

# **Comparison of Various QuEChERS Approaches for the Determination of Pesticides in Produces**

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# MULTIRESIDUE PESTICIDE ANALYSIS

# 1993 Multiresidue Analysis (Luke II)

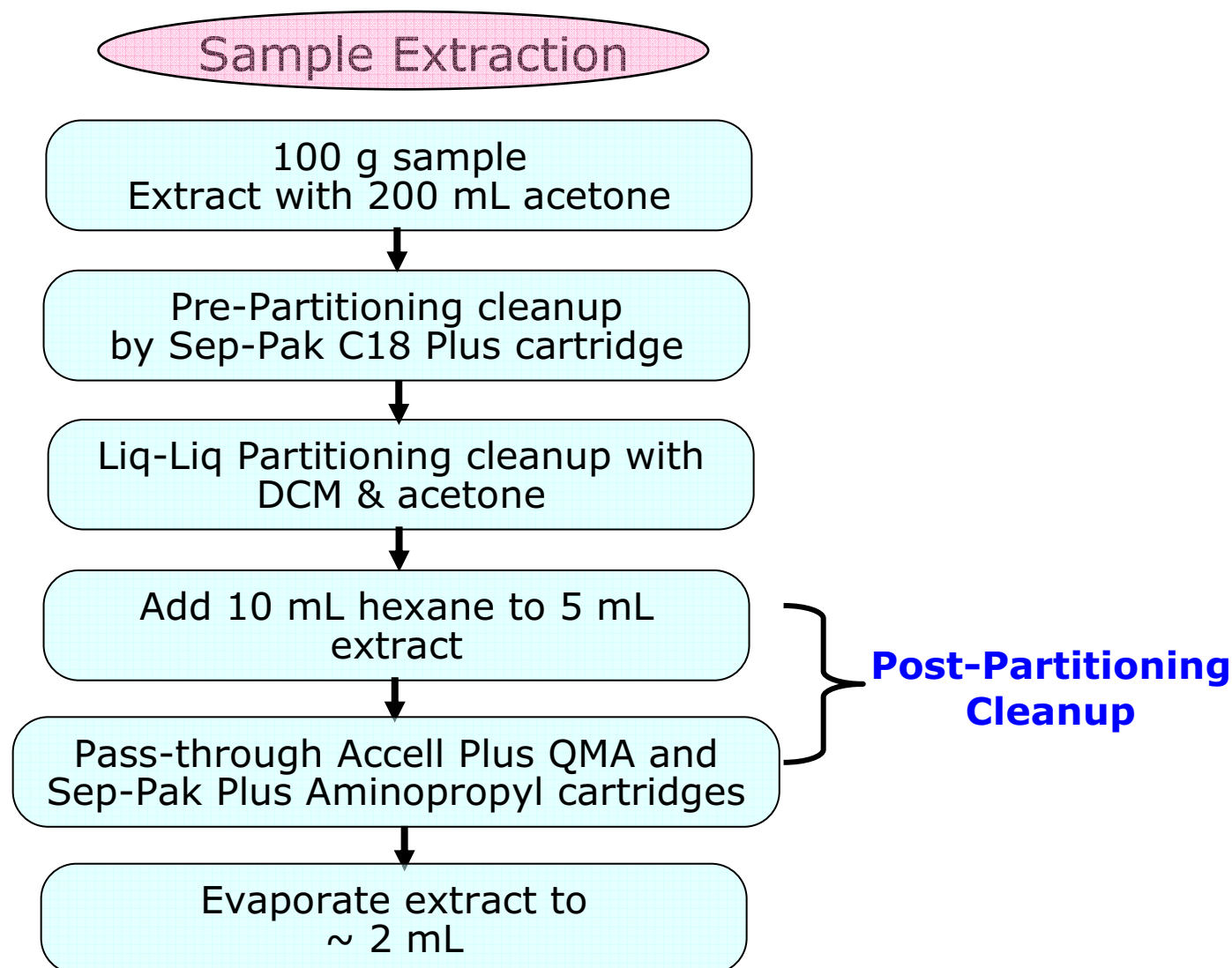
## Sample Preparation

- 100 g blended commodity
  - Extract with 200 mL acetone: add water if req. to 30%
- Take 40 mL of filtered extract
  - load onto Sep-Pak Plus C18 cartridge
    - collect eluent in sep funnel
  - elute with 10 mL 30:70 water/acetone
    - combine with prior eluent in sep funnel
- Partition Cleanup
  - Add 100 mL of methylene chloride (DCM), 50 mL acetone
    - Perform Extraction, collect organic layer, dry over  $\text{Na}_2\text{SO}_4$
  - Add 100 mL of methylene chloride (DCM), 50 mL acetone
    - Perform Extraction, collect organic layer, dry over  $\text{Na}_2\text{SO}_4$
  - Combine all extract in Kurdena-Danish (K-D) apparatus and evaporate to 5 mL
- Post-Partition Cleanup

# 1993 Multiresidue Analysis (Luke II) Sample Preparation (cont)

- Post-Partition Cleanup
  - Add 10 mL hexane to the 5 mL acetone sample
  - Pass-through Accell Plus QMA (anion exchange) and Sep-Pak Plus aminopropyl SPE cartridges
  - Wash/elute cartridges with 3 (x 10 mL) 33:67 acetone/hexane
  - Transfer to K-D flask
    - evaporate to ~ 2 mL
      - exchange to DCM (for GC-MS)
      - exchange to acetone (for GC-ECD, GC-NPD etc.)

# Luke II Sample Preparation - Summary Chart



# Total Analysis Time for 10 Samples Luke II

- About 1.5 day for sample preparation
  - ~ 400 mL solvent usage and evaporation to environment
  - 3 SPE cartridges
  - Elaborate use of glassware (must be scrupulously cleaned)
- 1-2 GC(MS) analyses at ~ 45 min each
- LOQ ~ 10 ppb

**Method:** Cairns, T, Luke, M.A., et. al., *Rapid. Commun. Mass Spectrom.* 7, 1993 (1070-1076)

# QuEChERS MgSO<sub>4</sub> Partition/Dispersive SPE

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## QuEChERS

- Quick
- Easy
- Cheap
- Effective
- Rugged
- Safe



**DisQUE**<sup>™</sup>  
Dispersive Sample Preparation

**Method:** Anastassiades, M., Lehotay, S. J., Stajnbaher, D., Schenck, F.J. *J. AOAC Int.*, **86**, 412-431 (2003)

# QuEChERS Compared to Traditional Approach

- Rapid and straightforward sample preparation
- Fast analysis and a large numbers of samples can be processed simultaneously
- High quality results with a minimal number of steps
- Multi-class multi-residue method
- Small sample size
- Low material costs
- No special equipment or glassware
- No chlorinated solvents and less solvents (“cheap” for both usage and disposal)
- Less chemical (“cheap” on both usage and disposal)
- Acetonitrile extract is compatible to LC and GC analysis

# Total Analysis Time for 10 Samples

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- About 1 hour for sample preparation
- 1 GC/MS analyses at ~ 40 min each
- 1-2 UPLC/MS analysis ~ 10 min
- LOQ ~ 1 ppb (0.1 ppb if 5 mL extract is concentrated to 0.5 mL toluene)

# Original QuEChERS Method - No buffer

	2003 QuEChERS Method
<b>Sample Extraction</b>	10 g sample/10 mL MeCN, 4g MgSO <sub>4</sub> + 1g NaCl
<b>Sample Cleanup</b>	1 mL extract + 150mg MgSO <sub>4</sub> + 25mg PSA
<b>pH of Extraction</b>	depending on the sample
<b>pH of d-SPE cleanup</b>	depending on the sample

# QuEChERS Methods - With Buffers

	AOAC Official Method 2007.01	EU prEN-15662 (CEN)
<b>Sample Extraction</b>	15 g sample/15 mL 1% HOAc in MeCN + 1.5g NaAc + 6g MgSO <sub>4</sub>	10 g sample/10 mL MeCN, 4g MgSO <sub>4</sub> + 1g NaCl, 1g Na <sub>3</sub> Citrate 2 H <sub>2</sub> O + 0.5g Na <sub>2</sub> HCitrate 1 ½ H <sub>2</sub> O
<b>Sample Cleanup</b>	1 mL extract + 150mg MgSO <sub>4</sub> + 50mg PSA	1 mL extract + 150mg MgSO <sub>4</sub> + 25mg PSA
<b>pH of Extraction</b>	3 – 4 (MeCN phase) 5 – 6 (Water phase)	~ 8 (MeCN phase) 5 – 5.5 (Water phase)
<b>pH of d-SPE cleanup</b>	3 – 4 (Slightly lower than before cleanup)	~ 8 (MeCN phase)

<b>Fungicides</b>	<b>Class</b>
<b>Azoxystrobin</b>	Strobilurin
<b>Chlorothalonil</b>	OC
<b>Cyprodinil</b>	Anilinopyrimidine
<b>Imazalil</b>	Imidazole
<b>Kresoxim-methyl</b>	Strobilium
<b><i>o</i>-Phenylphenol</b>	Phenol
<b>Procymidone</b>	Dicarboximide
<b>Tebuconazole</b>	Triazole
<b>Thiabendazole</b>	Benzimidazole
<b>Tolyfluanid</b>	<i>N</i> -Trihalomethylthio
<b>Hexachlorobenzene</b>	OC

**OC: Organochlorine**

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**Insecticides****Class**

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<b>Bifenthrin</b>	Pyrethroid
<b>Carbaryl</b>	Carbamate
<b>Chlorpyrifos</b>	OP
<b>Chlorpyrifos-methyl</b>	OP
<b>Dichlorvos</b>	OP
<b>Endosulfan sulfate</b>	OC
<b>Ethion</b>	OP
<b>Imidacloprid</b>	Neonicotinoid
<b>Methamidophos</b>	OP
<b>Methomyl</b>	Oxime carbamate
<b>Permethrins</b>	Pyrethroid
<b>Pymetrozine</b>	Pyridine

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**OC: Organochlorine**

**OP: Organophosphate**

# Pesticides of Interest

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<b>Herbicides</b>	<b>Class</b>
<b>Atrazine</b>	Triazine
<b>Linuron</b>	Phenylurea
<b>Trifluralin</b>	Dinitroaniline

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# QuEChERS Experimental Procedures

- Internal Standards – d5-Atrazine
- Calibration standards
  - Prepared in the extracted blank sample just prior to the instrument analysis
  - Six calibration standards were prepared so that they are equivalent to the concentrations of 5, 10, 50, 100, 250, and 1000 ng/g in the commodity.

- Fruit and vegetable sample is cut coarsely before pulverized by a food chopper or blender or other similar device.
- A sub-sample of 15 g or 10 g is transferred to tube 1 for extraction.
- For rolled oat sample, 7.5 g of sample is diluted with 15 mL water before extraction with 15 mL of acetonitrile.
- Prepare six spike samples at 40 ng/g for oats and 20 ng/g for the others

# Extraction Procedure – Tube 1

## AOAC Method

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### Extraction Tube 1

Homogenize Sample

Sample Extraction

15 g sample  
15 mL 1% Acetic Acid in ACN

Liquid Fractionation

Shake for 1 minute  
Centrifuge >1500 x g

Collection

Transfer the supernatant into  
tube 2 for clean-up



Analytes  
remain  
in  
Supernatant

Tube 1

# Dispersive-SPE Procedure - Tube 2

## AOAC Method

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Extraction  
Tube 1

### Prepare Sample

Homogenize

15 g sample

15 mL 1% Acetic Acid in ACN

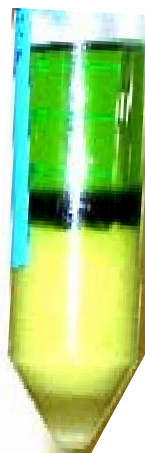
### Liquid Fractionation

Shake for 1 minute

Centrifuge >1500 x g

### Collection

Transfer the supernatant into  
tube 2 for clean-up



Tube 1

Transfer  
Supernatant  
to  
Tube 2



Tube 2



Analytes  
remain  
in  
Supernatant



Tube 2

d-SPE Clean-Up  
Tube 2

### Transfer

1 mL Extract  
From Tube 1 to Tube 2

Shake vigorously for 1 minute  
Centrifuge >1500 x g

### Transfer

Dilute extract for LC analysis  
Evaporate solvent and  
exchange into toluene for GC  
Analysis

- Follow the CEN prEN 15662 procedures
- The centrifugation time was adjusted according to the CEN method.

# Original QuEChERS Procedures

- Follow the procedures listed in the 2003 AOAC paper
- The tube 1 contains less salt mixture which is suitable for 10g/10mL format.
- The spiking volumes for standards were also reduced according to the sample weight

- For LC analysis:
  - Transfer 100  $\mu\text{L}$  extract to a vial.
  - For spike samples, add 10  $\mu\text{L}$  TPP solution and 5  $\mu\text{L}$  MeCN
  - For calibration samples, add 10  $\mu\text{L}$  TPP solution and 5  $\mu\text{L}$  of each spiking standard
  - Add 440  $\mu\text{L}$  of 0.1% FA in water
  
- If emulsion is observed in the vial, for examples, avocado and rolled oats samples, additional steps are needed to clarify solution
  - Centrifuge the extract for 5 min at  $>16,000$  rcf to clarify the solution
  - Pipette extract below the surface and transferred to LC vial

# Extract Handling for Analysis (Cont.)

- For GC analysis:
  - Pipet 500  $\mu\text{L}$  of extract to 15-mL graduated centrifuge tube  
Add 50  $\mu\text{L}$  TPP solution and 250  $\mu\text{L}$  toluene to the tube
  - Evaporate the extract in N-Evap at 50°C to less than 0.1 mL volume
  - For the 6 calibration standards, add 50  $\mu\text{L}$  of each respective calibration standard to the tube
  - Bring the final volume to 0.20 mL using toluene
  - Transfer the extract to vial for GC analysis

# UPLC/MS/MS Conditions

LC System: Waters ACQUITY UPLC® System

Column: ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm

Column Temp: 40 °C

Sample Temp: 4 °C

Flow Rate: 0.3 mL/min.

Mobile Phase A: Water + 0.1% formic acid

Mobile Phase B: Methanol + 0.1% formic acid

Gradient:

Time	A%	B%
0.00	75	25
0.25	75	25
7.75	0	100
8.50	0	100
8.51	75	25

Total Run Time: 11 min.

Injection Volume: 15 µL

# UPLC-MS-MS Conditions

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MS system: Waters ACQUITY® TQ Detector

Ionization mode: ESI positive polarity

Capillary voltage: 3 kV

Desolvation gas: Nitrogen, 800 L/Hr, 400 °C

Cone gas: Nitrogen, 5 L/Hr

Source temp: 150 °C

Acquisition: Multiple Reaction Monitoring (MRM)

Collision gas: Argon at  $3.5 \times 10^{-3}$  mBar



## GC-MS Conditions

Waters Quattro micro GC

Column: RTX 5MS, 30 m x 0.25 mm (0.25  $\mu$ m film)

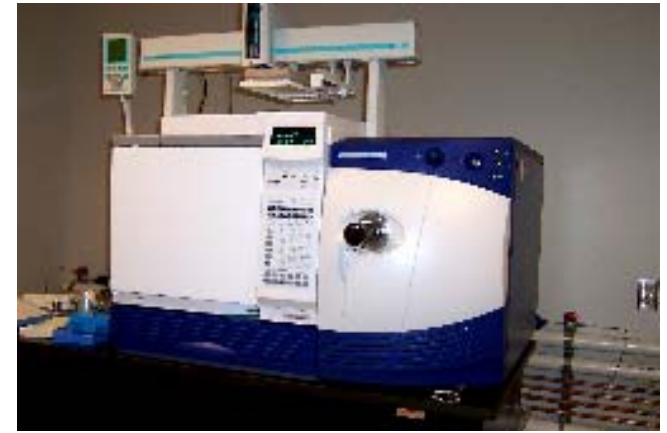
Carrier gas: Helium at 1 mL/min

Temperature program: 100° C initial, hold 1 min, then 10° C /minute to 320° C, hold for 7 minute

Injection: 2  $\mu$ L splitless

Mode: Electron Impact Ionization

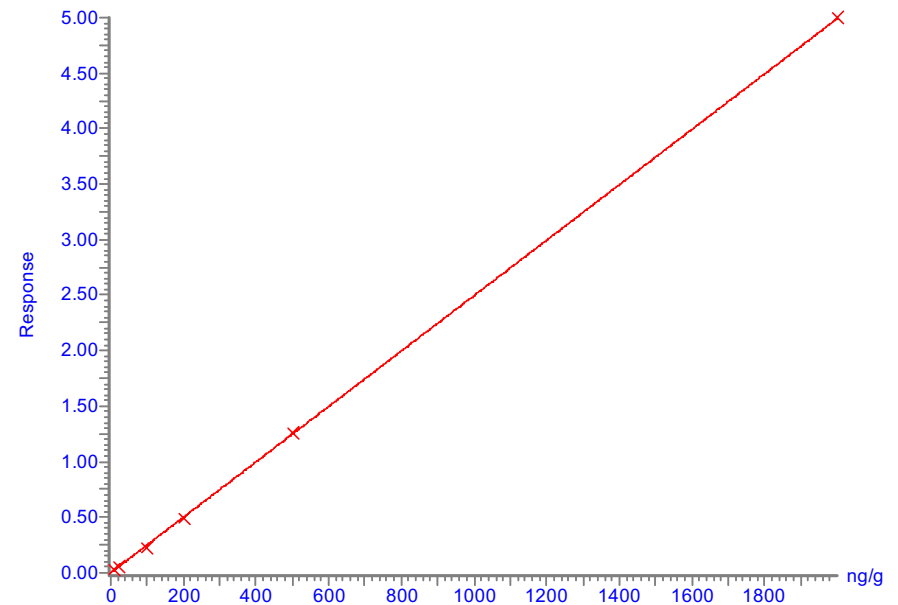
Acquisition: Single Ion Recording (SIR) Mode



- The correlation coefficient ( $r^2$ ) is  $> 0.995$  for majority of analytes. Some compounds with poor responses or susceptible to degradation may be difficult to reach the high linearity.

## Example of Calibration Curve

Compound name: Azoxystrobin  
Correlation coefficient:  $r = 0.999796$ ,  $r^2 = 0.999592$   
Calibration curve:  $0.0024955 * x + -0.000605938$   
Response type: Internal Std ( Ref 11 ), Area \* ( IS Conc. / IS Area )  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

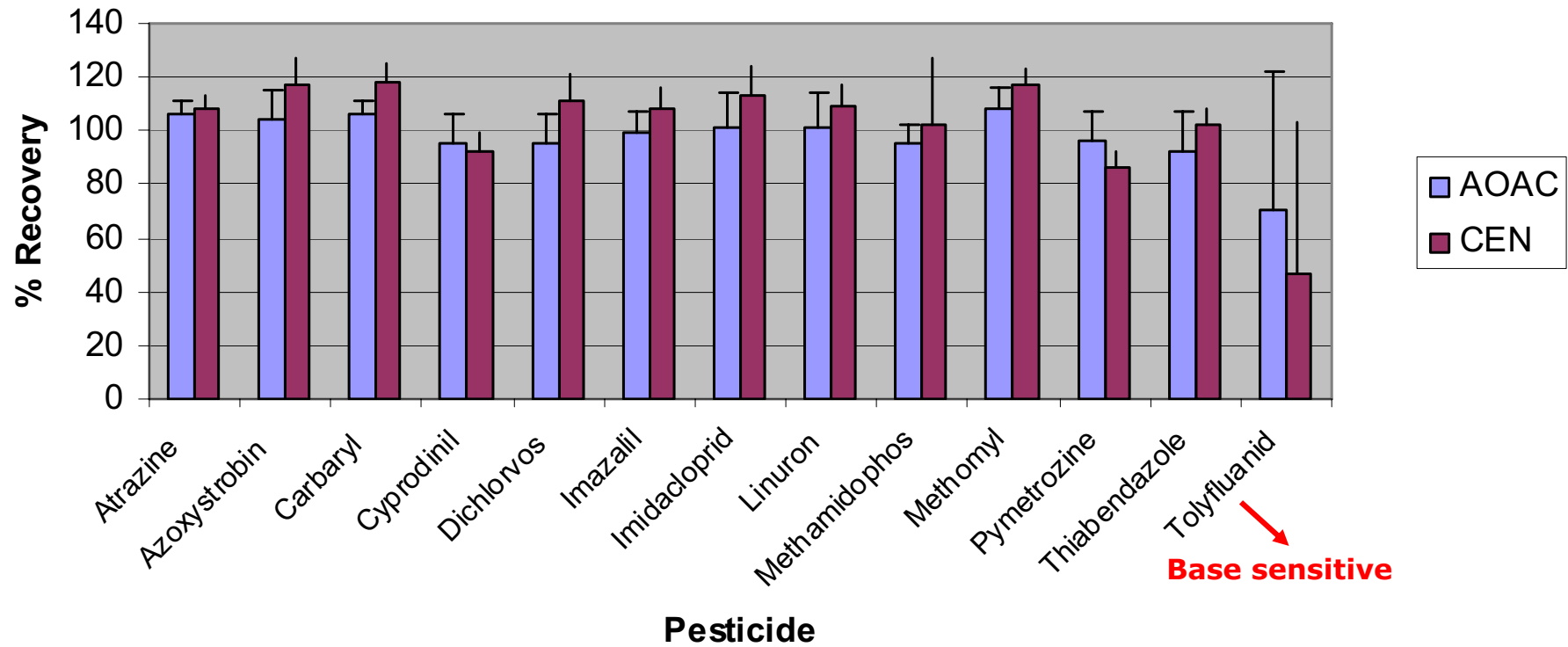




# Results

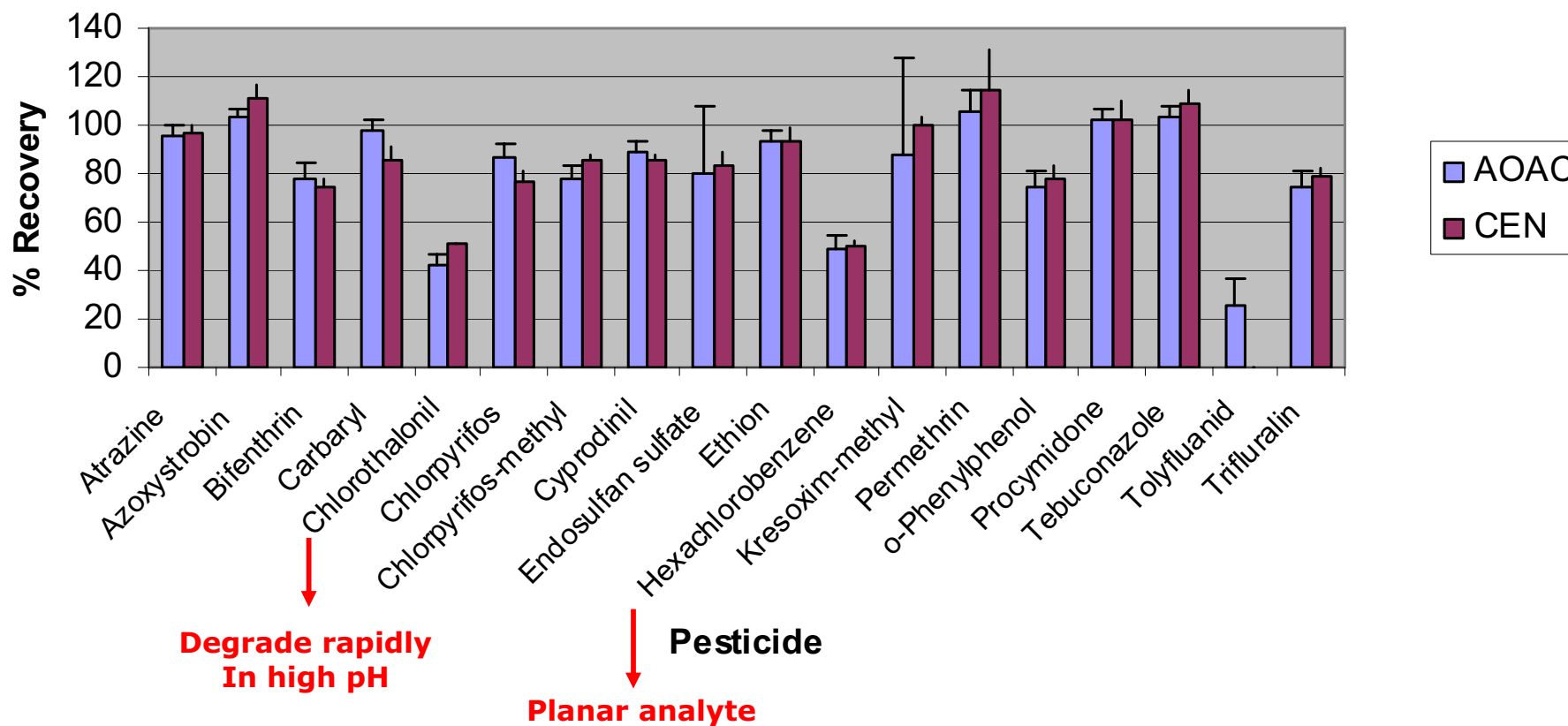
# Pesticide Recovery Rolled Oats Analysis by LC/MS/MS

## Pesticide Recovery in Oats by Various Methods



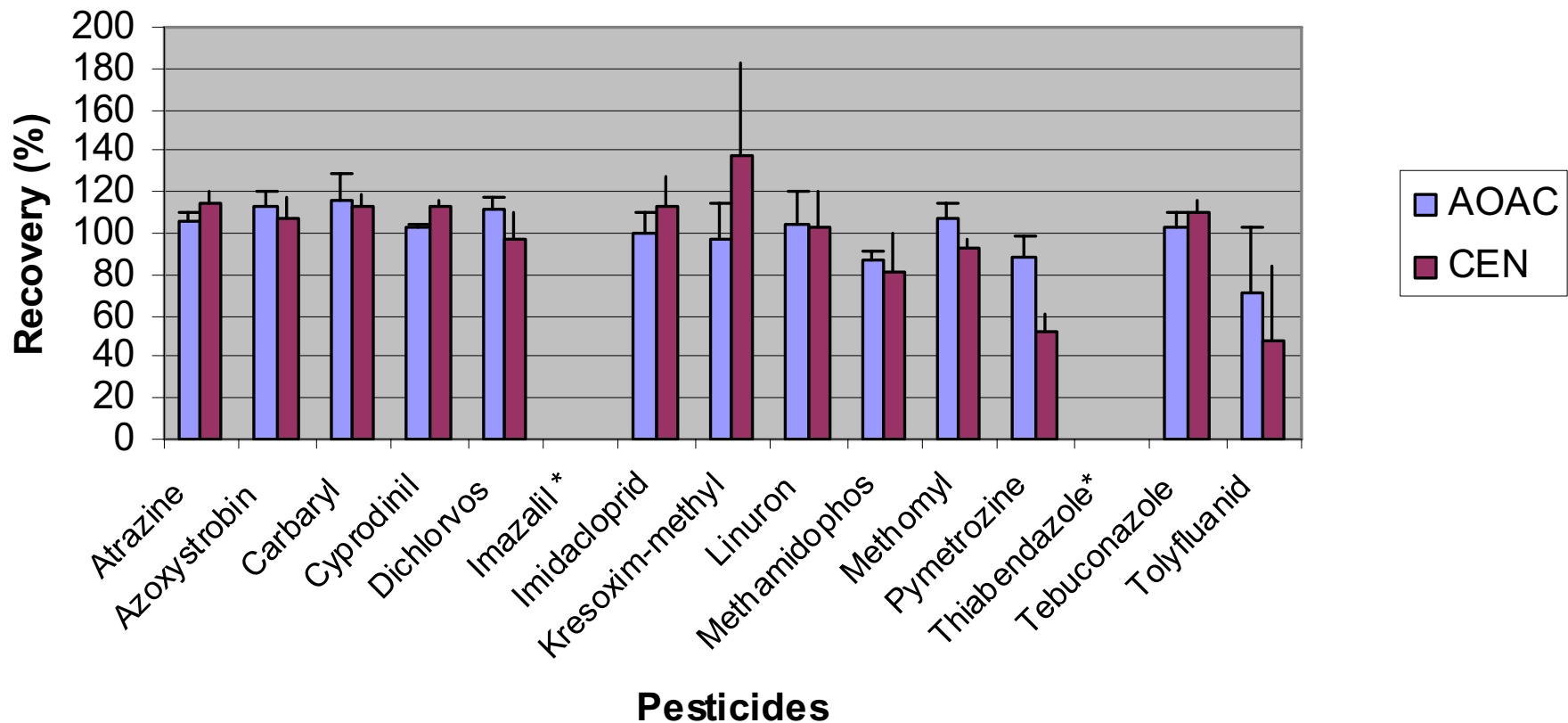
# Pesticide Recovery in Rolled Oats Analysis by GC-MS

## Pesticide Recovery in Oats by Various Method



# Pesticide Recovery in Orange Analysis by LC/MS/MS

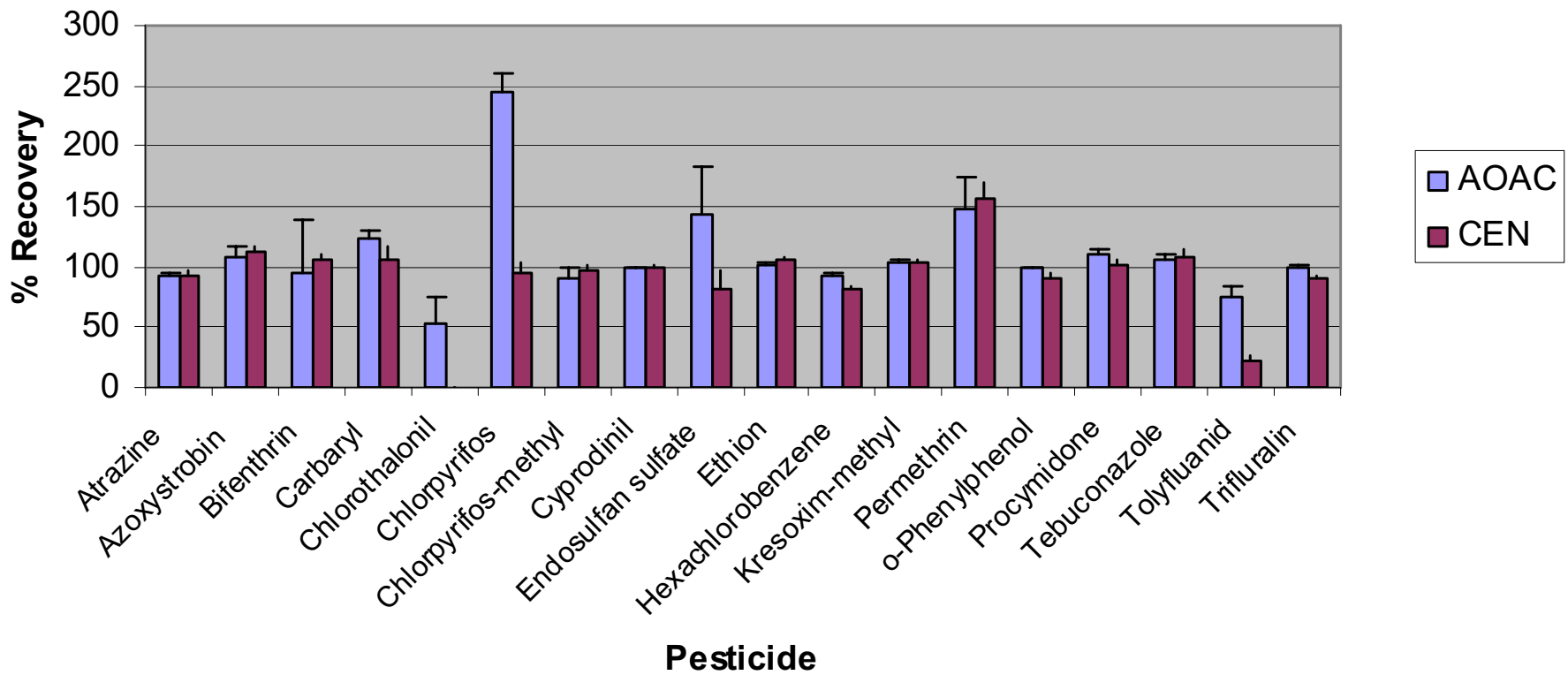
## Pesticide Recovery in Orange by Various Method



\* Pesticides incurred in orange  
Recovery not calculated

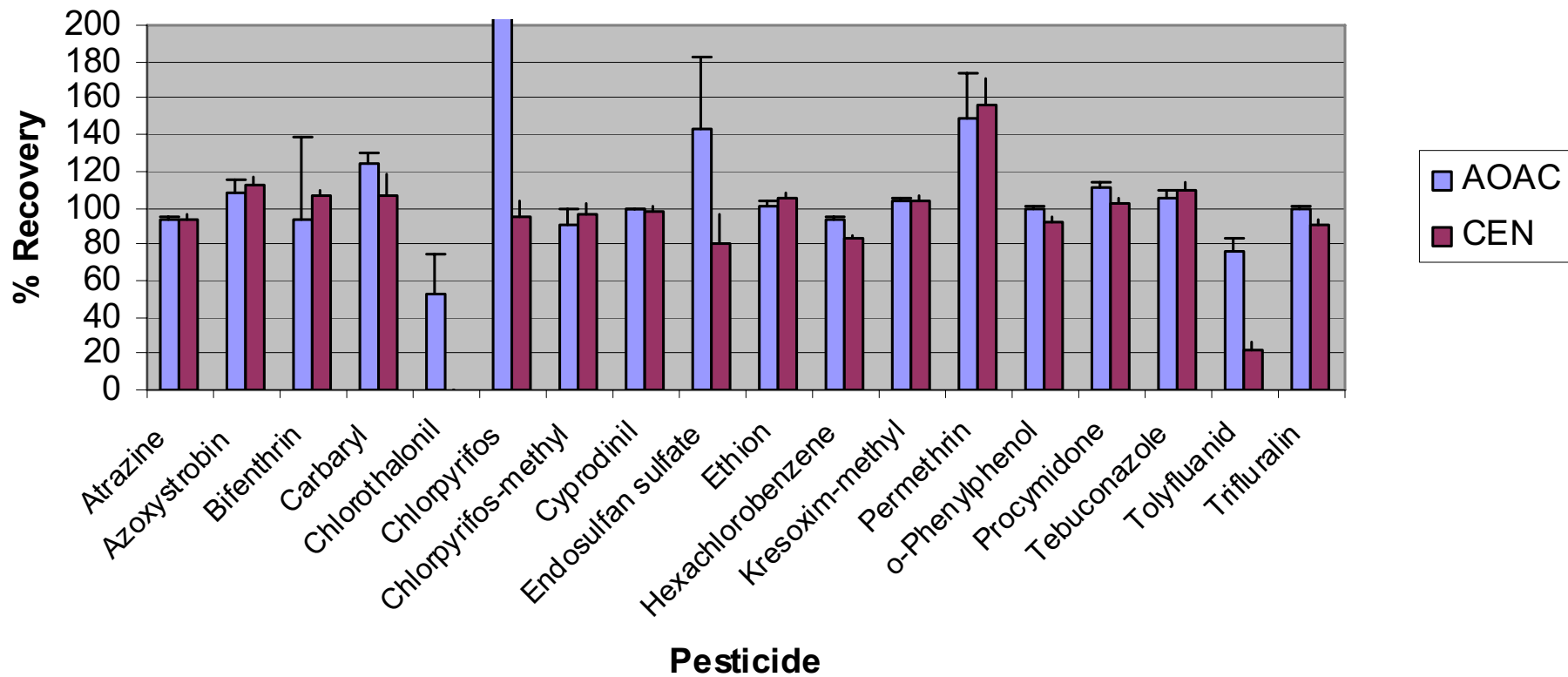
# Pesticide Recovery in Orange Analysis by GC/MS

## Pesticide Recovery in Orange by Various Method



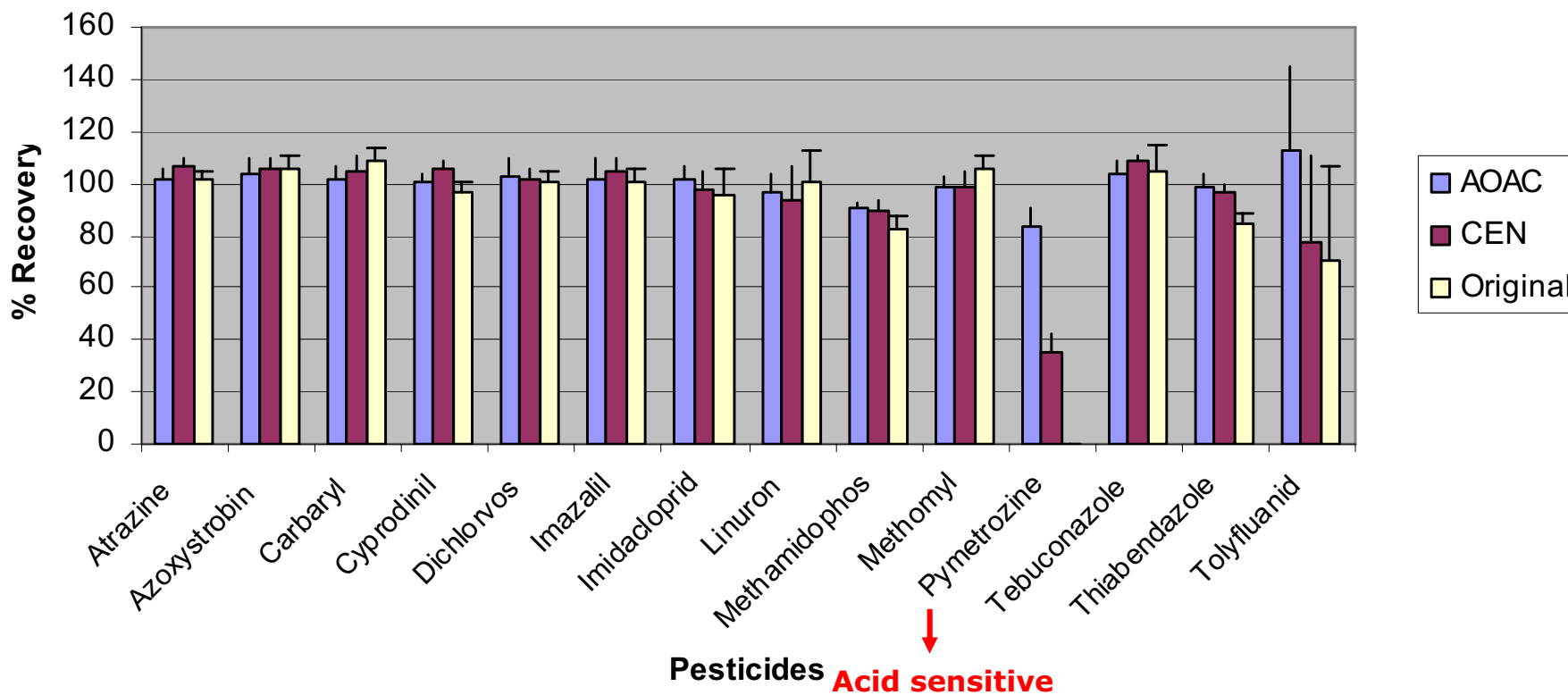
# Pesticide Recovery in Orange Analysis by GC/MS - Scale Up

## Pesticide Recovery in Orange by Various Method



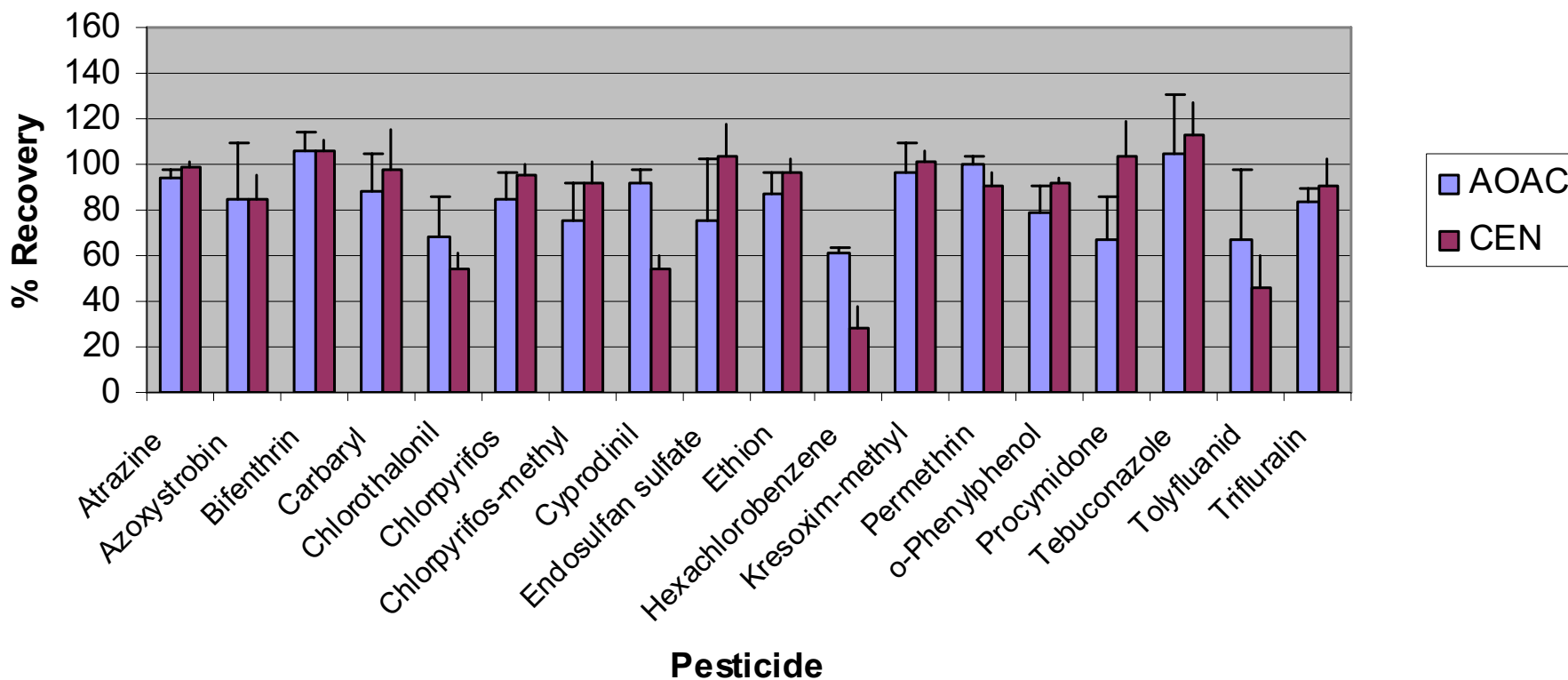
# Pesticide Recovery in Grape Analysis by LC/MS/MS

## Pesticide Recovery in Grape by Various Methods



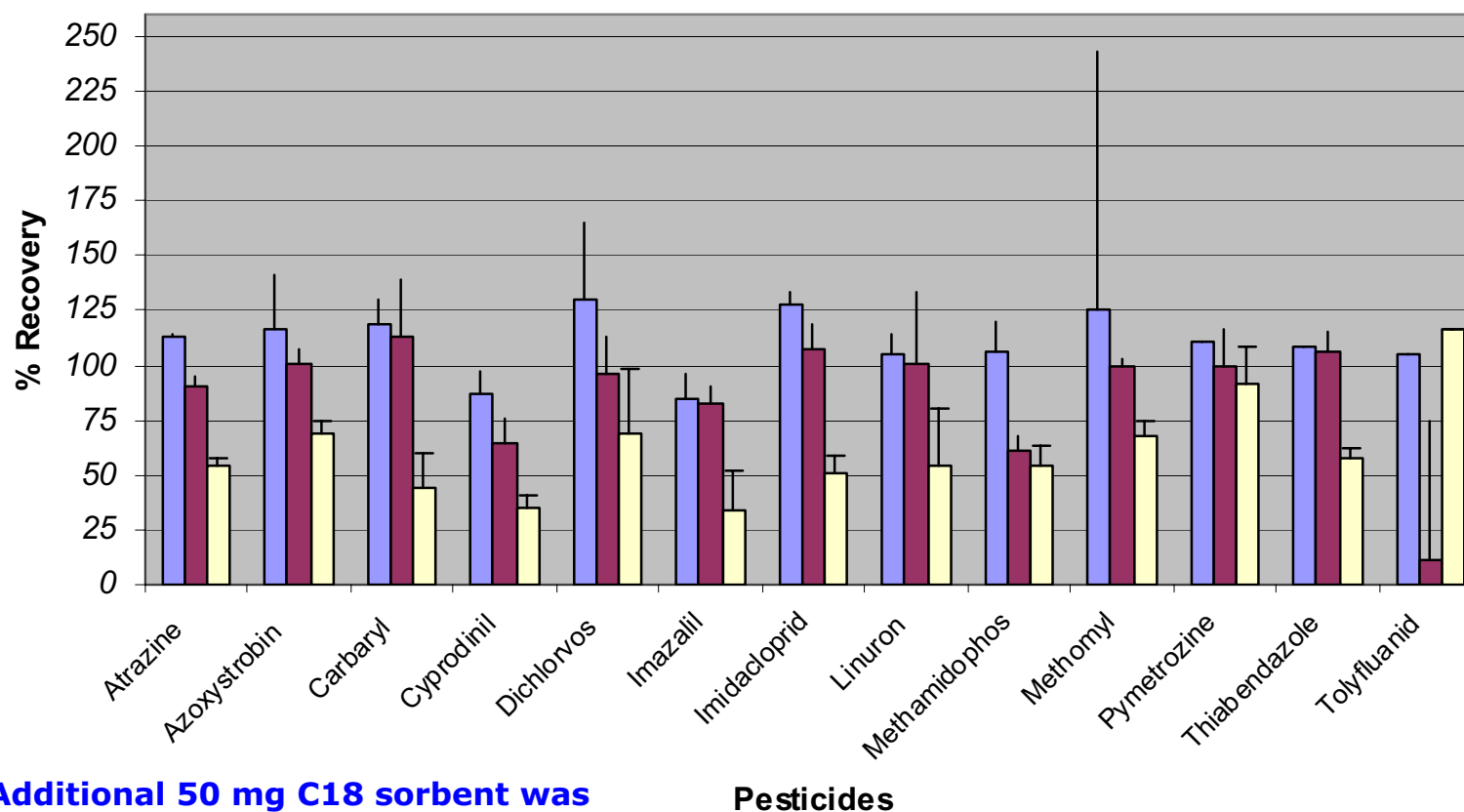
# Pesticide Recovery in Grape Analysis by GC-MS

## Pesticide Recovery in Grape by Various Method



# Pesticide Recovery in Avocado Analysis by LC/MS/MS

## Pesticide Recovery in Avocado by Various Method

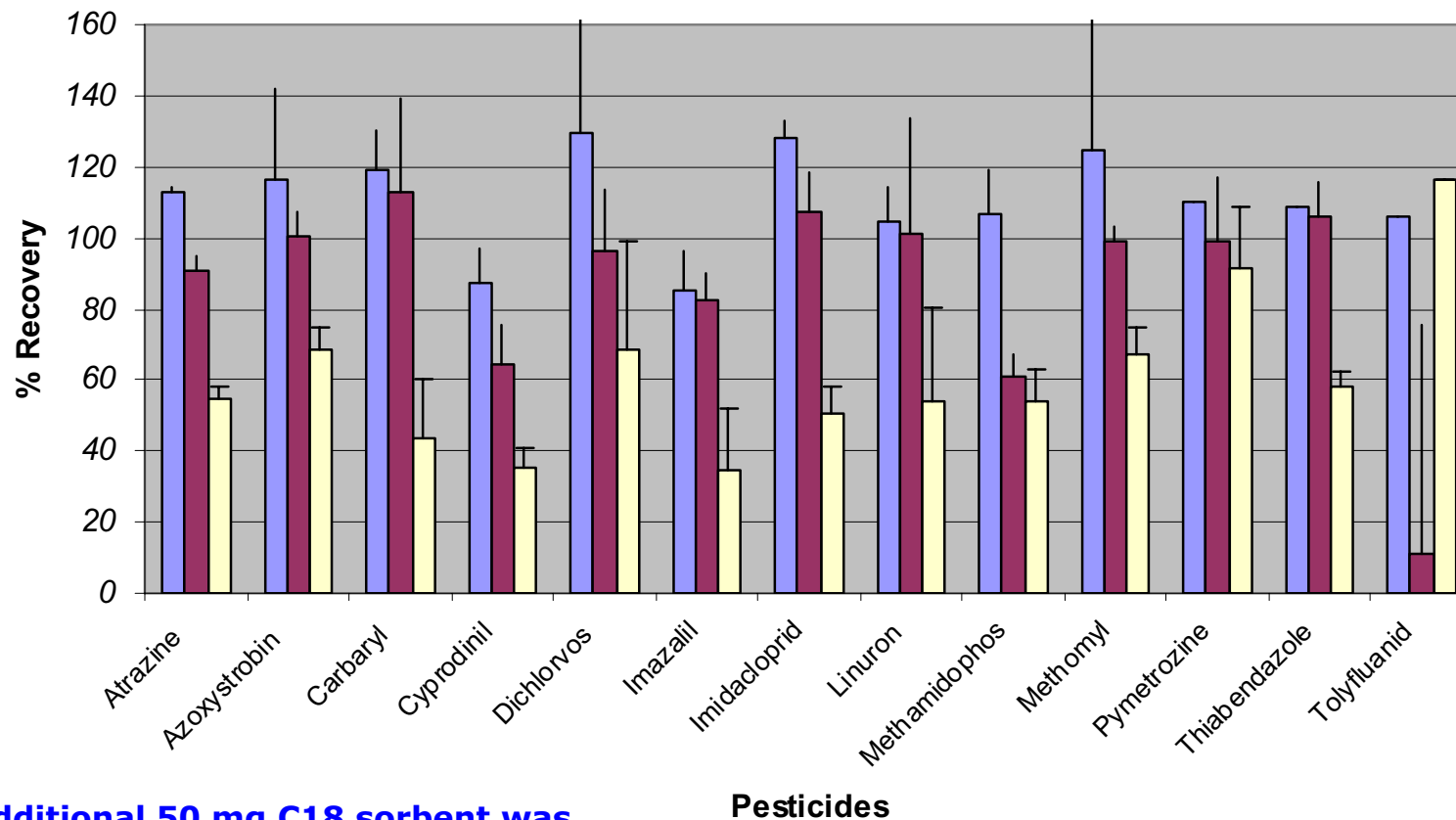


**Additional 50 mg C18 sorbent was added to the d-SPE cleanup tube**



# Pesticide Recovery in Avocado Analysis by LC/MS/MS – Scale Up

Pesticide Recovery in Avocado by Various Method

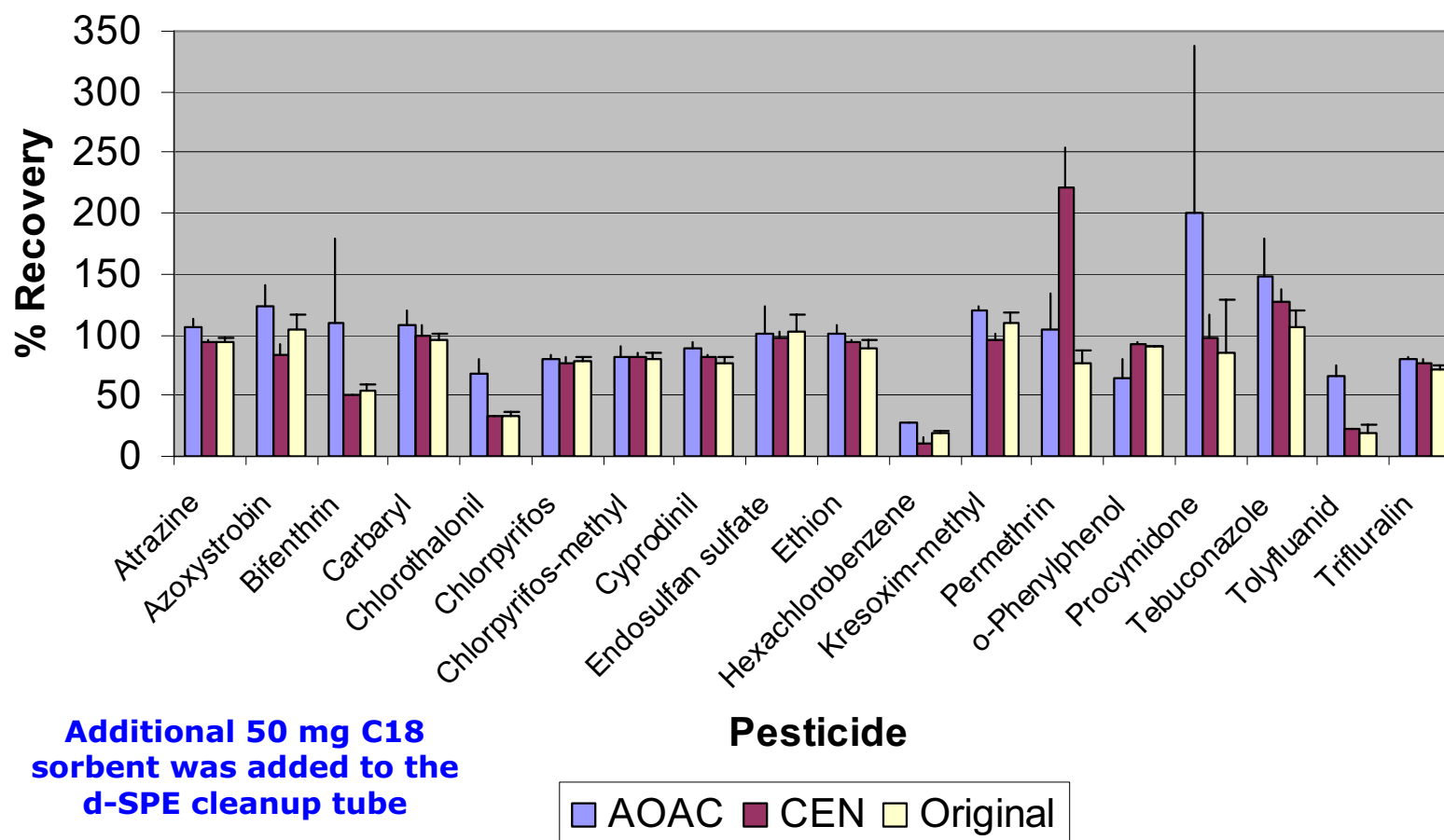


**Additional 50 mg C18 sorbent was added to the d-SPE cleanup tube**

AOAC CEN Original

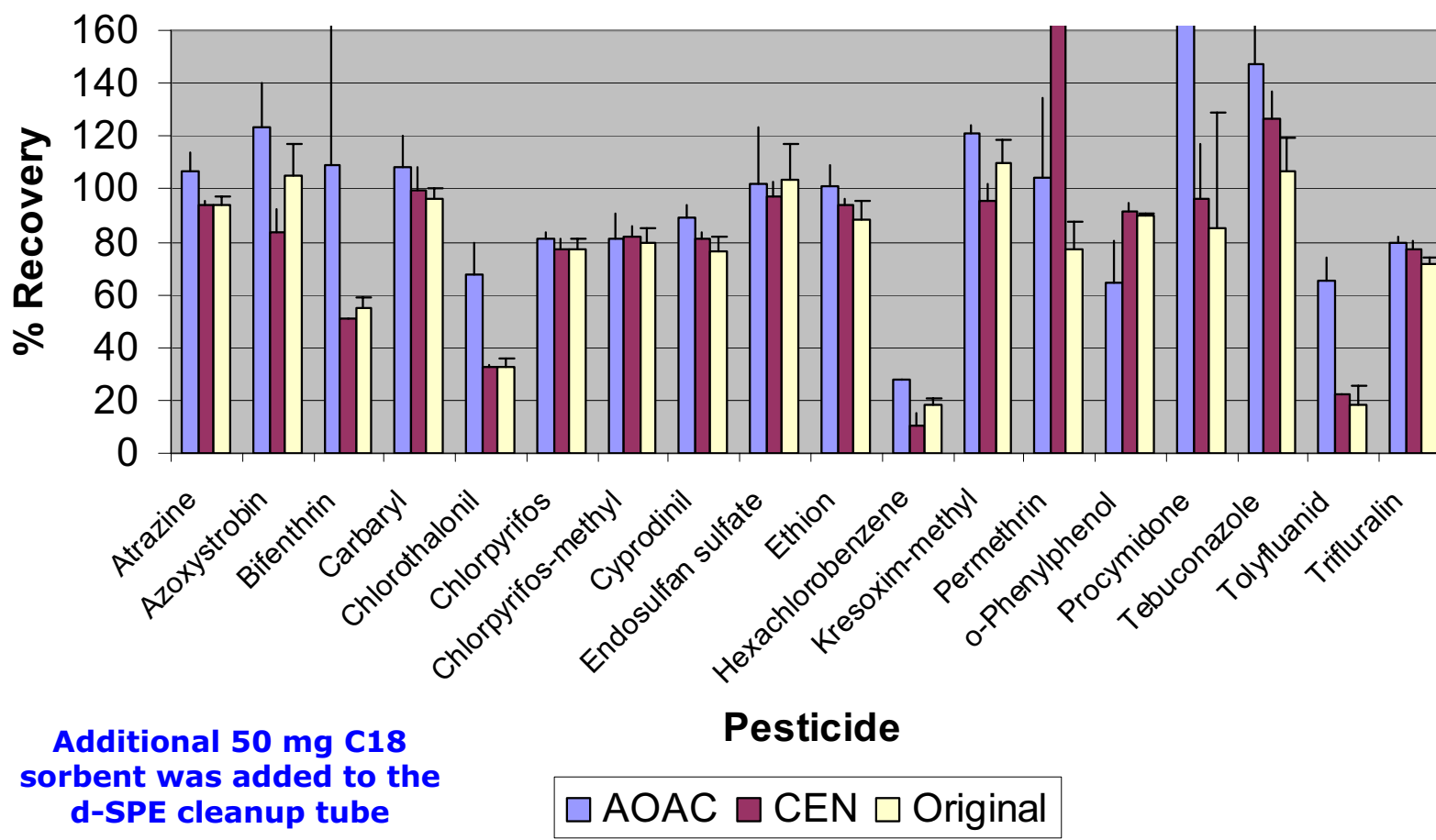
# Pesticide Recovery in Avocado Analysis by GC/MS

## Pesticide Recovery in Avocado by Various Method



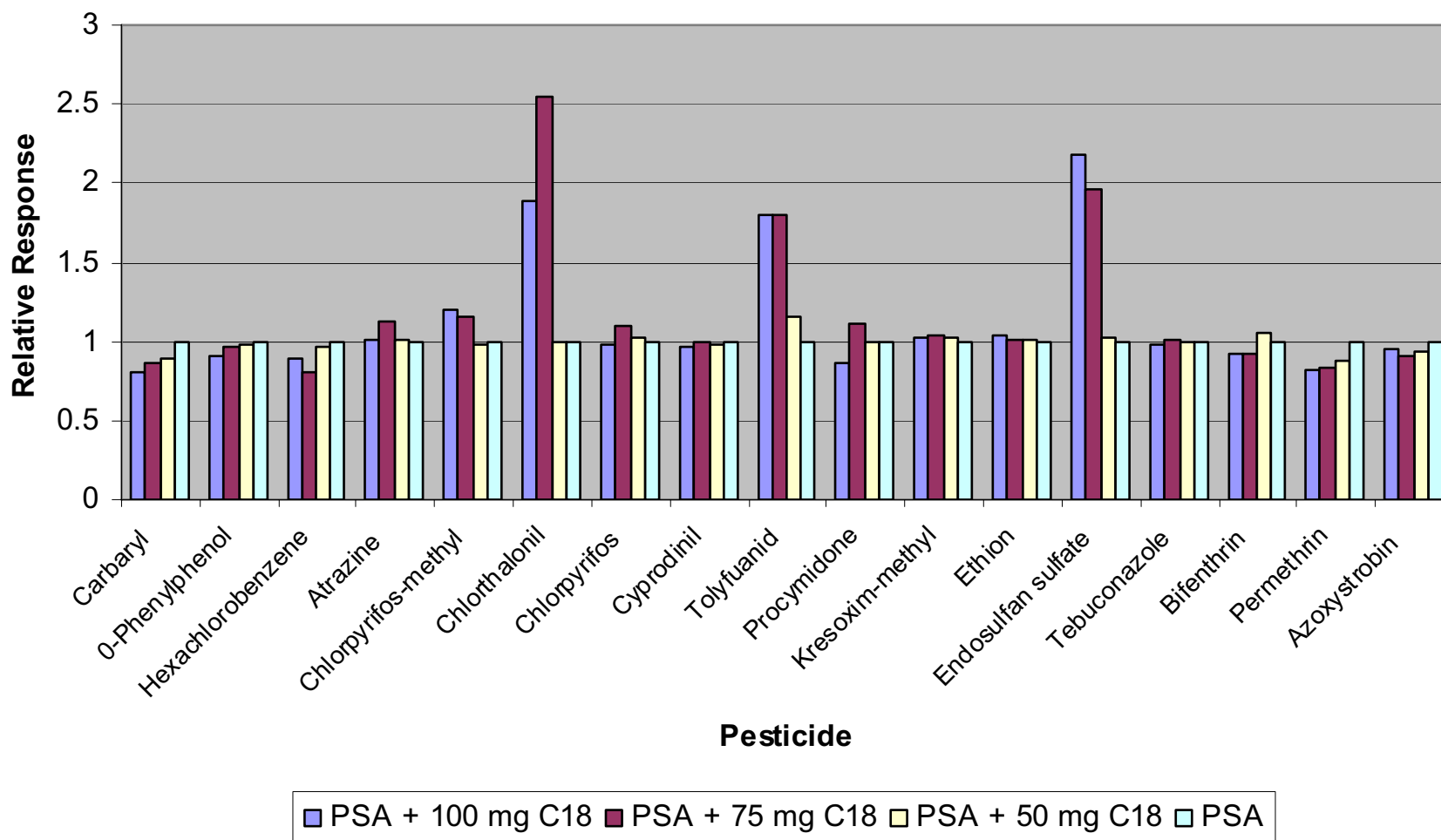
# Pesticide Recovery in Avocado Analysis by GC/MS Scale Up

## Pesticide Recovery in Avocado by Various Method



# How much of C18 Could be added to d-SPE Cleanup Tube?

## The Effect of C18 Amount on the Pesticide Response



- Among the matrix tested, no major discrepancy between the AOAC and CEN methods for most of the pesticides.
- For acid sensitive pesticide, e.g. pymetrozine, AOAC method with better buffer strength in acetonitrile give better recovery than CEN or original QuEChERS.
- Hexachlorobenzene, a planar analyte, were recovered low in all three matrix except in orange. It needs further investigation.
- C18 is used as additional sorbent during the d-SPE step to enable better cleanup for fatty sample such as avocado. It can be added as much as 100 mg without adversely effecting the signals of most pesticides. But it enhance the signals of chlorothalonil, tolyfluanid, and endosulfan sulfate significantly.

# Acknowledgement

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- Kim Van Tran
- Michael S. Young
- Kevin Jenkins
- Diane Diehl

# Question?