



Biomonitoring of Exposure to Pesticides

Dana B. Barr, Ph.D.
Chief, Pesticide Laboratory
*National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, GA USA 30341*

SAFER • HEALTHIER • PEOPLE™

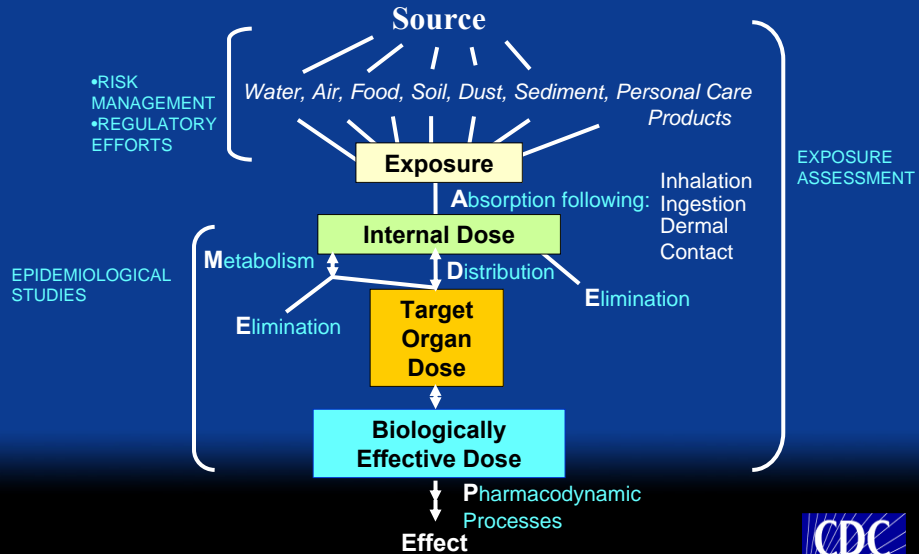
Overview



- Background
- CDC's Biomonitoring Program
- Pesticide Methods
 - ◆ Sulfonyl urea herbicides
 - ◆ Triazine herbicides
- Conclusions



Exposure-Effect Continuum for Environmental Chemicals



Angerer et al. *Tox Sci* **93**(1) 3-10 (2006)

Biomonitoring

Assessment of human exposure to an environmental chemical by measuring the parent chemical (or its metabolite or reaction product) in human blood, urine, milk, saliva, adipose, or other tissue.

History of Biomonitoring

- 1880s Occupational monitoring
- 1980s Selected “exposed” population
 - Pharmacokinetic studies
 - Close proximity to emissions
- 1990s General population exposures
 - Limited studies
 - Adult populations
 - Paraoccupational exposures
- 2000s Large scale studies
 - Epidemiologic studies
 - General population



CDC's Biomonitoring Program

- Almost 40 years old
- Pesticide biomonitoring is about 30 years old
- Pesticide biomonitoring has expanded exponentially over last decade
- State of the art instrumentation
- Selective instrumentation, isotope dilution
- Cutting edge in biomonitoring



Biomonitoring tells us:

RISK ASSESSMENT

- What we are exposed to and how much (with caveats)
- Whether minorities, children, and underserved populations are more highly exposed

RISK MANAGEMENT

- Whether interventions actually reduce exposure



Biomonitoring Data are Not Created Equally

- Specificity of biomarker (chemical or route)
- Biological persistence of the biomarker
- Analytical ability to accurately measure biomarker
- Interpretability of the biomarker data (biomarker be linked to exposure)
- Inter- and intraperson variability



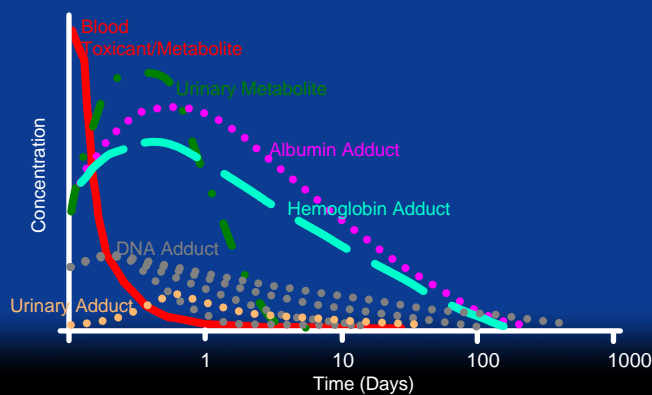
Biomonitoring Hinges on the Analytical Measurement

- All numbers are not created equally
 - ◆ Accuracy
 - ◆ Precision
 - ◆ Specificity
 - ◆ Linearity & Range
 - ◆ Limit of detection
 - ◆ Ruggedness/Robustness
- QA/QC Program
- Interlaboratory comparison



Needham et al. J Toxicol Environ Health A 65: 1893-1908 (2002)

Post-Exposure Fate of a Nonpersistent Toxicant in Blood and Urine



Needham and Sexton, JEAEE 10:611-629 (2000)



CDC's Pesticide Methodology

- Multiple human/animal matrices: urine, blood serum/plasma, breast milk, meconium, amniotic fluid, tissue homogenate
- Environmental matrices: water, dust, orange and apple juices



CDC's Pesticide Methodologies

- Metabolites
 - ◆ Insecticides (OP, pyrethroid, carbamate)
 - ◆ Herbicides (triazine, chloracetanilides, phenoxy acids)
 - ◆ Fungicides (dithiocarbamates, others)
 - ◆ Fumigants (2)
- Parent pesticides
 - ◆ Insecticides
 - ★ OP, pyrethroids
 - ◆ Fungicides
 - ◆ Herbicides



CDC's Pesticide Methodologies

- > 100 target analytes representing >200 pesticides or related chemicals
- >15 methods routinely applied
- Mass spectrometry-based (HPLC-MS/MS, GC-MS/MS, or GC-HRMS)
- Isotope dilution quantification
- CLIA certified (semi-annual proficiency testing, documentation of performance, calibration verification, annual recertification)



Methods Development and Maintenance

- Methods include diverse chemicals with different physio-chemical properties
- Highlight two methods with different complex considerations
 - ◆ Sulfonyl urea herbicides – analyte lability, validation considerations
 - ◆ Atrazine exposure assessment – are we measuring the correct analytes?



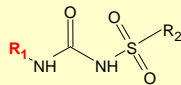
Background

- 1975 Dr. George Levitt discovered
- 1977 first SU herbicide was patented
- 1982 first SU herbicide marketed as “Glean”
- 23 commercialized SU herbicides (1998)
- Chemical scope very large (unlimited chemical variations)
- Developed for use on every major food crop
- Touted as “Safe”
- (ex. Oral rat LD₅₀ = 5000 mg/kg)
- Applied at 100th the rate of other herbicides
- Thermally labile



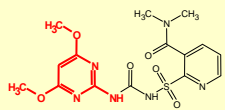
Question: What are we measuring?
Answer: Active ingredient (intact)

Generic structure

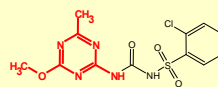


pyrimidinyl sulfonyl ureas

triazinyl sulfonyl ureas



Nicosulfuron



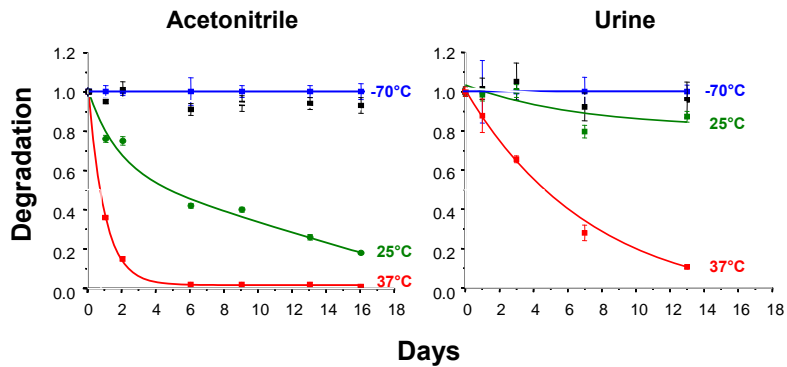
Chlorsulfuron

Urine metabolites



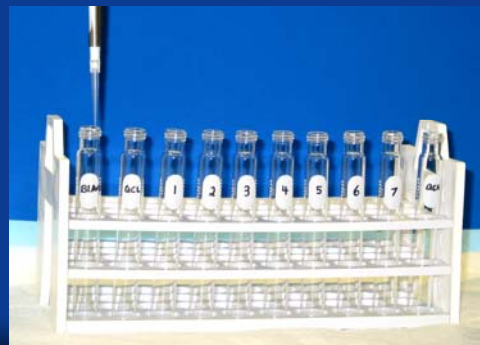
Degradation

Halosulfuron-methyl



Sample Prep Method Steps

- Spike Samples tubes with ISTD
- Aliquot 2 mL urine sample
- Add 1.5 mL buffer (pH 5)
- Vortex mix
- Condition SPE column 1 mL MeOH
- Equilibrate SPE column 1 mL H₂O
- Load sample
- Wash column 5% MeOH
- Elute 2 mL MeOH
- Blowdown 30 minutes (10psi/N₂)
- Add 0.35 mL MeOH
- Vortex mix
- Blowdown 7 minutes
- Reconstitute 50 uL acetonitrile
- Vortex mix
- Transfer to injection vials
- Load in to autosampler



LC Instrumentation

Instrument: Agilent 1100
1 mL/min.

Column: Phenomenex
Synergi
Polar – RP 80A
100 X 4.6 mm
4 μ particle

1 mL/min.

Gradient

35 Degrees C

Chromatogram



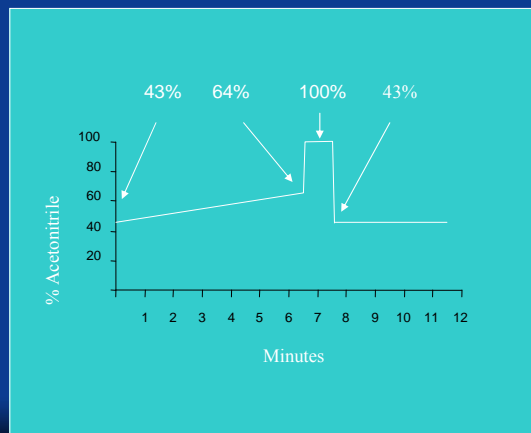
LC Instrumentation

Instrument: Agilent 1100

Column: Phenomenex
Synergi
Polar – RP 80A
100 X 4.6 mm
4 μ particle

Gradient

Chromatogram



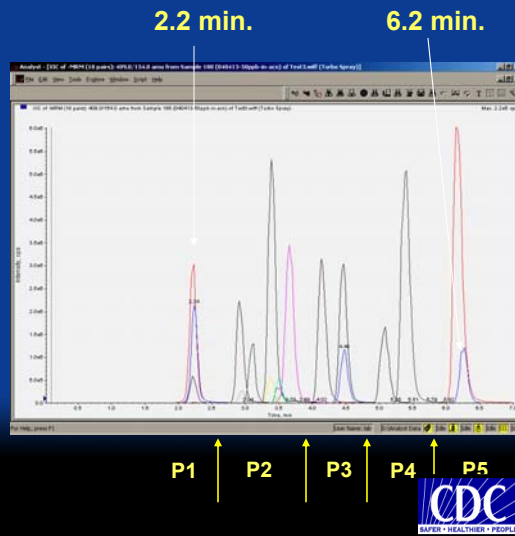
LC Instrumentation

Instrument: Agilent 1100

Column: Phenomenex
Synergi
Polar – RP 80A
100 X 4.6 mm
4 μ particle

Gradient

Chromatogram

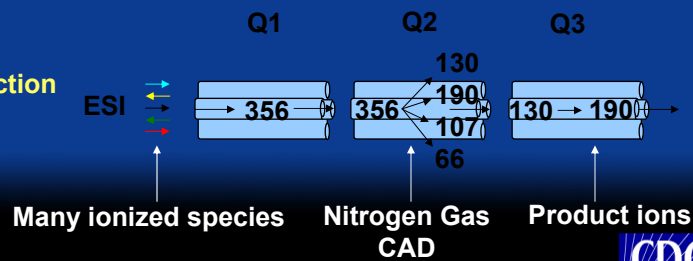


MS/MS Analysis

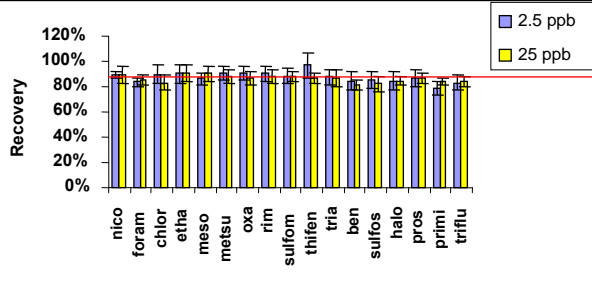
Instrument:
Applied Biosystems
API 4000

Ionization:
Pneumatically Assisted ESI
(Turbo Ionspray)
Negative ions

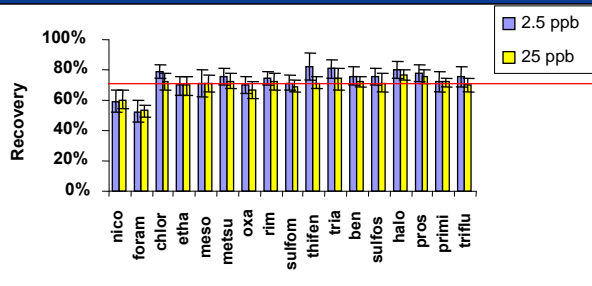
Mode:
Selective Reaction
Monitoring



Extraction and Total Analyte Recoveries



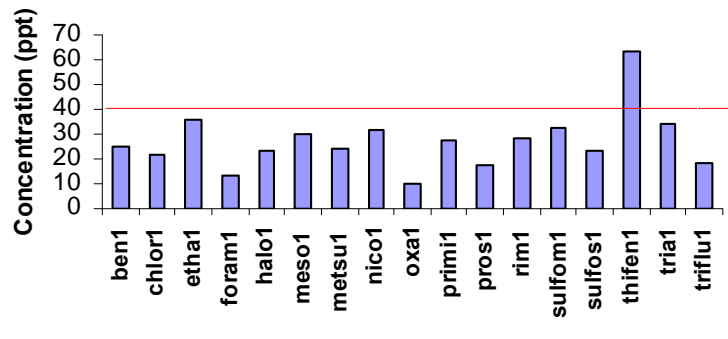
Average = 87%



Average = 72%



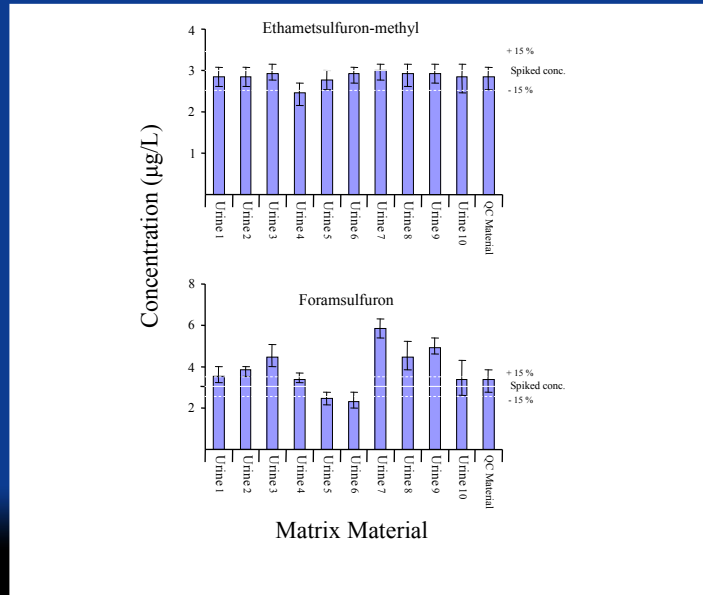
Limits of Detection



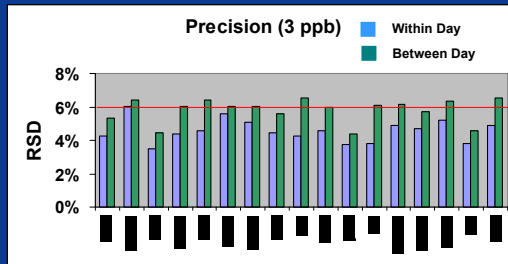
40 ppt



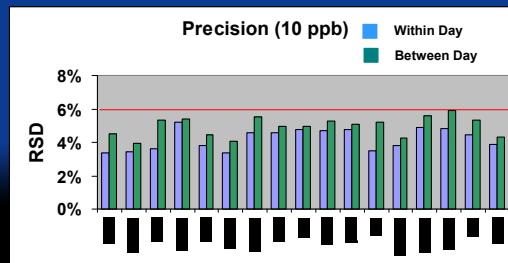
Accuracy: reevaluation of validation process



Precision



6 %



6 %



Sulfonyl Urea Summary

We can determine urinary concentrations of 17 of the 19 SU herbicides that are registered for use in US

LODs in the low ppt

Throughput = 100+ samples per day

Relative standard deviations less than 7%

Total recoveries average about 72%

Because lacking labeled standards for all compounds, matrix effects can contribute to inaccuracy for some analytes

Required more extensive validation

Requires rigorous monitoring including std addition



Atrazine Background

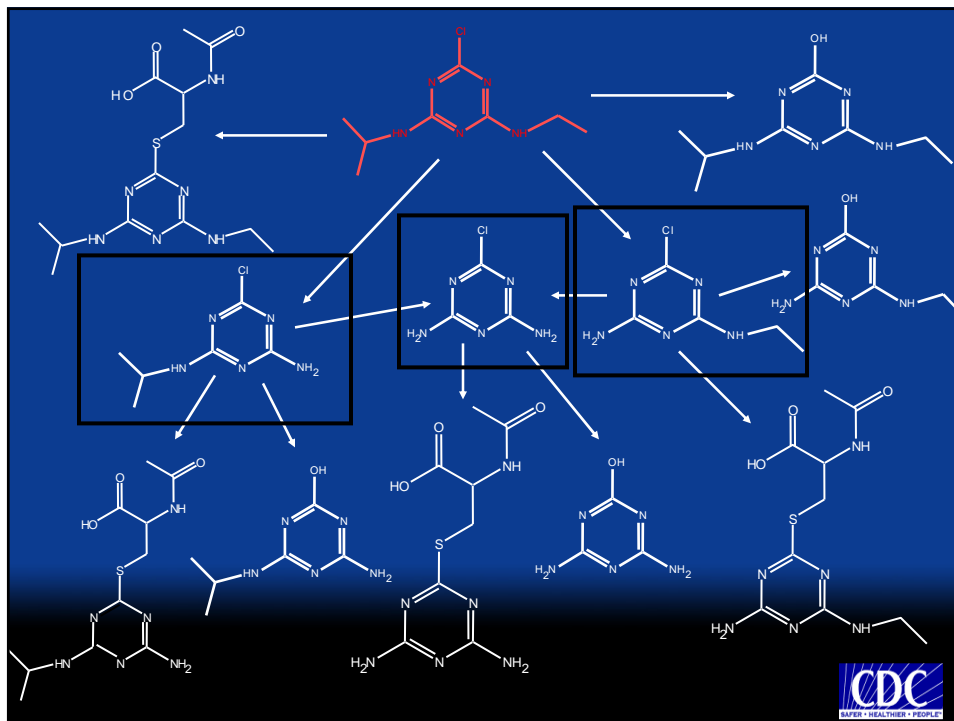
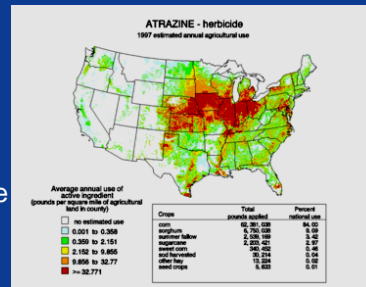
- Herbicide for controlling broadleaf and some grassy weeds by preventing photosynthesis
- In 2001, second most abundantly applied pesticide (75-80 million pounds)
- Most abundantly used over previous 15 years
- Pounds applied have remained consistent over 15 year span



Background

- Used predominantly in agriculture in Midwest (e.g., corn)
- In Southeast, predominant uses include sugar cane farming and commercial and residential turf applications
- Potential for contamination of surface and ground waters that supply municipal water systems

Source: USGS



Summary of Metabolite Studies

Study	Model	DACT	DIA	DEA	AM	ATZ	DACT-M	ATZ-OH	Ammeline
Novartis	Rat	1	minor	minor	ND	ND	1	ND	ND
Bakke	Rat	ND	1	1	ND	ND	ND	1	1
Bradway	Rat	1	1	1				1	1
Erickson	Swine			1					
Novartis	Monkey	1	4	2	3	ND	After IV	ND	ND
Catenacci	Human	1	minor	minor		minor			
Lucas	Human (D)	minor	minor	minor	1				
Buchholtz	Human (D)	2?	minor		1		2?		
Perry	Human	NA	NA	2	1	NA	NA	NA	NA
Catenacci	Human	1	minor	2		minor			



Analytical Methodology

- Simple, repeatable methodology
- Online SPE extraction using column switching
- 200 µl urine
- Analysis using HPLC-MS/MS
- 2 ion pairs monitored for each metabolite
- Isotope dilution quantification
- LODs range 0.1 – 2.8 ng/mL
- RSD typically < 10%
- Current throughput ~ 40 samples/day/instrument



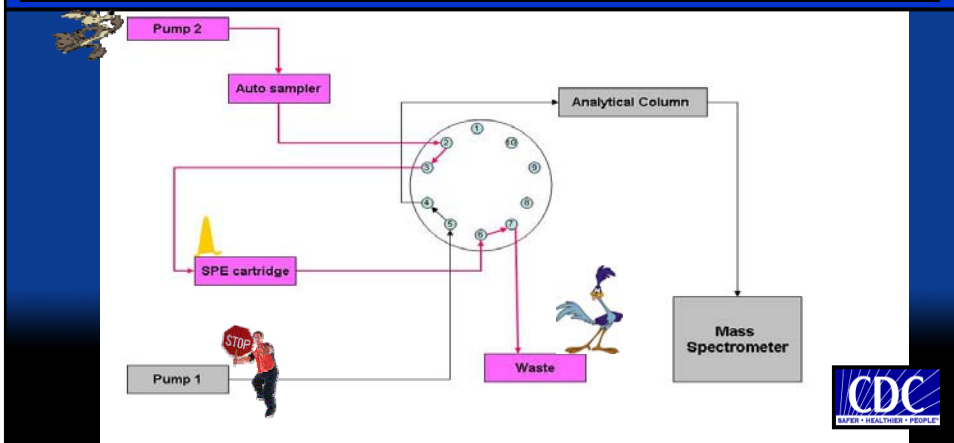
Analytical Methodology



2P/10Port Valve Switching System



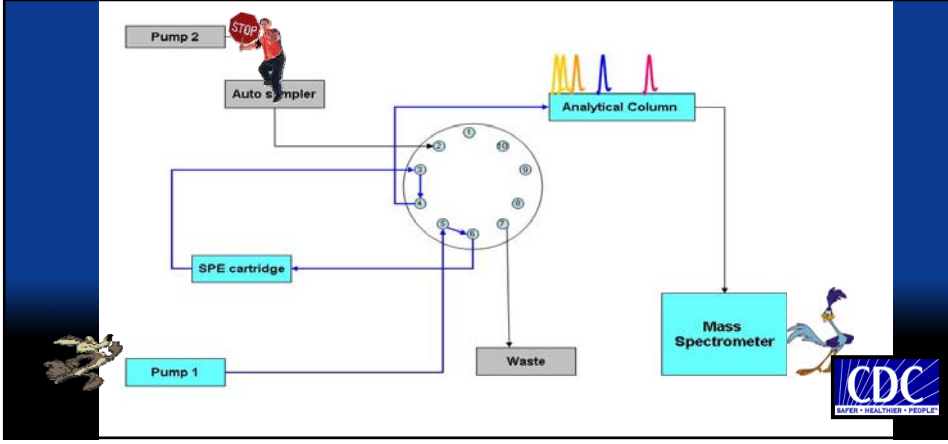
Time (min.)		0.00	1.50	1.51	3.00	3.01	10.00	13.00	14.00	17.00	18.00	20.00	20.01	22.00
Pump 1	0.1%formic acid/Water (%)	80	80	80	80	80	20	20	0	0	80	80	80	80
	0.1%formic acid/MeOH (%)	20	20	20	20	20	80	80	100	100	20	20	20	20
	Flow rate (mL/min.)	0.0	0.0	0.0	0.0	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.0
Pump 2	0.1%formic acid/Water (%)	100	100	90	90	100	100	100	100	100	100	100	100	100
	0.1%formic acid/MeOH (%)	0	0	10	10	0	0	0	0	0	0	0	0	0
	Flow rate (mL/min.)	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0



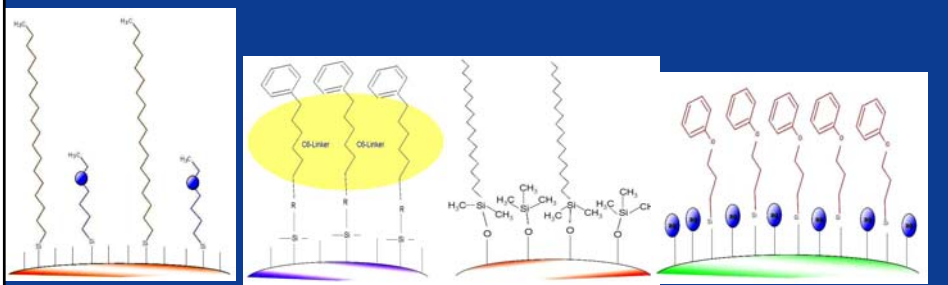
2P/10Port Valve Switching System



Time (min.)	0.00	1.50	1.51	3.00					18.00	20.00	20.01	22.00	
Pump 1	0.1%formic acid/Water (%)	80	80	80	80	20	20	20	20	80	80	80	80
	0.1%formic acid/MeOH (%)	20	20	20	20	80	80	80	80	20	20	20	20
	Flow rate (mL/min.)	0.0	0.0	0.0	0.0	0.2	0.3	0.3	0.3	0.3	0.3	0.0	0.0
Pump 2	0.1%formic acid/Water (%)	100	100	90	90	100	100	100	100	100	100	100	100
	0.1%formic acid/MeOH (%)	0	0	10	10	0	0	0	0	0	0	0	0
	Flow rate (mL/min.)	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0



HPLC Analytical Column Selection



“Fusion-RP” (a)
Polar embedded
C18 column
(2.5µm, 100Å, 3.0x100mm)

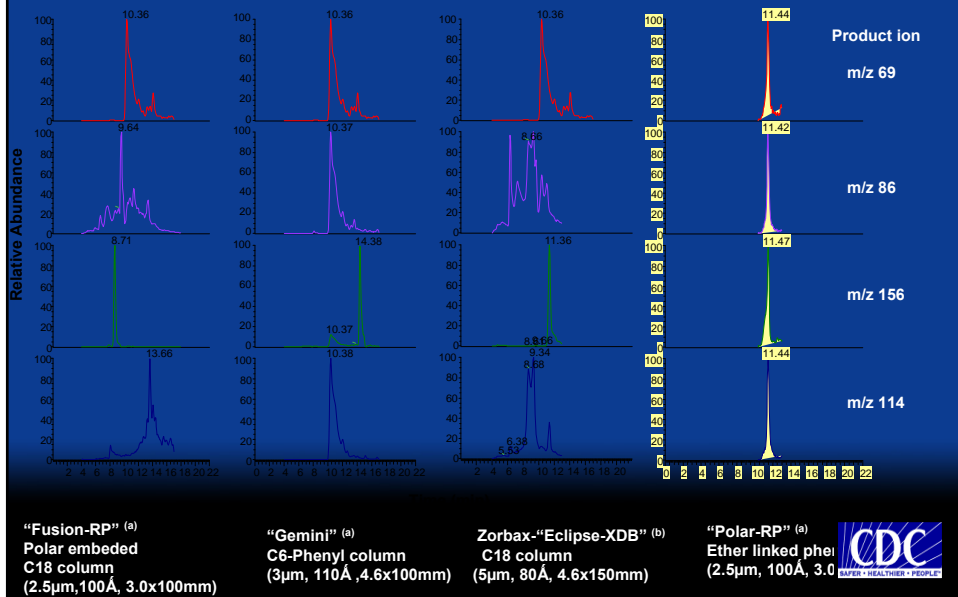
“Gemini” (a)
C6-Phenyl column
(3µm, 110Å, 4.6x100mm)

Zorbax-“Eclipse-XDB” (b)
C18 column
(5µm, 80Å, 4.6x150mm)

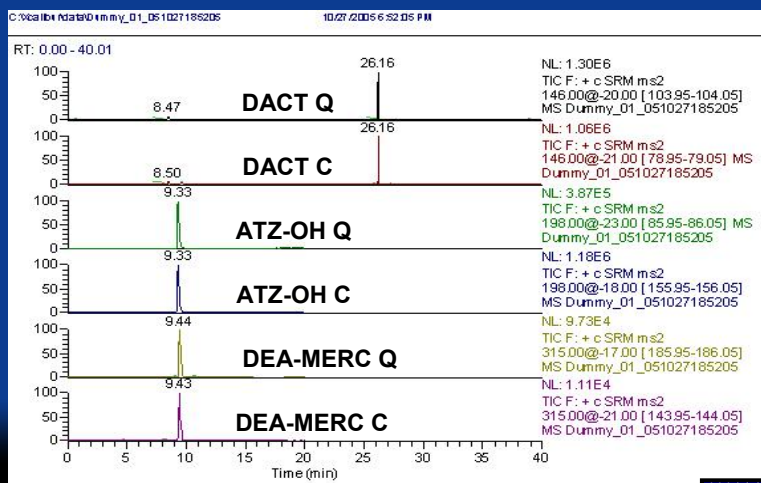
“Polar-RP” (a)
Ether linked phenyl
column
(2.5µm, 100Å, 3.0x100mm)



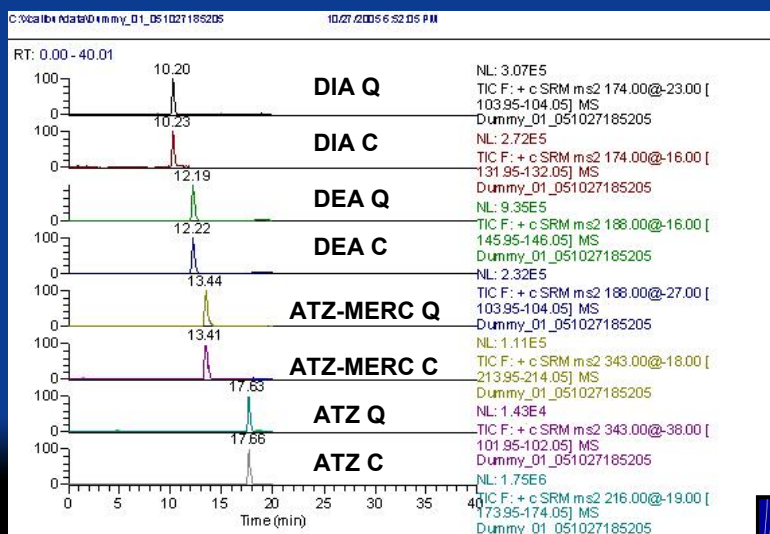
HPLC Analytical Column Selection



Filtered Ion Chromatogram



Filtered Ion Chromatograms



Method Specifications

Analyte	(% SPE recovery (N=3) Mean±SD		Accuracy (N=5) Mean±SD (% of expected level)				LODs ng/m L	MMD L ng/mL
	5 ng/mL	25 ng/mL	5 ng/mL	25 ng/mL	50 ng/mL	100 ng/mL		
ATZ-OH	94±13	92 ± 6	5.0±0.4 (100.8)	26.5±1.6 (105.8)	47.3±1.6 (94.5)	96.5±8.9 (96.5)	0.2	2.5
DACT	69±8	67±10	5.6±0.5 (112.7)	25.1±1.2 (100.4)	47.8±1.7 (95.7)	94.1±6.4 (94.1)	2.8	2.5
DEAM	100±14	103±16	4.3±0.4 (86.6)	25.3±1.3 (101.1)	48.3±2.0 (96.7)	98.2±6.6 (98.2)	0.4	0.5
DIA	97±10	99±14	4.9±0.1 (98.3)	25.0±1.6 (100.1)	48.8±1.9 (97.5)	94.6±7.8 (94.6)	0.3	0.5
DEA	86±3	94±3	5.0±0.2 (99.5)	25.3±1.3 (101.1)	47.8±1.8 (95.7)	97.3±9.3 (97.3)	0.5	0.5
ATZM	2 ng/mL	10 ng/mL	2 ng/mL	10 ng/mL	20 ng/mL	40 ng/mL		
	96±5	93±5	2.0±0.0 (100.8.)	10.0±0.3 (99.7)	19.0±0.7 (95.0)	39.0±2.1 (97.5)	0.1	0.2
ATZ	102±4	93±5	2.0±0.0 (98.7)	9.9±0.5 (98.6)	19.2±0.8 (96.1)	38.2±2.4 (95.6)	0.1	0.2

Analytic Precision

Analyte	QC Low		QC Medium		QC High	
	Mean (ng/mL)	RSD%	Mean (ng/mL)	RSD%	Mean (ng/mL)	RSD%
ATZ-OH	4.9±0.7	14.1	10.4±1.3	12.4	57.7±6.5	11.2
DACT	5.5±1.1	20.5	12.2±1.4	11.8	64.8±9.1	11.1
DEAM	5.3±0.8	14.7	11.5±0.9	7.9	57.7±4.8	8.2
DIA	5.3±0.6	12.1	10.5±0.6	5.3	51.2±4.2	8.3
DEA	4.7±0.5	10.2	10.0±0.6	5.6	50.6±4.8	9.4
ATZMM	5.1±0.5	9.9	10.5±0.4	4.1	51.8±4.5	8.6
ATZ	5.2±0.5	10.4	11.0±0.7	6.5	55.4±5.2	9.4

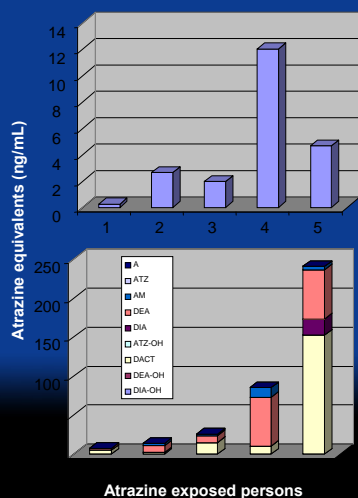


Requirements for Positive Identification

- Elute from HPLC at a specified time (+/- 10%)
- Coelute with stable isotopically labeled analogue
- Presence of both ions monitored
- Ions must be present in a specified ratio determined from standard materials



Lower Level Acute Exposures



- Measurement of only AM results in exposure misclassification
- Low level exposures may be missed if only AM measured
- DEA is a larger contributor to total metabolite level
- Large variation



Non-acute Environmental Exposure Scenario Complex

- Exposure to ATZ
- Exposure to chlorinated dealkylated degradates
- Exposure to hydroxylated degradates
- Parent chemical difficult to ascertain from biomonitoring data

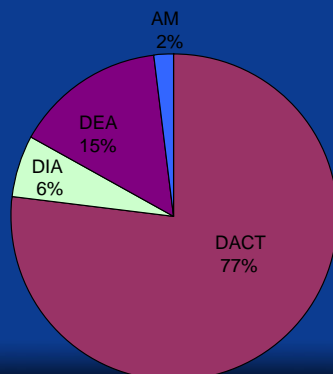


Metabolite Profile for Environmental Exposures

- DACT and DEA are predominant contaminants in drinking water
- Expect the alkylated metabolites to dominate based upon exposure pattern
- Dealkylated metabolites retaining the chlorine are considered equally as toxic as atrazine



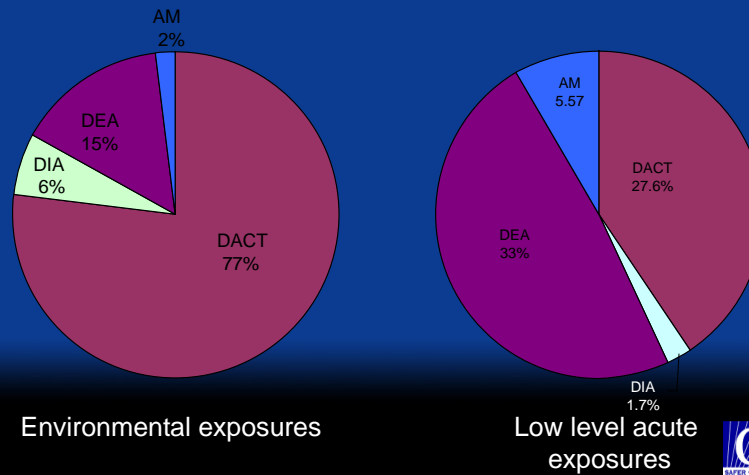
Environmental Exposure Metabolite Profile



- Data are preliminary
- N = 12
- Specifically selected samples with detectable AM
- Variable among samples
- Frequency of detection in US population cannot be ascertained until a full sample set is analyzed



Comparison of Metabolite Profiles from general population and low level acute exposures



Conclusions

- AM does not appear to be a major metabolite
- AM is likely most selective of exposure to ATZ itself
- DACT and DEA are most important metabolites
- Metabolite profile varies considerably among individuals
- Should measure all metabolites to accurately assess exposure to ATZ
- All metabolites should be measured when using biomonitoring to assess exposure in health effects studies
- ATZ-related exposures in the general population have likely been underestimated

Overall Conclusions

- Biomonitoring data have a purpose
- Biomonitoring data should be used with supporting data for interpretation
- Data gaps should be identified and studies conducted to supply these data
- Data gaps should be recognized as areas of uncertainty in data interpretation; however, incomplete data are better than no data at all
- Interpreting biomonitoring data for short-lived chemicals is challenging and subject to great uncertainty



Exposure Assessment Approaches



- Biomonitoring
- Personal Monitoring (air)
- Environmental Monitoring
- Questionnaire and other indirect means to complement all
- Exposure Modeling



Human data can markedly decrease the uncertainties associated with exposure and risk assessment

- Caveats should be recognized
- Data gaps should be filled where possible

