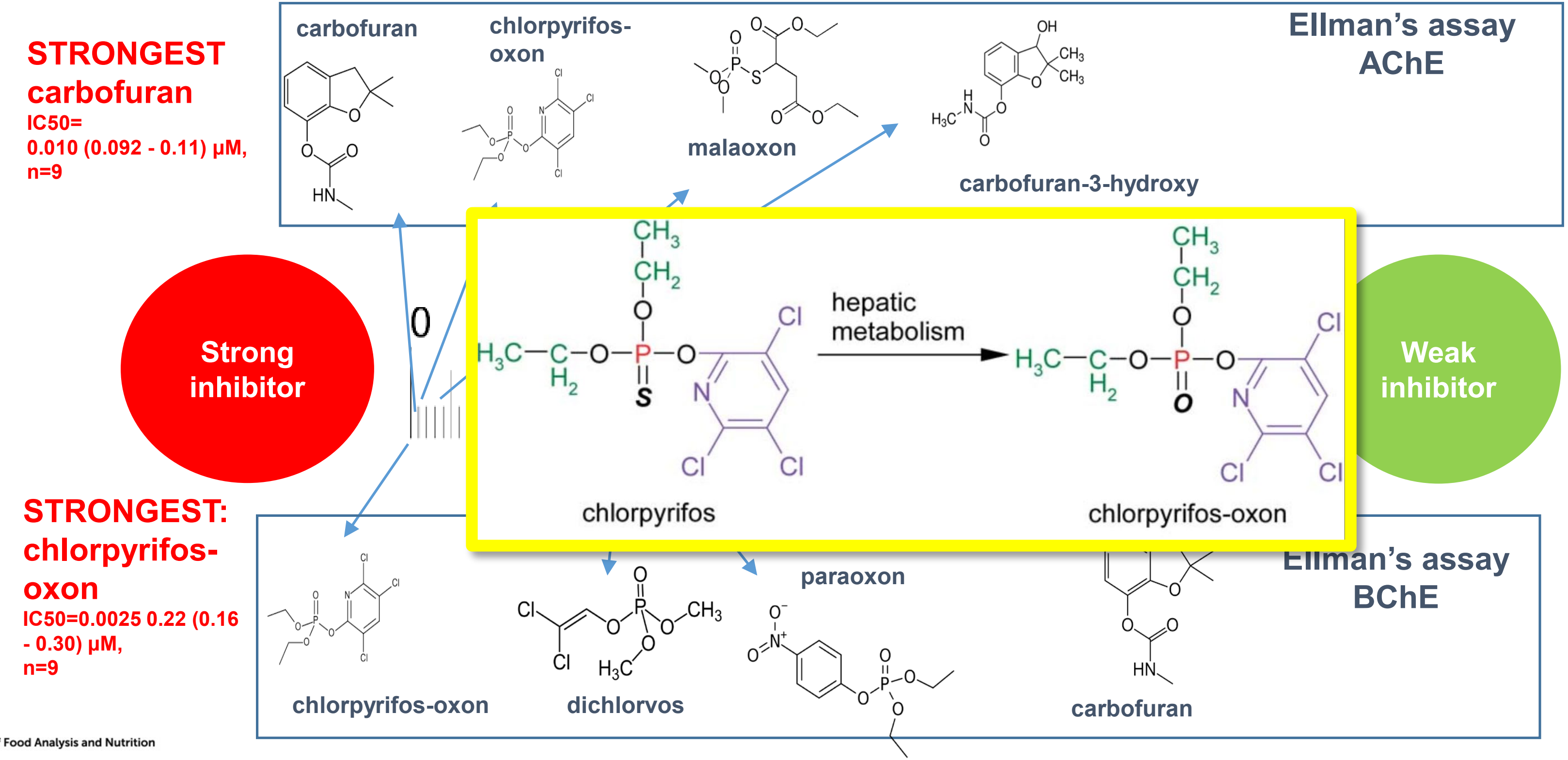


ChE inhibited by a pesticide

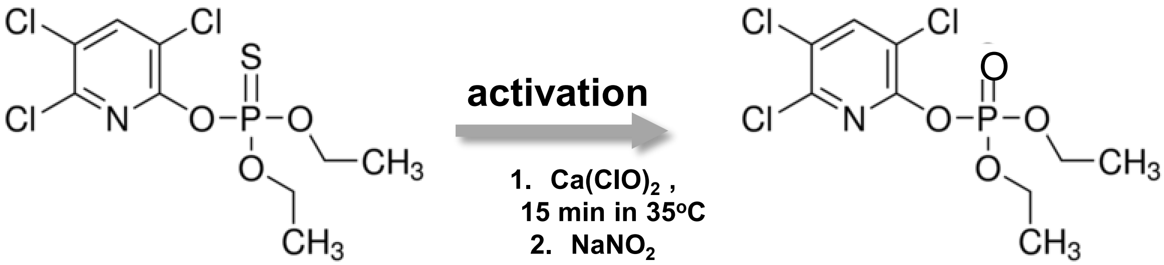
detection



Organophosphates and carbamates inhibition potency



P=S in-vitro activation

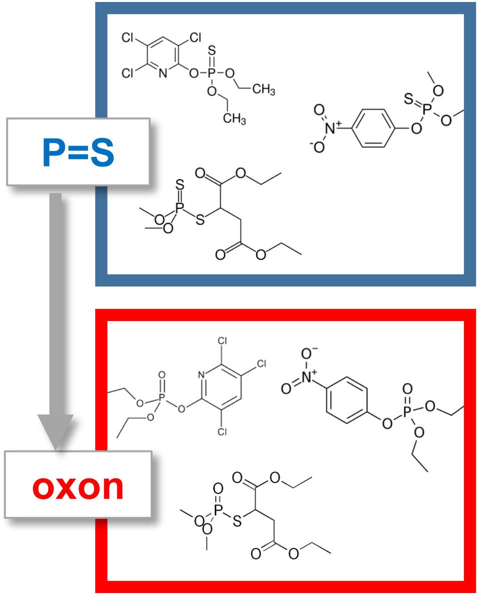


chlorpyrifos

chlorpyrifos-oxon

Activation is necessary to reveal the inhibitory potency !!!

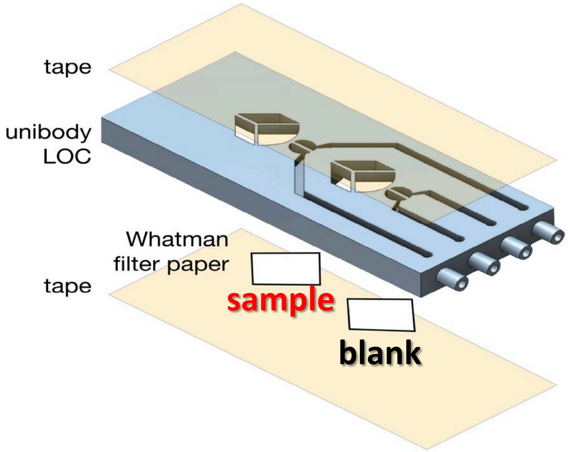
P=S/P=O in-vitro inhibitory potency

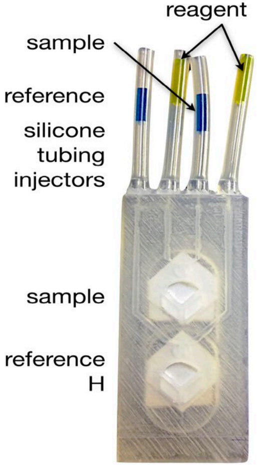


Compound	AChE IC_{50} (µM, n=9)	BChE IC_{50} (µM, n=9)
chlorpyrifos	420 (298 - 594)	**57** (41 - 79)
parathion	**294** (220 - 305)	2353 (2200 - 2450)
malathion	2356 (2157 - 2456)	**362** (295 - 386)
chlorpyrifos-oxon	0.093 (0.073 - 0.12)	0.0025 (0.0022 - 0.0030)
paraoxon	**0.10** (0.085 - 0.19)	4.3 (3.8 - 5.2)
malaoxon	13.73 (8.129 - 23.18)	**1.1** (0.54 - 2.3)

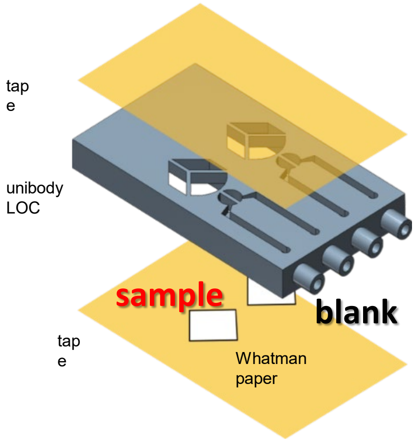
Paper-on-a-chip concept

Enzyme strips integrated to a 3D-printed chip



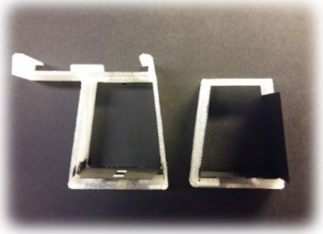


Last generation



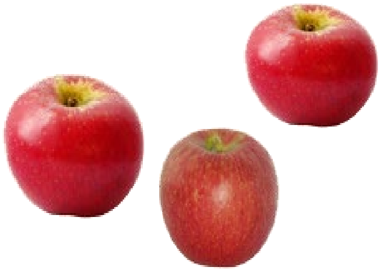
Ambient light elimination using a smartphone-reader

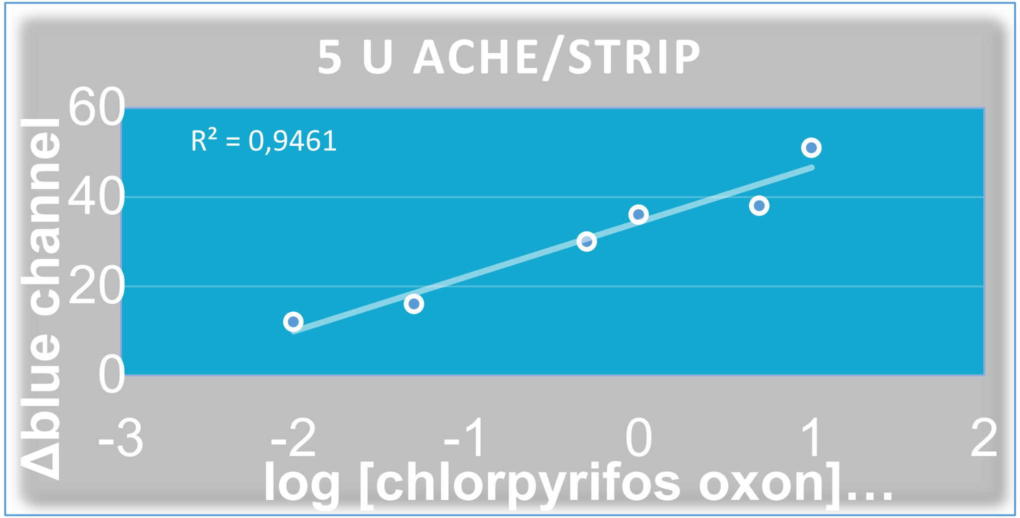
- Custom made 3D-printed coupler
- Elimination of any ambient light interference
- Camera flash constantly on to have a specific light source



Chlorpyrifos – oxon, calibration curve, apples

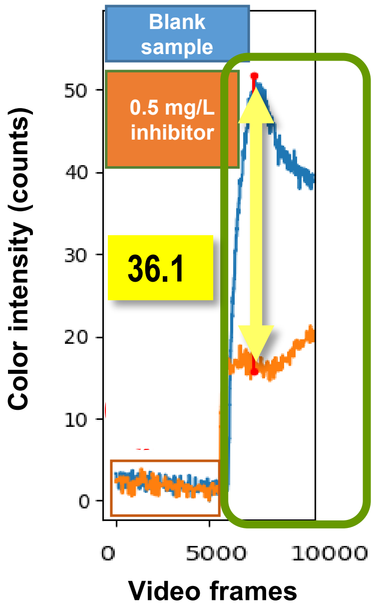
Sample preparation
1. QuEChERS extraction with d-SPE clean-up,
2. Evaporation under N2, reconstitution in PBS
3. Enzymatic paper-on-a-chip assay





0.01 - 10 mg/kg (n=6 calib. points)

Color intensity measurement using a smartphone



A video of the whole enzymatic reaction was recorded by smart phone app

- **Incubation with an inhibitor (NO color)**
- **Substrate addition (color development)**
- **Videos processed by in-house app**

Principle: the app finds the max color intensity of the blank sample and compares it with the tested sample

- **Numerical data enabling semi-quantitative results using calibration curve**

www.euchinasafe.eu

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