The 14th Annual Waste Testing & Quality Assurance Symposium

PROCEEDINGS

WTQA '98

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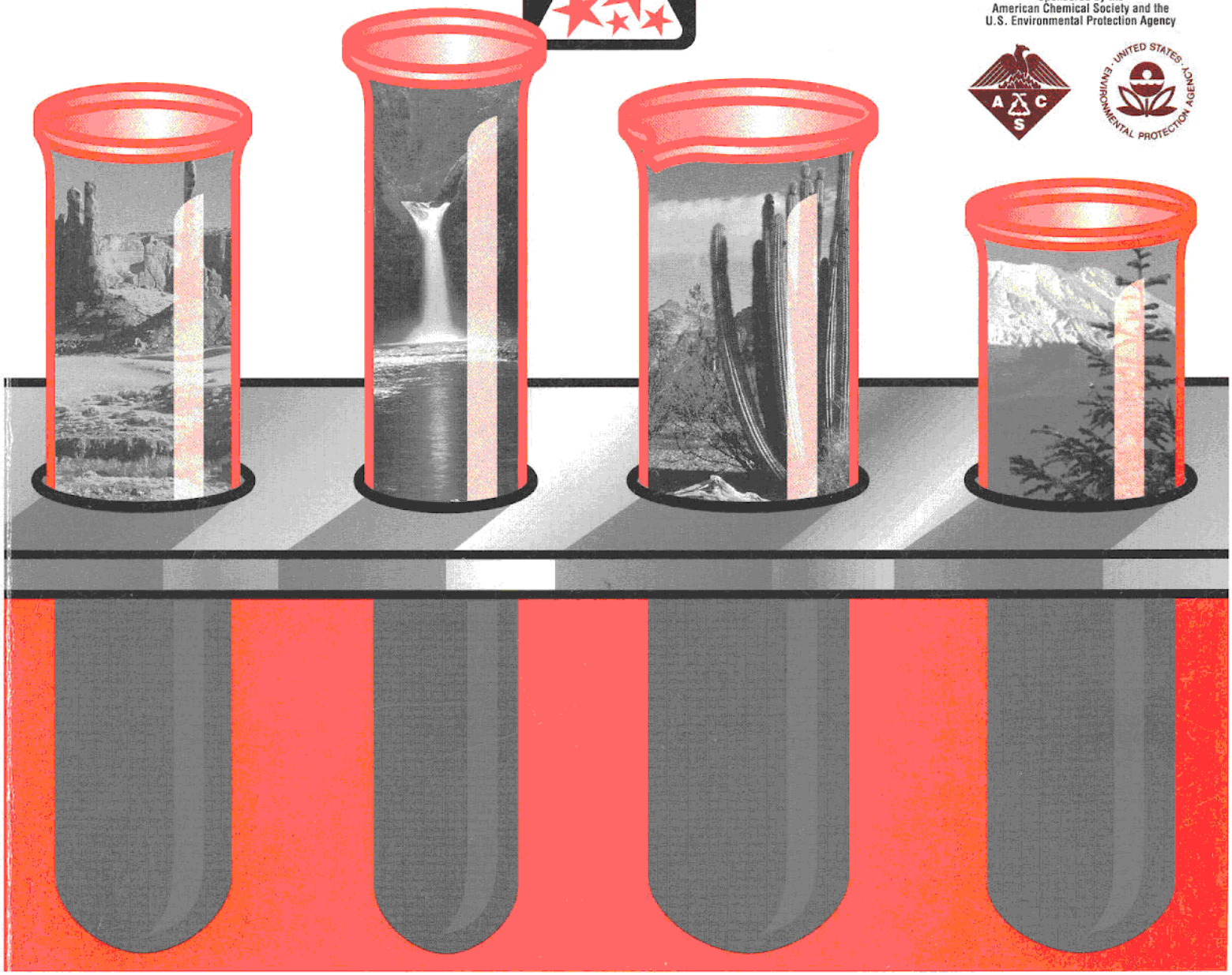

July 13-15, 1998 ■ Crystal Gateway Marriott ■ Arlington, VA

Proceedings

The Fourteenth Annual

Waste Testing & Quality Assurance Symposium (WTQA '98)

July 13-15, 1998

Crystal Gateway Marriott
Arlington, VA

HIGHLIGHTS

14th Annual Waste Testing & Quality Assurance Symposium
(WTQA '98)
Using A Performance-Based Measurement System (PBMS)

WTQA '98, cosponsored by the American Chemical Society (ACS) Division of Environmental Chemistry and the U.S. Environmental Protection Agency (EPA) Office of Solid Waste and Emergency Response (OSWER), will be held at the Crystal Gateway Marriott in Arlington, VA, July 13-15, 1998. This year's symposium continues the partnership between the regulated community and their supporting laboratories and state and federal regulators from the Research Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) programs.

Highlights
EPA is actively working to implement the President's program for "reinventing" government and reforming regulatory policy. As part of this endeavor, EPA has been trying to break down barriers to using new monitoring techniques. One barrier that OSWER is tackling is the requirement to use specific measurement methods or technologies in complying with Agency regulations. EPA's Environmental Monitoring Management Council (EMMC), members of the regulatory community, and Congress all agree that EPA needs to change the way it specifies monitoring requirements in regulations and permits. There is broad acceptance for use of a performance-based measurement system (PBMS). The EPA Office of Solid Waste and Emergency Response (OSWER) strongly supports this position and is committed to using this approach in both the RCRA and CERCLA monitoring programs. This year's plenary session speakers, including Fred Hanse, Deputy Administrator of EPA; Brad Campbell, Associate Director for Toxics & Environmental Protection; Steve Koorse, Hunton and Williams; Elizabeth Cotsworth, Acting Director, Office of Solid Waste; and Larry Keith, Waste Policy Institute, will focus their remarks on various aspects of PBMS.

PBMS Implementation Session
This session, organized by the International Association of Environmental Testing Laboratories Section of the American Council of Independent Laboratories (ACIL-IAETL), will include a presentation of EPA Program Office PBMS Implementation Plans, followed by speakers presenting the laboratory community, regulated community, and state regulatory agency perspectives on PBMS. The focus of the talks from the regulated community will be on the barriers they expect to face when trying to obtain the benefits of PBMS and what we all need to do to overcome the problems.

PBMS Methods Validation Session
Now that the EPA has moved towards implementing a PBMS approach to environmental monitoring, the regulated community needs to know how to validate methods under this new system. This session, also organized by ACIL-IAETL, will focus on how to tailor measurement system validation to the required data quality; how validation criteria should be specified in order that it not serve as a barrier to using alternative measurement technologies; and what is the minimum methods validation data set that one needs for the data obtained from the analysis to be of known and documented quality.

EPA's Environmental Monitoring Research Program
This session will focus on environmental monitoring methodology research supported under EPA's Science to Achieve Results (STAR) competitive, extramural grant program. The program has funded a number of research projects whose goal it is to develop unique or novel techniques for monitoring pollutants in the environment. Methodology to monitor air, water, soil and other media will be presented. The session will review the results from projects funded in prior years, and discuss the objectives and approaches to be undertaken in research studies that received funding this past year.

Superfund Session
The keynote for this Superfund session will be "Times Are Changing." Planned highlights include (1) a description of the Superfund pipeline over time, emphasizing the near and longer term future and how that relates to analytical service need; (2) information on how Superfund is working with other EPA program offices to enhance our operation; (3) the trend to encouraging economic redevelopment at Superfund sites; (4) how Superfund is implementing a

PBMS approach; (5) initiatives related to data quality and minimal Quality Systems; (6) electronic data delivery and validation; (7) usefulness of the National Environmental Laboratory Accreditation Conference (NELAC) accreditation for Superfund analytical work; (8) future direction for the Contract Laboratory Program (CLP); (9) Contracts 2000, Performance-Based Contracting, and what this means to the laboratory community, and (10) laboratory perspective on working for Superfund (a panel discussion).

SYMPOSIUM-AT-A-GLANCE

Sunday, July 12

8:00 a.m. - 9:30 a.m.	Registration for Short Courses
9:00 a.m. - 12:00 noon	Short Course: Analytical Strategy for the RCRA Program
9:00 a.m. - 4:00 p.m.	Short Course: An Introduction to Practical Ethics for Environmental Laboratories
1:00 p.m. - 5:00 p.m.	Short Course: Clean Chemistry for Trace Elemental Analysis

Monday, July 13

7:00 a.m. - 4:00 p.m.	Registration Open
8:00 a.m. - 12:00 noon	SW-846 Workgroups (closed)
8:00 a.m. - 12:00 noon	Short Course: Quality Systems, PBMS and NELAC: Putting It All Together
11:00 a.m. - 12:00 noon	Trial Exam for Environmental Analytical Chemists
2:00 p.m. - 4:30 p.m.	Opening Plenary Session David Friedman, Office of Research and Development Gail Hansen, Office of Solid Waste Fred Hansen, Deputy Administrator of EPA Brad Campbell, Associate Director for Toxics & Environmental Protection Steve Koorse, Hunton & Williams Elizabeth Cotsworth, Acting Director, Office of Solid Waste Larry Keith, Waste Policy Institute
5:00 p.m. - 7:00 p.m.	Opening Reception

Tuesday, July 14

7:00 a.m. - 5:00 p.m.	Registration Open
8:15 a.m. - 12:00 noon	Organic I Session
8:15 a.m. - 12:00 noon	Inorganic Session
10:00 a.m. - 10:30 a.m.	Break
12:00 noon - 1:00 p.m.	Lunch Break
1:15 p.m. - 5:00 p.m.	Organic II Session
1:15 p.m. - 5:00 p.m.	Environmental Monitoring Research Session
3:00 p.m. - 3:30 p.m.	Break
7:00 p.m. - 8:00 p.m.	Trial Exam for Environmental Analytical Chemists

Wednesday, July 15

7:00 a.m. - 5:00 p.m.	Registration Open
8:15 a.m. - 12:00 noon	PBMS Implementation Session
8:15 a.m. - 12:00 noon	QA Session
10:00 a.m. - 10:30 a.m.	Break
12:00 noon - 1:00 p.m.	Lunch Break
1:15 p.m. - 5:00 p.m.	PBMS Validation Session
1:15 p.m. - 5:00 p.m.	General Session
3:00 p.m. - 3:30 p.m.	Break
7:00 p.m. - 8:00 p.m.	Trial Exam for Environmental Analytical Chemists

Thursday, July 16

9:00 a.m. - 12:00 noon	Short Course: Analytical Strategy for the RCRA Program
9:00 a.m. - 4:00 p.m.	Superfund Session

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ORGANIC

INORGANIC

ORGANIC

OVERVIEW OF CURRENT STATUS OF RCRA ORGANIC METHODS PROGRAM

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NO ABSTRACT AVAILABLE

APPROPRIATE WAYS TO MODIFY EXISTING METHODS FOR NEW APPLICATIONS

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NO ABSTRACT AVAILABLE

QUESTIONABLE PRACTICES IN THE ORGANIC LABORATORY

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SW-846 is a collection of performance-based methods used by the US Army Corps of Engineers (USACE) for execution of environmental restoration projects. These methods provide guidance for the running of various organic and inorganic protocols that can be used for the analysis of samples from a variety of sample matrices. During recent laboratory audits conducted by the USACE, certain 'questionable practices' have been observed, especially in the organic analysis areas.

Most people have a relatively good idea of what constitutes a fraudulent activity today. The concepts of 'dry-labing,' 'peak shaving,' 'peak enhancing,' or 'time-traveling' are well understood. These practices clearly involve the deliberate direct manipulation and/or alteration of data, often to achieve or meet method QC criteria. However, there are a new group of 'questionable practices' now being observed that often involve the selective exclusion of data to achieve or meet method QC criteria.

This presentation will focus on several of these 'questionable practices'. Examples of some of these practices include the following: (1) One such practice involves the determination of initial calibration curves. Laboratories have been observed running eight or more standards for methods that state 'a minimum of five points should be used to establish the initial calibration curve.' Up to three points are then discarded, even from the middle of the curve, until the appropriate QC criteria can be met. No technical justification existed for the deletion of these points other than to meet the method QC criteria. (2) Another such practice involves the evaluation of the continuing calibration verification solution. Laboratories have been observed averaging the % difference or % drift across all target analytes even when several of the target analytes exceed the criteria by a significant amount such that the average still meets the criteria as stated in the method. (3) Another such practice involves the reporting of acceptance ranges for surrogates or laboratory control samples. Laboratories have been observed reporting a very tight range indicating that they have good method control. However, an examination of the actual control charts maintained by the laboratory shows a significantly wider range. (4) Another such practice involves the determination of the method detection limit (MDL). Laboratories have been observed running ten or more standards and then discarding points to achieve a lower MDL. No technical justification existed for the deletion of these points other than to achieve a lower MDL.

Should the above 'questionable practices' be considered as examples of fraudulent activities? Some of the laboratories have described these practices as 'the common approach used by everyone.' Yet when described to people within EPA, the clear response is that these approaches were never intended. The background history will be discussed as to how and why these practices have developed and what can be done to overcome them.

MODIFICATIONS TO SW-846 HPLC METHODS 8330 AND 8310

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Method 8000 of SW-846 allows for modification of the existing methodology, provided that the analyst has documented the ability to generate acceptable results by successfully performing an initial demonstration of proficiency with the altered conditions prior to sample analysis. Analysts may vary the extraction procedures and/or HPLC parameters (mobile phase composition, elution program, injection volume, etc) in order to improve sensitivity and chromatographic resolution, or to reduce interferences resulting in coelution with analytes of interest. The use of analytical columns different from those specified in the methods may require that different HPLC conditions and flow rates be used. Generally, the HPLC methods 8330 and 8310 have been found to perform acceptably as written. However, some method modifications need to be employed in order to improve analyte resolution, achieve desired quantitation limits and obtain definitive confirmation of the primary column results. Some of the modifications to Method 8330 and Method 8310 reported in this paper include: nitrogen blow-down to adequately concentrate the final acetonitrile extracts after the salting-out liquid-liquid extraction procedure or the solvent exchange procedures; reduced flow rates and gradient elution schemes for the C-18 primary columns; and, the use of an acetonitrile/water mobile phase for the cyano secondary column confirmations.

When reporting quantitated results for these analyses, it is more important to positively confirm the primary column results than it is to completely resolve all of the target compounds. The use of analytical columns different from those specified can produce coeluting pairs which may differ from those mentioned in the methods. This paper will also discuss the reporting of coeluting compounds as mixtures, and the use of confirmatory techniques (e.g. dissimilar secondary column analyses, spectral library matching, LC/MS, GC/MS and GC-ECD).

**PCB SEPARATIONS USING 2 DIMENSIONAL GC FOR CONFIRMATION:
USE OF A HEART-CUTTING SWITCHING VALVE**

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Environmental gas chromatography analysis continues to be a difficult but necessary effort for the detection and identification of PCBs. One approach is the use of a very good separation column, which would resolve most if not all of the 140-150 key congeners from one another. Another approach uses a more user-friendly approach to the congener specific analysis of PCBs. This approach is not a new or novel approach, but a revisit of a variation of a general column-confirmation column method. The use of two different stationary phase columns (orthogonal) allows a geometric gain in separation power compared to the algebraic gain by making the column longer or more efficient.

The approach we used involves a heart cutting switching valve, one injector and two detectors. Some discussion will be given concerning the choice of the stationary phases, the choice of carrier gas and especially the judicious combination of temperature programming rate and carrier linear velocity. Focus will be upon key critical PCB congeners as described by the European Union protocols, that is, congener specific detection and confirmation.

The configuration uses two columns and two detectors, with a valve between the two detectors and a second selective column before the second detector. In the initial analysis, the sample is nominally separated on a general-purpose column. As sets of unresolved PCBs elute, they are cut out of the first separation scheme and sent to the second more selective column. The front of the second column may be cooled cryogenically (we will also demonstrate what happens when the cooling of the second column is not used) to hold the analytes at the front of the second column until the analysis of the first column is complete. Then a second temperature program is begun to resolve previously unresolved pairs.

The first column is a 5 % phenyl phase and the second column we used was either a 35 % phenyl or a 50 % phenyl phase of intermediate polarity. Either column is useful, and a specific choice may be made depending upon the sensitivity level (the 35 % phenyl is a much lower bleed column) or whether additional selectivity is needed. There are a great many permutations of this approach possible, and we will outline what we did and what can be done if even greater resolution is required.

ESTIMATING THE TOTAL CONCENTRATION OF VOLATILE ORGANIC COMPOUNDS IN SOIL AT SAMPLING LOCATIONS: FIELD TRIALS

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ABSTRACT
This report describes a method for estimating the total concentration of volatile organic compounds (VOCs) in soil relative to a site-specific 0.2-mg/kg working standard and presents the results from three separate field trials. This method was developed to provide a decision tool for field or laboratory personnel so they can implement the appropriate soil sample preparation procedure for the selected method of instrumental analysis. Coupling a rapid method for estimating the total VOC concentration with sample collection, handling, and preparation procedures that limit substrate dissaggregation and exposure complements efforts to achieve site-representative estimates for vadose zone contamination.

INTRODUCTION
Since the beginning of the Superfund and the Resource Conservation and Recovery Act (RCRA) programs, gas chromatography/mass spectrometry (GC/MS) (via Methods 8260 and 8240) has served as the major laboratory instrument for identifying and quantifying VOCs in soils[1]. The principal reason for the selection of this analytical detection system is that it provides an unambiguous identification of analytes present. Unfortunately, this very desirable quality comes with the limitation that for quantification purposes the individual analytes must fall within a concentration range of 2 to 3 orders of magnitude. High analyte concentrations can degrade the performance of the MS detection system, which interrupts scheduled runs and may lead to expensive instrument repairs. Therefore, one of the challenges when using an MS is how to couple it with a sample collection, handling, and preparation protocol when analyte concentration can range over 7 orders of magnitude (percent levels to the current levels of instrumental detection, approximately 0.005 mg/kg). To cope with this concern, samples thought to be contaminated with VOCs at levels greater than 0.2 mg/kg are prepared by extraction (and perhaps further dilution) with methanol (MeOH), i.e., the high-level method. In contrast, samples thought to have concentrations less than 0.2 mg VOC/kg are analyzed directly, which is referred to as the low-level method. Many other

commonly used laboratory instruments and their respective methods for VOC detection (e.g., Methods 8015 and 8021B) also benefit from using these two approaches to sample preparation.

A second challenge is that VOCs in soils fail to maintain their concentration integrity if they are not collected and handled with limited disruption and exposure and if preventive measures are not taken to limit biological degradation of aromatic compounds. Today it is generally recognized that the sample collection and handling guidance, provided in the past by Method 5030, often resulted in a greater than 90% loss of the VOCs from soil samples prior to laboratory analysis[2-6]. To minimize losses due to volatilization and biodegradation, new sample collection and analysis protocols were included in the third update of SW-846[1]: Method 5035, "Modified purge-and-trap and extraction for volatile organics in soil and waste samples," and Method 5021, "Volatile organic compounds in soils and other solid matrices using equilibrium headspace analysis."

The two most effective collection and handling protocols that can be used with these new methods for preventing the loss of VOCs are 1) the on-site, rapid transfer of discrete samples with a small coring tool to a vessel that hermetically seals and already contains the appropriate dispersion/extractant solution for the chosen method of analysis[7], or 2) obtaining and temporarily storing (two days at 4ºC) a sample in an En Core™ (En Novative Technologies, Inc., 1241 Bellevue St., Green Bay, Wisc. 54302) sampler before transferring it into an appropriately prepared vessel[8]. In addition, it should be recognized that, if the sample is to be held for more than two days before analysis, then some form of chemical preservation may be necessary in addition to storage at 4ºC. For example, acidification can be used for low-level sample preparation procedures when carbonates are not present[6].

Because there is often no a priori knowledge of the VOC concentrations at a given location, the data quality objectives for site characterization activities often require that samples be collected and prepared for both the lowand high-level analysis procedures. To avoid collecting and processing samples through both of these preparation procedures for every location, it has been suggested that a rapid screening analysis be performed to establish an estimate for the total VOC concentration[9]. This screening indicates the levels of VOCs to expect, before the sample is prepared for analysis, and thus whether collocated sample(s) taken for laboratory analysis should be prepared using the low- or high- level procedures, or both. The method developed is based on the comparison of responses of a hand-held photoionization detector (PID) to a sample relative to a 0.2-mg VOC/kg site-specific working standard. Recognition of the potential effort and cost savings by using screening as a decision tool are two reasons why this method is being considered for inclusion in the fourth update of SW-846 (proposed Method 3815). This paper briefly outlines this screening method and presents the results from three case studies. Additional information concerning the development of this method for screening is available elsewhere[9].

SCREENING METHOD

Materials

The necessary equipment and reagents are as follows:

1) Modified VOA vials (40 or 44 mL), Teflon-lined septa with 5- to 6-mm hole punched through the middle and 3- x 3-cm squares of light-gauge aluminum foil for temporary covers (see Fig. 1).
2) Coring tool for the collection and transfer of discrete soil samples, e.g., disposable 10-mL plastic syringes with the Luer tip and rubber plunger cap removed, or an equivalent metal tube and plunger.
3) A portable photoionization detector (PID) analyzer with a 10.6-eV or greater electrode discharge tube, digital display, inlet flow rate greater than 200 mL/min, and sample inlet tube of 3 to 4 mm o.d. and at least 3 cm in length.
4) A 10-mL liquid syringe.
5) Reagent-grade water (i.e., water with no detectable VOCs), polypropylene glycol (PPG, or similarly low-vapor-pressure organic solvent), and principal VOC(s) of site interest.
6) A cylinder of calibration gas for the PID, e.g., 100 ppm isobutylene.

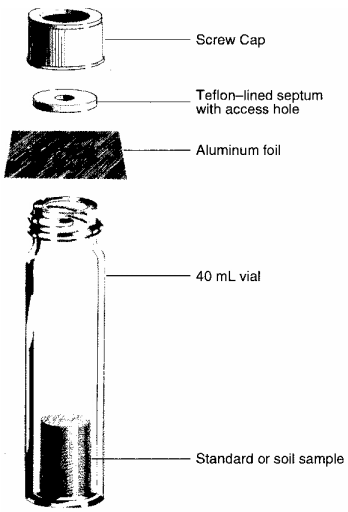

Figure 1. Modified VOA vials for rapid total VOC screening of soil samples.

Standards
A stock standard is prepared by transferring the VOC of interest into PPG. The stock standard concentration should be based on the density of the analyte of interest, so that a 1- to 3-µL volume transferred to a 40-mL VOA vial containing 10 mL of reagent water and 10 g of the site-specific soil matrix results in a 0.2-mg-VOC/kg working standard. For example,

Stock standard: 1.34 g/mL* x 2.0 µL/2.5 mL - 1.1 mg TCE/mL
Working standard: 1.1 mg TCE/mL x 1.8 µL/10 g soil = 0.2 mg TCE/kg
*Density of TCE

Immediately after spiking, these working standard vials are covered with a single sheet of aluminum foil that is held tightly in position with a septum with a hole punched in the middle and a screw cap (Fig. 1). The vial contents of the working standards should be thoroughly mixed by handshaking, then transported to the location of the sampling activity, stored out of direct sunlight, and allowed to equilibrate for 1 hr prior to use. Working standards should be prepared daily.

The PID response to the working standard should be at least 10x greater than its response to a blank (reagent water, contamination-free site-specific matrix, and appropriate volume of PPG). For analytes with high vapor pressures or low octanol water partition coefficients, or both, and soil matrices with low organic carbon contents, it may not be necessary to include the site-specific soil matrix in the working standards. This should be established on a site-by-site basis by comparing the means of triplicate working standards with and without the soil matrix. As a general rule, if the means differ by more than 20%, it is recommended that the soil matrix be included in the working standards.

Sample Collection and Analysis
Before field sampling, 10 mL of reagent water is added to the modified VOA vials. Once prepared, the VOA vials for screening samples should be transported to the sampling location and stored with the working standards until they are used. The native structure of the material being sampled for screening should be kept intact, thus experiencing as little disaggregration as possible during the collection and transfer process. This can often be accomplished with a coring tool designed to obtain a discrete sample. For example, a modified 10-mL syringe is a practical tool for obtaining up to a 10-g soil sample. If 10 g cannot be easily obtained in a single transfer, more than one corer can be used, or a couple of transfers with a single corer can be made. This coring device is transparent and comes with gradient markings so the volume/weight relationship for a given material can easily be established with a portable balance. The location of samples taken both for screening purposes and for laboratory analysis should be as close as possible to each other (generally within a 10-cm radius) and from the same stratum. Before preparing (or exposing) a fresh sampling surface, for instance, opening a split spoon or scraping away the top layer of a material, the cap and aluminum foil should be removed from the screening VOA vial. After retrieving a discrete sample, the core barrel should be inserted into the mouth of the screening VOA vial and the sample extruded. Once the sample has been extruded, the aluminum foil and cap should immediately be replaced on the vial. This collection and transfer process should take less than 10 seconds, and the sample weight only has to approximate 10 g (plus or minus 2 g).

Before a working standard or sample is analyzed, the VOA vial should be shaken by hand for 10 to 15 seconds. Cohesive materials, such as silts and clays, do not break apart rapidly upon shaking and may require more than 15 seconds for complete dispersion. The vial is then visually checked both for the complete dispersion of the sample matrix and for particles adhering to the aluminum foil cap liner (knock large particles off the aluminum foil if present). Then the inlet tube of the PID is pushed through the foil liner to a set position about 3 cm below the rim. A maximum response will be achieved within 2 to 3 seconds of punching through the foil liner. The maximum response for each sample screened and for the analysis of each working standard should be recorded.

Daily Operating Procedure for VOC Screening
The PID should be initially calibrated with a cylinder of standard gas (e.g., 100 ppm isobutylene) at the beginning of each day. This task can be performed before going to the sampling location. However, both the analysis of site-specific working standards and the screening of a sampling location should be performed under the same conditions, thereby normalizing meteorological influences on the performance of the PID. Site-specific working standards should be prepared daily and in sufficient quantity to satisfy the study's objectives. At a minimum, one working standard should be analyzed for every hour of site activity.

Collection of samples for VOC analysis should always be the first operation performed after a surface to be sampled has been exposed to the atmosphere. This includes samples both for screening and for laboratory analysis. To establish how to handle and prepare the discrete sample for laboratory analysis (low, high, or both procedures), a total VOC screening analysis should be performed at each sampling location. Therefore, before opening a split spoon, scraping a fresh surface on a pit wall, removing surface vegetation and the appropriate amount of top soil for a surface grid location, or removing the first several inches of some other type of waste material, the PID of choice should be operating. Furthermore, if a working standard is being utilized to verify performance of the PID for the sampling location, the analysis of a working standard should be completed before exposing a fresh sampling surface.

Once a fresh surface has been exposed, a sample should be quickly obtained, transferred to a screening VOA vial, dispersed, and analyzed. If the maximum response is greater than the working standard (or the running average), the sample or samples taken for laboratory analysis should be prepared using the high-level procedure (i.e., MeOH extraction). If the maximum response is below the working standard, the laboratory sample(s) should be prepared using a low-level procedure. The total elapsed time between exposing a fresh surface, screening a sample, and obtaining samples for laboratory analysis should be less than 2 minutes. As a precaution against false positive and false negative screening estimates relative to the decision point, locations where screening results are between 0.5 and 2x the working standard response should have samples prepared by both high- and low-level procedures.

Method Limitations

For this method of sample location screening to work, the VOC(s) of interest must be detectable by photoionization. If more than one analyte is of interest, and there are large discrepancies (greater than a factor of 2) in photoionization potentials, then the range around the decision point where samples are prepared by both high- and low-level procedures should be increased proportionally. That is, if the responses for the VOCs of interest differ by a factor of 3x, and the analyte with the highest response is used to make the working standard, then laboratory samples from locations where screening results are only 0.3x the working standard should be prepared by both procedures. However, this often will not be a problem for sites contaminated with common chlorinated and aromatic compounds because they have similar photoionization potentials. This approach may not be effective for sample matrices that are not readily dispersed in water (e.g., some clays and cementitious materials).

FIELD TRIALS

This method for rapidly estimating the total concentration of VOCs was tested during three different sampling activities performed under the supervision of personnel from EPA Region 1. At the sites visited,

Table 1. Field screening measurements and sample preparation procedures.

No.	Screening (response)*		Sample Preparation Method
	Working Std	Sample	
Site 1			
S1-1	6.5	0.0	5021, LL**
S1-2	6.5	0.0	5021, LL
S1-3	---	0.4	5021, L
S1-4	---	790	5035, HL†
S1-5	---	480	5035, HL
S1-6	7.0	1400	5035, HL
S1-7	8.0	26	5035, HL
S1-8	---	2.2	5021, LL
S1-9	---	12	5035, HL
S1-10	6.3	4.4	5021, LL
Site 2			
S2-1	2.3,2.5	0.0	5035, LL††
S2-2	3.2	0.0	5035, LL
S2-3	4.2	0.0	5035, LL
S2-4	3.9	0.0	5035, LL
S2-5	---	0.0	5035, LL
S2-6	3.9	0.0	5035, LL
S2-7	---	0.0	5035, LL
S2-8	---	0.0	5035, LL
S2-9	3.8	0.0	5035, LL
S2-10	4.8	0.0	5035, LL
Site 3			
S3-1	5.6	0.0	5021, LL
S3-2	4.8	0.0	5021, LL
S3-3	5.3, 5.5, 5.2, 4.9	0.0	5021, LL

* PID field screening: Working Std--Results of analyzing a site-specific working standard 0.2 mg TCE/kg for Site 1 and 0.2 mg PCE/kg for Sites 2 and 3. Sample --Results from rapidly (<30 s) screening 10±2 g soil.

** 5021, LL--Sample placed in 22-mL VOA vial containing 10 mL water.

† 5035, HL--Sample placed in 40-mL VOA vial containing 5 mL MeOH.

†† 5035,LL--Sample placed in 40-mL VOA vial containing 5 mL water.

samples were obtained from near the surface with the aid of hand tools and from split-spoon core barrels. All samples, whether collected for on-site screening or for off-site analysis, were transferred using a modified syringe. Samples collected for off-site analysis were placed into vials containing methanol or organic-free water, as appropriate for the intended method of sample preparation (Method 5035 or 5021) and analysis (Methods 8260, 8015, 8021), and analyzed within 48 hours.

The results of the screening analysis for both the working standards and samples are shown in Table 1, and the results of the laboratory analysis are shown in Table 2. During these field trials, the screening results were only used to decide whether to prepare samples by a low- or high-level procedure. With the possible exceptions of sampling locations S1-3 and S1-10, the samples were prepared appropriately for the intended method of analysis. That is, the analysis system was not exposed to an unexpectedly high analyte concentration. Indeed, this statement applies to all of the samples, since the concentrations were not much greater than 0.2 mg/kg for the individual analytes found in these two samples. Therefore, a scheduled run would not have been delayed nor would the detector have been damaged; however, there may have been individual analyte responses greater than the highest calibration standard.

Table 2. Laboratory results.

Site 1.

	HS/GC-PID/FID (mg/kg)							
No.	CDCE	TCE	Tol	PCE	EBen	p/m-Xyl	o-Xyl	Total
S1-1	---	---	---	<0.003*	---	---	---	<0.003
S1-2	---	---	---	<0.003	<0.003	<0.003	<0.009	
S1-3**	---	0.021	0.15	0.008	0.074	0.076	0.082	0.41
S1-4**	---	5.2	29	---	15	15	3.5	68
S1-5	---	---	---	140	---	---	---	140
S1-6	---	250	19	240	33	76	68	690
S1-7	---	---	---	7.4	---	---	---	7.4
S1-8	0.040	0.020	---	0.17	---	---	---	0.23
S1-9	---	---	---	1.0	---	---	---	1.0
S1-10	---	---	---	0.24	---	---	---	0.24

Site 2.

	PT/GC/MS† (mg/kg)		
No.	Trichlorofluoromethane	PCE	Total
S2-1	---	---	ND
S2-2	---	0.001	0.001
S2-3	---	0.001	0.001
S2-4	0.004	---	0.004
5	---	---	ND
6	---	---	ND
7	0.001	---	0.001
8	0.002	---	0.002
9	---	---	ND
10	---	---	ND

Site 3.

	HS/GC-PID/FID (mg/kg)	
No.	PCE	Total
1.	<0.003	<0.003
2.	---	ND
3.	---	ND

* <0.003--Peak identified but below quantation.

** Unidentified peaks present in chromatogram.

† Samples analyzed at EPA Region 1 Laboratory in Lexington, Mass.

In the case of sample S1-10, the recommendation that samples be processed through both procedures when sample screening results are within a factor of 2 to 0.5 of the working standards would have provided the necessary precaution. However, in the case of S1-3, where the screening results were well below 0.5x the working standard, this same logic would not have succeeded. Samples S1-3 and S1-4 were taken within 5 cm (vertical) of one another, and both the screening and laboratory results (Table 2) showed that this area had a large vertical gradient in VOC concentrations. In this case, a review of the data and perhaps the site history would alert the sample collectors to a potential problem and therefore the need to implement an additional precaution, so as not to overload the analytical

system. Two potential solutions that would have worked at the site where samples S1-3 and S1-4 were taken are 1) to have taken screening samples on either side of (just above and below) the sample taken for laboratory analysis, 2) or alternatively, to have automatically prepared samples by both low- and high-level procedures based on knowledge of where the source regions were on this site. Because of this experience, an additional recommendation is to use one of these two procedures (e.g., bracketing laboratory samples with screening samples or taking laboratory samples for both low- and high-level preparation procedures) when sampling near known or suspected source regions. Whenever samples are prepared by both a low- and high-level procedure, the sample prepared by the high-level method should be analyzed first. Furthermore, although not reported here, it has become evident that when a screening exceeds the scale of the PID, which is typically greater than 2000 ppm, further dilution of a sample processed by the high-level procedure is most likely warranted prior to analysis.

SUMMARY

The problems of underestimating the concentration of VOCs in samples taken from the vadose zone has facilitated the acceptance of new sample collection, handling, and preparation protocols (e.g., Methods 5035 and 5021). These changes not only present challenges to field sampling teams but to the laboratories responsible for sample analysis as well. For this reason both parties must be involved in the initial design of the sample collection plan and remain in contact throughout the project. To assist in deciding how samples should be prepared for instrumental analysis, a simple total VOC screening procedure has been developed. The main purpose of this screening method is to provide a decision tool during the sampling activity to help establish whether samples taken for laboratory analysis should be prepared by a low-level or a high-level procedure, or by both. This screening process is, however, not foolproof. Likewise, neither are any others that must contend with the possibility of a heterogeneous analyte distribution. For this reason, there are additional precautions that should be taken when using this method. One is for the case when screening results for samples are within a factor of 2 to 0.5 of the working standard results, another is for the case when sampling near known or suspected source regions, and the third is for when the PID's response to a screening sample is over range (greater than 2000 ppm). As demonstrated here, this screening procedure has the potential to greatly reduce the number of samples that would have to be collected and processed during a site investigation.

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FINAL EVALUATION OF METHOD 3546: A MICROWAVE-ASSISTED PROCESS (MAP™)* METHOD FOR THE EXTRACTION OF CONTAMINANTS UNDER CLOSED-VESSEL CONDITIONS

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ABSTRACT

Microwave-assisted extraction (MAP™) has been the subject of enhanced interest from the environmental sector in the past year as a result of the need for methodologies that will improve sample preparation without compromising the quality of the data while being sustainable environmentally. Liquid-phase microwave-assisted extraction (MAP™) offers such advantages: it is a very fast extraction technique, it consumes less solvent and energy, and it is cost effective. A preliminary validation study involving closed-vessel apparatus and contaminants such as PAHs, PCDDs/PCDFs, chlorinated pesticides, and PCBs was performed. Excellent performance and precision were achieved for these analytes. In order to fully evaluate the method for the range of analytes an inter-laboratory study was performed. A round-robin study was performed with five laboratories and involved thermally labile RCRA target analytes such as phenols, phenoxyacids herbicides and organophosphorus pesticides. Three split samples were used along with a single standard operational procedure (SOP). All analyses were performed by a single laboratory in order to minimise the variability of the results due to the determinative procedure. Clean up was performed using standard procedures and analysis was done according to the appropriate SW-846 methods. The broad range of applicability, the reduced sample preparation time and the reduced amount of solvent used all contribute to reach sustainable environmental protection goals. Furthermore, the reduced operational costs associated with the protocol compared to conventional Soxhlet for example - are significant and will prove valuable in these times where the "greening" of the laboratory usually gives rise to higher operating costs. Further work involving open-vessels apparatus is under way.

INTRODUCTION

The microwave-assisted process (MAP) is a technology patented by Environment Canada[1-3]. The most widely used applications to date make use of microwave energy to extract soluble materials from different matrices, mostly using organic solvents[4,5]. Microwave energy has been used in various ways to extract organic compounds from a variety of matrices[6]. For example, the technology has been applied to organochlorinated pesticides from sediments and PCBs from water[7], petroleum hydrocarbons from soil[8], and to herbicides from soils[9,10]. Lopez-Avila *et al*. used a MAP-approach to extract several groups of pollutants such as PAHs, PCBs, pesticides, phenols and base/neutral compounds in soils and sediments[11-13]. In all these studies, microwave-assisted extraction proved to be similar or more efficient than methods based upon the use of Soxhlet or ultrasound.

More recently, we reported on a preliminary validation of a draft method for inclusion into US EPA Test Methods for Evaluating Solid Waste Physical/Chemical Methods (SW-846)[14]. Table 1 presents the types of compounds that have been subjected to that preliminary work.

The method reported herein makes use of partially microwave-transparent solvents (or a mixture of such solvents) contained into a closed vessel. Although not as elegant and efficient as methods using open-vessel microwave-transparent solvents, it provides the possibility of combining the benefits of heat (enhanced solubility and diffusivity) to the action of the microwaves on the matrix. Work is currently underway to validate open-vessel methods and the results will be reported elsewhere in due time along with the parameters to be controlled to effect even more efficient extraction procedures using MAP. This paper reports on the final evaluation of a closed-vessel microwave-assisted extraction procedure for environmental pollutants from soils and sediments that recently met with the US EPA approval and will be referred to as Method 3546 under SW-846. It is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and other solid wastes. The method is applicable to the extraction of semi-volatile

* MAP is a Trade-mark of Her majesty The Queen in Right of Canada as represented by the Minister of the Environment.

organic compounds, organo-phosphorus pesticides, organo-chlorine pesticides, chlorinated herbicides, phenoxy acid herbicides, substituted phenols, PCBs, and PCDDs/PCDFs which may then be analyzed by a variety of chromatographic procedures.

TABLE 1. List of target analytes used in preliminary validation package

Base Neutral and Acid	**PAH**	**Organo-chlorines**
Hexachloropentadiene	Acenapthylene	PCB-I
Dimethylphthalate	d_{10}-*Acenaphtene (surrogate)*	PCB-2
Diethylphthalate	Fluorene	Hexachlorobenzene
Di-*n*-butylphthalate	d_{10}-*Phenantrene (surrogate)*	Simazine
Butylbenzylphthalate	Phenanthrene	Atrazine
bis(2-Ethylhexyl)adipate	Anthracene	Lindane
bis(2-ethylhexyl)phthalate	Pyrene	PCB-3
	Benzo(a)anthracene	Alachlor
Pentachlorophenol	d_{12}-*Chrysene (surrogate)*	Heptachlor
	Chrysene	PCB-4
	Benzo(b)fluoranthene	Heptachlor epoxide
	Benzo(k)fluoranthene	PCB-5
	Benzo(a)pyrene	*gamma*-Chlordane
	d_{12}-*Perylene (surrogate)*	*alpha*-Chlordane
	Indeno(123-cd)pyrene	*trans*-Nonachlor
	Dibenzo(ah)anthracene	PCB-6
	Benzo(ghi)perylene	Endrin
		PCB-7
	d_{14}-*Terphenyl (int. std)*	Methoxychlor
		PCB-8

This method has been validated for solid matrices containing 50 to 10,000 µg/kg of semi-volatile organic compounds, 250 to 2,500 µg/kg of organo-phosphorus pesticides, 10 to 5,000 µg/kg of organo-chlorine pesticides and chlorinated herbicides, 50 to 2,500 µg/kg of substituted phenols, 100 to 5,000 µg/kg of phenoxy acid herbicides, 1 to 5,000 µg/kg of PCBs, and 10 to 6000 ng/kg of PCDDs/PCDFs.

EXPERIMENTAL

The experimental procedures for the preliminary validation work has been presented elsewhere, hence all the text presented herein refers exclusively to the inter-laboratory work and is relevant to Method 3546 as approved.

This method is applicable to solid samples only with small particle sizes. If practical, soil/sediment samples may be air-dried and ground to a fine powder prior to extraction. Alternatively, if worker safety or the loss of analytes during drying is a concern, soil/sediment samples may be mixed with anhydrous sodium sulfate or pelletised diatomaceous earth. The total mass of material to be prepared depends on the specifications of the determinative method and the sensitivity required for the analysis, but 2 - 20 g of material are usually necessary and can be accommodated by this extraction procedure.

Safety

The use of solvents combined with the operational parameters associated with this method will raise temperatures and pressures in the extraction vessels to values that can be a safety concern in the laboratory. Only equipment designed for laboratory use and manufactured under legitimate rights should be used to ensure that proper safety devices are built into the apparatus. Common sense laboratory practices can be employed to minimize this concern. For example, the following sections describe some additional steps that should be taken.

The extraction vessels are at elevated temperatures and pressure after the extraction stage. Allow the vessels to cool before opening (the use of a water bath is recommended for this purpose) and always monitor the temperature and pressure by re-connecting the control vessel to the apparatus prior to opening the vessels.

During the heating step, some solvent vapors may escape through the vessel liner/seal cover. Follow the manufacturer's directions regarding the vessel assembly and instrument set up to prevent release of solvent vapors to the laboratory atmosphere. The instrument may contain flammable vapor sensors and should be

operated with all covers in place and doors closed to ensure proper operation of the sensors. If so equipped, follow the manufacturer's directions regarding replacement of extraction vessel seals when frequent vapor leaks are detected.

Extraction
Decant and discard any water layer on a sediment sample. Mix the sample thoroughly, especially composite samples. Discard any foreign objects (sticks, leaves, rocks, *etc*.). Air dry the sample at room temperature for 48 hours in a glass tray or on hexane-rinsed aluminum foil. Alternatively, mix the sample with an equal volume of anhydrous sodium sulfate or pelletised diatomaceous earth until a free-flowing powder is obtained.

If multiphase waste samples are used, then they must be prepared by the phase separation method in Chapter Two of SW-846 before extraction. Dry sediment/soil and dry waste samples amenable to grinding. Grind or otherwise reduce the particle size of the waste so that it either passes through a 1-mm sieve or can be extruded through a 1-mm hole. Disassemble grinder between samples, according to manufacturer's instructions, and decontaminate with soap and water, followed by acetone and hexane rinses.

Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise reduced in size to allow mixing and maximum exposure of the sample surfaces for the extraction. The analyst may add anhydrous sodium sulfate, pelletised diatomaceous earth, sand, or other clean, dry reagents to the sample to make it more amenable to grinding.

Grind a sufficient weight of the dried sample to yield the sample weight needed for the determinative method (usually 10 - 30 g). Grind the sample until it passes through a 10-mesh sieve. Prepare a method blank using an aliquot of a clean solid matrix such as quartz sand of the approximate weight of the samples. Add the surrogates listed in the determinative method to each sample and method blank. Add the surrogates and the matrix spike compounds appropriate for the project to the two additional aliquots of the sample selected for spiking.

A volume of about 30 mL of the appropriate solvent system is added to the vessel and sealed. The extraction vessel containing the sample and solvent system is heated to the extraction temperature and extracted for 10 minutes. The solvent systems used for this procedure vary with the analytes of interest and are listed below. The mixture is allowed to cool. The vessel is opened and the contents are filtered. The solid material is rinsed and the various solvent fractions are combined. The extract may be concentrated, if necessary, and, as needed, exchanged into a solvent compatible with the cleanup or determinative step being employed.

Six vessels were always placed in the microwave oven at any one time to standardise conditions. After extraction, the sample carousel was removed from the microwave and cooled in a water bath. To ensure that it was safe to proceed with the filtration step the control vessel was returned to the microwave oven and the temperature was monitored before opening. Solvent loss was checked randomly in some instances and found to be below 1%.

Interferences
Refer to Method 3500 of SW-846. If necessary, Florisil and/or sulfur cleanup procedures may be employed. In such cases, proceed with Method 3620 and/or Method 3660 of SW846.

Apparatus and Supplies
CEM Corporation (Matthews, NC) MAP™ solvent extraction systems equipped with appropriate microwave-transparent extraction vessels should be transparent to microwave energy and capable of withstanding the temperature and pressure requirements (200ºC and 200 psi) for this procedure. Models MES-1000 or MSP-1000 have been used for the present work.

Solvents Systems and Reagents
All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

Organo-chlorine pesticides, organo-phosphorus pesticides, semi-volatile organics may be extracted with acetone/hexane (1:1, v/v) or acetone/methylene chloride (1:1, v/v).

PAHs, PCBs, dioxins, and furans may be extracted with acetone/hexane (1:1, v/v), or acetone/methylene chloride (1:1, v/v), or hexane.

Phenoxy acid herbicides and phenols may be extracted with acetone/hexane (1:1, v/v) and the phosphate buffer solution.

Reagent grade chemicals shall be used in all tests. Organic-free reagent water should be used. Sodium sulfate (granular anhydrous), Na_2SO_4 and pelletised diatomaceous earth can be used as desiccant. They should be purified by heating at 400ºC for 4 hours in a shallow tray, or by extraction with methylene chloride. If the latter approach is used, then a reagent blank should be prepared to demonstrate that the drying agent is free of interferences.

Phosphate buffer solution - for use in extraction of phenols and phenoxyacid herbicides. Prepare a 0.1 M phosphate buffer solution by adding 1.2 g reagent grade sodium phosphate into a 250-mL beaker, add 100 mL of reagent water and thoroughly mix. Adjust the solution pH to 2 with the addition of reagent grade phosphoric acid

Quality Control
Chapter One and Method 8000 of SW-846 should be followed for specific Quality Control procedures and Method 3500 should be followed for sample preparation quality control procedures. Surrogate standards should be added to samples when listed in the appropriate determinative method.

RESULTS AND DISCUSSION
Reference 14 presents a large body of information and specific data on a number of analytes. It provides the basis for a major portion of the performance work associated with this procedure. References 12 and 15 are reports of similar, more specific studies. References 16 to 18 deal specifically with phenols. Representative data sets are presented in Tables 2 to 6. They are not exhaustive and are reported here as they are new data. Other data can be found in the references cited herein including references 19 and 20.

Chlorinated pesticides: Single-laboratory accuracy data were obtained for chlorinated pesticides using natural soils, glass-fiber, and sand matrices. Concentrations of each target analyte ranged between 0.5 to 10 µg/g. Four real-world split samples contaminated with pesticides and creosotes were also used (obtained from US EPA ERT, Edison, NJ). The latter were extracted by an independent laboratory using standard Soxhlet procedures and results compared to those obtained with this procedure. Extracts were analyzed by the appropriate method. Method blanks and five spiked replicates were included. Work was also carried out to assess the level of degradation of thermally labile pesticides and it was found that no significant degradation takes place under the procedure described herein. The data are reported in detail in Reference 4. Data summary tables are included in Method 8081.

TABLE 2. Single-laboratory organochlorine pesticides analysis data from a real contaminated soil

Compounds	Average (ppb)	Std. Dev.	RSD (%)	n	REAC value (ppb)
DDE+Dieldrin	3380	340	10.06	3	7100
Endrin	21500	2290	10.66	3	22000
*DDD	40000	5750	14.38	3	45000
*DDT	62670	8430	13.45	3	62000
*Methoxychlor	16500	1980	12.03	3	16000
-Chlordane	730	100	13.37	3	750
-Chlordane	720	90	12.47	3	910

*(dilution 1:5); Soil samples obtained from US EPA Emergency Response Center archive bank through their contract laboratory REAC (Edison, NJ). The standard Soxhlet extraction procedures were performed by REAC three years earlier; this long storage period is believed to account for the low DDE+Dieldrin recovery data in the present study. DDE+Dieldrin is the sum of the compounds since they were not resolved by chromatography.

Semivolatile organics: Single-laboratory accuracy data were obtained for semivolatile organics using natural soils, glass-fiber, and sand samples. Concentrations of each target analyte was about 0.5 µg/g. Extracts were analyzed by the appropriate method. Method blanks and five spike replicates were included. The data are reported in detail in Reference 14. Data summary tables are included in Method 8270.

PAHs: Single-laboratory accuracy data were obtained for PAHs using five reference materials comprising marine sediments (HS-3, HS-4, and HS-5, all from the National Research Council of Canada), lake sediments (SRM-1491, from the National Institute of Science and Technology), and a natural soil contaminated with creosote (SRS103-100, from Fisher Scientific, Fairlawn, NJ). Natural soils, glass-fiber, and sand samples were also used in spiked matrices work. Concentrations varied between 0.1 and 2000 µg/g. One real-world split sample contaminated with creosote and pesticides was also used (obtained from US EPA ERT, Edison, NJ). The latter was extracted by one laboratory using standard Soxhlet procedures and results compared to those obtained with this procedure. Extracts were analyzed by the appropriate method. Method blanks, spikes and five spiked replicates were included. Surrogates were used in real-world split sample. The data are reported in detail in Reference 14. Data summary tables are included in Method 8270.

PCBs: Single-laboratory accuracy data were obtained for PCBs using three reference materials EC-1, EC-2, EC-3 (from Environment Canada). Natural soils, glass-fibre, and sand samples were also used in spiked matrices work. Concentrations varied between 0.2 and 10 µg/g (total PCBs). Extracts were analyzed by the appropriate method. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in Reference 14. Data summary tables are included in Method 8082.

TABLE 3. Single-laboratory PCB recoveries data from certified Great Lake sediment materials

Sediment	Aroclor (ppb)	Std Dev.	RSD (%)	n	Certified value (ppb)
EC-1	1850	0.07	3.78	3	2000 ± 54
EC-2	1430	0.09	6.60	4	1160 ± 70
EC-3	670	0.02	3.12	3	660 ± 54

Sample size = 2 g extracted into a final volume of 4 mL; EC-2 and EC-3 certified values were provisional values only, at the time the work was conducted. The data presented herein were part of the validation data package used to confirm the certified values. Real samples were also tested when fortified with mixtures of native Aroclor (1242, 1254, and 1260) to a 600 ppb level. Recoveries were in the 88% range with a reproducibility of 2% RSD.

Chlorinated herbicides (phenoxyacid herbicides): Multi-laboratory accuracy data were obtained for chlorinated herbicides spiked at 100 ng/g in one soil type. A certified spiked material was used (obtained from ERA, Arvada, CO). Extracts were analyzed by Method 8151. Method blanks and three replicates from five laboratories were included. Data summary tables are included in Method 815 1.

Phenols: Single-laboratory accuracy data were obtained for phenols using a number of spiked natural soils and a number of real-world split soils. Concentrations varied between 0.2 and 10 µg/g. Extracts were analyzed by the appropriate method. The data are reported in detail in References 14 to 18. Data summary tables are included in Method 8041. Multi-laboratory accuracy data were obtained for phenols spiked at 250 µg/kg in one soil type. A certified spiked material was used (obtained from ERA, Arvada, CO). Extracts were analyzed by Method 8041. Method blanks and three replicates from five laboratories were included. Data summary tables are included in Method 8041.

TABLE 4. Multiple-laboratory phenoxyacid herbicides recoveries from certified spiked material

Compounds	**Average (µg/kg)**	**Recovery (%)**	**RSD**
2,4-D	81	81	13.0
2,4-DB	122	122	15.1
2,4,5-T	74	74	11.8
2,4,5-TP (Silvex)	68	68	17.9
Dicamba	50	50	17.6
Dichlorprop	87	87	20.7
Dinoseb	118	118	29.4

Material spiked at 100 µg/kg. Number of participating laboratories = 4. N = 3

TABLE 5. Multiple-laboratory phenols recoveries from certified spiked material

Compounds	Average (µg/kg)	Recovery (%)	RSD
2,4-D	81	81	13.0
2,4-DB	122	122	15.1
2,4,5-T	74	74	11.8
2,4,5-TP (Silvex)	68	68	17.9
Dicamba	50	50	17.6
Dichlorprop	87	87	20.7
Dinoseb	118	118	29.4

Material spiked at 250 µg/kg. Number of participating laboratories = 4. N = 3

Organophosphorus pesticides and chlorinated herbicides: Multi-laboratory accuracy data were obtained for organophosphorus pesticides spiked at 250 µg/kg in one soil type. A certified spiked material was used (obtained from ERA, Arvada, CO). Extracts were analyzed by Method 8141. Method blanks and three replicates from five laboratories were included. Data summary tables are included in Method 8151.

TABLE 6. Multiple-laboratory organophosphorus pesticides recoveries from certified spiked material

Compounds	Average (µg/kg)	Recovery (%)	RSD
Bolstar (Sulprofos)	74	30	8.0
Chlorpyrifos	70	28	7.7
Demeton O & S	nq	nq	nq
Diazinon	111	44	3.8
Dichlorvos	nq	nq	nq
Dimethoate	143	57	14.1
Disulfoton	nq	nq	nq
EPN	107	43	12.9
Ethoprop (Prophos)	198	79	6.8
Fensulfothion	207	83	7.5
Parathion ethyl	159	63	12.3
Parathion methyl	146	58	10.6
Phorate	117	47	13.5
Ronnel (Frenchlorphos)	145	58	5.0
Sulfotepp	nq	nq	nq
TEPP (Tetraethylpyrophosphate)	nq	nq	nq
Tetrachlorvinphos (Stirifos)	158	63	3.5
Tokuthion (Prothiofos)	153	61	5.5

Material spiked at 250 µg/kg. nq = not quantified. Number of participating laboratories = 4. N = 3.

Dioxins and furans: Single-laboratory accuracy data were obtained for dioxins and furans using two reference materials (DX-1 from Environment Canada and SRM-1944 from NIST). Concentrations varied between 0.01 and 6 µg/kg. Extracts were analyzed by the appropriate method. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in References 19 and 20. Data summary tables are included in Method 8280.

SUMMARY

A variety of simulated samples as well as real matrix materials have been analysed to evaluate and validate a well-defined microwave-assisted extraction process. Recoveries in all cases were satisfactory, problems encountered usually are in the actual instrumental analysis due to the complex nature of the matrix. Relatively low recoveries are all caused by volatility losses of the particular compounds during sample workup, and not due to the extraction process. Studies carried out using thermally labile organochlorine pesticides and organo-phosphorus pesticides showed that the use of microwaves, under the operational conditions presented herein, did not cause any significant degradation, if any, despite the relatively rigorous conditions prescribed in the proposed method. Commercially available extractors, such as those used herein, can process up to 12 samples in less than 1 hour while taking up much less space, solvent and energy than the currently used Soxhlet extractor. The rapid sample turn around time is a yet another advantage over traditional Soxhlet

techniques. We will report elsewhere on other approaches related to more precise control over extraction conditions and that will ensure even shorter extraction times as well as higher extraction efficiencies.

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PHENOXYACID HERBICIDE SCREENING

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Abstract
EPA SW-846 Methods 8150 and 8151 have been traditionally used for phenoxy acid herbicide analyses. These methods require the use of hazardous derivatization reagents and a highly flammable solvent. The sample preparation process is very long, complex, labor intensive and time consuming. Immunoassay, as used in EPA SW-846 Method 4015, uses aqueous based chemistry with minimal solvent volume and much simpler and faster sample preparation.

Quanterra has demonstrated that EPA SW-846 Method 4015 (2,4-D) can be extended to include 2,4,5-T and silvex by using both the 2,4-D and silvex kits from Strategic Diagnostics Inc. It is useful for screening both water and soil samples for phenoxyacid herbicides. Non-detect samples from the immunoassay (IA) screen are reported as nondetect at the applicable reporting limit. Samples which have responses greater than the threshold (i.e. positive response) are confirmed by the traditional methods. The overall false negative rates of 0.5% for waters and 1.0% for soils were well below the EPA Office of Solid Waste criteria of 5% at the reporting limits listed below. The false positive rates were 12.5% and 11.5% for waters and soils respectively. Water reporting limits were 2 µg/L, 10 µg/L and 10 µg/L for 2,4-D, silvex and 2,4,5-T respectively. Soil reporting limits were 1 mg/kg, 1.5 mg/kg and 1.5 mg/kg respectively.

Switching to IA improves laboratory safety, reduces organic solvent usage and disposal, improves turn-around-time and reduces analytical costs.

Introduction
Many herbicide analysis requests target three main analytes of interest: 2,4-D, 2,4,5-T and silvex (2,4,5-TP). Also, >95% of these samples are reported as "non-detect" for these analytes. Thus, it would be very useful to screen samples submitted for the. analysis of these herbicides. Only those samples with herbicides above the reporting limit would be subjected to quantitation by the more exhaustive traditional analysis methods. Immunoassay is capable of providing the screening information with an aqueous chemistry based method that is simpler, faster, safer and less expensive.

Currently Immunoassay Method 4015 is only applicable to 2,4-D. However, this validation study demonstrates that it is possible to extend the method by including a Strategic Diagnostics Incorporated (SDI) analysis kit developed specifically for silvex by Ohmicron. Both the 2,4-D and silvex immunoassay kits have cross reactivity for 2,4,5-T because of structural similarities. This cross reactivity enables the combined use of the two kits to effectively screen for all three compounds, The cross reactivity is shown below expressed as least detectable dose (LDD).

Table 1. Cross Reactivity

Compound	2,4-D Assay LDD (µg/L)	Silvex Assay LDD (µg/L)
2,4-D	0.70	100
2,4,5-T	3.0	1.0
Silvex	170	1.4

The Ohmicron (now part of SDI) 2,4-D kit has been validated following Office of Solid Waste (OSW) guidelines. It was approved by the OSW Organics workgroup and incorporated into Method 4015. Method 4015 was part of the Update III package for SW-846 promulgated in mid 1997. Ohmicron had established threshold test levels of 10 µg/L in water and 150 µg/kg in soil with their magnetic particle based assay kit. The sensitivity difference between the two matrices is mostly due the large dilution of the methanol soil extract needed to reduce antibody exposure to methanol.

The Ohmicron Silvex kit was validated similarly to the 2,4-D kit but the results were not submitted to the OSW workgroup. The silvex kit has comparable sensitivity to the 2,4-D kit. Ohmicron has documented that the silvex assay responds similarly to 2,4,5-T (i.e. cross reactivity).

Immunoassay Method Summary
The immunoassay form applied in these two kits is enzyme linked immunosorbent assay (ELISA). Information is available from SDI product literature and the EPA - Las Vegas (web site http://www.epa.gov/crdlvweb/asb/immuchem/forum.htm) describing IA basics.

For these SDI kits a subaliquot (250 µL) of aqueous sample is combined with 250 µL of enzyme conjugate solution. One portion of the conjugate molecule resembles the analyte of interest. Antibody which is bound to small magnetic particles is added to this analyte/conjugate mixture. The analyte and conjugate compete for antibody binding sites. The higher the analyte concentration the less conjugate is bound to the antibody. The lower the analyte concentration the more conjugate is bound to the antibody.

A magnet is used to retain the antibody particles and the analytes and conjugates that have bound to the antibodies. All other analytes, conjugates and matrix components are washed away. A color development reagent is added to the antibodies. This reagent reacts with another portion of the conjugate molecule and develops color proportional to the amount of bound conjugate present. Thus, the color (i.e. absorbance measured on the filter photometer) is highest (~1) for blanks and clean samples and the least absorbance is produced for high concentration standards and samples. This inverse relationship between analyte concentration and absorbance response has caused some confusion.

Thus, when using this IA format for threshold testing the results scenarios are:

a) if sample absorbance > threshold standard absorbance then analyte concentration is < threshold (e.g. non detect at 10 µg/L), b) if sample absorbance ≤ threshold standard absorbance then analyte concentration is ≥ threshold (e.g. positive analyte ≥ 10 µg/L)

Since the color response will always have some variability associated with both the threshold standard and the sample, it is customary to prepare the threshold standard at a concentration slightly below the reported test threshold. This "low bias" on the standard favors the assay toward producing false positives in order to reduce the false negative rate. The following 10 µg/L report threshold example illustrates:

Table 2. Example Assay Batch

Assay	Absorbance	Comment
threshold standard #1 (7 µg/L)	0.8	
threshold standard #2 (7 µg/L)	0.88	use the avg std response 0.84 for cutoff
LCS (10 µg/L)	0.6	pass
sample #1	0.97	ND <10
sample #2	0.92	ND <10
sample #3	1.02	ND <10
sample #4	0.86	ND <10
sample #5	0.4	positive >10
sample #6	0.55	positive >10
sample #7	0.7	positive >10*
NIS of ND sample #1(>10 µg/L)	0.57	pass
MS of ND sample #2(>10 µg/L)	0.86	fail - false negative**

* sample #7 may be a false positive at the test threshold since it falls between the. threshold standard and the LCS. In other words it may have herbicide present at 8 µg/L. It would be sent for 8150 confirmation.

** This MS illustrates a documented false negative which would trigger corrective action.

The threshold standard (7 µg/L) is intentionally lower than the reported test threshold (10 µpg/L) to reduce the false negative rate due to normal statistical variability and minor preparation losses and matrix interferences.

Quanterra intended to improve the sensitivity of the soil assay by reducing or eliminating the need for the large dilution used to reduce the impact of methanol on the antibodies. Evaporating the methanol from a small aliquot of sample extract, followed by reconstitution in water was effective at removing the methanol. However, this process also concentrated the interferences in the soil matrices and raised the level of non-specific antibody binding. Unfortunately, this raised the false positive rate to an unacceptable level. Thus, this preparation modification was not used in the validation study.

The silvex kit has not been "validated" for 2,4,5-T but the SDI cross reactivity data suggest that the silvex antibodies work similarly for 2,4,5-T threshold testing. 2,4,5-T was investigated along with 2,4-D and silvex for which the IA kits were designed. In fact, 2,4,5-T was detected by both the 2,4-D and silvex IA kits during the validation study. At least one kit produced an acceptable 2,4,5-T response for each sample included in the study.

This validation study builds on top of the existing studies and knowledge discussed above. Quanterra has demonstrated that the two IA herbicide kits are appropriate to screen out water and soil samples that have no detectable levels of 2,4-D, 2,4,5-T or silvex at the applicable reporting limits. This is not intended to replace Method 8151 for quantitation but to focus its use where it is most applicable. Since the extended IA method is not intended for quantitation and much validation work has already been completed for the IA kits very little additional direct comparison between IA and GC data is necessary to establish that the IA method is capable of screening out non-detect samples. We intended to utilize matrix spikes of well characterized matrices, matrix spikes of real samples (ND for herbicides by 8150/51) and a few real samples with herbicides that have been previously analyzed by 8150/51. Hereafter, herbicides that entered the sample by environmental transport mechanisms and have undergone aging and weathering are referred to as *native* herbicides, Unfortunately, the native herbicide samples that were available at the time of the validation study could not be used as intended because the analyte concentrations were much lower than the final test thresholds. To compensate, many of these samples were fortified with known amounts of each herbicide and included in the study as matrix spiked samples.

Validation Goal

The main goal was to demonstrate that the IA method responds to herbicide levels known to be at or above the IA reporting limit (threshold) for each analyte. Since samples with *native* herbicides above the final test thresholds were not available, the number of matrix spiked samples was significantly increased over the original validation plan. Water matrix spiked samples were increased from 6 to 18. Soil matrix spiked samples were increased from 7 to 12. Also, using matrix spikes allowed us to more accurately document performance at the critical area near the reporting limit. It was not necessary to analyze the matrix spiked samples with 8150/51 since the herbicide concentrations were already known. The matrix spiked soil samples were stored at 4ºC at least overnight and usually for several days between spiking and extraction in order to age them and more closely mimic *native* analytes.

Validation Protocol

Matrix spiked samples were prepared using techniques previously employed for SW-846 methods development work. Separate spike solutions containing known amounts of each of the three target analytes were prepared at appropriate concentrations in acetone. These high concentration standards were further diluted in reagent water. Water samples were spiked with these aqueous solutions, homogenized by shaking and immediately assayed. Soil samples were spiked, acetone solvent evaporated at room temperature and tumbled overnight in a rotary mixer. The spiked soil samples were stored at least overnight at 4ºC and usually for several days prior to extraction in order to "age" the samples and thus more closely mimic *native* soil samples. Obviously this process does not duplicate the extensive weathering which can occur in real environmental samples, but the combined use of short term "aging" and fuller's earth that is known to be difficult to extract should simulate many *difficult* samples. Each of the following sample-analyte combinations in Tables 3 & 4 was assayed at least once. Replicate analyses indicated in ().

Analytical Procedure Summaries

Water preparation

Allow particulates in water sample to settle, filter (0.45 µm PTFE) if sample is cloudy with suspended solids, aliquot 250 µL of sample into plastic assay tube. Aqueous matrix spike solution added as appropriate. TCLP buffers and samples were spiked as appropriate in a small vial. A 25 µL aliquot was transferred to the assay tube and 225 µL of diluent was added.

Soil preparation

Weigh 10 g of soil sample into 50 mL plastic centrifuge tube. Add acetone matrix spike solution as appropriate. Allow solvent to evaporate. Add 2-3 ball bearings. Tumble overnight (note: some wet clay samples formed large clumps during tumbling and were not tumbled when matrix spiking was required for additional analyses). After "aging", add 30 mL of extraction solvent (75% methanol, 23% reagent water, 2% acetic acid). Recap centrifuge tube, rotate onto side and mechanically shake at about 200 cycles/minute for 30 minutes. Stand centrifuge tubes up and allow soil to settle for 1 hour and/or centrifuge for 3-5 minutes. Filter (0.45 µm PTFE) a few milliliters of extract. Transfer 5 µL of extract into plastic assay tube and add 245 µL of diluent.

Table 3. Water Sample List and Matrix Spike Levels

Sample	No spike	Analyte					
		2,4-D µg/L		silvex µg/L		2,4,5-T µg/L	
		high	low	high	low	high	low
ground water	(10)	10	2(4)	10	5	10(27)	5
industrial waste water	(4)	10	2	10	5	10	5
TCLP buffer #1	(13)	100		100 (2)		100 (27)	
TCLP buffer #2		100 (7)		100 (7)			
TCLP Buffer #1 samples †							
X3		100		100			
02		100		100			
43		100		100			
01		100		100			
ZX		100		100			
Water samples †							
HN	(6)	10	2	10	5	10	5
HV	(7)	10	2	10	5	10	5
QK	(5)	10	2	10	5	10	5
5G	(4)	10	2	10	5	10	5
DA	(5)	10	2	10	5	10	5
W2	(6)	10	2 (8)	10	5	10	5
4JL	(5)	10	2	10	5	10	5
Performance Evaluations							
Wisc. PE	(3)						
WS038 PE	(3)						
Additional water samples screened for false positives †							
8H							
AH	(4)						
HK	(4)						
WC							
5V							
65							
6							
K1							
K2							
W3							
W6							
W7							
WA							
JW							
K0							

† Samples previously analyzed by 8150 but no herbicides detected.

Immunoassay

Allow all IA reagents (particularly the enzyme conjugate and antibodies) to warm to room temperature. Add sample or standard aliquot (250 µL final volume). Add 250 µL of enzyme conjugate. Add 500 µL of suspended antibody coupled magnetic particles and vortex mix for 1-2 seconds. Incubate at room temperature for 30 minutes. Apply magnetic rack base for 2 minutes to separate magnetic particles from bulk fluid in tubes. Pour out tube contents while the magnetic particles are retained at the bottom of the tubes. Rinse the antibodies twice with wash solution. Remove magnetic rack base. Add 500 µL of color development reagent and incubate for 20 minutes. Add 500 µL of sulfuric acid stop solution and read absorbance at 450 nm.

The assay tubes were arranged in a 3 X 10 layout in the SDI magnetic rack. Often two batches of 30 assays were performed simultaneously in the rack which holds a maximum of 60 tubes. A typical 30 tube layout is shown below.

It was quite common in the early batches of the validation study to assay standards prepared by both SDI and Quanterra® (QES) as a means of verifying the standards prepared in-house.

Table 4. Soil Sample List and Matrix Spike Levels

Sample	No spike	Analyte					
		2,4-D µg/kg		silvex µg/kg		2,4,5-T µg/kg	
		high	low	high	low	high	low
sandy soil	(5)	1.0	0.3 (2)	1.5		1.5 (2)	
loam soil	(4)	1.0	0.3 (2)	1.5		1.5 (2)	
fuller's earth* (dry)	(4)	1.0	0.3 (2)	1.5		1.5 (2)	
fuller's earth* (50% moisture)	(3)	1.0	0.3	1.5		1.5	
Soil -samples †							
03		1.0	0.3	1.5		1.5	
04		1.0	0.3	1.5		1.5	
2N		1.0	0.3	1.5		1.5	
36		1.0	0.3	1.5		1.5	
20		1.0	0.3	1.5		1.5	
20	(4)	1.0	0.3	1.5(8)		1.5 (8)	
22	(4)	1.0	0.3	1.5		1.5 (8)	
25		1.0	0.3	1.5		1.5	
1V		1.0		1.5		1.5	

* Fuller's earth is an absorptive clay known to *challenge* the efficiency of solid extraction procedures.
† Samples previously analyzed by 8150 but no herbicides detected above (or near) spike concentrations.

Raw Response Data

The average difference in response between duplicate SDI and QES standards was 4%. The response difference between silvex and 2,4,5-T was small (average 12.5%) and 2,4,5-T consistently produced less response. Thus, 2,4,5-T was used as the threshold compound for all silvex kit assays in order to reduce the false negative rate, particularly for 2,4,5-T containing samples. The test threshold standards were prepared at 70% of threshold concentration (0.7 x expected reporting level) in order to reduce false negatives for water matrices. Threshold standards were prepared at 50% of the threshold concentration for the soil assays.

Table 5. Example IA Batch Layout for 2,4-D kit assays - 10 µg/L Reporting Level and matrix spikes

	A	B	C	D	E	F	G	H	I	J
1	QES "10" std	GW	GW+ ***D***	TCLP	TCLP + ***D***	WW	WW + ***D***	W1	W1+ ***D***	SDI "10" std
2	W2	W2+ ***D***	W3	W3+ ***D***	QES LCS at 10 RL	SDI LCS at 10 RL	W4	W4+ ***D***	W5	W5+ ***D***
3	SDI "10" std	W6	W6+ ***D***	W7	W7+ ***D***	Wisc PE	Wisc PE	WS038 PE	WS038 PE	QES "10" std

Key:
QES "10" std = 7 µg/L 2,4-D standard prepared by Quanterra
SDI "10" std = 7 µg/L 2,4-D stock standard prepared by SDI
QES LCS at 10 RL = 10 µg/L 2,4-D standard prepared by Quanterra
SDI LCS at 10 RL = 10 µg/L 2,4-D stock standard prepared by SDI
D = 2,4-D spiked sample (10 µg/L)
GW = Ground water
TCLP = TCLP buffer #1 [10 X dilution]
WW = Industrial waste water
W1, W2, W3, W4, W5, W6, W7 = real water samples without *native* herbicides
PE = Performance evaluation samples from Wisconsin and EPA WS038 programs.

Results and Discussion

The key questions when evaluating the reliability of this screening method are:
1) If an analyte is present in a sample at a concentration greater than or equal to the threshold (i.e. reporting limit), what are the chances of the assay generating a false negative response?

2) If no analyte is present in a sample at or above the threshold concentration, what are the chances of the assay generating a false positive response?

Table 6. Threshold Standards

Threshold Level (reporting limit)	Compound	actual standard concentration
	water samples	
2.0 µg/L	2,4-D	1.4 µg/L 2,4-D
10. µg/L	silvex or 2,4,5-T	7.0 µg/L 2,4,5-T
	soil samples	
1.0 mg/kg	2,4-D	3.3 µg/L 2,4-D
1.5 mg/kg	silvex or 2,4,5-T	5.0 µg/L. 2,4,5-T

False negatives are undesirable since they would report a sample as "clean" with regard to the target herbicides when it was not and potentially increase environmental health risks. False positives are undesirable since they would needlessly "trigger" a batch of traditional 8151 analyses which would increase organic solvent usage, analyst exposure to hazardous reagents, turn-around time and cost.

Since the same number of replicates were not run for each sample it is not appropriate to simply divide the number of false negatives or positives by the total number of assays. The false negative or positive rate was determined for each sample - analyte combination or unspiked sample. These individual rates were then averaged to determine the overall false negative and positive rates. LCS results were not included in these calculations because they do not contain real sample matrix. The false negative rate must meet the normal Office of Solid Waste criteria, $\leq$5%. The false positive rate was expected to be $\leq$10%.

The matrix spike results from samples producing consistent false positive responses were not included when calculating the false negative rates for either water or soil samples.

Water Samples
Production threshold levels of 2 µg/L for 2,4-D and 10 µg/L for silvex and 2,4,5-T were selected after evaluating the initial water sample data. Table 7 summarizes the percentage of false positives and false negatives for each sample - analyte combination.

The overall false negative rate of 0.5% for water samples was excellent. Sample W2 had one false negative among 8 replicates at the 2 µg/L threshold for 2,4-D for a false negative rate of 12.5% for this sample - analyte combination. The ground water (GW) and TCLP buffer #1 produced many false negatives for 2,4,5-T when assayed with the silvex kit at the 10 µg/L (GW) and 100 µg/L (TCLP) levels. Keeping the spike levels and analyte the same but switching to the 2,4-D kit significantly improved the assay reliability. The false negative rates dropped to 16.7% and 5.9% for these two matrices respectively. Since 2,4,5-T is not a normal TCLP analyte these false negatives have little practical impact on assays of TCLP samples, although this does indicate that 2,4,5-T may be more susceptible to false negatives than the other two analytes. The ground water false negative rate was reduced by assaying for 2,4,5-T with the 2,4-D kit as noted above. This indicates that either immunoassay kit will respond to 2,4,5-T and that at least one kit is likely to produce a positive response at the 10 µg/L threshold when 2,4,5-T is present.

The false positive rate was determined by dividing the total number of "non-detect" samples included in the study into the number of samples that produced false positives for a false positive rate of 12.5%. Replicate assays were performed on two of the samples that produced false positives. This confirmed that a matrix interference existed which produced the false positive. One of the false positive sample responses was near the response of the threshold standard and probably would not produce a false positive in all instances if replicate assays were performed. It appears that false positives are primarily generated by matrix interferences. Thus, positive matrix interferences are likely to be site specific and the false positives that do occur in actual production use of the IA kits should be clustered together in a limited number of sample lots. This means that most of the sample lots assayed are expected to be free of false positives.

Table 7. False Negative and False Positive Rate Calculations for Water Samples

Matrix or sample	No spike % false positives	2,4-D % false negatives	Silvex % false negatives	2,4,5-T % false negatives
ground water	0	0	0	3/18 = 16.7
waste water	0	0	0	0
TCLP buffer #1	0	0	0	1/17 = 5.9
TCLP buffer #2	0	0	0	
X3	0	0	0	
02	0	0	0	
43	0	0	0	
01	0	0	0	
ZX	0	0	0	
HN	0	0	0	0
HV	0	0	0	0
QK	0	0	0	0
5G	0	0	0	0
DA	0	0	0	0
W2	0	1/8 = 12.5	0	0
JL	0	0	0	0
Wisc PE		0	0	
WS038 PE		0	0	
Matrix or sample	No spike % false positives	2,4-D % false negatives	Silvex % false negatives	2,4,5-T % false negatives
8H	0			
AH	100			
HK	100			
WC	0			
5V	0			
63	0			
65	0			
6	0			
K1	0			
K2	0			
W3	0			
W6	0			
W7	100			
WA	0			
JW	0			
K0	100			
Average rates	12.5%	0.7%	0%	2.3%
		overall false negative rate 0.5%		

Soil samples

Attempts to improve the sensitivity of the soil method were unsuccessful. Initial information from SDI indicated that the 50X extract dilution included in the SDI soil preparation method was designed to remove the deleterious effect that methanol has on the immunoassay. We intended to remove the methanol interference by evaporating a small aliquot of the extract to dryness then redisolving the analytes in diluent solution or water. Analyte recovery appeared acceptable and there was no visible methanol residue, but there was still some small positive interference which probably would have prevented reliable assays at the 30 µg/kg target threshold. Real soil extracts showed very large positive interferences when 250 µL of extract were concentrated for the assay. Thus, it was necessary to restrict to soil preparation to the original SDI/Ohmicron soil prep method. This was accomplished by diluting 5 µL of extract with 245 µL of diluent. When this 50X dilution was combined with higher analyte spike levels the interference problems were reduced to an acceptable level.

The following preparation method was used for most soils: 10 g soil + 30 mL extract solvent, shake for 30 minutes,

settle or centrifuge, filter, 5 µL of extract + 245 µL diluent. Thus, the final concentration of analytes presented to the assay were 150X more dilute than in the original soil sample. Quantities of some samples were limited so the preparation amounts were scaled back to 5 g soil and 15 mL of extraction solvent. The assay threshold standard concentrations reflected the dilution built into the sample prep. Most spike levels were 1.0 mg/kg for 2,4-D and 1.5 mg/kg for silvex and 2,4,5-T. Some low level (0.03 and 0.3 mg/kg) work was attempted, but the false positive rate was unacceptably high.

The concentration of the test threshold standard concentration was also reduced from 70% to 50% of threshold for soil assays. This decreased the false negative rate without unacceptably raising the false positive rate. Table 8 below shows 3 soil - analyte combinations had false negative results for silvex or 2,4,5-T when spiked initially at 1.5 mg/kg. Replicate sample aliquots were spiked, extracted and assayed. No additional false negative results were produced. Thus, the overall false negative rate was 1.0%, which is excellent. False positive results were reported for two samples at the 1.0 mg/kg 2,4-D or 1.5 mg/kg silvex/2,4,5-T assay levels. The overall false positive rate for soil samples was 11.5%. It is expected that false positives will be primarily caused by specific constituents in the soil samples and are thus more likely to be related to specific sites rather than be evenly distributed through all sample lots.

Table 8. False Negative and False Positive Rate Calculations for Soil Samples

Matrix or sample	No spike % false positives	2,4-D % false negatives	Silvex % false negatives	2,4,5-T % false negatives
ground water	0	0	0	0
loam soil	0	0	0	0
fullers earth dry	0	0	0	0
fullers earth wet	0	0	0	0
03	50	0	0	0
04	0	0	0	0
2N	0	0	0	0
36	0	0	0	0
2E	100			
20	0	0	1/8 = 12.5	1/8 = 12.5
22	0	0	0	1/8 = 12.5
25	0	0	0	0
1V	0	0	0	0
Average rates	11.5%	0%	1.0%	2.1%
		average false negative rate = 1.0%		

The soil extraction procedure did not appear to suffer any serious extraction efficiency problems despite its simplicity and short time frame. Even though the IA results were not quantitative, the low false negative rate indicates that analyte recovery was > 50% and many recoveries were > 70%. Previous Quanterra work with wet fullers earth with nonpolar analytes and solvents, showed very low (<20%) analyte recovery for hydrocarbons. Good extraction efficiency of the phenoxy acid herbicides with the simple shake extraction used with these IA kits was probably due to the following reasons: 1) The polar extraction solvent (methanol / water / acetic acid) readily permeated the wet clay matrix. 2) The polar solvent molecules could effectively displace polar analyte molecules from the polar sorption sites on the matrix. 3) The polar analytes were readily soluble in the polar extraction solvent.

Conclusion

The validation study results are summarized in Table 9 below. The false negative rates easily meet the normal EPA Office of Solid Waste criteria of ≤5%. The. false positive rates although slightly higher than the target 10%, are still acceptable. The water reporting limits are in the low part-per-billion range and meet the validation plan minimum objectives. In particular, analyses from TCLP buffers and samples demonstrated excellent performance for 2,4-D and silvex at levels well below the regulatory limits. The soil reporting limits although higher than originally expected should still be useful for many types of herbicide samples.

Table 9. Summary of Performance Results and Reporting Limits

Matrix	2,4-D	Silvex	2,4,5-T	False Negative	False Positive
water	2 µg/L	10 µg/L	10 µg/L	0.5%	12.5%
soil	1 mg/kg	1.5 mg/kg	1.5 mg/kg	1.0%	11.5%

Acknowledgments
The authors would like to thank Allen Debus from US EPA, Region 5, Craig Kostyshyn and Timothy Lawruk from Strategic Diagnostics and Susie Dempster, Carolynne Roach, Chris Lee and Richard Burrows from Quanterra Inc.

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FIELD DEMONSTRATION OF A PORTABLE IMMUNOSENSOR FOR EXPLOSIVES DETECTION

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Environmental biosensors are being developed at the Naval Research Laboratory for detection of the explosives TNT and RDX in groundwater and monitoring of cleanup progress for these compounds at remediation sites. Based on a displacement immunoassay, the portable sensor, known as the FAST 200, has been engineered by Research International (Woodinville, WA) to quantitate water samples with no sample preparation or reagent addition. Analysis is complete within five minutes. The sensor, along with a fiber optic biosensor, recently was extensively tested in field trials at several U.S. EPA Superfund sites to validate sensor performance. Results of these studies and application of the technology will be described.

ENVIRONMENTAL APPLICATIONS OF A FIBER OPTIC BIOSENSOR

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Detection and remediation monitoring of the explosives TNT and RDX on-site requires a sensitive and preferably portable method. The fiber optic biosensor is based on a competitive fluoroimmunoassay being performed on the core of an optical fiber probe. A portable version of the sensor was engineered by Research International (Woodinville, WA) and is known as the Analyte 2000. With this sensor, four optical probes can be monitored simultaneously and relatively Adirty@ samples can be employed. Analysis takes 16 minutes for the four probes. This sensor, along with the FAST 2000, was extensively evaluated at three on-site trials to validate sensor performance. Results of these studies and other applications for the fiber optic biosensor will be described.

DEVELOPMENT AND VALIDATION OF AN IMPROVED IMMUNOASSAY FOR SCREENING SOIL FOR POLYNUCLEAR AROMATIC HYDROCARBONS

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Polynuclear aromatic hydrocarbons (PAHs) are a group of fused ring compounds most typically found as combustion by-products. Over the past 4 - 5 years a number of immunoassay kit manufacturers have developed and commercialized soil screening method for PAHs. Briefly, there methods involve a rapid extraction of soils by shaking with methanol followed by analysis of the filtered sample extract using competitive enzyme immunoassay. We have developed an improved method for screening of PAHs in soil samples. The new method utilizes a modified sample extraction step that results in improved extraction efficiency compared to earlier methods. In addition, the specificity of the antibody used in the method allows for a better estimate of the total PAHs present. Immunogen design and antibody specificity will be described in detail. Results of concordance study with a gas chromatographic method will be presented.

A NEW DIOXIN/FURAN IMMUNOASSAY WITH LOW PICOGRAM SENSITIVITY AND SPECIFICITY APPROPRIATE FOR TEQ MEASUREMENT: APPLICATIONS DEVELOPMENT

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Since 1990 the commercial development of immunoassay kits his opened a new market in environmental analysis. The US EPA has approved more than 10 immunoassay screening methods under the 4000 series of Field Screening Methods within SW-846. These tests are now widely used in assessment and remediation of hazardous waste sites.

This recent success has not included the development of a useful immunoassay method for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs). The development and application of immunoassays for PCDD/Fs pose unique challenges not found in immunoassays for other analytes. First, the sensitivity requirements of PCDD/F analysis are typically in the ppt range rather than the high ppb to mid ppm range of the existing 4000 series screening methods. Second, if the test is to provide useful data, the EIA response must correlate to the relative toxicity of the 17 most toxic PCDD/F congeners. No previous immunoassay has demonstrated the necessary combination of sensitivity and specificity required for measurement of toxic equivalency (TEQ) at ppt levels. No immunoassay specific sample preparation methods have been developed because of the obvious lack of commercial potential demonstrated by all previous PCDD/F immunoassays .

A new enzyme immunoassay (EIA) for PCDD/Fs has been developed using novel chemistry. The sensitivity of this test is approximately 4 pg of 2378-TCDD, which is more than an order of magnitude better than previous PCDD/F immunoassays. Based on typical sample size, this sensitivity is sufficient to measure low ppt TEQ levels in solid samples or 0.1 ng/m^3 TEQ in stack gases using only a small fraction of the prepared sample extract. This sensitivity allows detection of 2378-TCDD in a 10 µL sample aliquot at the same concentration as the lowest calibration solutions typically used for HRGC/HRMS based PCDD/F methods such as EPA Methods 1613, 8290, and 23. These sensitivity comparisons indicate that the EIA is capable of screening samples prior to HRGC/HRMS analysis without consuming an unacceptably large proportion of the sample.

The dioxin/furan congener cross-reaction profile of this EIA is suitable for TEQ measurement. The test is most sensitive to the three most toxic congeners, 2378-TCDD, 12378-PnCDD, and 23478-PnCDF. EIA specificity data plus HRGC/HRMS data from previously analyzed samples have been utilized in a simple, additive response model to predict the EIA response for each sample. The resulting correlation between predicted EIA response and TEQ validates the concept of TEQ screening by EIA for a variety of samples.

A muliti-laboratory collaborative effort is now in progress for evaluation of kit performance and development of sample preparation methods. Results for fly ash and soil validate the additive response model for TEQ measurement by EIA. These results also validate the use of the kit for screening fully cleaned samples. Extension of this validation to partially cleaned samples is in progress, with positive initial results for fly ash and soil. Work on immunoassay specific sample preparation methods, including rapid extraction of soil and fly ash, is also in progress. The ultimate goal of this coli program is to develop sample preparation protocols which will maximize throughput and cost-effectiveness of immunoassay based PCDD/F screening.

DEVELOPMENT AND VALIDATION OF AN IMMUNOASSAY FOR SCREENING SOIL FOR POLYCHLORINATED BIPHENYLS

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Polychlorinated Biphenyls (PCBs) were commonly used in electrical applications due to their properties of high thermal transfer and low conductivity. Since being identified as toxic substances their use has been banned in the US. Due to the once widespread use and their stability in the environment a large number of sites are contaminated with PCB residues. A number of immunoassay kit manufacturers have developed and commercialized soil screening methods for PCBs. Briefly, these methods involve a rapid extraction of soils by shaking with methanol followed by analysis of the filtered sample extract using competitive enzyme immunoassay. We have developed an improved method for screening of PCBs in soil samples. The new method utilizes a modified sample extraction step that results in improved extraction efficiency compared to earlier methods. The immunoassays performed on the sample extract and yields qualitative results at 1, 5, 10 or 50 ppm. The test can be used for measuring Aroclors 1016, 1242, 1248, 1254 and 1260. Results of a concordance study with a gas chromatographic method will be presented.

GASOLINE RANGE AROMATIC/ALIPHATIC ANALYSIS USING PATTERN RECOGNITION

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Finding an analytical technique for the analysis of aromatic and aliphatic compounds in the gasoline range of hydrocarbons is of great interest to not only the laboratory but also the regulated community. In many states, including Alaska, great pressure to apply risk based cleanup standards is driving the need to separate "high risk compounds" from a given matrix in an economical manner with high confidence. False positives can mean an unneeded costly cleanup, while false negatives can mean non-compliance and possible fines if found. Analysis of aromatic and aliphatic compounds in the gasoline range has been accomplished using pattern recognition algorithms based on PCB matching criteria. Using existing methodologies for the analysis of gasoline range hydrocarbons in multimedia samples by GC-FID-PID this algorithm has been developed using widely available software to recognize and quantify aromatic hydrocarbons in a given sample. Soil, sediment, and water samples were analyzed using standard Alaska Department of Environmental Conservation methodology (AK101/EPA8021) and the results of pattern matching showed a significantly reduced number of false positives for the aromatic portion of the analysis.

BIOREMEDIATION ASSESSMENT USING CONSERVED INTERNAL BIOMARKERS

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ABSTRACT

The lack of homogeneity of field samples is often a primary concern in the analytical assessment of hydrocarbon bioremediation treatment efficacy. One approach to handling the sampling variability is to take a statistically significant number of samples to adequately represent the distribution of the contaminant. This approach is limited in that small biodegradation changes often cannot be seen within the inherent sampling variability. An additional drawback of this approach is the high cost of analyzing sufficiently large numbers of samples to draw conclusions.

An alternative approach, which can minimize the effects of sampling variability, is to use naturally occurring molecules, which resist biodegradation as conserved internal "biomarkers". There are a number of marker classes commonly found in petroleum products, which can be analyzed by GC/MS, these include hopanes, steranes and isoprenoids. These markers can be used as the basis for relative comparisons of target analytes before and after treatment. The ratios of two or more markers can also be used as a fingerprint to help identify the petroleum product source.

This poster demonstrates the application of biomarker normalized GC/MS data to evaluate the biodegradation of petroleum products from well-mixed refinery sludge waste. A comparison is shown tracking the treatment progress using absolute target analyte quantities and relative amounts normalized to biomarkers. The results show that the biomarker normalized data provided a good basis for determining the percent biodegradation of total oil as well as the molecular components, similar to the results obtained using absolute quantities.

The conclusions of the above study indicate that although significant amounts of 2, 3, 4 and 5 ring polynuclear aromatic hydrocarbons (PAHs) were removed during treatment the biomarkers used to normalize the data were conserved, resisting significant biodegradation. The implication of this work is that the use of biomarkers can be effective in situations that are less homogeneous to assess biodegradation of petroleum constituents from products with similar sources.

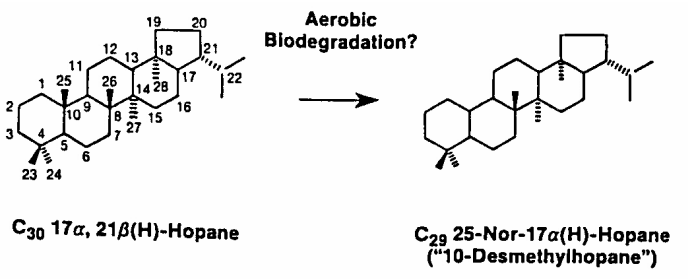
INTRODUCTION

The objective of the following study was to remediate a refinery process sewer sludge, through aerobic biodegradation, using composting to accelerate this process. Composting is a modification of the "biopile" process, which includes the addition of biodegradable ammendments (straw, wood chips, manure, etc.) to help aerate and supply energy for microbial growth. The laboratory scale evaluation was conducted in three-liter insulated glass beakers designed to minimize the loss of heat generated by the compost.

To minimize the effects of sludge heterogeneity on analytical results, a number of steps were taken which included mixing the sample well. An additional approach was to use naturally occurring "biomarker" molecules, which resist biodegradation, as internal standards. The latter approach was taken considering that future field applications would probably have much higher sampling variability due to the difficulty in homogenizing large volumes of compost.

The sludge used in these tests had significant amounts of two biomarkers typically found in petroleum products, C_{30} 17*a*, 21*B*(H)-Hopane ("hopane") and C_{29} 25-Nor-17*a*(H)-Hopane ("norhopane"). The above molecules are related by a mechanism proposed by Peters and Moldowan that suggests the origin of norhopane through the biological demethylation of hopane (see figure 1). Both biomarkers were found by selective ion (SIM) GC/MS at *m/z* 191, norhopane also has a characteristic peak at *m/z* 177.

The progress of the composting experiments were tracked by periodic sampling during the period of peak biological activity as indicated by naturally occurring elevated temperatures within the cells. The analytical tests performed on the extracted hydrocarbon included Total Petroleum Hydrocarbon (TPH, modified New Jersey method OQA-QAM-025-10/91) and GC/MS analysis for priority pollutant (PP) polynuclear aromatic hydrocarbons (PAHs). The TPH data was quantified by total ion GC/MS response using an external calibration of free oil collected from the



Proposed origin of 25-norhopanes by bacterial demethylation of 17 (H)-hopanes. The methyl group attached to the C-10 position in the C_{80} 17 (H)-hopane (left) is removed to produce the C_{29} 25-norhopane (right).

Figure 1. Proposed origin of norphopane from hopane

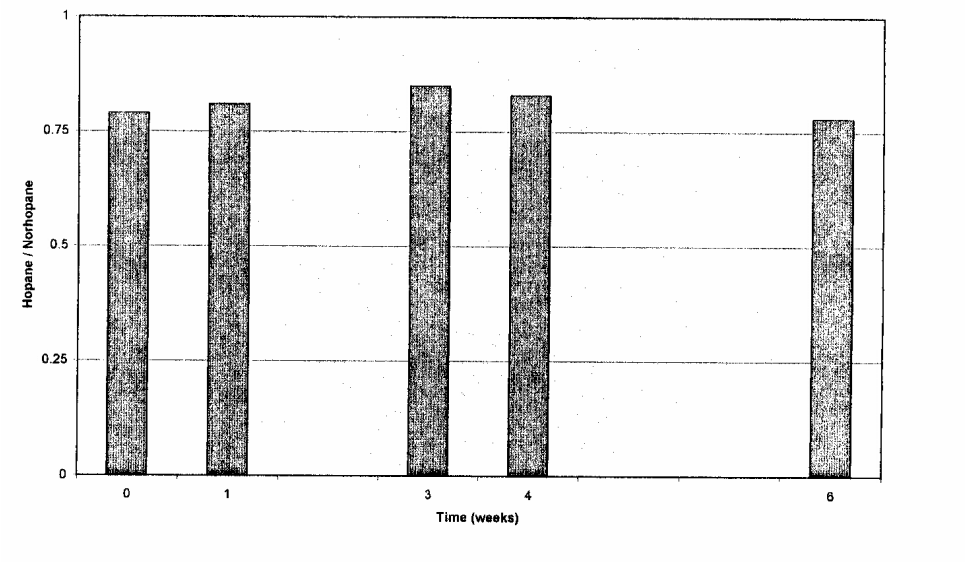


Figure 2. Biomarker ratio of hopane/norhopane

sludge. The PAHs were quantified using internal standards spiked into the extracts as prescribed in EPA SW846 method 8270.

The "biomarker normalized" data was obtained by dividing the GC/MS response of the analyte by that of hopane. The degradation measured relative to hopane was then compared to the "absolute" values obtained using the traditional approach described in the above paragraph. The conservation of hopane throughout the test, was monitored by comparing its GC/MS response relative to that of norhopane.

SUMMARY

The results show that rapid degradation of both TPH and PAHs occurred over the six-week period of the composting experiments, using both the absolute measurement and the hopane normalized approach. During this period there was no indication to suggest that significant hopane degradation occurred as shown by little change in the ratio of hopane to norhopane (see figure 2). Also qualitative examination of the biomarker's ion chromatograms at *m/z* 191 and 177 show little change before and after treatment (see figures 3 and 4). In contrast, the total ion chromatogram of the extracted hydrocarbon shows significant removal of the oil during treatment (see figure 5).

The TPH removal during treatment is shown in figure 6, which compares the absolute measurement to the hopane normalized results. These results show somewhat higher degradation estimates relative to hopane, ranging from 4% higher after one week to 30% higher after 6 weeks of treatment. The comparison of a selection of the more abundant 3, 4 and 5 ring PP PAHs using the absolute and hopane-normalized approaches is shown in figures 7 and 8 respectively. This comparison shows good agreement for phenanthrene and pyrene measurements using both approaches, with a somewhat lower estimate for the hopane normalized benzo(a)pyrene degradation (~ 30% lower). The lower concentration of benzo(a)pyrene (1 ppm) compared to phenanthrene (27 ppm) and pyrene (15 ppm) may

have attributed to the variability seen in its degradation measurements.

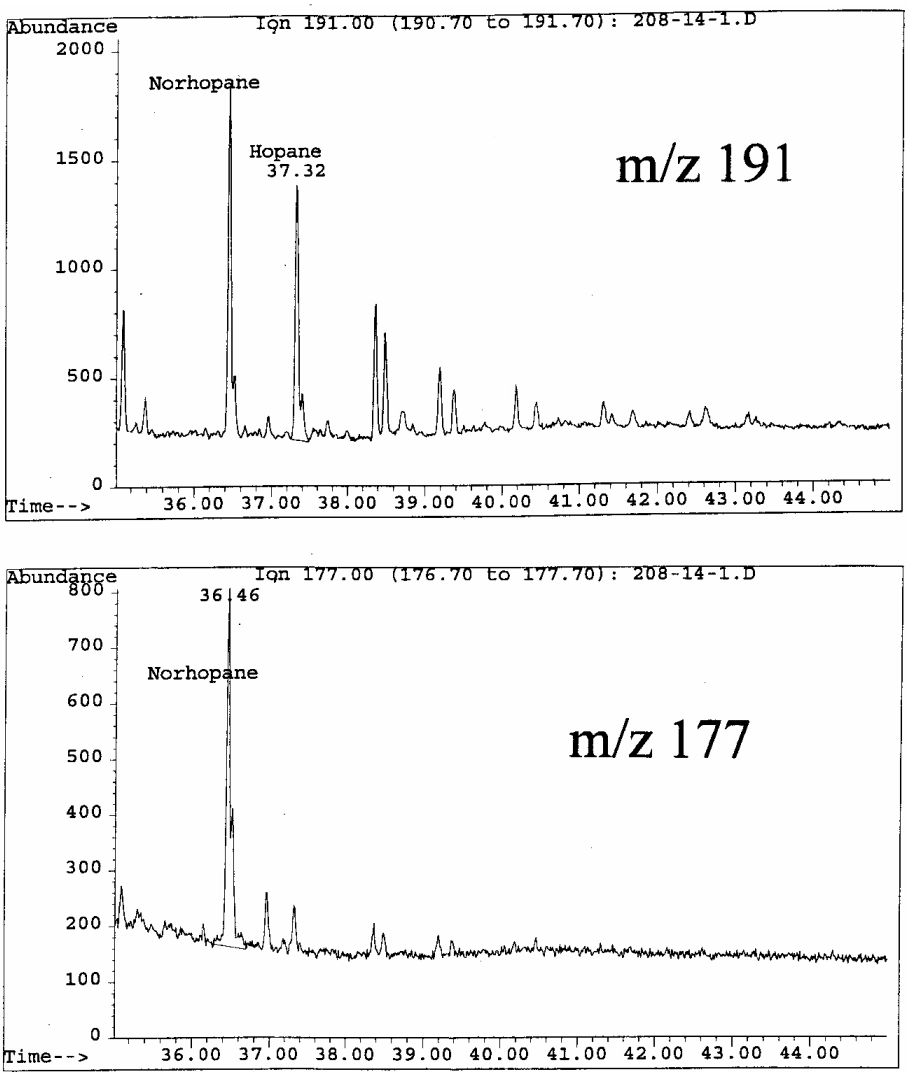


Figure 3. Before treatment ion chromatograms with hopane and norhopane

The degradation of the C1, C2 and C3 alkylated homologs of phenanthrene and anthracene are shown in figures 9, 10 and 11 respectively. This comparison shows very close agreement between the absolute and hopane normalized approaches (less than 10% difference) over the duration of the composting experiments.

CONCLUSIONS

This work demonstrates a practical application using selective ion GC/MS to characterize the biodegradation of petroleum contaminants relative to naturally occurring biomarkers. The hopane and norhopane biomarkers used in the above composting experiments showed no sign of significant biodegradation over the tests duration, while 2, 3, 4 and 5 ring PAHs as well as TPH showed rapid biodegradation. A comparison of biodegradation measurements using conventional quantification and biomarker normalized approaches showed good agreement for the most abundant analytes with somewhat higher variability for those in low concentrations.

The biomarker approach was easy to use and is independent of many sources of analytical and sampling variability associated with absolute measurements of analytes. Although this was a controlled experiment using a relatively homogeneous sludge, many pilot and field applications often deal with a wide range of contaminant distribution, resulting in sampling variability which is difficult to handle. The large numbers of samples needed to measure statistically significant changes can be avoided using biomarkers since analyte responses are normalized to stable (naturally occurring) internal standards.

An additional benefit of biomarkers is in site characterization of petroleum spills where ratios of two or more biomarkers can be used as a fingerprint to help identify the source of the contaminant. The degree of weathering and natural attenuation in various areas of a spill can also be assessed using this approach.

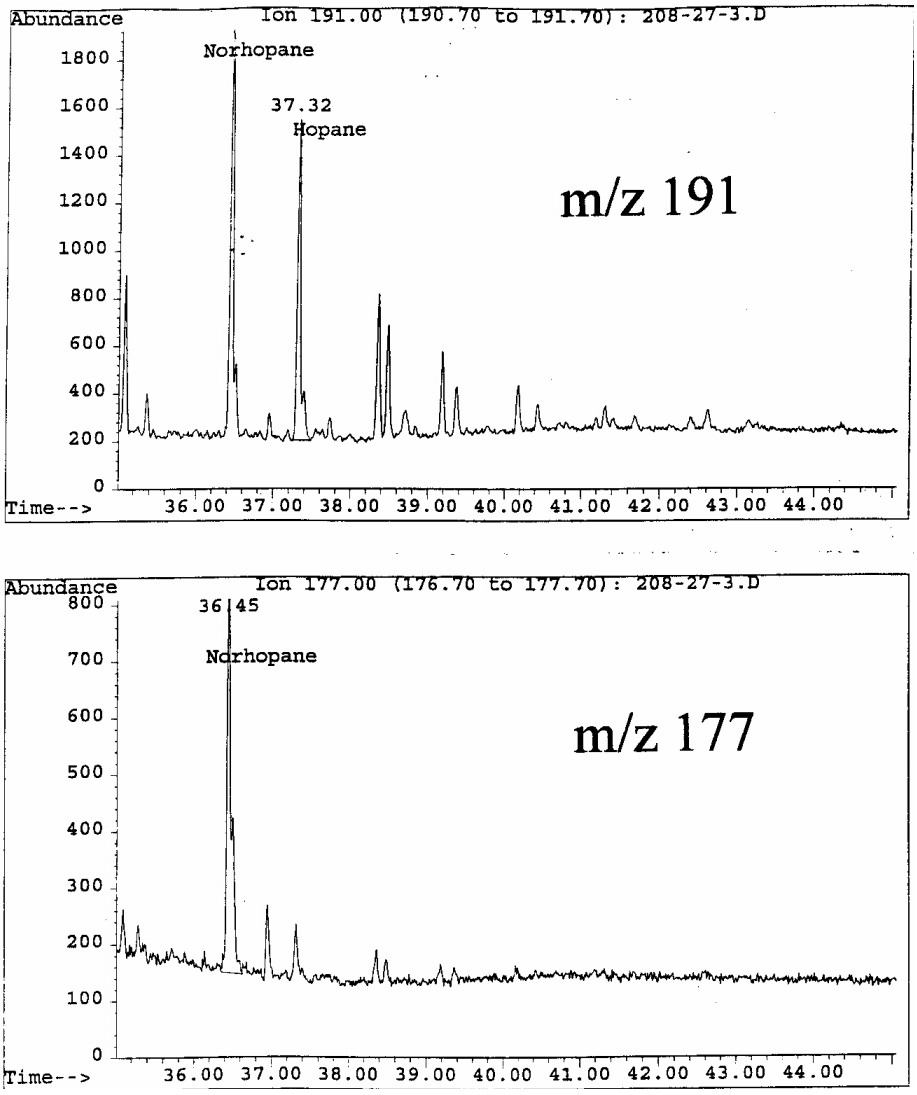


Figure 4. After treatment ion chrmatograms with hopane and norhopane

ACKNOWLEDGMENTS

Dr. Roger C. Prince for his insights into the application of biomarkers to oil spills.

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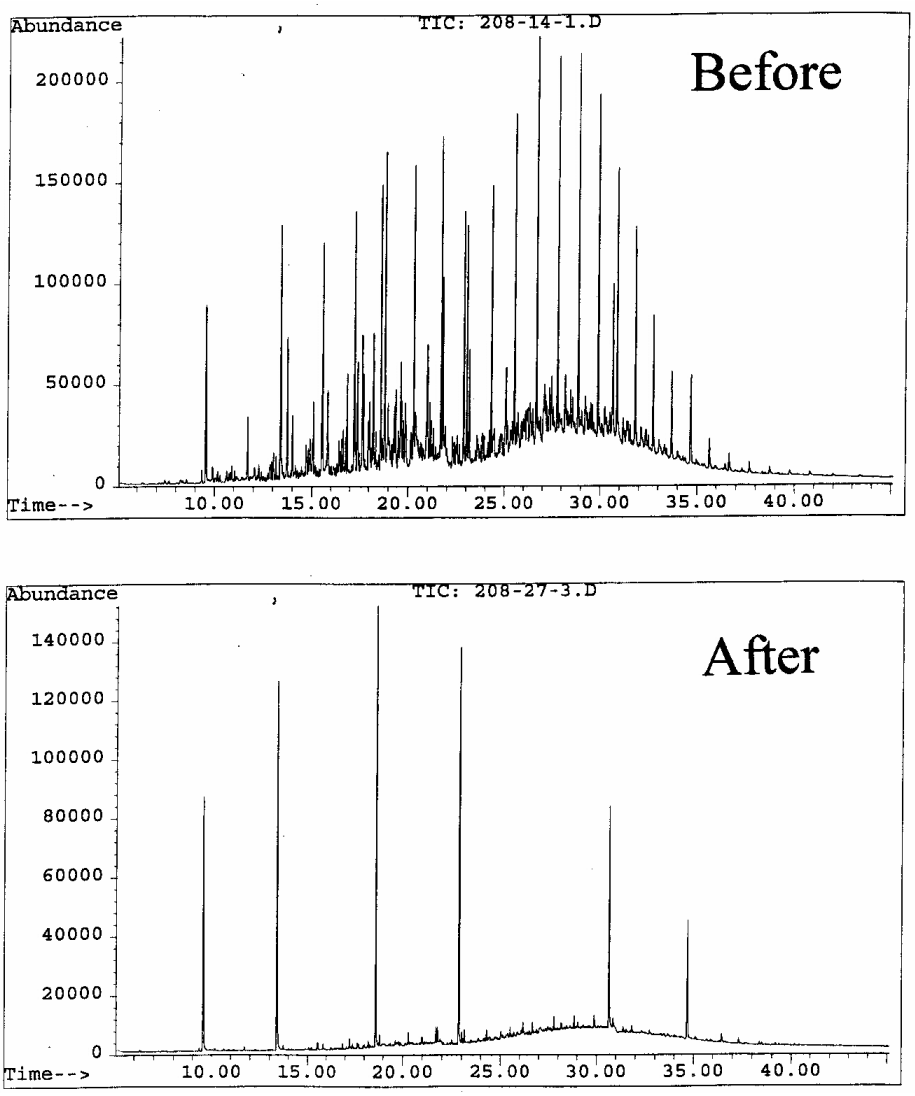

Figure 5. Before and after treament total ion chromatograms

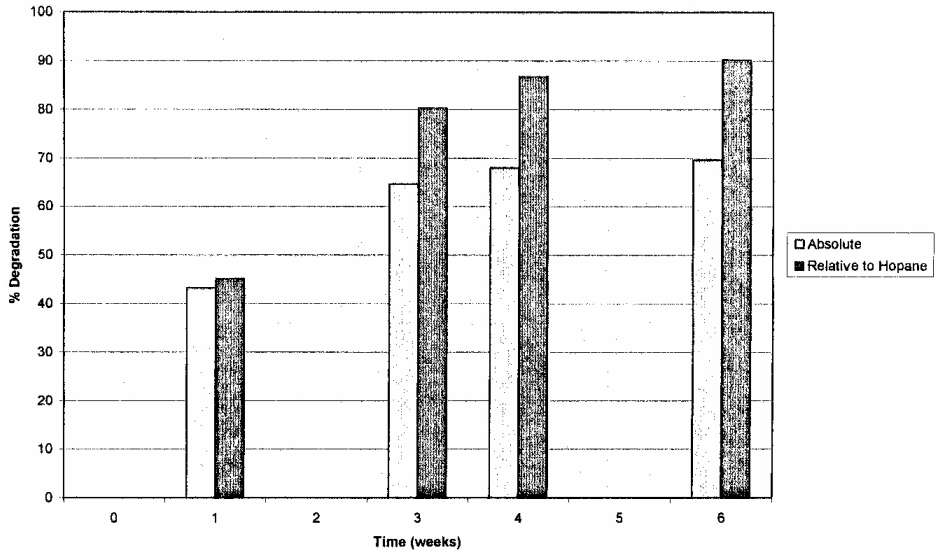

Figure 6. Comparison of absolute and relative TPH (GC) degradation

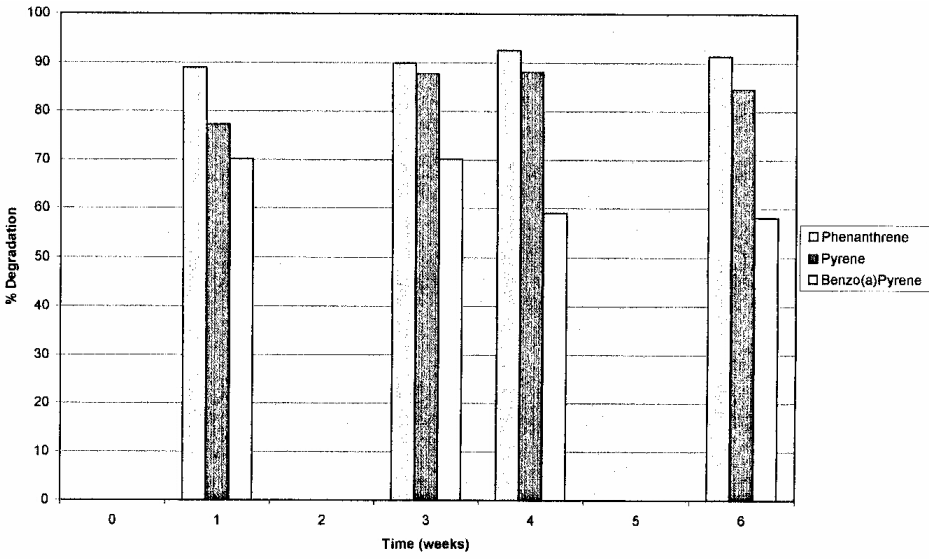

Figure 7. Degradation of 3, 4 and 5 ring PAHs absolute values

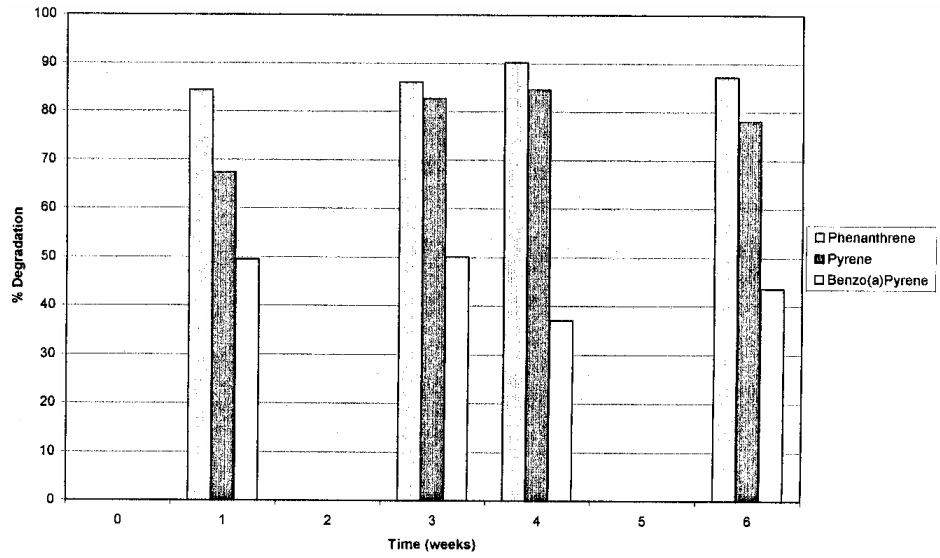

Figure 8. Degradation of 3, 4 and 5 ring PAHs relative to hopane

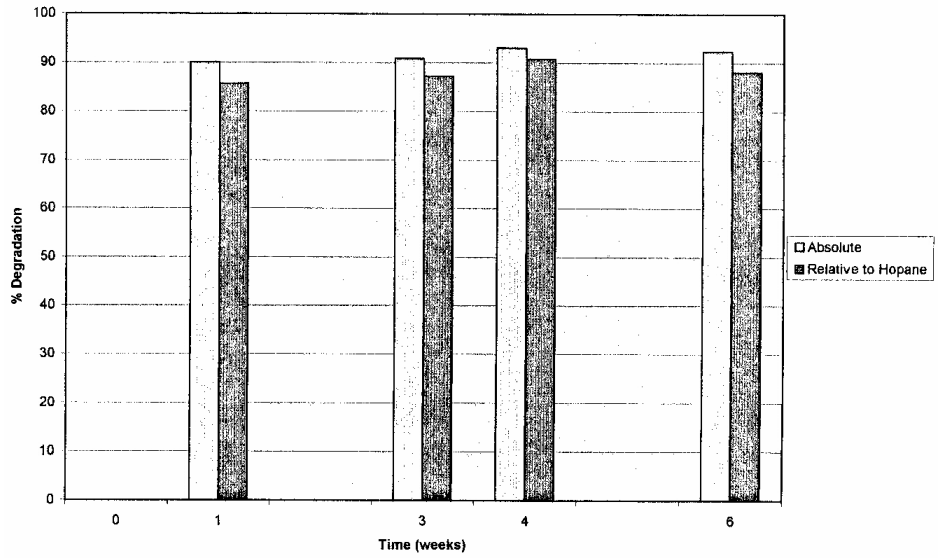

Figure 9. Comparison of absolute and relative C1-phenanthrene / anthracene degradation

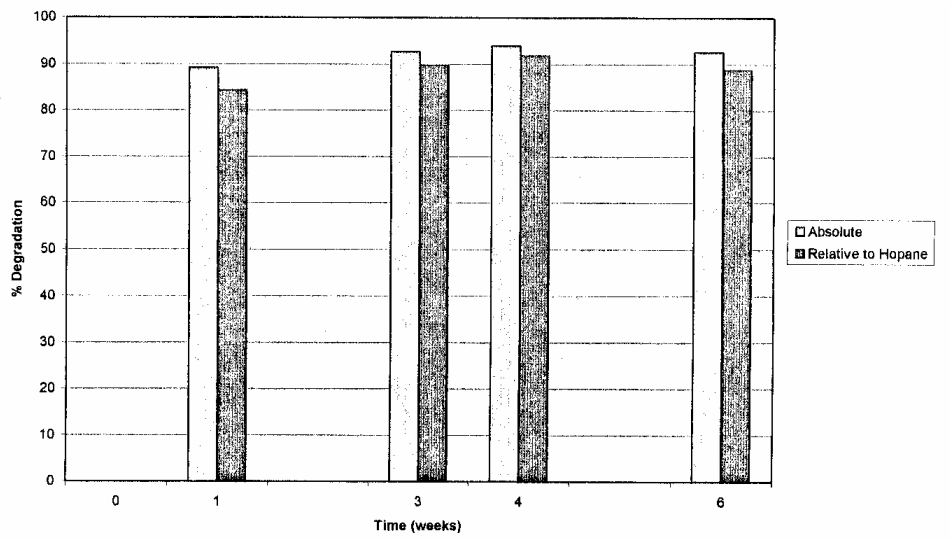

Figure 10. Comparison of absolute and relative C2-phenanthrenes / anthracenes degradation

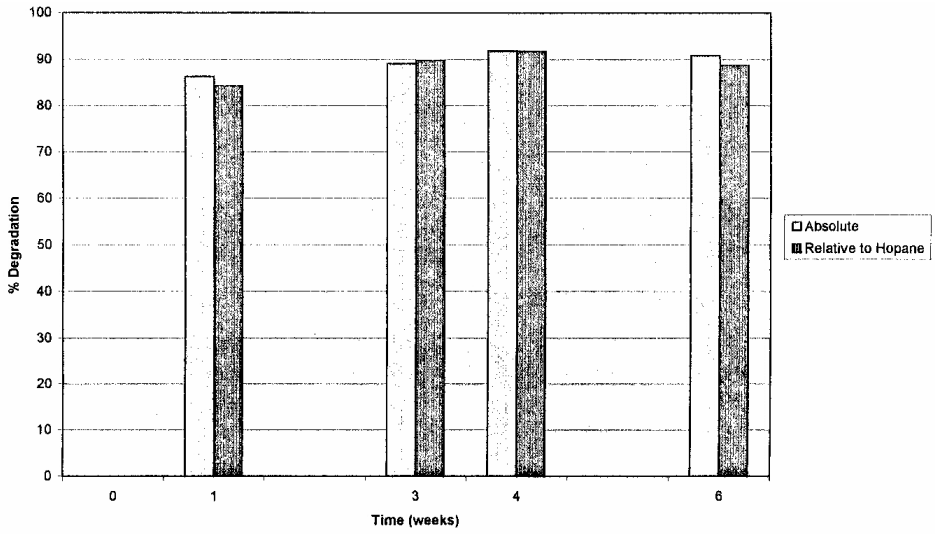

Figure 11. Comparison of asolute and relative C3-phenanthrenes / anthracenes degradation

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METHOD 8270 FOR MULTICOMPONENT ANALYTE ANALYSIS

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ABSTRACT

The identification and quantitation of multicomponent analytes (yielding more than one chromatographic peak) can be an analytical and productivity challenge. Multicomponent analytes such as Aroclors, Toxaphene, and technical Chlordane tentatively identified by another method may be confirmed using SW846 method 8270. Alternate confirmation of a tentative identification may be made using an electron capture detector (ECD) method such as 8081 or 8082 with a second column. For instruments with sufficient sensitivity, the mass spectrometer and ECD can be used in parallel for a simultaneous tentative identification and quantification. This paper will investigate the utility of a new mass spectrometer system for the quantitative identification of a mixture of multicomponent analytes. The method will be evaluated for detection limits, linearity, accuracy, and precision. The GC-MS method will be compared with the dual column method for analytical capability, productivity, and compliance.

INTRODUCTION

While the ability to positively identify sample analytes can be accomplished with the use of two columns, it is not necessarily the most desirable of options. In many cases the confirmational column alone is not sufficient and additional clean-up procedures need to be performed to eliminate co-eluting analytes. The additional equipment and analysis time required places productivity burdens on a laboratory. Gas Chromatography/Mass Spectrometry is widely used because its selectivity enables positive identification without additional sample processing. Along with the ability to make qualitative determinations, GC/MS is an invaluable tool for providing quantitative results. Mass Spec methods however, are generally considered less sensitive than conventional detector methods, although sensitive enough for most applications. The analysis of multicomponent analytes, such as Toxaphene and the Aroclors is more of a challange. EPA Method 8270C states, "In most cases, Method 8270 is not appropriate for the quantitation of multicomponent analytes, e.g., Aroclors, Toxaphene, Chlordane, etc., because of limited sensitivity for those analytes. When these analytes have been identified by another technique, Method 8270 is appropriate for confirmation of the presence of these analytes when concentration in the extract permits."[1] The development of more sensitive quadrupole mass spectrometry technology along with innovative sample introduction techniques, allow for the quantitation of many of these analytes at levels previously not achievable. The data to follow illustrates the ability of quadrupole mass spectrometry to quantitate multicomponent analytes at these lower levels. The ability to accurately identify and quantitate using GC/MS can eliminate the need for additional confirmatory analyses and reduce the amount of sample preparation required.

EXPERIMENT DESCRIPTION

Identical standards were analyzed using two sets of experimental conditions. A 50 µL Large Volume Injection was used in both cases. One set of standards was analyzed using the GC/MS Full Scan mode (FS-50) and the second using the Selected Ion Recording mode (SIR-50). Table 1 lists the chromatographic conditions used for both experiments, while Tables 2 and 3 list the Mass Spec conditions used for each set. The results are evaluated with respect to the accepted standard analytical techniques.

Perkin-Elmer AutoSystem XL	
Column:	PE-5MS 30 to x 0.25 mm; 0.25 µm film thickness
Pre-Column:	1 m x 0.32 mm deactivated fused silica
Oven Temperature Program:	55ºC for 5 min., 45ºC/min. to 160ºC; 6ºC/min to 320ºC
Programable Pneumatic Control (PPC):	Helium 1.0 mL/min.
Programable Split/Splitless (PSS) Injector:	55ºC for 4 min.; ballistic to 250ºC; Solvent Purge Mode
Injection Volume:	50 µL

Table 1. Chromatographic Conditions

FS-50

Perkin-Elmer TurboMass Mass Spectrometer	
Mass Scan Range:	50 - 350 m/z
Scan Speed:	2.0 scans/sec
Filament Delay:	5 min.
Ion Source Temperature:	150 ºC
Transfer Line Temperature:	250 ºC
Ionization Mode:	EI

Table 2. Full Scan Mass Spectrometer Conditions

SIR-50

Perkin-Elmer TurboMass Mass Spectrometer	
Selected Scan Masses:	159, 231, 233 m/z
Scan Speed:	2.0 scans/sec.
Filament Delay:	5 min.
Ion Source Temperature:	150ºC
Transfer Line Temperature:	250ºC
Ionization Mode:	EI

Table 3. Selected Ion Recording (SIR) Mass Spectrometer Conditions

RESULTS

Toxaphene standards at 0.10 ng/µL, 0.20 ng/µL, 0.50 ng/µL, 1.00 ng/µL, and 5.00 ng/µL concentrations were analyzed

using both methods. The chromatograms shown in Figure 1 were obtained using the SIR mode. All the calibration standards clearly exhibit the characteristic Toxaphene pattern.

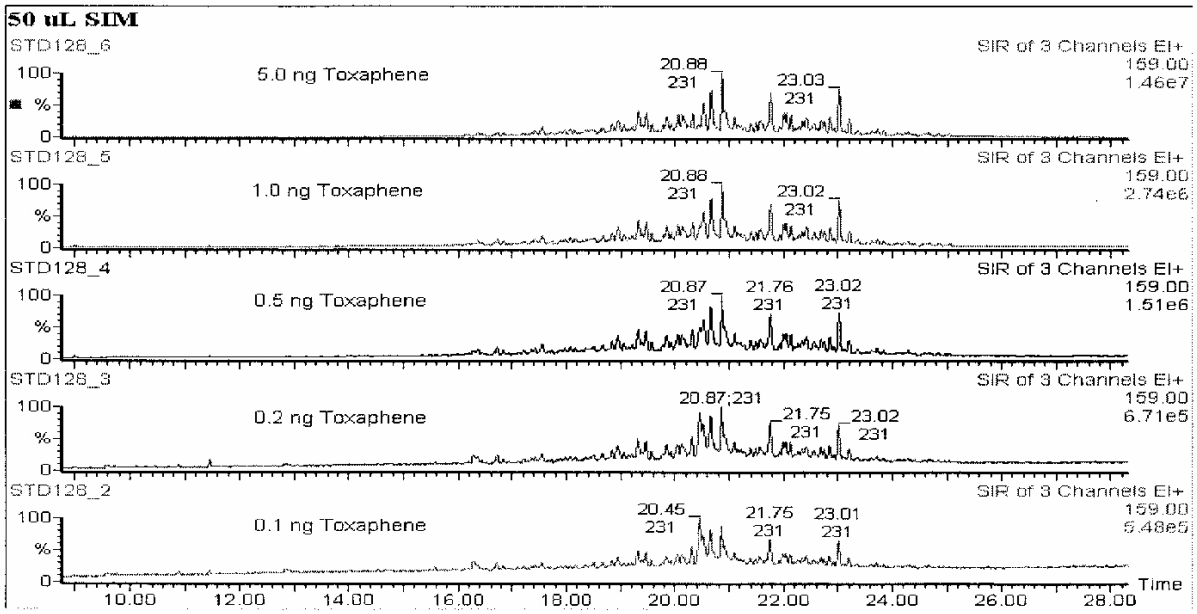


Figure 1. Calibration Standards show recognizable pattern for all levels.

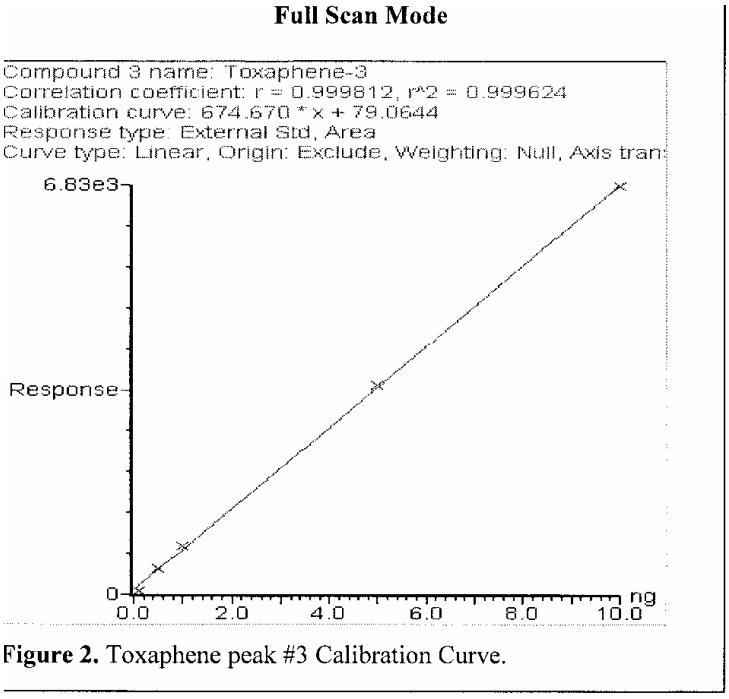
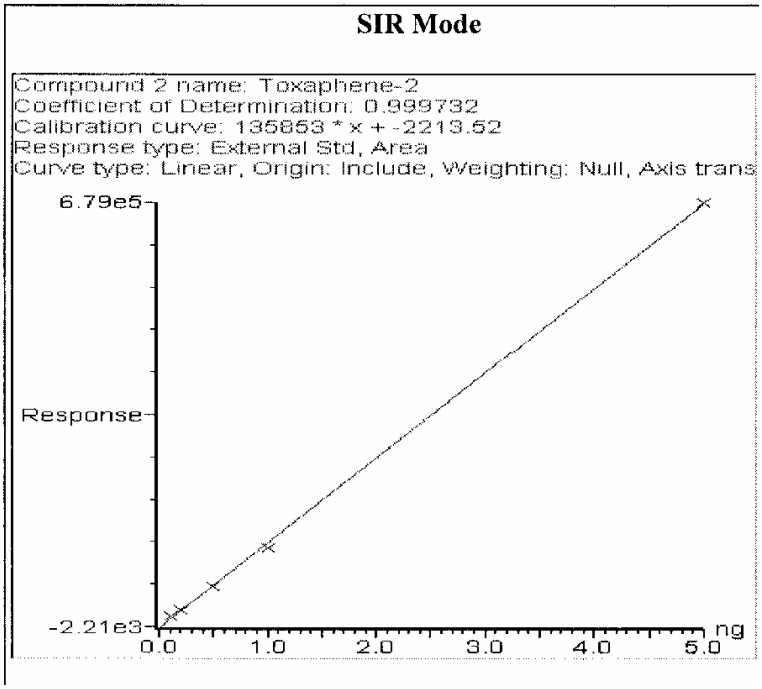


Figure 2. Toxaphene peak #3 Calibration Curve.

Four chromatographic peaks were selected and determined as representative of the multicomponent analyte Toxaphene. Calibration Factors (CF) were calculated based on the integrated peak areas and the known standard concentrations. From these results, the Relative Standard Deviation (RSD) for each multilevel concentration range was determined. These results were averaged providing a final Toxaphene RSD. Correlation coefficients were calculated in a similar fashion and are illustrated in Figures 2 and 3.

The results of all the calibration data and acceptance criteria are listed in Tables 4 and 5. Both experimental results easily comply with method performance specifications.



The Method Detection Limits (MDLs) listed in Table 6 are the result of seven (7) replicate injections of a 0.10 ng/µL standard using the standard deviation and the t-statistic. Integrated peaks representative of the entire calibration range can be seen in Figure 4. The bottom chromatogram was obtained from a 0.05 ng/µL standard which is below the lowest calibration standard of 0.10 ng/µL. The peaks are readily discernible above the noise and can be easily integrated.

Figure 3. Toxaphene peak #2 Calibration Curve.

Full Scan-50

Calibration Peaks	**RSD**		**Correlation Coefficient**	
	Actual	Acceptance Limit	Actual	Acceptance Limit
Peak #2	11.0		0.99934	
Peak #3	13.6		0.99949	
Peak #4	12.6		0.99962	
	8.4		0.99948	
Toxaphene (Average of 4 peaks)	**11.4**	**15.0**	**0.9995**	**0.99**

Table 4. Comparison with Calibration Acceptance Criteria using Full Scan mode.

SIR-50

Calibration Peaks	**RSD**		**Correlation Coefficient**	
	Actual	Acceptance Limit	Actual	Acceptance Limit
Peak #1	10.4		0.99936	
Peak #2	8.2		0.99973	
Peak #3	10.8		0.99967	
Peak #4	7.5		0.99934	
Toxaphene (Average of 4 peaks)	**9.2**	**15.0**	**0.9995**	**0.99**

Table 5. Comparison with Calibration Acceptance Criteria using Selected Ion mode.

Calibration Peaks	**Calculated Analytical Detection Limits**	
	FS-50	**SIR-50**
	(ng/µL)	(ng/µL)
Peak #1	0.073	0.065
Peak #2	0.089	0.009
Peak #3	0.105	0.021
Peak #4	0.035	0.014
Toxaphene (Average)	**0.07**	**0.02**

Table 6. Calculated Detection Limits

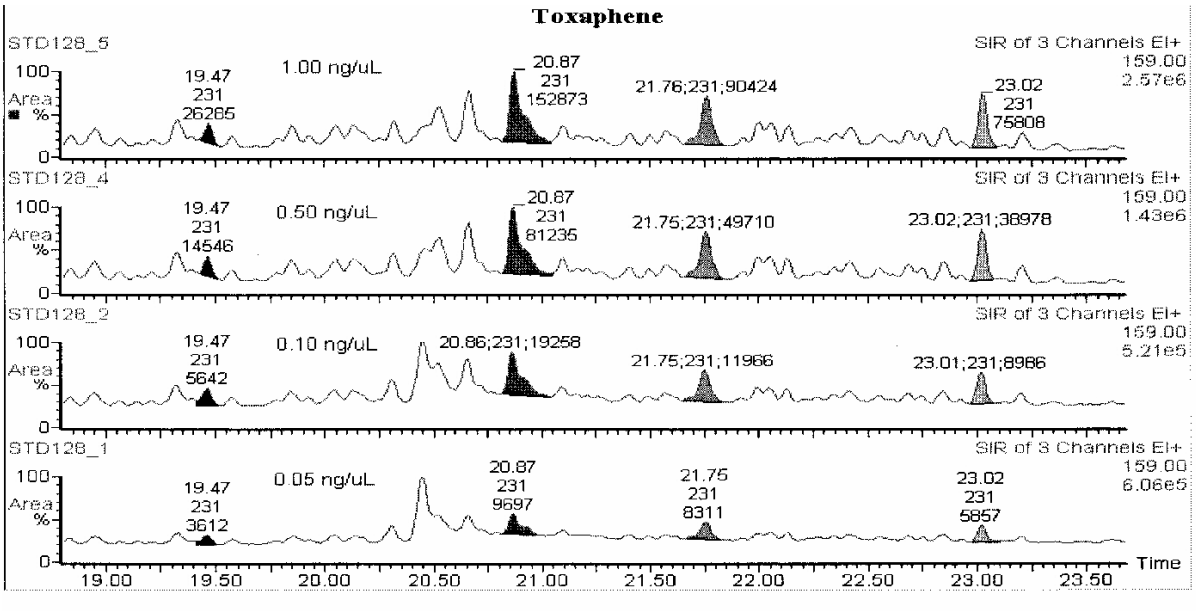


Figure 4. Integrated Toxaphene Peaks

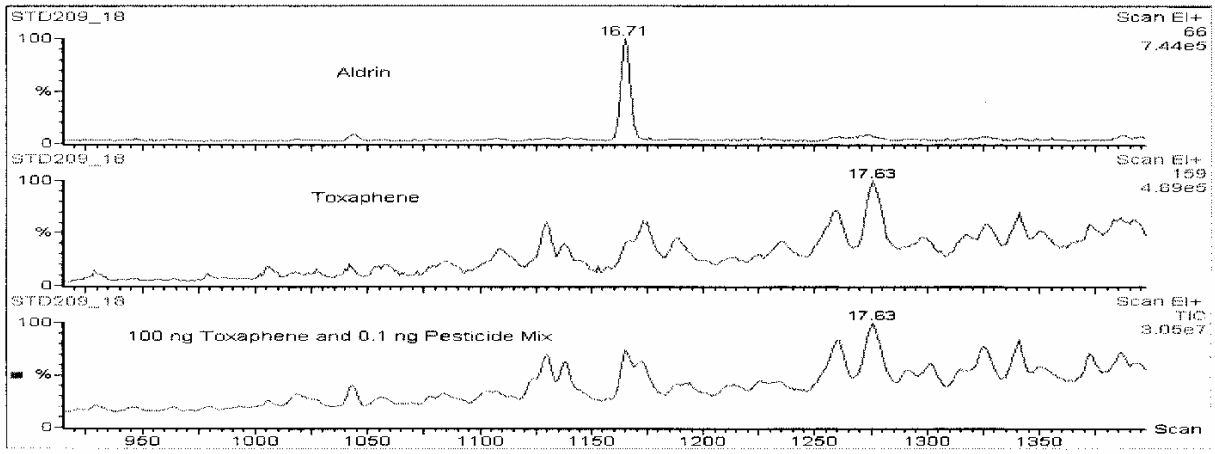


Figure 5. Aldrin and Toxaphene Extracted Ions

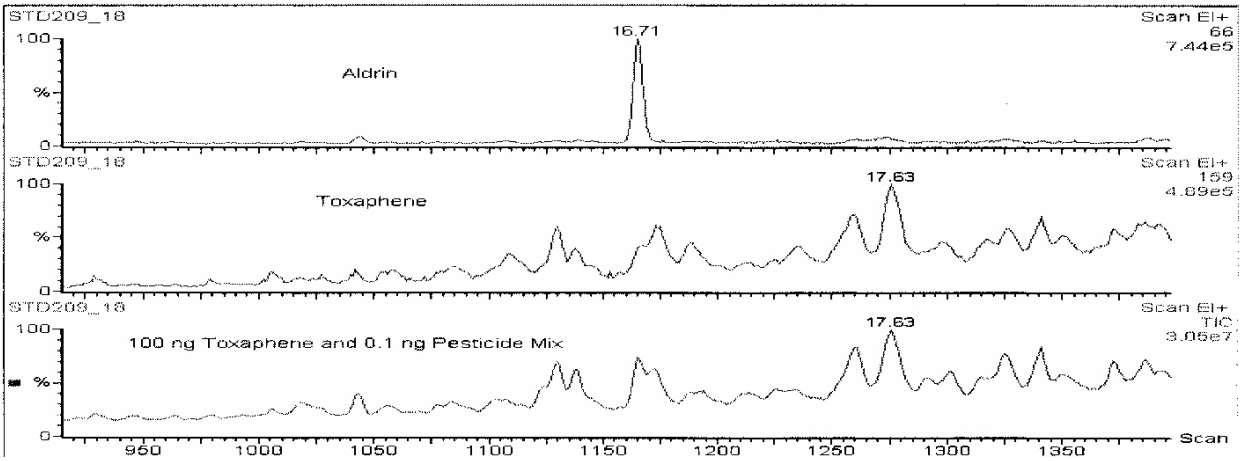


Figure 6. Library Searchable Spectra

What sets the Mass Spectrometer apart from other forms of detectors is the ability to selectively-identify individual masses. Figure 5 shows the Total Ion Chromatogram (TIC) of a mixture of 100 ng/µL Toxaphene and 0.10 ng/µL Pesticide Mix. The Extracted Ion (EI) mass 159 is Toxaphene, and the Extracted Ion (EI) mass 66 is Aldrin which was confirmed by a NIST library search as seen in Figure 6. Aldrin is easily identified and integrated without additional preparatory procedures.

The Contract Laboratory Program (CLP) lists 0.2 ng/µL as the quantitation limit for Aroclor 1221 using an Electron Capture Detector. Figure 7 shows Aroclor 1221 well above the noise level at the 0.20 ng/µL quantitation level, using GC/MS in the SIR mode and large volume injection.

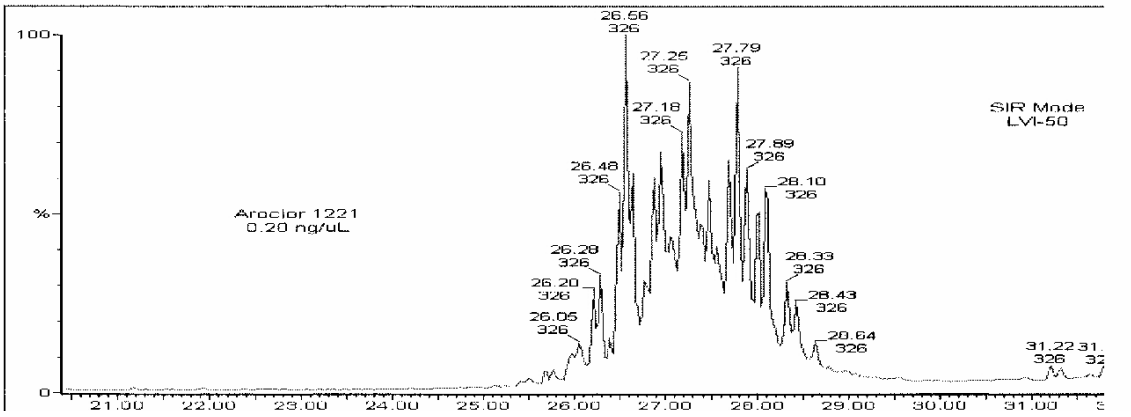


Figure 7. Quantitation Limit Pattern Recognition

SUMMARY

The ability of GC/MS to selectively identify a component based on an extracted ion chromatogram from a mixture of compounds not only assures a positive identification, but also saves time by eliminating additional cleanup and analyses. Recent technological advances in quadrupole Mass Spectrometry have increased the instrument's sensitivity. The use of Selected Ion Recording provides further sensitivity enhancements. In addition to the detector and it's mode of operation, the use of large volume injection with a programmable inlet system allow for introduction of larger sample volumes. The combination of these elements enhances the sensitivity of a GC/MS system so multicomponent analytes can be *identified and quantified* in an efficient and productive manner.

References

1. "Method 8270C, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)," in *Test Methods for Evaluating Solid Waste Physical/Chemical Methods,* SW-846, Third Edition, U.S. Government Printing Office, Springfield, VA (1996).

BENZIDINE? REALLY?

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ASI has been performing sample analysis using GC-MS Method 8270, with a variety of sample preparations from SW-846 for many years. In 1995 soil samples were received from a manufacturing site for analysis. The samples were part of a general site survey of the manufacturers facility to determine what may need remediation efforts in the future. ASI performed the requested analyses, and found and reported benzidine in several of the samples at levels approaching 1000 mg/kg. Unfortunately the data reports were simply filed by the manufacturer.

In late 1997, the manufacturing site became under consideration for sale to another company. As part of the pre-sale

investigation of the site, past analytical records were examined and the benzidine results came to light. As there had never been any benzidine used or stored on the site and the manufacturing processes involved no chemical syntheses, there were some questions about the validity of the reported results.

The raw data generated during the sample analyses was examined in detail. The initial calibration was acceptable, as were the daily calibration verifications and tunes. The system performance criteria were being met. The initial calibration was used for quantitation, with retention times and user generated library spectra being updated on a daily basis. Examination of the raw chromatograms (Figure 1) from the samples revealed a hump-o-gram. Random MS scans from the hump suggested a petroleum-based background interference. Although the benzidine hits were buried in the hump-o-gram, rather than being isolated well defined peaks, the candidates matched up perfectly with the retention time and daily generated mass spectrum of the benzidine standard.

The client had not requested a library search for TIC with the original analysis. By happenstance during the data review, a library search of the questioned peak was performed. Quite surprisingly the search generated a match for dibenzothiophene, rather than the expected benzidine. Spectra of both compounds were pulled from the database for examination.

It is rather startling how similar the mass spectra of benzidine and dibenzothiophene appear in a fast visual comparison (Figures 2 and 3). Both have a dominant peak at m/z 184 and an assortment of low intensity smaller m/z signals. The two compounds have the same unit mass molecular weight (184), the only difference in the molecular formulas being the two amino groups in benzidine and the sulfur in dibenzothiophene, $C_{12}H_8(NH_2)_2$ *vs*. $C_{12}H_8S$. By coincidence the mass of the two amino groups (32) is the same as the sulfur (32).

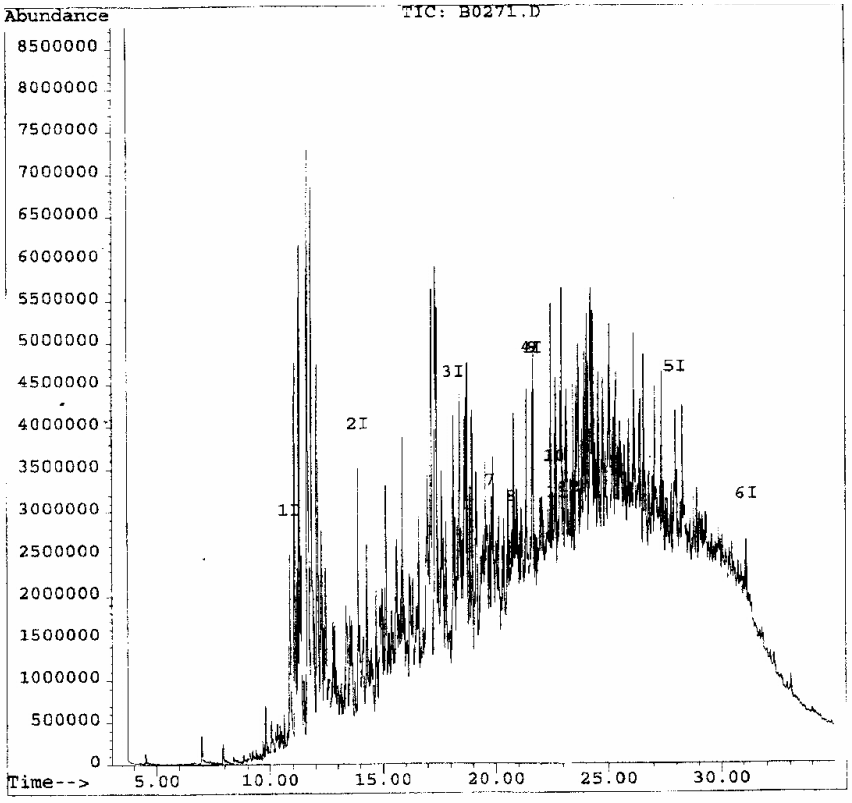
Detailed peak mass matching and relative abundance comparisons of select peaks reveal definitive mass spectral variances between the compounds that allows conclusive identification of either analyte. First off, both compounds have a M + I isotopic peak, however only dibenzothiophene has a very prominent M + 2 signal due to the sulfur. The fragmentation patterns of the molecular ions of the two compounds are nowhere near alike. Both compounds can loose a hydrogen to give M-1 signals, however only benzidine will continue to lose hydrogens giving the M-2 and M-3 peaks. Benzidine can also lose a $-NH_2$ group to generate the M-16 peak at m/z 168 or a NH_3 group, forming a benzyne at m/z 167 (M-17). Dibenzothiophene has no fragmentation pathway to generate either m/z 167 or 168. Extrusion of sulfur from dibenzothiophene gives rise to a prominent peak at m/z 152 (M-32).

The stability of the molecular ion of benzidine is probably enhanced through ring-expansion of one of the aromatic rings to include a nitrogen in a seven-membered aromatic ring (aza-tropyllium ion). Concerted ejection of a CNH_4 unit (M-30) from this ring generates m/z 154. A similar ring expansion, followed by concerted ejection of SCH from dibenzothiophene forms m/z 139 (M-45) as a significant signal that is quite undistinguished in the benzidine spectrum. Other important differences are indicated in Figures 2 and 3, and include m/z 65 and 77 in the benzidine spectrum, while Dibenzothiophene exhibits 69 and 79.

Although the distinguishing features of the two spectra are easy to overlook by eye, it was obvious that the computer spectral matching algorithm was having no such problem, and further investigation focused upon the user generated spectra. This is displayed in Figure 4, along with the spectra from one of the challenged identifications. What was in the sample matched up almost perfectly with what was stored as a spectrum of benzidine from the standard. Using the identification criteria listed above to examine the library spectra led to the inescapable conclusion that the standard used for initial and continuing calibration was dibenzothiophene rather than benzidine.

The retention time of dibenzothiophene is slightly less than that of benzidine, however not so much as to be really startling. Benzidine itself exhibits shifts in absolute and relative retention times as columns are changed in the GC-MS. As it is our habit to replace columns with recalibration, the slight shift in retention time from one initial calibration to the next was unexceptional. The quick visual examination of the mass spectra that accompanied the recalibration failed to detect any differences.

Dibenzothiophene is a naturally occurring substance commonly found in high sulfur crude oils. Discussions with the client revealed that the samples with "benzidine" all came from the soil underneath a storage area where several barrels of high sulfur Venezuelan fuel oil #6 had been placed. The high sulfur levels had made the oil unacceptable for use in the boilers at the facility. The GC-MS chromatograms contained hydrocarbon



hump-o-grams along with the "benzidine" and other sulfur-containing aromatics. As all the evidence was now consistent, the reports were re-issued deleting the benzidine hit.

The investigation was expanded to include examination of the calibration spectra both prior to and after these particular samples were analyzed. It was found that the problem began several months prior to October, 1995 surprisingly in the middle of a supplier's lot number. The problem continued after October, 1995, through the next lot number of benzidine standard purchased from the supplier. It was not until Summer, 1996 and a further lot number change that the spectra reverted back to the correct benzidine mass spectrum.

Figure 1. Chromatogram of sample

ASI contacted the supplier of the benzidine standard and presented them with the above evidence. The supplier was not making any spectral checks upon purchased stock standards as part of their QA program. Only the technically outdated melting point determination was being verified, and it was not being performed as a mixed melting point. There were no ampules of the particular lot numbers available for examination, however the supplier offered to reimburse the cost of the standards.

We went back through every sample analyzed during the year that the incorrect standard had been used. Fortunately we found that no other samples had been reported with hits for benzidine, thus there were no false positives. We are still in the process of doing a manual search of the tape archives for any false negative benzidine hits in these samples. None have been found to date.

How could we have caught the error and prevent it's happening again in the future? The corrective actions we have instituted are to: 1) inject new GC-MS standards under old calibrations prior to changing out the column and re-calibrating; 2) use second source standards to check each new calibration; and 3) closely examine the mass spectrum of each new benzidine standard that is purchased.

The lesson learned? Don't trust anyone's claims or documentation of purity or authenticity. Their acceptance standards may well be different than your own.

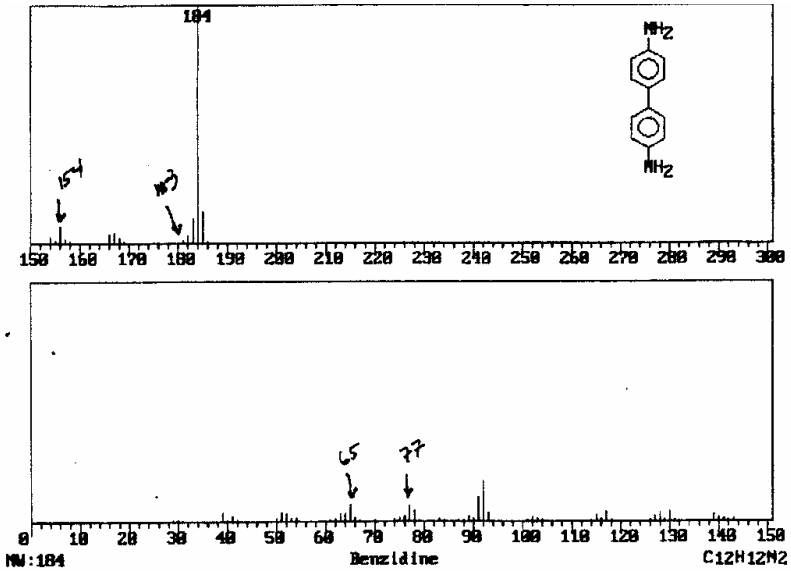


Figure 2. Mass spectrum of benzidine from Wiley-NIST library

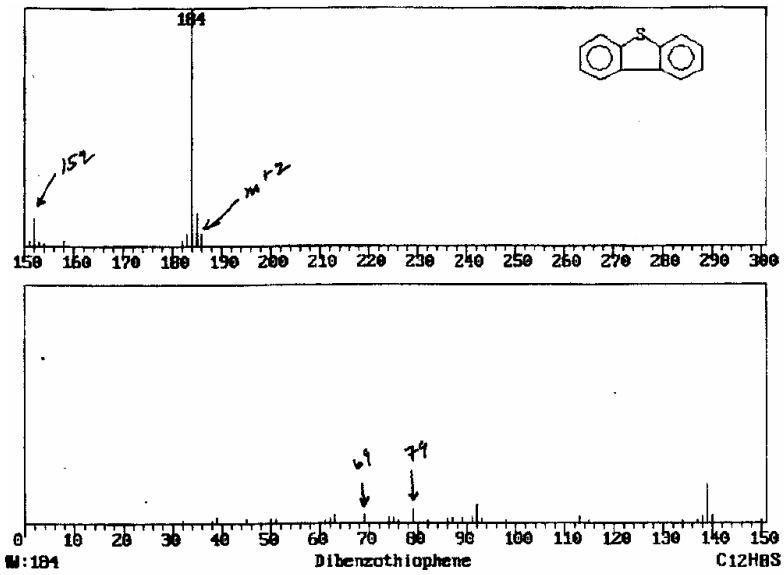


Figure 3. Mass spectrum of dibenzothiphene from Wiley-NIST library

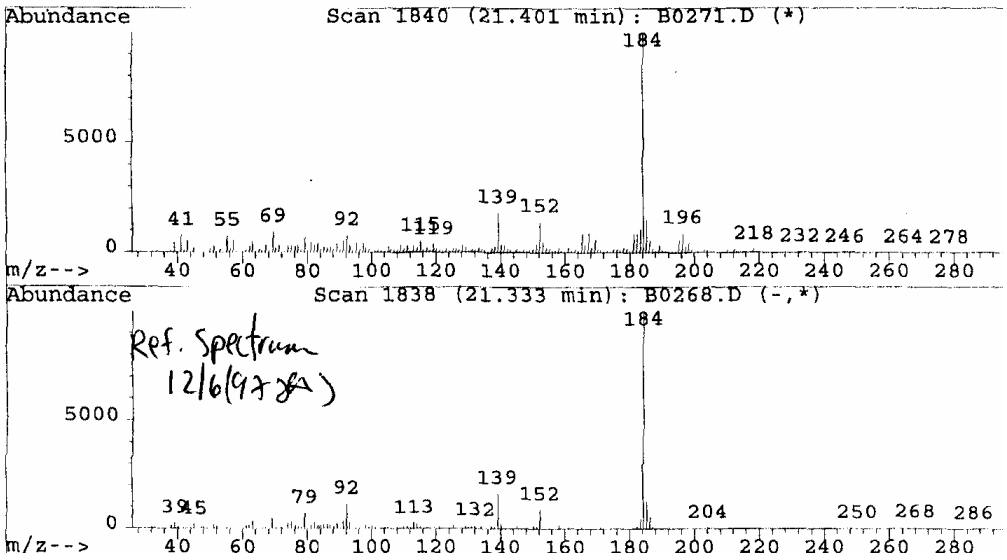


Figure 4. Mass spectra from sample and lab generated library

COMPARISON OF VOLATILE ORGANIC COMPOUND RESULTS BETWEEN METHOD 5030 AND METHOD 5035 ON A LARGE MULTI-STATE HYDROCARBON INVESTIGATION

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ABSTRACT

With the promulgation of SW-846 Update III during June of 1997, elimination of Method 5030 and implementation of Method 5035 have created significant challenges for the regulatory and laboratory communities (US EPA, 1997). Based on historical data, the results for volatile organics in certain sample types using the previously approved direct heated purge technologies were observed to be biased low (Hewitt, 1994). The loss of volatile organics was not observed to be the determinative process of Method 5030 but of the sampling, preservation, and preparatory aspects of the methodology (Hewitt, 1997, Siegrist, 1992). The promulgation of Method 5035 requires training of field samplers and a decision-tree approach to collecting and analyzing samples.

As recent as the first quarter 1998, for previously initiated on-going projects, various regulatory agencies have been issuing directives to either implement Method 5035 or continue with Method 5030 (US EPA, 1997). In order to understand the implications of implementing Method 5035 after several years of using "traditional" soil sampling methods and analysis by Method 5030 on a large multi-state hydrocarbon investigation, a 4-week study was performed to ascertain the differences in field activities, documentation issues, and analytical results.

A description of the comparative study, inclusive of observations between the traditional Method 5030 and the three Method 5035 options (e.g., Encore®, sodium bisulfate, and methanol), will be presented. In addition, some of the lessons learned from the study will be discussed.

INTRODUCTION

A two-phased study was performed to determine the comparability between investigative samples collected and analyzed by the "traditional" sampling/analytical method (SW-846 Method 5030) and investigative samples collected and analyzed by the recently promulgated sampling/analytical method (SW-846 Method 5035). Soil samples included in this study were collected from four states with different soil types and contaminant concentrations. The soil samples were analyzed for a list of 18 volatile compounds by gas chromatography/mass spectroscopy (SW-846 Method 8260A).

STUDY DESIGN

The study included the collection of soil samples in two phases. The first phase of sampling was conducted at select locations in Ohio, West Virginia, Pennsylvania, and Maryland from January 27 through February 11, 1998. Soil samples were collected at 56 sample locations. Nine field duplicates were collected during this phase. A trip blank (one sodium bisulfate and one deionized water) was collected for each day of sample collection. Based on previous analyses and remedial activities, samples collected during phase one were expected to either be "clean" or require low-level analysis. Accordingly, methanol samples were not collected for this phase. Specifically, for the first phase of sampling, samples were collected utilizing three techniques as follows:

- The traditional method of sample collection (in a 125 ml wide-mouthed glass jar);
- Utilizing a plastic syringe and placing five grams of soil into a 40 ml glass vial pre-preserved (by the laboratory) with sodium bisulfate; and
- Utilizing an Encore® sampler (the Encore® analyses for both phases were performed with the modifications recommended by the International Association of Environmental Testing Laboratories [IAETL]).

The second phase of sampling was performed the week of March 2, 1998. Samples were collected at 33 select locations (including two field duplicates) in West Virginia. The selection criteria for sample locations collected for the second phase of the project were based upon available historical sample concentration data. The sample locations were selected to include samples that contained low, medium, and high concentrations of volatile compounds (based on the traditional sample collection historical data).

Trip blanks (one sodium bisulfate, one deionized water, and one methanol) were collected for each day of sample collection. For the second phase of sampling, samples were collected utilizing four techniques as follows:

- The traditional method of sample collection (in a 125 ml wide-mouthed glass jar;
- Utilizing a plastic syringe and placing five grams of soil into a 40 ml glass vial pre-preserved (by the laboratory) with sodium bisulfate;
- Utilizing an Encore® sampler (the Encore® analyses for both phases were performed with the modifications recommended by the IAETL); and
- Placing five grams of soil into a vial containing methanol.

All samples were packed in coolers at 4ºC under Chain-of-Custody and shipped via overnight courier to a reputable commercial environmental laboratory. All samples were analyzed within a holding time of 14 days of samples

collection. In addition, each sample type (i.e., traditional, Encore® sodium bisulfate and methanol) collected at a given sample location was analyzed within 24 hours of the other sample types. In order to minimize possible confounding effects of analytical holding times, all sample types at a given location were analyzed within the sample 24-hour time period.

DATA REVIEW

Reduced data package deliverables were prepared by the laboratory for all samples. The reduced data package deliverables included a summary of the reported analytical results for all field samples (including samples, field duplicate samples and trip blanks), the associated method blank results, the associated laboratory control sample recoveries, and the surrogate recoveries. The analytical data for both phases were reviewed for completeness of the data package deliverables, compliance with the SW-846 Methods 5035 and 8260A, and usability of the reported analytical results (Clark and Vitale, 1996). Although important for comparability, compliance with methodologies often provides little information on data quality (BIye and Vitale, 1995).

The initial and continuing calibration criteria for Method 8260A were met for all study samples. With the exception of one compound in one laboratory control sample (LCS), the LCS recoveries were within study-specified limits (75-125%). The recoveries for one or more of the three volatile surrogate compounds were outside the limits specified (varying limits for each surrogate with limits between 70-121%) for many (38) of the study samples. Because the "true value" accuracy was not important for this study, the surrogate recoveries are not expected to affect the operational definition for comparing techniques on respective sample aliquots, which was most relevant in a comparative study. Analysis of the study trip blanks and laboratory method blanks did not reveal the presence of target analytes, with the exception of methylene chloride and acetone. The positive sample results for these two compounds were rejected from further consideration.

Eleven field duplicate samples were collected for the study; approximately one field duplicate sample was collected per day of sample collection. Field duplicates provide valuable information on precision and sample representativeness when evaluated properly (Zeiner, 1994). Acceptable precision (<50% RPD, as defined for this project) was noted between six field duplicate pairs. High RPDs (>50% RPD) were noted for five of the duplicate pairs. Such sample variability appears to have complicated a meaningful comparison of techniques as separate sample aliquots were collected and analyzed for each of the study sampling techniques.

SUMMARY OF RESULTS

A summary of the reported analytical results is provided on Table 1. All results are reported on a dry-weight basis. Variations between sample-specific quantitation limits were evident due to the sample collection volume, the percent moisture of the sample, and the sample-specific dilutions performed. The sample collection volume at a given sample location is different for each sample type due to the manner in which the sample was collected. The quantitation limits for samples preserved with methanol were raised by the laboratory due to the medium-level sample preparation.

Fourteen of the sodium bisulfate samples were not analyzed due to the observed concentrations of non-target compounds in these samples (samples containing sodium bisulfate cannot be analyzed using medium-level protocol). The corresponding traditional and Encore® samples at these sample locations were analyzed at a medium-level due to the same reason. In addition, six of the sodium bisulfate samples could not be analyzed due to observed sodium bisulfate effervescence.

In total, 79 Encore® samples, 79 traditional samples, 62 sodium bisulfate preserved samples, and 23 methanol-preserved samples were collected for the volatile pilot study. Of the 79 sample locations examined, positive results were reported in 33 of the sample locations. Positive results were reported for four aromatic compounds (benzene, toluene, ethylbenzene, and total xylenes); two ketones (4-methyl-2-pentanone and 2-butanone); and four chlorinated aliphatic hydrocarbons (1,1-dichloroethene, 1,1,2,2-tetrachloroethane, 1,1,1-trichloroethane, and tetrachloroethene).

TABLE 1. SUMMARY OF ANALYTICAL RESULTS

Sample Number	Encore™	Traditional	Sodium Bisulfate	Methanol
SAMPLE 1				
total xylenes	6U	6U	6	N/A
SAMPLE 2				
benzene	6U	6U	6	N/A
ethylbenzene	6U	6U	12	N/A
total xylenes	6U	15	14	N/A
SAMPLE 3				
toluene	6U	5U	8	N/A
2-butanone	95U	110	110U	N/A
SAMPLE 4				
toluene	6U	6U	7	N/A
SAMPLE 5				
toluene	6U	7U	12	N/A
SAMPLE 6				
tetrachloroethene	6U	6U	23	N/A
toluene	6U	6U	23	N/A
ethylbenzene	6U	6U	15	N/A
total xylenes	6U	6U	260	N/A
SAMPLE 7 (DUPLICATE OF SAMPLE 6)				
tetrachloroethene	5U	6U	13	N/A
toluene	5U	6U	9	N/A
total xylenes	5U	6U	7	N/A
SAMPLE 8				
total xylenes	36	140J	45	N/A
SAMPLE 9 (DUPLICATE OF SAMPLE 8)		0	0	
total xylenes	610	440U	N/A	N/A
SAMPLE 10		0	0	
total xylenes	760	130U	N/A	N/A
SAMPLE 11				
1,1-dichloroethene	200J	320U	N/A	N/A
1,1,1-trichloroethane	2200	570	N/A	N/A
toluene	1200	110J	N/A	N/A
ethylbenzene	210J	320U	N/A	N/A
total xylenes	930	160J	N/A	N/A
SAMPLE 12				
1,1,2,2-tetrachloroethane	310	330U	N/A	N/A
ethylbenzene	200J	280J	N/A	N/A
total xylenes	1200	2100	N/A	N/A
SAMPLE 13				
toluene	5U	6U	6	N/A
SAMPLE 14 (DUPLICATE OF SAMPLE 13)				
1,1,2,2-tetrachlorethane	6U	6U	16	N/A
SAMPLE 15				
4-methyl-2-pentanone	31,000	9700	N/A	N/A
SAMPLE 16				
4-methyl-2-pentanone	13,000	8400	N/A	N/A
SAMPLE 17				
toluene	1000	1600	15	N/A
ethylbenzene	6200	12,000	16	N/A
total xylenes	30,000	63,000	53	N/A
SAMPLE 18				
4-methyl-2-pentanone	2700U	6500U	1,300	N/A
ethylbenzene	2000	1400	790	N/A
total xylenes	20,000	18,000	240	N/A

TABLE 1. (Cont.)

Sample Number	Encore™	Traditional	Sodium Bisulfate	Methanol
SAMPLE 19				
toluene	63	34	61	N/A
ethylbenzene	20	11	31	N/A
total xylenes	160	59	280	N/A
SAMPLE 20				
tetrachloroethene	6300	5000	2900	N/A
toluene	5U	6U	14	N/A
ethylbenzene	5U	6U	9	N/A
total xylenes	11	6U	24	N/A
SAMPLE 21				
toluene	7U	6U	7	N/A
2-butanone	130U	120U	120	N/A
SAMPLE 22				
1,1-dichloroethene	5U	6U	16	530U
toluene	5U	6U	6U	100J
SAMPLE 23				
benzene	250J	220J	N/A	670
tetrachloroethene	901	300U	N/A	200J
toluene	1300	1100	N/A	3300
ethylbenzene	470	550	N/A	870
total xylenes	4600	5500	N/A	8400
SAMPLE 24 (DUPLICATE OF SAMPLE 23)				
benzene	250J	220J	N/A	400J
tetrachloroethene	80J	300U	N/A	200J
toluene	1200	1500	N/A	1900
ethylbenzene	570	430	N/A	740
total xylenes	5600	4500	N/A	7200
SAMPLE 25				
benzene	5U	6U	45	570U
toluene	5U	6U	90	570U
ethylbenzene	5U	6U	26	570U
total xylenes	5U	6U	130	300J
SAMPLE 26				
ethylbenzene	600	230J	N/A	1200
total xylenes	370	110J	N/A	930
SAMPLE 27				
benzene	6U	6U	12	610U
ethylbenzene	6U	15	7U	610U
SAMPLE 28				
benzene	2000	6U	N/A	650U
toluene	7400	6U	N/A	200J
ethylbenzene	1500	6U	N/A	650U
total xylenes	14,000	6U	N/A	600J
SAMPLE 29 (DUPLICATE OF SAMPLE 28)				
benzene	6U	7J	6U	540U
toluene	6U	30	6U	100J
total xylenes	6U	4600	23	780
SAMPLE 30				0
ethylbenzene	120J	120U	N/A	340U
total xylenes	60J	320U	N/A	80J
SAMPLE 31				
benzene	5U	13J	6U	260U
ethylbenzene	5U	23J	6U	60J
total xylenes	5U	33	7	670

TABLE 1. (Cont.)

Sample Number	Encore™	Traditional	Sodium Bisulfate	Methanol
SAMPLE 32				
toluene	210J	280U	N/A	470U
ethylbenzene	540	280U	N/A	470U
total xylenes	3300	280U	N/A	550
SAMPLE 33				
benzene	6U	6U	8	610U
toluene	6U	6U	14	610U
total xylenes	6U	6U	8	610U

Notes: U - Not detected at or above the associated numerical value
N/A - Not analyzed
J - Estimated value; result less than the quantitation limit
E - Estimated value; result exceeded the calibration range and not reanalyzed

COST ANALYSIS

For the reputable commercial laboratory that was utilized for this study, the cost of analysis of all four sample collection types is identical, and there are not any cost implications for analyzing a volatile soil sample by any of the four sample collection methods. The cost of sample bottleware preparation is different between the four sample collection types. The cost for traditional sample collection bottleware is built into the contract with the laboratory. The cost for the Encore® sampler is roughly $10.00 per sampler. Three Encore® samplers are filled at each sample location at a total of <$30.00 per sample location. The cost for a methanol-preserved vial (two vials are sampled at a given location when the methanol preserved sample is not collected in tandem with a sodium bisulfate preserved sample; otherwise, only one vial is submitted) is <$35.00 per sample or <$60.00 per sample location. The cost for a sodium bisulfate preserved vial is approximately $50.00 per sample. Two sodium bisulfate preserved vials are collected per sample location at a cost of approximately $100.00 per sample location.

The labor costs for the collection of the various sample types can vary and exact costs could not be precisely calculated based on information available. The Encore® sampler and the traditional method of sample collection were found to be the quickest and easiest sample types to collect. The sodium bisulfate samples were found to take the longest time to collect and provided the most difficulty in the field.

FIELD OBSERVATIONS

Sodium Bisulfate and Methanol Sampling Techniques

The sodium bisulfate and methanol techniques are very similar and are reviewed here jointly. These methods were by far the most cumbersome of the selected sampling techniques. The necessity of using an analytical balance under imperfect field conditions proved frustrating. The balance readings would fluctuate wildly with the slightest amount of wind or movement of the sample preparation surface. In addition, unless the sample preparation surface was perfectly horizontal, the scale could not calibrate nor zero out. Furthermore, calibration of syringes with soil matrix was time consuming in comparison to the traditional and Encore® methods. Using razor knives to cut syringe tips evenly required practice and care. Finally, preservative solution was prone to spillage unless great care was taken while placing the soil in the vials.

The methanol technique has an additional drawback. When shipping samples via popular air couriers, samples must be packaged according to IATA regulations. The regulations regarding shipping of flammable compounds are very strict and may result in lost or delayed delivery if the sample containers are not properly packaged. In addition, the appropriate paperwork and package labeling must be completed in a precise manner, or the shipment will be delayed or returned to the sender. From a field sampling perspective of the methods used during the pilot study, these were by far the most time consuming and frustrating.

Encore® Sampling Technique

The Encore™ sampling system was very straightforward in its approach and implementation, although some minor problems were encountered during the pilot study. Problems were encountered when trying to place loose and/or wet soils into the Encore® sampler. Soils of this type had to be manipulated into the Encore® with another sampling device (such as a spatula). The only other sample collection issue involved improper seating of the cap on the plunger. However, by pushing down on a hard surface with the T-handle, the cap could be seated properly. Overall, the Encore® system appeared to be easy to use even under adverse field conditions.

Traditional Sampling Technique

This sampling method is well-known to most field technicians and has been employed under a wide variety of field conditions. It was apparent during the field study that this technique was easily implemented and rivaled the Encore® sampling technique for ease of use. However, from a field perspective, some clays, silts and other tightly compacted soils are difficult to place into a sample container so that no head space is allowed. Breaking up soils to place into sample containers may result in loss of volatiles, thereby lowering detectable concentrations. However, from a field perspective, this technique has been historically easily implemented.

CONCLUSIONS

The following observations can be made from the overall study. With the exception of the first bullet item, the conclusions presented below should not be interpreted to be applied to other sites, soil types, and concentrations of analytes. These are general conclusions regarding this particular data set; there may not be an equivalent trend noted in other data sets. Our findings suggest that inherent difficulties associated with analyzing soil samples makes definitive states regarding data comparability difficult. Furthermore, the number of positive data points and the disparity observed for half of the collected field duplicates makes statistical trend analysis problematic at best.

- At sample locations where methanol-preserved samples were collected and where the concentration of target analytes was within range of the medium-level analysis, the concentration of target analytes of the methanol-preserved sample type was greater than the concentration of the analytes in the other sample types at the same sample location. This is consistent with other studies appearing in peer-reviewed literature.

- At sample locations where methanol-preserved samples were not collected and where the concentration of target analytes was within range of the medium-level analysis, the concentration of the aromatic analytes appeared to be greater in the traditional sample collection type than the other sample collection types at the same location.

- At sample locations where methanol-preserved samples were not collected and where the concentration of analytes was within range of the medium-level analysis, the concentration of the non-aromatic target compounds appeared to be greater in the Encore sample collection type than the other sample collection types at the same location.

- At sample locations where methanol-preserved samples were not collected and where the concentration of target analytes was not within range of the medium-level analysis, the concentration of the analytes appeared to be greater in the sodium bisulfate sample collection type than the other sample collection types at the same location.

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INORGANIC

SW-846 INORGANIC METHODS UPDATE

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NO ABSTRACT AVAILABLE

DIRECT MERCURY ANALYSIS: FIELD AND LABORATORY APPLICATIONS

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ABSTRACT
EPA Method 7473 is designed for the determination of total mercury in solid and aqueous samples. This method is based on the instrumental methodology of the Direct Mercury Analyzer-80 (DMA-80) (Milestone, Inc.) in which sample preparation and analysis are essentially integrated into a single analytical step. The method's unique capability for direct analysis allows for application in either laboratory or field settings. Method 7473 has been validated by analysis of various Standard Reference Materials (SRMs) in both the laboratory and in the field. This validation data has been presented[1]. Results from Method 7473 have also been confirmed by independent analysis using traditional methods. Method 7473 has been used on-site in conjunction with mercury remediation. Real-time analysis using this technique has provided an accurate and cost-effective risk assessment of mercury contaminated sites.

INTRODUCTION
There are several analytical techniques that may be applied for the determination of mercury in solid waste. Existing EPA methods for the analysis of mercury include inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Method 6010B), cold vapor atomic absorption spectroscopy (CV-AAS) (Method 7471A), and anodic stripping voltammetry (ASV). Regardless of the method used, sample preparation is required. "Soils, sludges, sediments, and other solid wastes require digestion prior to analysis"[2]. Method 7473 has an advantage over traditional mercury analysis because it eliminates the need for a discrete sample preparation step. Direct analysis is performed by integration of thermal decomposition, amalgamation, and atomic absorption spectroscopy. While the fundamental theory for this type of analysis has been available in the literature, the DMA-80 is the firs instrumental implementation of these integrated concepts.

A schematic of the DMA-80 is shown in Figure 1. The sample is automatically inserted into the quartz deomposition tube, where it is first dried and then thermally decomposed. The gaseous decomposition products are carried by a flow of oxygen to the catalytic core, which is maintained at a temperature of 750 ºC to ensure complete thermal decomposition. The oxygen flow continues to carry the gases to the gold amalgamator, where mercury is selectively trapped. Continuous oxygen flow removes any remaining decomposition products. The amalgamator is subsequently heated, releasing the mercury vapor to the absorbance cuvettes where peak height is measured at 253.7 nm as a function of ng of mercury.

Calibration for Method 7473 can be performed in two ways. One method is by the traditional analysis of aqueous standards. The ability for direct analysis also allows for unique calibration using solid standards with a certified mercury content. Method 7473 provides the option to perform calibration using solid samples, "An alternative calibration using standard reference materials may be used..."[3]. This option is beneficial, especially for on-site analysis, when transport and storage of aqueous standards may be problematic.

Subsequent to validation of Method 7473 in both laboratory and field settings, the method was applied to the laboratory analysis of a series of contaminated soils. Duplicate soil samples were sent to a commercial laboratory for independent analysis. Only those soils with a mercury content less than 10 mg/kg were analyzed directly due to the extreme sensitivity of the instrument. Soils above 10 mg/kg were leached using EPA Method 3051A prior to analysis. Mercury content in these soils ranged from 1-700 mg/kg. Regardless of the mercury content, results using Method 7473 agree with results using the traditional cold vapor Method 7471A and show greater precision. Average

RSDs for Methods 7473 and 7471A were less than 10% and 15%, respectively.

In collaboration with a local engineering firm, Method 7473 has been used on-site to evaluate the remediation efforts of a large national utility company. A site near Gettysburg, Pennsylvania, was first evaluated. A sample was taken from each wall and the floor of the 124 ft^3 excavated area. It was determined that remediation efforts were successful, as the mercury content in all samples was below the action level of 20 mg/kg. These results have been confirmed by independent commercial laboratory analysis.

A second on-site evaluation was performed in Hocking County, Ohio. Real-time results were used to direct the extent of excavation. The original scope of work based exclusively on the site characterization was an excavation area of 750 ft^3. Use of Method 7473 on-site produced real-time information as to the level of remediation required and allowed remediatiors to reduce the planned excavation area by more than half to 250 ft^3. Real-time Method 7473 results indicated that the reduced excavation was adequate in all areas except one. Further excavation in that location was thus performed, providing a more accurate remediation and eliminating the need for a return trip to the site. Approximately $9,000 in savings resulted from the reduced amount of soil remediated and elimination of a return trip.

SUMMARY

A method for the direct determination of total mercury in both laboratory and field environments has been established. Method 7473 has been validated by analysis of SRMs as well as by independent analysis. Data indicates that Method 7473 can achieve lab-quality results in a field setting. use of Method 7473 in the field can lead to more accurate and cost-effective risk assessment of mercury contaminated sites. The next step will be to investigate compatibility of Method 7473 with speciation technology.

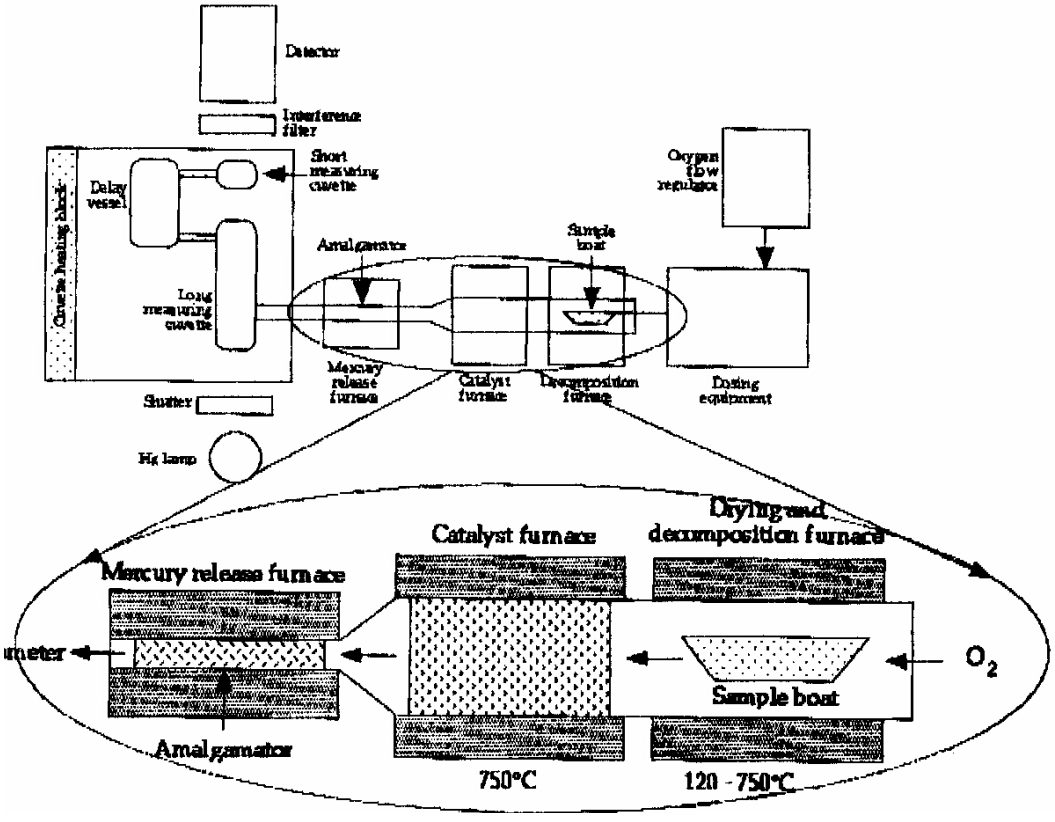

Figure 1. Schematic of the DMA-80

Table 1. Comparison of techniques use for method validation ($n \geq 5$).

EPA Method	7473	7471A	6020
Technique	direct mercury analysis	CV-AAS	ICP-MS
Average RSD	<10%	<15%	~15%
Agreement?	✓	✓	✓

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MERCURY IN SOIL SCREENING BY IMMUNOASSAY

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Abstract

EPA SW-846 Method 7471 (Cold Vapor Atomic Absorption, CVAA) has been traditionally used for mercury analysis. This method requires several hours of labor and total processing time to prepare soil samples and analyze them. The sequential nature of the analysis step limits sample throughput and capacity. Also, the method is difficult to perform at a remediation site. In contrast, immunoassay (IA), as used in proposed Method 4500, prepares and analyzes the samples in parallel and can provide very large sample capacity in a 2-3 hour processing time. Also, the IA kit is much more field portable.

Quanterra performed an evaluation of the BiMelyze Soil Extraction and Mercury Immunoassay kit from BioNebraska for the Olin Corporation. Four different site soil samples were analyzed in quadruplicate by CVAA and IA following the New York State Department of Health (NYSDOH) Alternate Testing Procedure guidelines. Threshold standards were setup at 1 mg/kg and 15 mg/kg. No false negatives were observed. Thefalse positive rate was 6%. The overall accuracy rate was 94%.

Switching to immunoassay improves turn-around-time, sample capacity and field portability.

Introduction

Community, environmental and economic concerns are exerting pressure on the environmental market to reduce analysis turn-around time and cost. At the same time, some data end users have realized that traditional test methodologies and their QA/QC requirements are not necessary for all environmental decisions. There is a growing need to provide reliable, quick turn-around field testing in order to expedite site remediation. Accelerated testing can be instrumental in reducing the impact that remediation activities may have on the local community. It also facilitates faster site closure, thus reducing the time the excavation site is exposed to the effects of weathering.

Immunoassay has been widely used in biochemical testing for health services for decades. Several companies have adapted this technology to test environmental pollutants including organic compounds and metals. Immunoassay has proven to be a low cost, fast turn-around, high capacity analysis for the health sciences. These same characteristics make it very attractive for the environmental market. Immunoassay (IA) for metals does not provide data that is identical to the traditional inductively coupled plasma or atomic absorption tests. The specificity of IA should make it less susceptible to the interferences that limit spectroscopic analyses. However, the biochemical nature of IA may make it sensitive to new interferences. The QA/QC data normally available from IA can include replicates and matrix spikes.

This study compared the results from proposed US EPA SW-846 Method 4500 (Mercury in Soil Sample by Immunoassay) with EPA SW-846 Method 7471 (Cold Vapor Atomic Absorption, CVAA). Method 4500 was performed using the BioNebraska BiMelyze Soil Extraction and Mercury Immunoassay kit. Actual field samples from

a remediation site containing the analyte of interest (mercury) were used for this study.

Immunoassay Method Summary (excerpted from Section 2.2 of Method 4500)
"Solid samples are prepared by extraction with a mixture of hydrochloric and nitric acids for ten minutes and then buffered prior to analysis. The sample is added to a tube (treated with BSA-glutathione) and incubated at ambient temperatures for five minutes. The mercuric ions bound to the sulfhydryl groups of the BSA-glutathione are now reacted with a reconstituted antibody specific for mercury and incubated for five more minutes. A peroxidase conjugate is added to the sample, reacting with any mercury specific antibody. The substrate is then added forming a color that is in proportion to the amount of mercury originally present in the sample. The color produced is then spectrophotometrically compared with the control standards."

Experimental
This project evaluated the BioNebraska BiMelyze Soil Extraction and Mercury Immunoassay kits (BN-IA-Hg), proposed Method 4500 by comparison to the traditional mercury analysis method (cold vapor atomic absorption, SW-846 7471). NYSDOH Alternative Testing Procedure guidelines (4/1/86) were followed.

The NYSDOH Alternative Testing Procedure specifies that limited approval for a "new" test method requires the following analytical work:

1) Three samples from the soil source.
2) Analyze four aliquots of each sample with the approved method (7471).
3) Analyze four aliquots of each sample with the alternative method (4500).
4) Analyte concentrations should range from the detection limit to 20% greater than the regulatory limit.
5) The result from the alternative method must fall within the confidence interval of the approved method (based on two times the published standard deviation).

An additional sample from the soil source which had a mercury concentration previously determined to be well below the lowest action threshold was evaluated to test the likelihood of the IA kit producing false positives. False positives occur when interferences or analytical error produces a test result that is greater than the control threshold when the analyte concentration is actually below the threshold. A false positive can result in unnecessary remediation work.

Also, it is common to intentionally bias the results of semi-quantitative field tests slightly high. This reduces the false negative rate. Many organic immunoassays use a bias of 30%. A 20% bias had been used previously by others working with the BioNebraska mercury test kit. A 30% bias on the threshold standards was selected for this study.

The NYSDOH requirements described above were met as follows:

1) Four samples from the soil sample source were selected based on previous CVAA analysis to cover the appropriate concentration ranges. Approximately 150 g of each sample was frozen and then homogenized cold to reduce analyte losses. The samples were stored at 4ºC until appropriately sized aliquots were removed from the sample containers for each test method. No elemental mercury was visible and the samples appeared visually to be homogeneous before sub-aliquots were removed.
2) Four aliquots of each of the 4 samples were analyzed by Method 7471.
3) Four aliquots of each of the 4 samples were analyzed by Method 4500 following the procedure described in the BioNebraska test kit product literature. The two control thresholds were 1 and 15 mg/kg since those correspond to the two action limits for theapplicable site.
4) Ideally one sample would have been available for the middle of the following ranges: x < 0.5 ppm, 1 ppm < x < 5 ppm, 10 ppm < x < 15 ppm, 15 ppm < x < 20 ppm
   The quadruplicate CVAA results show that 3 of the 4 ranges were covered by the samples selected.
5) Since the alternative method is semi-quantitative, the range determined for each individual sample was compared to the corresponding average quantitative result from Method 7471.

Results and Discussion
Accuracy
The mercury field test kit is semi-quantitative for this site (e.g. x < 1 mg/kg or 1 mg/kg < x <15 mg/kg or 15 mg/kg < x). The concentration range determined by method 4500 (IA) was compared to the concentration measured by 7471

(CVAA). IA results which placed the sample in the correct range were considered "accurate". Results that were too low were labeled "false negative". Results that were too high were labeled "false positive". Normal US EPA criteria for field test kits specify the false negative rate should not exceed 5%. NYSDOH criteria were not available.

Table 1. Comparison of Average Results, CVAA vs IA

Sample ID	CVAA avg mg/kg	IA avg abs	IA interpretation (mg/kg) x<1	1<x<15	15<x	Test Agreement
B41-24-102297	0.12±0.02	0.21	X			OK
B11-02-102197	9.4 ±0.88	0.43		X		OK
B44-02-102197	35.2 ±9.2	1.20			X	OK
B1-24-102197	16.9±14	0.77			X	OK

±2 standard deviation confidence interval

Table 2. Comparison of Detailed Results, CVAA vs IA

Sample ID	CVAA mg/kg	IA abs	IA interpretation X<1	1<x<15	15<x	Test Agreement
blank soil		0.07				
1 ppm threshold std*		0.24				
15 ppm threshold std*		0.70				
B41-24-102297	0.12					
	0.12					
	0.12					
	0.10					
		0.24		x		false +
		0.21	x			OK
		0.20	x			OK
		0.19	x			OK
B11-02-102197	9.6					
	9.2					
	8.8					
	9.8					
		0.45		x		OK
		0.40		x		OK
		0.43		x		OK
		0.42		x		OK
B44-02-102197	41					
	36					
	33					
	31					
		1.26			x	OK
		1.47			x	OK
		0.93			x	OK
		1.13			x	OK
B1-24-102197	27					
	16					
	12					
	13					
		1.03			x	general
		0.62		x		data
		0.77			x	set
		0.67		x		agreement

*The actual concentration of the two threshold standards was 30% lower than the stated thresholds in order to reduce the likelihood of false negative results

The average IA results agreed with the average CVAA results for each of the four samples.

As expected the individual results showed greater variability and slightly less agreement between the two analysis types. One immunoassay result from B41-24-102297 (x < 1 ppm) was a false positive since its absorbance equaled that of the 1 ppm threshold standard. Occasional results of this type are to be expected when using low biased threshold standards to reduce the false negative rate. The IA and CVAA results for samples B11-02-102197 (1 < x < 15 ppm) and B44-02-102197 (15 ppm < x) showed excellent agreement.

Sample B1-24-102197 had a concentration near the 15 ppm threshold and was apparently more heterogeneous than the other samples (despite the extensive homogenization that had been performed). Both the IA and CVAA results were more variable and were evenly split on either side of the 15 ppm threshold. As a general data set the results from the two methods are in agreement. Please note that since the individual IA and CVAA analyses were performed on separate aliquots of the same sample, it is not appropriate to directly compare the individual test results in order. Rather the quadruplicate results from each method must be considered as a set.

Given the results discussed above and shown in the table below, no false negatives (0%) and only one false positive (6%) were observed in the 16 sample assays. The agreement rate between the two tests was 94%.

The average IA kit absorbances correlated well with the CVAA average results. This indicates it may be possible to use the IA kit for quantitative analysis, particularly if standards were prepared in or made from soil samples on-site. The chart below shows IA absorbances plotted relative to the CVAA results. The IA standard soils (from BioNebraska) are also shown relative to their actual prepared concentrations. The least squares linear equation for the 4 soil samples was :

IA abs = 0.02879 (Hg conc mg/kg) + 0.2089 with an R^2 value of 0.9848.

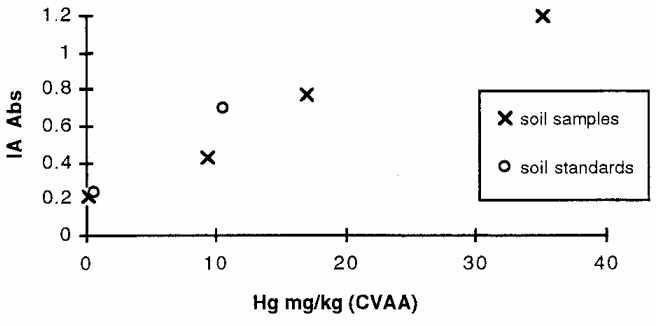

Figure 1. Comparison of IA Absorbance vs CVAA Concentration

Precision

The standard deviation of the absorbances produced by the IA test were useful when interpreting results near a control threshold. It is not practical to calculate the standard deviation of the concentration since this is a semi-quantitative test in its current configuration.

The percent relative standard deviation (%RSD) of the result (conc for CVAA and absorbance for IA) for each method was comparable. This indicates the two methods have the same precision for this group of samples. Most likely sample homogeneity was the factor which limited the precision of both methods.

Table 3. Comparison of Precision, CVAA vs IA

Sample ID	CVAA		IA	
	Std Dev	%RSD	Std Dev	%RSD
B41-24-102297	0.01	8.6	0.02	10
B11-02-102197	0.44	4.7	0.02	4.9
B44-02-102197	4.6	13	0.23	19
B1-24-102197	7.1	42	0.18	24
average %RSD		17		

Information from a BioNebraska representative indicates that the use of volumetric pipettes instead of the "eye dropper" volume measurements described in the product literature may have improved precision by a few percent.

Sample Turn-Around Time

Complete kit preparation, extraction and analysis of a batch of soil samples took approximately 3 hours for a batch

of 19 assays (16 samples). Kit preparation (including reagent prep and sample container labeling) took about 0.5 hours. Extraction and filtration of the soil samples took 1.75 hours. Performing the immunoassay took 0.75 hours. Two analysts were working together performing the soil filtrations. If a single analyst was performing all the filtrations with the filters supplied in the kit, add about 0.5 hours to the total time. Obviously smaller batches of samples would reduce the time required for kit preparation, extraction and filtration but the assay step would be only slightly shorter. Also, autopipettes and repeating pipettes were used (at client request) to improve accuracy and precision. This equipment (not included in the field kit) also shortened the time necessary for some of the liquid handling steps in the immunoassay.

Sample Capacity

The field portable version of the test kit is designed to process 13 soil samples in each batch. Smaller batches can be processed but the cost per sample increases because the test kits are used less efficiently and additional control soil samples must be purchased. The laboratory version of the test can be configured for much larger batch sizes. Using laboratory pipettes and previous experience with immunoassay, two field kits were combined to process all 16 tests (4 samples in quadruplicate) in the same batch. Laboratory kits are available to process up to 96 assays (~80 samples) in a batch.

Analyst Skill

The field test kit was designed for use by field technicians with average manual dexterity and attention to detail but limited experience with scientific instrumentation. Based on the experience during this study, this expectation is true. Appropriate hands-on training with the IA kit on known samples is essential before beginning work on unknown samples. Also, two problems occurred during sample preparation which might happen for other samples on this site.

1) B44-02-102197 produced a large amount of foam when the acid was applied to the sample at the being of the extraction. During the study, the acid was added very slowly and the bottle continuously tapped on the bench top to break the foam bubbles. Even so, 2 of 4 aliquots lost about 1% of the sample due to foaming out of the extraction bottle. This did not appear to affect the assay results but required considerable attention, persistence and time to avoid significant sample loss. An experiment conducted after the assays were complete showed that adding the recommended amount of acid solution to a 5g sample aliquot resulted in foam overflowing the normal 32 mL sample bottle and a 67 mL bottle. When a 140 mL bottle was used the foam filled 80% of the bottle before bursting and settling into the bottom of the bottle. Thus, for samples similar to this one it would be advisable to use a 140 mL bottle to avoid accidental sample loss, slow processing and increasing the skill requirements.

2) The extract filtration step was much slower for the samples collected at the site than for the standard soils supplied by BioNebraska. Sample B41-24-102297 in particular was very difficult to filter. Several samples required squeezing the sample bottle with pliers in order to force sufficient extract filter. This would make reproducible results difficult using "eye dropper" volume measurements at this point in the process. Other types of IA kits have more "user-friendly" filtration processes. It may also be possible to adapt the current filtration process to improve its performance.

Conclusion

Method 4500 (IA) produced semi quantitative results which matched the Method 7471 (CVAA) results for 15 of the 16 tests (94%) performed. There was one false positive where the immunoassay overestimated the mercury concentration. The false negative rate in this four sample evaluation study was 0%, while the false positive rate was 6%. This meets the requirements of the NY DEC Alternative Testing Procedure. In addition, the average IA absorbances from each quadruplicate set of data correlated very well with the CVAA results. This indicates that the IA kit may also be useful for quantitative analyses in the future.

Summary of Reporting Limits and Performance Results

Matrix	Mercury	Accuracy	False Negative	False Positive
soil	1 mg/kg	94%	0%	6%

Acknowledgments

The authors would like to thank Veronica Bortot from Quanterra - Pittsburgh for overall project coordination and Craig Schweitzer from BioNebraska for IA product and technical support.

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UTILIZATION OF A FIELD METHOD FOR THE SEMIQUANTITATIVE DETECTION OF SILVER IN ENVIRONMENTAL SAMPLES IN THE 0 - 50 ppb RANGE

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Abstract
Silver is commonly used in industry, and its bactericidal properties have also lead to its use for water disinfection purposes. Excess concentrations of silver may damage human health and be toxic to aquatic life. Current methods of silver analysis, in the ppb range, require expensive equipment and careful technique. There is a need for a quick, easy screening method for silver at these levels. The procedure described employs the bromopyrogallol red/1,10-phenanthroline method, described by Dagnall and West (1964), combined with a novel concentration/detection method. At a pH of 7, a ternary complex is formed with two 1,10-phenanthroline molecules binding to each silver ion, and then two of these complexes bind to a bromopyrogallol red molecule. This results in a blue precipitate. The colored precipitate is caught on a 13 µm pore size filter, and the filter is compared to a precalibrated (0, 5, 10, 25, and 50 ppb) printed color chart for quantification. All the reagents are combined in a single powder that contains both dyes, a buffer, and a masking reagent. The system is easy to use, fast, portable, and all reagents are stable for at least one year making the system ideal for field testing. This method has been evaluated on a variety of tap water, pool & spa water, river water, and sewage effluent samples that have been spiked with known amounts of silver. Some of the river water samples and the sewage effluent required a sulfuric acid digestion, but all samples resulted in good recovery of the spikes and correlated well with numbers generated using AA techniques. Various soil samples that were spiked with 25 ppb Ag resulted in good recoveries that corresponded to the appropriate color spot on the chart. Use of this method as a screening process may help to save time and money by cutting down on the need to do more accurate analysis of all samples.

Introduction
Silver is a common contaminant of industrial process and wastewater. In private industry, silver is used in applications such as jewelry, coins, dentalware, silverware, solder, electroplating, photography, and battery production. In low concentrations, silver's antibiotic properties make it desirable for use as a fungicide and for drinking water disinfection purposes, and it has been gaining in popularity as a pool and spa biocide. However, according to the World Health Organization (WHO), continuous exposure to silver in drinking water (0.4 mg or more) in humans causes arygaria, an irreversible condition which produces a bluish-gray discoloration of the skin, hair, nails and eyes.[4] Long term continuous exposure to silver has also been implicated in liver damage and enzyme inactivation in humans.[4]

Unpolluted surface water levels of silver usually range between 0.1 - 4 µg/L. Drinking water levels range between 0 - 2 µg/L; average = 0.13 µg/L.[3] The WHO has not as yet set limits for safe silver concentrations in drinking water.[5] The USEPA has adopted the Public Health Service (PHS) standard that silver in domestic water not exceed 50 µg/L.[3] The USEPA-adopted PHS standard was set to protect aquatic life and human health. Canada has adopted a similar 50 µg/L standard while the EEC standard is 10 µg/L.[5] Silver is also on the list of seven priority pollutant metals that must be monitored in landfill leachate.[7]

There are many current methods to measure silver in the ppb ranges. These include atomic adsorption by flame or electrothermal techniques, inductively coupled plasma, or colorimetry. Each of these methods requires complicated and expensive apparatus, hazardous chemicals and/or a large investment in time and equipment. Each also has its drawbacks. AA is accurate at moderate concentrations, but displays sensitivity to ion interference. ICP techniques have higher minimum detection limits and are sensitive to refractory elements. Colorimetry loses sensitivity at these