



Health
Canada Santé
Canada

Your health and
safety... our priority.

Votre santé et votre
sécurité... notre priorité.

Application of the QuEChERS extraction method for the analysis of pyrethrin and pyrethroid pesticides in fin and non-fin fish.

Veronica Roscoe¹, Judy Judge¹, Dorothea F. K. Rawn²

¹Health Products and Food Program, Winnipeg, Manitoba

²Bureau of Chemical Safety, Food Research Division, Ottawa, Ontario

Presentation: Florida Pesticide Residue Workshop, July 2009



Canada 

Overview

- Background
 - Goals of project
 - Sampling details
- Method of analysis
- Method performance data
- Survey and QC Results
- Conclusions



Project: Organic and Inorganic Contaminants in Retail Freshwater Fish and Seafood Products

- Establish if trace levels of the natural pyrethrins and synthetic pyrethroids (cypermethrin & deltamethrin) are present in fish and if differences in concentration levels exist between fish collected from the wild relative to levels in fish raised in farm situations.
- The data developed will be used for the determination of Canadian exposure of these pesticides through consumption of fish and non-fin fish grown domestically and internationally.



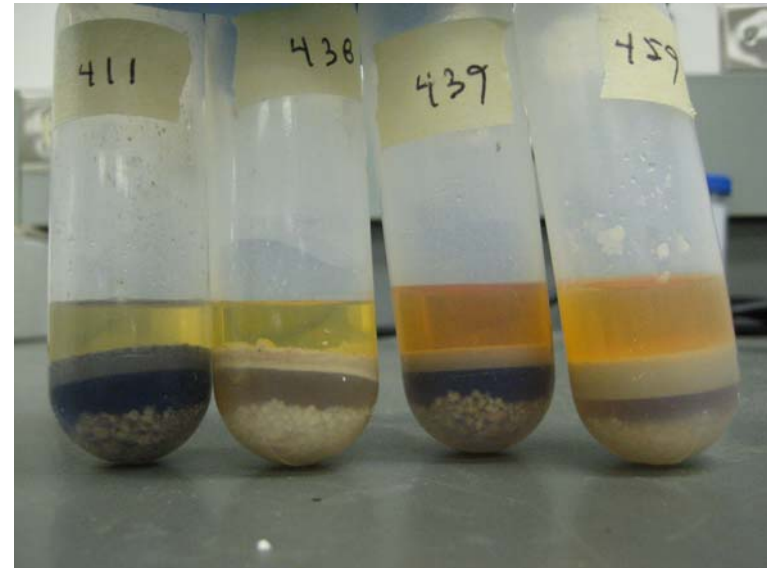
Survey: Sample collection and preparation

- Samples of fresh and salt water fin and non-fin fish (141) were collected at retail stores in BC, ON and NS during winter and spring of 2002
 - Arctic char, crab, marlin, mussels, oysters, prawns, salmon, shark, shrimp, tilapia, trout and tuna
- Country of origin
 - Canada (77% of samples), Chile, China, Ecuador, India, Jamaica, Taiwan, Thailand and US
- Farmed (72% of samples) and wild
- Fresh, frozen, previously frozen or live
- Edible portions were homogenized



QuEChERS Extraction Method

- Weigh 5 g sample into 50 mL FEP centrifuge tube
- Add 250 ng *cis*-permethrin (phenoxy- $^{13}\text{C}_6$) surrogate standard
- Add 5 mL 1% acetic acid in acetonitrile
- Add 2 g anhydrous MgSO_4 and 0.5 g NaAc
- Shake and centrifuge



QuEChERS Clean-up Method

- Transfer 1 mL of the extract to the QuEChERS dispersive tubes*
 - 150 mg MgSO_4
 - 50 mg PSA
 - 50 mg C18
- Shake and centrifuge

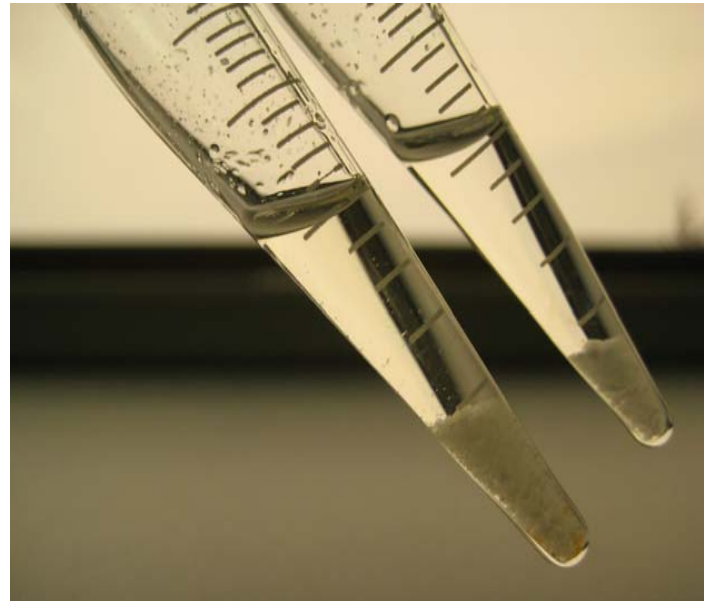


* Chromatographic Specialties p/n UCTCUMPSC18CT or Restek p/n 26124



Final cleanup

- Transfer 0.5 mL extract to graduated tube and evaporate solvent just-to-dryness
- Add 50 ng *trans*-permethrin (phenoxy- $^{13}\text{C}_6$) and bring to 0.5 mL with TMP
- Add MgSO_4 to reach the 0.2 mL mark on tube and vortex.
- Inject 2 μL



Instrumental Analysis

Thermo Trace GC/MS in negative CI mode

GC Conditions

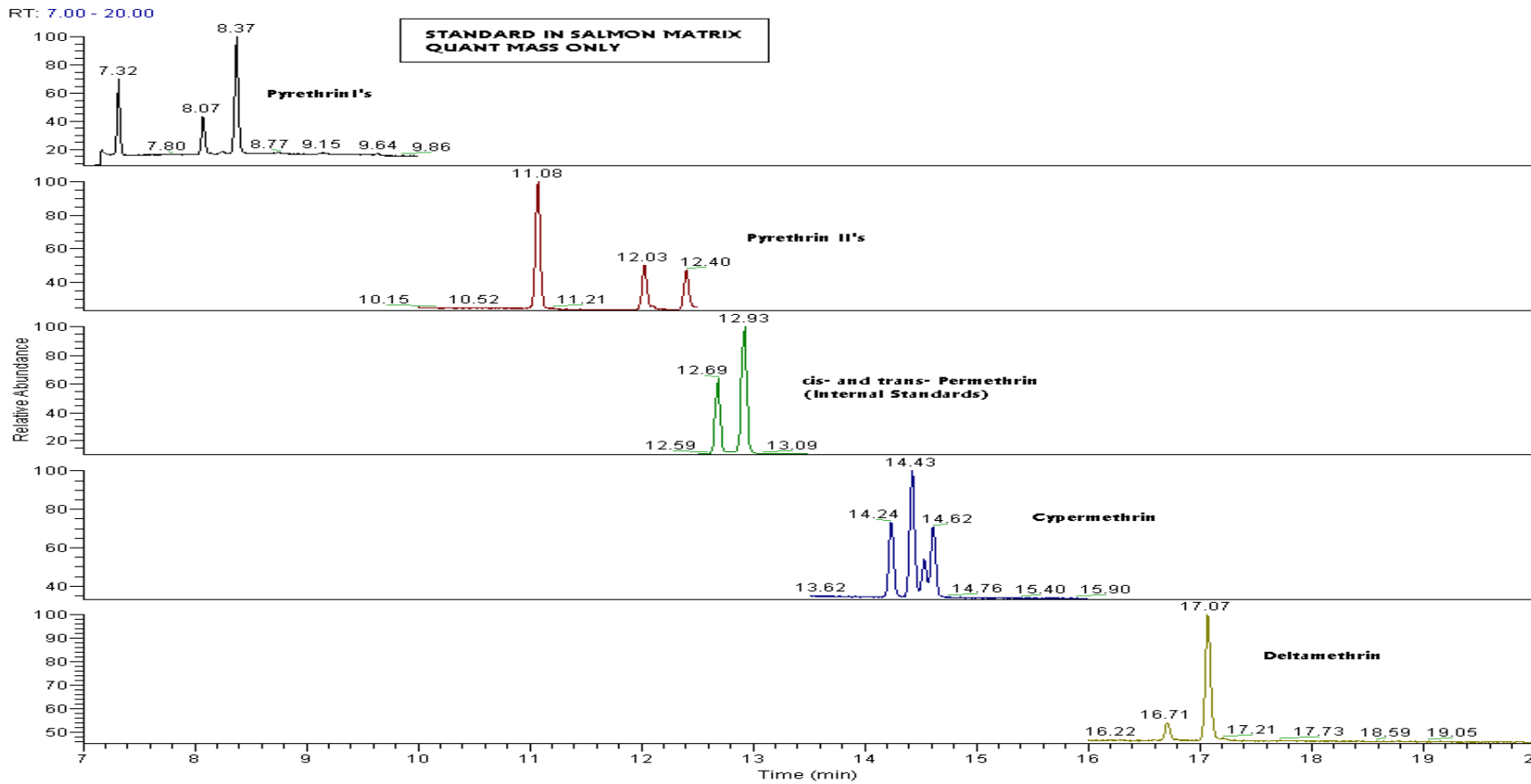
- HP-5, 30m X 0.32 mm with 0.25 μm film
- Splitless, 240° C,
- Oven program
 - 80° C, hold 1 min
 - 50° C/min to 200° C
 - 5° C/min to 285° C
 - 50° C/min to 325° C, hold 5 minutes
- Transfer line 250° C

MS Conditions

- Source 150° C
- Methane reagent gas
- Selected Ion Monitoring mode



Extracted Ion Chromatogram: Standard at 20 ng/mL



Method performance data

Limits of detection (LOD) & quantification (LOQ)

Bias and repeatability

Precision



Limits of Detection and Quantification

- Standards were prepared in matrix at 4 ng/mL and 1 ng/mL.
- n=7 injections of 4 ng/mL std, n=3 injections of 1 ng/mL std
- LOD = 3 x S/N
- LOQ = 10 x S/N

Method LOD/LOQ in Salmon Matrix		
Analyte	LOD (ng/g)	LOQ (ng/g)
Total pyrethrins	0.5	2
Cypermethrin	0.3	1
Deltamethrin	0.3	1



Assessing Bias and Reproducibility

- salmon samples containing no detectable amount of analyte were fortified at three levels
- each sample was additionally fortified with 50 ng/g surrogate internal standard (*cis*-permethrin (phenoxy- $^{13}\text{C}_6$)) prior to extraction
- trans*-permethrin (phenoxy- $^{13}\text{C}_6$) was added to each sample prior to preparing at final volume (performance internal standard)

<i>Mean Recovery of cis-permethrin (phenoxy-$^{13}\text{C}_6$)</i>		
Sample Set	% Recovery	% RSD
4 ng/g (n=5)	77	6.0
20 ng/g (n=7)	71	4.8
40 ng/g (n=7)	79	7.5



Assessing Bias and Reproducibility cont'd

Analyte	Spike level (ng/g)	Mean Calc'd Amt (ng/g)	% RSD	Corrected Recovery (%)
Pyrethrins	3.89	4.48	6.2	115
	19.4	22.4	4.0	115
	38.9	44.6	3.9	114
Cypermethrin	3.85	4.12	2.9	107
	19.3	19.7	4.3	102
	38.5	39.9	14	104
Deltamethrin	4.13	4.16	2.5	101
	20.6	21.4	3.3	104
	41.3	28.8	12	70



Assessing Precision, within- and between-run

- 3 salmon samples were spiked at 4 ng/g and at 20 ng/g.
- sets completed on 5 different days

Analyte	Spike level (ng/g)	Mean Calc'd Amt (ng/g)	Within-run %RSD	Between-run %RSD
Pyrethrins	3.89	3.95	6	9
	19.4	19.5	5	12
Cypermethrin	3.85	3.86	7	10
	19.3	19.3	6	5
Deltamethrin	4.13	3.41	6	16
	20.6	17.8	5	16



Survey results

- No analytes detected except in salmon
- **7 of 22 samples of salmon were found to contain cypermethrin**
 - Domestic
 - Farmed
 - Range: 0.26 – 6.44 ng/g
 - Mean = 1.87 ng/g



QC Results: Mean Spike Recovery data (20 ng/g)

Matrix	Pyrethrins (%)	Cypermethrin (%)	Deltamethrin (%)	<i>cis</i> -permethrin (¹³ C ₆) (%)
Salmon (n=11)	117	110	106	70
Arctic Char (n=1)	131	111	98	82
Trout (n=2)	110	107	102	80
Mussel (n=1)	113	105	113	98
Oysters (n=2)	111	113	101	84
Marlin (n=1)*	176	157	165	64
Shrimp (n=3)*	174	169	216	107
Tilapia (n=2)*	200	181	202	72
Crab (n=2)*	137	176	190	82
Mean (w/o *)	115	110	105	78
RSD (w/o *)	9	16	14	14



QC Results: Comparison of recoveries quantified using matched matrix vs (salmon matrix)

Commodity	Pyrethrins	Cypermethrin	Deltamethrin	<i>cis</i> -permethrin ($^{13}\text{C}_6$)
Marlin	78% (176%)	127% (157%)	132% (165%)	77% (65%)
Shrimp	76% (171%)	98% (172%)	100% (237%)	88% (109%)
Tilapia	122% (239%)	117% (220%)	121% (256%)	88% (72%)
Tilapia	83% (160%)	79% (142%)	69% (147%)	88% (72%)



Conclusions

- **QuEChERS extraction method has been successfully demonstrated to apply to the analysis of the natural pyrethrins and synthetic pyrethroid pesticides in fin and non-fin fish tissue.**
- **GC/MS in negative Chemical Ionization mode provided the required sensitivity to detect at low ppb levels.**
 - **Limits of detection were established at 0.3 ng/g for cypermethrin and deltamethrin and at 0.5 ng/g for total pyrethrins.**
- **The use of standards prepared in matrix and a surrogate internal standard provided reproducible results however, for some matrices, quantification using standards prepared in the exact same matrix would be required.**
 - **Average recoveries achieved during survey for a variety of species compared well with method validation data using salmon.**



Conclusions cont'd

- Of the residues studied in this project, cypermethrin was the only detected compound, at concentrations ranging from 0.26 ng/g to 6.4 ng/g with a mean level of 1.9 ng/g.
- In all cases, cypermethrin residues were well below the General Maximum Residue Limit (MRL) of 100 ng/g established in the Canadian Food and Drug Regulations for chemicals not listed in Table 2 of Division 15.



Questions?

