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FOOD CONTAMINANTS ANALYSIS IN CEREALS

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PROGRAMA DE INVESTIGACIÓN Y ANÁLISIS DE RESIDUOS Y CONTAMINANTES QUÍMICOS

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INTRODUCTION

- FOOD-SOURCE FOR MAN AND ANIMALS
- TRADED ON THE INTERNATIONAL MARKET
- QUANTITIES CONSUMED

CONSUMER HEALTH

AGRI-FOOD ECONOMY

CHEMICAL CONTAMINATION

CEREALS

Wheat – Rice - Maize - Other “coarse grains” (barley, oats, rye, etc.).

World’s grain production in 2015/16 was in the order of 2.4 thousand million tons (US Department of Agriculture, 2016).

Where produced cereals are consumed???

a substantial amount (370 million tons, 2015/16, US Department of Agriculture, 2016) is exported and traded internationally

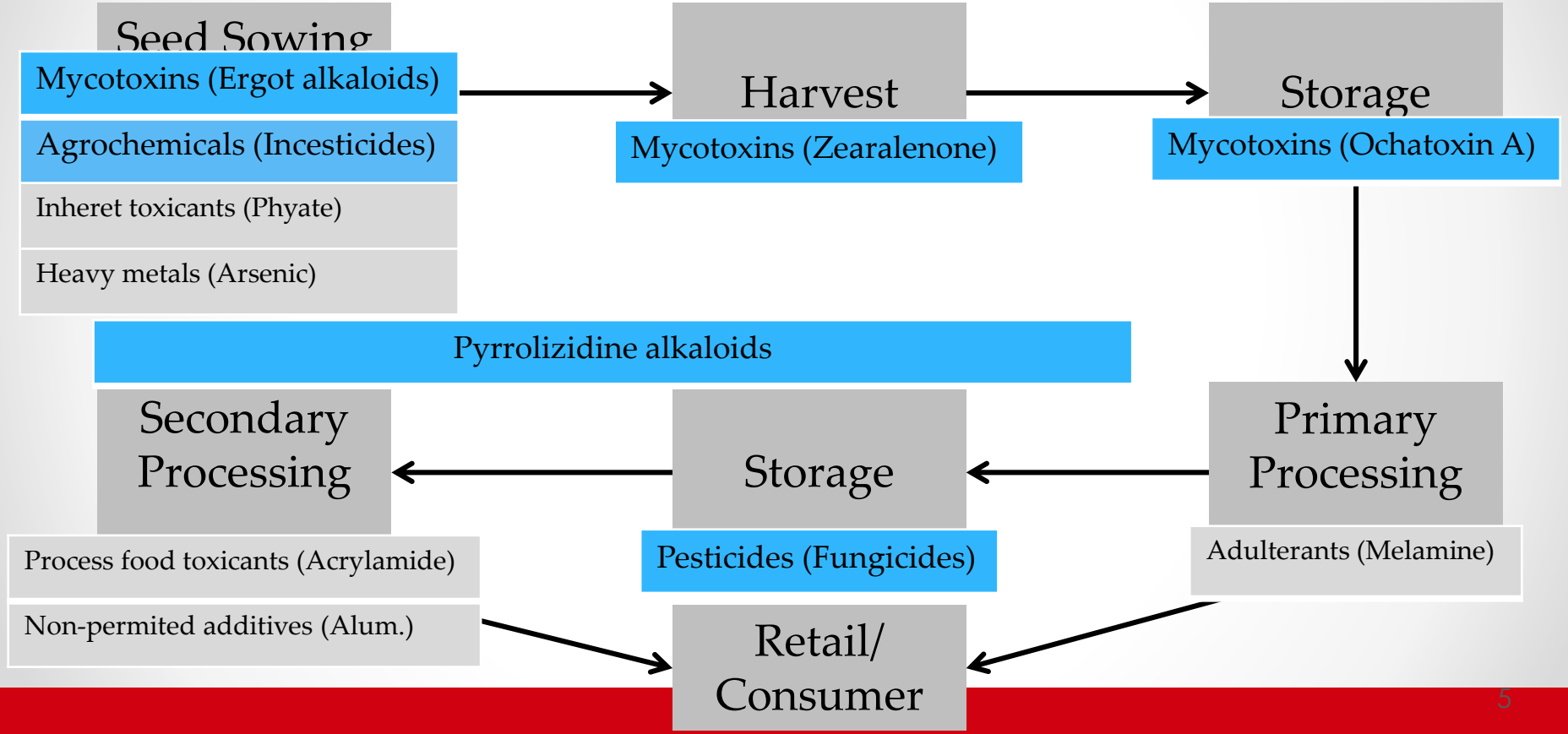
Argentinean scenario overview

ARGENTINA

- 32 Mha sown
- 111 Mton production
- Soy, maize, sunflower, wheat, & other.

(2016/17)

GRAIN-BASED FOOD FROM FIELD TO CONSUMER



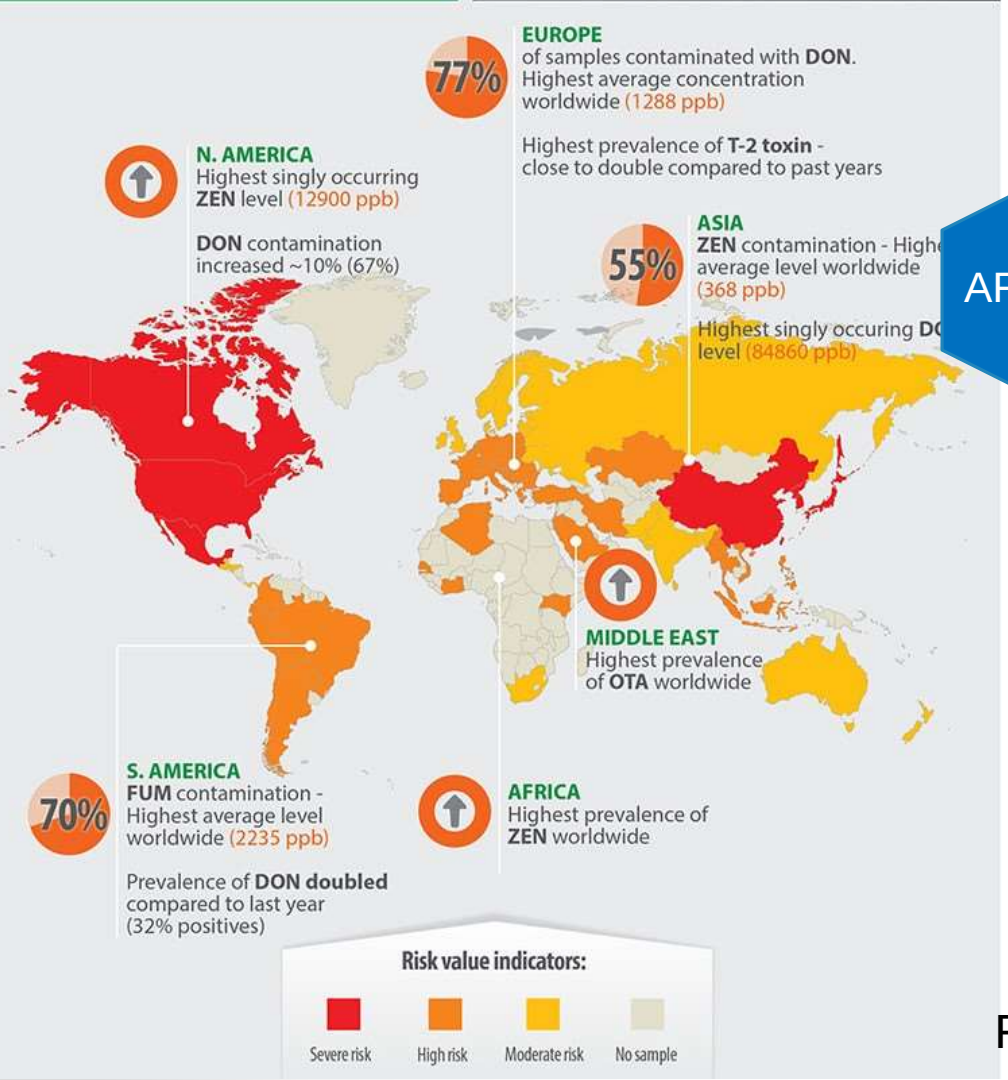
Mycotoxins

Secondary metabolites produced by fungi.

humidity – temperature – organic materials → cereals

Mycotoxins:

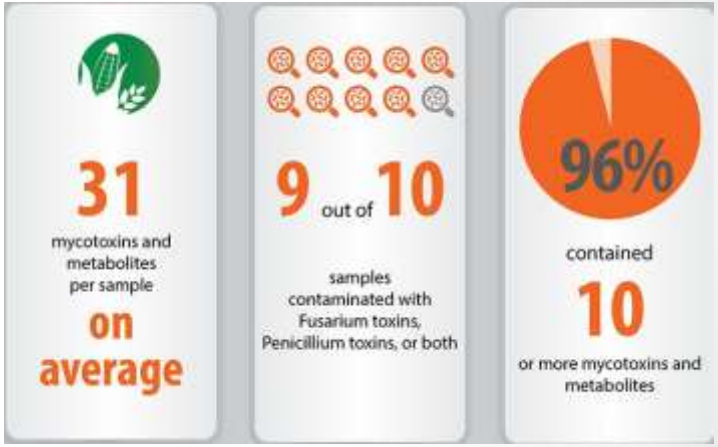
- can be very stable during food processing, and can be found in final products.
- fungi belong to the *Fusarium genus*: can infect cereals either on the field or during storage.
- fungi, belonging to *Aspergillus* and *Penicillium* genus, do not infect cereals before harvest, their reproduction and mycotoxin production is the consequence of inappropriate storage.



Muestras 8271

Países 75

AFLA | ZEA | DON | T-2 | FUM | OTA



Aumento en la prevalencia de FUM y DON

Fuente: 2015 Mycotoxin survey. BIOMIN

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Modified mycotoxins (mascarede)

- These modified forms of mycotoxins can be produced by fungi or generated as part of the defense mechanism of the infected plant.

- Modified mycotoxins are formed by:
  - reactions during processing,
  - by microorganisms, or
  - by the metabolism of plants.

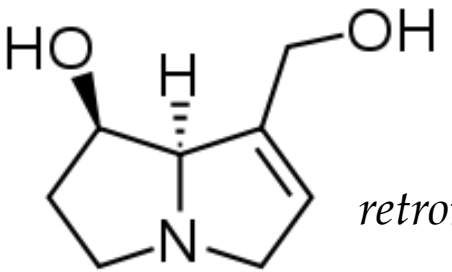
  - Conjugadas
  - Derivadas
  - Unidas covalentemente

INTRODUCTION

	In the World:	In Argentina:
PESTICIDES	❑ >900 a.i.	❑ 447 a.i. registred ❑ MRLs for 427 a.i.
MYCOTOXINS	❑ >300	❑ MLs only for Aflatoxins

INTRODUCTION

pyrrolizidine alcaloids (PAs)



retronesine

Are produced by plants as a defense mechanism against insect herbivores

It has been estimated that 3% of the world's flowering plants contain PAs

Risk for human health related to the presence of PAs in honey, tea, herbal infusions and food supplements

https://www.efsa.europa.eu/en/efsajournal/pub/4908

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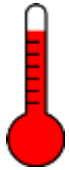
SAMPLES DIFFICULTY

CEREAL BASE

- ✓ DIFFERENT BASIC COMPOSITION

CEREAL	LIPIDS	STARCH	BRUT FIBER	SOLUB. FIBER	WATER	PROTEINS
OAT	5.7	42.5	1.56	22.8	13	12.6
WHEAT	2.2	59.2	2.0	10.1	13.2	11.7
MAIZE	3.8	62.6	2.2	8.4	12.5	9.2
RICE	2.4	70.4	0.7	5	13.1	7.4

- ✓ DIFFERENT INDUSTRIAL PROCESSINGS



- ✓ DIFFERENT ADDED INGREDIENTS

- ✓ DIFFERENT FINAL PRODUCTS

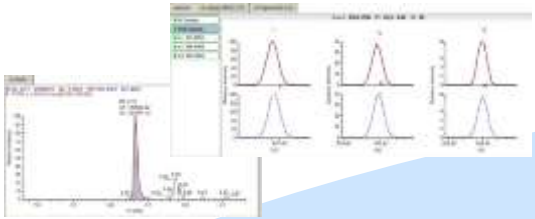
+ . . .

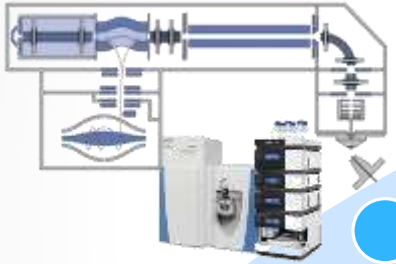
- ✓ Strict regulations
- ✓ Low MRLs
- ✓ Low LOD needed

ANALYTICAL METHODOLOGIES

ANALYTICAL METHODOLOGY

FULLY OPTIMIZED & VALIDATED METHOD
[Dzuman et al. Anal.Chim.Acta 863 (2015) 29-40]





DATA EVALUATION

323 PESTICIDES

55 MYCOTOXINS

11 PYRROL. ALKALOIDS

INSTRUMENTAL ANALYSIS

- UHPLC: Dionex UltraMate® 3000.
- Accucore™ aQ; 150x2.1 mm i.d., 2.6µm).
- HRMS/MS: Thermo Q-Exactive™
- Heated electrospray ionization (HESI-II)
- Operation mode: Full MS-dd-MS/MS

QuEChERS Extraction

- 2 g sample
- No clean-up

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QuEChERS Extraction

EXTRACCIÓN

2 g sample

10 mL H_2O w/2% formic acid — hydration 30 min

10 mL MeCN — mechanic shaking 30 min

4 g $MgSO_4$ anh. + 1 g NaCl — vig. manual shaking 1 min

Centrifugation 5 min 10,000 rpm

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CLEAN UP

2 mL ext. MeCN

300mg $MgSO_4$ + 150mg PSA + 100 mg C18

1 min agitation

5 min centrifugation

LC-HRMS/MS

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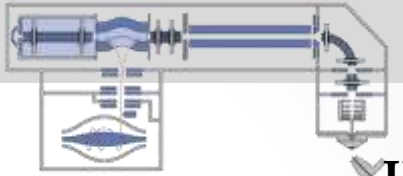

INSTRUMENTAL ANALYSIS

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Quality assurance

1. VALIDATION
   - ✓ Pesticides
   - ✓ Mycotoxins:
   - ✓ PAs

Commission Regulation 2006/401/EC

Commission Decision 2002/657/EC (criteria for LC-MS/MS, but only for feed and animal products)

2. Proficiency Tests
3. Official CEN methods
4. Accreditation according to ISO 17025

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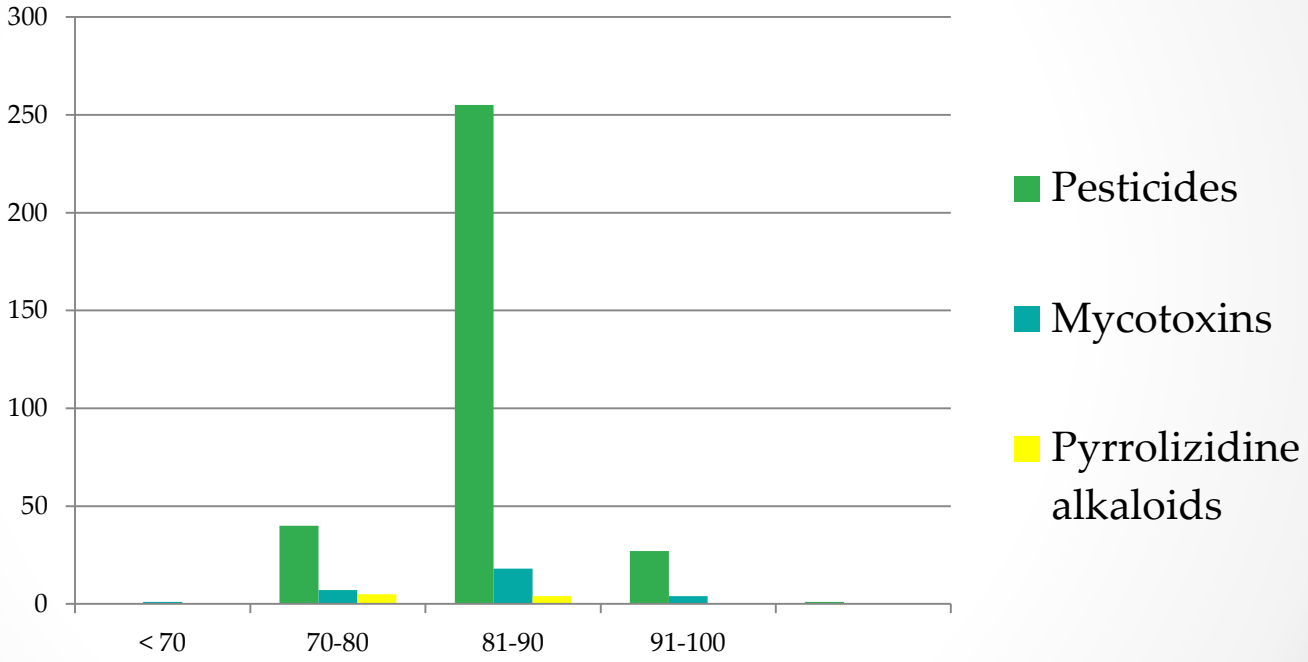
METHODOLOGY PERFORMANCE

RECOVERIES



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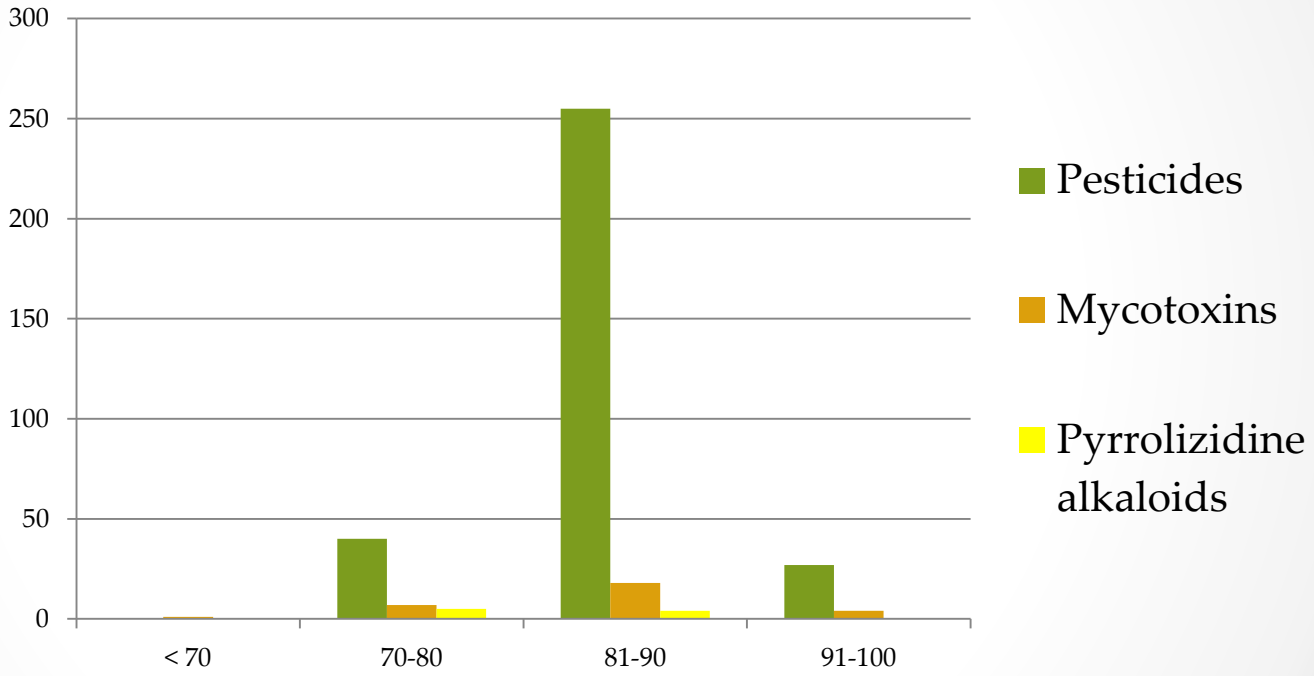
METHODOLOGY PERFORMANCE

LOQs



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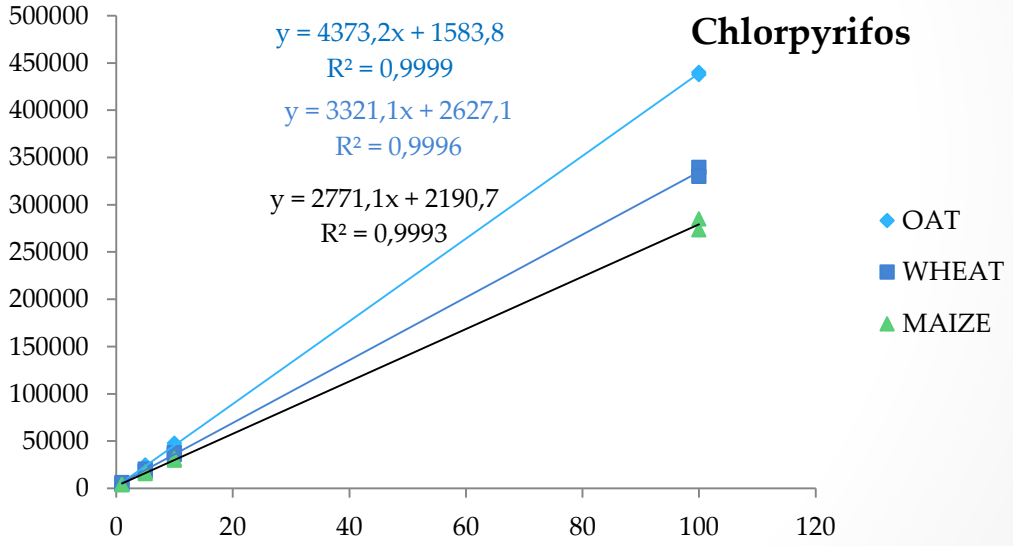
METHODOLOGY PERFORMANCE

CALIBRATION CURVES



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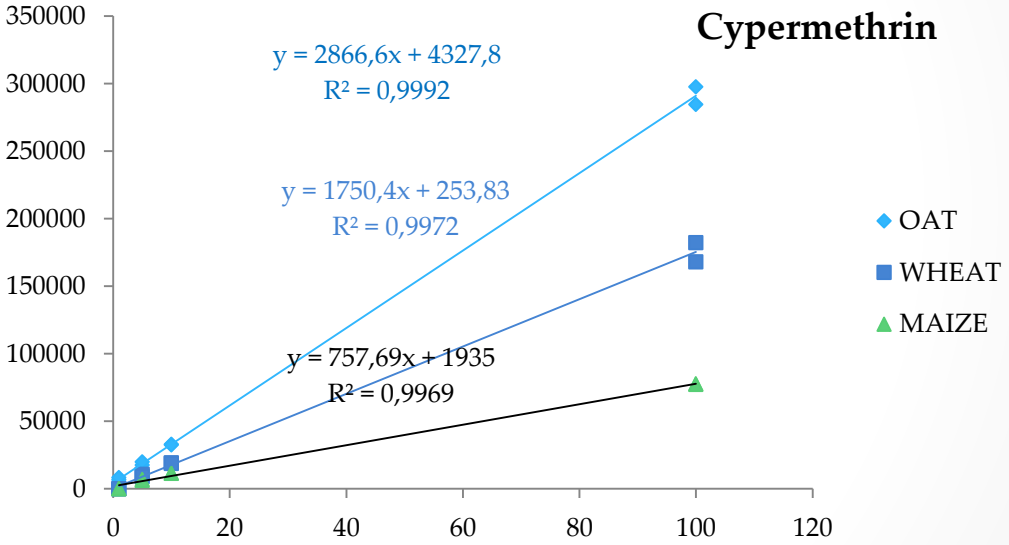
METHODOLOGY PERFORMANCE

CALIBRATION CURVES



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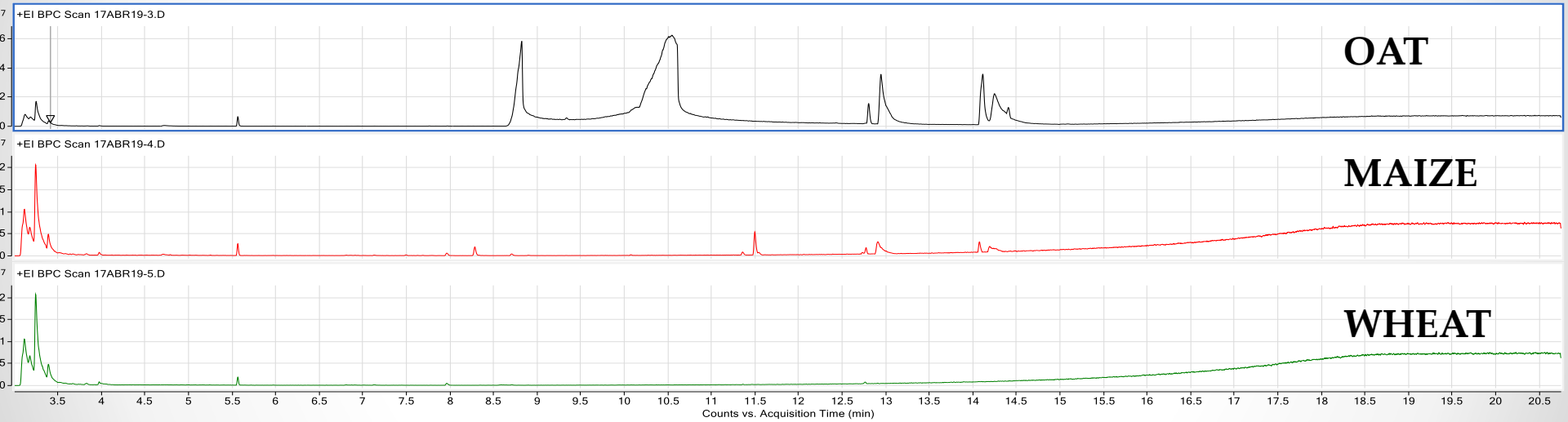
METHODOLOGY PERFORMANCE

GC – MS/MS
Full Scan - MATRIX



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ANALYZED SAMPLES

17 baby and infant cereal-based foods:

- ✓ 8 baby and infant cereal-based food from 6 or 8 months.

- ✓ 2 infant cereal-based food from 1 years old.

- ✓ 7 infant cereal-based food.

- ✓ 1 organic rice.

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ANALYZED SAMPLES

Pesticides, mycotoxins and PAs by QuEChERS – LC – HRMS/MS

Short Oral Presentation 3

Wide-Scope Pesticide Residues and Contaminants in Cereal-Based Infant Formulas

Nicolás Michlig, PRINARC – FIQ/UNL – Argentina

May 6th, 2019 (MONDAY)

12:05 - 12:20

Samples

- 2017_03_06_MARIA_POS
  - STD_MTX_MYC_0,2ppb_A
  - STD_MTX_MYC_5ppb_A
  - STD_MTX_MYC_30ppb_A
  - STD_MTX_MYC_200ppb_A
  - STD_MTX_PEST_2ppb_A
  - STD_MTX_PEST_50ppb_B
  - STD_MTX_PEST_100ppb_A
  - BLANK_MYCOT
  - BLANK_PEST
  - BF01_2017
  - BF02_2017
  - BF03_2017
  - BF04_2017

Compounds

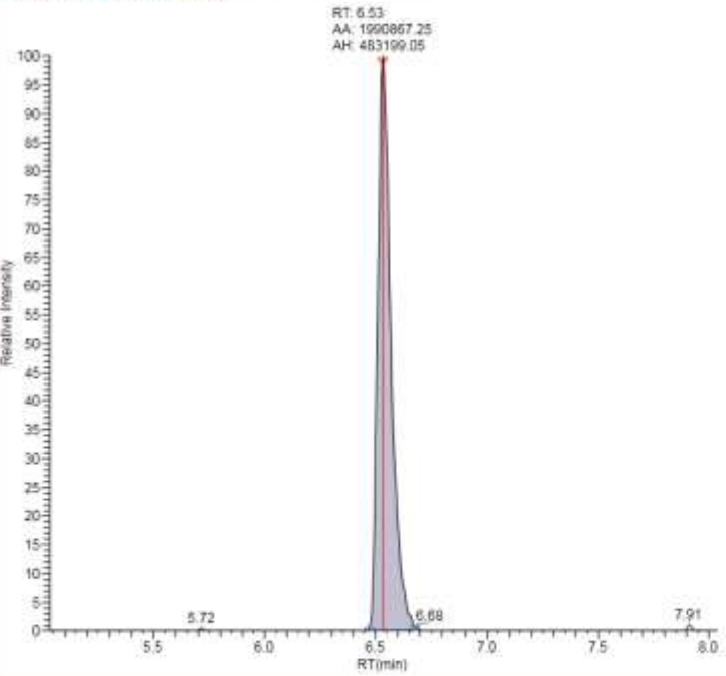
1 10_4_2016_multimethod-new-spice_pos

	Flag	MZ	RT	IP	FI	LS	Confirmed	RT (Expected)	RT (Measured)	RT (Delta)	Compound Name	Formula	Adduct	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))	Measured Area
							≥ 1										
1							3 out of 3	3.91	4.04	0.13	DEET	C12H17NO	M+H+	192.1383	192.1381	-0.8441	3.2754E06
2							3 out of 3	8.06	8.32	0.26	enniatin B	C33H57N3O9	M+NH4+	657.4433	657.4412	-3.2518	7.8099E05
3							3 out of 3	2.08	2.10	0.02	penicillic acid	C8H10O4	M+H+	171.0652	171.065	-1.2441	5.7868E05
4							3 out of 3	6.49	6.53	0.04	pirimiphos-methyl	C11H20N3O3PS	M+H+	306.1036	306.1031	-1.7711	[illegible]
5							3 out of 3	8.54	8.50	-0.04	pyrethrin I	C21H28O3	M+H+	329.2111	329.2086	-7.6314	3.1883E05
6							2 out of 3	2.22	2.27	0.05	2-NOA	C12H10O3	M+NH4+	220.0968	220.0969	0.3546	2.6365E06

Chromatogram

M+H+

BF02_2017 pirimiphos-methyl NL: 4.84E5 m/z: 306.1005 - 306.1067
F: FTMS + p Full ms [80.00-1200.00]



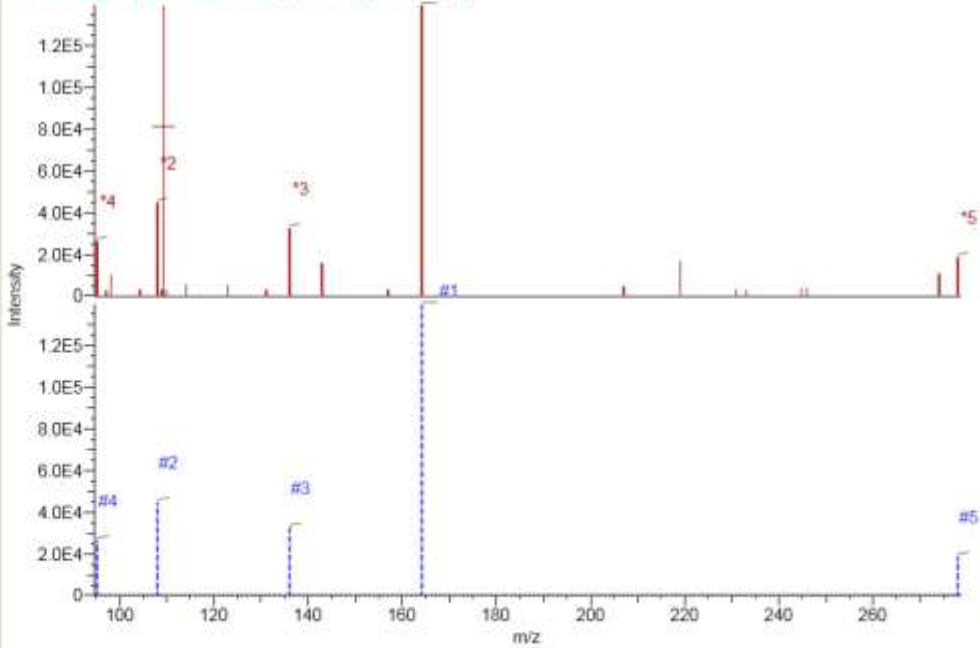
Spectrum

Spectrum | Isotopes 91% (3 of 4) | Fragments (5 of 5)

- All Fragments
- #1: 164.11820
- #2: 108.05560
- #3: 136.08690
- #4: 95.06040
- #5: 279.07230

Minimum # of fragments needed: 1

BF02_2017 #: 1210 RT: 6.54
F: FTMS + p ESI d Full ms2 306.10@hcd40.00 [50.00-330.00]



ANALYZED SAMPLES

Pesticides, mycotoxins and Pas by QuEChERS – LC – HRMS/MS

- ✓ Clorpyrifos, pirimiphos-methyl, metholcarb insecticides
- ✓ Tebuconazole fungicide showed major occurrence.
- ✓ Zearalenone and fumonisins were mycotoxins mostly found.
- ✓ Unexpectable emerging toxins (ergot alkaloids and ennantins) were also found.

METHODOLOGY PERFORMANCE - RESULTS

PYRETROIDS

multi-component

QuEChERS

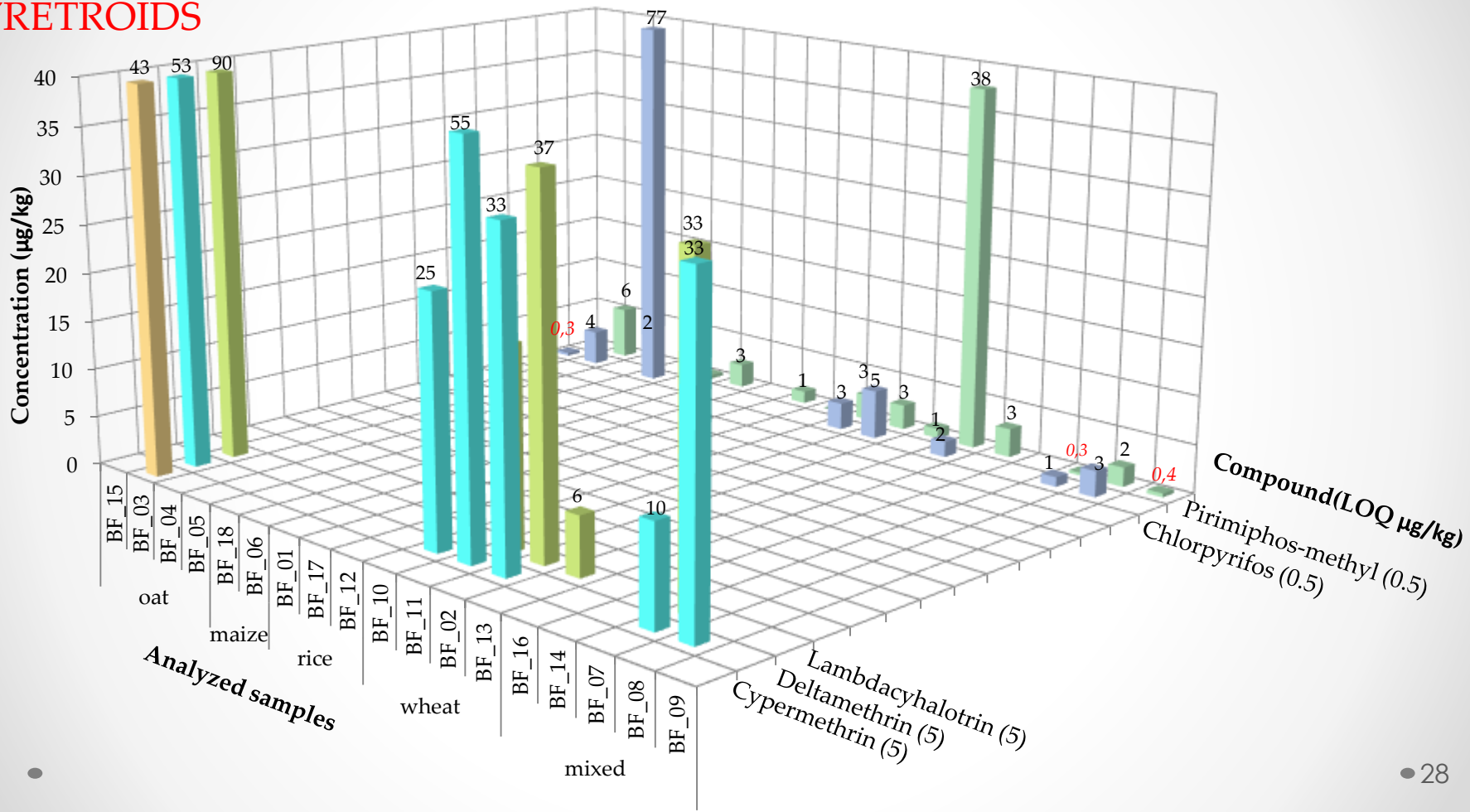
- ✓ 2 g sample
- ✓ 10 mL H_2O → 30 min hydration
- ✓ 10 mL MeCN → 30 min extraction
- ✓ 4g $MgSO_4$, 1g NaCl → 1 min partition

Extract (MeCN)

Pesticides, GC-MS/MS
Agilent 7890
Agilent 7000C
160 compounds

METHODOLOGY PERFORMANCE - RESULTS

PYRETROIDS



CONCLUSIONS

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- ✓ In Argentina there is scarce information about pesticide and mycotoxin contamination in cereal and cereal base food.
- ✓ Only the most common compounds are controlled.
- ✓ The use of multi-component and wide scope analytical techniques to assess the quality of cereal based food is an innovative contribution to study the current Argentinean situation.
- ✓ Presence of multi-contaminants in one same sample was frequent.

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- ✓ The HRMS/MS availability of multiple confirmatory steps (accurate mass, isotopic profile and MS/MS fragmentation spectra) significantly improved the confidence in the results.

- ✓ Except for the organic rice sample, pesticide residues and mycotoxins were found in 100% of the commercial samples, verifying multi-incurrence in 95% of samples.

- ✓ Clorpyrifos, pirimiphos-methyl, metholcarb insecticides and tebuconazole fungicide showed major occurrence. Zearalenone and fumonisins were mycotoxins mostly found. Unexpectable emerging toxins (ergot alkaloids and ennantins) were also found.

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