

Emerging Environmental Contaminants and Solid Phase Microextraction

Janusz Pawliszyn's Legacy in the Environmental Arena

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U.S. Environmental Protection Agency

Office of Research & Development

Advantages of SPME...



Torsten Schmidt

Traditional: Liquid-Liquid Extraction (LLE) or Solid-Phase Extraction (SPE)

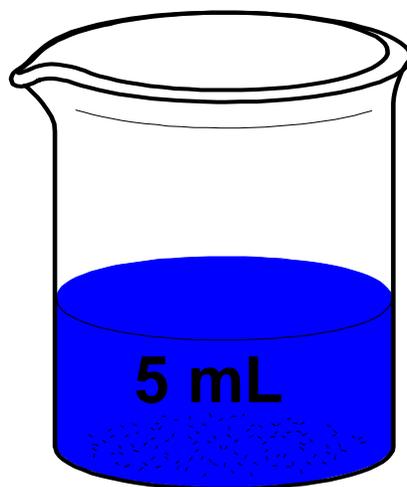
- Large volumes of often toxic organic solvents used per sample
- Expensive because of required solvent purity (LLE) and disposable extraction cartridges (SPE)
- Time-consuming sample preparation procedures, multiple extractions required (LLE)
- Analyte losses during solvent evaporation for pre-concentration
- Clogging of extraction cartridges (SPE)
- Formation of emulsions, difficult phase separation (LLE)

Solvent Volume Requirements for 1000 Samples

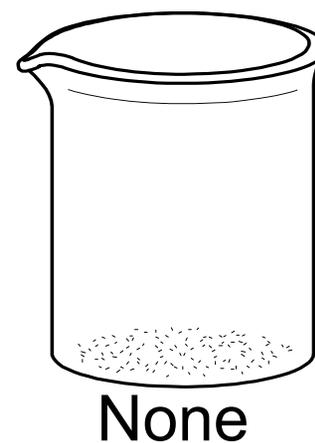
**Liquid-Liquid
Extraction**



**Liquid Phase
Microextraction**



**Solventless
Microextraction
Methods**



Comparison of Sample Preparation Procedure using Solid-Phase Extraction or Solid-Phase Microextraction

SPE

measurement of sample (e.g. by volume)

conditioning of the phase

extraction

adsorbent drying

elution of analytes

solvent concentration

transfer of extract into autosampler vial

start of GC-MS process

SPME

transferring of samples into HS-vials

adding of salt

start of GC-MS process

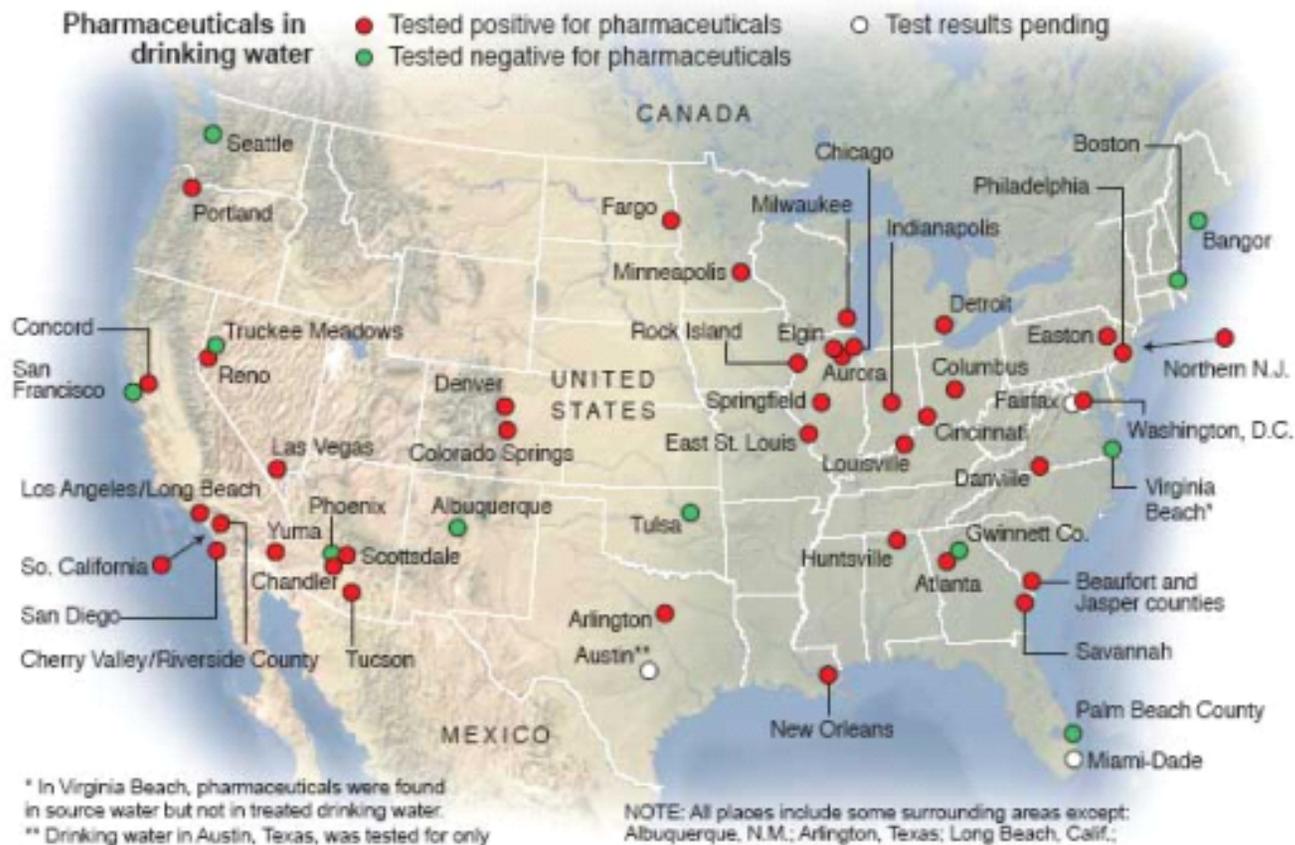
Emerging Environmental Contaminants and SPME

- Pharmaceuticals
- EDCs
- DBPs
- New pesticides and pesticide transformation products
- Brominated flame retardants
- Chiral contaminants
- Musks
- Dioxane
- Algal toxins

Pharmaceuticals

46 million in U.S. have drugs in drinking water

Testing shows traces of meds in water greater than previously reported



updated 4:29 p.m. ET, Thurs., Sept. 11, 2008

Testing prompted by an Associated Press story that revealed trace amounts of pharmaceuticals in drinking water supplies has shown that more Americans are affected by the problem than previously thought — at least 46 million. That's up from 41 million people reported by the AP in March as part of an investigation into the presence of pharmaceuticals in the nation's waterways.

Pharmaceuticals

Several included on the new CCL-3; will also be included in new Water Quality Criteria

- Potential estrogenic effects on biota (e.g., feminization of fish)
- Potential antibiotic resistance
- May affect aquatic organism populations (decreased food sources)
- Transformation in drinking water treatment



Emerging Contaminants What are They? Or a Paradigm Shift?

An Example of a Whole Lake Ethynylestradiol Dosing Experiment at Canada's Experimental Lake Area



**Jim Lazorchak, U.S. EPA,
NERL, Cincinnati, OH**

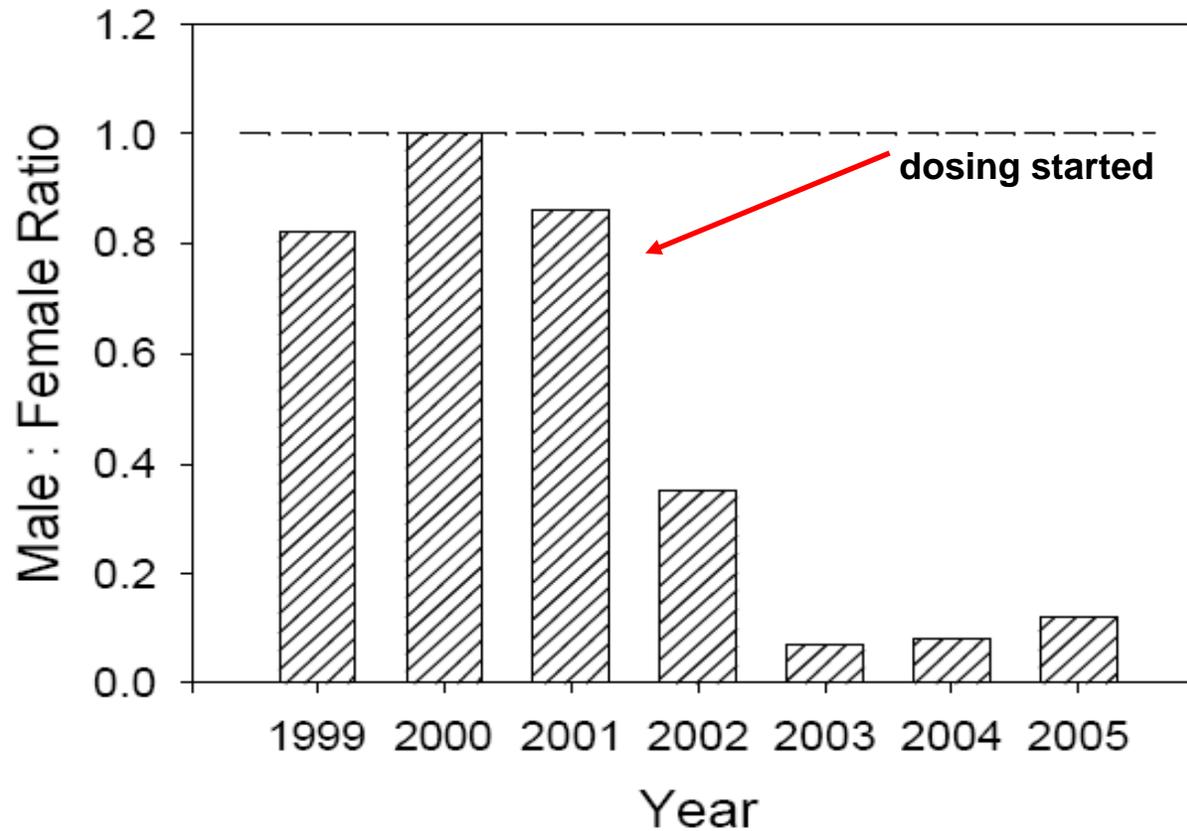
Kidd, Blanchfield, Mills, Palace, Evans, Lazorchak, Flick. 2007. Collapse of a fish population after exposure to a synthetic estrogen. *PNAS* 104, 8897-8901.

Summary - Fathead Minnow

- 
- **Spring 2001** - EE2 additions began
 - Vg gene expression induction in deployed 114 fish in 260 in 24-hrs
 - significant vitellogenin plasma induction after 7 weeks
 - **Fall 2001 (4 months)**
 - proteinaceous accumulation in kidney
 - liver cell size increased
 - **Spring 2002 (12 months)**
 - disorganized testes, immature ovaries
 - decreased spawning aggression, fewer & less-developed eggs
 - reduction in secondary sex characteristics
 - No fish population impacts observed
 - **Fall 2002 (17 months)** - reproductive failure, few age 0 fish
 - **Spring 2003 (2 years)** - only age 2 fish remaining
 - one male found, females with large ovipositors
 - **2006 (3 yrs post additions)** – Fathead population recovered



Pearl Dace Sex Ratio

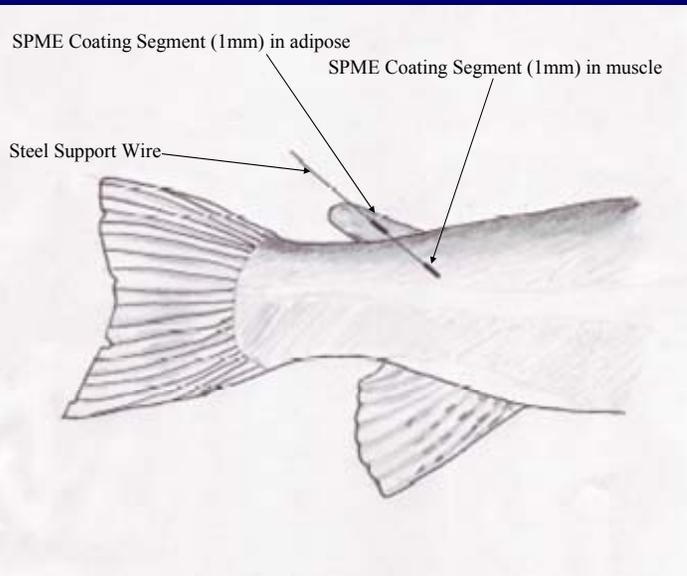


K Mills – FW Inst

Tissue-specific *In Vivo* Bioconcentration of Pharmaceuticals in Rainbow Trout Using Space-Resolved Solid-Phase Microextraction



Mark Servos
Univ. Waterloo



- Immature rainbow trout exposed to a PPCP mixture of 3 $\mu\text{g/L}$ each (atorvastatin, bisphenol A, carbamazepine, diclofenac, fluoxetine, gemfibrozil, ibuprofen, and naproxen)
- Water samples collected throughout the 8 day exposure and analyzed by both SPME and solid phase extraction (SPE).
- SPME samples taken every second day in both adipose fin and dorsal-epaxial muscle

Drinking Water DBPs

- Formed by the reaction of disinfectants with natural organic matter

Concern over possible human health risk:

- Epidemiologic studies: **risk of bladder cancer** some cause cancer in laboratory animals
- Recent concerns about possible **reproductive & developmental effects** (from epi studies)
- Huge movement toward alternative disinfectants
- Use of UV and membranes increasing dramatically
- By 2025, 70% of drinking water plants expected to use membranes



DBPs Regulated by the U.S. EPA

DBP	MCL ($\mu\text{g/L}$)
Total THMs	80
5 Haloacetic acids	60
Bromate	10
Chlorite	1000

But more than 600 DBPs have been identified

Little known about occurrence & toxicity of unregulated DBPs

Measuring Iodo-THMs in Tap Water and Blood



Ben Blount, CDC

“I have never met him but his SPME work has revolutionized my analysis of VOCs in biological fluids.

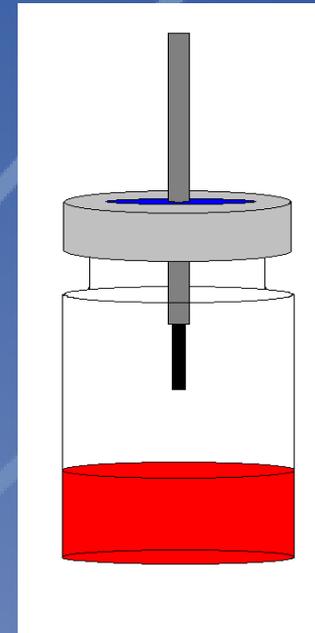
Automated SPME increased my throughput by an order of magnitude while improving safety and reducing costs.”

Measuring Iodo-THMs in Tap Water and Blood



Ben Blount

1. Spike sample with internal standards ($^2\text{H}_1$ -iodoTHMs)
2. **SPME extraction (Carboxen/PDMS fiber)**
3. GC (DB-624, 25m x 0.20mm x 1.12 μm)
 - Cryotrapping and cryo-oven
- High Resolution Mass Spectrometry
 - MAT 95 magnetic sector mass spectrometer
 - 10,000 Resolution
4. Quantify using stable isotope dilution



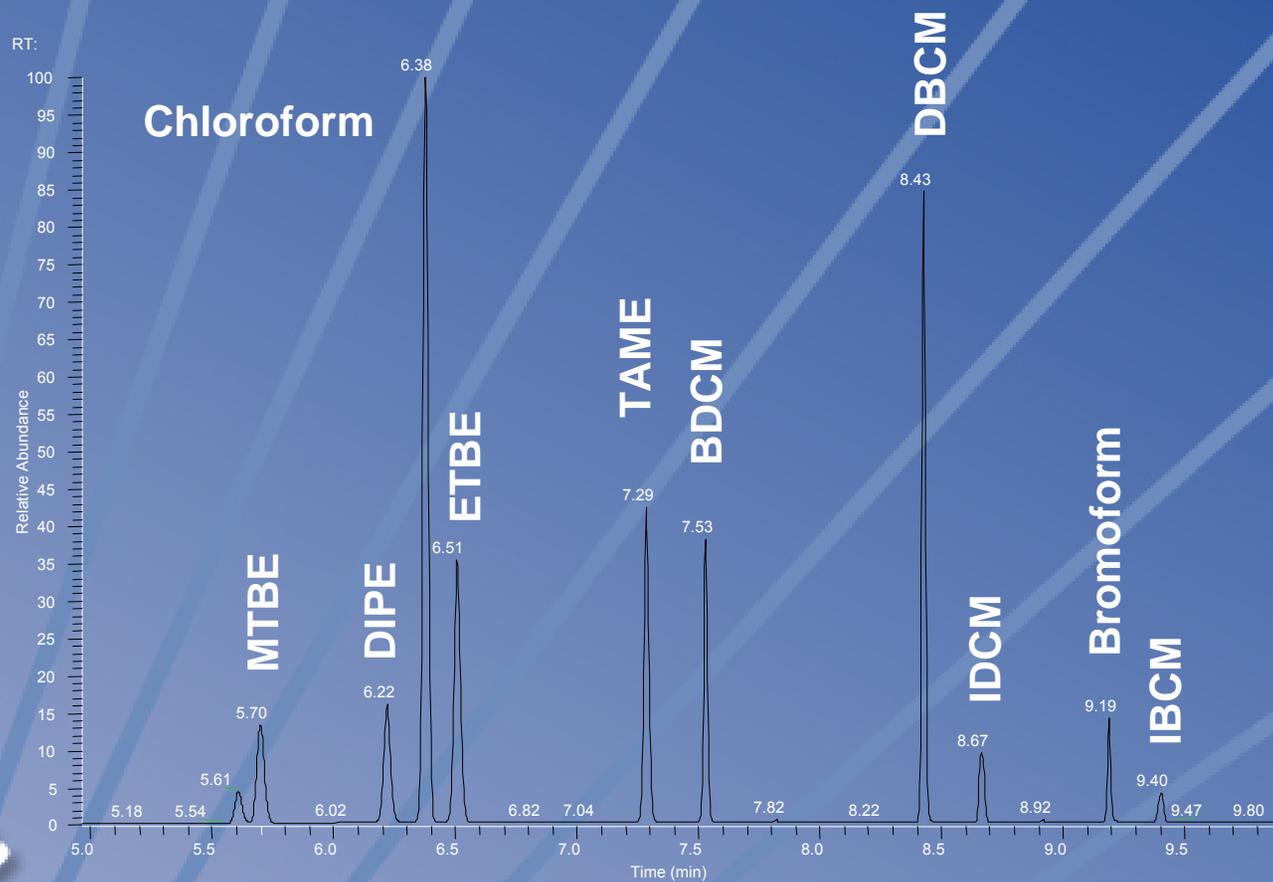
Slide courtesy of Ben Blount, CDC



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IodoTHMs: standards



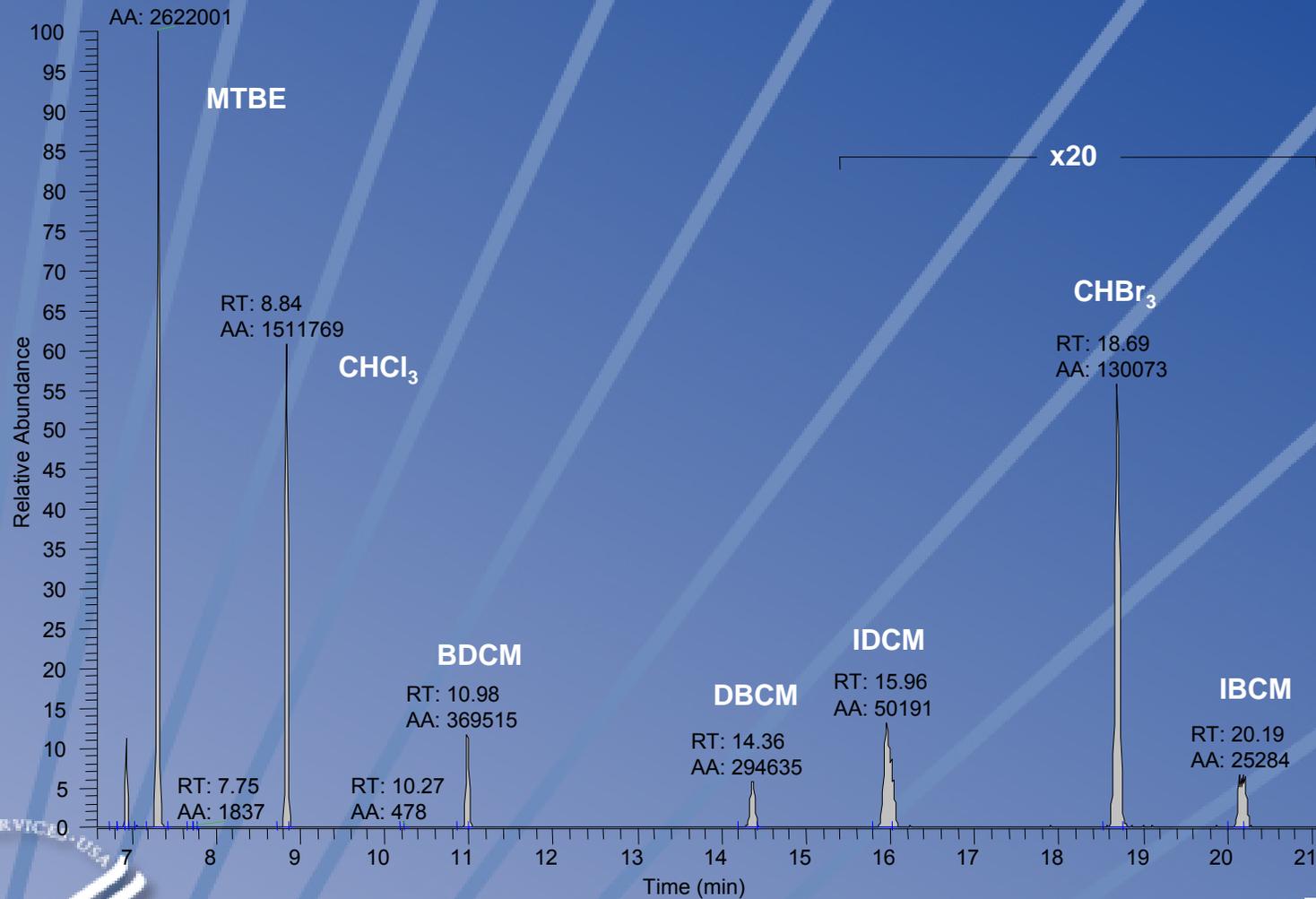
Slide courtesy of Ben Blount, CDC



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Blood THM Analysis



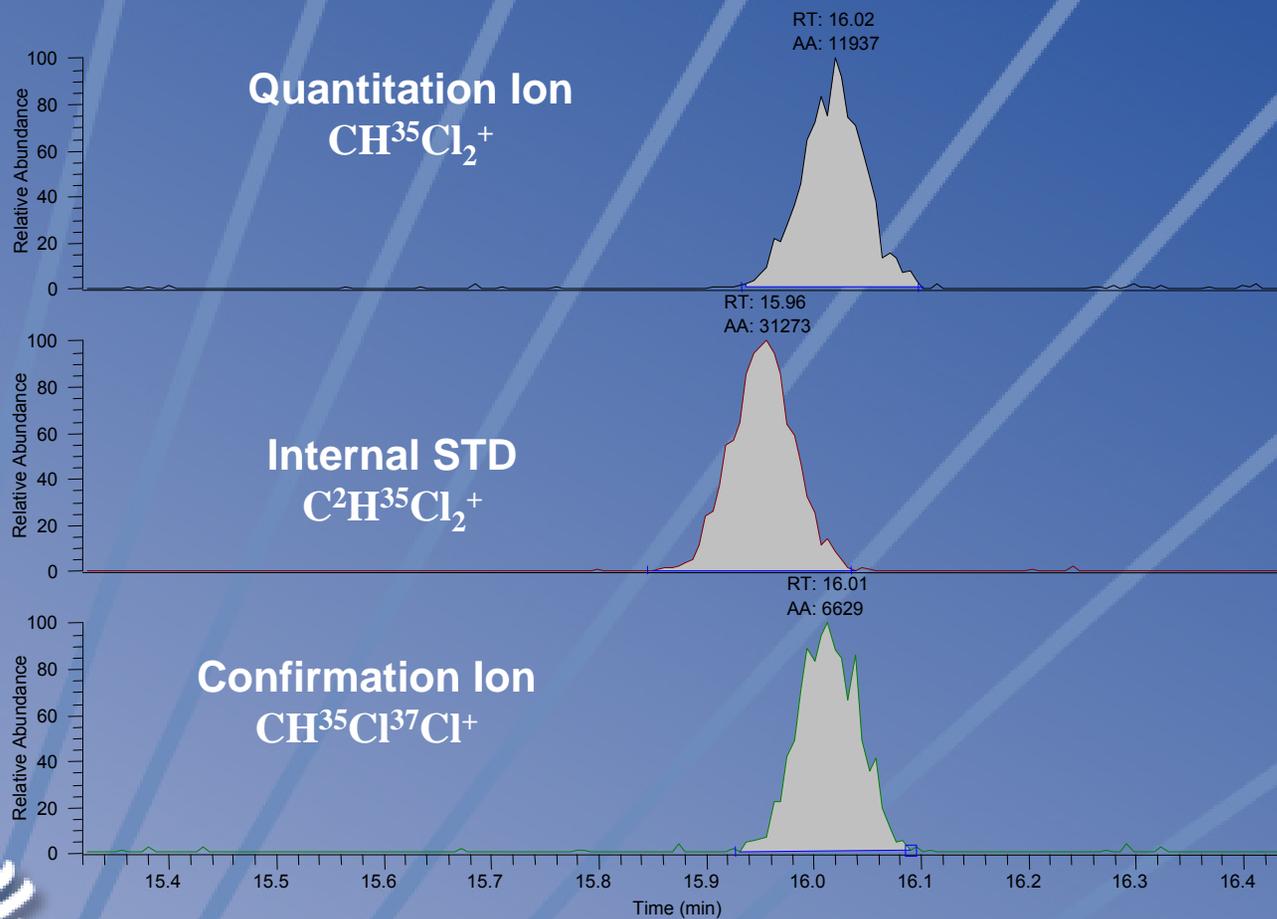
Slide courtesy of Ben Blount, CDC



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IDCM in Blood (19.8 pptr)



Slide courtesy of Ben Blount, CDC



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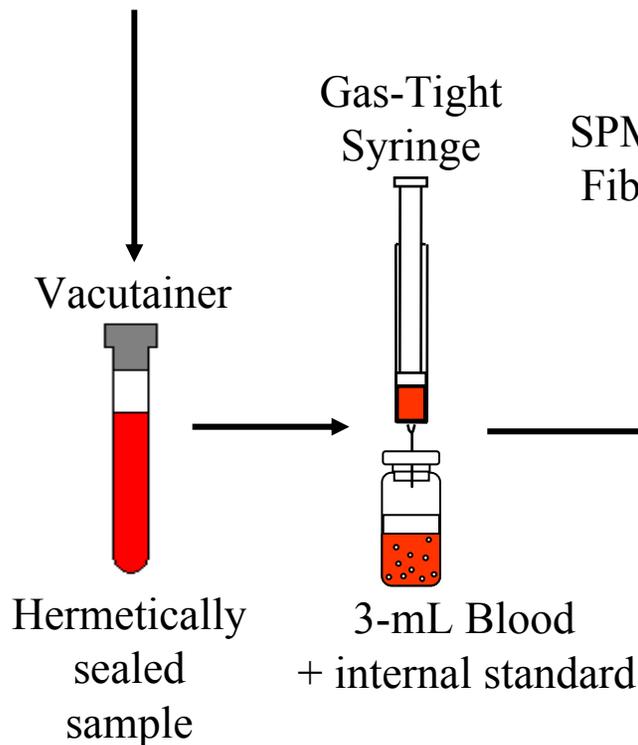
NHANES Study—50 VOCs using SPME



SPME Headspace/
Cryotrap/GC/SIM MS

SPME
Syringe

SPME
Fiber



Subjects

- Blood samples were collected from 4584 NHANES 2007-2009 study participants.

Sample Collection & Storage

- Blood is collected into a pre-cleaned VOC free, evacuated blood collection tube
- Samples are stored at 10°C for under for 4 weeks but no longer than 10 weeks

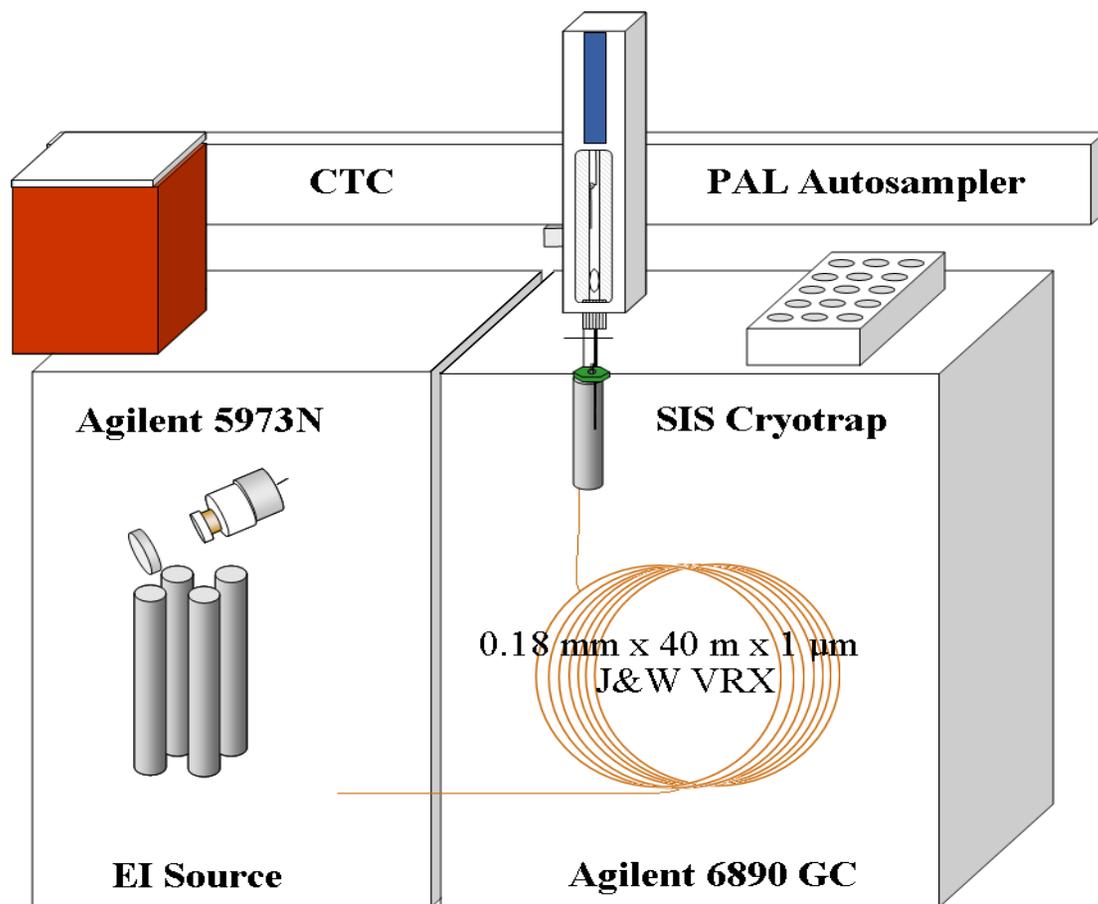
Sample Preparation

- Samples are equilibrated and mixed in 10-ml blood collection tube at room temperature
- 3-ml of blood is drawn using a gas-tight glass syringe and placed into a 10-ml headspace vial
- 40 μ l internal standard is then pipetted into vial
- Vial is sealed with reprocessed PTFE barrier silicone septum

Analyte Collection

- 75- μ m Carboxen-PDMS SPME fiber is exposed for 15 min at 40°C while agitating at 500 rpm

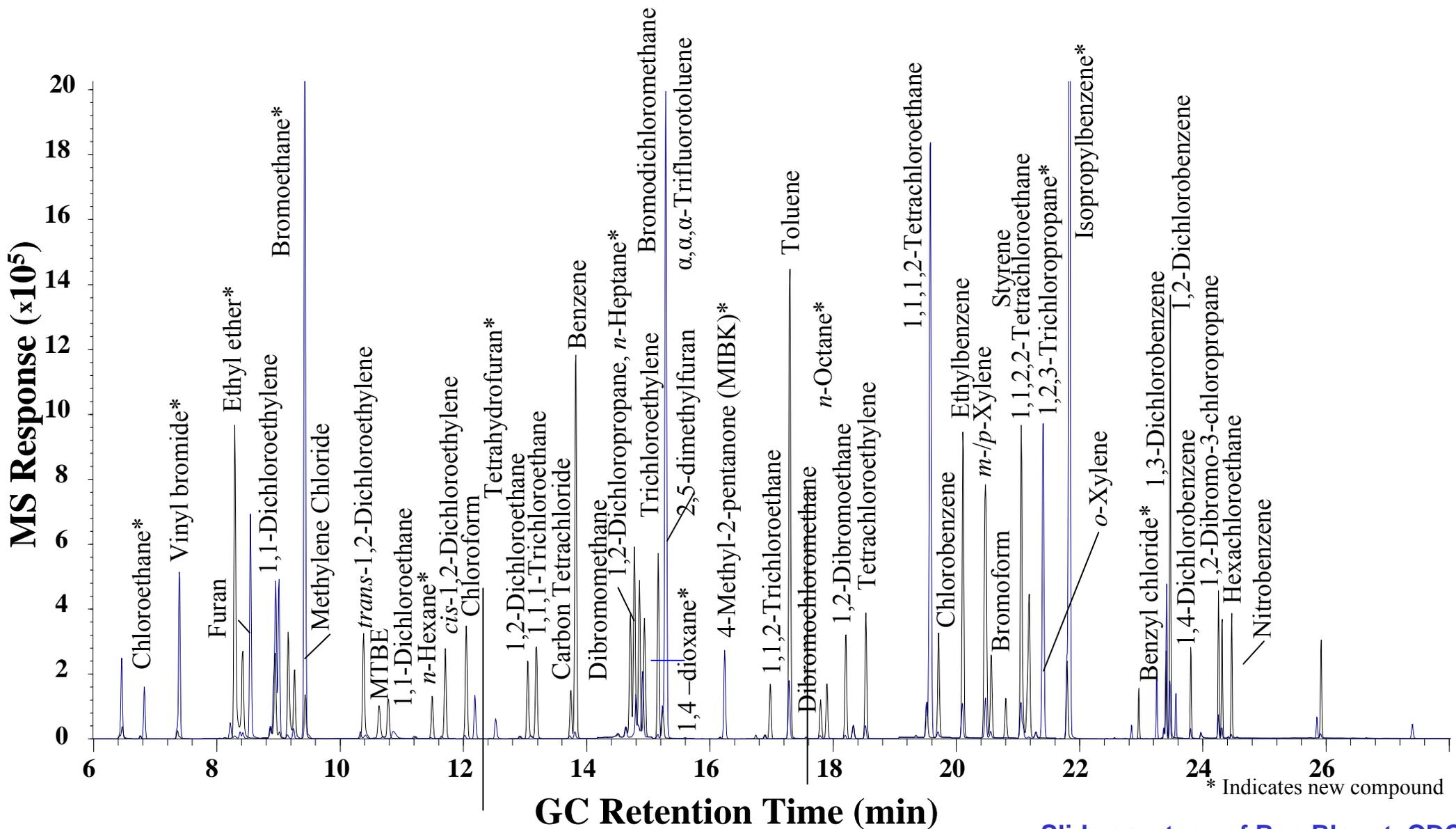
Analytical System



Method Parameters

- SPME adsorption: 15 min at 40°C while agitating at 500 rpm
- SPME desorption: 1.5 min at 250°C pulsed splitless, remaining in inlet throughout GC run while GC is held in splitless mode
- Cryofocusing: 1 min at -100°C then ballistically heated to 225°C
- GC Program: 1.5 min at 0°C, ramp 7°/min to 140°C, 40°/min to 220°C, hold 8.5 min (32 min total)
- MS Detection: Selective Ion Monitoring (SIM) mode

Mass Chromatogram of 50 VOCs



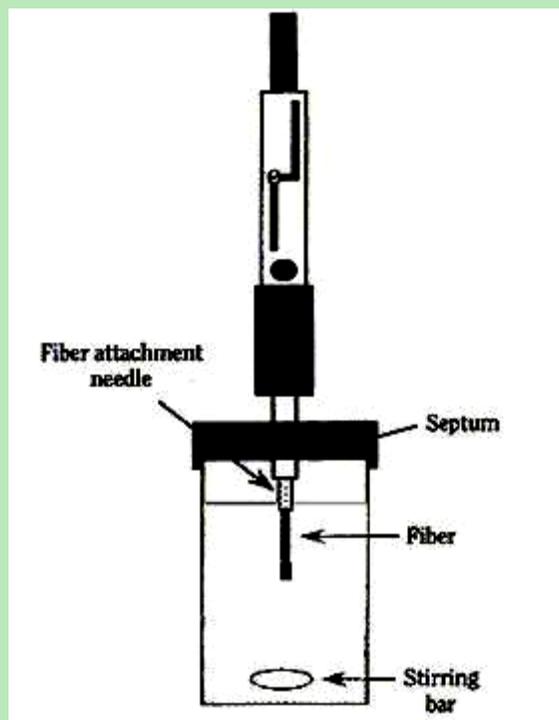
Slide courtesy of Ben Blount, CDC

Pesticide Degradation Products

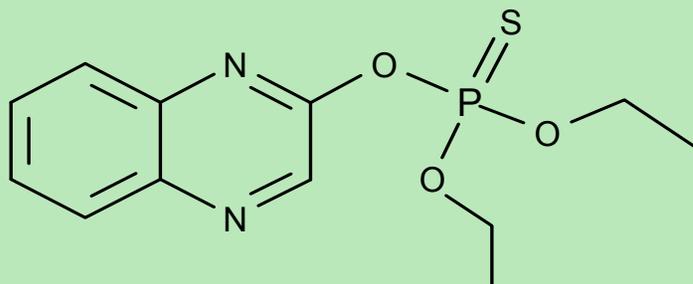
- May be more environmentally relevant than parent species (and some more toxic than parent)
- Previously overlooked
- Most highly polar; require LC/MS or LC/MS/MS
- Some on the **CCL-3**:
 - Alachlor ESA & OA
 - Acetochlor ESA & OA
 - Metolachlor ESA & OA

Photodegradation of Quinalphos in water

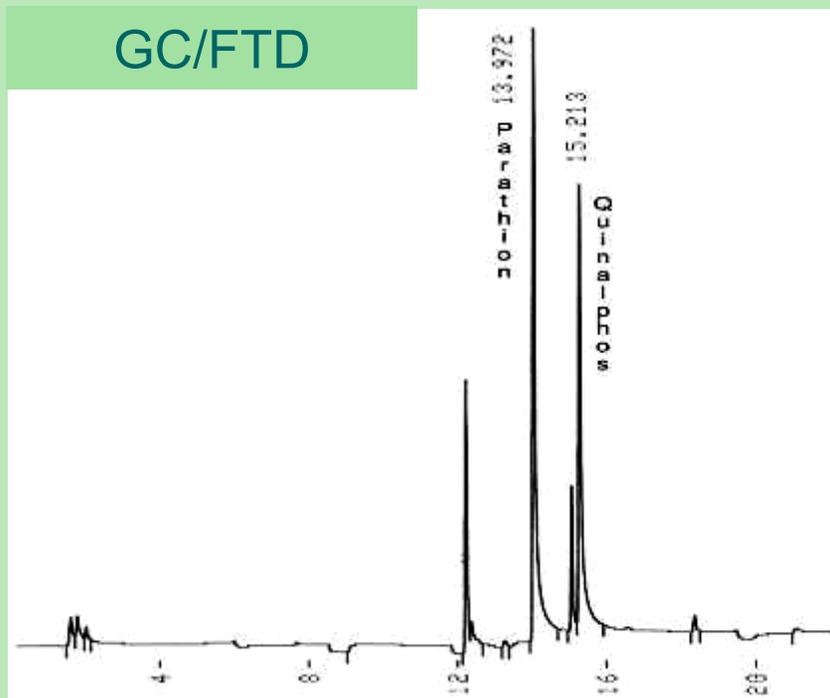
SPME device



85 μm Polyacrylate fiber
Parathion 1 ppb int. std
4 ml sample, agitation
20 min, 60 $^{\circ}\text{C}$



GC/FTD



Goncalves, C.; Dimou, A.; Sakkas, V.; Alpendurada, M. F.; Albanis, T. A.
Chemosphere 2006, 64 (8), 1375–1382.



Vasilis Sakkas

Slide courtesy of Vasilis Sakkas, Univ. of Ioannina, Ioannina, Greece

Photodegradation in water

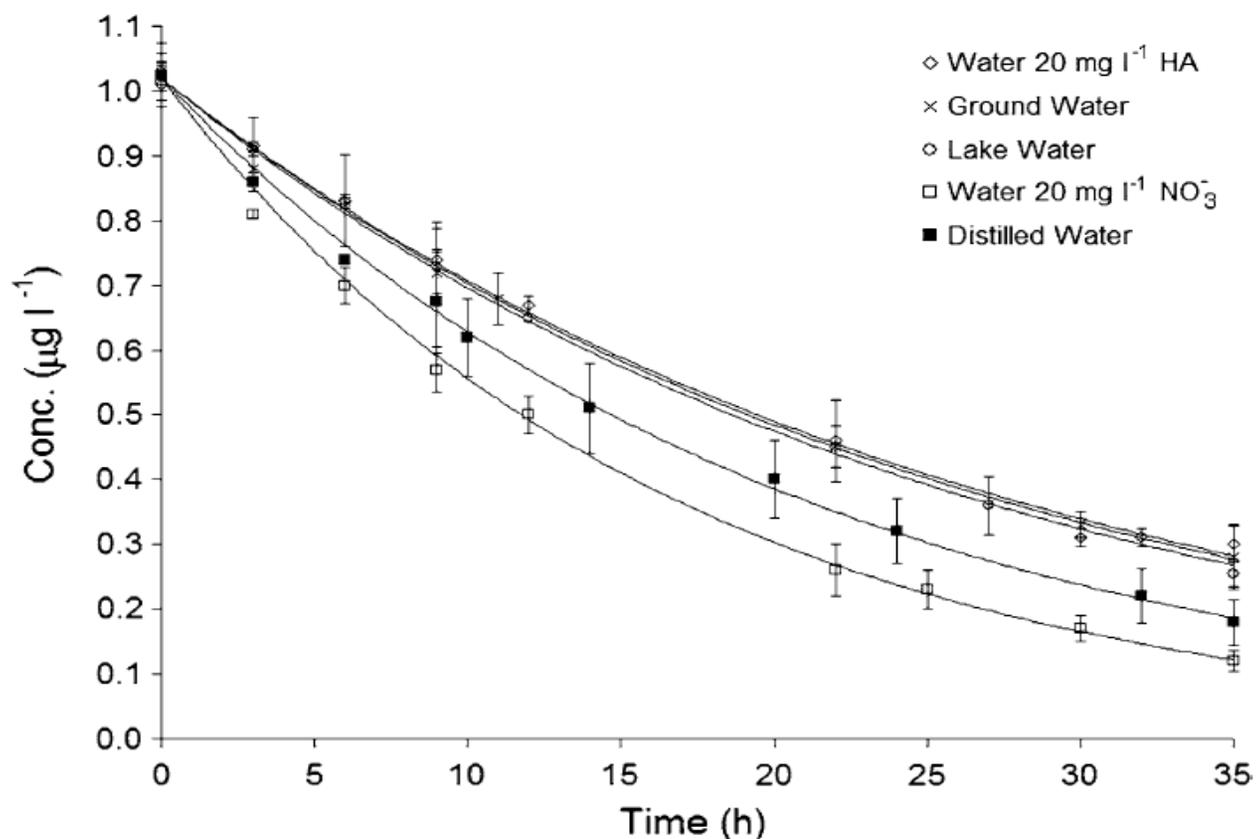


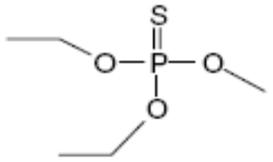
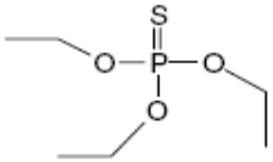
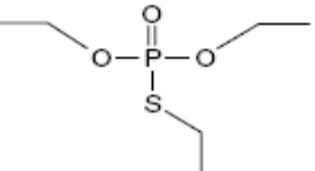
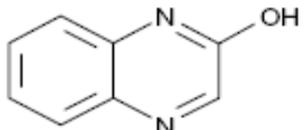
Fig. 1. Typical first-order degradation plots obtained in the photolysis of quinalphos in aqueous phase and under simulated solar irradiation conditions.

Photolysis by-products

Identified by GC/MS

Table 4

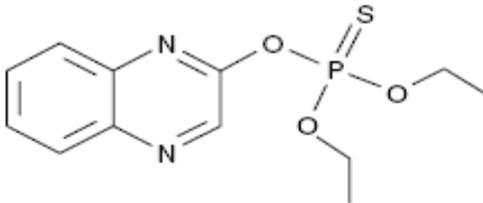
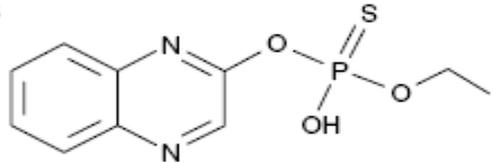
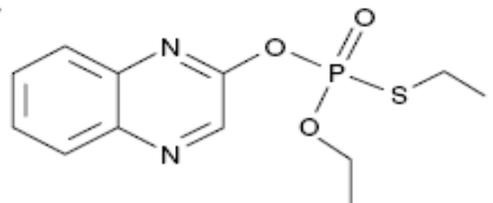
Mass spectrum characteristics of tentative by-products as identified in photolysis studies carried out in water and soil matrices

Degradation product	R_t (min) ^a	M^+	Main fragment ions (m/z)
(1) <i>O,O</i> -diethyl- <i>O</i> -methylphosphorothioate 	16.4	184	156, 140, 129, 111, 107, 95, 79
(2) Triethylphosphorothioate 	18.3	198	170, 154, 143, 126, 121, 115
(3) <i>O,O',S</i> -triethylthiophosphate 	22.1	198	170, 154, 142, 138, 126, 109
(4) 2-Hydroxyquinoxaline 	32.5	146	118, 103, 91

Continued...

Slide courtesy of Vasilis Sakkas, Univ. of Ioannina, Ioannina, Greece

Photolysis by-products

(5) Quinalphos		41.3	298	270, 241, 193, 173, 157, 146, 129, 118
(6) Desethylquinalphos		43.2	270	241, 193, 178, 146, 129, 118
(7) Quinalphos <i>S</i> -ethyl isomer		45.3	298	269, 241, 173, 157, 146, 129, 118

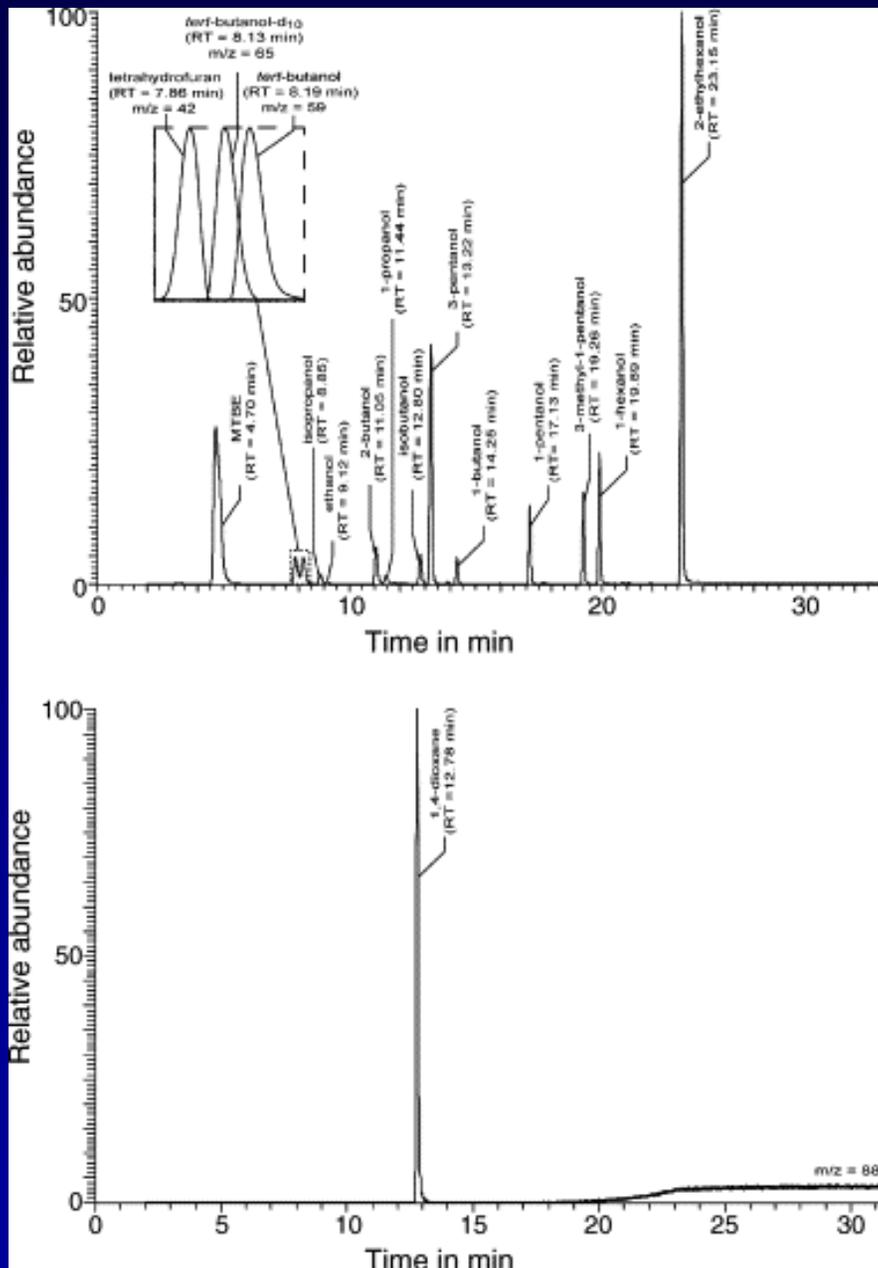
^a R_t —retention time.

✓ Due to its high sensitivity, SPME allowed to test a low pesticide concentration, equivalent to environmental contamination levels, and using small sample volumes which is particularly important in this kind of studies

1,4-Dioxane

- On CCL-3 (also for consideration on the new UCMR-3)
- High production chemical used as a solvent stabilizer in the manufacture and processing of **paper, cotton, textiles, automotive coolants, cosmetics, shampoos**, and for 1,1,1-trichloroethane (TCA)—a popular vapor degreasing solvent
- Probable human carcinogen
- Widespread contaminant in groundwater (often exceeding water quality criteria and guidelines)
- Highly water soluble; problematic to extract and measure

1,4-Dioxane



Torsten Schmidt

- Headspace-SPDE-GC/MS
- SPDE a variation of SPME (inside of syringe needle coated with extraction phase)
- 4-6× larger extraction phase volumes than 100 μm SPME fibers
- Detection limits of 0.8 $\mu\text{g/L}$

Slide courtesy of Torsten Schmidt, Univ. of Duisburg-Essen, Germany

Jochmann et al., J. Chromatogr. A, 2008, 1179, 96-105.

New EPA Method 522

EPA/600/R-08/101

**METHOD 522 DETERMINATION OF 1,4-DIOXANE IN DRINKING WATER BY
SOLID PHASE EXTRACTION (SPE) AND GAS CHROMATOGRAPHY/
MASS SPECTROMETRY (GC/MS) WITH SELECTED ION
MONITORING (SIM)**

**Version 1.0
September, 2008**

Jean W. Munch and Paul E. Grimmett

**NATIONAL EXPOSURE RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268**

www.epa.gov/nerlcwww/ordmeth.htm

Perchlorate (ClO_4^-)

Recent EPA Methods

- Developed to overcome matrix interferences in high ionic strength waters and to lower detection limits
- EPA Method 314.0: IC-conductivity (MRL 4 $\mu\text{g/L}$)
- EPA Method 330.0: IC/ESI-MS (MRL 0.02-0.05 $\mu\text{g/L}$)
- EPA Method 331.0: LC/ESI-MS/MS (MRL 0.02 $\mu\text{g/L}$)

www.epa.gov/safewater/methods/sourcalt.html

www.epa.gov/nerlcwww/ordmethod.htm

CCL-3: www.epa.gov/safewater/ccl

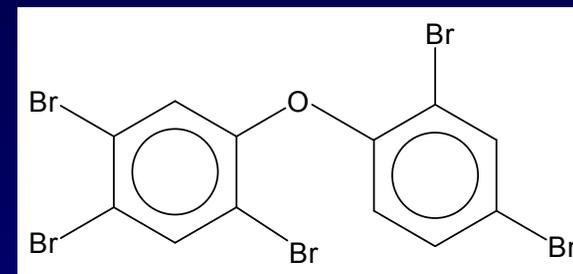
Brominated Flame Retardants (e.g., PBDEs)

on the UCMR-2

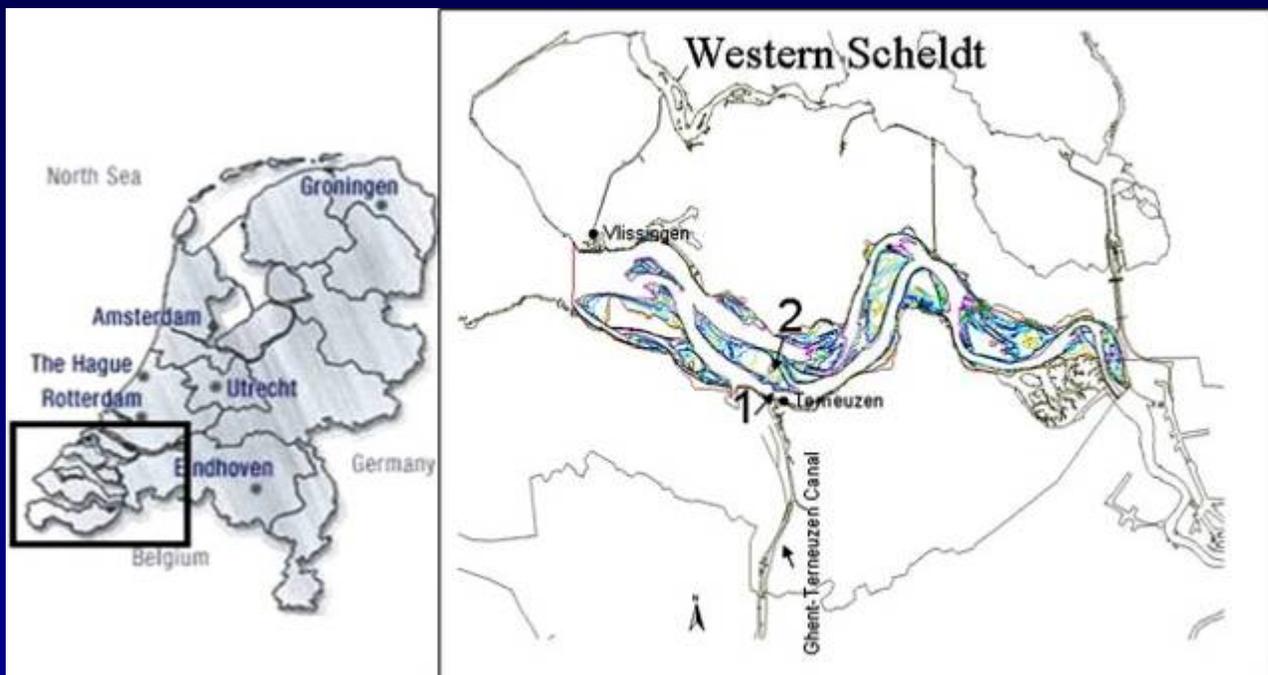
www.epa.gov/ogwdw/ucmr

- Found in human samples—worldwide
- Potential adverse developmental effects, cancer
- Global production: 200,000 metric tons (most used in U.S. and Canada)
- Added to plastics, textiles, particularly furniture, consumer electronics
- Environmentally persistent; ubiquitous
- Octa- and penta- phased out in North America in 2004, but deca- still made
- EPA Method 527 (GC/MS; mid-ng/L detection limits)

www.epa.gov/safewater/methods/analyticalmethods_ogwdw.html



Brominated Flame Retardants



SPME used with GC/NCI-MS
and GC/EI-MS to measure brominated flame
retardants in environmental waters

Lopez, P.; Brandsma, S. A.; Leonards, P. E. G.; De Boer, J. J. *Chromatogr., A* **2009**, *1216* (3), 334–345.

Patricia Lopez, VU University Amsterdam

Journal of Chromatography A, 1216 (2009) 334–345
Contents lists available at ScienceDirect
Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

Methods for the determination of phenolic brominated flame retardants, and by-products, formulation intermediates and decomposition products of brominated flame retardants in water

P. López*, S.A. Brandsma, P.E.G. Leonards, J. De Boer
Institute for Environmental Studies (IVM), VU University Amsterdam, De Boelelaan 1087, 1083 HV Amsterdam, The Netherlands

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Keywords:
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Bromobenzenes
Bromonitriles
Bromocyclohexanes
Water analysis
REACH

ABSTRACT
Brominated flame retardants (BFRs) are the chemicals of high importance within the REACH framework. In addition to polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobiphenyl A (TBBPA), other BFRs such as bromophenols, intermediates in PB formulation like bromoanilines, and their brominated and non-brominated by-products such as bromoaniloles, bromotoluenes, bromoanilanes and 1,5,9-cyclododecatrienes, respectively should be monitored and controlled because of their toxicity and their very low odour and taste thresholds, below sub-nanogram-per-liter levels. In the present study several analytical methods for the simultaneous determination, i.e., combining one single sample treatment and one analysis step, of these compounds in water have been developed, optimized and evaluated. The methods involve a (pre-concentration)-extraction technique, such as liquid-liquid (LLE), solid-phase (SPE), headspace (HS) extraction or solid-phase microextraction (SPME), followed by gas chromatography (GC)-mass spectrometry (MS) analysis with either electron capture negative ionization (ECNI) or electron impact (EI) as ionization techniques. ECNI is more sensitive than EI for analytes with more than one bromine atom. HS and SPME were previously optimized by means of a multifactorial experimental design. Extraction temperature and the liquid/headsapce volume ratio were the most significant factors in HS extraction. In SPME, the variables studied were the nature of the fiber, the mode of extraction and the extraction temperature. Polydimethylsiloxane (PDMS) fibers appeared to be more suitable than carbon-coated-polydimethylsiloxane (CAR-PDMS) for the analysis of the target compounds with more than one bromine atom. The extraction of 2,4-dibromoaniline was only achieved in a direct immersion mode, in which the optimal extraction temperature was 60°C. The methods LLE-ECNI/MS, LLE-GC-EI/MS, SPE-ECNI/MS, SPE-GC-EI/MS, HS-GC-EI/MS and SPME-GC-EI/MS were evaluated. In terms of linearity, precision, detection limits and trueness. All methods, with the exception of HS-GC-EI/MS, were linear in a range of at least two orders of magnitude, giving recoveries above 75% and detection limits at the low level for most of the target analytes. SPE-GC-ECNI/MS is the most sensitive and reliable method for the determination of most of the bromine compounds, whereas SPE-GC-EI/MS is the most suitable to quantify the three isomers of 1,5,9-cyclododecatrienes. Both methods together with SPME-GC-EI/MS (for qualitative confirmation) were applied to water samples from the Western Scheldt (The Netherlands), where 2,6-dibromophenol and 2,4,6-tribromoanisole could be detected at levels higher than their respective odour thresholds.

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1. Introduction
On 29 October 2003, the European Commission adopted a proposal for a new EU regulatory framework for chemicals called REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), which entered into force on 1st June 2007 [1]. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances, while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry. A Chemicals Agency will act as the central point in the REACH system: it will run the databases necessary to operate the system, co-ordinate the in-depth evaluation of suspicious chemicals and run a public database in which consumers and professionals can find hazard information. The Regulation also calls for the progressive substitution of the

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E-mail address: patricia.lopez@vu.nl (P. López).
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doi:10.1016/j.chroma.2008.08.041

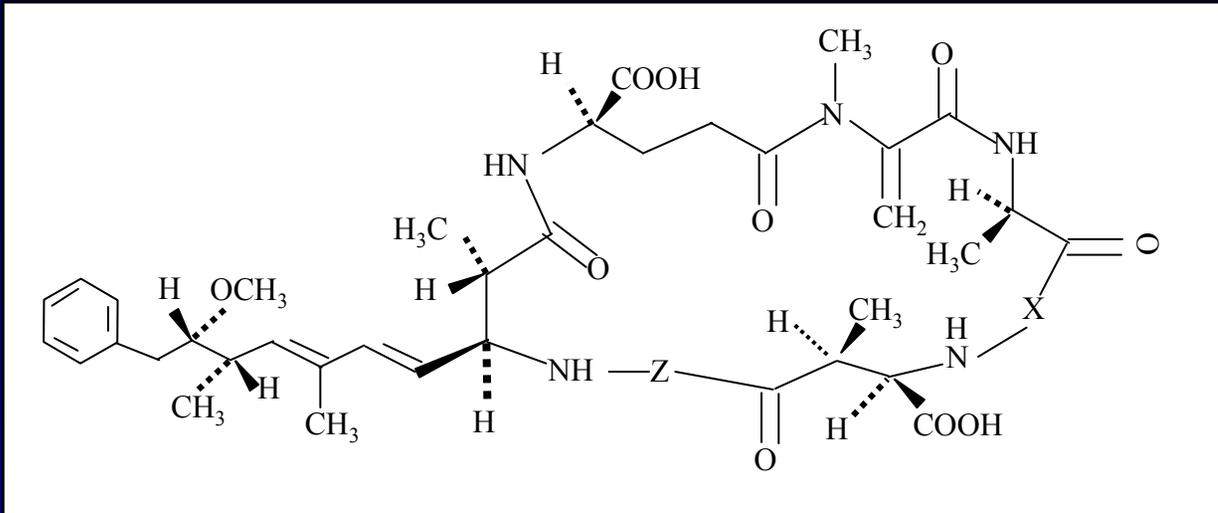
Algal Toxins

- **Included on the new CCL-3**
(microcystin-LR, anatoxin-a, cylindrospermopsin)
- **EU:** guideline (0.1 µg/L)
- **WHO:** provisional guideline (1.0 µg/L)
for microcystin-LR
- Responsible for large fish kills, poisoning of shellfish, illness in people

Examples:

- Microcystins, nodularins, saxitoxins, anatoxins, brevetoxins
- Some found in finished drinking water from Florida (2003)

Microcystins



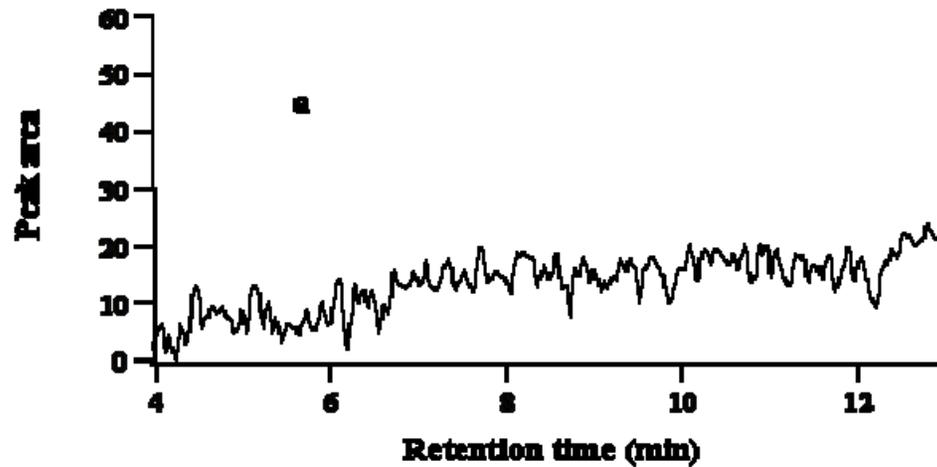
Xingfang Li

MC-LR: X=Leu, Z=Arg
MC-RR: X=Arg, Z=Arg

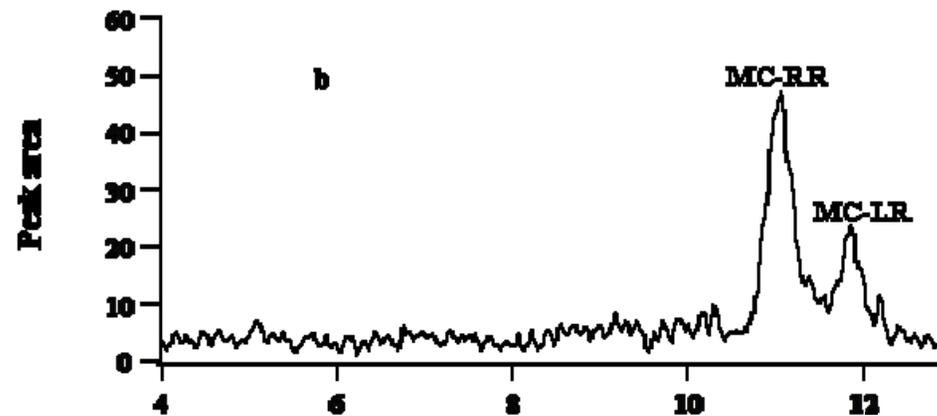
- SPME-microbore-LC-QTOF-MS
- 0.6-1.6 pg detection limits
- 70-86% recoveries
- Small sample volumes (12 mL)
- Minimized the use of solvents

Slide courtesy of Xingfang Li, Univ. Alberta, Canada

Sample Analysis



blank



Spiked lake water
1 $\mu\text{g/L}$

Zhao, Y.-Y.; Hrudey, S.; Li, X.-F. *J. Chromatogr. Sci.* 2006, 44, 359–365.

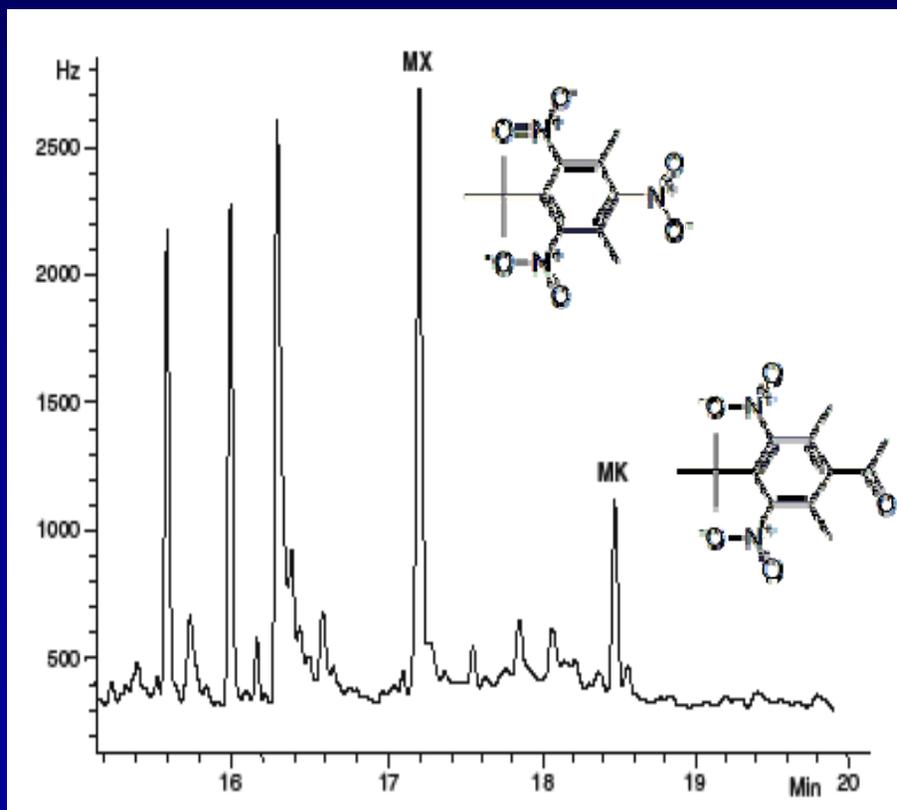
Musks

- Synthetic musks widely used as fragrance additives in many consumer products, including perfumes, lotions, sunscreens, deodorants, and laundry detergents
- Widely present in environmental samples, including wildlife and humans
- Accumulate in sediments, sludges, and biota
- Up to 190 ng/g lipid in humans



Musks

- SPME-GC/ μ -ECD used to measure musks in wastewater and sewage samples at low ng/L detection limits



Anal Bioanal Chem (2007) 388:1789–1798
DOI 10.1007/s00216-007-1359-z

ORIGINAL PAPER

Optimization of a sensitive method for the determination of nitro musk fragrances in waters by solid-phase microextraction and gas chromatography with micro electron capture detection using factorial experimental design

María Polo · Carmen García-Jares ·
María Llompart · Rafael Cela

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Abstract A solid-phase microextraction method (SPME) followed by gas chromatography with micro electron capture detection for determining trace levels of nitro musk fragrances in residual waters was optimized. Four nitro musks, musk xylene, musk moskene, musk tibetene and musk ketone, were selected for the optimization of the method. Factors affecting the extraction process were studied using a multi-variate approach. Two extraction modes (direct SPME and headspace SPME) were tried at different extraction temperatures using two fiber coatings [Carboxen–polydimethylsiloxane (CAR/PDMS) and polydimethylsiloxane–divinylbenzene (PDMS/DVB)] selected among five commercial tested fibers. Sample agitation and the salting-out effect were also factors studied. The main effects and interactions between the factors were studied for all the target compounds. An extraction temperature of 100 °C and sampling the headspace over the sample, using either CAR/PDMS or PDMS/DVB as fiber coatings, were found to be the experimental conditions that led to a more effective extraction. High sensitivity, with detection limits in the low nanogram per liter range, and good linearity and repeatability were achieved for all nitro musks. Since the method proposed performed well for real samples, it was applied to different water samples, including wastewater and sewage, in which some of the target compounds (musk xylene and musk ketone) were detected and quantified.

Keywords Nitro musk fragrances · Solid-phase microextraction · Gas chromatography–micro electron capture detection · Water analysis · Factorial design

Introduction

In the last few years, increasing attention has been given to a class of emerging toxic substances like some components of the so-called pharmaceuticals and personal care products (PPCPs). Numerous products like soaps, detergents, cosmetics and other consumer products are being continuously released into the environment. Since fragrances are constituents of many of these products, their musk components can be characterized by their ubiquity [1]. Three families of musks, nitro musks, polycyclic musks and macrocyclic musks, were developed to substitute the extremely expensive natural musks.

Among the synthetic musk compounds, the nitro musk family includes five members: musk xylene (MX), musk ambrette, musk moskene (MM), musk tibetene (MT), and musk ketone (MK) [2]. In particular, MX and MK are the most widely used nitromusks, mainly in detergents and cosmetics, so their primary method of release into the environment is through discharges of domestic wastewater. Since the time when nitro musk compounds were detected by Yamagishi et al. [3, 4] in marine animals and humans from Japan, MX has no longer been used in that country. Subsequently, MX and MK were mostly detected in Europe in aquatic biota [5–7], as well as in human milk and adipose tissue [8], demonstrating the lipophilic nature and the high bioaccumulation potential of nitro musk compounds. On

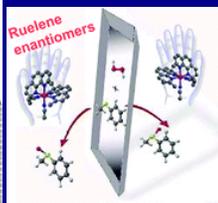
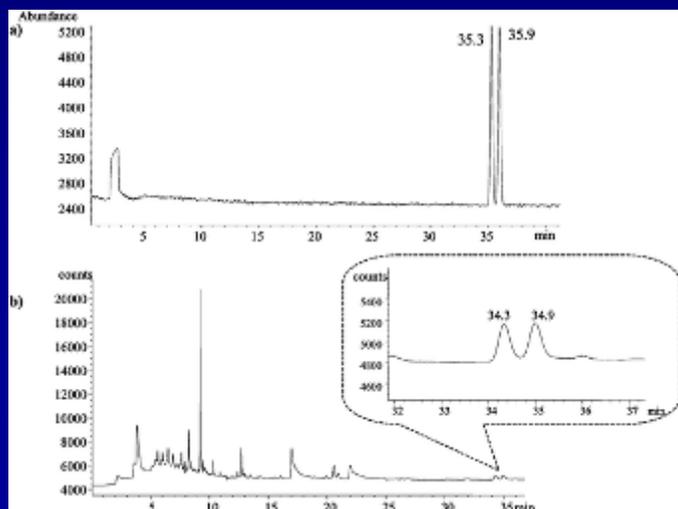
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Chiral Contaminants

- Chiral contaminants are similar chemically, but can behave differently in the environment and in biological systems
- SPME used with enantioselective GC-ICP-MS to measure chiral speciation of the pesticide ruelene in environmental samples



SPME-enantioselective gas chromatography with ECD and ICP-MS detection for the chiral speciation of the pesticide ruelene in environmental samples†

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Most chiral pesticides are used as racemates despite the fact that the pesticidal activity is generally due to just one enantiomer while the other one may have toxic effects for nontarget organisms. Accordingly, a separation of the two enantiomers of the organophosphorus pesticide ruelene by capillary gas chromatography (GC) has been developed, using a commercial chiral column (CP-Chirasil-Dex CB) and a temperature program from 50 °C (held for 1 min) to 190 °C at 40 °C min⁻¹ with He as carrier gas. This enantioselective gas chromatographic separation of ruelene can be enhanced by a previous solid-phase microextraction (SPME) sample preparation. Experimental SPME conditions such as extraction time, extraction temperature, salt additives and pH have been optimised. The developed SPME-enantioselective GC procedure has been evaluated using two different detection systems: a classical electron capture detector (ECD) and a P-specific detection with inductively coupled plasma mass spectrometry (ICP-MS). The ICP-MS detector has proved to be superior to the ECD in terms of selectivity and sensitivity with excellent detection limits of 27 ng L⁻¹ (3 ng L⁻¹ as P) for each enantiomer of ruelene. The final determination of enantiomers of ruelene in different spiked samples (river water, red wine, orange and tomato juices) has been worked out. The general advantages of ICP-MS detection for chiral pesticides determinations in complex environmental samples are discussed.

Introduction

Organophosphorus pesticides (OPPs) are widely used nowadays as they play an important role in agriculture for crop protection. OPPs are used as pesticides because of their property of inhibiting acetylcholinesterase (AChE) in insects, but they are also toxic to mammals by the same mode of action. The large and often inadequate use of OPPs may result in the presence of residues of these substances in soil, surface and ground waters, drinking waters and agricultural commodities including food and beverages, thus compromising the safety of such products.²⁻⁴

Living organisms possess many vital chiral biomolecules such as amino acids, sugars and enzymes. In fact, they show different biological responses to one of a pair of enantiomers in drugs, pesticides, waste compounds, etc. Numerous pesticides such as OPPs are enantiomeric compounds with phosphorus or carbon atoms as chiral centers. They are produced and released into the environment as racemates despite the usually different bioactivity, toxicity, metabolism and degradation behaviours of the individual enantiomers (as a result of their differential interaction with enzymes or other naturally occurring chiral molecules).⁵⁻⁸ In these cases, the assessment

of the environmental impact of racemic OPPs residues in soil, water, food, beverages and other environmental matrices only based on the data obtained by traditional methods can be misleading. In order to perform a more complete evaluation of the risks posed to environment and public health, it is necessary to provide adequate information of the particular enantiomer in which phosphorus is occurring (so-called "chiral speciation").^{9,10}

Traditionally, the determination of OPPs in environmental samples requires the application of sample preparation procedures to extract the analytes from the matrix, bring them to a suitable concentration level and remove possible interferences (clean-up) prior to final analysis. Solid-phase microextraction (SPME) is a solvent-free extraction technique^{11,12} that has been successfully applied for the determination of OPPs in various samples usually by gas chromatography with different detectors: mass spectrometry (MS),^{13,14} flame photometric (FPD),^{4,15} nitrogen-phosphorus (GC-NPD)^{4,17} and electron capture detector (ECD).⁷ Recently, we have reported that the combination of SPME-GC with inductively coupled plasma mass spectrometry (ICP-MS) enables sensitive and selective detection of phosphorus in pesticides.¹⁸

Ruelene (also called crufomate) is a moderately toxic chiral OPP banned in USA but still widely used in other countries.¹⁹ Both enantiomers of ruelene are insecticidal, but the (+) enantiomer is four times more toxic.¹⁹ Moreover, ruelene is enantioselectively degraded in soil.^{19,20} Therefore, to evaluate the real environmental toxicity of ruelene and to reduce its

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EDCs and Steroid Hormones

- Concern about possible estrogenic or androgenic and other effects, both to wildlife and humans
- SPME-GC-MS used to measure EDCs and hormones in river water and fish serum
- Diethylstilbestrol (DES), octylphenol, nonylphenol, dehydroisoandrosterone (DEHA), estrone (E1), 17 β -estradiol (E2), testosterone, and pregnenolone

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SHORT COMMUNICATION

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Full automation of solid-phase microextraction/on-fiber derivatization for simultaneous determination of endocrine-disrupting chemicals and steroid hormones by gas chromatography–mass spectrometry

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Abstract A fully automated method using direct immersion solid-phase microextraction (DI-SPME) and headspace on-fiber silylation for simultaneous determinations of exogenous endocrine-disrupting chemicals (EDCs) and endogenous steroid hormones in environmental aqueous and biological samples by gas chromatography–mass spectrometry (GC-MS) was developed and compared to a previously reported manual method. Three EDCs and five endocrine steroid hormones were selected to evaluate this method. The extraction and derivatization time, ion strength, pH, incubation temperature, sample volume, and extraction solvent were optimized. Satisfactory results in pure water were obtained in terms of linearity of calibration curve ($R^2=0.9932\text{--}1.0000$), dynamic range (3 orders of magnitude), precision (4–9% RSD), as well as LOD (0.001–0.124 $\mu\text{g L}^{-1}$) and LOQ (0.004–0.413 $\mu\text{g L}^{-1}$), respectively. These results were similar to those obtained using a manual method, and moreover, the precision was improved. This new automated method has been applied to the determinations of target compounds in real samples used in our previous study on a manual SPME method. Exogenous octylphenol (OP), technical grade nonylphenol (t-NP), and diethylstilbestrol (DES) were at 0.13, 5.03, and 0.02 $\mu\text{g L}^{-1}$ in river water and 3.76, 13.25, and 0.10 $\mu\text{g L}^{-1}$ in fish serum, respectively. Natural steroid hormones estrone (E₁), 17 β -estradiol (E₂), and testosterone (T) were at 0.19, 0.11, and 6.22 $\mu\text{g L}^{-1}$ in river water; and in female fish serum E₁, E₂, and pregnenolone (PREG) were at 1.37, 1.95, and 6.25 $\mu\text{g L}^{-1}$, respectively. These results were confirmed by the manual method. The developed fully automated SPME and on-fiber silylation procedures showed satisfactory

applications in environmental analysis and the performances show improved precision and a reduced analysis time compared to the manual method.

Keywords Automated silylation · Nonylphenol · Octylphenol · Diethylstilbestrol · Steroid hormones · Water and biological samples

Introduction

Endocrine-disrupting chemicals (EDCs), which include a diverse range of synthetic and natural compounds, have recently been attracting increased attention due to their ability to disrupt or alter the functions of the endocrine system and consequently their adverse health effects in an intact organism [1]. Growing concerns have also been voiced with respect to another group of biologically active compounds, the so-called steroid hormones, which are low molecular weight lipids naturally synthesized from cholesterol [2]. In previous research, it has been shown that natural steroid hormones excreted by humans or wildlife are released into the aquatic environment through discharges from sewage treatment and may act as EDCs [3, 4]. Moreover, the EDCs are delivered into target organs via the blood flow and pose some adverse effects by disrupting the balance of natural steroid hormones in blood [5, 6]. Since both kinds of compounds have been linked in nature, simultaneous determinations of EDCs and steroid hormones in water and blood are essential not only for monitoring EDCs but also for clarifying their functions in the body.

To achieve an efficient, practical, and reliable method for the simultaneous determinations of EDCs and steroid hormones, appropriate sample preparation is very important. Traditional sample preparations such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been used for cleanup of the samples in the simultaneous determination of EDCs and steroid hormones [7]. However, these methods are laborious, time-consuming, and use relatively large amounts of sample and organic solvent;

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