



Methods for Extractable Nonvolatile Compounds Analyzed by Liquid Chromatography/Mass Spectrometry for Environmental Restoration Following Homeland Security Events



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Mention of Vendor Names Does Not Constitute Product Endorsement



SAM Document

- How Chicago Regional Lab Became Involved
 - Reviewed the LC/MS Standard Analytical Protocol (SAP)
- Matrices
 - **Water**
 - Ambient Air
 - **Wipes**
 - **Soil**
 - Residential
 - Industrial



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Split up the Analytes into Different Methods and Optimized Conditions

- Methods Indicate Specific Analytical Columns and Conditions for Best Separation.
- Met Risk Criteria Goals in Environmental Water
- Easily Transferable to Environmental Laboratories.
- External Calibration with relevant surrogates for each method, try to keep cost of analysis low.
- Initial Precision and Accuracy Data generated in reagent and nearby Chicago surface water.
- Methods Presently in Multi-laboratory Study for Incorporation into SW-846 and Drinking Water

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Multi-laboratory Participants

- Read the SOPs- Can they be followed? Suggest comments to make them more helpful.
- Verify calibration curves (linear or quadratic)
- Verify that the reporting and detection limits are achievable in their labs on various LC/MS/MS systems
- Generate recovery data at 4 points over the calibration curves in both reagent water and their local surface water.
- All surface waters are characterized by EPA Region 5 CRL

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New LC/MS/MS Water Methods Overview

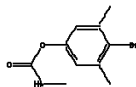
Method Number	Target Compound	Time of Analysis		Risk Criteria (PPB)	Limit of Detection (PPB)	Reporting Limit (PPB)
		Including Sample Prep (Min)				
1	Aldicarb			35	0.1	1
	Bromacilone			0.7	0.5	1
	Carbofuran	28		180	0.1	1
	Oxamyl			10	0.1	1
	Methomyl			NA	0.1	1
2	Diisopropyl methylphosphonate			2800	1	5
	Ethyl methylphosphonic acid			880	10	50
	Isopropyl methylphosphonic acid	25		3500	20	50
	Methylphosphonic acid			700	10	100
	Ethyl hydrogen dimethylamidophosphate			NA	0.1	5
	Pinacolyl methylphosphonic acid			NA	10	50
3	Diethanolamine			NA	5	25
	Triethanolamine	32		NA	5	25
	n-Ethylethanolamine			NA	5	25
	n-Methylethanolamine			NA	20	25
4	Thiodiglycol	21		16000	50	250

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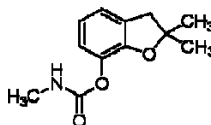
*Risk Based Criteria are based upon US EPA Region 3 risk based concentration methodology.



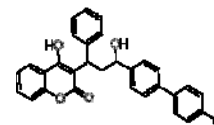
Aldicarb, Bromadiolone, Carbofuran, Methomyl, Oxamyl and BDMC (Surrogate) Method One



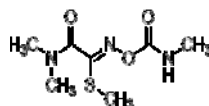
BDMC



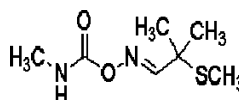
Carbofuran



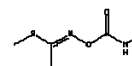
Bromadiolone



Oxamyl



Aldicarb



Methomyl

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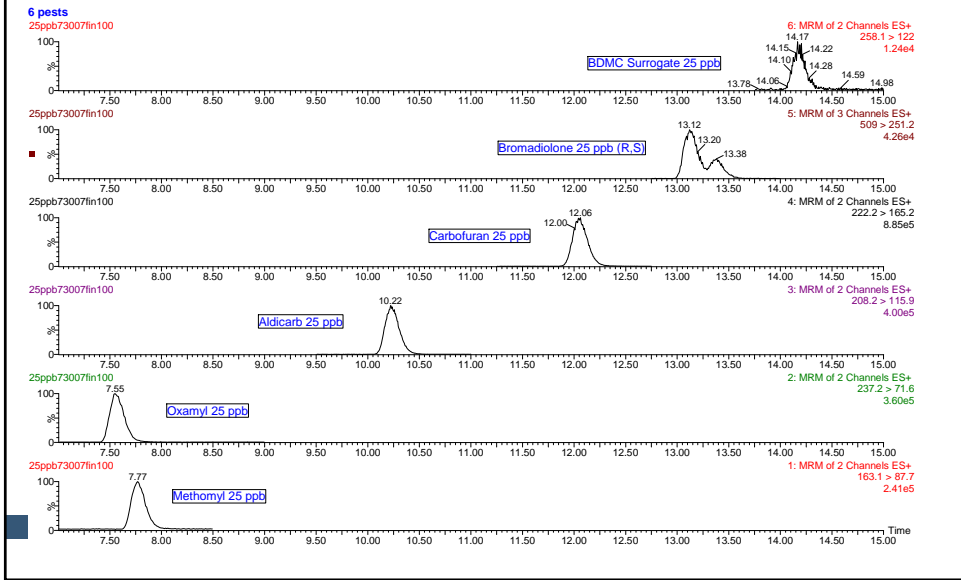
LC and MS/MS Conditions

- Sample Prep
 - Add Surrogates
 - Filter
- LC/MS/MS
 - Waters 2695 LC and Quattro Micro API MS/MS
- XBridge C₁₈ Column
 - 2.1 x 150 mm, 3.5 μm particle
- Modifier
 - 2.5 mmolar NH₄OAc/NH₄OH
- Gradient
 - 100% Water to 90% CH₃CN

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Chromatography, all at 25 PPB



Signal/Noise (Limit of Detection)

Analyte	Concentration (ppt)	S/N
Aldicarb	250	16.5
Bromadiolone	250	22
BDMC (Surr)	2500	6.6
Carbofuran	100	5.2
Methomyl	750	6.2
Oxamyl	250	5.2

Precision and Accuracy Data

Analyte	Reagent Water	Chicago River Water
	25 ppb Spike*	25 ppb Spike*
	<u>% Recovery (Std Dev)</u>	<u>% Recovery (Std Dev)</u>
Aldicarb	99.8 (1.4)	99.7 (0.98)
BDMC (50 ppb*)	103.5 (2.4)	93.6 (2.3)
Bromadiolone	111.1 (5.7)	99.1 (2.9)
Carbofuran	102.8 (1.7)	102.6 (0.49)
Methomyl	97.3 (2.5)	70.2 (0.96)
Oxamyl	98.2 (2.4)	65.1 (0.54)

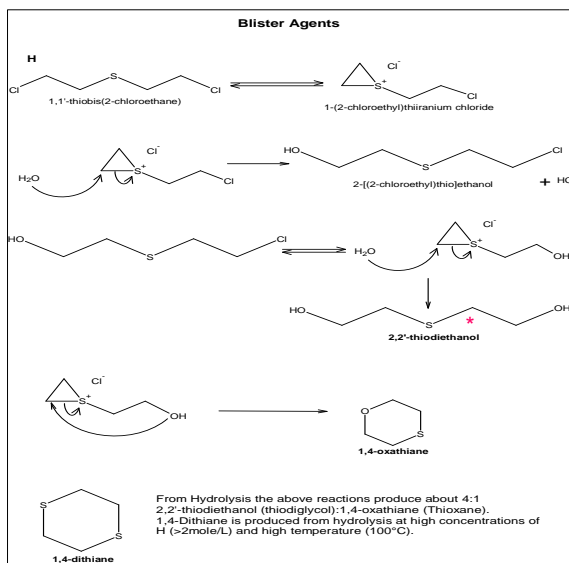
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Thiodiglycol (TDG) and 3,3'-Thiodipropanol (Surrogate, 3,3'-TDP)



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Blister Agent Background



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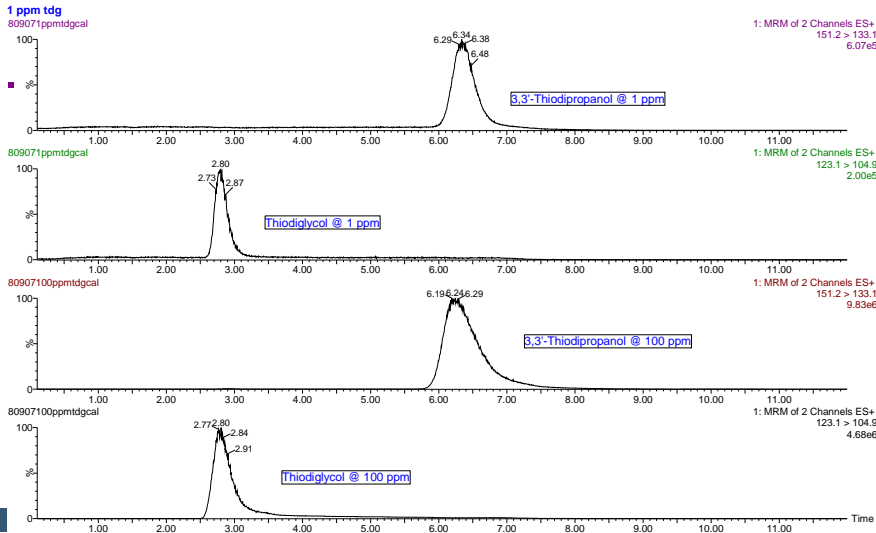
LC and MS/MS Conditions

- LC/MS/MS
 - Waters 2695 LC and Quattro Micro API MS/MS
- Primesep SB Column
 - 2.1 x 150 mm, 5 μ m particle
- Modifier
 - 0.1% Formic Acid/25 mmolar $\text{NH}_4\text{CO}_2\text{H}$
- Gradient
 - 100% Water to 90% CH_3CN

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Chromatography



Precision and Accuracy Data Using 2.5 to 100 ppm Calibration Curves

Analyte	Reagent Water		Chicago River Water	
	25 ppm Spike % Recovery (Std Dev)	2.5 ppm Spike % Recovery (Std Dev)	25 ppm Spike % Recovery (Std Dev)	2.5 ppm Spike % Recovery (Std Dev)
TDG	98.1 (8.8)	100.2 (4.7)	102.3 (0.63)	92.2 (4.8)
3,3'-TDP (Surr)	106.8 (2)	95 (3.7)	111.2 (1.6)	114.2 (11.1)

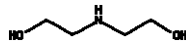
Precision and Accuracy Data Using 250 ppb to 10 ppm Calibration Curves

Analyte	Reagent Water 2.5 ppm Spike % Recovery (Std Dev)	Chicago River Water 2.5 ppm Spike % Recovery (Std Dev)
TDG	114.6 (3)	106.3 (2.9)
3,3'-TDP (Surr)	114.9 (1.6)	124.7 (6.7)

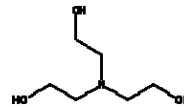
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Ethanolamines

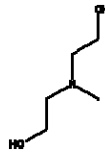
Method Three



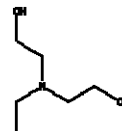
Diethanolamine



Triethanolamine



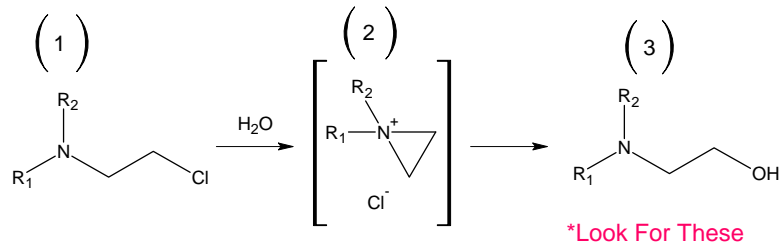
n-Methyldiethanolamine



n-Ethyldiethanolamine

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Nitrogen Mustard Background



	R ¹	R ²	Nitrogen Mustard
HN1	C ₂ H ₅	CH ₂ CH ₂ Cl	Steps 2-3 repeated for each CH ₂ CH ₂ Cl group.
HN2	CH ₃	CH ₂ CH ₂ Cl	
HN3	CH ₂ CH ₂ Cl	CH ₂ CH ₂ Cl	

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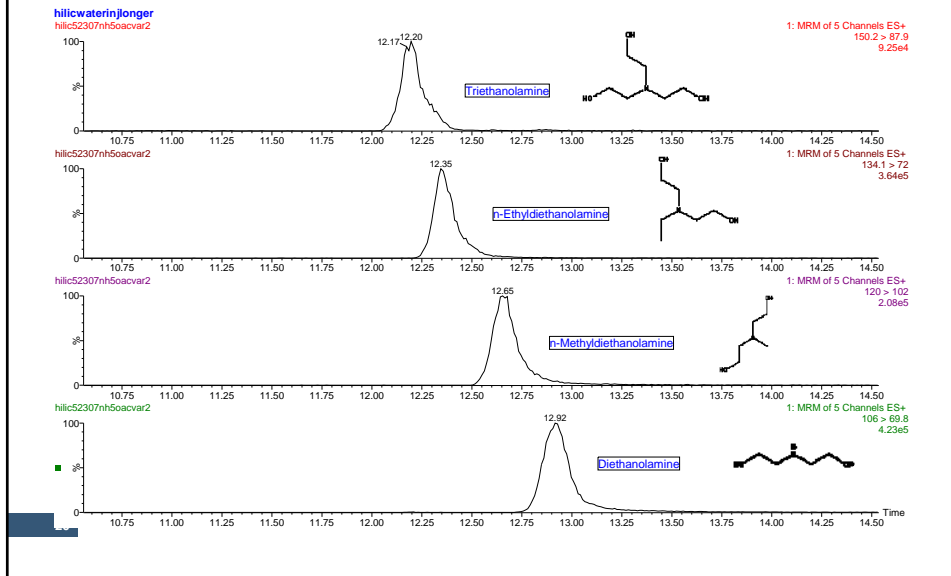
Separation of the Ethanolamines

- MS/MS ESI Positive
- 20 mmolar Ammonium Acetate
- CH₃CN/H₂O Gradient
 - 0.3 mL/min
 - Variable Gradient-25 minute total run time
 - 95% CH₃CN to 60% Water/40% CH₃CN
- Column- Atlantis HILIC Silica, 3 μm, 2.1 x 100 mm
- 25-50 μL Direct Injection of Water

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Separation of the Ethanolamines (25 μ L Water Injection)

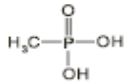


Ethanolamine Parameters

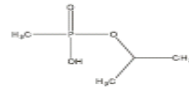
PARAMETER	% Recovery* LCS/LCSD	% Recovery** MS/MSD
	Reagent Water	Environmental Water
Diethanolamine	63-123	86-146
Triethanolamine	54-114	42-102
n-Ethyldiethanolamine	73-133	31-91
n-Methyldiethanolamine	62-122	20-120
Diethanolamine-d ₈ (Surrogate)	75-135	95-155

Organophosphonic Acids

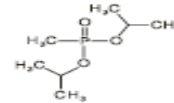
Method Four



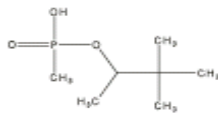
Methylphosphonic acid



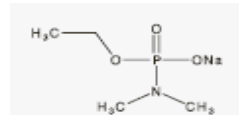
Isopropyl methylphosphonic acid



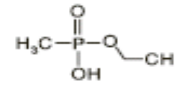
Diisopropyl methylphosphonate



Pinacolyl methylphosphonic acid



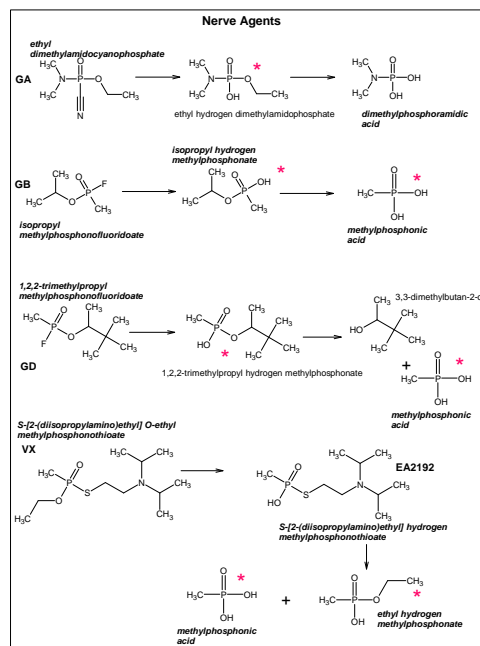
Ethyl hydrogen dimethylamidophosphate



Ethyl methylphosphonic acid

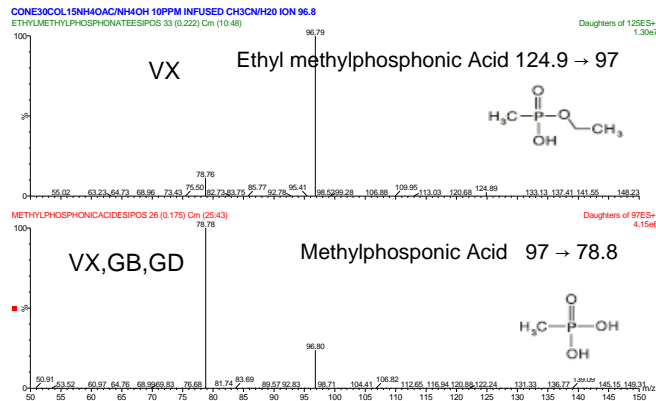
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Nerve Agent Background



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Common Precursor Compound Coupled with no Chromatography



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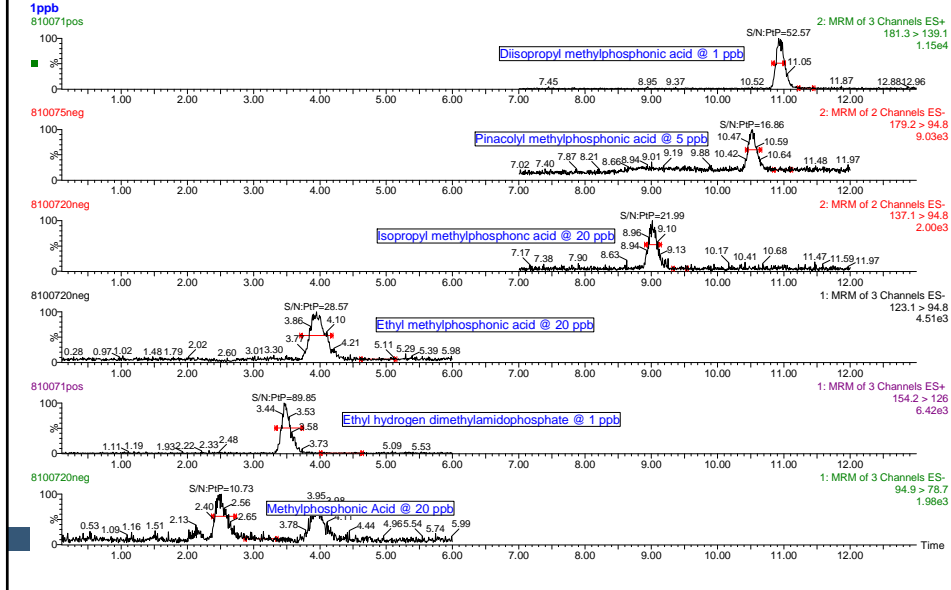
Separation of the Phosphonic Acids

- MS/MS ESI Negative and Positive
- 0.1% Formic Acid
- CH₃CN/H₂O Gradient
 - 0.3 mL/min
 - Variable Gradient-20 minute total run time
 - Water to 95% CH₃CN
- Column- Atlantis dC₁₈, 3 μm, 2.1 x 150 mm
- 50-100 μL Direct Injection

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Chromatography and S/N



PARAMETER	ESI Mode (Positive/Negative)	% Recovery* LCS/LCSD Reagent Water	% Recovery* MS/MSD Environmental Water
Diisopropyl methylphosphonate	POS	75-135	74-134
Diisopropyl methylphosphonate	NEG	NA	NA
Ethyl hydrogen dimethylamidophosphate	POS	57-117	54-124
Ethyl hydrogen dimethylamidophosphate	NEG	60-120	54-114
Ethyl methylphosphonic acid	POS	70-130	70-130
Ethyl methylphosphonic acid	NEG	73-103	78-108
Isopropyl methylphosphonic acid	POS	71-131	65-125
Isopropyl methylphosphonic acid	NEG	70-130	74-134
Methylphosphonic acid	POS	60-120	110-170
Methylphosphonic acid	NEG	64-124	0
Pinacolyl methylphosphonic acid	POS	76-136	78-138
Pinacolyl methylphosphonic acid	NEG	74-134	80-110
Diisopropyl methylphosphonate-D ₁₄ (Surrogate)	POS	74-134	74-134
Diisopropyl methylphosphonate-D ₁₄ (Surrogate)	NEG	NA	NA
Methylphosphonic acid-D ₃ (Surrogate)	POS	65-125	92-152
Methylphosphonic acid-D ₃ (Surrogate)	NEG	66.8-126.8	0-47.2
Pinacolyl methylphosphonic acid- ¹³ C ₆ (Surrogate)	POS	NA	NA
Pinacolyl methylphosphonic acid- ¹³ C ₆ (Surrogate)	NEG	70-130	75-105



LC/MS SAP Conclusions/Current Status/Future Work

- 4 New Methods
- Met Risk Criteria Goals
- Verification and Validation in Process for Incorporation into SW-846
- Ten Individual Laboratories are currently testing reagent water and local surface waters to create control limit criteria and validate the methods.
- Developing wipe and soil methods
- Inclusion of more analytes of interest
- Ultra Performance Liquid Chromatography- MS/MS
- Update outdated EPA methods

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Acknowledgements

- **ORD- National Homeland Security Research Center**
- **EPA National Enforcement Investigations Center**
- **US EPA ORD/National Exposure Research Laboratory**
- **Office of Pesticide Programs, Stennis Space Center, MS**
- **Virginia Division of Consolidated Laboratory Services**
- **US EPA Regions 1, 6 and 8**
- **Lawrence Livermore National Laboratory**
- **US EPA Office of Pesticide Programs Biological and Economic Analysis Division Analytical Chemistry Branch, Ft. Meade, MD**

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