# Multi-Residue Pesticide Analysis on Incurred Produce Samples Using Variations of Disposable Pipette Extraction

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## DPX Movie of Dye Extraction

Demonstration of Dispersive SPE in a Tip

## Outline of Presentation

I. AOAC QuEChERS Study—DPX comparison

II. Multi-Residue Study Using GC/NPD and GC/ECD

III. DPX-Q—QuEChERS in a Tip

IV. Future Studies: Automation (GERSTEL MPS-2)

## I. DPX in QuEChERS AOAC Study

Extract in acetonitrile following QuEChERS procedure
- 15 g sample plus 15 mL ACN
- Add salt ($MgSO_4$ and NaCl)
- Shake, centrifuge

## I. DPX in QuEChERS AOAC Study

**DPX-RP (nonpolar compounds):**

Take 1 mL and add 2.4 mL $H_2O$ plus 0.8 mL sat'd NaCl
Perform DPX extraction (app. 3-4 minutes)
Water wash step; elution with hexanes/ethyl acetate
Inject into **GC-ECD** (*no solvent evaporation*)

## I. DPX in QuEChERS AOAC Study

**DPX-CU (polar compounds):**

Take 1 mL ACN solution
Aspirate in-and-out of DPX cleanup (polyamino) tip
Solvent exchange into "acetone"
Inject into **GC-NPD**

(Acetone should have been replaced with toluene.)

# I. DPX Results

DPX-RP

| Orange (GC-ECD) | type | ECD | %RSD |
|---|---|---|---|
| HCB | OC | 49.9 | 3.2 |
| chlorothalonil | OC | 63.9 | 6.8 |
| chlopyriphos | OC/OP | 58.6 | 5.2 |
| endosulfan-I | OC | 62.1 | 6.9 |
| p,p'-DDE | OC | 52.4 | 6.0 |
| ethion | OP | 64.2 | 4.4 |
| endofulfan-II | OC | 63.0 | 6.7 |
| endofulfan-SO4 | OC | 67.9 | 7.9 |
| phosmet | OP | 66.4 | 6.4 |
| azinphos-methyl | OP | 111.2 | 8.4 |
| trans-permethrin | OC | 105.9 | 12.6 |

DPX-Polyamino (cleanup)

| Orange (GC-NPD) | type | NPD | RSD |
|---|---|---|---|
| methamidophos | OP | 69.7 | 8.2 |
| Acephate | OP | 80.3 | 12.8 |
| Chlopyriphos | OC/OP | 88.7 | 6.1 |
| TPP (int std) | OP | 89.6 | 7.1 |

**Lower recoveries with elutions from the "**bottom**" (similar results with spinach).** Elute from the top and obtain app. 100% recoveries of nonpolar pesticides (see charts in next slides).

# I. DPX Results

DPX-RP

| Carrots (GC-ECD) | type | ECD | %RSD |
|---|---|---|---|
| HCB | OC | 91.7 | 4.5 |
| chlorothalonil | OC | 111.9 | 4.9 |
| chlopyriphos | OC/OP | 103.2 | 3.5 |
| endosulfan-I | OC | 99.2 | 4.4 |
| p,p'-DDE | OC | 89 | 1 |
| endofulfan-II | OC | 99.9 | 2.9 |
| ethion | OP | 98.7 | 3.6 |
| endofulfan-SO4 | OC | 103.8 | 1.7 |
| phosmet | OP | 108 | 0.2 |
| azinphos-methyl | OP | 107.1 | 1.5 |
| trans-permethrin | OC | 81 | 8.3 |

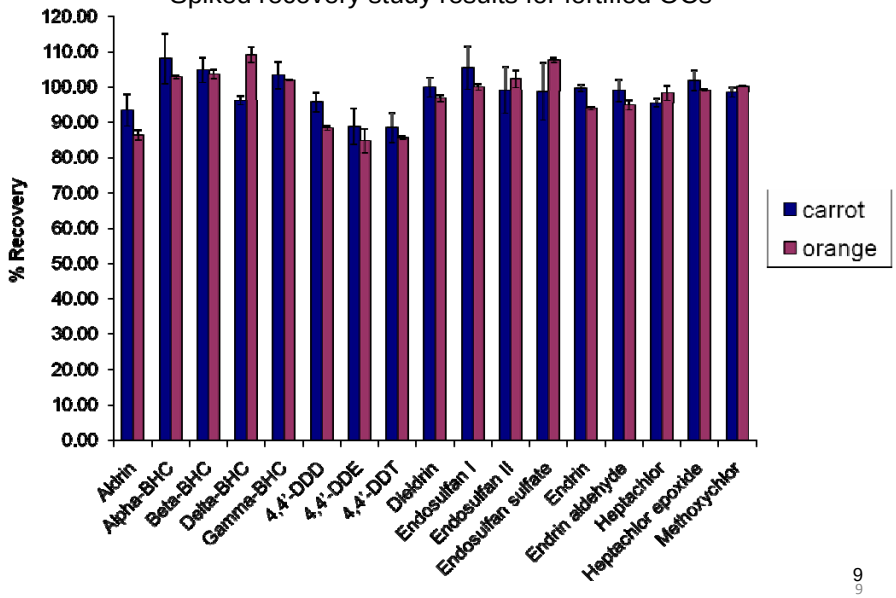
DPX-Polyamino (cleanup)

| Carrots (GC-NPD) | type | NPD | %RSD |
|---|---|---|---|
| methamidophos | OP | 58.9 | 7.3 |
| acephate | OP | 58.3 | 16.9 |
| chlopyriphos | OC/OP | 77.4 | 3.9 |
| TPP (int std) | OP | 78.8 | 3.7 |

Top elution provides highest recoveries! Polyamino cleanup (to remove fatty acids) can be improved with additional solvent or by replacing with PSA. Polyamino has more active sites and acts as a stronger anion exchange sorbent..—better for removing fatty acids (gastric contents, tissue, grain, etc.).

# Results and discussion

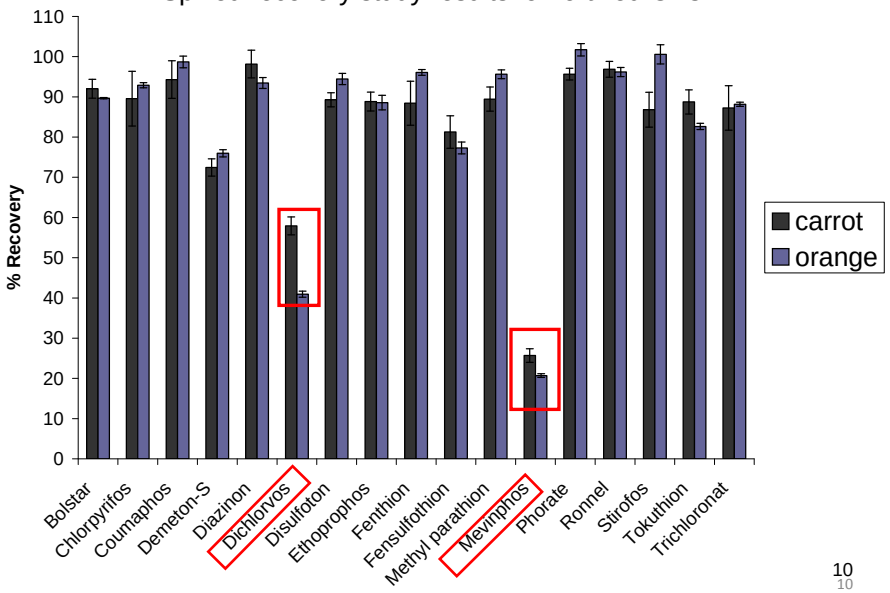
Spiked recovery study results for fortified OCs





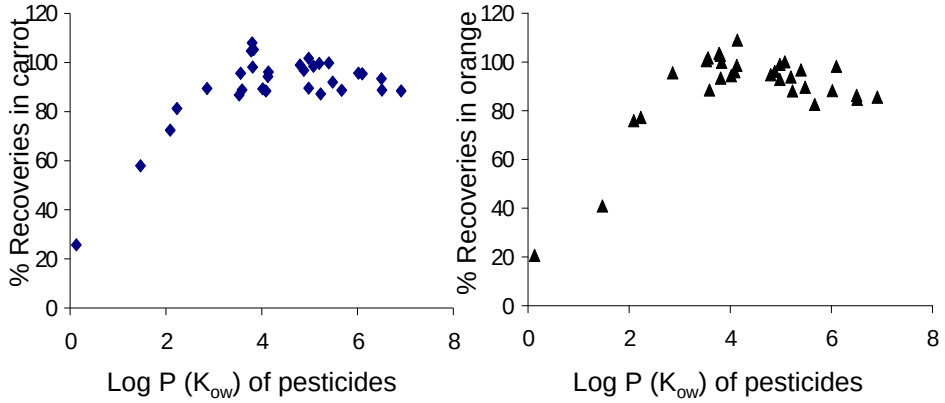

# Results and discussion

Spiked recovery study results for fortified OPs



# Results and discussion

Hydrophobicity and recovery




It is possible to estimate the recovery using the log P ($K_{ow}$) value of the pesticide.

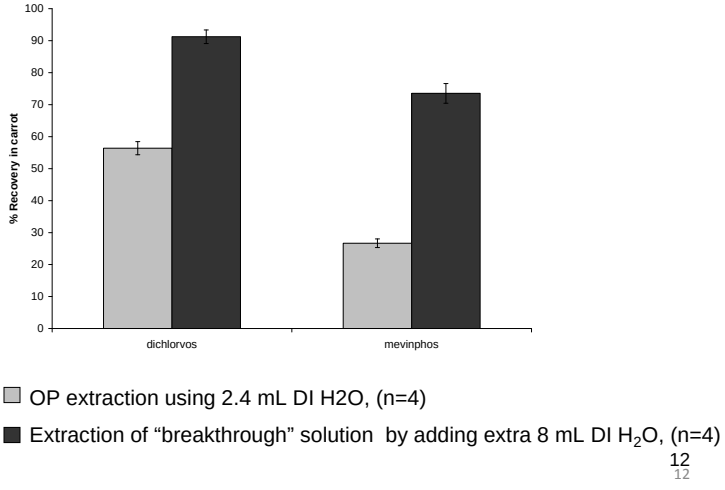


Database: U.S. National Library of Medicine.http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM

# Results and discussion

What did we do to improve recoveries for polar OPs ?

Adding large volume of DI $H_2O$ to "breakthrough solution"



# Linearity and LOD for studied OCs

| Pesticide | $r^2$ | | LOD (ppm) | |
|---|---|---|---|---|
| | carrot | orange | carrot | orange |
| Aldrin | 0.9979 | 0.9984 | 0.0172 | 0.0308 |
| Alpha-BHC | 0.9988 | 0.9988 | 0.0072 | 0.0216 |
| Beta-BHC | 0.9991 | 0.9986 | 0.0069 | 0.0234 |
| Delta-BHC | 0.9990 | 0.9983 | 0.0329 | 0.0333 |
| Gamma-BHC | 0.9991 | 0.9987 | 0.0100 | 0.0119 |
| 4,4′-DDD | 0.9986 | 0.9968 | 0.0039 | 0.0741 |
| 4,4′-DDE | 0.9955 | 0.9981 | 0.0141 | 0.0324 |
| 4,4′-DDT | 0.9974 | 0.9957 | 0.0220 | 0.0629 |
| Dieldrin | 0.9991 | 0.9990 | 0.0170 | 0.0388 |
| Endosulfan I | 0.9990 | 0.9987 | 0.0099 | 0.0334 |
| Endosulfan II | 0.9974 | 0.9984 | 0.0136 | 0.0592 |
| Endosulfan sulfate | 0.9990 | 0.9954 | 0.0182 | 0.0806 |
| Endrin | 0.9992 | 0.9986 | 0.0246 | 0.0624 |
| Endrin aldehyde | 0.9980 | 0.9987 | 0.0481 | 0.0272 |
| Heptachlor | 0.9984 | 0.9984 | 0.0116 | 0.0460 |
| Heptachlor epoxide | 0.9978 | 0.9986 | 0.0224 | 0.0221 |
| Methoxychlor | 0.9982 | 0.9965 | 0.0051 | 0.0506 |

$r^2$: coefficient of determination, LOD: limit of detection.

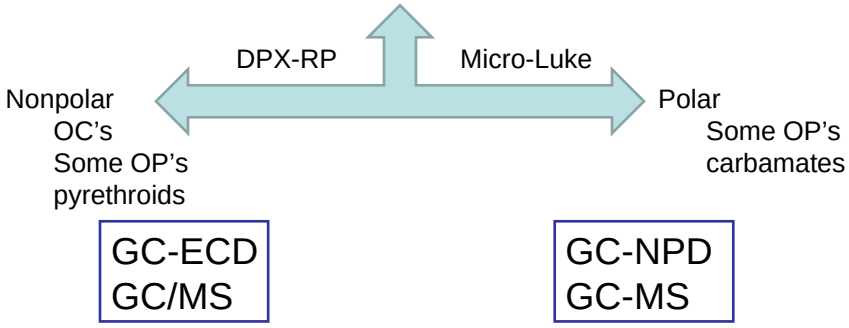


# Linearity and LOD for studied OPs

| Pesticide | $r^2$ | | LOD (ppm) | |
|---|---|---|---|---|
| | carrot | orange | carrot | orange |
| Bolstar | 0.9977 | 0.9983 | 0.0061 | 0.0277 |
| Chlorpyrifos | 0.9990 | 0.9996 | 0.0134 | 0.0374 |
| Coumaphos | 0.9976 | 0.9984 | 0.0066 | 0.0576 |
| Demeton-S | 0.9972 | 0.9995 | 0.0201 | 0.0201 |
| Diazinon | 0.9996 | 0.9997 | 0.0331 | 0.0213 |
| Dichlorvos | 0.9984 | 0.9967 | 0.0120 | 0.0153 |
| Disulfoton | 0.9995 | 0.9978 | 0.0101 | 0.0128 |
| Ethoprophos | 0.9997 | 0.9992 | 0.0143 | 0.0246 |
| Fenthion | 0.9990 | 0.9995 | 0.0051 | 0.0227 |
| Fensulfothion | 0.9977 | 0.9990 | 0.0093 | 0.0270 |
| Methyl parathion | 0.9991 | 0.9978 | 0.0139 | 0.0187 |
| Mevinphos | 0.9945 | 0.9989 | 0.0228 | 0.0352 |
| Phorate | 0.9996 | 0.9990 | 0.0049 | 0.0208 |
| Ronnel | 0.9993 | 0.9995 | 0.0058 | 0.0213 |
| Stirofos | 0.9992 | 0.9956 | 0.0224 | 0.0718 |
| Tokuthion | 0.9988 | 0.9983 | 0.0129 | 0.0355 |
| Trichloronat | 0.9994 | 0.9993 | 0.0189 | 0.0141 |

# II. DPX v. Luke Method

ACETONE solution




GC-ECD
GC/MS

GC-NPD
GC-MS

# II. DPX v. Luke Method

•Blend 100 grams of produce with 200 mls of Acetone
• Filter Extract through glass fiber filter paper

•NPD detector—no solvent exchange necessary (acetonitrile)

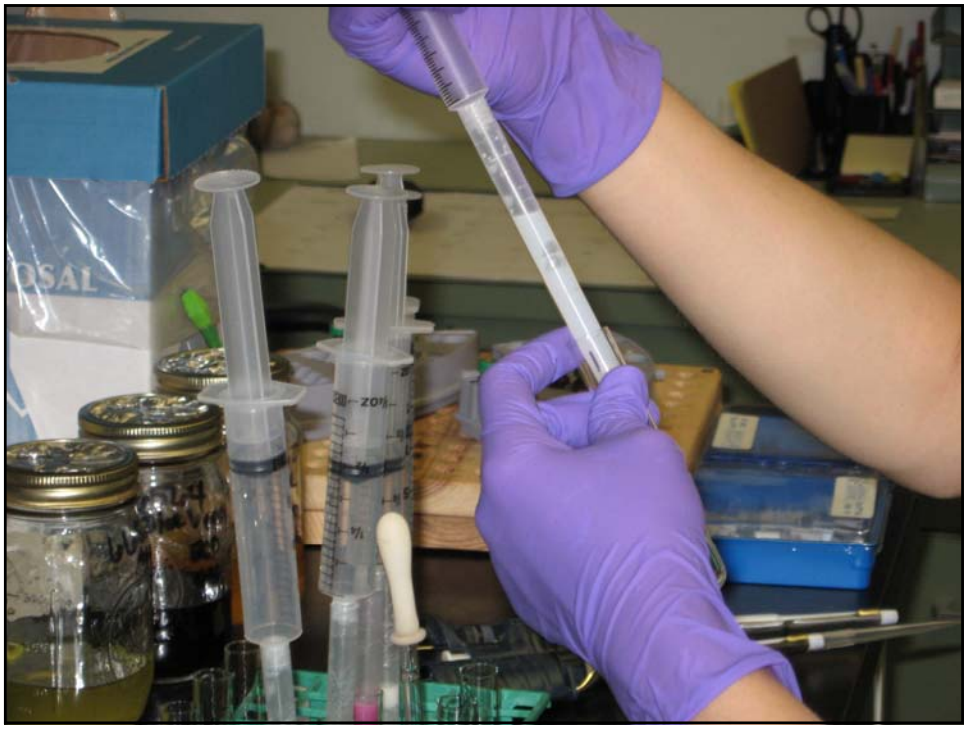
## II. DPX procedure ---- ECD method

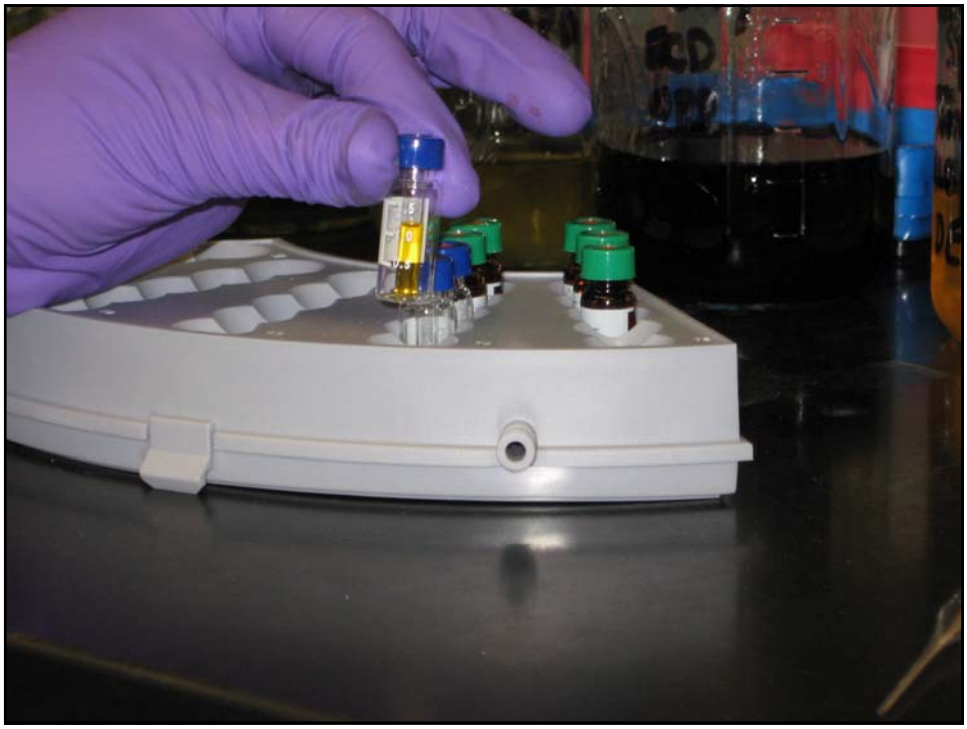
1. Place 2.5 mls of acetone extract into a glass test tube
2. Add 6.0 mls of D.I. water and 2.0 mls of saturated NaCl
3. Draw in about 3.5 mls of total solution to a DPX-RP tip, draw in about 10 mls of air to mix.
4. Stand for 30 sec. to 1 min, then dispense into a waste container
5. Repeat two more times and draw in all the solution to the same tip
6. Add 1.0 ml of D.I. water to DPX top and dispense to waste
7. Add 0.7 ml of Hexane-ethyl acetate (1+1, v/v) to DPX top and elute

Inject "top" organic layer (small volume of immiscible water at bottom).

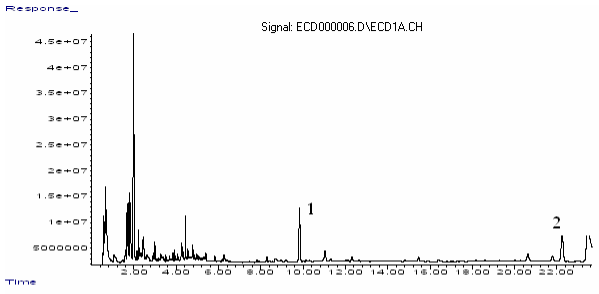
## II. MicroLUKE procedure ---- NPD method

1. Place 2.5 ml of acetone extract into a glass test tube
2. Add 1.0 ml of saturated NaCl, vortex mix 10 sec.
3. Add 2.5 ml of petroleum ether, vortex mix 10 sec.
4. Add 7.5 mls of methylene chloride, vortex mix 10 sec.
5. Stand for 5 min to let the two layers separated clearly
6. Upper layer drain through about 4.0 g of anhydrous sodium sulfate
7. Wash the sodium sulfate with 1 ml of methylene chloride
8. Transfer all the extract to C-tube and dry down to 0.5 ml



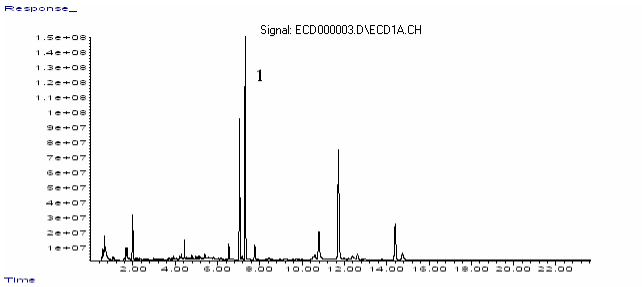


## II. GC/ECD data using DPX-RP



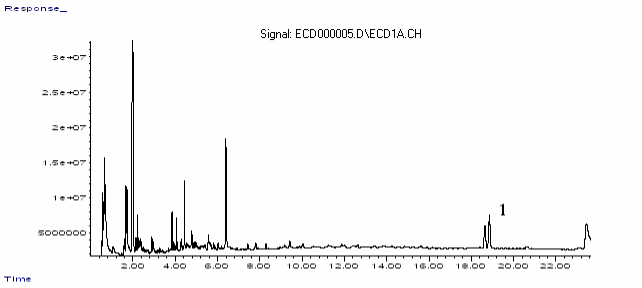

Blueberries
1-malathion
2-esfenvalerate

## II. GC/ECD data using DPX-RP



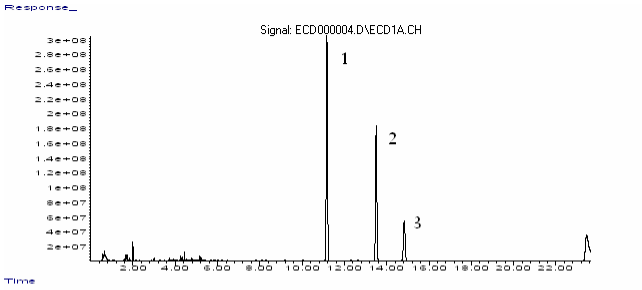

Celery
1-dicloran

## II. GC/ECD data using DPX-RP




Spinach
1- permethrin

# II. GC/ECD data using DPX-RP




Tomatoes
1-endosulfan I
2-endosulfan II
3-endosulfan sulfate and bifenthrin

# II. Results of DPX-RP and Micro-Luke (week 2)

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 65885 | SPK Strawberries @ 0.05 | ECD | 0.022 | 0.160 | Chlorothalonil | NT |
| | SPK Strawberries @ 20.0 | NPD | 20.72 | 27.85 | Carbaryl | 10.0 |
| | Strawberries - Incurred | ECD | 0.03 | 0.06 | Captan | 25.0 |
| | " | ECD | 0.27 | 0.41 | Bifenthrin | 3.0 |
| 65497 | Apple Bites | ECD | 0.22 | 0.29 | Captan | 25.0 |
| | " | NPD | 0.55 | 0.34 | DPA | 10.0 |
| 65498 | Red Apples | ECD | 0.42 | 0.45 | Captan | 25.0 |
| | " | ECD | 0.02 | 0.03 | Endosulfan | 2.0 |
| | " | NPD | 0.30 | 1.64 | DPA | 10.0 |
| 65881 | Celery | ECD | 0.28 | 0.38 | Dicloran | 15.0 |
| 65883 | Pears | ECD | 0.75 | 0.99 | Captan | 25.0 |
| | " | ECD | 0.03 | 0.05 | Bifenthrin | 0.5 |
| | " | ECD | 0.03 | 0.04 | L. Cyhalothrin | 2.5 |
| 62875 | Red Grapes | ECD | 0.14 | 0.20 | Dursban | 1.5 |
| 65579 | Yellow Squash | ECD | 0.0152 | 0.0238 | Endo I | 2.0 |
| | " | ECD | NR | 0.0036 | Endo II | 2.0 |
| | (Endo Sulfate and Bifenthrin | ECD | 0.0106 | 0.0327 | Endo Sulfate | 2.0 |
| | co-elute on DPX) | ECD | 0.1900 | NR | Bifentrhin | 0.4 |
| | " | ECD | 0.100 | NR | Chlorothalonil | 5.0 |

# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 47323 | Strawberries | ECD | 4.80 | 8.39 | Captan | 25.0 |
| | " | ECD | 0.0066 | 0.0142 | Endo I | 2.0 |
| | " | ECD | 0.0315 | 0.0666 | Endo II | 2.0 |
| | " | ECD | 0.1037 | 0.0224 | Endo Sulfate | 2.0 |
| 64288 | Tomatoes | ECD | 0.02 | 0.06 | Chlorothalonil | 5.0 |
| 65521 | Bell Peppers | ECD | 0.06 | 0.08 | Chlorpyrifos (Dursban) | 1.0 |
| | " | NPD | 0.14 | 0.21 | Chlorpyrifos (Dursban) | 1.0 |
| 65525 | Grape Tomatoes | ECD | 0.06 | 0.22 | Chlorothalonil | 5.0 |
| 66006 | Strawberries | ECD | 0.60 | 0.85 | Captan | 25.0 |
| 64287 | SPK - Orange @ 0.05 ppm | ECD | 0.0082 | 0.0314 | Chlorothalonil | |
| | SPK - Orange @ 5.0 ppm | NPD | 2.37 | 0.26 | Acephate | |

Week 3

# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 66126 | Blueberries | ECD | 0.08 | 0.15 | Esfenvalerate | 3.0 |
| 66129 | Baking Potatoes | ECD | 0.006 | 0.005 | p,p, DDE | NT - A.L. 1.0 |
| 63645 | Peaches | ECD | 0.17 | 0.32 | Phosmet | 10.0 |
| | " | NPD | 0.09 | 0.06 | L/L Phosmet - no clean-up | 10.0 |
| 65198 | Apricots | ECD | 0.09 | 0.20 | Phosmet | 5.0 |
| | " | NPD | 0.03 | 0.03 | L/L Phosmet - no clean-up | 5.0 |
| 63643 | Yellow Squash spiked @ 20.0 ppm | ECD | 13.96 | 20.07 | Chlorpropham | |
| | " | NPD | 13.68 | 21.22 | Chlorpropham | |
| | (Started NPD macro vs micro Luke | Liquid / Liquid micro Luke Extraction - no clean-up for NPD | | | | |
| 63643 | Yellow Squash spiked @ 10.0 ppm | NPD | 10.90 | 16.03 | Carbofuran | |

Week 4

# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 61824 | Cucumbers spiked @ 1.0 ppm | ECD | 0.733 | 0.975 | Chlorpyrifos ethyl (Dursban) | |
| | (NPD macro vs micro Luke) | NPD | 0.079 | 1.070 | Chlorpyrifos ethyl (Dursban) | |
| 61824 | Cucumbers spiked @ 1.0 ppm | ECD | 0.740 | 0.855 | Cyfluthrin | |

Week 5

# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 62931 | Celery | ECD | 4.202 | 5.329 | Dicloran | 15.0 |
| 66560 | Tomatoes | ECD | 0.473 | 0.437 | Bifenthrin | 0.2 |
| | " | ECD | 0.183 | 0.191 | Endo I | 2.0 |
| | " | ECD | 0.110 | 0.131 | Endo II | 2.0 |
| | " | ECD | 0.010 | 0.005 | Endo Sulfate | 2.0 |
| 66561 | Spinach | ECD | 2.792 | 2.572 | Permethrin c & t | 20.0 |
| 66563 | Apple Slices | NPD | 0.493 | 0.732 | DPA | 10.0 |
| | (NPD macro vs micro Luke) | NPD | 0.564 | 0.788 | Thiabendazole | 10.0 |
| 66564 | Blueberries | ECD | 0.079 | 0.071 | Esfenvalerate | 3.0 |
| | " | ECD | 0.075 | 0.122 | Malathion | 8.0 |
| | | NPD | 0.136 | 0.188 | Malathion | 8.0 |
| 66560 | Tomato-SPK @ 0.5 ppm L. Cyhalothrin | ECD | 0.393 | 0.370 | L. Cyhalothrin | |
| | Tomato-SPK @ 1.0 ppm DEF | ECD | 0.638 | 0.648 | DEF | |
| | " | NPD | 0.760 | 1.068 | DEF | |

Week 6

# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 60081 | Gala Apples | ECD | 0.0095 | 0.0129 | Dursban | 1.5 |
| | (NPD macro vs micro-Luke) | NPD | 0.0274 | 0.0471 | Dursban | 1.5 |
| | " | NPD | 0.7869 | 1.1844 | Thiabendazole | 10.0 |
| | " | NPD | NR | 0.3652 | Azinphos - methyl | 2.0 |
| | | ECD | NR | 0.2243 | Azinphos - methyl | 2.0 |
| 66251 | Peaches | ECD | 0.2464 | 0.4411 | Phosmet | 10.0 |
| | ( NPD macro vs. micro Luke) | NPD | 0.0758 | 0.0858 | Phosmet | 10.0 |
| 66638 | Snap Beans | ECD | 0.1232 | 0.158 | Cypermethrin c & t | 0.5 |
| 60082 | Cukes-SPK @ 2.0 ppm | ECD | 1.396 | 1.5526 | Cypermethrin c & t | |
| | Cukes-SPK @ 2.0 ppm | NPD | 0.4318 | 2.0617 | Demeton | |
| | (NPD - macro vs. micro Luke) | | | | | |

Week 7

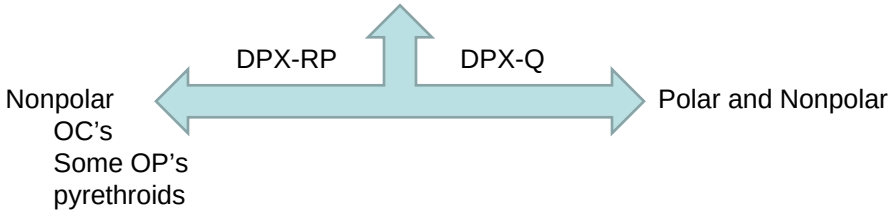
# II. Results of DPX-RP and Micro-Luke

| SAMPLE ID | MATRIX | DETECTOR | LUKE (PPM) | DPX (PPM) | COMPOUND | EPA TOLERANCE (PPM) |
|---|---|---|---|---|---|---|
| 65871 | spiked Tomatoes @ | | | | | |
| | 1.0 ppm Captan | ECD | 1.15 | 0.85 | Captan | |
| | 0.05 ppm Chlorothanonil | ECD | 0.01 | 0.03 | Chlorothalonil | |
| | 5.0 ppm Acephate | NPD | 2.25 | 2.46 | Acephate | |
| | 4.0 ppm Thiabendazole | NPD | 5.56 | 6.99 | Thiabendazole | |
| | (NPD macro vs micro Luke) | | | | | |

Week 8

# III. DPX-Q scheme

ACN solution




GC-ECD
GC/MS
HPLC/MS/MS

GC-NPD (FPD)
GC-MS
HPLC/MS/MS

# III. DPX-Q
# QuEChERS Tips

**DPX-Q (polar compounds):**

1. Take 2.5 mL ACN solution from original pre-sample preparation
2. Extract in and out of "DPX-Q" 2-3 times
   Rapidly remove water and salts (app. 1 minute)
3. Inject into **HPLC/MS/MS or GC/MS**

**OR solvent exchange and inject into GC-NPD**

*Takes several minutes (app. 20 min) to solvent exchange*

# III. DPX-Q for polar OP's (GC/MS)

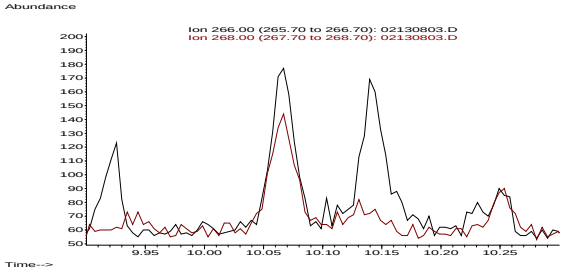
| Pesticides | Vegetable/fruit | Spiked conc. (ppm) | DPX method | % recovery (n=4) | %RSD (n=4) |
|---|---|---|---|---|---|
| Acephate | Spinach | 5.0 | GCB/$MgSO_4$/PSA[a] | 99.88 | 3.92 |
| | Orange | 5.0 | | 109.5 | 1.38 |
| Dichlorphos | Spinach | 0.5 | GCB/$MgSO_4$/PSA[a] | 91.56 | 2.09 |
| | Orange | 0.5 | | 100.55 | 2.00 |
| Mevinphos | Spinach | 0.5 | GCB/$MgSO_4$/PSA[a] | 90.38 | 1.86 |
| | Orange | 0.5 | | 94.51 | 3.79 |
| Methamidophos | Spinach | 2.0 | GCB/$MgSO_4$/PSA[a] | 96.32 | 9.60 |
| | Orange | 2.0 | | 103.06 | 2.59 |

Procedures:
•1ml acetonitrile solution, add 300μL of toluene (total 1.3 mL sample solution).
•Draw in DPX (150 mg mixed GCB/$MgSO_4$/PSA) three times from the bottom to remove sample matrix.
•Dispense and collect after cleanup (total about 1 mL).
•Add "external" standard.
•Dry down to 0.5 mL.
•Inject.

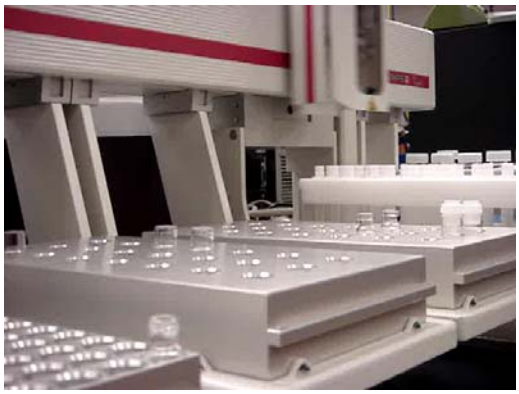
# III. DPX-RP for captan and chlorothalonil

| pesticides | Vegetable/fruit | Spiked conc. (ppm | DPX method | %recovery | % RSD |
|---|---|---|---|---|---|
| Captan | Spinach | 2.0 | SDVB | 104.48 | 1.19 |
| | Orange | 2.0 | SDVB | 91.81 | 5.35 |
| chlorothalonil | Spinach | 0.5 | SDVB | 113.57 | 1.91 |
| | Orange | 0.5 | SDVB | 115.28 | 9.80 |

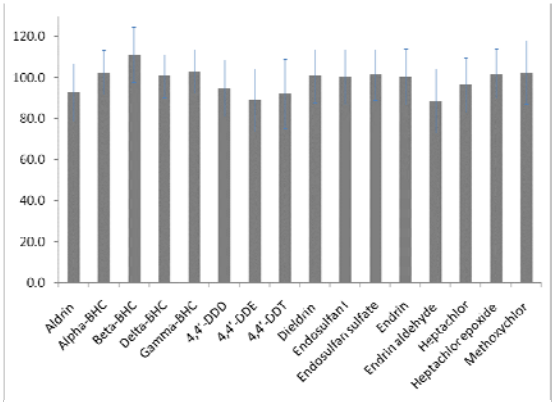
10 ppb chlorothalonil extracted by DPX-RP using GC/MS (SIM)



## IV. DPX -- GERSTELIZED



## IV. % Recovery of OCs in carrot extracted using automated DPX with GERSTEL MPS=2




OCs: spiked 0.5ppm in carrot

# Conclusions

DPX-RP provided “cleaner” extracts than QuEChERS (modified) for GC-ECD data.

High recoveries were achieved for all of the studied pesticides by combining DPX-RP and DPX-Q, with %RSD lower than 10%. This indicates a good accuracy and precision of the current method.

DPX method is more environmentally friendly.

Average coefficient of determination, $r^2 > 0.995$, indicates a good linearity of the current DPX method.

Current research focuses on full automation of DPX using GERSTEL MPS with:

- Large volume injection (LVI, GERSTEL PTV)
- fast GC (ECD, FPD)
- HPLC/MS/MS



# Acknowledgments

Sherry Garris, SC Dept. of Agriculture; participation in QuEChERS Study

GERSTEL, MPS-2

DPX Labs, LLC, DPX supplies

University of South Carolina, Dept. of Chemistry and Biochemistry