Development and validation of a UHPLC-ToFMS and UHPLC-MS/MS based approach for screening pesticide residues in fruit and vegetables

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http://www.fera.defra.gov.uk

Outline of Presentation

- Background and Objectives
- Method development and results
- Conclusions and future work

Background

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- Objective: to Increase the scope of the UK monitoring programme
- Currently based on targeted pesticide analysis to answers the question:

  which pesticides from a predefined list are present in the sample at or above a specified concentration?

- Only detects pesticides in the 'predefined list'
- Other residues present will not be detected!

Consumer expectations

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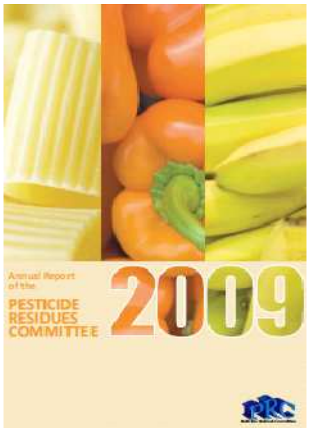
Annual Report of the PESTICIDE RESIDUES COMMITTEE 2009

http://www.pesticides.gov.uk/prc_home.asp

The more demanding question?

- Are there **any** pesticides in the samples and if so, what are the identities and concentrations?
- MS acquisition across the full mass range can increase the scope of analytes
- Possibility for retrospective screening
- But Is LC-ToF is a realistic and practical option for monitoring?

Project to validate LC-MS/MS LC-ToF based methods for ~350 pesticides in grapes and lettuce

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ToF MS– Initial questions

- Mass resolution and mass accuracy
- Acquisition rate *(speed)?*
- Dynamic linear range *(repeat analysis/drop-out)?*
- Sensitivity *(final solvent/detection rate)?*
- Quantification *(target limits for precision)?*
- Identification of analytes?
- Software provides automated peak detection?
- Management of large volumes of data?
- Reliability of instrumentation?

ToFMS Instrumentation

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- Agilent 6230 ToFMS Instrumentation
- 1200 series LC
- Jet stream source
- Acquisition setting 1.41
- mass accuracy typically < 2 ppm (lockmass))

- Mass resolution

  ~11,000 at *m/z* 118 .086285

  ~14,000 at *m/z* 322.0483
- Dynamic range 5 orders
- Mass Hunter software

  - *automatic peak detection and assignment*

Experimental – first steps

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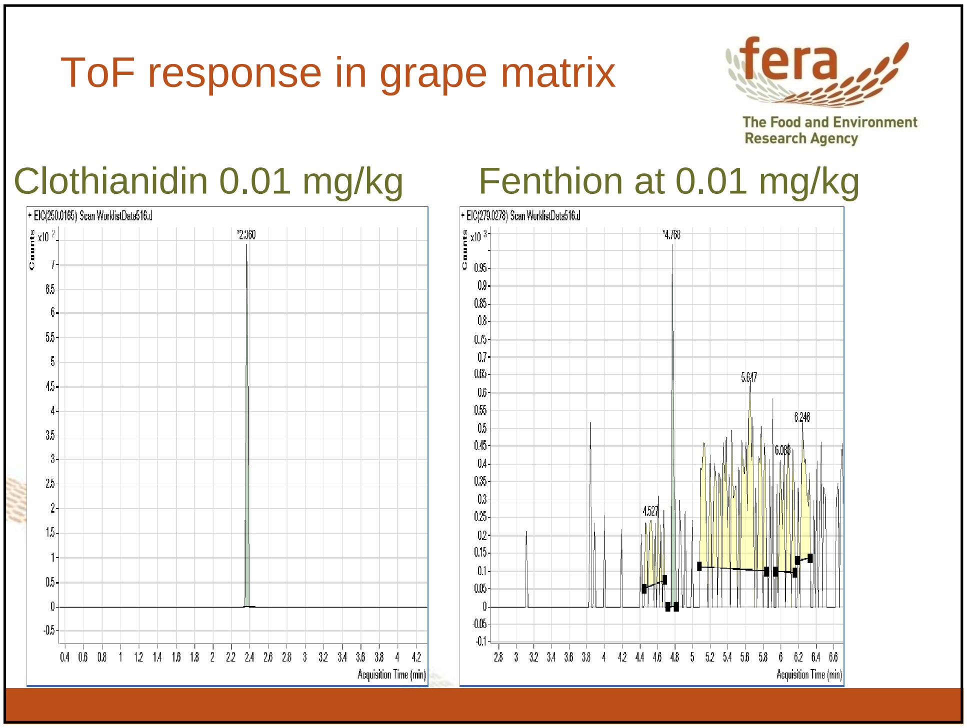
- Optimise chromatography an detection parameters
- Inject standards to get information on retention time and response
- Build data base
- Or use manufacturer conditions/database
- Select a ‘generic extraction’ method



ToF response in grape matrix

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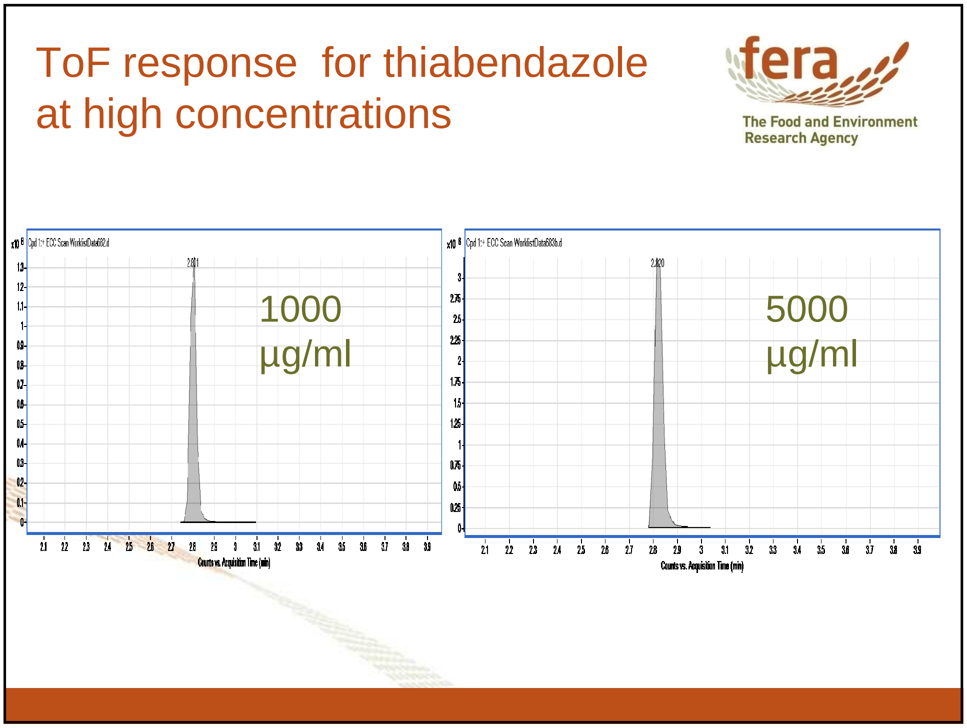
Clothianidin 0.01 mg/kg

Fenthion at 0.01 mg/kg



ToF response for thiabendazole at high concentrations

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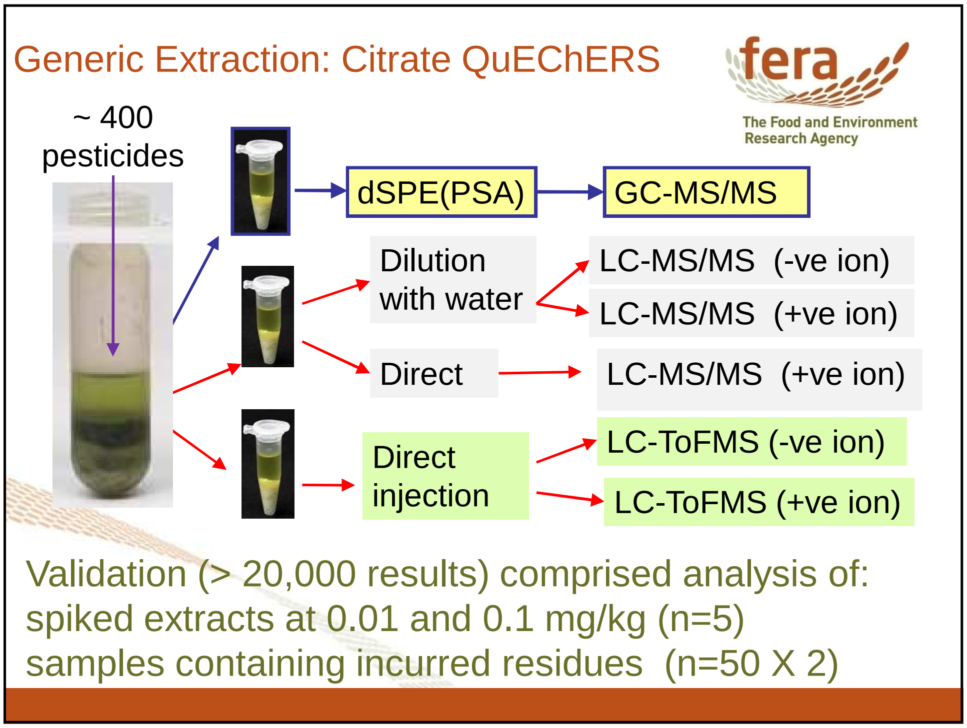
1000 µg/ml

5000 µg/ml



Generic Extraction: Citrate QuEChERS

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~ 400 pesticides

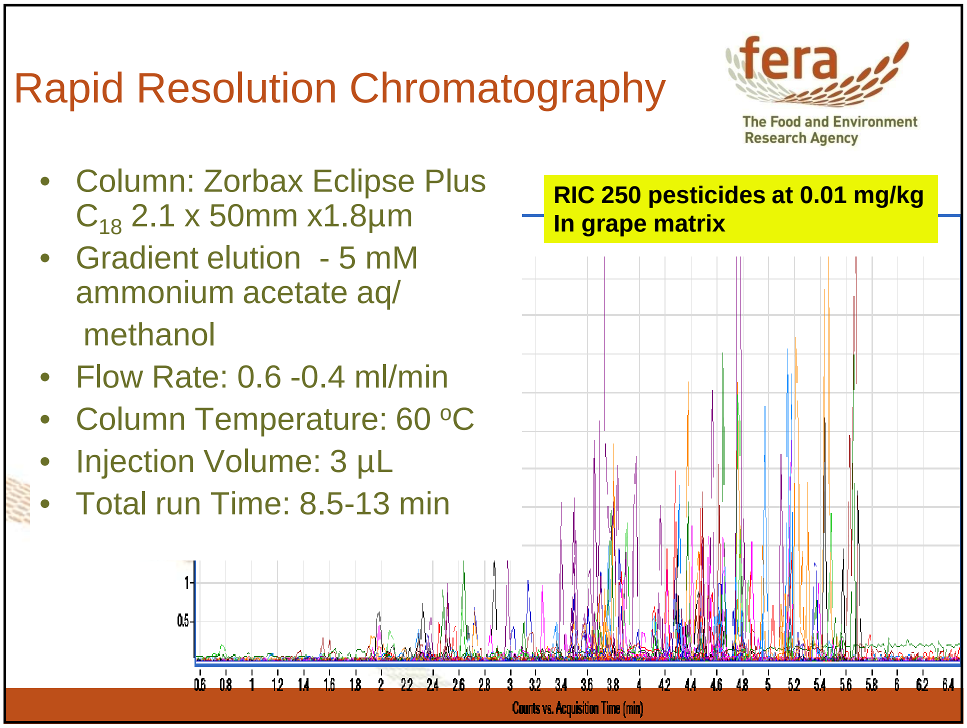
dSPE(PSA) → GC-MS/MS

Dilution with water → LC-MS/MS (-ve ion)
LC-MS/MS (+ve ion)

Direct → LC-MS/MS (+ve ion)

Direct injection → LC-ToFMS (-ve ion)
LC-ToFMS (+ve ion)

Validation (> 20,000 results) comprised analysis of:
spiked extracts at 0.01 and 0.1 mg/kg (n=5)
samples containing incurred residues (n=50 X 2)



Rapid Resolution Chromatography

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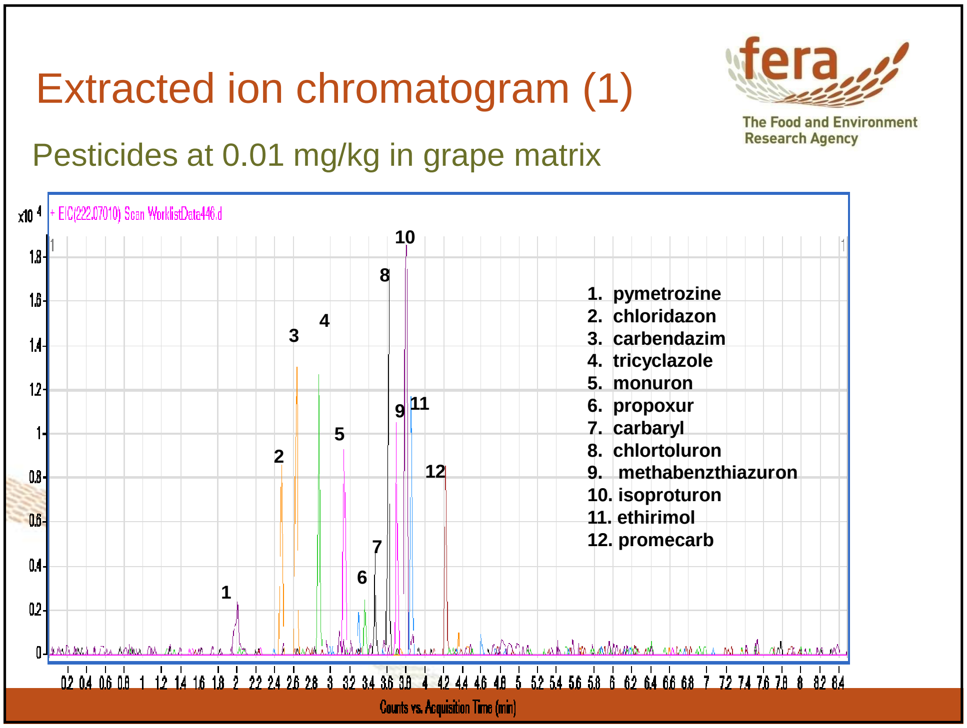
- Column: Zorbax Eclipse Plus C_{18} 2.1 x 50mm x1.8µm
- Gradient elution - 5 mM ammonium acetate aq/ methanol
- Flow Rate: 0.6 -0.4 ml/min
- Column Temperature: 60 ^{o}C
- Injection Volume: 3 µL
- Total run Time: 8.5-13 min

RIC 250 pesticides at 0.01 mg/kg
In grape matrix



Extracted ion chromatogram (1)

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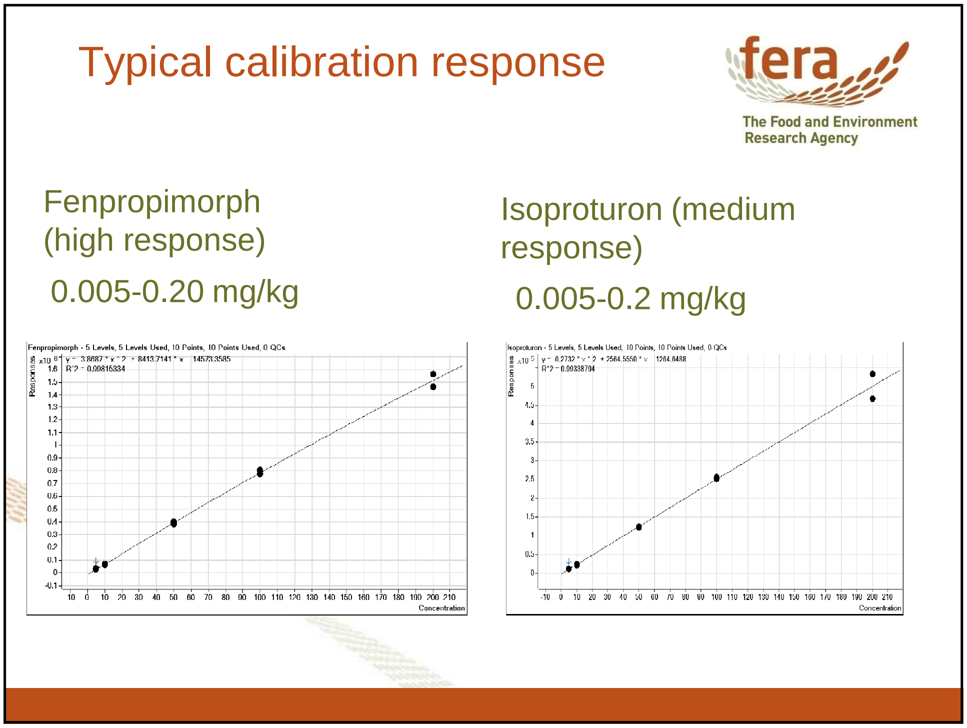
Pesticides at 0.01 mg/kg in grape matrix

1. pymetrozine
2. chloridazon
3. carbendazim
4. tricyclazole
5. monuron
6. propoxur
7. carbaryl
8. chlortoluron
9. methabenzthiazuron
10. isoproturon
11. ethirimol
12. promecarb



Typical calibration response

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Fenpropimorph (high response)

0.005-0.20 mg/kg

Isoproturon (medium response)

0.005-0.2 mg/kg

Fenpropimorph - 5 Levels, 5 Levels Used, 10 Points, 10 Points Used, 0 QCs

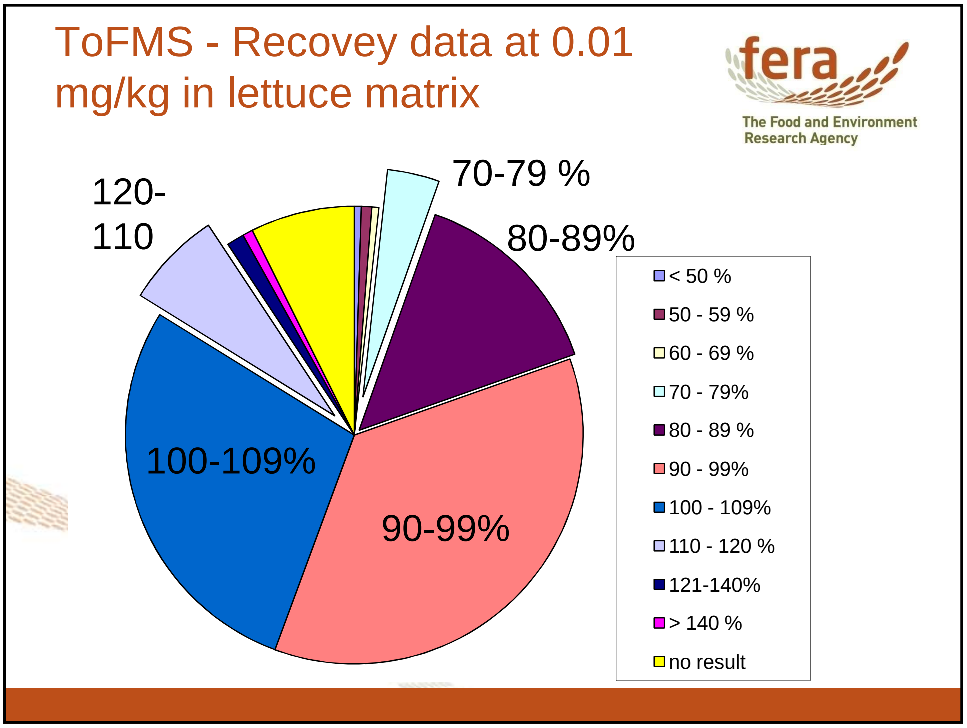
y = 3.8687 * x ^ 2 + 8413.7141 * x 14573.3585

R^2 = 0.99815334

Isoproturon - 5 Levels, 5 Levels Used, 10 Points, 10 Points Used, 0 QCs

y = 0.2732 * x ^ 2 + 2564.5550 * x 1264.6488

R^2 = 0.99338794



ToFMS - Recovey data at 0.01 mg/kg in lettuce matrix

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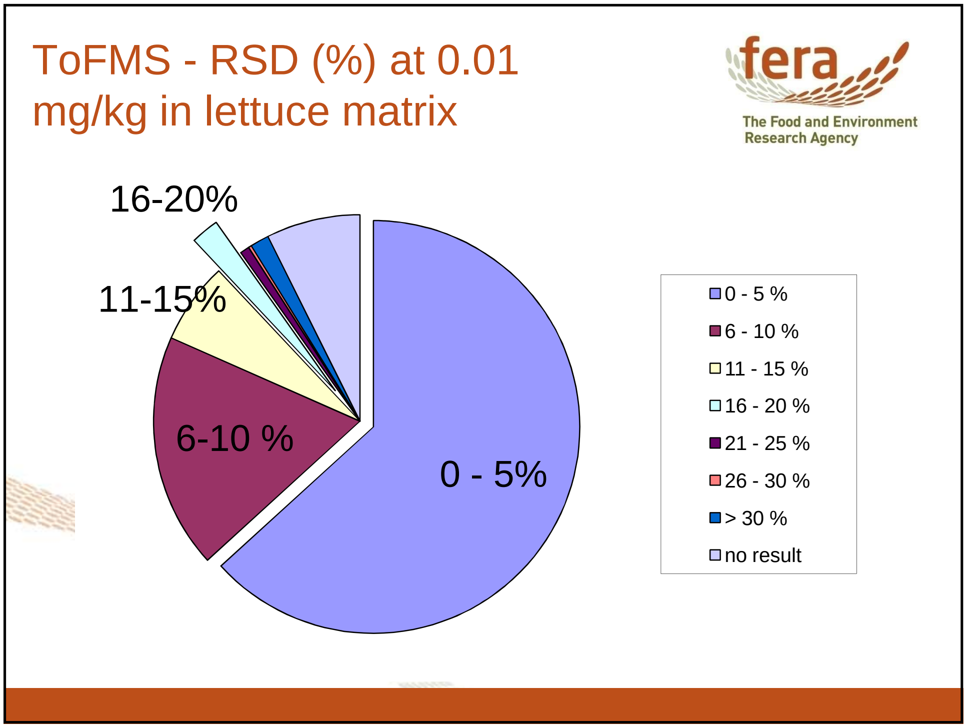

ToFMS - RSD (%) at 0.01 mg/kg in lettuce matrix

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Results Summary

commodity	No of pesticides	
	Validated at 0.01 mg/kg*	Screening at 0.01 mg/kg
Lettuce (ToF)	357 (88%)	378 (93%)
Lettuce (MS/MS)	374 (91%)	396 (96%)
Grape (ToF)	333 (81%)	375 (92%)
Grapes (MS/MS)	380 (92%)	398 (96%)

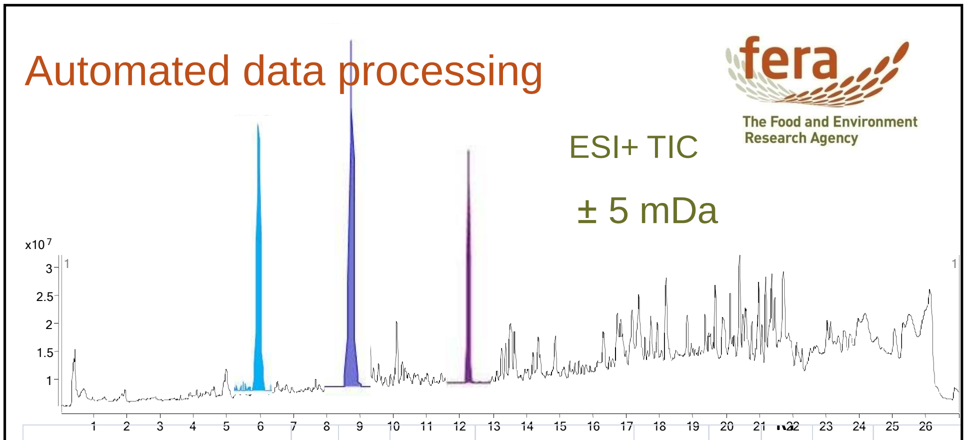
In accordance with *EU Method validation criteria

- Next step is to use the method for samples containing incurred residues

Automated data processing

ESI+ TIC

± 5 mDa



Label	Hits (DB)	Mass (DB)	Diff (DB, mDa)	Formula (DB)	Diff (DB, ppm)	RT (DB)	Diff (DB)	Score (DB)	Base Peak
Cpd 89: Ethiofencarb sulphoxide	2	241	-0.7	C11 H15 N O3 S	-2.89	4.8	-0.65	56.2	242.0853
Cpd 204: Terbufos sulphone	2	320	0.26	C9 H21 O4 P S3	0.8	11.6	0.01	91.94	321.041
Cpd 20: Butocarboxim sulfoxide	2	206	0.2	C7 H14 N2 O3 S	0.99	1.9	-0.4	60.5	207.0796
Cpd 172: Isoprocarb	2	193	-0.42	C11 H15 N O2	-2.18	10	-0.01	63.9	194.118
Cpd 148: Carbaryl	2	201	-0.44	C12 H11 N O2	-2.17	8.9	0.03	88.24	202.0867
Cpd 102: Triasulfuron	2	401	-0.71	C14 H16 Cl N5 O5 S	-1.78	6.2	-0.02	97.1	402.0641
Cpd 96: Picolinafen	1	376	1.02	C19 H12 N2 O2 F4	2.72	5.2	-0.52	41.13	377.0898
Cpd 93: Carbendazim	1	191	-0.5	C9 H9 N3 O2	-2.64	5.5	-0.13	88.25	192.0773

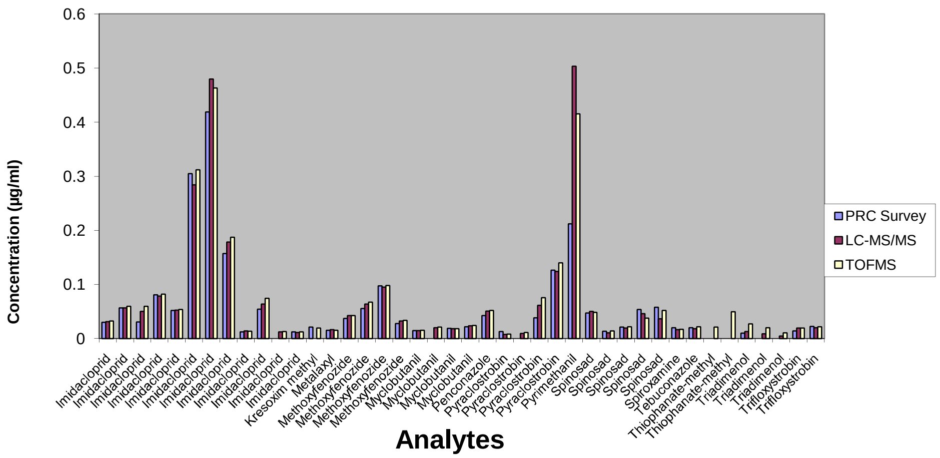
Analysis of samples containing incurred residues

Samples of lettuce (10), grapes (20) and Pears (20) containing 35 different pesticides (0.01 – 0.78 mg/kg)

	Target compound analysis							ToF Screening		Difference
Pesticide	PRC	LC-MS/MS			TOF (Quan data analysis			Auto-	Software	in Averages
	Result	Rep 1	Rep 2	Average	Rep 1	Rep 2	Average	detection	Fit'	MS/MS v ToFMS
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	Yes/No	%	%
GRAPE SAMPLES CONTAINING INCURRED RESIDUES										
boscalid	0.03	0.04	0.03	0.04	0.04	0.03	0.03	✓	46	109
fenhexamid	0.05	0.06	0.05	0.05	0.05	0.04	0.04	✓	46	118
fenhexamid	0.36	0.71	0.65	0.68	0.59	0.55	0.57	✓	99	120
cyprodinil	0.07	0.09	0.09	0.09	0.08	0.08	0.08	✓	84	111
fludioxinil	0.02	0.03	0.03	0.03	0.03	0.02	0.03	✓	41	127
fenhexamid	0.10	0.08	0.09	0.09	0.08	0.08	0.08	✓	72	109
imidacloprid	0.08	0.07	0.08	0.08	0.08	0.09	0.08	✓	46	95
kresoxim-methyl	0.02	<0.005	<0.005	<0.01	0.02	0.02	0.02	✓	46	n/a

http://randd.defra.gov.uk/Document.aspx?Document=PS2541_10023_FRP.pdf

Grape: incurred residues-comparison



- Original LC-MS/MS data – QuEChERS acetate method
- Repeat analyses (this project) –citrate QuEChERS

ToFMS Results; incurred residues – detection rates

Commodity	**Incurred samples**	
	data processing + analyst intervention	***Automated - no intervention** (± 5 mDa)
Lettuce (ToF)	29/30	28/30
Grape ToF)	67/68	63/68
Pear (ToF)	67/71	66/71

Data filtered by database (mass and retention time)

False negatives

- False negatives in EU screening ring tests, because:
  - Low response (detected by LC-MS/MS)
  - Matrix effects
  - Pesticides not present in data base
- Retropective searching using data base (updated using CRL data):
  - detected suspected residues which were false negatives on initial TOF screen and by LC-MS/MS analysis

False positive detects

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Impacts on efficiency of delivery

- Screening lettuce without database usually detects ~ 35,000 ions
- Database filtering decreases this <<<number
- Residues below the required reporting limit (hence some calibration is important)

Observations

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- Acquisition rate – satisfactory for RRLC
- Linearity is good
- Sensitivity – never satisfied!
- Quantification – validated
- Reliability – now improved with 6224
- Mass accuracy – stable
- identification of analyte?
- Automated data processing -promising
- How to deal with data volume – portable hard drives - SDMS/investment

Conclusion

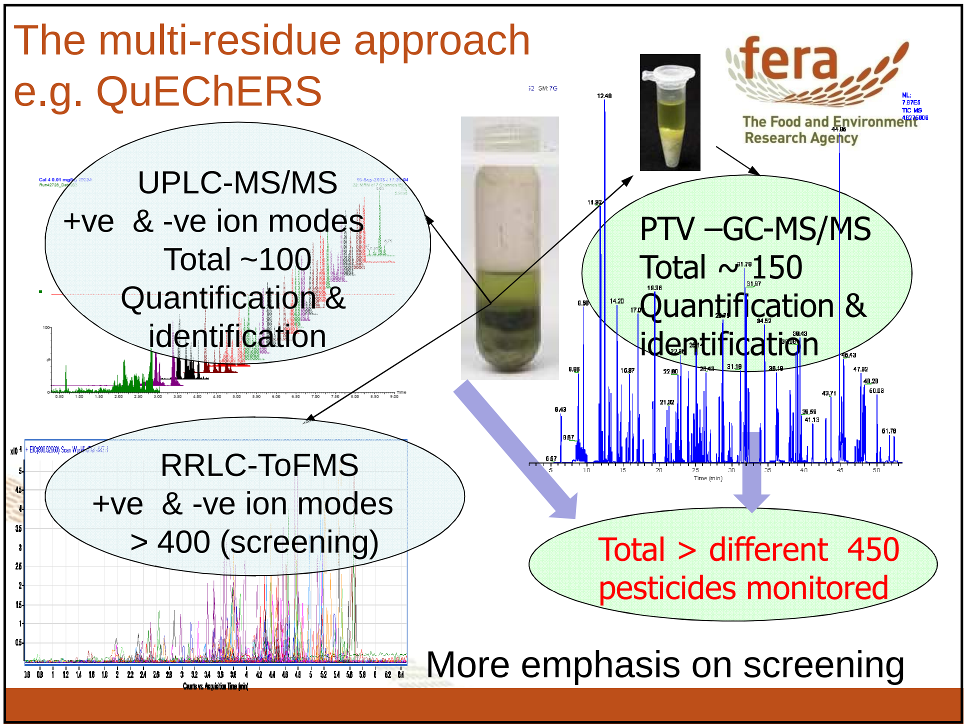
Are there **any** pesticides in the samples and if so, what are the identities and concentrations?

- Not possible to screen for 'unknown residues' which requires a different strategy, software etc.
- Benefit of ToF - retrospective investigation

Should use as complementary techniques

i) detection (screening) and identification (Tof supporting evidence)

ii) > scope against bigger list, data-base(s)



The multi-residue approach e.g. QuEChERS

UPLC-MS/MS
+ve & -ve ion modes
Total ~100
Quantification & identification

PTV –GC-MS/MS
Total ~150
Quantification & identification

RRLC-ToFMS
+ve & -ve ion modes
> 400 (screening)

Total > different 450 pesticides monitored

More emphasis on screening

Future work

- Implementation of ToF is a step closer (depends on developments in software and in LC-MS/MS techniques)
- Further work to evaluate ToF automated detection rates (screening approach) for more (complex) matrices
- Further work to develop appropriate AQC procedures

Acknowledgements

Fera colleagues
Dawn Findlay
Antony Lloyd
Monika Sehnalova

Agilent
Thomas Glauner
John Lee

Thank you for your attention