Fast Screening of Over 1000 Pesticides and Toxins Using Multiple Techniques at the US Food and Drug Administration

3rd Latin American Pesticide Residue Workshop
May 8-11, 2011

Chris A. Sack, David Eide, Michael Smoker, Jon Wong, Kai Zhang, Greg Mercer, Lora Lin, Sara Edison
Introduction

- Overview
- Residue methods
- Future
- Pesticide Residue Analysis Pilot Course
Overview - Challenges

- Food and feed matrices
  - Imports: > 11 million per year
  - Domestic: ???

- Pesticides and other contaminants
  - 1000s that are known
  - Range: 10 ppb - ???

- Analyses
  - Up to 50 samples per day per lab
  - Timeframe: 1 day for imports
Overview

6 PESTICIDE LABORATORIES

SEATTLE
KANSAS CITY
LOS ANGELES
JEFFERSON
NEW YORK
ATLANTA
Analytical Strategy

Pesticides and Toxins

Expected

Unexpected

Unknown

Targeted analysis
LC-MS/MS & GC-MS/MS

Non-targeted screening
GC-MS, LC-MS/LIT
LC-HRMS, DART-HRMS

Fast Screening of Over 1000 Pesticides and Toxins Using Multiple Techniques at the US FDA
Analytical Strategy

- Targeted Quantitative Analysis
  - LC-MS/MS and GC-MS/MS
  - >350 Selected analytes
    - Historical findings
    - Anticipated findings
- Calibration Standard Mixtures
  - Designed by FDA
  - Prepared by commercial vendors
  - Available for purchase
Analytical Strategy

- Qualitative Screening Analysis
  - No standards required
- Spectral Library Techniques
  - GC-MS (fullscan mode)
  - LC-MS/LIT (IDA-EPI)
- Exact Mass Techniques (Exactive Orbitrap)
  - LC-HRMS
  - DART-HRMS
LC-MS/MS Determination

- **Scope:** >240 pesticides in 15 minutes
- **Chromatography:** reverse phase
- **Columns:** C\textsubscript{18}
  - 100 x 2.1 mm, ~3 µm
  - 50 cm x 4.6 mm, 1.9 µm
- **Mass Spectrometer parameters**
  - **Ionization:** Positive electrospray
  - **Detection:** scheduled MRM (two transitions)
- **Detection Limit:** < 10 ppb for most compounds
LC-MS/MS Determination

XIC of +MRM (358 pairs): 184.1/143.0 amu Expected RT: 3.5 ID: Acephate.1 from Sample 21 (A-J 200 pp... Max. 3.4e6 cps.

NYK 200 ng/mL
## LC-MS/MS Determination

### Residues in strawberry sample

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>PPB</th>
<th>Pesticide</th>
<th>PPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boscalid</td>
<td>128</td>
<td>Novaluron</td>
<td>9</td>
</tr>
<tr>
<td>Pyraclostrobin</td>
<td>54</td>
<td>Hexythiazox</td>
<td>5</td>
</tr>
<tr>
<td>Cyprodinil</td>
<td>47</td>
<td>Spiromesifen</td>
<td>2</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>45</td>
<td>Propiconazole</td>
<td>1</td>
</tr>
<tr>
<td>Pyrimethanil</td>
<td>41</td>
<td>Acetamiprid</td>
<td>1</td>
</tr>
<tr>
<td>Chlorantranilaprole</td>
<td>23</td>
<td>Carbendazim</td>
<td>1</td>
</tr>
<tr>
<td>Fludioxinil</td>
<td>22</td>
<td>Methomyl</td>
<td>0.7</td>
</tr>
<tr>
<td>Fenhexamid</td>
<td>20</td>
<td>Thiophanate methyl</td>
<td>0.5</td>
</tr>
<tr>
<td>Bifenazate</td>
<td>9</td>
<td>Methoxyfenozide</td>
<td>0.3</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>9</td>
<td>Fenpyroximate</td>
<td>0.2</td>
</tr>
</tbody>
</table>
LC-MS/MS Determination

Trifloxystrobin 1

Trifloxystrobin 2

Trifloxystrobin @ 0.2 ppb in applesauce
GC-MS/MS Determination

- **Scope:** > 200 pesticides in 20 minutes
- **GC**
  - Retention time locking
  - Backflushing mid column
- **MS/MS detection**
  - MRM (2-3 transitions/compound)
- Detection Limit: 1 - 10 ppb for most analytes
## GC-MS/MS Determination

### Residues in strawberry sample

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>PPB</th>
<th>Pesticide</th>
<th>PPB</th>
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</thead>
<tbody>
<tr>
<td>THPI</td>
<td>237</td>
<td>Bifenthrin</td>
<td>7</td>
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<tr>
<td>Boscalid</td>
<td>128</td>
<td>Quinoxyfen</td>
<td>3</td>
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<tr>
<td>Pyraclostrobin</td>
<td>54</td>
<td>Spiromesifen</td>
<td>2</td>
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<tr>
<td>Cyprodinil</td>
<td>47</td>
<td>Propiconazole</td>
<td>1</td>
</tr>
<tr>
<td>Pyrimethanil</td>
<td>41</td>
<td>Fenpropathrin</td>
<td>0.7</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>37</td>
<td>Biphenyl</td>
<td>0.3</td>
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<tr>
<td>Captan</td>
<td>27</td>
<td>Chlorpyrifos</td>
<td>0.1</td>
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<tr>
<td>Fludioxinil</td>
<td>22</td>
<td>Folpet</td>
<td>0.1</td>
</tr>
<tr>
<td>Fenhexamid</td>
<td>20</td>
<td>p,p’-DDE</td>
<td>0.1</td>
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<tr>
<td>Bifenazate</td>
<td>9</td>
<td>p,p’-DDT</td>
<td>0.04</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>9</td>
<td>o,p’-DDT</td>
<td>0.03</td>
</tr>
<tr>
<td>Malathion</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
GC-MS/MS Determination

o,p’-DDT in strawberry

Standard: 50 ng/mL

Incurred residue @ 0.03 ppb
What’s New with the Pesticide Residue Program in the FDA?

**GC-MS Fullscan Screen**

- **Scope:** > 900 compounds in 20 minutes
- **Screen for library matches - no standards**
- **Agilent GC-MSD pesticide library**
- **Identification:**
  - Spectral matching of AMDIS deconvoluted spectra
  - Retention time
What’s New with the Pesticide Residue Program in the FDA?

GC-MS Fullscan Screen

Celery fullscan TIC

Dieldrin @ 20 ppb buried under matrix
What’s New with the Pesticide Residue Program in the FDA?

**GC-MS Fullscan Screen**

**Celery fullscan TIC**

---

**Fullscan spectra**

**Deconvoluted spectra**

**Library Spectra - Dieldrin**
What’s New with the Pesticide Residue Program in the FDA?

**GC-MS Fullscan Screen**

**Celery deconvolution report**

<table>
<thead>
<tr>
<th>R.T.</th>
<th>Compound Name</th>
<th>AMDIS</th>
<th>NIST</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Match</td>
<td>R.T. Diff</td>
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<tr>
<td>2.5859</td>
<td>Naphthalene-d8</td>
<td>99</td>
<td>-8.0</td>
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<tr>
<td>2.842</td>
<td>Carvone</td>
<td>75</td>
<td>-8.3</td>
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<tr>
<td>3.341</td>
<td>EPTC</td>
<td>94</td>
<td>-7.2</td>
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<tr>
<td>6.977</td>
<td>Anthracene</td>
<td>80</td>
<td>-5.4</td>
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<tr>
<td>7.9576</td>
<td>Diisobutyl phthalate</td>
<td>75</td>
<td>1.2</td>
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<tr>
<td>9.214</td>
<td>Di-n-butyphthalate</td>
<td>65</td>
<td>0.8</td>
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<tr>
<td>9.658</td>
<td>Parathion</td>
<td>82</td>
<td>2.5</td>
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<tr>
<td>11.925</td>
<td>Dieldrin</td>
<td>79</td>
<td>1.5</td>
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<tr>
<td>14.3189</td>
<td>Chrysene-d12</td>
<td>79</td>
<td>-12.1</td>
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<tr>
<td>14.8515</td>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>96</td>
<td>3.3</td>
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<tr>
<td>15.7388</td>
<td>Permethrin I</td>
<td>60</td>
<td>6.5</td>
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<tr>
<td>15.833</td>
<td>Permethrin II</td>
<td>63</td>
<td>7.0</td>
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<tr>
<td>16.518</td>
<td>Cypermethrin II</td>
<td>43</td>
<td>11.7</td>
</tr>
</tbody>
</table>
What’s New with the Pesticide Residue Program in the FDA?

GC-MS Fullscan Screen

Residues discovered and transferred to the LC-MS/MS and GC-MS/MS targeted analyses:

- Fluopicolide
- Spiromesifen
- Spirodiclofen
- Flonicamide
- Chlorfenapyr
- Etoxazole
- Bifenazate
- Etoxazole
- Fenamidone
- Famoxadone
- Quinoxyfen
- Sudan I*

*Food color prohibited in US - not added to target analysis
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

- One instrument and Two Approaches
  - MRM by LC-MS/MS
  - MS library matching using LC-MS-Linear Ion Trap Hybrid Mass Spectrometry
- Targeted and library screening

Kai Zhang\textsuperscript{1}, Jon W. Wong\textsuperscript{1}, Paul Yang\textsuperscript{2}
1. Food and Drug Administration, College Park, MD, U.S.A.
2. Ontario Ministry of the Environment, Ontario, Canada
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

Targeted screening: information dependent acquisition-enhanced product ion (IDA-EPI) method
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

Carbaryl confirmed in peach sample

Carbaryl 202.1>145

Fit: 93
RevFit: 94
Purity: 89
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

False Positive: Pyrimethanil?

- RT match
- Two Pyrimethanil MRMs
  - 200 → 107
  - 200 → 82
- Ion Ratios (200→107/200→82)
  - Requirement: ± 20 %
  - Sample/Standard: ~ 60 %
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

Unknown

Reference
Pyrimethanil

Fit: 35
RevFit: 30
Purity 22

200 > 107

EPI spectrum
Looking to the Future

LC-MS/LIT Mass Spectral Libraries

- Currently building MS libraries for targeted compounds
- Plan to increase screening capability by adding other non-targeted compounds
Looking to the Future

**Exactive Orbitrap® High Resolution MS**

- **Resolution**
  - 100,000 @ 1 scan per second
  - 10,000 at 10 scans per second
- **Mass accuracy:** <1 ppm
- **Scan speed:** → 10 per second
- **Scan range:** 50 – 4000 m/z
- **Detection Limit:** < 10 ng/ml (100 ppb) most compounds
- **Sample introduction**
  - LC (shown here)
  - DART
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

**Exactive Orbitrap® High Resolution MS**
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-HRMS Pesticide Screening Analysis

- Screen > 500 pesticides
- Sample introduction by reverse phase uHPLC
  - Column: C18, 100 x 2.1 mm, 1.9 µm particles
- Identification
  - Retention time
  - HRMS Resolution of 100,000
- Limit of Detection : < 10 ng/ml (100 ppb)
- Plan to expand to other chemical contaminants: mycotoxins, plant toxins, veterinary drugs, dyes, emerging organic pollutants, etc…
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-HRMS Pesticide Screening Analysis

QuEChERS extract of a Peach Sample analyzed by uHPLC-single stage Orbitrap MS (full scan analysis)
What’s New with the Pesticide Residue Program in the FDA?

Looking to the Future

LC-HRMS Pesticide Screening Analysis

Residues extracted from peaches
Looking to the Future

Screening using DART-HRMS

- Sample introduction by Direct Analysis in Real Time (DART)
- HRMS: Resolution = 100,000
- Scope: > 500 pesticides and toxins
- Analysis time: 7.5 minutes/sample
Method:

- Surface of raw agricultural commodities are moistened with solvent and swabbed with foam
  - No sample preparation or extraction!
- Swabs are directly analyzed using a DART ionization source combined with high resolution mass spectrometry
  - No chromatography!
Looking to the Future

Screening using DART-HRMS

Custom foam rail autosampler
Looking to the Future

DART Ionization

\[
\text{He}(2^3\text{S}) + \text{nH}_2\text{O} \rightarrow [(\text{H}_2\text{O})_{n-1} + \text{H}]^+ + \text{OH}^- \text{ He}(1^1\text{S})
\]

\[
[(\text{H}_2\text{O})_n + \text{H}]^+ + \text{M} \rightarrow [\text{M} + \text{H}]^+ + \text{nH}_2\text{O}
\]
Looking to the Future

Screening using DART-HRMS

Diazinon

Carbaryl

Diazinon 202.08627 m/z

Carbaryl 305.10831 m/z
Looking to the Future

Screening using DART-HRMS

Findings
- Up to 10 pesticides per sample
- Average 2-3 pesticides per sample

Publications
  - ¹USFDA, Forensic Chemistry Center, Cincinnati, OH, USA
  - ²USFDA, Center for Food Safety and Applied Nutrition, College Park, MD, USA
- Practical considerations for rapid screening for pesticides using ambient pressure desorption ionization with high-resolution mass spectrometry, Sara E. Edison¹*, Lora A. Lin¹, Lenin Parrales² (Submitted to Food Additives and Contaminants)
  - ¹Food and Drug Administration, Forensic Chemistry Center, Cincinnati, OH
  - ²Food and Drug Administration, San Francisco District Office, Alameda, CA
Pesticide Residue Analysis Pilot Course
June 6-10, 2011

- Location: University of Maryland
  College Park, MD, USA
- By invitation only: Latin American countries
- Scope: Focus on fresh fruits and vegetables
- Hands-on laboratory training
- Instrumental Techniques: LC-MS/MS
- Certificate of Accomplishment of Pesticide Residue Analysis in Fresh Fruit and Produce
# Agenda

<table>
<thead>
<tr>
<th>Monday</th>
<th>Morning -Lecture</th>
<th>Afternoon -Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pesticide compliance in LA Sample preparation</td>
<td>Laboratory SOPs Sample preparation</td>
</tr>
<tr>
<td>Tuesday</td>
<td>Principles of GC and LC Detection: Element and Mass LC-MS</td>
<td>Group A: LC-MS training Group B: Calibration and other sample preparation</td>
</tr>
<tr>
<td>Wed</td>
<td>Quantitative analysis Identification and confirmation QC/QA</td>
<td>Group A: Calibration and other sample preparation Group B: LC-MS training</td>
</tr>
<tr>
<td>Thursday</td>
<td>Method Development Validation of single lab method Regulations</td>
<td>Sample analysis Results interpretation</td>
</tr>
<tr>
<td>Friday</td>
<td>Review and evaluation</td>
<td></td>
</tr>
</tbody>
</table>
Pesticide Residue Analysis Pilot Course

Course Instructors

Dr. Alexander Krynitsky
Supervisory Chemist, Office of Regulatory Science, FDA

Dr. Jon Wong
Chemist, Office of Regulatory Science, FDA

Dr. Kai Zhang
Chemist, Office of Regulatory Science, FDA

Dr. Steven Robbs
Chemist, Office of Center Director CFSAN, FDA

Mrs Doriliz De Leon
Consumer Safety Officer, Office of Compliance, FDA
Pesticide Residue Analysis Pilot Course

Registration

contact Dr. Janie Dubois at:

jifsantraining@umd.edu
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- David Eide, Michael Smoker (USFDA-Kansas City)
- Jon Wong, Ronald Roy, Kai Zhang (USFDA-CFSAN)
- Greg Mercer, Barb Neuhaus (USFDA-Seattle)
- Luis Suguiyama, US Environmental Protection Agency
- US Department of Agriculture - Foreign Agriculture Service