The 13th Annual Waste Testing & Quality Assurance Symposium

PROCEEDINGS

WTQA '97

Sponsored by the
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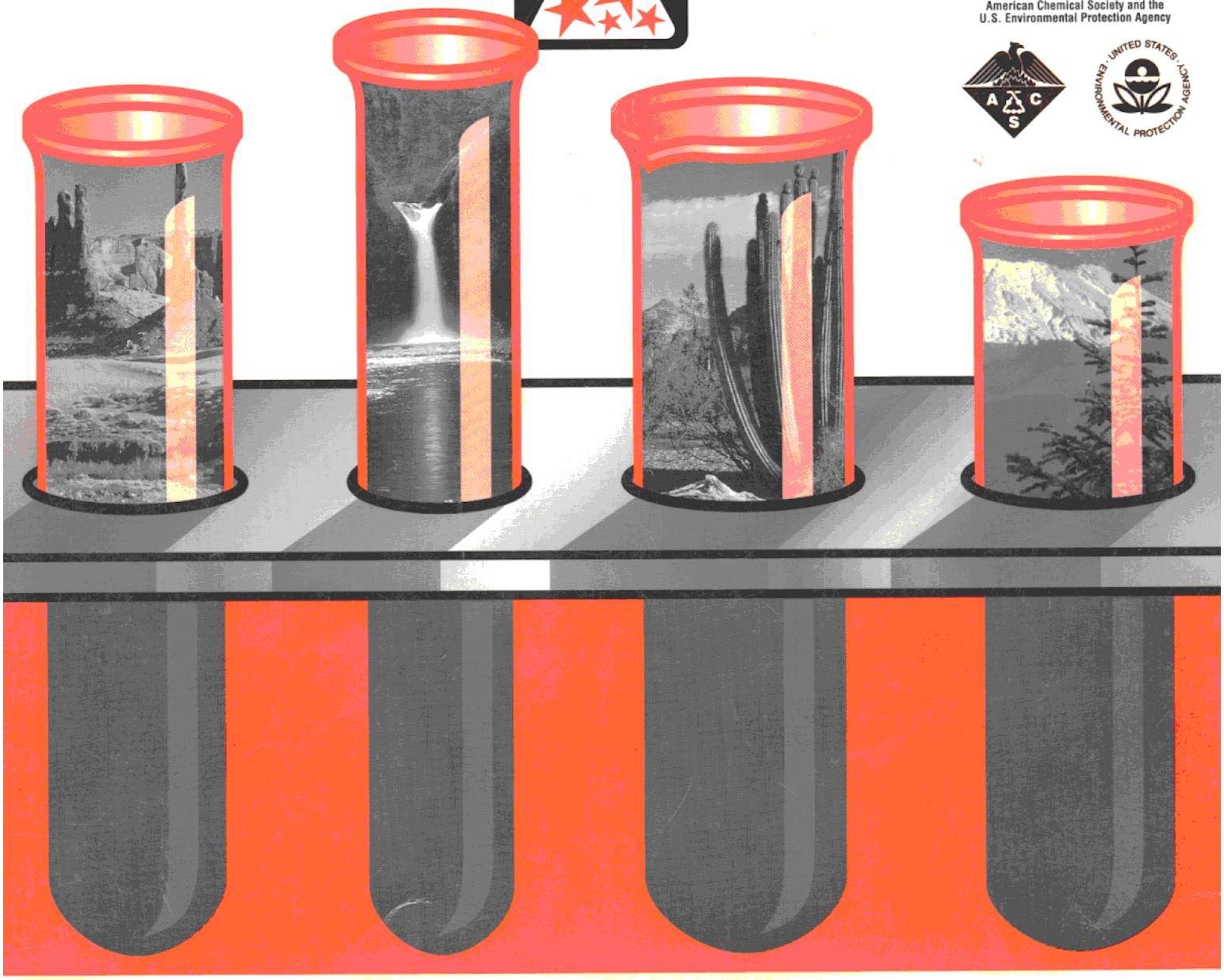
U.S. Environmental Protection Agency



July 6-9, 1997 ■ Crystal Gateway Marriott ■ Arlington, VA

Proceedings

The Thirteenth Annual

Waste Testing & Quality Assurance Symposium (WTQA '97)

July 6-9, 1997

Crystal Gateway Marriott
Arlington, VA

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ENDOCRINE DISRUPTORS

ENVIRONMENTAL ENDOCRINE DISRUPTORS: AN ACS OVERVIEW OF THE ANALYTICAL CHALLENGE

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INTRODUCTION

Environmental Endocrine Disruptors (EEDs), endocrine modifying chemicals which are also environmental pollutants, are the subject of a special session at the 13th Annual Waste Testing & Quality Assurance Symposium sponsored by the U.S. Environmental Protection Agency (EPA) and the American Chemical Society (ACS) Division of Environmental Chemistry. While most recent technical symposia on this topic have, and continue, to focus on the *effects* of EEDs, the focus of this special session is on the *analysis* of EEDs. The analysis of EEDs in environmental matrices (e.g., water, air, soil, wastes, and biota including both plants and animals) is critical to future regulatory monitoring of them as well as to an understanding of their occurrence, transport and migration within the environment, and their ultimate degradation.

In order to be able to analyze for EEDs several prerequisites are necessary:

The EEDs subject to monitoring and/or regulation need to be identified*;

2. Methods for EEDs need to be effective at concentration levels desired for monitoring**; and

3. Analytical reference materials must be available to calibrate the instruments used for the analyses.***

DEFINITION OF ENDOCRINE DISRUPTING CHEMICALS

The endocrine system refers to the complex system that involves the brain and associated organs and tissues of the body. These include the pituitary, thyroid, and adrenal glands and the male and female reproductive systems, all of which release hormones into the bloodstream. In particular the sex hormones include estrogens in females and androgens in males. Endocrine disrupting chemicals (EDCs) consist of synthetic and naturally occurring chemicals that affect the balance of normal hormonal functions in animals. Depending on their activity they may be characterized as estrogen modulators or androgen modulators. They may mimic the sex hormones estrogen or androgen (thereby producing similar responses to them) or they may block the activities of estrogen or androgen. (i.e., be anti-estrogens or anti-androgens).[1]

There are three categorical sources of EDCs:[2]

1. Pharmaceuticals - One of the first recognized synthetic EDCs was diethylstilbestrol (DES), a pharmaceutical product given to pregnant women from 1948 to 1972 to help prevent miscarriages. It caused clear-cell carcinoma in the vagina, reproductive abnormalities in female offspring, and a much higher than normal rate of genital defects in male babies.

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* Developing screening methods is an important effort that hopes to bypass initial needs to identify specific individual EEDs. However, as discussed below, screening methods for EEDs will have to accommodate a wider variety of diverse chemicals than have ever been subjected to screening methods before. This is an extremely complex and difficult challenge.

** Concentration levels at which EEDs are presumed to be effective, and therefore potentially monitored, are at those of hormones. These are typically a thousand or more times below levels at which most current analytical methods are able to measure analytes in the environment with reasonable rates (e.g., <10% at a 95% confidence level) of false positive and false negative identifications. Thus, EED levels of concern may be at parts per trillion (**10^{-12}**) or below.

*** Analytical reference materials with certifications of identity, purity, and homogeneity are necessary for both qualitative and quantitative analysis.

2. Naturally Occurring EDCs - This source of EDCs, collectively called "phyto-estrogens," includes foods such as soybeans, apples, cherries, wheat, and peas.

3. Environmental EDCs - The third group of EDCs are some environmental pollutants. These *environmental endocrine disruptors* (EEDs) are the subject of this symposium.

ANALYTICAL CHALLENGES

The analytical challenges can be summarized as:

1. Determining which analyses are EEDs so that their effects can be studied and those of importance can be monitored and/or regulated in the environment and in food.

2. Developing screening analytical methods that will accommodate a wide variety of analytical functional groups at extremely low detection levels.

3. Developing qualitative and quantitative analytical methods to confirm, as necessary, the identity and quantity of EEDs in the environment and in food.

4. Assuring, through appropriate use of analytical reference materials and QA/QC procedures, an acceptable level of false positive and false negative determinations at regulatory levels.

Analytical Challenge #1 is the subject of this paper. The other authors in this symposium will address the other three analytical challenges.

CHALLENGE #1 - DETERMINING WHICH POLLUTANTS ARE ENVIRONMENTAL ENDOCRINE DISRUPTORS

One of the major problems we face is determining which chemicals in the environment should be labeled as environmental endocrine disruptors. This is a critical question because these materials will be the subject of future regulations. Obtaining information on them will also require very significant expenditures of time and money. In addition, traditional environmental analytical methods require specific known analytes for identification and quantification. If you don't know what chemical(s) to analyze for then you can't analyze for them -- a trivial statement but a difficult problem with EEDs. Thus, identifying which chemicals are EEDs is the first of four analytical challenges. Currently, the process for deciding if a chemical is an environmental endocrine disruptor is to determine the effects of that chemical on the endocrine systems of humans and other animals.

Much of the research on EEDs to date has focused on the effects of EEDs on wildlife. There are many studies involving certain pesticides, polychlorobiphenyls (PCBs) and polychlorodibenzo-p-dioxins (e.g., 2,3,7,8-TCDD) that link them to birth defects and aberrant sexual behavior. Laboratory tests have also produced genital defects, reduced testicular weights and low sperm counts in rats fed with DDE, PCBs, Vinclozolin, and 2,3,7,8-TCDD.[3]

It is also believed by some researchers that EEDs may be the cause of similar types of recent observations in humans. The CDC in Atlanta has performed surveys that show that the average US resident has hundreds of chemicals accumulated in their fat tissues including polychlorodibenzo-p-dioxin and polychlorodibenzofuran isomers ("dioxins" and "furans").[4]

Some chemicals, including many EEDs, can "bioaccumulate" or build up in animals. Once they are incorporated into the tissues and fat of animals and humans, they can remain there for long periods of time until they are ultimately metabolized. Thus, an embryo, the most sensitive stage of life, can be damaged by chemicals the mother was exposed to weeks or years earlier.[5]

Another factor that is important with EEDs is that timing of exposure to them can be critical. In fact, timing of exposure may be more important than the dose or concentration of their exposure. A single exposure at a vulnerable moment for a developing embryo has the potential to cause damage and, of course, long term exposure to relatively small amounts of them also could cause damage.[5]

It has also been discovered that some combinations of two or three EEDs can be many times more potent than any

one of them by themselves. Thus, in addition to the timing of exposure, it appears that the combinations of EEDs that one is exposed to can cause a synergistic effect that can magnify the damage they can cause. These are all unresolved issues that will have to be worked out with time and additional research.[1]

Lastly, there is the problem of the naturally-occurring EDCs. How does the exposure of EEDs relate to exposure to naturally-occurring EDCs? How do humans and animals metabolize or neutralize the effects of each categorical source of EDCs? These effects are briefly described later.

Table 1 reflects the enormity of the first analytical challenge: deciding which environmental pollutants are EDCs. There are at least 103 suspect EEDs identified to date by various organizations; no doubt this will change. The EPA lists 60 suspect EEDs, 25 of which are targeted by the EPA NERL Endocrine Disruptor Exposure Team for multi-media environmental analysis this year.[6] The Centers for Disease Control & Prevention (CDC) in Atlanta, GA has identified 48 suspect EEDs that are of interest to that agency.[7] Finally, the World Wildlife Fund Canada (WWF) has expanded on the approximately 50 suspect EEDs listed in *Our Stolen Future*[8] and now lists 68 suspect EEDs.[4] Many of these chemicals are on all three lists, some are on only two of the three lists, and others are on only one of the three lists. In addition, some are listed by other known names or synonyms so it is very important to characterize any list of these chemicals by their (almost) unique Chemical Abstract Service (CAS) numbers. Even using CAS numbers two of the chemicals were discovered to have two different CAS numbers and "parathion" was interpreted by this author to be the ethyl ester rather than the methyl ester.

USAGE CLASSIFICATIONS OF EEDS

One of the characteristics of EEDs mentioned in the introduction is the wide variability of their chemical class characteristics. This is also reflected in a classification of their uses. Some suspected EEDs are various types of pesticides, others are common metals, and many fall into the classification of useful organic industrial chemicals. The net result provides a real challenge for developing screening methods.

There are at least nine different usage classifications of ECDs and these are listed below and also in Table 1.

1. Biocides.
2. Insecticides,
3. Herbicides,
4. Nematocides,
5. Fungicides,
6. Industrial Chemicals (e.g., solvents, plasticizers, etc.),
7. Metals
8. PCBs (i.e., Specific PCB isomers), and
9. No Commercial Use (i.e., compounds that are a degradation product or impurity of other chemicals).

EFFECTS OF EEDS

Earlier it was mentioned that the determination of which pollutants are environmental endocrine disruptors depends on their effects on the endocrine systems. However, the exact effect of hormone exposure, both natural and unnatural, is greatly dependent on factors such as species, age, and gender.

Generally, the offspring of exposed adults are the most vulnerable to these effects. Fetuses and newborns are especially susceptible to environmental contaminants. In addition to regulating sexual differentiation during fetal development, sex hormones play a role in the organization of specific areas of the brain. Less is known about this action, but studies have shown a correlation between levels of estrogen and brain morphology, as well as with sexual behavior in male rats and mice. The brain and central nervous system continue development throughout the fetal stage and early natal period making them particularly susceptible to chemical exposure.[5]

EEDs affect humans as well as wildlife. There are many documented effects on humans and even more suspected effects. These include decrease in male fertility, defects in male sexual development, increases in prostate cancer, female reproductive problems, increases in breast cancer, endometriosis, immune system damage, increased incidence of goiters, and behavioral and developmental problems in children. Some examples of these are described below.[1]

Male Fertility

The most likely effect of endocrine disruption in men may be a reduction in sperm production and also in the sperm's ability to fertilize an egg. In normal human males, the number of sperm produced per ejaculate is normally close to the level required for fertility. Thus, even a small reduction in daily sperm production can lead to infertility.[5] Sperm production by the average man in western countries, including the U.S., today is reported by some to be half of what it was in 1940. One report indicates that average sperm count has declined 42% and average volume of semen diminished by 20%.[9,5] Another report showed an increase in infertility in the last twenty years concluding that one in twenty men are either subfertile or infertile.[10,5] However, these may be an over simplification and later reports have questioned these kind of conclusions.[1]

Male Sexual Development Defects and Cancer

There appears to be an increase in sexual development defects in recent years and some of these may be linked to exposure to EEDs. For example, more baby boys have to undergo operations to correct undescended testicles ("cryptorchidism") now than 30 years ago; the rate appears to have increased 2- to 3-fold during the past 30 years. A birth defect called "hypospadias," in which the male urinary canal is open on the underside of the penis, also is increasing. "Inter-sex" features in baby boys, where the penis is covered with a layer of fat and genitals have a cleft resembling female features, also appear to be increasing. In some cases where pregnant mothers were exposed to very high levels of toxic chemicals, the mothers' boys have shorter than normal penises, similar to Lake Apopka's alligators in Florida. Boys born to women who were exposed to PCB-poisoned rice bran cooking oil in 1978-79 in central Taiwan, the so-called "Yucheng" boys, were found to have significantly shorter penis lengths at ages 11 to 14.[11,5]

Studies in some industrialized western nations show that cancer of the testicles, relatively more common in young men than older men, has increased at least 3-fold in the past 30 years. Another possible effect of exposure to estrogen-like contaminants is prostate enlargement in older men. This condition affects 80% of men 70 years and older. The exact cause of prostate enlargement, however, is often unknown. Prostate cancer in men also has increased by 80% in the last 20 years.[12,5]

Female Reproductive Effects

Women normally are exposed to estrogen, but the effects of EEDs on females are more difficult to track due to the estrous cycle and the resulting huge differences in circulating hormone concentrations at different stages of the cycle. The presence of estrogen mimicking compounds in adult women can impair reproductive capacity by interfering with natural hormone cycles, potentially rendering women unable to conceive or to maintain pregnancy.[5]

Female Breast Cancer

Breast cancer may also have links to the estrogenic contaminants.[5] Women in the US and Canada who live to age 85 have a one in nine risk of contracting breast cancer in their lifetime, double the risk in 1940.[13] Furthermore, breast cancer mortality since the 1940s has increased by 1% per year.[14] Two leading theories of the primary risk factors for breast cancer are exposure to estrogen and high fat diets.[5] It also may be possible that some chemicals are promoters or inducers of cancer rather than being direct carcinogens. This theory is supported by the findings that some EEDs have estrogenic properties, and that estrogen is known to promote abnormal cell growth. If estrogen exposure after maturation plays a role in the full expression of early developmental changes then this could provide an explanation for both the increased risk of breast cancer to women exposed to estrogens in utero and the rare cancers initiated at maturation in the women whose mothers took DES.[5]

Endometriosis

Recent animal studies strongly suggest that human exposure to dioxin may be linked to endometriosis, a painful disease currently affecting 10% of reproductive-age women. Endometriosis causes bits of uterine lining to migrate generally to other pelvic organs and can cause infertility, internal bleeding and other serious problems. The disease appears to becoming more common and afflicting women at younger ages.[5]

Immune System Damages

Associations between endocrine-disrupting pollutants and immune system damages in wildlife are well established.[15] Similar associations are being discovered in humans.[5]

Goiters

Another effect of endocrine disruption in both adult males and females may be thyroid gland enlargement, more commonly known as goiter. The thyroid gland controls growth hormones and the hormones that regulate metabolism, and enlargement of the thyroid gland can disrupt metabolism. EEDs that have been implicated in a syndrome known as the "wasting syndrome" include PCBs, dioxin, DDT, toxaphene and lead.[5]

Hyperactivity, Learning, and Attention Problems With Children

Recent studies found a dose-response relationship between the quantity of contaminated Great Lakes fish consumed by the mother and such measures in newborn infants as abnormally weak reflexes, reduced responsiveness, motor coordination and muscle tone.[16,17,5] Further studies indicated that more highly exposed children had slower reaction times to visual stimuli, made more errors on a memory test and took longer to solve problems. Hyperactivity and learning deficits are among the likely effects in children exposed in utero to endocrine-disrupting chemicals, based on many related studies. If only a small part of the learning and behavioral problems of children can be attributed to endocrine, immune, or nervous system damages caused by maternal or childhood exposure to EEDs, the implications are profound.[5]

SUMMARY

In summary, the analytical challenges are complex and depend first on determining which pollutants are to be labeled as environmental endocrine disruptors and thus studied, analyzed, and perhaps regulated. Currently, the only means of determining which pollutants are EEDs is from observing their effects on the endocrine systems of humans and other animals but that also has a separate set of complex factors that affect those decisions (this is where most research is currently focused but it is only the first stage of the process). Once chemicals are selected for analysis as EEDs then the problems of developing appropriate screening and/or individual chemical confirmatory analyses must be solved. Methods must be developed and validated for identification and quantification of EEDs at concentration levels a thousand times lower than most environmental methods can currently function. At those levels there will be more interferences and thus greater possibilities for false positive and false negative conclusions from the data. Thus, programs that incorporate appropriate QA/QC data and reliable analytical reference materials for both qualitative (identification) and quantitative analysis will be critical in order to avoid basing decisions and regulations on data of unknown or unreliable quality. EPA's Data Quality Objective (DQO) process and associated data quality assessments will be important tools to facilitate the measurement and use of reliable analytical data as environmental chemists cross a new threshold of analytical challenges. It won't be easy.

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Table 1. Comparative List of Environmental Endocrine Disruptors

Compound	Type	EPA	CDC	WWF	CAS Number
Tributyl tin chloride	B,F	x*	X		56-35-9
Triphenyl tin acetate	B	X			900-95-8
Triphenyl tin hydroxide	B	X			76-87-9
2-Acetylaminofluorene	C	X			53-96-3
Acenaphthene	C	X			83-32-9
Anthracene	C	X			120-12-7
Benz(a)anthracene	C	X			56-55-3
Benzo(a)pyrene	C	X*		X	50-32-8
Benzo(b)fluoranthene	C	X			205-99-2
Benzo(k)fluoranthene	C	X			207-08-9
Benzophenone	C			X	119-61-9
Bisphenol-A	C	X	X	X	80-05-7
n-Butyl benzene	C			X	104-51-8
Butyl benzyl phthalate	C	X*	X	X	85-68-7
Butylated hydroxyanisole (BHA)	C	X			25013-16-5
Butylated hydroxytoluene (BHT)	C	X			128-37-0
Chrysene	C	X			218-01-9
2,4-Dichlorophenol	C			X	120-83-2
Dicyclohexyl phthalate	C			X	84-61 -7
Diethyl phthalate	C			X	84-66-2
Diethylhexyl adipate	C			X	103-23-1
Diethylhexyl phthalate	C			X	117-81-7
Dihexyl phthalate	C			X	84-75-3
Di-n-butyl phthalate	C	X		X	84-74-2
Di-n-pentyl phthalate	C			X	131-18-0
Dipropyl phthalate	C			X	131-16-8
Indeno(1,2,3-cd)pyrene	C	X			193-39-5
Octachlorostyrene	C			X	29082-74-4
p-Nitrotoluene	C			X	99-99-0
p-Nonylphenol	C	X*	X		25154-52-3
PCBs	C	X*	X	X	1336-36-3
Pentachlorophenol	C	X*	X	X	87-86-5
Phenanthrene	C	X			85-01-8
Pyrene	C	X			129-00-0
Styrene	C		X		100-42-5
Tributyltin oxide	C			X	56-35-9
2,3,7,8-TCDD	C#		X	X	1746-01-6

Dimethyl mercury	C#	X			593-74-8
Benomyl	F		X	X	17804-35-2
Chlorothalonil	F	X			1897-45-6
Hexachlorobenzene	F		X	X	118-74-1
Mancozeb	F		X	X	8018-01-7
Maneb	F		X	X	12427-38-2
Metiram	F		X	X	9006-42-2
Pentachloronitrobenzene	F	X*			82-68-8
Zineb	F		X	X	12122-67-7
Ziram	F		X	X	137-30-4
Alachlor	H	X	X	X	15972-60-8
Amitrole	H		X	X	61-82-5
Atrazine	H	X*	X	X	1912-24-9
2,4-D	H	X	X	X	94-75-7
Metolachlor	H	X			51218-45-2
Metribuzin	H		X	X	21087-64-9
Nitrofen	H		X	X	1836-75-5
Simizine	H	X			122-34-9
2,4,5-T	H		X	X	93-76-5
Trifluralin	H	X	X	X	1582-09-8
Aldrin	I	X			309-00-2
Allethrin	I	X			584-79-2
alpha-BHC	I	X			319-84-6
beta-BHC	I	X	X	X	319-85-7
Carbaryl	I		X	X	63-25-2
Chlordane	I	X*	X	X	57-74-9
Chlorpyrifos	I	X*			2921-88-2
Cyhexatin	I	X			13121-70-5
Cypermethrin	I			X	52315-07-8
Dicofol	I		X	X	115-32-2
Dieldrin	I	X	X	X	60-57-1
Endosulfan	I	X*	X	X	115-29-7
Endrin	I	X*			72-20-8
Esfenvalerate	I			X	66230-04-4
Fenvalerate	I			X	51630-58-1
Heptachlor	I	X	X	X	76-44-8
Heptachlor epoxide	I		X	X	1024-57-3
Kelthane	I			X	115-32-2
Kepone	I			X	143-50-0
Lindane (gamma-BHC)	I	X*	X	X	58-89-9
Malathion	I			X	121-75-5
Methomyl	I		X	X	16752-77-5
Methoxychlor	I		X	X	72-43-5
Mirex	I		X	X	2385-85-5
Oxychlordane	I	X	X	X	27304-13-8
p,p'-DDD	I	X	X	X	72-5-8
p,p'-DDE	I	X*	X	X	72-55-9
p,p'-DDT	I	X*	X	X	50-29-3
Parathion (ethyl)	I		X	X	56-38-2
Permethrin	I	X		X	52645-53- 1
Pyrethroids (synthetic)	I		X	X	NA
Toxaphene	I		X	X	8001-35-2
trans-Nonachlor	I	X	X	X	39765-80-5
Vinclozolin	I	X		X	50471-44-8
Arsenic	M	X*			7440-38-2
Cadmium	M	X*	X	X	7440-43-9
Copper	M	X*			7440-50-8
Lead	M	X*	X	X	7439-92-1

Manganese	M	X*			7439-96-5
Mercury	M	X*	X	X	7439-97-6
Tin	M	X*			7440-31-5
1,2-Dibromo-3-chloropropane	N		X	X	96-12-8
Aldicarb	N		X	X	116-06-3
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB	X*			26601-64-9
3,3',4,4',5-Pentachlorobiphenyl	PCB	X*			25429-29-2
3,3',4,4'-Tetrachlorobiphenyl	PCB	X*			32598-13-3
Total Number in Column	103	60	48	68	

Abbreviations Used in Table 1:

EPA = NERL Endocrine Exposure Team List, October 24, 1996 from Tammy Jones
CDC = List from Larry Needham @ CDC 8/25/96
WWF = List from World Wildlife Fund Canada from the Internet 8/27/96
X = Present on this list
B = Biocide
I = Insecticide
H = Herbicide
N = Nematocide
F = Fungacide
C = Industrial Organic Chemical
M = Metal
PCB = Specific PCB isomer
* = Analytes that the EPA NERL Endocrine Disruptor Exposure Team plans to examine in a multi-media environment in 1997.
# = No Commercial Use; compound is a degradation product or impurity of other chemicals.

CDC'S ANALYTICAL APPROACH

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The Toxicology Branch of the Centers for Disease Control and Prevention (CDC) is collaborating with other Federal and international investigators to determine the relationship between human exposure to selected environmental toxicants and health effects involving the endocrine system. Several case-control studies are underway; in general, these studies involve the use of serum that was given for another purpose in the past (e.g., 1970s) by healthy adults-some of these adults have since developed certain diseases (e.g., breast cancer) while others have not. Those cases and controls are then matched (based on certain demographic criteria), and the serum from each is analyzed for the environmental toxicants, such as polychlorinated dibenzo-p-dioxins, furans, and biphenyls as well as chlorinated pesticides. The results are then analyzed to determine if the cases have significantly higher levels of these compounds than the controls. In order to perform these assessments, we have developed methods to measure these toxicants either separately or sequentially from one milliliter of human serum. These studies, the analytical methods, and some of the findings to date will be discussed. Analytical approaches for assessing human exposure to other potential endocrine disruptors, such as the phthlate esters and bisphenol-A will also be discussed.

MULTIMEDIA ANALYTICAL APPROACHES TO MONITORING AND MEASURING SUSPECT ENDOCRINE DISRUPTING COMPOUNDS

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To develop a quantitative health and environmental risk assessment of endocrine disrupting compounds (EDCs), information on exposures is essential. Since the late 1980's, considerable literature has evolved concerning EDCs and the role they may be playing in decreasing fertility of mammalian and reptilian species and in increasing the incidence of breast and other reproductive-tract cancers. Over 45 environmental contaminants or classes of agents have been reported to cause changes in reproductive and hormonal systems. The U.S. Environmental Protection Agency (EPA) needs to be able to identify these suspect agents and estimate the exposure of susceptible populations to them. A full exposure assessment has complex requirements that require preliminary information to direct further research in this area. Such research begins with determining the levels of suspect EDCs in the environment. The EDCs can be broadly classified into two categories: those that can be analyzed by conventional means (e.g., gas chromatography for organics and various elemental analyzers for inorganics), and those that are non-volatile/non-extractable/thermally labile (unconventional). Although many of the EDCs can be measured in dilute standards, few, if any, EPA methods exist for their measurement in biota or complex environmental media. Current methodologies also may not be sensitive enough to measure low levels of the EDCs in ambient environmental media. New analytical methodologies will be needed to deal with the monitoring and measurement of the EDCs in ambient multimedia environment.

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STATUS OF EPA LABORATORY METHODS FOR MEASURING ENDOCRINE DISRUPTORS

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ABSTRACT

Over the past 20 years, EPA has published hundreds of analytical laboratory procedures for measuring pollutants in air, water, and soil. Unfortunately, these procedures may not be applicable to the current need of providing baseline data for assessing the fate and transport of endocrine disruptor chemicals (EDCs) in the environment. This presentation will focus on the capability of existing EPA procedures to provide measurement data for EDCs in environmental samples. A suggested EDC analyte list will be presented listing existing EPA method performance data (sensitivity and accuracy). This table can be used to determine gaps in EPA methods.

INTRODUCTION

Within the last decade, the first generation of children whose parents were first exposed to organochlorine pesticides during fetal development have become adults and reached their reproductive stage of life. There is growing evidence that a number of synthetic chemicals may be disrupting the endocrine (hormonal) systems of mammals.[1-4] These endocrine disruptor chemicals (EDCs) may cause a variety of problems with development, behavior, and reproduction. Thus, the current reproductive human population may be at risk due to environmental activities conducted 50 years ago.

The endocrine system helps guide development, growth, reproduction, behavior and other bodily functions. It is

comprised of endocrine glands and hormones. Some of the major endocrine glands are the pituitary, thyroid, pancreas, adrenal, and the male and female gonads (testes and ovaries). Endocrine glands produce hormones and secrete them directly into the bloodstream. Hormones (estrogen, testosterone, adrelin, etc.) act as chemical messengers, traveling through the blood to distant tissues and organs, where they can bind to specific cell sites called receptors. By binding to receptors, hormones trigger various responses in the tissues containing the receptors. For example, PCBs can bind to the receptor for estrogen, yielding an estrogenic effect such as feminization.

An endocrine disruptor chemical has been defined as "an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior."[5]

A variety of chemicals, including certain pesticides, have been found to cause endocrine disruption in laboratory animal studies. Observed effects have included disruption of female and male reproductive function (such as disruption of normal sexual differentiation, ovarian function, sperm production, and pregnancy) and effects on the thyroid gland.

Some of the research indicates that effects can occur at trace levels. For example, levels of bisphenol A in the low microgram range have been shown to have an estrogenic effect in animals.[6]

At this time, conclusive evidence does not exist which can establish a causal relationship between ambient levels of EDC exposure and observed adverse effects.[5] However, all of the research indicates that much more information is needed.

Relative to environmental monitoring activities, the following questions need to be answered:

- What specific chemicals are of concern?
- What matrices need to be measured?
- How low do the measurements need to be made?
- What quality of data are needed?

These issues are discussed in more detail below.

ANALYTE LIST

Existing research indicates that many synthetic chemicals may adversely affect the endocrine system. Since studies for this type of effect have not been routinely performed, it is likely that the list of analyses of concern will substantially grow.

Table 1 provides a list of chemicals which reflects the current research. The list was compiled from the literature sources in the bibliography, including the Internet sources listed.

The table also has a column showing relative risk, as established by the Illinois EPA.[7]

MATRICES

For the past 25 years, the environmental testing industry has focused primarily on environmental media--air, water soil. This focus was due to concerns relative to determining the concentration of pollutants in the ambient environment.

With the new emphasis on EDCs, the focus will likely shift to ingestion pathways and to a better understanding of ambient levels in the exposed population. Thus, we will need to establish rugged methods for measuring EDCs at trace levels in foods and tissue.

As shown in Table 1, EPA methods have not been established for many EDC analyses. However, many of the existing methods can likely be adapted to provide reliable measurements. Other technology widely available (e.g. LC/MS) may be necessary for some analyses.

Based on the current list, there appear to be no "impossible" analyses.

DETECTION LEVELS

At this time, the levels at which measurements need to be made are not known. Current research indicates that levels at or below our current technology will be needed.

Table 1 list typical levels of measurement which are achievable using current EPA methods.

Better sample preparation techniques, better cleanup techniques, and more sensitive instrumentation may be needed.

DATA QUALITY

Given the importance of EDC measurements, I believe we need to move away from a concept of "data of known quality" to a concept which establishes a data quality goal. In my opinion, we will need methods which can provide quantitative results. One measure of this level of data quality is an accuracy of 65-135%.[8-9]

As shown in Table 1, for many EDC analyses for which EPA methods exist, the accuracy of the methods is at best semi-quantitative. The accuracy of the methods can be improved by procedural changes such as better instrument calibration, isotope dilution, etc.

SUMMARY

The concern over EDCs in the environment will require knowledge—What chemicals are present, and at what concentrations, in what species, and via what pathways?

The importance of these measurements is such that we must establish rugged, reliable methods for measuring EDCs in a variety of challenging matrices at very low levels with very good data quality.

I call on EPA and all of those who develop methods to begin to fill in the gaps in Table 1.

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http://www.epa.gov/opptintr/opptendo/index.htm
http://www.OSF-facts.com

Table 1. Capabilities of Existing EPA Methods to Determine EDCs in the Environment

EDC	IEPA Category	Methodology	Example EPA Methods(s)	Sensitivity (ppb)		Accuracy
				Water	Solids	
Alachlor	P	GC/MS	525	1	20	SQ
Aldicarb	S	LC/MS	8321	5	100	NA
Aldrin	P	GC/ECD	8080	0.05	1	SQ
Amitrole	P	NA	NA	NA	NA	NA
Atrazine	K	GC/MS	525	0.1	2	SQ
Benomyl	P	LC/MS	8321	2	40	NA
Benzophenone	-	NA	NA	NA	NA	NA
BHCs	K/P	GC/ECD	608, 8080	0.05	1	SQ
Bisphenol-A	P	NA	NA	NA	NA	NA
bis (2-Ethylhexyl) phthalate	P	GC/MS	625, 8270	10	200	SQ
n-Butyl benzene	-	GC/MS	524, 8260	5	100	SQ
Butyl benzyl phthalate	S	GC/MS	625, 8270	10	200	SQ
tert-Butylheydroxy anisole	S	NA	NA	NA	NA	NA
p-sec-Butyl phenol	S	NA	NA	NA	NA	NA
p-tert-Butyl phenol	S	NA	NA	NA	NA	NA
Cadmium	P	ICP/MS	200.8, 6020	0.02	2	Q
Carbaryl	S	GC/MS	8270	10	200	SQ
Chlordane	K	GE/ECD	608,8080	0.05	1	SQ
Cypermethrin	S	NA	NA	NA	NA	NA
2,4-D	P	GC/ECD	615, 8150	1	20	SQ
Dacthal		NA	NA	NA	NA	NA
DBCP	K	GC/MS	524, 8260	1	100	SQ
DDD	K	GC/ECD	608, 8080	0.1	2	SQ
DDE	K	GC/ECD	608, 8080	0.1	2	SQ
DDT	K	GC/ECD	608, 8080	0.1	2	SQ
~~DES*~~	K	NA	NA	NA	NA	NA
2,4-Dichlorophenol	S	GC/MS	615, 8270	10	200	SQ
Di (2-Ethylhexyl) Adipate	S	NA	NA	NA	NA	NA
Dicofol (kelthane)	K	GC/ECD	8081	NA	NA	NA
Dicyclohexyl phtholate	S	NA	NA	NA	NA	NA
Dieldrin	K	GC/ECD	608, 8080	0.1	2	SQ
Dioxins/furans	K	GC/HRMS	8290, 1613	0.001	0.2	SQ
Di-n-butyl phthalate	S	GC/MS	625, 8270	10	200	SQ
Di-n-hexyl phthalate	S	NA	NA	NA	NA	NA
Di-n-pentyl phthalate	S	NA	NA	NA	NA	NA
Di-n-propyl phthalyte	S	NA	NA	NA	NA	NA
EDB	-	GC/ECD	504	0.05	10	SQ

Endosulfans	K	GC/ECD	608, 8080	0.1	5	SQ
Endrin	P	GC/ECD	608, 8080	0.1	2	SQ
Esfenvalerate	S	NA	NA	NA	NA	NA
Ethylparathion	P	GC/NPD	614, 8140	0.1	5	SQ
Fenvalerate	S	NA	NA	NA	NA	NA
Heptachlor	P	GC/ECD	608, 8080	0.05	2	SQ
Heptachlor epoxide	P	GC/ECD	608, 8080	0.05	2	SQ
Hexachlorobenzene	P	GC/MS	625, 8270	10	200	SQ
Kepone	K	GC/MS	8270	NA	NA	NA
Lead	P	ICP/MS	200.8, 6020	0.02	2	Q
Malathion	S	GC/FPD	614, 8140	0.1	5	SQ
Mancozeb	P	NA	NA	NA	NA	NA
Maneb	P	NA	NA	NA	NA	NA
Mercury	P	CVAA	245.2, 7470	0.2	20	Q
Methomyl	S	LC/MS	8321	5	100	NA
Methoxychlor	K	GC/ECD	608, 8080	0.5	10	SQ
Methyl parathian	P	GC/FPD	614, 8140	0.1	5	SQ
Metiram	P	NA	NA	NA	NA	NA
Metribuzin	S	NA	NA	NA	NA	NA
Mirex	P	GC/ECD	8081	NA	NA	NA
Nitrofen	S	GC/ECD	8081	NA	NA	NA
4-Nitrotoluene	-	HPLC	8330	10	200	SQ
p-Nonylphenol	K	NA	NA	NA	NA	NA
Octachlorostyrene	S	NA	NA	NA	NA	NA
p-Octylphenol	P	NA	NA	NA	NA	NA
Oxychlordane	-	NA	NA	NA	NA	NA
PAHs	S	GC/MS	615, 8270	10	200	SQ
PBBs	P	NA	NA	NA	NA	NA
PCBs	K	GC/ECD	608, 8080	1	20	SQ
Pentachlorophenol	P	GC/MS	625, 8270	50	1000	SQ
p-iso-Pentyl phenol	S	NA	NA	NA	NA	NA
p-tert-Pentyl phenol	S	NA	NA	NA	NA	NA
Permethrin	S	GC/ECD	8081	NA	NA	NA
Pyrimidine carbinol	-	NA	NA	NA	NA	NA
Styrene	P	GC/MS	624, 8260	5	50	
2,4,5-T	P	GC/ECD	615, 8150	1	20	
Toxaphene	K	GC/ECD	608, 8080	5	200	
Transnonachlor	-	GC/MS	525	0.5	10	NA
Triazines	-	GC/NPD	619	0.05	1	
Tributyl tin	K	NA	NA	NA	NA	NA
Tifluralin	P	NA	NA	NA	NA	NA

Vinclozolin	P	NA	NA	NA	NA	NA
Zineb	P	NA	NA	NA	NA	NA
Ziram	S	NA	NA	NA	NA	NA

* Best documented evidence of endocrine effect in humans. Unlikely compound for monitoring due to ban on use and manufacture.

NOTES:
Sensitivity and accuracy values are rough averages of data in EPA Methods
CVAA: Cold Vapor Atomic Adsorption
GC/ECD: Gas Chromatography Electron Capture Detection
GC/PPD: Gas Chromatography Flame Photometric Detector
GC/HRMS: Gas Chromatography High Resolution Mass Spectrometry
GC/MS: Gas Chromatography Mass Spectrometry
GC/NPD: Gas Chromatography Nitrogen Phosphorous Detector
HPLC: High Pressure Liquid Chromatography
ICP/MS: Inductively Coupled Plasma Mass Spectrometry
LC/MS: Liquid Chromatography Mass Spectrometry
Q: Quantitative; accuracy is generally +/- 35%
SQ: Semiquantitative; accuracy is generally +/- 50%
NA: No routine EPA method exists for this analyte; no published performance data
IEPA Categories: K- Known; P - Probable; S - Suspect

REFERENCE MATERIALS FOR ENDOCRINE DISRUPTING COMPOUND (EDC) ANALYSIS: AN OVERVIEW

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ABSTRACT

Analytical reference materials will be a critical part of Endocrine Disrupting Compound (EDC) analysis. Aspects of research and testing for EDCs, including toxicology studies, environmental testing, screening of chemicals for endocrine disrupting effects, and in food analysis, will require accurate high quality reference materials. Neat reference materials must be of high purity and correct identity, and standard solutions must be accurate, consistent, and reliable. Sensitivity and reliability of analytical methods will be important. Isotope dilution methodology has emerged as the analytical method of choice for many environmental pollutants. This technique provides many advantages resulting in higher quality data. The availability of stable isotope-labeled reference materials will help to make this methodology a viable alternative to existing analytical methods for EDC analysis.

INTRODUCTION

There is a rising interest in environmental pollutants that affect hormonal functions in animals. Many of these chemicals appear to adversely affect the endocrine system by either mimicking hormones or by blocking their activity. The activity of these chemicals may cause a number of defects including: malformations of newborns, abnormal sperm or low sperm counts, feminization of males, masculinization of females, thyroid dysfunction, goiter, learning disabilities, and hyperactivity in children.[1] The effects appear to be strongest on the developing fetus, and the time of exposure is more critical than the concentration level.

This new class of chemicals, called endocrine disrupting chemicals (EDCs), consists of many synthetic and naturally occurring substances. Some of these materials are ubiquitous and have been known to exist in the environment for a number years. The sources of possible EDCs include: naturally occurring substances, pharmaceutical products, synthetic industrial chemicals, synthetic pesticides, and other environmental contaminants (such as banned pesticides, PCBs, and dioxins). The World Wildlife Fund (WWF), Centers for Disease Control (CDC), and the U.S. Environmental Protection Agency (EPA) have each identified compounds as

possible EDCs. A comprehensive list is shown in Table 1. The list does not include many of the hundreds of registered pesticides and thousands of industrial chemicals currently being produced.

The interest in EDCs is widespread, and the U.S. Government is funding over 400 research projects to explore their chemical properties and effects.[2] Several challenges in EDC research include development of: toxicological research programs to understand the complexities of endocrine disruptors; screening tests to identify potential EDCs; and analytical methods to measure EDCs in the environment.[3,4] Recent legislation passed by the U.S. Congress provided the EPA with a timeline for developing a screening program.[3] Toxicological studies and development of screening methods are complicated because there does not appear to be a common structural link between suspected EDCs. Because the compounds appear to exhibit their effects at very low levels, lower detection limits may be necessary in environmental testing and may present analytical challenges.

As toxicological studies, screening methods, and analytical protocols are developed, the need for reference materials will increase dramatically. Highly pure authentic compounds and accurate standard reference solutions (SRSs) will be an integral part of all aspects of EDC research. Meaningful toxicological results and development of screening tests will require using compounds with confirmed identity and free from impurities. Establishing sensitive analytical testing methods will not be possible without the availability of appropriate and accurate SRSs. A number of factors which should be considered in developing reference materials include: types (neat, SRS, or other matrix) needed; analytical specifications for each type; and applicability for intended uses.

CHARACTERISTICS OF NEAT REFERENCE MATERIALS

EDC research and testing will require high purity neat materials. In toxicological studies and screening method development, neat materials will be needed for both in vivo and in vitro assays. In environmental testing, neat materials will be needed for preparation of quantitative analytical solutions. Each area will have a variety of specifications for neat materials. For example, a material used for toxicological testing should be of very high purity (99+%) and impurities that may adversely affect a sensitive assay should be minimized. Some impurities may also exhibit endocrine disrupting properties that would lead to false conclusions about the original compound. Purity requirements for a neat material used to prepare an SRS for environmental analysis may not be as stringent since minor impurities typically do not affect analytical results. In these cases, 98% chemical purity may be acceptable. An analytical method may require a specific isotopically labeled material that would not be necessary in developing screening assays. The two common factors for all neat reference material needs are that the substance must be of known identity and purity.

Identity of a neat reference material should be unequivocally established to ensure studies are not performed on the wrong compound. A common occurrence is the misidentification of isomeric compounds. Isomers (for example, cis and trans isomers) often have very different toxicological and analytical properties, and using the incorrect isomer would yield erroneous results and conclusions.

One potential EDC is the banned pesticide chlordane. Technical chlordane is actually a mixture of a number of similar chlorinated compounds. Cis- and trans-chlordane are two major components of the technical mixture. These two materials have very similar physical properties but may have very different toxicological properties. Positive structure identification is critical in reference material production. Figure 1 illustrates very similar mass spectra of cis- and trans-chlordane and unequivocal structure determination is not possible. Detailed analysis of the ^{1}H NMR spectra (Figure 2) indicates differences in coupling constants of cis and trans hydrogens and allows for positive identification of isomers.

The chlordane example illustrates the value of performing multiple analytical methods to determine identity, and that relying only on a single method may lead to misidentification of the reference material. Common techniques used in combination to establish identity include: gas chromatography-mass spectrometry (GC-MS), fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscopy (NMR), melting point determination, refractive index determination, and gas chromatography (GC).

Determining purity is also critical to the quality of the reference material. Purity specifications must be adequate for the material's intended use as illustrated above. Some of the common impurities found in neat reference materials include: residual solvents; water; inert materials or surfactants; and reaction by-products, intermediates, and starting materials.

Simazine is a herbicide and a suspected EDC that was procured for use as a neat reference material. Purity analysis by GC with a flame ionization detector (FID) indicated a purity of 99%. A second purity assay by thin layer chromatography (TLC) indicated a spot with an R_f of 0.76 and an additional spot at the baseline using neutral alumina and a chloroform mobile phase. Additionally, elemental analysis for carbon and hydrogen did not agree with theoretical values (C, theoretical-41.69%, found-38.65%; H, theoretical-6.00%, found-5.42%). By chromatographic purification, it was determined that the simazine contained greater than 10% of an impurity. In this case using only gas chromatographic methods for purity determination would have resulted in an incorrect purity assay. The impurity may have contributed to a false conclusion in a toxicological study.

Table 2 contains four representative compounds that are suspected EDCs. These materials were procured for use as reference materials. Each compound was analyzed for chemical purity by multiple methods including GC-FID and elemental analysis (EA). Carbaryl is an example of a material that required no additional purification and purity data was consistent by all methods. Nitrofen is an example where GC data indicated impurities, but EA data was acceptable. In this case further purification was required. Using only EA would have resulted in an inaccurate purity assignment. Parathion and vinclozolin are examples where GC data was acceptable but EA data was not. The two materials contained residual solvent and/or water and further drying was necessary. Gravimetric preparation using impure neat materials would have resulted in solution concentrations with a low bias.

As with identity, the above examples illustrate that multiple methods must be used for purity assays. Some of the common techniques used for purity determination include: GC-FID, high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC), EA, TLC, and melting point or refractive index determinations. Recent publications include more detailed discussions of neat reference materials and their properties.[5,6]

CHARACTERISTICS OF STANDARD REFERENCE SOLUTIONS (SRSs)

Standard reference solutions of EDCs will primarily be used for applications in analytical method development and environmental testing and quantitation. Traditionally, SRSs have been required for: calibration of instrumentation; calibration checks; and internal, surrogate, matrix spiking, and quality control (QC) standards. The USEPA has published methods including the SW-846 series that describe in detail the various types and uses of standards. Many of these standards are commercially available.

Accuracy, traceability, consistency, homogeneity, and stability should always be considered when preparing and verifying an SRS. Accuracy is the nearness of a solution's true concentration to its nominal or theoretical concentration. Traceability is the documented unbroken chain of comparisons back to a recognized national or international, or an *ab initio* source. Consistency is a measure of batch-to-batch variability. Homogeneity is a measure of variability within a batch. Stability refers to the variability of analyte concentration in a solution over time.

Obtaining an accurate SRS begins with verification of the neat material as described above. Analytes should be weighed using balances calibrated with NIST traceable weights to provide gravimetric verification. Dilutions should be performed using Class A glassware at a minimum. Further verification of accuracy should include comparison to independently prepared calibration solutions, or at minimum to a second independently prepared solution.

Consistency and homogeneity of an SRS can be verified by comparison studies. Comparing each new batch of standard to the previous batch will demonstrate consistency. When a batch of standard solution is packaged into multiple containers, homogeneity should be verified. This can be done by removing random containers from the batch and analyzing for concentration differences.

Stability issues should be considered to help determine a valid shelf life of the SRS. Some analyses may decompose over time or react with the solvent, moisture, or oxygen. On-going stability studies will help identify these problems. Testing can be achieved by storing archive samples over time and then comparing to a new solution. Accelerated stability studies are often performed by placing a test standard at elevated temperatures and comparing to a new solution. Many suspected EDCs such as PCBs and polycyclic aromatic hydrocarbons (PAHs) should be quite stable in solution. Others (e.g., pesticides) may have stability problems in solution. A more detailed discussion of standard solution verification has recently been published.[7]

REFERENCE MATERIALS AND ANALYTICAL METHOD DEVELOPMENT

Establishing specifications for SRSs used in EDC analysis will be dependent on the analytical method. For example, a gas chromatography/electron capture detector (GC-ECD) method will require a different set of calibration standards than a GC-MS method. While there are a number of existing analytical methods for many analyses currently listed as potential EDCs, there will be a challenge to develop protocols for additional analyses and to meet the requirements for lower detection limits. Historically, analytical method development has been somewhat dependent on the development of analytical equipment and the availability of reference materials. This is exemplified by Clement's discussion of method development for trace analysis of chlorinated dibenzo-p-dioxins and dibenzofurans[8] and the development of EPA method 1613 discussed below.

Requirements for low detection limits in EDC analysis will lead to new method development. One technique that has proven to be very effective in environmental analysis is stable isotope dilution mass spectrometry.[8,9] Theoretical aspects of this methodology are discussed in detail by Pickup and McPherson.[10] The basis of the technique is that stable isotope-labeled compounds are used as internal standards in quantitation of analyses.

Internal standards are commonly used in analytical testing. A brief description of the method follows. Calibration solutions are prepared containing known amounts of the analyses of interest. A known amount of internal standard is added and a response factor is calculated based on the response of the internal standard and the analyte of interest. Samples are then "spiked" with a known amount of internal standard and analyses of interest can be quantitated using the response of the unknown and the response factor for that analyte. The challenge of the technique is to identify appropriate internal standards that have similar properties to the analyses of interest and do not interfere with the analysis.

In isotope dilution mass spectrometry the internal standard is a stable isotope-labeled analog of the analyte of interest. The internal standard and the analyte of interest differ only in their molecular mass. Stable isotopes such as ^{2}H, ^{13}C, and ^{15}N are commonly used. This technique addresses many of the challenges of selecting an internal standard since the two compounds will exhibit very similar properties. It also takes advantage of mass spectrometry which has seen many advances in the last few years. Using high resolution mass spectrometry (HRMS) in the selective ion monitoring (SIM) mode provides a very sensitive technique. A disadvantage to using isotope-dilution methods is that a labeled analog is needed for each analyte of interest, whereas only representative compounds are needed for traditional internal standard techniques.
This disadvantage has hindered the development of isotope-dilution method development. A good example of this is in the development of EPA Method 1613 for analysis of dioxins and furans. Figure 3 represents a timeline in the development of this method. Isotope dilution was discussed in detail in the mid 1970s.[10] Gas chromatographic separations were greatly improved by the development of capillary column technology in the early 1980s. Even though the analytical tools were in place, Method 1613 was not developed for another decade. The method could only be established after ^{13}C-labeled analogs of all 17 chlorinated dioxin and furan toxic congeners were made available in the late 1980s.

Isotope dilution mass spectrometry is ideally suited for environmental analysis of EDCs because of its sensitivity and past use in trace analysis. It may be possible to adapt existing methods to many EDCs. While obtaining isotope-labeled analogs for all potential EDCs may be a challenge, many labeled analogs of compounds in Table 1 are currently available and are listed in Table 3. As additional EDCs are identified, labeled analogs will most certainly be developed to meet the demands for method development and testing.

CONCLUSION

The adverse effects of some chemicals on endocrine systems of wildlife and humans appears to be real, and research and development for toxicology, screening methods and environmental testing are rapidly growing. The availability of reference materials will greatly assist these studies. Appropriate reference materials must be available for each area of research and testing.

Neat reference materials will be needed for all aspects of EDCs research and testing. They must be fully characterized, and data for identity and purity should accompany each material. Multiple complimentary analytical methods should be used to establish purity and identity. Relying on a single method may lead to errors and bias results.

Standard reference solutions will be used extensively in environmental testing. Preparing and verifying SRSs should include components for accuracy, traceability, consistency, homogeneity, and stability.

Developing analytical methods for EDC testing will be dependent on the availability of reference materials. To gain the necessary sensitivity, methods such as isotope dilution mass spectrometry should be employed. This will be possible because current methods
may be adapted and isotope-labeled reference materials are currently available for use in developing new methods.

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Table 1. Possible Endocrine Disrupting Chemicals (EDCs)

Compound	CAS No	Compound	CAS No
Acenaphthene	83-32-9	Endrin	72-20-8
2-Acetylaminofluorene	53-96-3	Esfenvalerate	66230-04-4
Alachlor	15972-60-8	Fenvalerate	51630-58-1
Aldicarb	116-06-3	Heptachlor	76-44-8
Aldrin	309-00-2	Heptachlor epoxide	1024-57-3
Allethrin	584-79-2	Hexachlorobenze	118-74-1
Amitrole	61-82-5	3,3',4,4',5,5'-Hexachlorobiphenyl	26601-64-9
Anthracene	120-12-7	Indeno(1,2,3-c,d)pyrene	193-39-5
Arsenic	7440-38-2	Kelthane	115-32-2
Atrazine	1912-24-9	Kepone	143-50-0
Benomyl	17804-35-2	Lead	7439-92-1
Benz(a)anthracene	56-55-3	Lindane (gamma-BHC)	58-89-9
Benzo(a)pyrene	50-32-8	Malathion	121-75-5
Benzo(b)fluoranthene	205-99-2	Mancozeb	8018-07-7
Benzo(k)fluoranthene	207-08-9	Maneb	12427-38-2
Benzophenone	119-61-9	Manganese	7439-96-5
alpha-BHC	319-84-6	Mercury	7439-97-6
beta-BHC	319-85-7	Methomyl	16752-77-5
Bisphenol-A	80-05-7	Methoxychlor	72-43-5
Butylated hydroxyanisole (BHA)	25013-16-5	Metiram	9006-42-2
Butylate hydroxytoluene (BHT)	128-37-0	Metolachlor	51218-45-2
n-Butylbenzene	104-51-8	Metribuzin	21087-64-9

Butyl benzyl phthalate	85-68-7	Mirex	2385-85-5
Cadmium	7440-43-9	Nitrofen	1836-75-5
Carbaryl	63-25-2	4-Nitrotoluene	99-99-0
Chlordane (tech)	57-74-9	trans-Nonachlor	39765-80-5
Chlorothanlonil	1897-45-6	p-Nonylphenol	25154-52-3
Chlorpyrifos	2921-88-2	Octachlorostyrene	29082-74-4
Chrysene	218-01-9	Oxychlordane	27304-13-8
Copper	7440-50-8	Parathion (ethyl)	56-38-2
Cyhexatin	13121-70-5	3,3'4,4',5-Pentachlorobiphenyl	25429-29-2
Cypermethrin	52315-07-8	Pentachloronitrobeneze	82-68-8
2,4-D	94-75-7	Pentachlorophenol	87-86-5
p,p'-DDD	72-5-8	Permethrin	52645-53-1
p,p'-DDE	72-55-9	Phenanthrene	85-01-8
p.p'-DDT	50-29-3	Pyrene	129-00-0
1,2-Dibromo-3-chlorpropane	96-12-8	Simazine	122-34-9
2,4-Dichlorophenol	120-83-2	Styrene	100-42-5
Dicofol	115-32-2	2,4,5-T	93-76-5
Dicyclohexyl phthalate	84-61-7	2,3,7,8-TCDD	1746-01-6
Compound	**CAS No**	**Compound**	**CAS No**
Dieldrin	60-57-1	3,3'4,4'-Tetrachlorobiphenyl	32598-13-3
Diethyl phthalate	84-66-2	Toxaphene	8001-35-2
Diethylhexyl adipate	103-23-1	Tin	7440-31-5
Diethylhexyl phthalate	117-81-7	Tributyltin chloride	56-35-9
Dihexyl phthalate	84-75-3	Trifluralin	1582-09-8
Dimethyl mercury	593-74-8	Triphenyl tin acetate	900-95-8
Di-n-butyl phthalate	84-74-2	Triphenyl tin hydroxide	76-87-9
Di-n-pentyl phthalate	131-18-0	Vinclozalin	50471-44-8
Dipropyl phthalate	131-16-8	Zineb	12122-67-7
Endosulfan	115-29-7	Ziram	137-30-4

Table 2. Purity Assays by GC-FID and Elemental Analysis of Some Suspected EDCs

	GC-FID (% Purity)		Theoretical		Found A[1]		Found B[2]	
Compound	A[1]	B[2]	% C	% H	% C	% H	% C	% H
Carbaryl	99.9	---	71.63	5.51	71.66	5.47	---	---
Nitrofen	95.1	99.1	50.73	2.48	50.61	2.53	50.77	2.53
Parathion	99.7	99.8	41.24	4.84	41.75	4.98	41.31	4.91
Vinclozolin	99.7	99.6	50.38	3.17	49.40	3.01	50.39	3.12

[1]Analysis before purification
[2]Analysis after purification

Table 3. Available Stable-Isotope Labeled Compounds for EDC Analysis

Acenaphthene-$^{13}C_6$	Di-n-butyl phthalate-D_4
Alachlor-$^{13}C_6$	2,4-Dichlorophenol-$^{13}C_6$
Aldrin-$^{13}C_4$	Dieldrin-$^{13}C_4$
Anthracene-$^{13}C_6$	Diethyl phthalate-D_4
Atrazine-$^{13}C_3$	Endosulfan I-D_4 (α isomer)
Benz(a)anthracene-$^{13}C_6$	Heptachlor-$^{13}C_4$
Benzo(a)pyrene-$^{13}C_4$	Heptachlor epoxide-$^{13}C_1$
Benzo(b)fluoranthene-$^{13}C_6$	Hexachlorobenzene-$^{13}C_6$
Benzo(k)fluoranthene-$^{13}C_6$	3,3',4,4',5,5'-Hexachlorobiphenyl-$^{13}C_{12}$
Benzophenone-D_{10}	Indeno(1,2,3-c,d)pyrene-$^{13}C_6$
β-BHC-$^{13}C_6$	Kepone®-$^{13}C_8$
($\pm$)-α-BHC-$^{13}C_6$	Mirex-$^{13}C_8$
γ-BHC-$^{13}C_6$ [Lindane-$^{13}C_6$]	4-Nitrotoluene-$^{13}C_6$
Bis(2-ethylhexyl) phthalate-D_4	Parathion-D_{10}
Bisphenol-A-$^{13}C_{12}$	3,3',4,4',5-Pentachlorobiphenyl-$^{13}C_{12}$
Butyl benzyl phthalate-D_4	Pentachloronitrobenzene-$^{13}C_6$
Chlordane-$^{13}C_1$ (random)	Pentachlorophenol-$^{13}C_6$
Chrysene-$^{13}C_6$	Phenanthrene-$^{13}C_6$
2,4-D-$^{13}C_6$	Pyrene-$^{13}C_3$
p,p'-DDD-D_8	Styrene-D_8
p,p'-DDE-$^{13}C_{12}$	3,3',4,4'-Tetrachlorobiphenyl-$^{13}C_{12}$
p,p'-DDT-$^{13}C_{12}$	

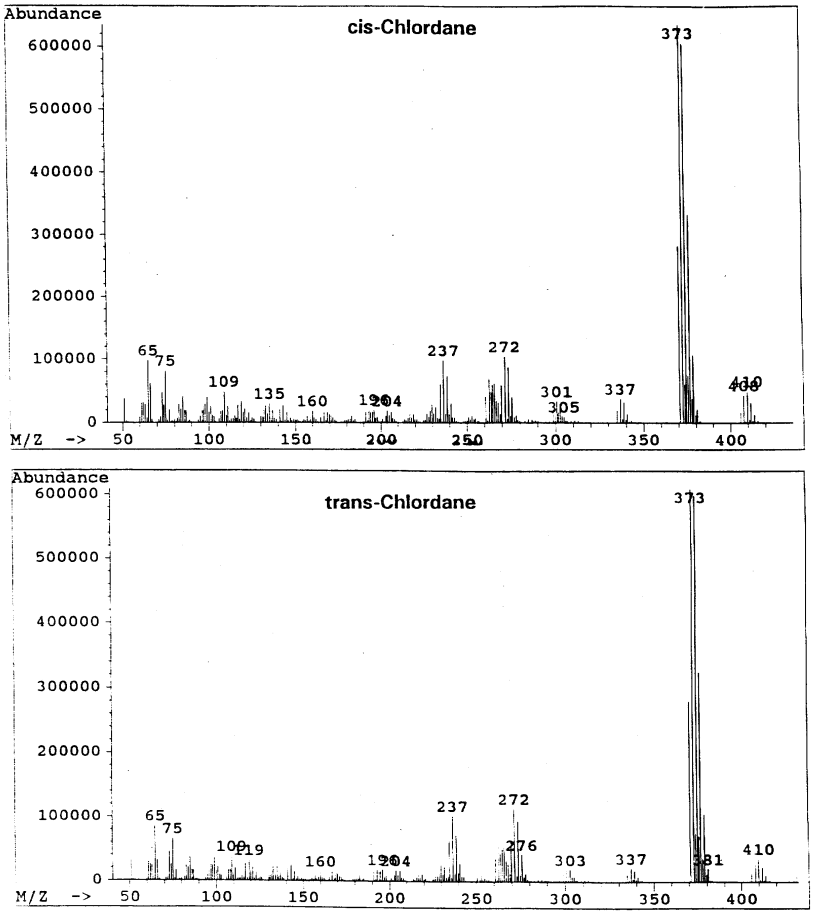

Figure 1. Mass Spectra of cis- and trans-Chlordane

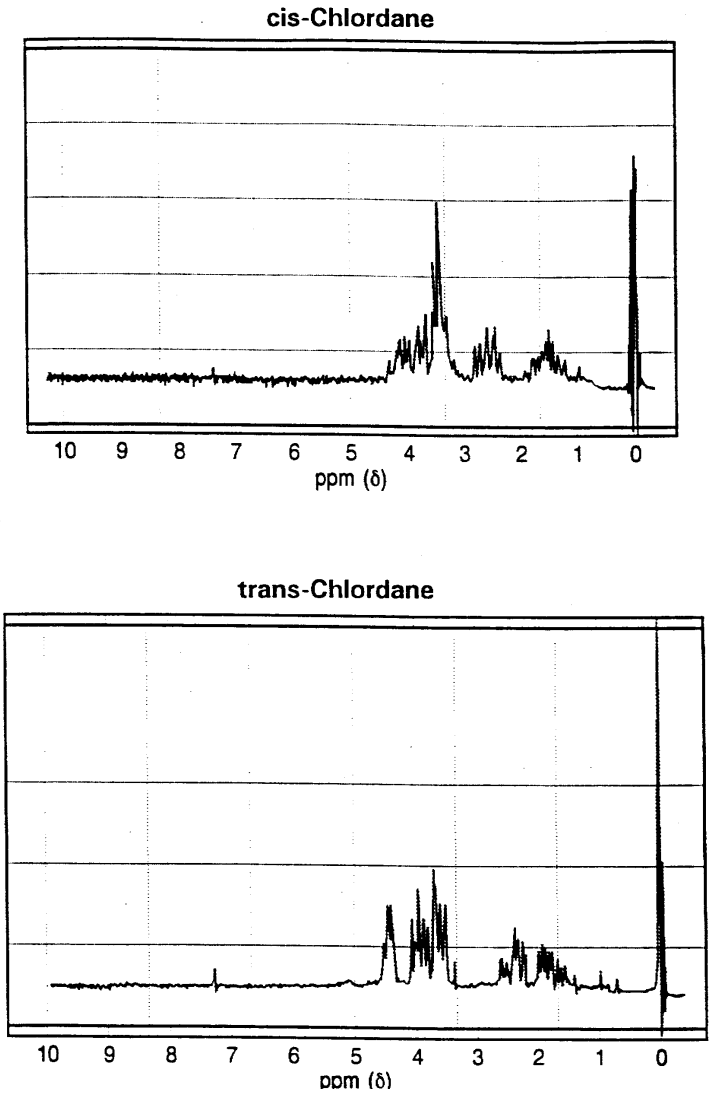

Figure 2. 60 mHz ^{1}H NMR of cis-and trans-Chlordane

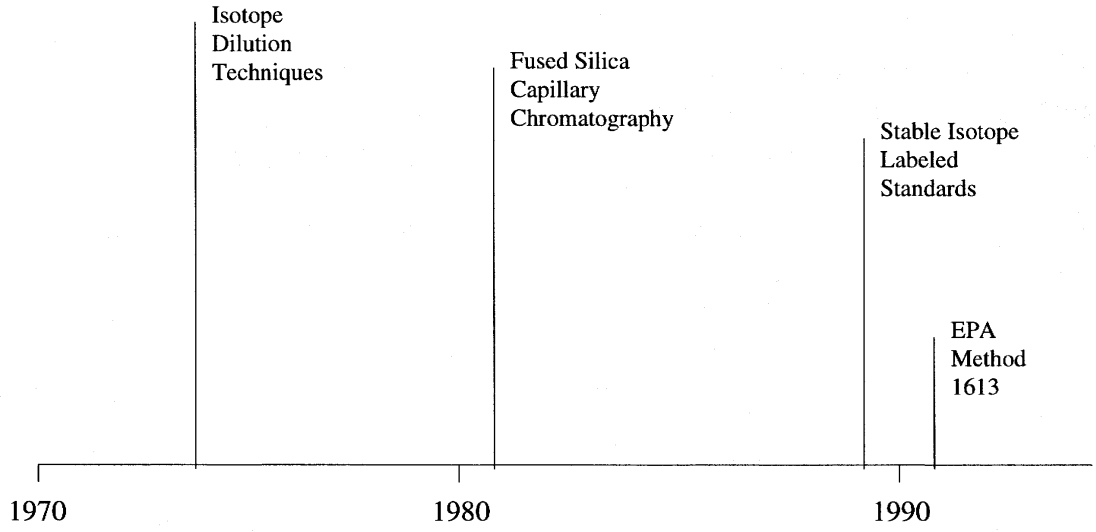

Figure 3. Timeline for Development of Isotope Dilution Techniques for Trace Dioxin Analysis

DEVELOPING A METHOD USED TO SCREEN FOR MORE THAN 400 PESTICIDES AND ENDOCRINE DISRUPTERS

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ABSTRACT

A gas chromatographic (GC) method has been developed that can be used to screen for more than 400 pesticides and suspected endocrine disrupters. In principle, it can be used to screen for any GC-amenable pesticide, metabolite, or endocrine disrupter. The method relies on a new technique called retention time locking, a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with same stationary phase, nominal phase ratio and diameter are used. Because retention time locking increases retention time precision and predictability, raw retention times can be used as a more reliable indicator of compound identity. The chromatographer first locks the GC method so that all retention times match those listed in a 408-compound pesticide retention time database. Using GC/AED or other element-selective detectors, the analyst enters a peak's retention time and known elemental content (presence or absence of heteroatoms) into a dialog box. Software then searches the pesticide database for those compounds that elute at the correct retention time and have the right elemental content. The software usually finds from one to five compounds that meet these criteria. Confirmation is performed by GC/MS or by calculation of elemental ratios using GC/AED data. With retention time locking, pesticides have the same retention time on all GC systems; this makes GC/MS confirmation much easier because the analyte' s retention time is already known.

INTRODUCTION

More than 700 pesticides are currently registered for use in the world[1] and many more continue to persist in the environment, even after being banned. For the protection of human health and the environment, acceptable limits in food and water have been set by governmental bureaus such as the United States Environmental Protection Agency (USEPA) and the Codex Alimentarius Commission[2]. Numerous methods have been developed to screen for pesticide contamination in food[3-7] and the environment[8-10] to ensure that these standards are met.

Certain pesticides and other synthetic chemicals have been suspected of behaving as pseudo hormones, disrupting normal functions of the endocrine system in wildlife and humans. Maladies such as birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence have been blamed on exposure to endocrine disrupters[11]. The 1996 publication of *Our Stolen Future,* a book by Colborn, Dumanoski, and Myers[11], brought these concerns to the attention of the public. Recently-passed legislation in the US calls for more testing of suspected endocrine disrupters and monitoring of them in food[12] and water[13] supplies. In order to facilitate more research into the endocrine disrupter issue, methods are needed to detect suspected compounds at trace levels.

Because so many pesticides are in use, it is usually impractical to screen for large numbers of them individually and, therefore, multiresidue methods are preferred. Most laboratories that analyze for pesticides in foods, screen for only a few dozen compounds because it is often very difficult to screen for more. Recently however, methods have been developed using gas chromatography with mass spectral detection (GC/MS), that can screen for more than 200 [4] or even 300 [5] pesticide residues.

Still, there is no universal method to analyze for all GC-amenable pesticides. While GC/MS methods are gaining in popularity, there are still some limitations. When methods employ selected ion monitoring (SIM) or tandem mass spectrometry (MS/MS), method development is more tedious and any shift in GC retention times requires that individual analyte retention time windows be shifted accordingly. These methods are only capable of detecting compounds on the target list, there are still hundreds of pesticides, metabolites, and suspected endocrine disrupters that could be missed. On the other hand, methods based on scanning GC/MS alone require more sample cleanup to avoid interferences from coextracted indigenous compounds. Typically, these methods do not screen for many pesticide metabolites, endocrine disrupters, or other environmental contaminants. A method that could be used to screen for endocrine disrupters and almost all of the volatile pesticides and metabolites would offer a better means of monitoring the food supply and the environment.

This paper describes a universal method that, in principle, could be used to screen for any pesticide, metabolite, or endocrine disrupter that can be eluted from a gas chromatograph. As a first test of the concept, a method was developed to screen for 408 pesticides. The method is being expanded to include virtually all of the volatile pesticides, metabolites, and suspected endocrine disrupters. The screening procedure relies on a new gas chromatographic technique called "retention time locking"[14] with database searching based on retention time and elemental content. This technique is used to narrow an analyte's identity to a few possibilities. Confirmation is performed by GC/MS or by calculation of a compound's elemental ratio using GC with atomic emission detection (GC/AED).

EXPERIMENTAL

Samples Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee) and from the Canadian Pest Management Regulatory Agency, Laboratory Services Subdivision (Ottawa). Samples from Florida were extracted using the Luke procedure[15-17] while those from Canada were prepared according to the method described by Fillion, et al.[5]

Instrumentation. Table 1 lists the instrumentation and chromatographic conditions used for GC/AED screening and GC/MS confirmation.

Table 1. Instrumentation and conditions of analysis.

GC/AED System	
Gas chromatograph	HP 6890
Automatic sampler	HP 6890 Series Automatic Sampler
Atomic Emission detector	HP G2350A Atomic Emission Detector
Computer for data acquisition & analysis	HP Vectra XU Series 4 5/150
Software	HP G2360AA GC/AED Software running on MS Windows 3.11
Column	30 m X 0.25 mm X 0.25 µm HP-5MS
GC Inlet	Split/splitless, 250°C or 280°C
Injection volumes	2 µL splitless or 5 µL pulsed splitless
Inlet pressure (splitless)[a]	27.6 psi, constant pressure for 2-µL injections
Inlet pressure program (pulsed splitless)[a]	60 psi (2.01 min), 10 psi/min to 27.9 psi (hold)
Oven temperature program	50°C (1.13 min), 30°C/min to 150°C (2 min), 3°C/min to 205°C (0 min), 10°C/min to 250°C (20 min)
AED transfer line temperature	260°C
AED cavity temperature	260°C
AED elements & wavelengths (nm)	Group 1: C 496, Cl 479, Br 478 Group 2: C 193, S 181, N 174 Group 3: P 178
GC/MS System	
Gas chromatograph	HP 6890
Automatic sampler	HP 6890 Series Automatic Sampler
Mass spectral detector	HP 5973 MSD
Computer for data acquisition & analysis	HP Vectra XU 6/200
Software	HP G1701AA Version A.03.00 running on MS Windows 95
Column	30 m X 0.25 mm X 0.25 µm HP-5MS
Inlet	Split/splitless, 250°C
Injection volume	2 µL
Inlet pressure[a]	14 psi (constant pressure)

Oven temperature program	Same as GC/AED
MSD Parameters	
Acquisition mode	Scan (35-550 amu)
Temperatures	Transfer line = 280°C, MS quad = 150°C, MS source = 230°C

a) The column head pressures shown are typical values. Exact values were determined as part of the retention time locking procedure.

Software for Method Translation Software for use in translating the GC method from one GC column to another column (same phase but different dimensions) was obtained from Hewlett-Packard Co. (Wilmington, DE); the software is available on the world wide web at the following address: http://www.dmo.hp.com/apg/servsup/usersoft/main.html

RESULTS AND DISCUSSION

Retention Time Locking Key to the development of this method is a new concept in gas chromatography called retention time locking. This is a procedure that allows the chromatographer to match analyte retention times from run to run, independent of the GC system, detector, or manufacturing variations in column dimensions; the only requirement is that the columns used have the same stationary phase and the same nominal diameter and phase ratio. For example, with retention time locking, it is possible to match analyte retention times on a GC/AED and a GC/MS even though the column outlet pressures are much different: 1.5 psi above ambient pressure for the AED and vacuum for the MSD. The procedure also compensates for differences in GC column length resulting from variations in manufacturing or from column cutting required during routine maintenance.

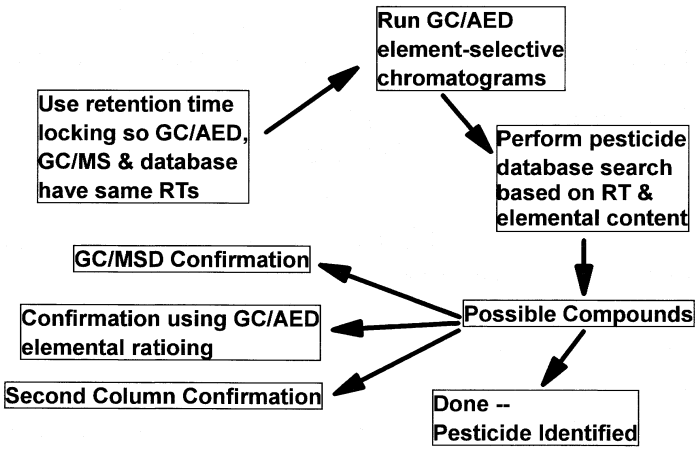
Retention time locking is accomplished by adjusting the GC column head pressure until a given analyte, such as an internal standard, has the required retention time. When this is done, all other analyses in the chromatogram will have the correct retention times as well. Software has been developed that can be used to determine the column head pressure that will correctly lock the retention times after a single "scouting" run.

With retention time locking, it is possible to measure pesticide retention times using a given GC method and then reproduce those retention times in subsequent runs on the same or different instruments. With this increased retention time precision and predictability, raw retention times become a far more useful indicator of analyte identity. For many years relative retention times[3,6] or retention indices[18-19] have been used to identify compounds; these techniques were developed to compensate for the fact that retention times were not generally predictable from day to day, column to column, or instrument to instrument. With modern instrumentation and retention time locking, it seemed that raw retention times could be used for compound identification in much the same way that retention indices have been used in the past, albeit with much less effort. The chromatographer could simply scan a table of pesticide retention times, eliminating all possibilities but those with close elusion times under the same locked GC conditions.

Pesticides almost always contain heteroatoms and often have several in a single molecule; the most frequently encountered heteroatoms are O, P, S, N, Cl, Br, and F. GC/AED has been shown to be a useful tool for pesticide screening because it is selective for all of the elements found in these compounds[20-22]. Thus, GC/AED screening provides valuable information about the elemental content of an unknown molecule. By including this elemental information along with the retention time, it should be possible to narrow pesticide "hits" to just a few possibilities.

Pesticide Retention Time Table To test this pesticide screening concept, a table of pesticide retention times and molecular formulas was required. Stan and Linkerhagner[7] recently published a list of 408 pesticides with their molecular formulas and their GC/AED retention times using a 25 m X 0.32 mm X 0.17 µm HP-5 column. While their retention time table and GC method could have been used as published, their column was not an ideal choice for GC/MS. Therefore, the GC method and all of the pesticide retention times were translated to a 30 m X 0.25 mm X 0.25 µm HP-5MS column which could be used for both GC/AED screening and GC/MS confirmation. These conversions were made using software for GC method translation developed by Blumberg[23-24]. Further adjustments to the 408 retention times were made by curve fitting the actual retention times for 60 known compounds and applying the correction to the table.

Pesticide Screening Method Figure 1 diagrams the pesticide screening method. First, retention time locking was



used to match GC/AED and GC/MS analyte retention times to those listed in the translated pesticide database. Prototype software for retention time locking was used to determine the column head pressure needed to produce a retention time of 25.216 min for p,p'-DDE. Using the GC/AED, element selective chromatograms were obtained for C, Cl, Br, N, S, P, and sometimes F and I. Prototype software was then used to search the database by retention time and elemental content.

Figure 1. Diagram of the pesticide screening method that uses retention time locking and retention time database searching

Figure 2 is a screen capture from this software showing the dialog box used to input the search criteria. One must choose a search time window wide enough to be sure to include the correct analyte, but narrow enough to eliminate as many extraneous "hits" as possible. A value of 0.8 min was chosen because tests with several dozen compounds showed that, under locked condi- tions, pesticide retention times always fell within +/-0.3 min of the tabulated value. This time window would be smaller if one were to use a database generated on the same column under locked conditions. Of course, a narrower time window would generate fewer hits and a more accurate screening method.

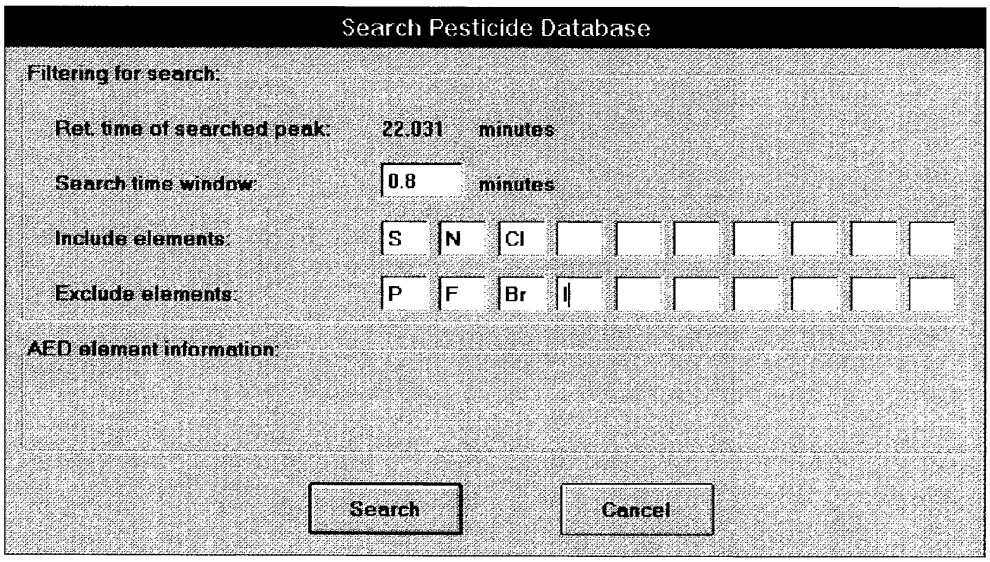


Figure 2. Dialog box used in the pesticide database searching software.

From the GC/AED chromatograms it is usually possible to determine which heteroatoms are present or absent in the suspected pesticide peak. All available information is added to the dialog box and this is used to focus the search on those pesticides that fall within the retention time window and have the specified elemental content. The search produces a list of pesticides that meet these criteria.

Confirmation is usually done by GC/MS under locked conditions so that all GC/MS retention times match the GC/AED values. Alternatively, when there is adequate signal to quantitate the analyte in multiple AED element-selective chromatograms, it is often possible to confirm a pesticide's identity simply by calculating its heteroatom ratio. GC/AED software for element ratioing facilitates this procedure. Although not yet tried, it should also be possible to use a second column with a different phase for confirmation. This would require developing a new retention time database on the confirmation column, but that is not yet available. Figure 3 shows a set of GC/AED element-selective chromatograms obtained for a strawberry extract. Peaks in the S, N, P, and Cl chromatograms suggest the presence of several pesticides. The peak at 22.031 min contains S, N, and Cl but does not appear to have any P, F, Br, or I. When the database was searched only on the basis of the peak's retention time using a 0.8 min window, 20 possibilities were reported (Figure 4). However, when the elemental information was included in the search, only three of the 408 pesticides in the database met the criteria (Figure 5).

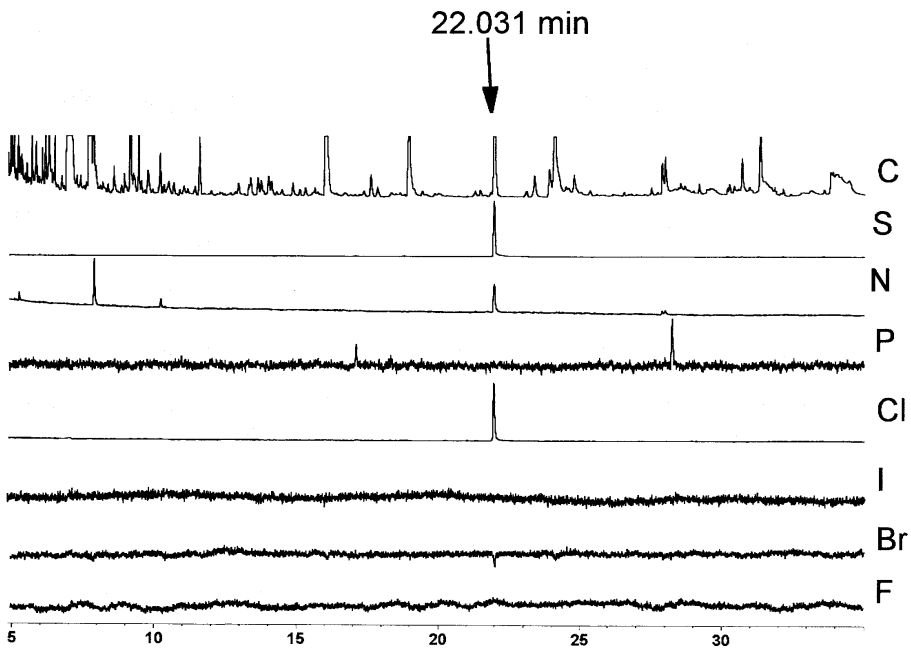


Figure 3. GC/AED element-selective chromatograms obtained for a strawberry extract. The GC/AED method was locked to the pesticide database.

Pesticide Search Results

#	Ret_Time	Mol_Formula	Compound_Name	Mol_Weight
1	21.76	C:15,H:23,N:3,O:4,	Isopropalin	309.37
2	21.79	C:14,H:16,Cl:1,N:5,O:5,S:1,	Triasulfuron	401.82
3	21.8	C:10,H:5,Cl:7,O:1,	Heptachlorepoxide-cis	389.32
4	21.81	C:14,H:16,Cl:1,N:3,O:1,	Metazachlor	277.75
5	21.84	C:19,H:26,O:3,	Allethrin	302.41
6	21.9	C:9,H:10,Br:1,Cl:1,N:2,O:2,	Chlorbromuron	293.55
7	21.9	C:10,H:5,Cl:7,O:1,	Heptachlorepoxide-trans	389.32
8	21.93	C:11,H:10,Cl:1,N:1,O:3,S:1,	Benazolin-ethyl	271.72
9	21.96	C:13,H:19,N:3,O:4,	Pendimethalin	281.31
10	21.99	C:12,H:14,N:4,O:4,S:2,	Thiophanate-methyl	342.39
11	22.06	C:13,H:15,Cl:2,N:3,	Penconazole	284.19
12	22.06	C:14,H:18,N:4,O:4,S:2,	Thiophanate-ethyl	370.44
13	22.08	C:11,H:21,N:5,S:1,	Dimethametryn	255.38
14	22.1	C:12,H:27,P:1,S:3,	Merphos I	298.5
15	22.11	C:9,H:5,Cl:3,N:4,	Anilazine	275.52
16	22.15	C:14,H:12,Cl:2,O:1,	Chlorfenethol	267.15
17	22.18	C:11,H:13,F:3,N:2,O:3,S:1,	Mefluidide	310.29
18	22.18	C:10,H:13,Cl:2,F:1,N:2,O:2,S:2,	Tolylfluanid	347.25
19	22.23	C:9,H:8,Cl:3,N:1,O:2,S:1,	Captan	300.59
20	22.36	C:13,H:11,Cl:2,N:1,O:5,	Chlozolinate	332.14

Figure 4. Database search results for the peak at 22.031 min (Figure 3). The database search used only retention time; no elemental information was entered into the search dialog box.

Pesticide Search Results

#	Ret_Time	Mol_Formula	Compound_Name	Mol_Weight
1	21.79	C:14,H:16,Cl:1,N:5,O:5,S:1,	Triasulfuron	401.82
2	21.93	C:11,H:10,Cl:1,N:1,O:3,S:1,	Benazolin-ethyl	271.72
3	22.23	C:9,H:8,Cl:3,N:1,O:2,S:1,	Captan	300.59

Figure 5. Database search results for the peak in the strawberry extract found at 22.031 min (Figure 3) when both retention time and elemental content were entered into the dialog box as shown in Figure 2

Confirmation was first done by calculating the Cl:N:S ratio in the molecule using the GC/AED elemental ratioing software. Using chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) as the element-specific calibration standard, the Cl:N:S ratio in the unknown peak was calculated to be 3.07:0.95:1.00. Though approximate, this heteroatom ratio is only consistent with captan (Figure 5).

The same sample was then analyzed by GC/MS under locked conditions so that all suspect pesticides would have GC/MS retention times very close to their GC/AED values. Captan was found at 21.979 min, just 0.052 min away from its GC/AED retention time and close to the database value of 22.23 min (Figure 6). With retention time locking, it was possible to make the GC/AED, GC/MS, and database retention times all agree to within 0.25 min. This made it much easier to find the suspected pesticide in the total ion current chromatogram (TIC), because the compound's retention time was already known. Moreover, since the possibilities had been narrowed to just three compounds, their characteristic ions could be extracted to reduce the background contribution from coextracted indigenous materials.

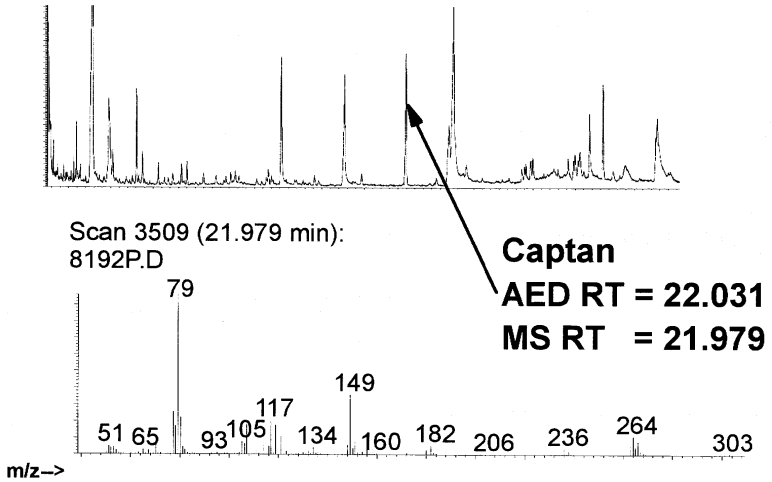


Figure 6. Scanning GC/MS analysis of the strawberry extract shown in Figure 3. The GC/MS method was locked so that retention times would match both the pesticide database and the GC/AED. Captan was identified at 21.979 min. Its retention times in the GC/AED and pesticide database were 22.031 min and 22.23 min, respectively.

Figure 7 shows S-, N-, P-, and Cl-selective chromatograms of an orange extract. When the database was searched for the chlorine-containing compound labeled 1, two possibilities were listed - aldrin and PCB-152 (Table 2). However GC/MS could not confirm either of these possibilities nor were any other compounds suggested. Several explanations are possible for this discrepancy: a) the compound is not a pesticide or PCB, b) the compound is a pesticide or metabolite that does not appear in either the retention time or mass spectral databases. or c) one or both of the suggested compounds is not contained in the mass spectral libraries.

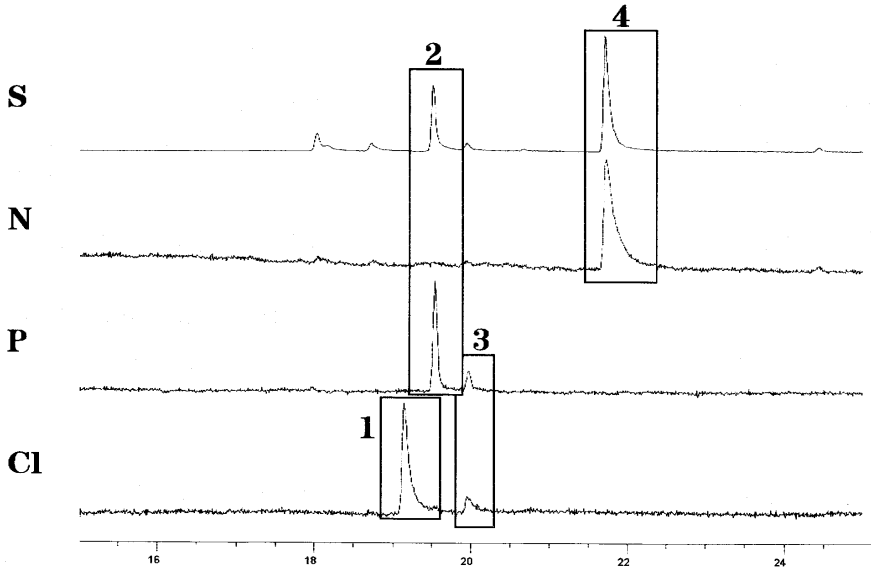


Figure 7. GC/AED element-selective chromatograms for an orange extract. The GC/AED method was locked to the pesticide database retention times. Peaks labeled 1-4 are identified in Table 2.

Table 2. Pesticides initially suggested by searching the pesticide database on the basis of retention times and elemental content. Identities as determined by GC/MS are shown along with pesticide concentrations determined using compound-independent calibration. Peak numbers refer to peaks labeled in Figure 7.

Peak Number	GC/AED Retention Time	Database Search Results Using AED Element Information	MS Confirmation	Concentration Using CIC (ppm)
1	19.152	Aldrin PCB-152	not found	0.6 (Cl only)
2	19.519	Malathion	Malathion	0.8
3	19.957	Chlorpyrifos Dicapthon	Chlorpyrifos	0.26
4	21.717	Thiophenate-methyl Thiophenate-ethyl Dimethametrynel	Thiabendazole (not in database)	2.28

Malathion (Table 2) was the only choice suggested for peak 2 (Figure 7) and this was easily confirmed by MS library searching. On the basis of retention time and elemental content, chlorpyrifos and dicapthon were suggested for peak 3. Chlorpyrifos was confirmed by GC/MS. Three possibilities were offered for peak 4 - thiophenate-methyl, thiophenate-ethyl, and dimethametrynel. However, at that retention time, GC/MS found thiabendazole. Thiabendazole was not in the original retention time database and this is why it was not given as a possibility. The database searching software allows one to add or edit entries, so thiabendazole was added to list. Even though thiabendazole was not in the original retention time database, it was still much easier to find and identify this compound in the TIC because retention time locking ensured that its GC/MS and GC/AED retention times were nearly the same.

Compound-independent calibration (CIC) is a GC/AED technique that allows one to use a single analyte as a calibration standard for all others that contain the same elements. It is the first step in calculating elemental ratios. Using chlorpyrifos as the element-selective calibration standard, the concentrations of malathion, chlorpyrifos, and thiabendazole in the orange extract were determined (Table 2). Even though peak 1 could not be identified, CIC could still be used to determine the concentration of Cl (0.6 ppm). For regulatory purposes this might be very useful. If the Cl level is found to be very low further investigation may not be required; however, if the Cl level is high, it may be necessary to work harder at compound identification.

SUMMARY

Most pesticide screening procedures are capable of finding only a fraction of the pesticides that are registered for use. This procedure has the capability of screening for virtually any volatile pesticide, metabolite, or endocrine disrupter. Although confirmation is usually required, GC/MS analysis is made much easier and more reliable because the pesticide's retention time is already known.

The pesticide database used to demonstrate the feasibility of this method was originally developed by Stan and Linkerhagner on a different gas chromatograph (HP 5890 Series II), using a different column, and without the benefit of retention time locking. Experience in this laboratory shows that retention time locking and the new generation of electronic pneumatic control should allow one to prepare a database with even more precise retention times. Moreover, retention time locking would allow chromatographers to match those retention times with far more accuracy than was routinely possible in the past. By creating a database from the beginning on the preferred GC column under locked conditions, it should be possible to narrow the required search window which would result in fewer hits and greater accuracy. A project is underway in this laboratory to create such a database on two different GC columns that will contain several hundred volatile pesticides, metabolites, and suspected endocrine disrupters.

While GC/AED has been shown to be an ideal tool for element-selective pesticide screening[20-22], many laboratories rely on a combination of other selective detectors. It should still be possible to apply this method if each GC system runs the same method under the same locked conditions. Whatever elemental data that is available could be entered into the search dialog box. It may often be possible to identify a pesticide by using two different GC columns each configured with the same type of element-selective detector. For example, a pair of flame photometric detectors could be used to isolate organophosphorus pesticides on two different GC columns. Two databases could

be searched and only those compounds that appear in both lists would be possibilities. Future work in this laboratory will test this possibility.

Retention time locking with database searching could easily be applied to similar types of analyses. For example, one might use the procedure to identify polychlorinated biphenyls, polynuclear aromatics, or flavor and fragrance compounds.

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ADVANCED ENVIRONMENTAL MONITORING RESEARCH

EPA'S EXTRAMURAL MONITORING RESEARCH PROGRAM

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For Fiscal Year 1996, the Environmental Protection Agency (EPA) increased funding for its investigator-initiated research grants program and identified research on field analytical methods and continuous monitoring methods as ones that are important to the future of EPA and therefore worthy of funding. The purpose of the program is to advance measurement science by stimulating research on radically new approaches to solving environmental monitoring problems. EPA solicited proposals in these areas from the academic, state government, and non-profit research communities. Approximately 78 proposals were received

In the first phase of the proposal review process, EPA convened a panel of outside experts representing academia, industry, other government agencies, and other research institutions to evaluate and rate the proposals based on originality and creativity of the proposed research, training and demonstrated experience of the investigators, availability and adequacy of the facilities and equipment to conduct the proposed work, and responsiveness of the proposal to the indicated research needs that were set forth in the solicitation. Only those proposals that receive a rating of excellent or very good, move to the second phase.

In the second phase, EPA monitoring experts focused on: how well does the proposed study fit into EPA's overall research strategy or regulatory program in this area; did the study have the potential to strengthen the scientific basis for risk assessmen/risk management in the subject area by addressing uncertainties in the supporting science; would the research enhance or complement EPA's in-house research program; would the results of the study have broad applicability or impact large segments of the population; and would the study eventually produce data or methods which can be utilized by the public, states, and EPA to better assess or manage environmental problems?

Based on the results of these reviews, EPA awarded grants to 11 researchers for a total of $2.5 M. In this paper, the author will discuss the extramural research program and review the specific to be conducted. The author will emphasize research dealing with potential applicability to RCRA and CERCLA monitoring.

INTRODUCTION

EPA, as a regulatory agency, is charged with protecting human health and the environment through regulatory programs and other avenues. In order to effectively carry out its mission, EPA relies on the application of sound science in the assessment of environmental problems and solutions.

As a means of helping the nation's academic institutions to focus the creativity of their physical, biological and social scientists and engineers on developing new environmental methods, techniques, and information, Agency has undertaken a major expansion of its extramural environmental science research program.

The FY 96 EPA academic and not-for-profit research support program identified nine priority areas of environmental study:

- Ecological assessment
- Exposure of children to pesticides
- Air quality
- ***Analytical and Monitoring Methods***
- Drinking water
- Environmental fate and treatment of toxics and hazardous wastes
- Environmental statistics
- High-performance computing
- Exploratory research, including Early Career Research Awards

Because quality analytical and monitoring data is critical to the decision making process, in this age of increasingly scarce resources, a significant challenge that the environmental community faces is how to gather the needed date in the most efficient manner. To that end, the Agency identified analytical and monitoring science as an important area of research.

While it is obvious that one needs accurate monitoring data in order to assess the quality of the environment and the effectiveness of Agency programs, the importance of analytical and monitoring methods cuts across almost all of the areas in which EPA conducts research. For instance, ecological assessment cannot be successfully undertaken if high quality data are not available on the concentrations of toxic chemicals in the environment. Exposure of children to pesticides cannot be adequately determined, and therefore not properly controlled, unless accurate measurement of pesticide concentrations are available. Air quality cannot be assessed or improved unless there is the capability to measure reliably the current and future pollutants in the ambient air, as well as emissions that affect air quality. Consequently, analytical methods are the hingepin upon which many of EPA' s other programs pivot.

ANALYTICAL MONITORING PRIORITIES

The primary purpose of the Program is to advance measurement science by stimulating research on radically new approaches to solving real-world environmental monitoring problems. To that end, for the FY 1996 solicitation, EPA identified two general areas where current environmental monitoring technology are known to be inadequate.

- Field portable methodology, and
- Continuous measurement methods

In both of these areas, there is a critical need for equipment and methods that are inexpensive to procure, operate, and maintain. Additionally, EPA was seeking technologies that did not use toxic chemicals, and/or did not generate hazardous waste. Given that decision making can only be as good as the quality of the data on which it is based, the methods should produce measurement data for which EPA, the regulated community, and the public can have a high degree of confidence in the results. EPA favors, in this Program, proposals that are new and innovative rather than proposals that are essentially improvements upon existing methodology. However, existing methods adapted from the non-environmental arena were also considered.

Field Portable Measurement Techniques - There is a serious need for generating analytical data as close to real-time as possible. To this end, methodology that can develop reliable analytical data in the field has become a priority. Having data available immediately allows personnel to make management and operating decisions, without having to wait days to weeks for laboratory analysis. Such waits frequently necessitate additional site visits, and the repetition of complex sampling events (such as moblilizing/demobilizing for the boring of contaminated soil samples or stack sampling). Additionally, the development of analytical data in the field will save the cost of transporting samples, avoid problems with holding times, and alleviate chain-of-custody requirements. Further; if analytical data obtained in the field appear suspect, additional confirmatory analysis can be run immediately. To make field-adaptable monitoring methods reality these methods must use equipment that is relatively light, rugged, and will yield reliable data at the required sensitivity levels quickly (preferably within 4 hours). Examples of field-portable, rapid results monitoring technology that have now become commercially available include immunochemistry-based assays (e.g., for analyzing for petroleum contaminants or solvents in soils), and hand-held x-ray fluorescence spectrophotometers for wastewater analyses.

Continuous Measurement Methods - Periodic analyses of contaminants provides only a "snapshot" of the condition of the sampled stream. Even though EPA-approved analytical methods applied to the periodic samples can provide extremely analytical accurate data, there is no guarantee that the "snapshot" data are representative of discharges that take place continuously. The ability to monitor continuously would also allow for trend analysis, which in turn might allow for the correlation of emissions with process changes. Specific areas where EPA believes continuous sampling would benefit the public are:

- analysis of organic and inorganic contaminants in municipal and industrial waste water to support the design of appropriately sized treatment facilities;
- metal emissions (particularly mercury) from high temperature processes, such as incinerators,
- release of volatile organic compounds from complex point or area source, such as tanks, pipes, valves, landfills, and contaminated soils,
- concentrations of toxic chemicals in the ambient air near hazardous waste sites and industrial facilities,
- deposition or emission flux of toxic air pollutants, especially semi-volatile substances that exist both in the gas phase and attached to particulate matter, and
- the mass of inhalable particulate matter (both $PM_{2.5}$ and PM_{10}), including semivolatile organics, ammonium nitrate (i.e., semivolatile inorganics), and particle-bound water.

This paper will next discuss the research grant selection process and review those grants that focus on field portable methodology and continuous measurement methods.

PROPOSAL SELECTION PROCESS

The focus of the grant selection process is to ensure that the research that is funded represents both high quality of science and will have a significant positive impact on the nation's environmental programs if successful. To that end, EPA follows a rigorous protocol for selecting award recipients. The protocol has three major components.

In the first phase, experts from EPA's Regulatory Program Offices, Regions, and the Office of Research and Development identify environmental issues or health problems that are of the highest priority to the Agency. These issues are based on the regulatory and strategic research needs of the Agency as well as the areas that the Agency feels methodology or data gaps limits the Agency's ability to address the issue or problem. Based on this premise, a technical working group is established in order to develop a grant solicitation for response that represents the goals and needs of the Agency; as in the case of this paper, the area of concern involves a grant solicitation that focuses on Air, RCRA and CERCLA monitoring problems.

Once the proposals are received EPA conducts a peer review by convening a panel of outside experts to evaluate the proposals. These experts represent academia, industry and other government agencies and research institutions. They evaluate and rate the proposals based on:

- originality and creativity of the proposed project,
- training and demonstrated experience of the investigators,
- availability and adequacy of the facilities and equipment with which the research will be conducted, and
- responsiveness of the proposal to the indicated research needs that were set forth in the solicitations

Only those proposals that are deemed excellent or very good by the outside peer review pane! receive further evaluation

The next step in the selection process consists of an in-house relevancy review. The focus of this review, which is conducted by a panel of appropriate EPA experts, is to rate the proposals on their:

- fit into EPA's overall research strategy or regulatory program,
- potential to strengthen the scientific basis for risk assessment/risk management by addressing uncertainties in the supporting science,
- ability to enhance or complement EPA's in-house research program,
- applicability to broad segments of the population, and
- ability to produce data/information that can be utilized by the public, states, and the federal government to better assess and manage environmental programs.

The result of this review is a ranking of the proposals as to their relevance and potential to have a significant impact on environmental monitoring. At this point an assessment is made as to the total amount of resources that is available, whether any of the highly ranked proposals are duplicative of one another or of already ongoing research EPA is conducting and whether the requested level of EPA funding is reasonable. Funds are then allocated to the proposals in order of their final ranking after taking these factors into account.

In response to the FY 1996 solicitation, EPA received approximately 78 proposals in the Analytical and Monitoring Methods research category. The proposals covered several relevant areas of research. Five of these areas that received a significant number of proposals were: sampling (14 proposals), particle monitoring (9), biosensors (5), spectroscopy (22), and electrochemistry (11). The outside peer review was conducted by a panel of 27 persons. Based on their review, 17 proposals which received a rating of either "excellent" or "very good" were then submitted to the EPA panel for relevancy review. 0f the 17 "very good to excellent" proposals, for FY 1996, EPA awarded eleven research grants for analytical and monitoring methods totaling about $3.2 million. Most of the grants fund research that will take several years to complete. Six of these eleven proposals relate directly to field portable methodology and/or continuous measurement for RCRA and CERCLA-type pollutants, or air emissions that may exist at RCRA/CERCLA sites.

If one looks at totals, the overall success rate for proposals was 14% in the analytical and monitoring methods category. For the proposals that received high marks from the peer review process, the success rate was very high being almost 65%; for instance all the proposals that received an excellent rating were funded. The 6 proposals dealing with RCRA and CERCLA-related concerns will be discussed next.

SUMMARY OF SIX ENVIRONMENTAL MONITORING PROJECTS

1. Development of a New Biosensing System for Environmental Monitoring of PCBs, Heavy Metals, and Atrazines, in Aqueous Streams, Using Conductive Electroactive Polymer (CEP)-Based Sensing (Dr. Omowunmi Sadik - State University of New York at Binghamton)

Conventional chromatographic-based approaches to immunoassay screening analyses of aqueous contaminants are not very sensitive, and require substantial time (minutes to hours) to complete. The proposed project will develop a CEP-based selective methodology to give rapid (i.e., real-time) detection of PCBs, heavy metals, and atrazines, with the potential for application to other chemicals. In addition, the equipment and procedures involved are purported to be less expensive, more compact, and more user friendly than current immunoassay techniques The principle upon which this technology is based is that the electronic conductivity of CEP materials allows electrical potentials to be applied. Measurement of the reaction to these potentials in combination with enzyme-based biological reagents then allows the chemical properties of the samples to be determined.

This project proposes to develop the CEP-based monitoring technique, and will describe the application of the technology for field demonstration.

2. Real-Time Trace Detection of Elemental Mercury and Its Compounds (Dr. Robert Barat - New Jersey Institute of Technology)

Currently, commercial devices are not sensitive enough to detect Mercury (Hg) in ambient air or gaseous emissions at all levels of interest (1 - 1000µg/m^3) in real-time, and certainly there is no technology that will rapidly speciate the various Hg compounds. This project will research the capabilities for measurement of Hg and its compounds, using a novel hybrid instrument combining Doppler-shifted Resonant Fluorescence (RF) and Photofragment Fluorescence (PFF) with test gases expanded in a supersonic jet. The instrument will be called a Supersonic Jet Spectrometer (SJS). This project will build the SJS, and evaluate its detection capabilities on Hg-containing air streams. The detection limits, range of linearity, relative accuracy, and response time will be evaluated. If performance is satisfactory, the SJS can subsequently be developed into a relatively low cost instrument for field use.

3. Field-Usable Compact Capillary-Based Ion/Liquid Chromatographs. Real-Time Gas/Aerosol Analyzers (Purnendu Dasupta - Texas Tech University)

Currently, ion and liquid chromatography, two widely used techniques for analysis of organic constituents in the environment (aqueous streams as well as atmospheric samples), remain relegated to the laboratory due to lack of true portability. This project proposes to develop a briefcase-sized (< 20 pound) packed capillary and open tubular ion/liquid chromatographic instrument, including suppressed conductometric and optical detectors, as well as accompanying software for a laptop computer. The instrument is expected to be far more sensitive than its predecessors (in the sub-ppt range for gases and pg/m^3 range for most aerosol constituents). The project will construct two complete systems, and will be field-tested for pesticide analysis in runoffs from cotton fields local to the University.

4. Rapid Determination of Organic Contaminants in Water by Solid Phase Microextraction (SPME) and Infrared Spectroscopy (IR) (David Tilotta - University of North Dakota)

The objective of this study is to develop a simple, sensitive, and rapid method for the field determination of organic contaminants in water. The method will combine SPME and Fourier transformed IR techniques. Current conventional analytical techniques are typically time-consuming, labor-intensive, and require expensive instrumentation. Hence, they are not readily field-adaptable. The proposed research will construct a reusable aluminum SPME "dip-stick", which is coated with a sorbent film, such as poly(dimethylsilaxane) and paraffin-impregnated poly(butylene). The sorbent is expected to have relatively large partitioning coefficients (2 - 2000) for many environmentally-important

compounds. The dipstick will be placed in the contaminated water, and will selectively partition the organics into the coating. Initial studies show that the SPME has a partitioning time as fast as 40 minutes, and that the combined SPME/IR methodology is sensitive (ppm - ppb range) as well as reproducible (RSDs of 1 - 11%). In addition, the method is expected to cost less than 20c per film, and because it is a solid phase extraction, will not generate solvent waste.

The study proposes to identify 5 to 10 solid phases for use with the dipstick, and will test the methodology on volatile organic compounds, crop protection chemicals, and PAHs. The proposed method will then be compared to standard methods for determining target organics in water, and will then be applied in the field, at actual contaminant sites

5. Field Determination of Organics for Soil and Sludge Using Sub-Critical Water Extraction Coupled with Solid Phase Extraction (Dr. Steven Hawthorne - (University of North Dakota)

Currently, there are no field-portable water-based extraction methods available for quantitative analysis of both polar and non-polar organics from soils and sludges. Currently, the well-known sorption methods, solid phase extraction (SPE) and solid phase microextraction (SPME), are only applicable to water samples. This proposal will investigate the use of subcritical water (up to 250°C and only a few bars [or atmospheres] pressure) to quantitatively extract organics into water in preparation for SPE or SPME. Preliminary work suggests that subcritical water can solubilize from soils and sludges even non-polar organics, such as PAHs, more than 10^6-fold as compared to relatively low temperature water. Additionally, with proper internal standards, quantitative results for PAHs, PCBs, and aromatic amines have been obtained with total sample preparation time of about 30 minutes (including subcritical extraction from the solid sample and SPE or SPME sorption), yielding detection limits of less than ppb-level sensitivity.

The study proposes to further investigate the use of subcritical water extraction combined with SPE and SPME for the rapid and quantitative determination of organics in soils and sludges. It intends to optimize the selectivity of the extractions (based on the temperature of the water used for extraction, and on SPE/SPME sorbent selectivity). It will compare analysis of the extracts in the field as opposed to sending the SPE/SPME devices back to a laboratory, and will also compare this method with conventional EPA extraction/analysis methods for soils and sludges.

6. Supercritical Fluid Chromatography Directly Coupled to Dynamic Nuclear Polarization (SFC/DNP) (Harry Dorn - Virginia Polytechnic Institute and State University)

The objective of this research will be to develop an SFC/DNP instrument which will be used for analysis of nonvolatile organic and inorganic contaminants in soils and aqueous sources. The technology will directly couple supercritical fluid chromatography (SFC) with dynamic nuclear polarization (DNP). DNP is a variant of nuclear magnetic resonance (NMR). The DNP will allow much greater sensitivity than (NMR). In ultimate applications, the organic and inorganic contaminants will be collected using adsorbent traps (Tenax, XAD, etc.). The extract from the adsorbents will then be injected onto a supercritical fluid (carbon dioxide) chromatographic column with a DNP detector. The technology is expected to allow for rapid, continuous or nearly continuous, and sensitive analysis of most contaminants, including chlorocarbons, organophosphates, pesticides, and petroleum compounds. The method will also allow determination of molecular structure information, such as isomer identification.

CONCLUSION

The Agency's FY 1996 research support program is funding a significant amount of research with the potential for having a meaningful positive impact on the nation's ability to monitor for environmental contamination. In FY 1997, EPA will increase its overall level of support to its extramural research program. While the FY 1997 solicitation does not call specifically for research in the Analytical and Monitoring Methods, significant levels of funding for measurement research are contained in the technical areas that are specified in the FY 97 Research Grants Announcement. For example, the Ecosystem Indicators research area has funding available for projects which focus on the development of monitoring methods for sampling and measuring the ecosystem indicators.

EPA has and continues to provide significant funding for analytical and monitoring methods research projects. The

objective of the research should be focused on innovative technologies that fill existing gaps in current analytical and monitoring protocols and which can have significant impact on future monitoring programs.

FIELD DETERMINATION OF ORGANICS FOR SOIL AND SLUDGE USING SUB-CRITICAL WATER EXTRACTION COUPLED WITH SOLID PHASE EXTRACTION

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The primary purpose of the proposed investigations is to couple well known extraction methds for water, solid phase extraction (SPE) and solid phase microextraction (SPME), with subcritical water extraction of soils and sludges to allow field-portable water methods to be applied to contaminated solids.

For water samples, both SPE and SPME can be used to extract and concentrate organics in the field for subsequent analysis (e.g., field-portable GC), but are not applicable to extracting organic pollutants from solid samples. If organic pollulants on soils and sludges could be efficiently transferred to water, both SPE and SPME could be very useful for field determinations of organic pollutants from solids. We have demonstrated that subcritical water (hot water maintained as a liquid by a few bar pressure) is an excellent solvent to quantitatively extract polar and non-polar organics from soils and sludges. Subcritical water extractions can be highly selective; polar organics extract at lower temperatures (e.g, phenols and amines extract at 50 to 100 °C), and non-polar organics extract at high temperatures (e.g., 200 to 250 °C). By heating water under low pressure, solubilities of polar organics increase dramatically, and even non-polar organics such as PAHs can increase solubilities by >106-fold.

Initial experiments have demonstrated that coupling subcritical water extraction with SPE and SPME can provide an extremely simple, rapid, and inexpensive method to determine organic pollutants found on soils, sediments, and sludges. With proper internal standards, quantitative results for PAHs, PCBs, and aromatic amines have been obtained with total sample preparation (including extraction from the solid and SPE or SPME sorption) of ca. 30 minutes. Detection limits of <ppb are obtained.

During this three-year study we will:

1. Investigate and develop the use of subcritical water coupled to SPE (exhaustive extraction) for the quantitanve determination of polar and non-polar organics from soils and sludges.

2. Investigate and develop the use of subcritical water extraction coupled with SPME (equilibrium extraction) for the rapid and qualitative determination of polar and non-polar organics from soils and sludges.

3. Optimize the selectivity of the extractions based on water extraction temperature and on SPE and SPME sorbent selectivity.

4. Compare analyzing extracts in the field to shipping SPE and SPME devices to the lab.

5. Demonstrate the best approaches in the field and compare results to conventional EPA extraction and analysis methods.

Based on initial results, it is expected that the proposed investigations will yield an extremely simple (requiring only an extraction cell and oven), inexpensive and field-portable approach to utilizing SPE and SPME with subcritical water for the extraction and quantitative determination of polar and non-polar organics from contaminated solids and semi-solids.

SOLID PHASE MICROEXTRACTION COUPLED WITH INFRARED SPECTROSCOPY FOR THE DETERMINATION OF ORGANIC POLLUTANTS IN WATER

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The current standard methods for determining organic compounds in water include liquid-liquid extraction, solid phase extraction, solid phase microextraction (SPME), and purge and trap gas chromatography. Although each of these methods have certain advantages and disadvantages, many of them are time-consuming, labor-intensive, difficult to implement, and require relatively expensive instrumentation. In addition, most are not readily adaptable to field detections. Field determination of contamination is attractive because It eliminates many of the problems connected with collection and transporting samples (e.g., representative sampling, contamination, loss of volatiles. storage, etc.).

Work in this laboratory is focusing on the development of a simple, solventless method of analysis that combines SPME and infrared (IR) spectroscopy. Infrared spectroscopy is a proven sensitive and selective analytical technique that can potentially be used to detect organic contaminants. However, the direct detection of organics in water by IR spectroscopy yields poor results due to the severe spectral interference of the water. SPME is a relatively new analytical technique used to selectively partition organic compounds from an aqueous phase into a solid sorbent. Because the partition coefficients for many environmentally-important compounds are large (e.g., in the 2-2,000 range), a substantial preconcentration enhancement is enjoyed.

We are constructing, simple, reusable "dipsticks" that contain a thin film of a chromatographic solid phase that is capable of partitioning target organics from water After the organics partition into the solid phase, a portable FT-IR (Fourier transform infrared) spectrometer is used to determine the organics partitioned in the solid phase. This solventless preconcentration step minimizes water and matrix interferences and makes it possible to detect organic compounds in the ppm-ppb range. Additionally, because the SPME/IR method uses no solvents or cumbersome equipment, it is field portable.

This presentation will discuss recent work in our lab on the examination of various polymer films for suitability as SPME/IR sorbents. Selection criteria examined include: optical transparency, equilibration times, rigidity, reusability, and analyte selectivity. Additionally. we will also discuss our primary work on the application of this method to determining selected organic contaminants (e.g., benzene, ethylbenzene, trichloroethylene, etc.) in aqueous samples. Preliminary results obtained from sorbent films such as poly(dimethylsiloxane) and paraffin-impregnated poly(butylene) show that the SPME/IR method is fast (partitioning times as fast as 40 min), and reproducible (RSDs of 1-11%) with detection limits ranging from ca. 15 ppb - 1 ppm. In addition, we will show that the SPME/IR results are in good agreement with those obtained from standard EPA protocols. We anticipate that the initial laboratory tests will be completed by the end of 1997, and field testing of this new method in 1998.

REAL-TIME TRACE DETECTION OF ELEMENTAL MERCURY AND ITS COMPOUNDS

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INTRODUCTION

Emission of mercury vapor from combustion and other processes is now considered to be a major environmental issue [Von Burg and Greenwood, 1991]. It can be emitted directly to the air in its highly volatile elemental form. In addition, mercury compounds [e.g. $HgCl_2$, $Hg(CH_3)_2$] are quite volatile, and have been detected in incinerator effluent and the biosphere.

The present research addresses the need to develop real time stack monitoring of emissions of Hg and its compounds. Such technology will enable combustion facilities to identify peak emission events and take corrective

action. It will replace the current uncertainty in emission estimations based on feed mercury content. Realistic emission inventory will enable regulatory agencies to better assess health risks associated with future siting of waste incinerators.

To be applicable to a wide range of applications, an instrument should be capable of detecting Hg in the range of 1-5000 $\mu g/m^3$, with an ultimate sensitivity limit on the order of 0.1 $\mu g/m^3$ (ca. 10 pptv). Current best technology for mercury detection uses a collection / concentration step followed by off-line analysis using atomic absorption, atomic fluorescence, or plasma emission spectroscopy [Baeyens, 1992]. None of these three detection methods in their commercially available configurations are suitable for real-time monitoring of elemental mercury.

The detection concept for elemental mercury involves Doppler-shifted resonant atomic fluorescence excited by a UV laser or a low pressure mercury lamp. The Doppler-shifted fluorescence will be separated from the unshifted background signal by use of a mercury vapor filter precisely matched to the spectral linewidth of the source. The detection concept for compounded mercury will use the photofragment fluorescence (PFF) excited by deep UV light. Examination of the fluorescence spectrum will permit identification of the original mercury compounds. Photo-fragment fluorescence has been successfully applied for the gas-phase analysis of $HgCl_2$, $Hg(CH_3)Cl$, and HgI_2 (Poulos and Barat, 1997).

APPROACH -- ELEMENTAL HG

Atomic Fluorescence Spectroscopy (AFS) is a highly sensitive spectroscopic marker for elemental Hg detection. Current AFS instruments use a cold vapor trap for collection/concentration of the air sample followed by purging and excitation with an atomic vapor mercury lamp (253.7 nm) and measurement of the resonant fluorescence (at the same wavelength). Sensitivity is limited by the elastically scattered light from the exciting source. In the case of 253.7 nm Hg fluorescence, this limitation is problematic because the fluorescence signal is already reduced due to quenching by air.

The technique under study, shown in Figure 1, will expand the Hg-contaminated air stream across a supersonic nozzle into a high vacuum chamber. Light at 253.4 nm will be directed across the jet. Atomic Hg fluorescence will be Doppler-shifted by between 1 and 3 GHz due to the jet motion (Barat, 1996). Total collected light, comprised of the shifted fluorescence and stray elastic scattering, will be passed out of the vacuum chamber and through a sharp cutoff atomic mercury vapor filter to attenuate stray elastic scattering while transmitting the fluorescence signal.

As illustrated in Figure 2, when narrow bandwidth radiation is incident upon a high speed flow field, elastically scattered background light is superimposed upon the Doppler-shifted mercury vapor fluorescence. If the excitation source coincides exactly with the absorption band of an optically thick mercury vapor, and, if a cell filled with mercury vapor is placed in front of a detector, then the elastically scattered background light will be strongly attenuated (Miles, 1991; Finkelstein, et al., 1994).

The expansion reduces the collision rate of excited mercury with the air, so collisional quenching and collisional broadening are both substantially reduced. The low temperature associated with this expansion further reduces the fluorescence linewidth, enhancing the performance of the mercury vapor filter.

APPROACH -- COMPOUNDED HG

In general, mercury compounds absorb light strongly below 250 nm [Gowenlock and Trotman, 1955], and these absorption bands are dissociative. In PFF, a photolyzing UV photon dissociates the target molecule into fragments, some of which are imparted with excess energy. The energy might then be lost by fluorescence:

$$A\text{-}B \xrightarrow{h\nu} A + B^* \qquad (1a)$$

$$B^* \longrightarrow B + h\nu' \qquad (1b)$$

where A and B can be atoms or polyatomic species. The fragment identities and distributions, as revealed in the fluorescence spectrum, can, in principle, provide information on the composition of the target, in a manner analogous to mass spectrometry and other fragmentation spectroscopies.

For example, Figure 3 shows the PFF spectrum from 193 nm excimer laser excitation of $Hg(CH_3)Cl$ vapor in a static cell (Poulos and Barat, 1997). Two features are evident: atomic Hg emission lines at 546, 577, and 579 nm; and a broad continuum assigned to the B-->X system of HgCl** excited state. Some of the following photochemical processes could explain these findings:

$$Hg(CH_3)Cl \xrightarrow{h\nu} HgCl^{**} + CH_3 \qquad (2)$$

$$HgCl^{**} \longrightarrow HgCl + h\nu' \qquad (2a)$$

$$\longrightarrow Hg + Cl \qquad (2b)$$

Processes (2a) and (2b) are clearly implicated, but the origin of the Hg atomic emission lines is open to question.

The concentration of the target compound may be related to the fluorescence intensity from a hot fragment. For example, excitation laser energy of 2 mj/pulse at a repetition rate of 10 Hz at 229 nm was applied to HgCl: vapor in a static cell (Poulos and Barat, 1997). Analyte concentrations were varied by changing the bath temperature around an analyte reservoir of the static optical cell used. The PFF signal was monitored at 253.7 nm, the strongest fluorescence line. As shown in Figure 4, signal linearity was observed over at least 2.5 orders of magnitude of concentration. The concentrations studied overlap the range of inorganic mercury levels measured exiting from stacks of coal-powered utilities and municipal waste incinerators by standard methods.

The supersonic jet spectroscopy are expected to further improve sensitivity of PFF detection. Fluorescence spectra will be sharpened, leading to better discrimination of fragment vibrational structure. Quenching by O_2 will be reduced due to the low pressure. The UV source will penetrate deeper into the sample because there is no optical filtering of the light by O_2 from the window to the jet, and reduced filtering in the jet.

QUALITY ASSURANCE/PERFORMANCE ASSESSMENT

For comparison of time-averaged test concentrations, the mercury-containing sample stream will be diverted to a reference method, such as optical absorption. Testing of the research technology will consist of obtaining data on five performance measures:

Relative Accuracy: the absolute mean difference between the metals concentration determined by the monitor and that determined by the reference method, plus a 2.5 percent uncertainty confidence coefficient based on a test series.

Calibration Drift: the difference in the monitor output reading from the established reference value after a stated period of operation. The reference value is established by a calibration standard which has a concentration nominally 80 percent or greater of the full scale reading capability of the monitor.

Zero Drift: calibration drift when the reference value is zero.

Response Time: the time interval between the start of a step change in the concentration of the monitored gas stream and the time when the output signal reaches 95 percent of the final value.

Detection Limit: three times the standard deviation of nine repeated measurements of a low-level (near blank) sample.

IN CONCLUSION

This program will determine the capabilities and limitations of mercury detection in real-time by the proposed techniques. It is anticipated that the detection limits for elemental mercury will be on the order of 0.1 $\mu g/m^3$ (ca. 10 pptv). It is desirable that response be linear up to cat 5000 $\mu g/m^3$. It is anticipated that PFF signals will successfully discriminate between species such as $HgCl_2$, HgO, $Hg(CH_3)_2$, and $Hg(CH_3)Cl$, at detection levels below 1 $\mu g/m^3$.

ACKNOWLEDGMENT

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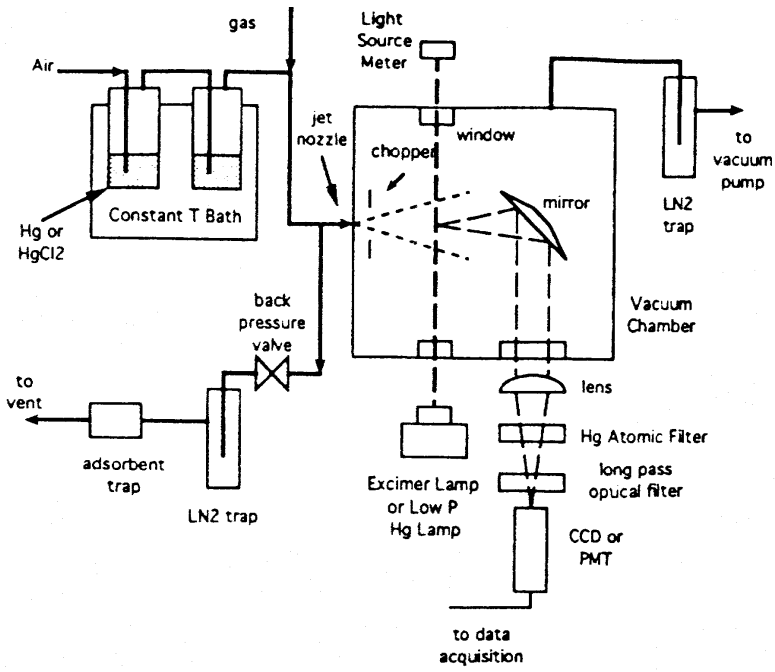

Figure 1. Experimental Test Apparatus

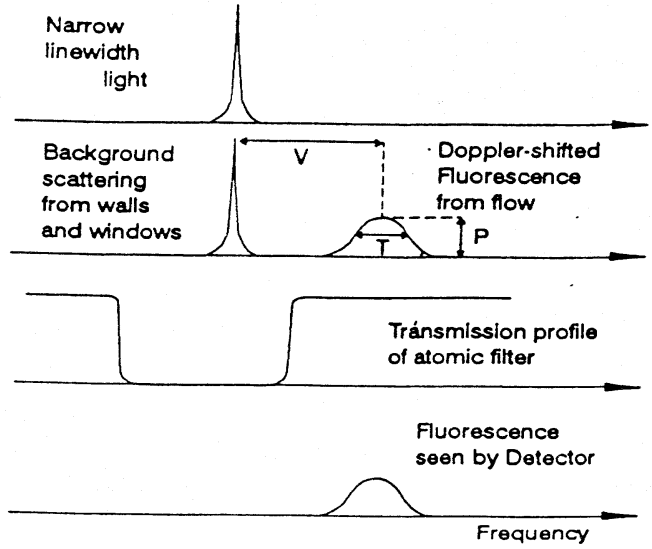

Figure 2. Mercury Atomic Filter Concept

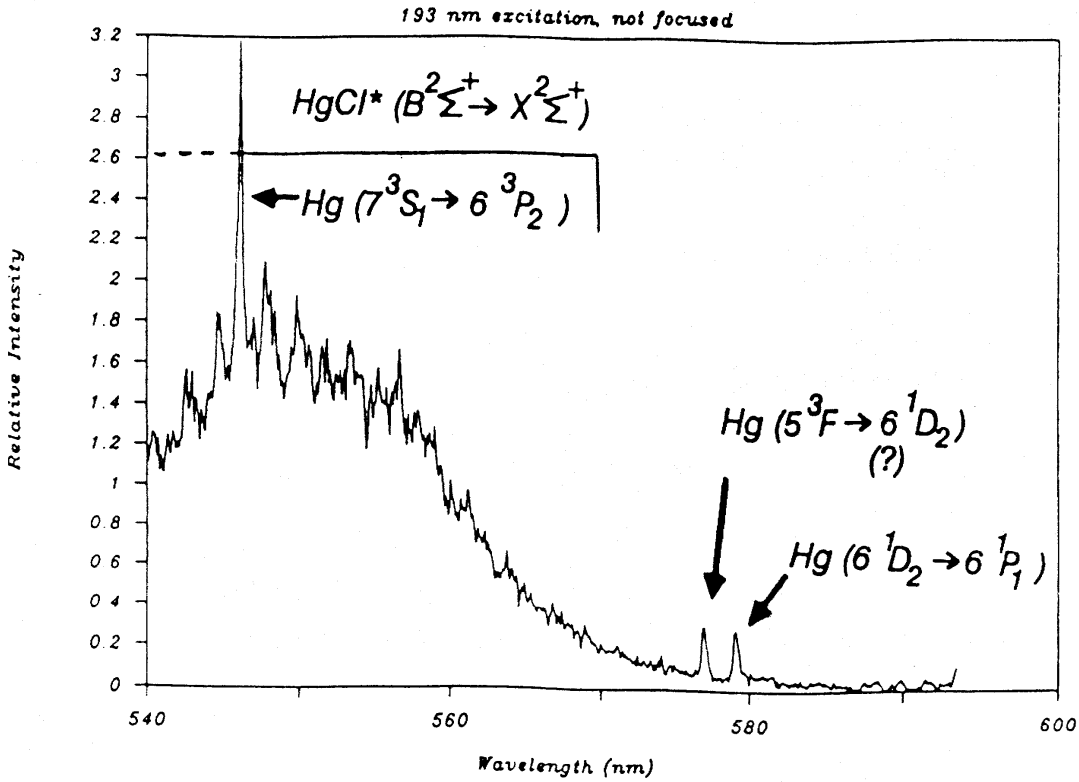

Figure 3. PFF Spectrum of $Hg(CH_3)Cl$

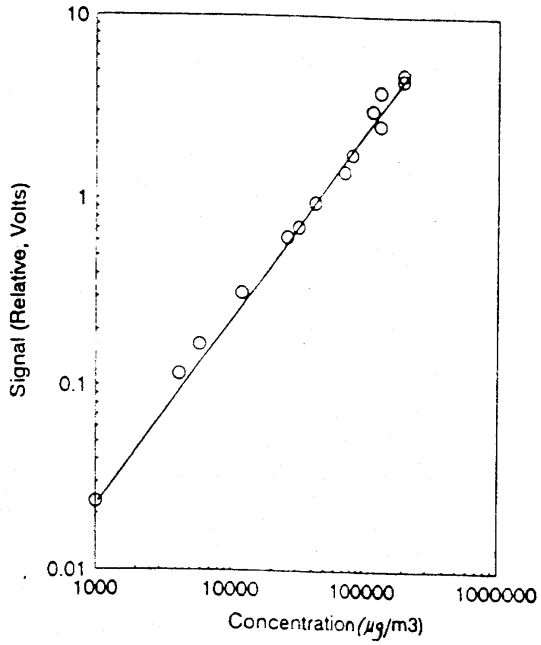

Figure 4. PFF Intensity (at 254 nm) vs. $HgCl_2$ Concentration

ELECTRODIALYTIC NAOH ELUENT PRODUCTION AND GRADIENT GENERATION

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A small inexpensive system is described that allows high performance suppressed anion chromatography on a capillary scale. A fully computer controlled stepper motor driven syringe type dispenser, equipped with a 500 mL capacity glass syringe is capable of pumping at pressures up to 1000 psi when equipped with an appropriate inlet check valve. Fused silica capillary columns ~50 cm in length and 180 mm in i.d., packed in-house with a commercial packing, provide excellent performance, significantly exceeding the efficiencies observed for the same packing in commercially available 2 mm bore format. The system operates with a pressure drop of <800 psi at a flow rate of 2 mL/min. The system utilizes a novel electrodialytic NaOH eluent generator that is deployed on the high pressure side of the pump and thus requires no special measures for electrolytic gas removal This device permits both isocratic and gradient operation with excellent eluent purity; the NaOH concentration is generated linearly with applied current with near-Faradaic efficiency up to a concentration of at least 100 mM.

SENSORS FOR DIRECT MONITORING OF ENVIRONMENTAL POLLUTANTS

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The considerable amount of time and costs involved in carrying, out field sampling and laboratory analysis of environmental samples has resulted in the need to develop alternative screening methods. Immunosensors are capable of "decentralizing" environmental testing through on-site rapid screening. Current research and development efforts are directcd towards more compact cheap, and user-friendly devices. In our laboratories we are pursuing the development of portable, rapid, cost-effective, and in-situ, multi-layer electro-optical sensors for continuous detection of environmental samples in real time. In our previous work, we have shown that conducting electroactive polymer (CEP)-based sensors possess significant advantages over conventional methods of detection and quantitation of environmental analyses. Also the uniqueness of CEP-based sensors in combining the role of transducers required for measuring immunological reactions with that of antibody (Ab) entrapment matrices translates into substantial equipment miniturization as well as huge reduction in response time. This promises to open up new horizons in environmnental monitoring, medical and clinical applications. This paper will discuss the development of a direct multi-electrode array detection and quantitation of polychlorinated biphenyls (PCBs), heavy metals, and atrazines, using CEP-based sensors. Preliminary results obtained with enzyme-based electro-optical detection methods will aIso be analyzed and discussed. The advantages of utilizing these types of assay formats to provide unparalleled rapid, sensitive, and cost-effective options for environmental analsis of pesticides will be presented.

INORGANIC

PRACTICAL CLEAN CHEMISTRY TECHNIQUES FOR TRACE AND ULTRA-TRACE ELEMENTAL ANALYSIS

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Trace and ultra-trace analysis is as much dependent on the control of the analytical blank as it is on the accuracy and precision of the instrument making the measurement. Inability to control contamination that is external to the sample, or those contributions of the analyte coming from all other sources than the sample, is frequently the limiting factor in trace (parts per million (ppm) to parts per billion (ppb)) and ultra-trace analysis (ppb to parts per trillion (ppt)). Analytical blank contributions occur from four major sources: surroundings, reagents, vessels, and the analyst's technique.

The analytical blank can be dramatically reduced through the combination of clean chemistry and microwave sample preparation. Microwave sample preparation systems minimizes continuous sustained transfer of elements from the air to the sample solution by providing a controlled, closed, or restricted sample container during decomposition[1] It prevents the entry of the laboratory atmosphere and thus prevents the majority of environmental airborne contamination. This leaves only the air within the vessel, captured during transfer, and digestion/extraction. To prevent this volume of air from contaminating the sample, the sample must be transferred in a clean environment.

Portable class 100 clean benches were designed and produced to allow for addition of sample and reagents, and to open or close vessels of both the atmospheric and closed vessel design. These carts can be constructed for $1,000-$1,600, depending on whether they need to be exhausted or are just islands of clean laboratory air. Similar portable clean systems are becoming available from analytical instrument manufacturers. A clean laboratory of dimensions 18' x 13.5' was designed and constructed for approximately $30,000 using readily available materials and specialized HEPA clean hood components. This clean facility is used for both microwave vessel sample preparation and ICP-MS analysis. The ICP-MS is located in the clean laboratory, so its extremely low detection capability may be utilized.

95% of the capability of a cleanroom can be made available to standard environmental laboratories for between $1,500 and $15,000. The quality of the analysis can be improved for ultra-trace analysis of many type for modest cost. Some of these relatively low cost systems will be described for both their effectiveness and method of implementation. These modest cost methods will be proposed for inclusion in chapter 3 of SW-846 as guidelines to improve environmental analytical analysis.

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FLAME ATOMIC ABSORPTION FOR THE DETERMINATION OF ARSENIC AND SELENIUM IN TCLP EXTRACTS

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ABSTRACT

Toxicity Characteristic testing is performed to assess the potential of a material to leach hazardous constituents after disposal in a landfill. The Toxicity Characteristic Leaching Procedure (TCLP) extract solution is analyzed for 31 organic components and 8 metals and if the regulated limits are exceeded the material is considered hazardous and must be treated appropriately.

Currently approved EPA Methods for the determination of As and Se include graphite furnace atomic absorption (GFAA) (7060A, 7740) and hydride generation atomic absorption (7061A, 7741A). With the advent of modern instrumentation, including electrodeless discharge lamp light sources, more stable electronics, background correction, and improved sample introduction systems, flame atomic absorption analysis for low levels of these elements becomes viable.

Detection limits ten times below the MCL are generally accepted as necessary to ensure reliability at the decision-making point. Method detection limits were calculated for As and Se based on the EPA procedure published at 40CFR, part 136. The method detection limit obtained for As was 0.12 ppm and for Se was 0.04, using the high sensitivity nebulizer. The detection limit for As would also be lower using the high sensitivity nebulizer (0.04), but meets our goal of ten times below the MCL (0.5 ppm needed) with the universal nebulizer.

Calibration is an important component of an analysis and we will discuss the choice of calibration standards and the resulting calibration. The linear range will be documented and the value of extending the calibration curve past the linear range will be discussed.

The developed flame atomic absorption method will be applied to the analysis of As and Se in real TCLP samples.

INTRODUCTION

One of the more popular tests performed under the EPA Resource Conservation and Recovery Act (RCRA) program is the Toxicity Characteristic Leaching Procedure (TCLP). TCLP testing is performed to assess the potential of a material to leach hazardous constituents after disposal in a landfill. The first step involves extraction of the potentially hazardous material (method 1311) with a mildly acidic buffered solution. The extract is then analyzed for 31 organic compounds and 8 metals and if any of the analyses are present at levels exceeding the maximum allowed, the material is considered hazardous and must be treated as such. Table I lists the maximum contaminant levels (MCLs) permitted in the extract for metals.

Table I. TCLP Metal Limits

Element	MCL (mg/L)
As	5
Ba	100
Cd	1.0
Cr (total)	5.0
Pb	5.0
Hg	0.2
Se	1.0
Ag	5.0

The methods for contaminant analysis can come from many reputable sources but the EPA RCRA publication *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods; SW-846* is a primary source for many organizations performing RCRA measurements. The methods available in SW-846 for As and Se determination are listed in Table II.

Table II. RCRA SW-846 Methods

	GFAA	Hydride	ICP-OES	ICP-MS
As	7060A	7061A	6010	6020
Se	7740	7741A	6010	--

With the advent of modern instrumentation including electrodeless discharge lamp light sources more stable electronics background correction and improved sample introduction systems flame atomic absorption determination of these elements becomes viable. Flame atomic absorption is an inexpensive technique to acquire and operate and

many laboratories might prefer the choice if a method was readily available.

The goal of this paper is to evaluate the possibility of FLAA for the determination of As and Se in TCLP extracts. The detection limits will be determined to assess the analytical feasibility. The linear ranges and potential interferences will be evaluated. Stability over the course of an unattended analytical run and the recovery of spikes in real matrices will be presented.

EXPERIMENTAL

The Perkin-Elmer® AAnalyst™ 100 atomic absorption spectrometer was used for all measurements. The analytical parameters are shown in Table III. The high sensitivity GemTip™ nebulizer was used exclusively for Se because the estimated detection limits indicated that the additional sensitivity would be required. The universal and high sensitivity GemTip™ nebulizers were both evaluated for As. Standards were prepared to contain approximately 1% HNO_3 and 2% HCl (GFS Chemicals®, Columbus, OH)

Table III. AAnalyst 100 Instrumental Conditions

Parameter	As	Se
Wavelength (nm)	193.7	196.0
Slit (nm)	0.7	2.0
Mode	AA-BG	AA-BG
Flame	Air-Acet.	Air-Acet.
Nebulizer	Universal or High Sensitivity	High Sensitivity
Calibration	Linear	Linear
Standards (mg/L)	0.2, 2.0, 5.0, 10.0	0.1, 0.5, 1.0, 2.0

Keith Hutchinson of NET Midwest, Inc. provided the digested TCLP extracts. The synthetic sample was prepared to approximate the provided samples by combining single element stock solutions to create a matrix of 1500 mg/L Na, 20 mg/L Ca, and 70 mg/L Fe.

RESULTS AND DISCUSSION

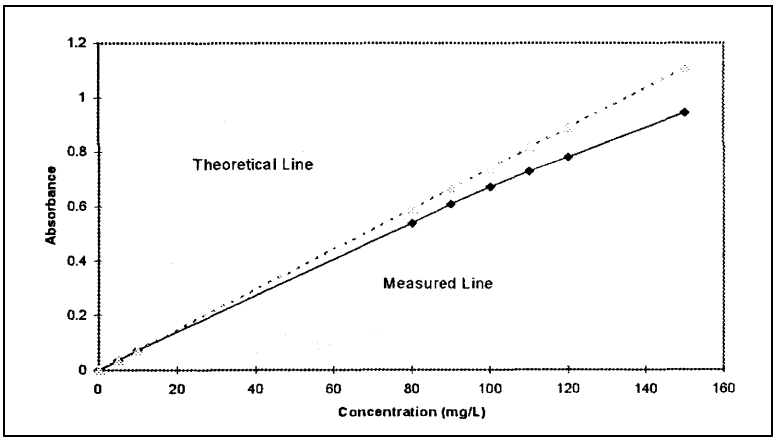
Detection limits ten times below the MCL are generally accepted as necessary to ensure reliability at the decision-making point. This means that we would need a detection limit for As of 0.5 mg/L and 0.1 mg/L for Se to meet the goal. Method detection limits were calculated based on the EPA procedure published at 40CFR, part 136 [1]. The detection limits shown in Table IV meet the goals described. The universal nebulizer is adequate for the measurement of As or the high sensitivity can be used for additional margin. The high sensitivity nebulizer is necessary to meet the goals for the determination of Se.

Table IV. Method Detection Limits (MDLs)

Nebulizer	As	Se
Universal	0.12	--
High Sensitivity	0.043	0.041

Linear ranges must be determined initially to characterize instrumental performance. It is generally accepted that a calibration is no longer linear when the measured concentration based on a lower level calibration deviated from the the value by more than 5%. Using a spreadsheet program to calculate the percentage difference at increasing concentrations the linear range was rounded off to 20 mg/L for Se and 60 mglL for As. Figure 1 shows the curves for Se and Figure 2 shows the analogous curves for As (universal nebulizer).

Modern instruments using algorithms to reliably model the curves obtained at higher concentration can give accurate and precise measurements above the linear range. The choice of calibration standards is more critical and three standards should be used in a ratio of 6:3:1, with 6 representing the highest concentration[2]. If performance can be



verified through the use of quality control checks for calibration verification precision and recovery this extends the useful working range of the instrument. For atomic absorption with one of the shorter working ranges this can improve the range four-six times above the concentration at the top of the linear range.

Figure 1. Se linear range.

Before measuring real samples a synthetic sample was evaluated to determine if matrix effects were present. As and Se were spiked at a low level and at the MCL into a synthetic matrix approximated from ICP-MS analysis of a digested sample. Table V shows the results indicating that the matrix was not a problem for the sample introduction system.

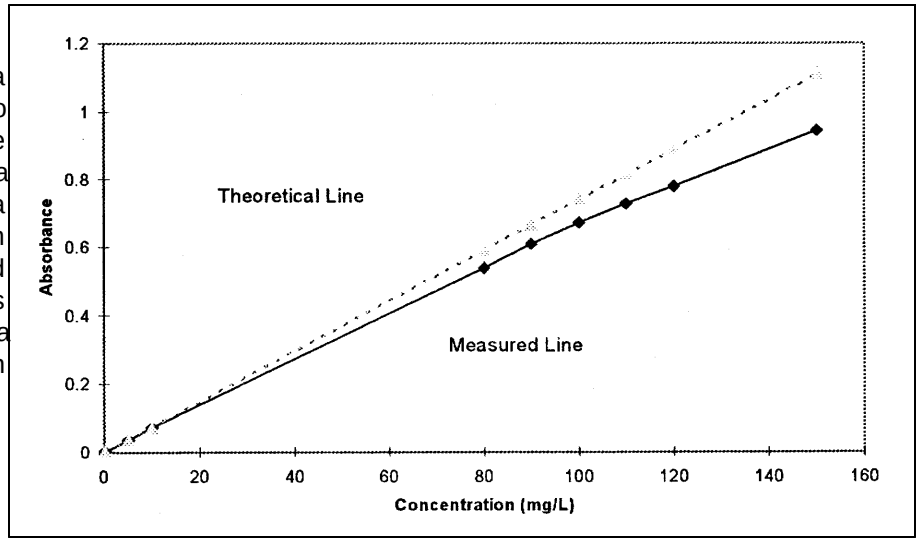


Figure 2. Arsenic linear range.

Table V. Synthetic Sample Results

Element	Low Spike (mg/L)	% Recovery	High Spike (mg/L)	% Recovery
As	0.5	113	5.0	92.2
Se	0.5	93.0	1.0	95.2

The analysis of samples spiked before the digestion process can indicate the entire process is in control. This includes the digestion instrument calibration, and method. Laboratory control samples provided from NET spiked at 1 mg/L prior to digestion are shown in Table VI.

Table VI. Predigestion Spikes
% Recovery of 1 mg/L Spike

Sample	As	Se
4-19E	99.3	102
4-23B	109	108

The percentage recovery falls between 99.3 to 108% well within the EPA limits of 80-120% recovery of the expected value.

Post digestion spikes were added to TCLP samples at a low level and high level to confirm the method performance with a variety of matrices. The results are shown in Table VII and demonstrate acceptable recoveries in all cases.

Table VII. Post Digestion Spike Results

Sample	Arsenic		Selenium	
	Spike Added (mg/L)	% Recovery	Spike Added (mg/L)	% Recovery
3-16M LCS	1.0	107	0.5	99.0
333130	1.0	106	0.5	102
333131	1.0	95.5	0.5	98.6
333132	5.0	89.8	1.0	105
333133	5.0	98.1	1.0	93.2

Productivity is an important component of an environmental analysis and TCLP digests contain a fairly high amount of dissolved material. The stability of a standard was monitored during an autosampler analysis of 160 samples at regular intervals. The 5-mg/L As standard was recovered within ±5% of the expected value during the more than 2 hour run. The 1-mg/L Se standard was recovered within ±6% of the expected value.

CONCLUSIONS

Modern flame atomic absorption can meet the analytical requirements of a TCLP-digest analysis. The method detection limits are more than ten times below the MCL where a decision is made. The linear range has been characterized and provides a useful range for the amounts of As and Se expected in a typical digested sample. The useful concentration could be extended with the use of nonlinear calibration curves. Recoveries in synthetic and real samples demonstrate freedom from interferences and the utility of the method.

Flame atomic absorption is less time-consuming and labor intensive than GFAA or hydride analysis. If used in the determination of the full suite of TCLP metals, it can help reduce the number of techniques required for the analysis of 8 metals. Consolidation of techniques can reduce the analysis time for a sample and improve turnaround time.

The EPA should be encouraged to include a flame atomic absorption method in SW-846 that includes these elements. Data demonstrating performance at low levels will give laboratories additional flexibility in choosing the method best suited for the analytical scenario and business requirements.

ACKNOWLEDGMENTS

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FIELD AND LABORATORY ANALYSIS OF MERCURY

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Mercury is a toxic element in many chemical forms whose analysis is hampered by its intrinsic mobility and volatility. There is a potential for loss of mercury during every stage of analysis including: collection, storage, sample preparation, and analysis. Collection and storage of the sample can have adverse effects on the mercury concentration and its species. Sample preparation can lose mercury during drying, digestion, and reactions of the mercury with vessel walls. Analyses of mercury are also hampered by mercury reacting or sticking to components of

the analysis system. Two approaches to solving these problems are evaluated: analysis of mercury through direct analysis of solid materials, requiring little or no sample preparation; and alternatively identification of sources of mercury loss during every stage of sample preparation[1].

Using an automated mercury analyzer (AMA-254, Milestone S.r.l), the solid or liquid sample can be directly analyzed without sample preparation. This technique is capable of on-line decomposition of solid samples in an oxygen furnace while collecting the mercury as an amalgam[2]. Alternatively, a liquid sample can be introduced into the oxygen furnace with the mercury collected as the amalgam. Subsequent release of the mercury into a detector leads to total mercury analysis at the nano-gram level. Due to the instruments rugid and compact design this instrument will be demonstrated as a field technique as well as being compatible for direct and decomposed samples. This is one of the first field capable instruments that has the potential to produce laboratory quality data in field environments.

Traditional sample preparation steps are evaluated for mercury quantitation. Drying of the sample in an oven, vacuum drying, and other techniques rely on different mechanisms for drying which show varying degrees of mercury loss. Typically, acid digestion of a sample in a closed vessel retains the mercury. Standard EPA SW-846 Methods 3052, 3051A, 3015A, and 3050B will be evaluated for their recovery of mercury[3]. Many analysis techniques require matrix conversion after digestion. This conversion may inadvertently unstabilize the mercury ions, leading to elemental loss This study will demonstrate that a mercury analyzer is capable of analyzing solid and liquid samples, either the collected sample or the acid digest solution, at trace levels in the laboratory or in the field. For these reasons, we have proposed EPA standard method 7473 for the analysis of solid and liquid samples either in the laboratory or the field.

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LEGALLY DEFENSIBLE SPECIATED MEASUREMENTS USING SIDMS

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A new method for the accurate determination of chemical species in the environment has been developed: Speciated Isotope Dilution Mass Spectrometry (SIDMS)[1]. This method utilizes isotopically enriched speciated spikes combined with isotope dilution to accurately determine and correct for specie transformations that occur in sample processing. The errors in the measurement are those that are limited by the ability of the ratio measurement and the equilibrium of the species. It was specifically developed to address the problems of accurately quantifying different species in complicated matrices. Additionally, it is a diagnostic tool for identifying both the error and bias inherent in specific methods of sampling process, storage, sample preparation, and measurement. SIDMS is applicable to most non-monoisotopic elements and extends to various oxidation states, organometallics, and molecular forms of species.

Data for species such as the highly reactive Cr(VI) and Cr(III) demonstrate the capabilities of this method. Currently these species are extracted from soil using Method 3060A and are analyzed with Method 7196A. Both extraction and analysis methods are biased in the speciation measurement process. SIDMS is capable of accurately correcting back to the original concentration samples in which > 50% degradation of Cr(VI) to Cr(III) during sample processing. These unique capabilities will eventually make it possible to establish standard speciation measurement methods and to develop standard sampling procedures for speciation. The method permits bias correction for degradation of the analyte species during collection, storage, extraction and chemical manipulation.

A new reference method based on Speciated Isotope Dilution Mass Spectrometry will be proposed to permit the implementation of this procedure. The objective is to provide a legally defensible reference method for measurements that have high degrees of uncertainty and error due to highly reactive analyses such as species.

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EPA METHODS 3015A AND 3051A: VALIDATION STUDIES FOR UPDATED MICROWAVE LEACH METHODS

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ABSTRACT

Validation studies for two recently proposed updated microwave leach methods (Method 3051A and Method 3015A) have been conducted. These updated methods allow for the inclusion of hydrochloric acid in the leaching mixture for complexation and stabilization of certain RCRA-regulated elements. Experimental details of the validation procedure are discussed. Experimental data demonstrates the effectiveness of inclusion of HCl in recovering analyses like antimony, silver and iron. Final validation data for Method 3051A is presented. Validation for Method 3015A has also progressed, with enhancements in "problem" analyte recoveries also being shown.

INTRODUCTION

Recently, updated versions of two microwave extraction methods have been proposed for acceptance by the EPA to be included in the next update of SW-846. These updated methods are EPA Method 3051A, an acid leach method for sediments, sludges, soils, and oils; and EPA Method 3015A, an acid leach method for aqueous samples. EPA microwave leach Method 3051 was proposed as an alternative to EPA hot-plate leach Method 3050 (B). However, because Method 3051 is limited to the use of nitric acid only, it does not recover certain "problem" analyses as completely as Method 3050, which also includes hydrochloric acid and hydrogen peroxide. Some examples of these "problem" analyses are antimony, silver, and high concentrations of iron. These metals, and others, are not stable in the strong oxidizing environment of nitric acid-only digestions. The updated microwave methods provide the analyst with options for using alternate reagent combinations to enhance the performance and appropriateness of the methods for certain analyses. For example, preliminary results have demonstrated that the recovery of antimony increases from 0% in an all-nitric digest to almost 80% in a digest using both nitric acid and hydrochloric acid[1]. The chemistry of the updated methods has been modified to accurately reproduce the chemistry of the standard EPA hot-plate methods. The option to combine hydrochloric acid with the nitric acid in the optimized ratio of 9 mL HNO_3 to 3 mL HCl, is provided to enable the complexation and stabilization of elements such as aluminum, antimony, iron, and silver, when needed. This acid ratio has demonstrated optimum recoveries for all 26 RCRA-regulated elements. It increases recoveries of certain analyses without sacrificing the recoveries of other RCRA-regulated metals. Data demonstrating the need for inclusion of HCl in the microwave methods will be presented. The enhanced performance of this updated microwave leach method will be demonstrated for a variety of matrices, namely a sediment sample (SRM 2704, Buffalo River sediment), a sludge sample (a 1-to-1 mixture of SRM 2704 and SRM 1634c, Trace Elements in Fuel Oil), a soil sample (SRM 4355, Peruvian soil), and an oil sample (SRM 1084a, Wear Metals in Oil). Final validation for Method 3051A will be demonstrated by comparing the recovery data from these matrices using the all-nitric Method 3051 digest with the data using the nitric-and-hydrochloric mixed acid Method 3051A digest.

EPA Method 3015 is also a nitric acid-only leach, and does not allow for inclusion of HCl for complexation and

stabilization of certain analyses. In proposed EPA Method 3015A, the inclusion of HCl along with HNO_3 in the leaching acid mixture should overcome the complexation difficulties demonstrated in nitric-only leaches. Digestions using both the nitric-only Method 3015 and those using the nitric-and-hydrochloric mixed acid Method 3015A are compared. The results of including HCl in the leaching mixture for aqueous samples parallel those for solid samples. Recoveries of problem analyses increase while recoveries of other analyses are preserved. In addition, validation for the elements boron, mercury, and strontium was not provided for Method 3015. By analyzing for these elements in the current validation study of EPA Method 3015A, possible validation for these three RCRA-regulated elements will also be examined.

EXPERIMENTAL

The validation study for Method 3051A consisted of an initial re-optimization of the acid ratio to be used in the method. This was accomplished by comparing the results of four leaches of SRM 2704 (Buffalo River sediment) using different acid combinations. Initially, 10 mL HNO_3 only was used (10:0). Subsequent acid mixtures consisted of 9 mL HNO_3 + 1 mL HCl (9:1), 9 mL HNO_3 + 3 mL HCl (9:3), and 9 mL HNO_3 + 5 mL HCl (9:5). The goal of these acid ratio studies was to determine the optimum amount of HCl for maximum complexation while still achieving similar oxidizing strength of the acid mixture. Excessive dilution of the HNO_3 with the HCl would lead to incomplete recoveries of analyses, thus introducing another bias. Also, as the method must be valid for all 26 RCRA-regulated elements, it is not appropriate to optimize the method for only a few of the analyses (i.e. antimony or iron). The final method must be optimized for recovering all RCRA-regulated analyses. Elemental analysis was performed either by Inductively-Coupled Plasma-Mass Spectrometry (ICP-MS) or by Flame Atomic Absorption Spectrometry (FAAS).

Upon re-optimizing the acid ratio, the remainder of the validation study consisted of comparing the recoveries using the nitric-only Method 3051 versus using the nitric-and-hydrochloric mixed acid 3051A. Comparisons were made using the three remaining types of matrices for which 3051 is applicable, namely sludge, soil, and oil. Recoveries using the 10:0 versus using the 9:3 digests were compared, as well as recoveries from the hot-plate Method 3050. Data for the hot-plate method was taken from the original report for the validation of Method 3050 and 3051 [2]. The results demonstrated by the 9:3 3051A leach method suggest that it is a valid alternative leach method, achieving comparable results to those of the hot-plate leach Method 3050.

The validation experiments for the aqueous leach Method 3015A paralleled those for the solid sample leach method 3051A. The samples used were simulated wastewater samples prepared just prior to the leach digestion. Simulated wastewaters were used because of the lack of appropriate standard reference materials for wastewater type matrices. Additionally, the validation study for the original aqueous leach method 3015 was performed using a simulated wastewater. The wastewaters were prepared from the same SRM's used in the 3051A validation study, combining approximately 0.35 grams of the solid samples with 45 mL double-deionized water in each digestion vessel just prior to acid addition. The SRM's used were SRM 2704 (Buffalo River sediment), SRM 4355 (Peruvian soil), SRM 1084a (Wear Metals in Oil), and a 1-to-1 mixture of SRM's 2704 and 1634c (Trace Metals in Fuel Oil).

Digestions were performed for the wastewater samples using either 5 mL HNO_3 only (Method 3015) or 4 mL HNO_3 and 1 mL HCl (3015A). Elemental analysis was performed using either ICP-MS or FAAS. The recoveries of analyses using each acid mixture were compared. Once again, the addition of hydrochloric acid provided better complexation and stabilization of problem analyses while preserving the recoveries of other analyses. Also, the proposed Method 3015A has demonstrated that it is effective for the elements boron, mercury, and strontium. Validation for these three RCRA-regulated elements was not provided in the original validation of Method 3015.

RESULTS AND DISCUSSION

The following figures illustrate the data for the re-optimization of the 3051A acid ratio. As discussed, analyses were leached from SRM 2704 using these four acid ratios: 10:0, 9:1, 9:3, and 9:5. For the "problem" analyses shown, the acid ratio of 9:3 provides optimum complexation. The most dramatic case is for antimony, whose recovery increases from approximately 0% in the nitric-only digest to greater than 80% in the 9:3 digest. Other recovery increases are shown for iron and vanadium. The remaining figures demonstrate that the 9:3 mixture also retains the overall oxidizing strength of the acid mixture, hence preserving the recoveries of the other analyses.

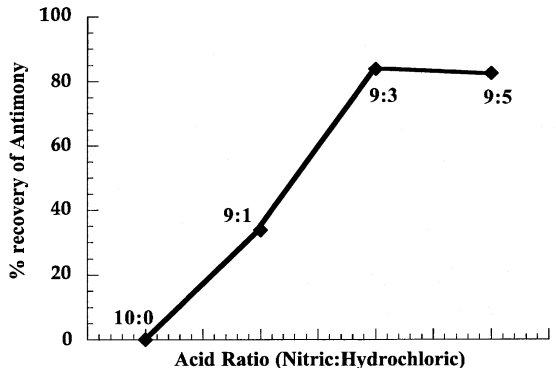

Figure 1. Recovery of antimony vs. acid ratio used in leach digest.

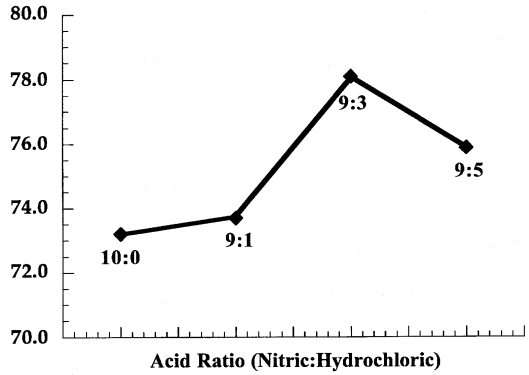

Figure 2. Recovery of iron vs. acid ratio used in leach digest.

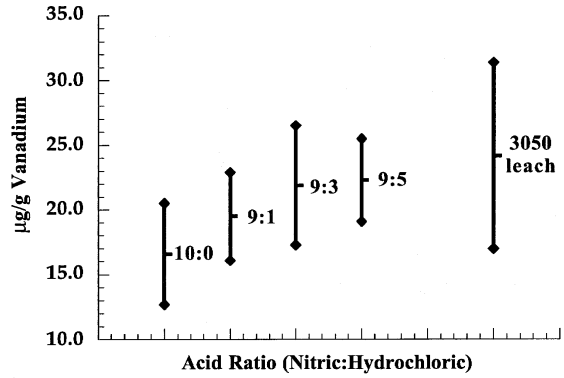

Figure 3. Recovery of vanadium vs. acid ratio used in leach digest.

The above figures illustrate the increased recoveries of Sb, Fe, and V as HCl is added to the leaching mixture. Also demonstrated is the optimization of the acid ratio at 9 mL HNO_3 to 3 mL HCl. The analyte recoveries using the 9:5 acid ratio either decrease slightly, or provide no further recovery enhancement. This indicates that the 9:5 ratio is not as appropriate and that the 9:3 ratio offers the highest level of complexation without compromising the overall oxidizing strength of the acid mixture. The figures below also illustrate that the oxidizing strength is not lowered, as the recovery of non-biased analyses remains virtually the same when the 9:3 ratio is used.

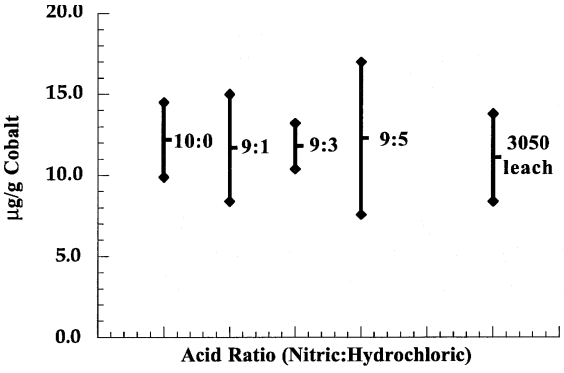

Figure 4. Recovery of cobalt vs. acid ratio used in leach digest.

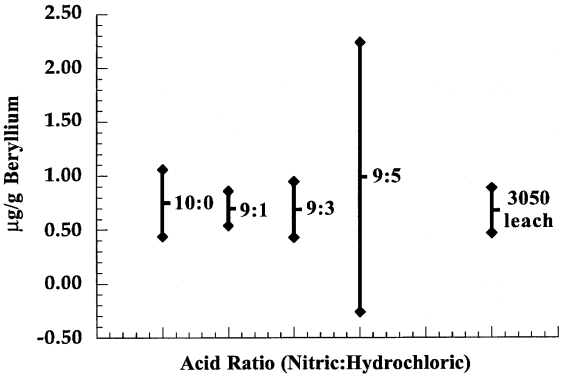

Figure 5. Recovery of beryllium vs. acid ratio used in leach digest.

With the acid ratio, re-optimized at 9:3 using leach data from SRM 2704, the remainder of the validation study continued. Three remaining matrices, a soil (SRM 4355), an oil (SRM 1084a), and a simulated sludge (1-to-1 mixture of SRM 2704 and SRM 1643c), were digested using both the nitric-only Method 3051 and the nitric and hydrochloric mixed-acid Method 3051A. The data demonstrates the increased recoveries of biased analytes with preservation of

other analyte recoveries. The recoveries for Method 3051A are comparable to those of Method 3050, the hot-plate digestion that uses nitric acid, hydrochloric acid, and hydrogen peroxide. The following tables will demonstrate these improved recoveries using the microwave leach and their comparability to the recoveries using the hot-plate leach. Analyte recoveries are expressed in µg/g unless otherwise indicated.

Table 1. Comparison of analyte recoveries from SRM 4355 (Peruvian Soil) for alternative EPA leach methods (µg/g ± 95% confidence interval, unless otherwise noted)

Element	3051	3051A	3050	Total
Sb	0.202 ± 0.347	10.4 ± 0.826	---*	14.8 ± 0.76
V	65.5 ± 2.12	123 ± 7.78	81.4 ± 17.3	(151)**
Fe	2.8 ± 0.09%	3.28 ± 0.18%	2.98 ± 0.342%	4.45 ± 0.19%
Cd	1.11 ± 0.31	1.09 ± 0.27	1.03 ± 0.202	(1.50)**
Cr	14.6 ± 0.68	18.7 ± 0.68	17.1 ± 2.37	28.9 ± 2.8
Pb	126.7 ± 9.3	130.6 ± 5.1	131 ± 14.2	129 ± 26

* Antimony was not analyzed in the 3050 validation study.
** Values in parenthesis are not certified concentrations and are for reference only.

The first three elements demonstrate the enhanced recoveries upon adding 3 mL HCl to the acid mixture. Especially dramatic is the enhancement for antimony, showing only 1% recovery for the nitric-only digest but rising to approximately 70% upon adding 3 mL HCl. The remaining entries in the table demonstrate that recoveries of other analyses are preserved when the mixed acid is used.

Table 2. Comparison of analyte recoveries from SRM 1084a (Wear Metals in Oil) for alternative EPA leach methods (µg/g ± 95% confidence interval)

Element	3051	3051A	Total
Cr	91.2 ± 4.8	93.5 ± 3.1	98.3 ± 0.8
Al	98.0 ± 4.8	101.1 ± 6.6	(104)*
Mg	92.9 ± 6.3	96.6 ± 4.9	99.5 ± 1.7
Ni	93.6 ± 2.0	92.9 ± 4.6	99.7 ± 1.6
Cu	92.2 ± 4.2	91.8 ± 3.6	100.0 ± 1.9
Mo	90.3 ± 2.4	93.2 ± 3.4	100.3 ± 1.4
Ag	92.5 ± 3.1	96.4 ± 7.0	101.4 ± 1.5
Pb	99.8 ± 6.5	100.8 ± 7.8	101.1 ± 1.3

* Values in parenthesis are not certified concentrations and are for reference only.

The recoveries of all analyses for the oil matrix are similar for both acid combinations. However, most recoveries for the mixed acid digest are slightly higher than the nitric-only digest.

Table 3. Comparison of analyte recoveries from "simulated sludge" mixture of ~ 0.25 g SRM 2704 (Buffalo River Sediment) and ~ 0.25 g SRM 1634c (Trace Metals in Fuel Oil) for alternative EPA leach methods (µg/g ± 95% confidence interval)

Element	3051	3051A	3050
Cr	49.7 ± 3.73	44.7 ± 2.99	42.3 ± 6.04
Co	7.69 ± 1.85	6.18 ± 1.18	5.89 ± 1.43
Ni	32.3 ± 6.06	29.3 ± 9.60	31.4 ± 5.56
Mo	1.53 ± 0.20	1.71 ± 0.61	<2.5
Ag	0.209 ± 0.067	0.216 ± 0.191	<4.0
Cd	1.81 ± 0.478	1.87 ± 0.431	1.67 ± 0.196
Pb	83.4 ± 7.05	78.2 ± 6.41	76.4 ± 8.17

Again, we see the, preservation of recoveries for the mixed acid, as well as good agreement with the hot-plate 3050 recoveries. This simulated sludge matrix is highly heterogeneous, which may account for the greater variation seen in the data for this matrix.

The data presented serve as the final results of the validation study for EPA Method 3051A. It has demonstrated effectiveness for enhancing the recovery of "problem" analyses from four different matrix types while at the same time preserving the recovery of the remaining analyses. It has demonstrated comparable recoveries with hot-plate Method 3050.

Validation for proposed Method 3015A proceeded using similar experimental procedures. Comparisons were made using an all-nitric digest (5 mL HNO_3) versus using a nitric-and-hydrochloric mixed acid digest (4 mL HNO_3 + 1 mL HCl) on four "simulated-wastewater" matrices. These matrices consisted of approximately 0.35 grams of solid sample mixed with 45 mL double-deionized water (per digestion vessel) immediately prior to acid addition and digestion. The solid samples used parallel those used for the 3051a study, namely SRM 2704, SRM 4355, SRM 1084a, and the mixture of SRM 2704 and SRM 1634c. The mixed acid digest should complex and stabilize certain problem analyses, leading to increased recoveries of these analyses. The leach recovery data for the 2704-wastewater and the 4355-wastewater are presented in the following tables.

Table 4. Comparison of analyte recoveries from "simulated wastewater" mixture of SRM 2704 (Buffalo River Sediment) and double-deionized water for EPA leach methods (µg/g ± 95% confidence interval)

Element	3015	3015A	Total
V	32.6 ± 4.83	51.6 ± 2.23	95 ± 4
Mo	2.07 ± 1.71	2.92 ± 1.19	---*
Be	0.85 ± 0.116	0.95 ± 0.170	---*
Co	11.0 ± 0.47	11.9 ± 0.45	14.0 ± 0.6
Zn	392 ± 5.6	406 ± 8.7	438 ± 12
Ni	38.6 ± 4.24	39.9 ± 4.64	44.1 ± 3.0
Ag	0.33 ± 0.037	0.36 ± 0.115	---*

* The total concentration of this analyte is not certified for SRM 2704.

The results for this "sediment-wastewater" matrix demonstrate the enhanced recovery for certain analyses (i.e. vanadium, molybdenum, and beryllium) and similar recoveries for other analyses using the nitric-and-hydrochloric mixed acid Method 3015A digest.

Table 5. Comparison of analyte recoveries from "simulated wastewater" mixture of SRM 4355 (Peruvian Soil) and double-deionized water for EPA leach methods (µg/g ± 95% confidence interval)

Element	3015	3015A	Total
V	33.9 ± 3.57	38.7 ± 5.94	(151)*
Sb	3.70 ± 0.37	5.20 ± 0.67	14.3 ± 2.2
Ag	1.31 ± 0.16	1.62 ± 0.14	(1.9)*
Co	10.5 ± 0.42	10.4 ± 0.51	14.8 ± 0.76
Zn	415 ± 16.2	418 ± 16.4	368 ± 8.2
Ni	11.1 ± 1.44	10.8 ± 2.40	(13)*
Mo	0.98 ± 0.07	1.09 ± 0.13	(1.7)*
Pb	135 ± 4.6	136 ± 4.7	129 ± 26

* Values in parenthesis are not certified concentrations and are for reference only.

This set of digestions also demonstrates that inclusion of HCl enhances the recovery of certain analyses (V, Sb, Ag) while preserving the recovery of other analyses.

Two additional types of simulated wastewater matrices are currently in the digestion and data acquisition process for the validation of proposed Method 3015A. These are the oil-wastewater (SRM 1084a and DDI water) and the "sludge"-wastewater (SRM's 2704 and 1634c and DDI water). It is expected that similar enhancements in "problem" analyte recoveries, as well as preservation of other analyte recoveries will be demonstrated by the mixed acid digest.

SUMMARY

The final validation data for proposed EPA Method 3051A has been given. The data shows the effectiveness of including HCl in the leaching acid mixture for enhancing the recoveries of "problem" RCRA-regulated metals such as antimony and iron. This mixed acid also shows comparable recoveries for other non-biased analyses. This updated microwave leach method minimizes the reagent-induced biases between the two alternative leach methods 3050 (hot-plate) and 3051 (microwave). This study also demonstrates the effectiveness of adding HCl in Method 3015A for the leaching of aqueous samples (wastewaters). Data for digestions of two matrix types using the mixed-acid leach Method 3015A has demonstrated similar enhancements in recoveries. Digestions for the remaining two wastewater matrix types are in progress, with similar enhancements in the recoveries of problem analyses expected.

A paper explaining the depth of the information in this validation study is in preparation. The discussion will include chemical equilibria and other data documenting the reactions and summarizing these results.

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LONG-TERM STABILITY OF ICP SPECTRAL REGISTRATION BY MANAGEMENT OF THE MODELS: APPLICATION TO QUANTITATION USING MULTIVARIATE ANALYSIS

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ABSTRACT

A mathematical technique is described which provides constancy of multivariate analysis models in ICP emission spectroscopy. Once an analysis protocol has been developed, the profiles of all active spectral components are saved in an archive. Through the use of well-defined spectral standards, this archive can be mathematically transformed at any time to correspond to any new conditions for an instrument. This results in analytical measurements which are completely independent of wavelength accuracy and wavelength calibration, providing not only exceptional long-term accuracy, but also complete transferability of ICP emission methods between instruments. Preliminary analytical results are shown which illustrate the utility of this technique.

INTRODUCTION

ICP emission spectroscopy is frequently applied for the determination of metals and metalloids in a wide variety of environmental matrices. Mathematical procedures are often required for the correction of background shifts and spectral overlaps, and are well represented in promulgated regulations (particularly the use of interelement correction factors, or IECs). While the IEC technique works properly in well defined circumstances, it does not lend itself well to all situations[1]. Some examples are: 1) IECs require the careful selection of background correction points, which often requires a lengthy and somewhat subjective selection process; 2) slight differences in plasma conditions in the course of an analysis can render the IEC factors nonviable due to changing line intensity ratios; 3) IEC factors are not always linear across all concentration ranges of an interferent.

Multivariate calibration techniques have been used in ICP emission spectroscopy for interference correction, particularly when array-based solid-state detectors are used[2-4]. Multivariate calibration techniques provide

exceptional capacity for resolving spectral overlaps in a straightforward manner[2], and provide improved analytical precision through the process of redundant sampling[3]. Numerous suggestions for improved multivariate methods have been made during the most recent public comment period for ICP emission Method 6010B. These multivariate methods are expected to be given strong consideration in the final version of the Method.

All spectroscopic interference correction techniques require that the mathematical correction models accurately reflect current wavelength axis conditions. In the case of ICP emission analyses, this is independent of whether IECs or multivariate techniques are used, and is the case for array-based and non-array detection. For example, if the spectral peak for an interferent occurs at one wavelength on day one and at a slightly different wavelength on day two, the interference correction models must be redefined (or "rebuilt") to reflect these new conditions. For this reason, the true requirements for wavelength accuracy and stability are largely unrealistic.

A unique aspect of multivariate calibration is that spectral shape information is part of the correction model. By contrast, IECs employ wavelength position and relative intensities but ignore shape. Shape information provides certain advantages over IECs in terms of analytical precision, accuracy, etc., and have been presented[2, 4]. Of particular significance is that shape-based correction models can be mathematically "shifted" (through mathematical interpolation) once they are built. Thus, any particular wavelength condition can be accommodated. This capacity adds a number of hitherto unachievable features to ICP emission determinations. Interference correction models, and hence analytical accuracy, are independent of wavelength conditions. Furthermore, true transferability of analytical methods, leading to "turnkey" conditions, is achievable. Ultimately, the potential for transferring spectral archives between instruments, thus creating more "absolute" analytical conditions within ICP emission analyses, will be within reach.

Example data for the technique described above will be presented. This example was selected due to its strong illustration of the necessity for wavelength-model coherence. The implications for this technique will also be presented and discussed.

EXPERIMENTAL

A Perkin-Elmer Optima 3000 DV was used for all experiments. A cross-flow nebulizer (1 mL/min) in a Scott-type double-pass spray chamber was used for sample introduction, and plasma conditions were: 0.8 mL/min nebulizer flow; 0.5 mL/min auxiliary flow; 15.0 mL/min plasma flow; 1450 W power. All spectra were collected with the "normal" spectral resolution setting. For collection of the archival model spectra, slit-scanning was applied for the purposes of giving data densities high enough for interpolative model shifting. Conversely, the sample data to which the models were applied was not slit-scanned, as the additional data density is not required. All data were collected using ICP WinLab software operating on a Digital Pentium 100 MHz personal computer. All mathematical operations were programmed either in MatLab (MathWorks, Natick, MA) or in Microsoft Excel (Microsoft Corporation, Redmond, WA).

Pure component spectra (molybdenum, chromium, vanadium) were collected by diluting and analyzing appropriate concentrations of PE Pure standard solutions. These pure component solutions were then combined to provide an appropriate test sample. All data was collected at the spectral region of the Optima detector corresponding to 270.028-270.160 nm. A wavelength monitoring solution was prepared which provided isolated lines over representative regions of the detector surface. Upon running this wavelength monitoring solution, it is possible to calculate current wavelength registration status for any and all regions of the detector surface.

RESULTS AND DISCUSSION

The test sample's individual components (each scaled to approximate equal intensity) are shown at the top of Figure 1. This combination of spectral components, particularly at the levels shown, is most useful for this demonstration since very small changes in wavelength registration can lead to significant analytical errors (> 10%). The reason for this high degree of dependence on wavelength registration is that the analyte (vanadium) is positioned on the shoulder of interferent 1 (chromium), which in turn is positioned on the shoulder of interferent 2 (molybdenum), and is clearly shown at the bottom of Figure 1. Furthermore, both interferents are present at levels in great excess (1,000-fold) of the analyte. This spectral positioning results in an amplification of analytical error brought about by changes in wavelength registration.

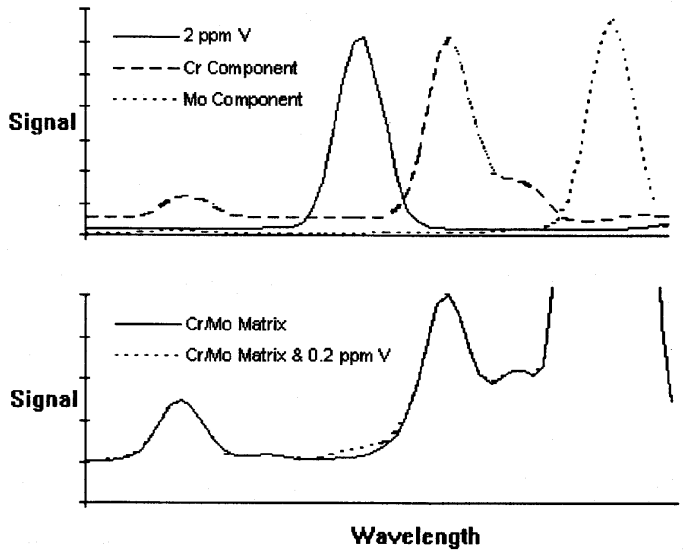

Figure 1. Vanadium, chromium and molybdenum components; individually and mixed.

Once the multivariate models were built with the pure spectral components, the mixed sample was analyzed and 100% recovery of the vanadium analyte was achieved. After 24 hours, the thermal conditions of the instrument were deliberately changed such that the wavelength registration was altered. The sample mixture was reanalyzed, and there was a dramatic loss in analyte recovery. At this time the degree of change in wavelength registration was measured by analyzing the wavelength monitoring solution. The pure archival spectra which make up the multivariate models were then "shifted" through interpolation, to an extent equal to that determined with the wavelength monitoring solution. For example, if the wavelength monitoring solution indicates a change in wavelength registration equal to *x*, then the archival component spectra are interpolatively "shifted" *x*. Once shifted, the multivariate models are rebuilt and used for subsequent samples. In the case of the sample described above, the shifting of the model spectra resulted in complete restoration of 100% analyte recovery. The results of these operations are shown in Table 1.

Table 1. Analytical results for vanadium in a highly interfering matrix; with and without wavelength registration compensation

Time	Condition	V recovery (%)
0 hours	---	100 %
24 hours	Induced drift	20 %
24 hours	Induced drift, shifted models	100 %

CONCLUSIONS

The use of this technique shows that it is possible to perform ICP emission analyses in a manner that is independent of the wavelength axis. Provided there is a means of determining current wavelength registration status, it is possible to update multivariate calibration models to the newer status, thus eliminating wavelength registration changes as a source of miscalculation. It is then possible to have true transferability of analytical methods, as well as the possible transfer of spectral component archives.

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ORGANIC

SOLID PHASE MICROEXTRACTION PREPARATIVE APPLICATIONS IN THE ANALYSIS OF ORGANIC COMPONENTS IN RADIOACTIVE WASTES

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ABSTRACT

The analytical chemistry of radioactive materials is complicated by numerous challenges. Primarily, the radiation level of material of interest may be sufficient to prohibit direct human contact. Additionally, materials used in the analysis may result in the production of mixed waste streams, which contain radionuclides in addition to hazardous wastes.

Solid Phase Microextraction (SPME), allows preparation of aqueous radioactive samples for analysis without the introduction of hazardous solvents while simultaneously achieving bulk separation from the radioactive components in the sample.

SPME does not generate conventional extraction solvent residues which are regulated by federal and state agencies as "hazardous" wastes These solvent wastes typically require treatment by incinerators operating under state and federal operating permits. Utilization of SPME eliminates the need for an auxiliary solvent, and the subsequent need for treatment and disposal of the solvent waste stream.

Isolation of organic materials from sample matrices by traditional extraction techniques has been extensively studied and developed to optimize analyte recovery and removal of interfering components. Radioactive components in samples may make these methods inappropriate or inapplicable. Methods which require lengthy contact with the sample may be prohibitive due to radiation exposure. SPME is a technique which reduces the effects of varying sample matrices and simultaneously achieves decontamination from the inorganic radioactive components.

The advantages of SPME as a sample preparation technique for the analysis of organic constituents in radioactive wastes are summarized, with data presented on the demonstration of these advantages

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SOLID PHASE EXTRACTION APPLICATIONS IN THE SAMPLING OF ORGANIC COMPONENTS IN RADIOACTIVE WASTES

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ABSTRACT

The recent advancement and maturation of the technology used in solid phase extraction leads to new and interesting opportunities in the analytical chemistry of radioactive sample matrices. Solid phase extraction (SPE) disks are readily applicable to the sampling of surface films in radioactive materials which must be accessed and handled remotely.

Remote sampling of unknown surface films from liquid radioactive waste storage tanks at the Savannah River Site using SPE disks has been shown to accomplish sampling objectives while reducing radiation exposure from the resulting samples.

Conventional sampling of surface films is limited by the ability to isolate the uppermost layer of the liquid into the sampling container. This inability is complicated by the hazards associated with exposures to highly radioactive materials. Sampling of these materials must be done remotely to minimize radiation exposure. Most often, this

remote handling is performed with mechanical devices such as cable activated sample grabbers, robotic instruments, or conventional "dip-bucket" grab samplers.

In order to selectively sample the uppermost layer of the liquid, the sampling device must be accurately positioned to avoid collecting lower layers of the tank contents. In most applications, grab sampling is adequate to capture an adequate quantity of the surface film for analysis. However, in the case of HLW storage tank sampling, obtaining an adequate sample is extremely expensive and time consuming because of the high radiation field.

A properly conditioned SPE disk has the ability to pass through the surface film of a liquid while selectively absorbing the organic species of interest. Samples obtained by this method sorb little of the aqueous liquid phase and can be sufficiently rinsed free of most of the radioactive contaminants to allow human contact while preserving the analysts ability to characterize the organic components in the film.

Recent experiences using SPE disk sampling methods at the Savannah River Site will be presented, summarizing the potential advantages of the technique.

A NEW PULSED FLAME PHOTOMETRIC DETECTOR FOR THE ANALYSIS OF PESTICIDES

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Many of the commonly pesticides contain sulfur, nitrogen, or phosphorus heteroatoms. In many of the pesticides more than one of the heteroatoms is present. There are a variety of detectors used to analyzed these pesticides, but all of these detectors have serious limitations. Flame photometric detectors suffer from interferences and quenching. Nitrogen - phosphorus detector have an unstable baseline and a short active element lifetime. Chemiluminescence detectors are very selective and sensitive but are complex and difficult to maintain at peak performance.

The pulsed flame photometric detector addresses these problems. The emissions from the flame are time resolved are well as wavelength resolved, so the detector is very selective. The increase in selectivity increased the sensitivity of the detector. The pulsed flame photometric detector is less affected by quenching than a conventional flame photometric detector. The detector is stable over a long period of time and requires minimum maintenance.

The pulsed flame photometric detector has dual channel data acquisition capability. Chromatograms for two different elements may be collected simultaneously from one injection and one column. This can be a very powerful tool for the confirmation of the identity of compounds containing more than one heteroatom.

The pulsed flame photometric detector offers significant advantages in selectivity, sensitivity, low maintenance, and the ability to acquire two independent chromatograms simultaneously.

EASIER AND FASTER GC/ECD ANALYSES OF PESTICIDES AND PCB'S

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(302) 633-8616

The generation of environmental data for pesticides and PCB's in various matrices can be a very time-consuming process for laboratories and engineering firms. In order to keep a GC/ECD system operating within control limits, precious analytical time must be spent on tasks such as: recalibrations, reinjection of samples, cleaning detectors,

reintegration of chromatography peaks, etc. All of these tasks take time away from running billable samples, and adversely affect the throughput or profitability of the lab.

Hewlett-Packard has developed a "new" micro-ECD that shows improved performance in several key areas: increased linear working range, increased sensitivity, more robust and less prone to contamination. The practical result of these features is that more GC analysis time can be spent analyzing billable samples instead of cleaning up and recalibrating the system. In addition, this detector is compatible with small id columns for fast chromatography.

Data will be presented from several laboratories to illustrate each of these features. In addition, some practical examples of how profitability (sample throughput) can be increased by the use of fast GC will be shown.

THE DEVELOPMENT OF AN ION CHROMATOGRAPHY METHOD TO MONITOR ORGANIC AND INORGANIC INDICATORS OF INTRINSIC BIOREMEDIATION AT HAZARDOUS WASTE SITES

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ABSTRACT

Many studies have demonstrated that the natural attenuation processes present at certain landfills and hazardous waste sites can effectively limit the migration of contaminant plumes. Indigenous microbial populations present in aquifer and soil systems often control contaminant migration by metabolic or co-metabolic degradation mechanisms. This process is defined as intrinsic bioremediation and has been utilized as a plume management strategy at many DOD, NPL, and state hazardous waste sites. During the intrinsic bioremediation process, contaminants are degraded to CO_2 and simple organic acids such as formic, acetic, and propionic. Electron acceptor compounds such as sulfate and nitrate are also involved and undergo reduction to sulfide and ammonia. The demonstration and implementation of intrinsic bioremediation depends on a comprehensive characterization of site conditions and a long term monitoring program that documents plume containment and reduction. The analysis of organic acid intermediates and electron acceptor compounds are important components of site characterization and monitoring programs.

This paper describes the development and validation of a single ion chromatography method for the analysis of low molecular weight organic acids and electron acceptor compounds in groundwater samples. Method 9056 cannot be used for all of these parameters due to limitations of the carbonate eluent. The method described uses a Dionex AS-11 column with a nonlinear sodium hydroxide gradient from 0.35 mM to 26.5 mM. Compounds analyzed by the method include: fluoride, chloride, formate, acetate, propionate, nitrate, sulfate, phosphate, pyruvate, fumarate, benzoate, and succinate. The method was validated by using deionized water, surface water, and groundwater as test matrices. Method detection limits for the compounds of interest range from 0.01 mg/l to 0.05 mg/l. Highly buffered groundwaters were found to interfere with the analysis of fluoride, acetate, and formate. This interference was removed by the use of a modified pretreatment resin cartridge (Dionex OnGuard-Ag™). A complete separation of the above analyses requires approximately 20 min.

The method was also tested on groundwater samples from a petroleum release, a landfill, and a spill of chlorinated solvents. Precision and accuracy results for MS/MSD analyses were 70% - 110%. Data for organic acids and electron acceptors were important components in the demonstration of intrinsic bioremediation at the above sites. Laboratories with ion chromatography equipment can readily implement the method. It provides similar performance to Method 9056 for common anions.

INTRODUCTION

In recent years, intrinsic bioremediation has become increasingly accepted as a plume management strategy for

organic compounds dissolved in groundwater. Intrinsic bioremediation is defined as the sum of naturally occurring biodegradation, dispersion, dilution, sorption, and chemical transformation reactions that effectively reduce the toxicity, mobility, and volume of a contaminant to levels that are protective of human health and the environment[1]. Protocols for data collection and analysis are available for fuels, aromatic hydrocarbons, and chlorinated solvents[2,3]. Central to the evaluation of natural attenuation is a rigorous monitoring program that demonstrates the reduction of a contaminant and the presence of indicators that reflect the degradation process. Inorganic anions such as nitrate and sulfate are important investigative parameters as they serve as electron acceptors in the anaerobic degradation process of chlorinated and aromatic solvents[4,5]. These compounds are reduced to ammonia and sulfide by microorganisms during anaerobic degradation. Other anions such as fluoride and chloride can be used as conservative ions to determine diffusion and dispersion[6]. Low molecular weight organic acids such as acetate, formate, and propionate are also important indicators of the degradation process as they represent intermediates in the metabolic and co-metabolic processes that convert organic solvents to CO_2 and CH_4 [2,3,5]. While physical/chemical mechanisms such as volatilization, sorption, and dispersion can account for the reduction of contaminants in groundwater, the presence of organic acids provides direct evidence of the biological degradation process.

Inorganic anions can be analyzed by a variety of wet chemical and ion chromatography methods[7]. Ion chromatography can also be used for the measurement of low molecular weight organic acids[8,9,10]. The ion chromatography method listed in SW-846. Method 9025, cannot be effectively used for the determination of organic acids due to limitations of the carbonate eluent. This paper describes the development of an ion chromatography method that can be used for the simultaneous analysis of the inorganic anions and organic acids commonly measured in the demonstration of intrinsic bioremediation at hazardous waste sites. The method includes a sample pretreatment step that removes matrix interference problems related to high levels of chloride and alkalinity. Data for precision, accuracy, and method detection limit are presented along with information related to the application of ion chromatography methods to the demonstration of intrinsic bioremediation.

EXPERIMENTAL

The ion chromatography method was developed on a Dionex DX-500 equipped with an electrochemical detector operated in conductivity mode. A complete description of instrumental conditions is presented in Table 1. The NaOH gradient described in the Dionex Technical Bulletin[11] was modified to provide an improved separation of early and middle eluting analyses. The gradient program is described in Table 1.

A chromatogram showing the separation of the inorganic anions and organic acids is shown in Figure 1. While acceptable chromatography was obtained in samples with low alkalinity, the concentrations of carbonate and bicarbonate typically found in natural waters resulted in the loss of resolution of fluoride, acetate, formate, and propionate. The concentration of carbonate species in natural water is sufficient to change the acid/base chemistry of the dilute NaOH eluent at the beginning of the gradient program. Carbonate also elutes as an interfering peak in the middle of the analysis. A typical chromatogram showing the effect of carbonate/bicarbonate on the analysis of organic acids and inorganic anions is shown in Figure 2. High levels of chloride can also interfere with the analysis since this anion is often present in the 100 mg/l range while organic acids are found at 50 µg/l concentrations.

In order to eliminate the interference problems related to sample alkalinity and chloride, a modified sample pretreatment system was developed. The use of pretreatment cartridges for the removal of interferences in ion chromatography has been described previously[12]. Cartridges are commercially available for the removal of interferences from high levels of carbonate, chloride, sulfate, and organic compounds. To remove carbonate/bicarbonate and chloride interferences, two cartridges used in series would be required. A silver resin cartridge would first be required to remove chloride interferences. The sample would then be treated with a protonated resin cartridge to reduce the pH to 4 and be sparged with helium to remove CO_2. These cartridges are generally viewed as disposable items and must be added to the cost of analysis. As a component of the development of this method, a Dionex OnGuard-Ag™ cartridge was modified by rinsing with a small amount of 5% nitric acid. During this process the resin becomes partially protonated while retaining some sites substituted with silver. The preparation procedure for the modified resin is described in Figure 3. Cartridges can be used for multiple injections by rinsing with 150 ml of deionized water at a rate of 10 ml/min. It is recommended that the cartridge be regenerated after 10 samples according to the procedure outlined in Figure 3. We have noticed no degradation of cartridge performance or carry over when this sequence is followed. The performance of the modified cartridge was

compared to the Dionex OnGuard-H™ which functions as a protonated resin to remove carbonate interferences.

Table 1. Instrumental Conditions for the Analysis of Inorganic Anions and Organic Acids

Instrument	Dionex DX-500			
Detector	Dionex ED40 Electrochemical Conductivity Mode			
Column	AS-11 with AG-11 Guard			
Suppressor	ASRS-1			
Injection	100 µl			
Flow	2 ml/min NaOH			
Gradient	0.35 mM - 26.5 mM			
	Eluent A Deionized			
	Water Eluent B 100 mM NaOH			
	Eluent C 5 mM NaOH			
	Time	%A	%B	% C
	0 min	93	0	7
	2 min	93	0	7
	8 min	30	0	70
	20 min	7	23	70
	21 min	93	0	7
Analytes (concentration range)	Chloride			0.2 to 10 mg/l
	Fluoride			0.02 to 1 mg/l
	Nitrate			0.02 to 1 mg/l
	Sulfate			0.2 to 10 mg/l
	Phosphate			0.02 to 1 mg/l
	Acetate			0.02 to 1 mg/l
	Formate			0.02 to 1 mg/l
	Propionate			0.02 to 1 mg/l
	Benzoate			0.05 to 5 mg/l
	Pyruvate			0.05 to 5 mg/l
	Glutarate			0.02 to 1 mg/1
	Succinate			0.02 to 1 mg/l
	Fumarate			0.02 to 1 mg/l

For sample analysis, the modified cartridge was rinsed with 10 ml of deionized water. A 10 ml aliquot of the sample was then passed through the cartridge. The first 3 mls were discarded and the remaining 7 mls were collected in a small test tube. The treated sample was the sparged with helium at a rate of 10 ml/min for 10 min to remove CO_2. After helium sparging, the sample was ready for analysis. A five point initial calibration curve was used for quantitation. Instrument calibration was verified by the analysis of a check standard after 10 samples. Analytical standards were prepared from neat materials at the concentration ranges are listed in Table 1. A 100 µg/l concentration was used for MS/MSD analysis. All standards and samples were pretreated with the modified cartridge.

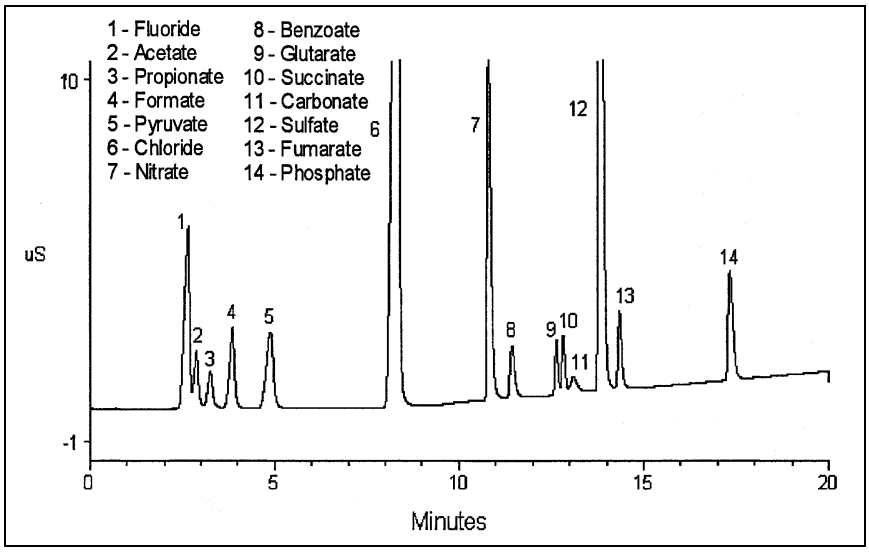
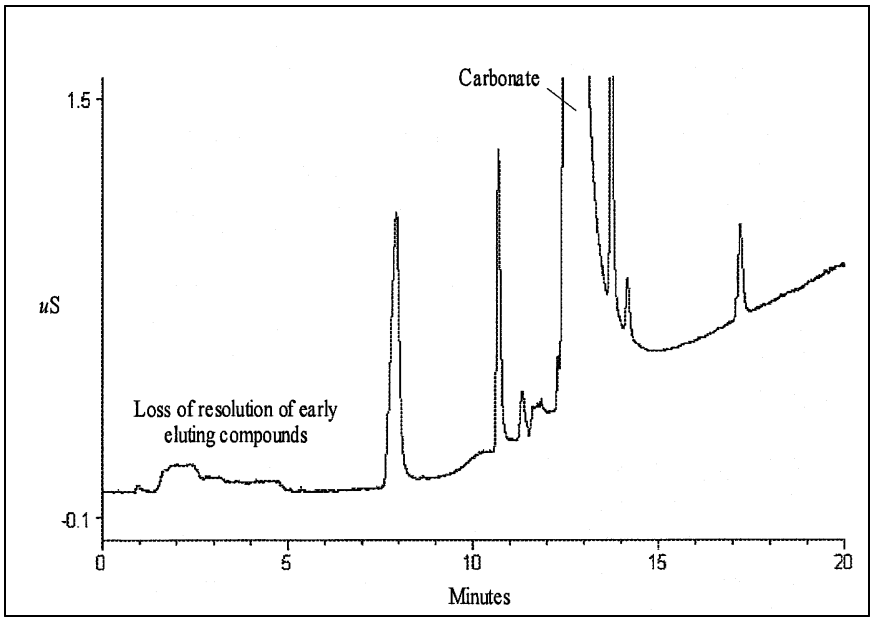


Figure 1. Standard chromatogram of inorganic anions and organic acids.



Since the cartridge removes chloride, a separate analysis of an untreated sample is required for this parameter. Method detection limits were calculated by the analysis of 7 replicates as described in 40CFR136. Method detection limit evaluations were performed in reagent water, surface water, and groundwater. Samples from hazardous waste sites were frozen in the field using dry ice to prevent the degradation of organic acids.

Figure 2. The effect of high carbonate/bicarbonate concentrations on early eluting inorganic anions and organic acids.

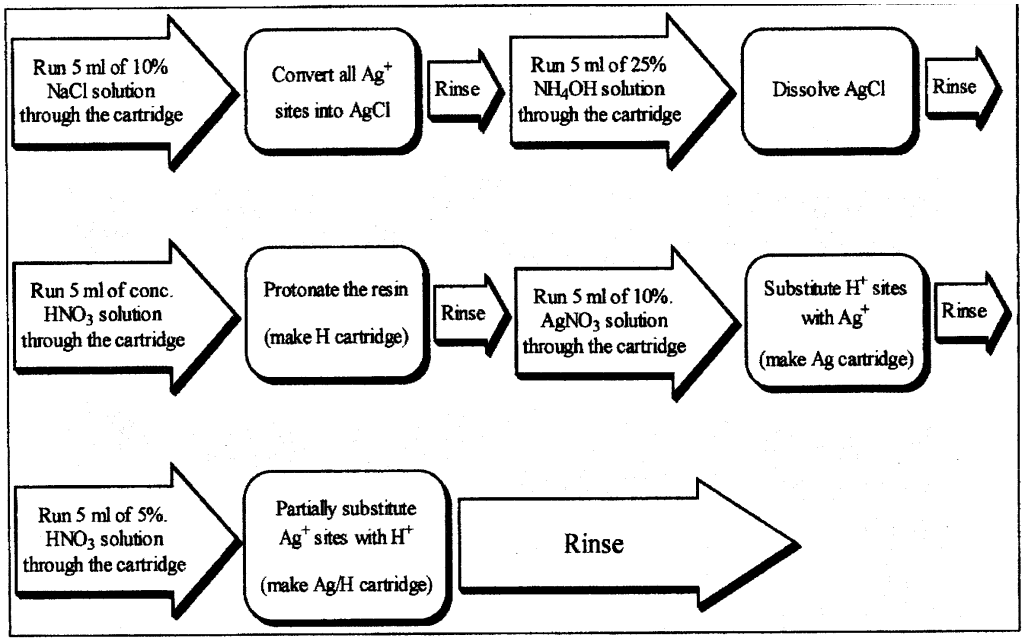


Figure 3. Preparation and regeneration sequence for modified sample pretreatment cartridges. Sequence begins with a Dionex OnGuard-AG™ cartridge.

RESULTS AND DISCUSSION

The effects of the cartridge pretreatment on a groundwater sample spiked with organic acids is shown in Figure 4. Severe peak broadening occurs during the analysis of the untreated sample due to the interaction of the natural carbonate buffering system with the NaOH eluent. The Dionex OnGuard-H™ cartridge improves the resolution however; fluoride and acetate are not adequately separated. The modified cartridge provides a separation of acetate and fluoride that can be quantitated by tangent skimming algorithms. The mechanism for the improved performance of the modified cartridge has not been determined. It is possible that the addition of silver to the resin results in sites that can decompose humic materials that are common in groundwater and can also influence the resolution of early eluting compounds.

The results of method detection limit (MDL) studies are summarized in Table 2. All analyses reported received cartridge pretreatment. With the exception of fluoride, MDLs for reagent water were comparable to results obtained in surface water and groundwater. The fluoride result was influenced by background concentrations found in the water samples. Based on the MDLs, reporting limits were set at the level of the lowest standard.

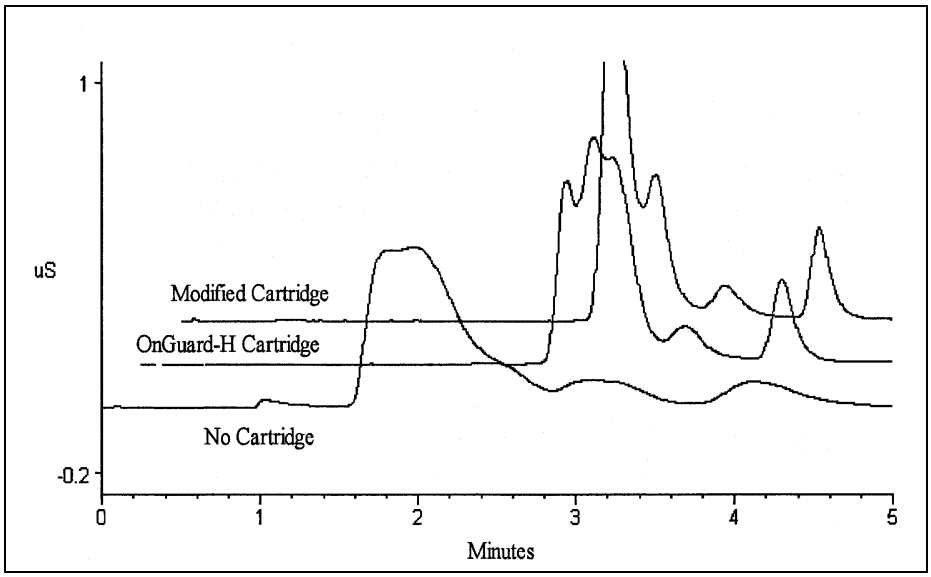


Figure 4. The effect of cartridge pretreatment on the chromatographic resolution of fluoride, acetate, formate, and propionate in a groundwater sample spiked with organic acids.

Table 2. Results of the Method Detection Limit Study

Parameter	Spiked Concentration	Reagent Water		Groundwater		Surface Water	
		S.D.	MDL (mg/l)	S.D.	MDL (mg/l)	S.D.	MDL (mg/l)
Fluoride	0.02 mg/l	0.002	0.005	0.006	0.020	0.008	0.024
Acetate	0.02 mg/l	0.001	0.003	0.003	0.009	0.002	0.007
Propionate	0.02 mg/l	0.002	0.006	0.002	0.007	0.001	0.004
Formate	0.02 mg/l	0.002	0.006	0.003	0.008	0.002	0.006
Pyruvate	0.05 mg/l	0.003	0.010	0.005	0.015	0.005	0.017
Nltrate	0.02 mg/l	0.002	0.008	0.003	0.008	0.002	0.006
Benzoate	0.05 mg/l	0.002	0.007	0.003	0.009	0.004	0.012
Glutarate	0.02 mg/l	0.001	0.004	0.002	0.007	0.001	0.004
Succinate	0.02 mg/l	0.001	0.004	0.001	0.004	0.002	0.005
Fumarate	0.02 mg/l	0.002	0.006	0.003	0.010	0.001	0.003
Phosphate	0.02 mg/l	0.002	0.005	0.001	0.002	0.005	0.017

S.D. = Standard Deviation
MDL = Method Detection Limit calculated from 7 replicates as described in 40CFR136.

Samples from ten hazardous waste sites were analyzed for inorganic anions and organic acids using the ion chromatography method with cartridge pretreatment. These sites represented a variety of solvent and fuel releases and a municipal solid waste landfill. The precision and accuracy results for MS/MSD analyses performed on groundwater samples from these sites are summarized in Table 3. Duplicate precision varied from ± 12 % to ± 20 % for the target analyte list. Accuracy varied from 70% to 100% for all analyses except phosphate. During sample analysis, it was noted that high levels of ferrous iron caused the cartridge to retain phosphate. Some of the ferrous iron was retained by the cartridge, resulting in active sites that would precipitate phosphate. Samples with visible ferrous iron require the use of a freshly prepared cartridges for improved recovery of phosphate. After analysis, the cartridge must be regenerated.

Sample chromatograms for three of the hazardous waste sites are included in Figures 5, 6, and 7. The sample represented in Figure 5 was from a solid waste landfill that had groundwater plume with low levels of chlorinated solvents. Formic acid was detected in a number of wells at the site using this method. Gas chromatographic analysis detected dissolved methane in the same wells. The oxidation of methane to formic acid is the result of a microbial process that produces an enzyme that degrades chlorinated solvents[3,5]. These results were used along with a groundwater model to demonstrate that intrinsic bioremediation was occurring at the site and that the process was reducing and containing the chlorinated solvents in the groundwater. The sample shown in Figure 6 was from a

release of 1,1,1-trichloroethane used for parts washing. Acetate, dichloroacetic acid, and trichloroacetic acid were detected in the groundwater plume. These compounds are known microbial metabolites and chemical hydrolysis products of 1,1,1-trichloroethane[4]. These analytical results and groundwater modeling were used to show that the plume was contained by intrinsic bioremediation and that continued operation of the groundwater treatment system at the site was not necessary. In a similar manner, the presence of acetate and large reductions in nitrate and sulfate in the groundwater plume of a toluene release (Figure 7) were used to show that indigenous microbial populations were actively degrading toluene in the aquifer.

Table 3. Precision and Accuracy Data for Organic Anions and Organic Acids

Parameter	Spiked Concentration	Precision % RPD	Accuracy % Recovery
Fluoride	0.50 mg/l	± 15 %	88 ±12%
Acetate	0.10 mg/l	± 18 %	86 ±15%
Propionate	0.10 mg/l	± 17 %	89 ±11%
Formate	0.10 mg/l	± 19 %	84 ±14%
Pyruvate	0.25 mg/l	± 20 %	88 ±10%
Nitrate	1.0 mg/l	± 12 %	92 ±10%
Benzoate	0.25 mg/l	± 15 %	85 ±12%
Glutarate	0.10 mg/l	± 14 %	88 ±14%
Succinate	0.10 mg/l	± 18 %	89 ±15%
Fumarate	0.10 mg/l	± 16 %	84 ±12%
Phosphate	0.50 mg/l	± 13 %	83 ±15%

RPD = Relative Percent Difference
% RPD and % Recovery are based on the average of 10 MS/MSD pairs

SUMMARY

The cartridge pretreatment step was an essential component of the ion chromatography method described in this paper. These cartridges can be regenerated and used for multiple analyses, which reduces the cost of consumables. The method yields results that are comparable with other ion chromatography and wet chemical methods for inorganic anions. It also provides data on organic acids that are useful indicators of intrinsic bioremediation. Laboratories using this method can provide a "value added" service to consultants developing intrinsic bioremediation demonstrations at hazardous waste sites.

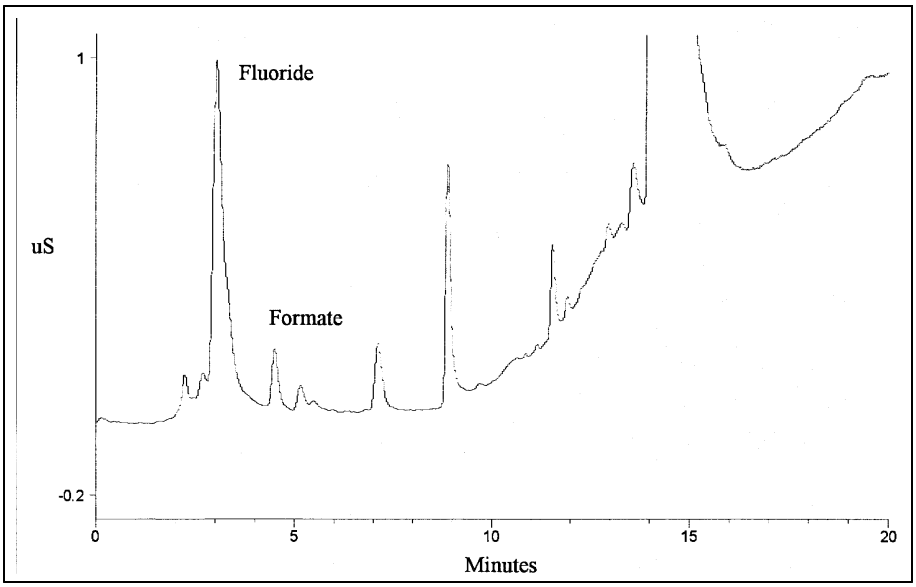


Figure 5. Sample from a solid waste landfill. The presence of formate was used to indicate methane oxidizing bacteria which can degrade chlorinated solvents.

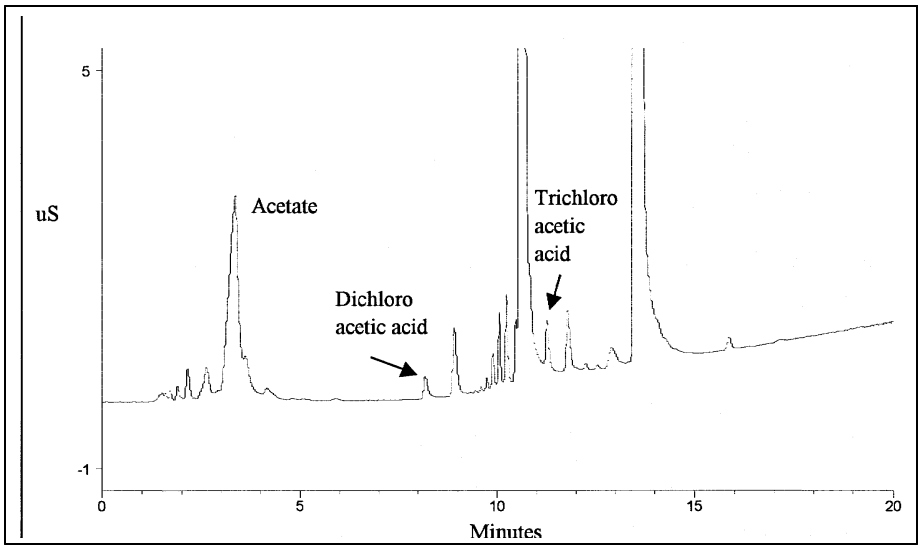


Figure 6. Sample from a release of 1,1,1-trichloroethane. The presence of acetate, dichloroacetic acid, and trichloroacetic acid were used to demonstrate intrinsic biodegradation.

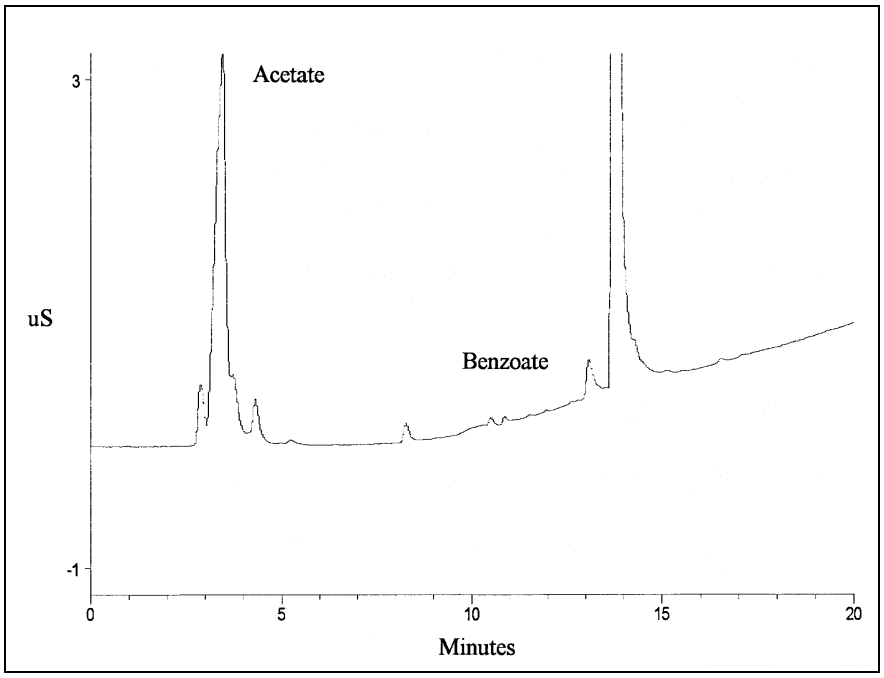


Figure 7. Sample from a toluene release. The presence of benzoate and acetate were used to document intrinsic bioremediation.

ACKNOWLEDGMENTS

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DETERMINATION OF CHLORINATED HYDROCARBON CONCENTRATIONS IN SOIL USING A TOTAL ORGANIC HALOGEN METHOD

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ABSTRACT

Total organic halogen screening has been used extensively to quantify chlorinated organic compounds in soil and is the basis for a new EPA method - SW-846 Draft Method 9078 "Screening Test Method for Polychlorinated Biphenyls (PCB) in Soil". This method uses an organic solvent to extract the chlorinated organics from the soil and a Florisil column to remove any inorganic chloride from the extract. The extracted chlorinated organics are then reacted with metallic sodium and the resulting chloride ions are quantified using a chloride specific electrode. Using a commercially available field test kit (the L-2000 PCB/Chloride Analyzer™), the ability of this technology to measure concentrations of chlorinated pesticides and chlorinated solvents in soil was determined. The compounds investigated were: DDT, pentachlorophenol (PCP), toxaphene, chlordane, trichloroethylene, tetrachloroethylene. The L2000 response was found to be linear over the range 0-100 ppm for all analyses and the method detection limits for these analyses ranged from a low of 2.7 ppm for Chlordane to a high of 4.8 ppm for Trichloroethylene. The average extraction efficiency varied from 39% for PCP to greater than 90% for the chlorinated solvents.

INTRODUCTION

The procedure for total organic chloride analysis was originally developed for use on PCB contaminated soils and the L2000 has been used extensively since 1990 for this purpose. There is a fairly large body of data amassed demonstrating the effectiveness of the L2000 at quantifying PCB in soil[1-7]. The underlying principals, however, are equally applicable to other chlorinated organic compounds such as chlorinated solvents and chlorinated pesticides, most of which are regulated in some way.

The L2000 has, in fact, been used to measure other chlorinated compounds in soil. In the majority of these cases the end user has undertaken to validate the feasibility of the technology for their particular use. This validation information is usually site specific and not available to the general public. With the growing interest in the remediation of other chlorinated compounds in soil and the increase in information requests for L2000 chlorinated organics applications, we have undertaken a validation program for these applications of the L2000.

The first and most important step in a total organic halogen analysis, or any chemical analysis, is the extraction of the chlorinated compounds, quantitatively, from the soil matrix. Performing this step in the field, quickly and reproducibly, on the broad range of soil matrices typically encountered is not a simple task. The solvent system must be designed to handle everything from wet clay to bone dry organic material. Unlike other field analytical methods, the organic chlorine is converted to inorganic chloride in a non-aqueous solvent. (The chloride ions are then extracted for quantification using a chloride specific electrode.) The solvent can, therefore, be easily tailored and optimized for a particular application.

The standard L2000 procedure uses a proprietary organic solvent that is polar enough to penetrate a wet clay matrix to solvate the PCB, but is itself not soluble in water. Water is added to the system to help partition the inorganic chloride into the water layer and away from the solvent layer. A Florisil column is used to remove any residual water and inorganic chloride from the extract.

This solvent/clean-up procedure has been shown to be effective at extracting PCB from most types of soils[1-7]. In some types of heavy clay soils with high water content, the extraction efficiency may be lowered and some of the more polar chlorinated organic compounds are removed by the Florisil column. Dexsil has developed an improved alternative two-step extraction procedure that has been shown to efficiently extract PCB from wet clay soils and can also be used on polar compounds such as PCP.[8] This system uses both a polar and a non-polar organic solvent combination and an aqueous/ organic solvent partition step. An optional Florisil clean-up step can be added if the analyte is not one of the polar chlorinated organics such as PCP or if PCP is considered an interfering compound.

In this study all of the non-polar compounds were analyzed using the standard solvent system and the alternative system was used to analyze the PCP contaminated soils. PCP was analyzed in this study using the alternative solvent system to demonstrate the flexibility of the L2000 solvent system.

Following the solvent extraction and clean-up (if necessary), the extract is reacted with metallic sodium in the presence of a catalyst. This removes the covalently bonded chlorine from the organic backbone producing chloride ions. The chloride is then extracted into an aqueous buffer and then quantified using a chloride specific electrode. The user can select a standard conversion factor for one of the typical PCB Aroclors to quantify the chloride ions as "equivalent Aroclor". The actual chlorine content of the original sample can be also be displayed. Using the chlorine content of the specific analyte, the equivalent concentration of the specific contaminant can be determined. Because the response of the instrument follows the standard Nernst equation and the quantified result is the chlorine content of the sample, any chlorinated organic compound can be quantified knowing only the percent chlorine in the compound. If the contaminant is unknown at the time of measurement, the results can later be converted using a simple linear transform, once the contaminant has been identified.

This study is the first in a series documenting the performance of the L2000 in new applications. Starting with the fundamental information required to determine if the L2000 technology is suitable for a particular application we have limited the scope of this investigation to determining: the method MDL, the range of linearity, and the extraction efficiency for a few of the most commonly encountered, regulated, chlorinated compounds. We have used laboratory spiked soils to simplify the experimental considerations.

EXPERIMENTAL

Preparation of Spiked Soil Samples

To ensure a consistent soil matrix throughout the spiking experiments, a large batch of composite soil was prepared prior to beginning. To simulate soils found in uncontrolled waste contaminated environments, the soil composite was prepared by mixing two types of clay and one type of sand. Each of the three soils were sifted through an 0.850 µm sieve, and then combined in a 1:1:2 ratio to form the composite.

The method of spiking depended on the particular analyte characteristics. For the non-polar, semi-volatile compounds, DDT, toxaphene, and chlordane, a 1% stock solution in chlorine free mineral oil was prepared. PCP, being more polar, is not soluble in mineral oil; therefore, methanol was used to make up the 1% stock solution. The soils were spiked at 100 ppm by adding 5 grams of the 1% stock solutions to 500 gram aliquots in aluminum pans. The spiked soil aliquots were then slurried with hexane (or, in the case of PCP, methanol) and allowed to evaporate

overnight in a hood space. The soils were then transferred to 16 oz glass jars and tumbled for one hour. The jars were then stored at room temperature for later use.

For each experiment, soils were prepared with the desired contaminant concentration by mixing together the correct proportions of the 100 ppm spiked soil and clean composite soil. The mixture was then tumbled for an hour prior to use.

Spiking soils with volatile solvents, uniformly and reproducibly, presented a formidable challenge. In previous work, all attempts to produce a quantity of soil, uniformly spiked, without loss of the analyte proved to be ineffective. Therefore, for the volatile solvents, trichloroethylene and tetrachloroethylene, each 10 gram soil sample was spiked, using a microliter-syringe, just prior to analysis.

Method Detection Limit Determination

The L2000 method detection limits for each of the chlorinated compounds were determined from replicate analysis using the method prescribed by the EPA[9]. An estimate of each of the detection limits was made using the concentration equivalent of three times the standard deviation of replicate measurements of the analyses in the composite soils. Soil was then spiked at the estimated detection limit. The spiking concentration for each of the chlorinated compounds are listed in Table 1 below:

Table 1. MDL Soil Spiking Concentrations

Analyte	Spiked Level	Percent Chlorine	Soil Chlorine Content
DDT	5 ppm	50.0	2.5 ppm
PCP	30 ppm	66.6	20 ppm
Toxaphene	5 ppm	~68	3.4 ppm
Chlordane	5 ppm	69.2	3.5 ppm
Trichloroethylene	16 ppm	81.0	13 ppm
Tetrachloroethylene	24 ppm	85.5	20.5 ppm

Each of the spiked soils were analyzed seven times using the standard extraction method, or the alternative solvent method in the case of the PCP contaminated soils. Seven matrix blanks were also analyzed using each method. The average blank measurements were subtracted from the respective sample measurements. The MDL was then computed using the following formula:

$$MDL = t_{(n-1*1-\alpha=0.99)}*S$$

where: t = the students t value
S = the standard deviation of the replicate analyses

The student's t value for 6 degrees of freedom at a 99% confidence interval used was 3.143. The mean recovery for each analyte was calculated by dividing the measured concentration by the theoretical concentration of analyte.

Response Curve Determination

In addition to the stock soil spiked at 100 ppm, standards were prepared in the composite soil at 2, 5, 10, 20, and 50 ppm of each of the chlorinated solvents and pesticides. Standards were analyzed on the L2000 using the standard extraction method, except for PCP which was analyzed using the alternative extraction method. A reagent blank was run with each analyte. These data were then compared to analysis by gas chromatography. The extraction for the DDT, PCP, chlordane and toxaphene samples to be measured by gas chromatography at the following concentrations: 2, 5, 10, 20, 50, and 100 ppm was performed by adding three 10 mL aliquots of 1:1 acetone-hexane solvent to 5 gram aliquots of each of the spiked semivolatile soils while rinsing each soil with each addition. The solvent was then removed from the soil and run through a polypropylene filter into a 25 mL volumetric flask. The volume was filled to the mark with excess 1:1 acetone-hexane. The solvent was then transferred to another 25 mL glass test tube and capped with a teflon cap, then centrifuged to remove remaining soil particles, and

prepared for gas chromatography analysis. The extraction method used for the soils spiked with volatile analyses, trichloroethylene and tetrachloroethylene, utilized 10 mL methanol mixed with 5 grams of soil at each of the concentrations 2, 5, 10, 20, 50 and 100 ppm. The methanol was then removed from the soil and the samples were then prepared for gas chromatography. The results were then analyzed and compared to the results obtained from the L-2000 analysis.

RESULTS AND DISCUSSION

Method Detection Limits

The MDLs calculated from the replicate analysis of spiked soils were within the recommended range for all analyses. (See Table 2 below). The MDLs calculated for the non-polar compounds using the standard analysis method ranged from a low of 2.7 ppm for Chlordane to a high of 4.8 ppm for Trichloroethylene and 4.4 ppm for Tetrachloroethylene. The semi-volatile MDLs being all lower than the MDLs for the volatile compounds. A contributing factor to the higher MDLs for the two volatile compounds was the difficulty in preparing the spiked soils. This was not unexpected, given the difficulty of working with volatile compounds in the field.

The MDL of 8.7 ppm calculated for the analysis of PCP was higher than expected. This may have been due to low extraction efficiency of the new solvent system on polar compounds. A low extraction efficiency indicates that the combination solvent was not able to penetrate the soil matrix to completely solvate the more polar PCP. In this type of a situation the analyte recovery is very sensitive to the exact handling of each sample replicate. Small changes in the shaking of the extraction tube or the length of extraction will have a larger effect on the recovery than is acceptable.

While the new solvent system facilitated the analysis of polar compounds, this sensitivity to extraction conditions is not a desirable characteristic. It produces variable results in the field and it indicates that the extraction efficiency will vary excessively with soil matrix. A second generation two-step alternative solvent system has been developed[8] and will be the subject of the next phase of this project.

Table 2. MDL Results

Analyte	Spiked Level	Mean Recovery	Replicate Standard Deviation	Calculated MDL
DDT	5 ppm	54%	1.15 ppm	3.6 ppm
PCP	30 ppm	56%	2.8 ppm	8.7 ppm*
Toxaphene	5 ppm	37%	0.91 ppm	2.8 ppm
Chlordane	5 ppm	57%	0.85 ppm	2.7 ppm
Trichloroethylene	21 ppm	102%	1.54 ppm	4.8 ppm†
Tetrachloroethylene	23 ppm	110%	1.3 ppm	4.4 ppm†

*Determined using the alternative extraction method.
†Determined using a direct spiking method.

Response Linearity

For each of the analyses investigated, the response of the L2000 using either solvent system was found to be linear over the range of concentrations studied. The resulting R^2 was greater than 0.96 for all analyses. (See Figures 1- 6). This indicates that the extraction efficiency is consistent over this analyte range. The results from the L2000 can, therefore, be corrected using the known recovery. There is no indication from this data that the range of linearity is limited to 100 ppm.

Extraction Efficiency

Data on the extraction efficiency of both solvent systems were obtained from the MDL determinations at a single point and from the response curve determination. The single point and the response curve determination of average extraction efficiency correlated well over the range 0-100 ppm.

Table 3. Extraction Efficiency

Analyte	MDL Mean Recovery	Average Recovery (from slope)
DDT	54%	52%
PCP	56%	39%
Toxaphene	37%	65%
Chlordane	57%	70%
Trichloroethylene	102%	89%
Tetrachloroethylene	110%	98%

Summary

In this study it has been shown that the L2000 can be used effectively to analyze soil for chlorinated volatiles and semi-volatiles. The method detection limits were shown to be in the low ppm range. This should be adequate for most contaminated sites. The response has been shown to be linear over the range of concentrations studied and a good correlation with lab methods demonstrated. A new solvent system suitable for polar organic compounds was shown to be promising. The effectiveness of a second generation two-step solvent system, demonstrated in a separate study, will be the subject of phase two of this project. Furthermore, the list of suitable chlorinated compounds will be expanded and the effectiveness of the new alternative solvent system on other chlorinated compounds will be investigated. The analyte concentration range will also be extended to 2000 ppm, the upper limit on the L2000's quantification range.

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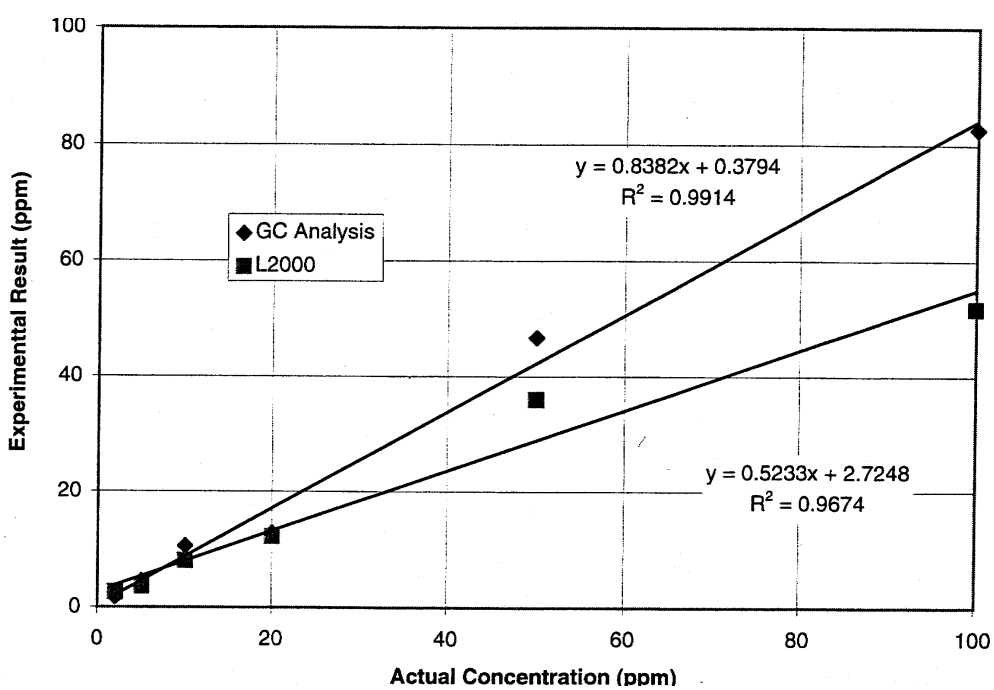

Figure 1. Response Curve for DDT

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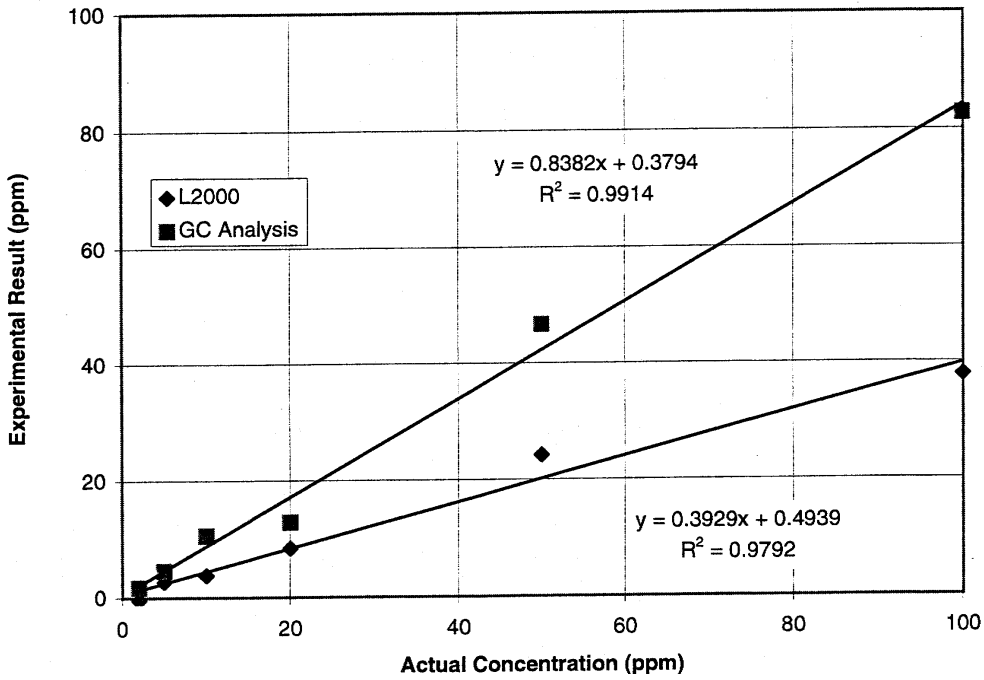

Figure 2. Response Curve for PCP

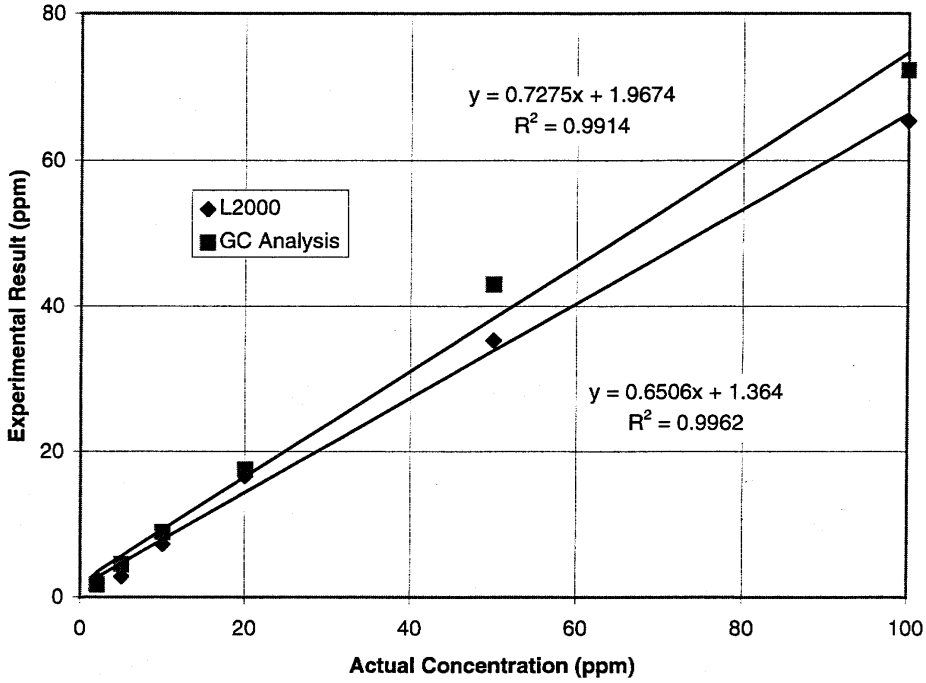

Figure 3. Response Curve for Toxaphene

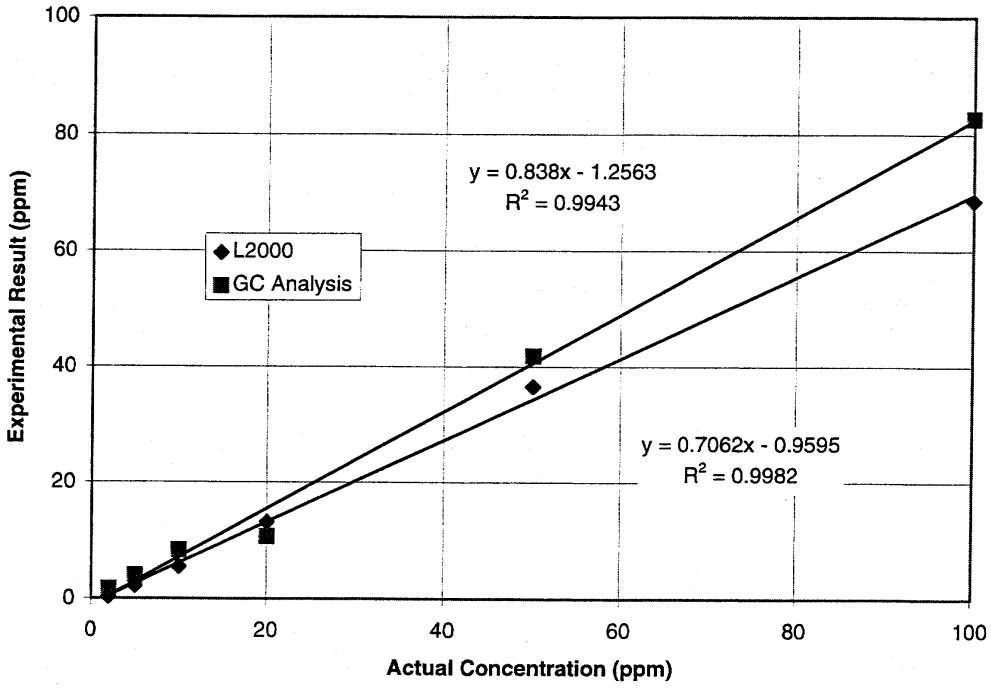

Figure 4. Response Curve for Chlordane

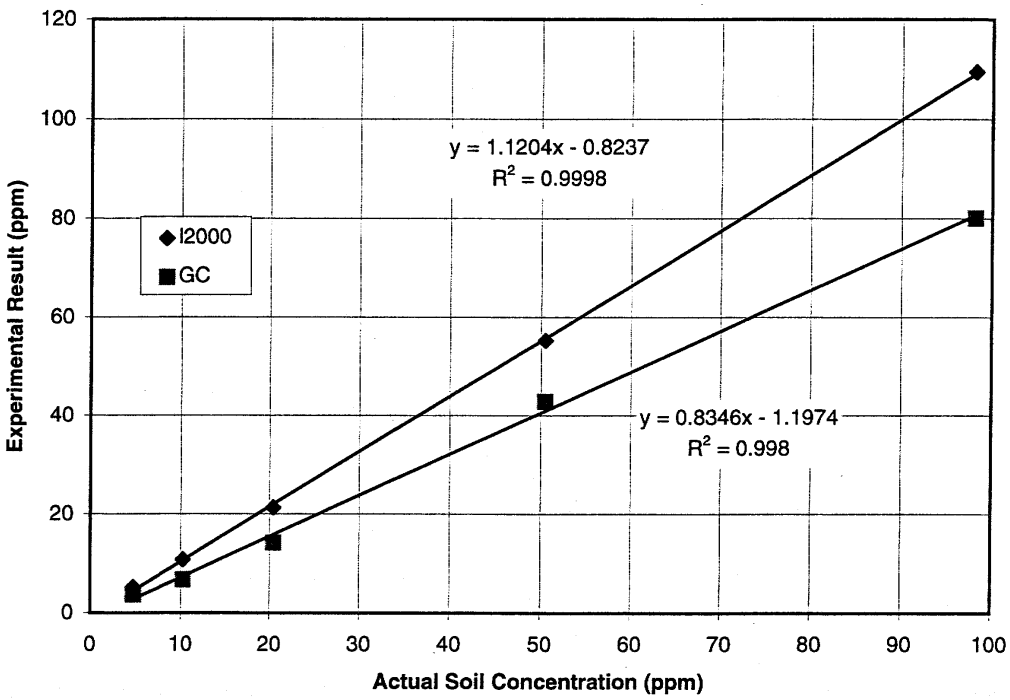

Figure 5. Response Curve for Trichloroethylene

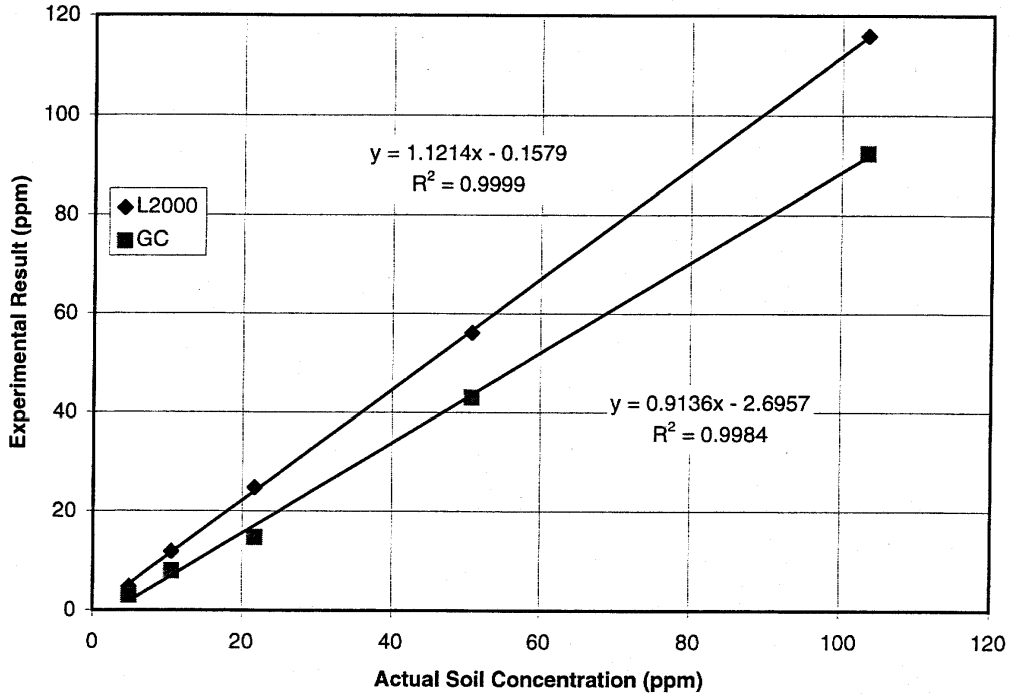

Figure 6. Response Curve for Tetrachloroethylene

A FIELD USEABLE METHOD FOR TOXICITY SCREENING OF WASTE STREAMS GENERATED DURING DESTRUCTION OF CHEMICAL WARFARE AGENTS

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A program to chemically neutralize chemical warfare agents (CWA) was carried out, and involved the neutralization of bis(2-chloroethyl)sulfide (HD) with monoethanolamine:water solutions. This program supports an effort to develop and deploy field transportable systems which can destroy CWA recovered from small burial sites. To ensure operator safety and completeness of destruction, a fieldable second tier screening method was required to estimate toxicity levels of the resulting reaction masses. This second tier screening is in addition to conventional GC/MSD methods for quantitation of residual CWA. In this case, the toxicity characteristic of the HD was its vesiccant, or alkylating capacity.

Alkylating capacity is defined as those compounds which react with 4-(4-nitrobenzyl)pyridine (4-NBP), and yield a product which absorbs in the range of 536 to 546 nm. Alkylating capacity is expressed as µmoles of alkylating sites per liter of sample, or µM. Quantitation is accomplished using a UV/Vis spectrophotometer, with external standard calibration. The method was determined to have a linear range of 98.7 to 987 µM of alkylating capacity, with the response beginning to exhibit non-linear behavior after approximately 1000 µM of alkylating capacity.

The use of 4-NBP for the detection of alkylating agents is well documented, and has been extensively reviewed. The method presented here has been optimized for a particular sample matrix, and consists of reaction of 4-NBP with acidified (pH 4 to 5) sample to form a dye precursor. The solution is then made basic with K_2CO_3 to generate a final product with an absorption maxima at approximately 540 nm. The reaction product is then extracted into 2M isopropylamine in toluene, and alkylating capacity is quantitatively estimated using standard methods of colorimetric analysis.

The analytical parameters were optimized with respect to acid type, base type, extraction solvent, reagent amounts, and heating time. Parameter selection was based on that value which gave a maximum absorbance and a minimum relative standard deviation. Examples of optimization experiments will be presented. In addition, results from experiments evaluating spiking procedure, response of method to other alkylating agents, and stability of reaction product will also be presented.

The method was validated by spiking and analyzing seven replicates at each of four spike levels. All four spike levels were performed in simulated matrix, and one spike level was also performed in an actual sample. There was not a significant difference in percent recoveries when the spike was performed in simulated matrix or an actual sample. The average percent recovery at 537 µM alkylating capacity was 102% (n=14, CV = 11.7%). The method limits of detection and quantitation will be discussed.

The method was used to evaluate three independent reactor runs, and gave consistent patterns for each kinetic series within a reactor run. In addition, a sample was monitored for approximately 90 hours after reactor drain to follow the continued breakdown of HD. Parallel analyses using the standard GC/MSD methods were also conducted. Results from both sets of analyses will be presented and compared.

DETERMINATION OF OIL CONTENT OF CONTAMINATED SOILS AND SLUDGES

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ABSTRACT

This paper reports potential applications of liquid phase agglomeration techniques in the removal of oil from contaminated soil and sediment samples for subsequent quantitative measurements. The use of liquid phase agglomeration techniques greatly improves the efficiency of solids-solvent separation. As a result, better contaminant recoveries were achieved in a shorter period of time compared with the conventional Soxhlet - Dean and Stark solvent extraction method. Quantitative determination of the contaminant was carried out using gas chromatographic and spectrophotometric methods. Two case studies are discussed. The first deals with the quantitative determination of diesel fuel from a contaminated diesel invert drilling mud sample. The second evaluates the extraction and subsequent quantification of heavy oil from a contaminated soil sample

The objective of this study was to develop a quick and efficient procedure for the extraction and subsequent quantification of total petroleum hydrocarbons in contaminated wastes. The proposed method can be adapted to the extraction and subsequent quantification of a variety of hydrocarbon pollutants from contaminated soils and sediments.

INTRODUCTION

Both the design and planning of a soil reclamation program and the evaluation of disposal, reprocessing or reclamation options for sludges, oily wastes and tailings depend upon a reliable and accurate means of evaluating the total oil content of polluted soils. A precise estimate of the amount of hydrocarbons is essential to determine the extent of contamination and the success of the reclamation program. Treatment technologies and site remediation progress lean heavily on analytical techniques that are accurate, reproducible and of real time value. Current analytical methods are inadequate because of the following concerns:

- The recovery of oil from contaminated samples is influenced by the sample preparation method and the oil extraction methods.
- The solvent extraction methods, used to remove oil from contaminated samples for subsequent measurements, have limitations with respect to the water content of the sample and the particle size distribution of the matrix. Usually these methods are less efficient for fine textured materials. Also, water is known to reduce the extraction efficiency of organic solvents because of the formation of oil-in-water emulsions[1-2].
- Loss of volatile organics, during solvent removal for sample concentration, results in significant bias.

- Spectroscopic determination of contaminants has the disadvantage that degradation of pollutants in the soil results in altered spectral characteristics. Absorbance at certain wavelengths may increase with time even though the total amount of oil in the soil would be decreasing. Also, in clay soils where oil components may be absorbed, spectral properties of the extracted oil may also change[1].
- A wide variety of laboratory techniques used to measure TPH provide data of varying quality.

In our previous work we demonstrated the potential applications of proton NMR in the quantitative determination of bitumen and solvents extracted from oil sands and fine tailings[3-4]. The NMR method can be used to estimate the amount of any residual solvent in the oil. The retention of low boiling components, normally lost during solvent removal, produces a more representative sample of the oil for calibration purposes.

Liquid phase agglomeration techniques, in combination with solvent extraction have been successfully used for the removal of hydrocarbon contaminants from fine textured, organic contaminated soils[5]. Agglomeration of fines with the coarse particles is achieved by the addition of water to a vigorously mixed slurry of soil in the selected solvent. Water acts as a solids bridging liquid and dense soil agglomerates are formed under appropriate conditions. The bridging liquid remains in the agglomerate pores where interfacial tension provides the forces holding the aggregates together. Judicious selection of agglomerate size, by controlling water content, greatly improves the efficiency of solids-solvent separation.

The objective of this study was to explore the use of liquid phase agglomeration techniques in the development of a more efficient solvent extraction procedure. Two hydrocarbon contaminants, one associated with a fine textured soil and the other with a coarser matrix, were selected for subsequent quantitative measurements.

EXPERIMENTAL

Samples. One of the samples used for this study was a used Diesel Invert Drilling Mud (DIDM) from Alberta. This sample contained over 70 w/w% of solids < 41 µm diameter. The diesel content of this sample was estimated by extraction with toluene using the Dean and Stark Soxhlet method[6]. The extract was characterized and subsequently quantitated by GC. The GC chromatogram of the extract was a good match with the chromatogram for a sample of diesel obtained from a local gas station in the summer of 1995. This commercial diesel sample was used for preparing calibration standards.

The second sample used in this study was a highly saline soil sample provided courtesy of Newalta Corporation, Calgary, Alberta. It was a mixture of tank bottoms, frac sand and spill material containing a range of organic and inorganic contaminants. The organic contaminant in this sample was a high boiling heavy oil.

Proton NMR measurements. Proton NMR measurements were performed on a Brucker AM-400 NMR spectrometer (400 MHz); 500 µL of solution in a 5 mm outer diameter tube was used in each case. A repetition time of 2 seconds was selected. Each spectrum was the Fourier transform of 1000 free induction decay curves. Once adjusted all parameters were kept constant for subsequent measurements.

GC determination of diesel. The extract was analyzed employing a Varian Model 3300 GC equipped with an FID detector and a temperature gradient program. A DB-5, megabore, capillary column, 30 meters in length with a 0.53 mm internal diameter and a 1.5 micron film thickness (J & W Scientific) was used. The initial operating temperature was 50 degrees Celsius with an initial hold-time of 5 minutes. The temperature was then increased at the rate of 10 degrees per minute to a final temperature of 300 degrees Celsius where it was held for 5 minutes. Calibration curves were prepared using the purchased sample of #2 Diesel Fuel.

Soxhlet Dean and Stark Method. The extraction of heavy oil from the Newalta sample was carried out for 20 hours using the Soxhlet-Dean and Stark method[6]. Both toluene and methylene chloride were used separately for extraction.

Spectrophotometric estimation of heavy oil. The quantitation of the oil component was carried out using a spectrophotometric method[7] based on the linear relationship between the absorbance at 530 nm and the concentration of oil in solution.

For calibration purpose oil samples were obtained from a methylene chloride extract of the soil. Non-filterable solids were removed from the oil extract by centrifugation. The solvent was removed at 40°C in a Brinkmann rotary evaporator under reduced pressure. The amount of residual solvent in the oil was quantitatively measured using proton NMR[4]. A correction for solvent content was applied to the amount of oil used in the preparation of standard solutions. For spectrophotometric measurements, absorbances at 530 nm were determined for toluene solutions of the oil for concentrations ranging from 0.01 - 0.4 w/w%. Plots of the percent oil vs. absorbance produced a straight line passing through the origin.

Liquid Phase Agglomeration Procedure. In a typical test a polypropylene Waring Blendor Jar (500mL), equipped with Teflon washers and a plastic cover, was accurately weighed. To the jar were added: contaminated solids (20g), and solvent (50mL). The contents were agitated at high shear for 1 minute. The solution was carefully drained into another 500 mL polypropylene jar. About 1w/w% of an additive (sodium meta phosphate, calcium hydroxide or scrubber sludge), additional water and fresh solvent (50 mL) were added to the original Blendor jar still containing the extracted solids from the primary extraction. The contents were agitated-at high shear for 1 minute followed by 5-10 minutes at low shear until the slurry became clear as discrete agglomerates were formed. The supernatant solution from the second treatment was drained into the polypropylene jar containing solution from the primary extraction. The procedure was repeated three more times and all extracts were combined. The extracted solids were surface washed and the washings were added to the solution in the polypropylene bottle. The contents of the bottle were centrifuged and the clear solution was transferred to a 500 mL glass measuring flask. The residue in the polypropylene bottle was agitated with fresh solvent and then centrifuged. The solution was combined with the solution in the measuring flask. The procedure was repeated until the washings became colorless. The solution in the measuring flask was made up to the mark. The amount of oil was estimated in this solution using the spectrophotometric method.

RESULTS AND DISCUSSION

The Amount of Extractable Oil from Contaminated Solids

The extraction of diesel from a used diesel invert mud sample and a heavy oil from Newalta contaminated soil sample were carried out using both the conventional Dean & Stark Soxhlet and Solvent Extraction Soil Agglomeration (SESA) methods. Subsequent quantification was carried out using GC/FID for the diesel and spectrophotometric method for the heavy oil. The results are summarized in the Table. It is obvious from the results that not only better recoveries of the contaminants were obtained using the SESA method but the total turn around time was also much reduced.

Method	**Extraction plus analysis turn around time (Hrs)**	**Amount of oil extracted (g/100g of wet solids)***	
		DIDM* **	**NEWALTA**
Dean & Stark Soxhlet Method	21	4.84 ± 0.1 (5)	12.9 ± 0.2 (5)
SESA **	2	4.99 ± 0.22 (3)	13.38 ± 0.49 (3)

* Figures in parenthesis represent number of tests carried out.
** Solvent Extraction Soil Agglomeration process.
*** Diesel Invert Drilling Mud sample

CONCLUSION

Liquid phase agglomeration has potential for use as a quick and efficient analytical procedure for the extraction and subsequent quantification of a variety of petroleum contaminants from contaminated wastes.

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IMPROVED EXTRACTION EFFICIENCY OF POLYCHLORINATED BIPHENYLS FROM CONTAMINATED SOIL USING A TOTAL HALOGEN SCREENING METHOD

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ABSTRACT

Polychlorinated biphenyls (PCBs) are strictly regulated on the state and federal levels. Responsible parties must determine the concentration and extent of contamination to make appropriate decisions regarding remediation of PCB contaminated soils. Gas chromatography (GC) analysis has been traditionally used to delineate PCB contamination in soil. On-site, field screening techniques have been developed within the last decade to reduce the number of samples requiring laboratory confirmation. All field screening methods require the use of organic solvents to extract the contamination from soil. Soils sampled for analysis frequently contain water which, with some solvents, may result in poor extraction efficiencies and can subsequently produce false negatives. In this study, GZA GeoEnvironmental, Inc. (GZA) utilized a total organic halogen (TOX) screening kit to characterize complex, multi-component PCB contaminated soil at an industrial property located in the midwest. Furthermore, the results demonstrated the effectiveness of two novel solvent systems to extract PCBs from wet clay soil for reliable quantification with a portable field analyzer. Preliminary GC analyses indicated that the soil was contaminated with Aroclor 1248 as well as diesel range organics. The excavated soil consisted of wet, red clay. Two types of field test methods are available for PCB screening, both of which may exhibit lower extraction efficiencies for wet clay soils. One method relies on immuno-assay chemistry, and the second method involves chemical dehalogenation of the PCBs followed by analysis with a colorimetric reaction or chloride-specific electrode. Immuno-assay PCB kits suffer severe negative interferences in the presence of hydrocarbon co-contaminants and are not suitable for this site. Dexsil Corporation's L2000® PCB Analyzer (U.S. EPA SW-846 Draft Method 9078, "Screening Test Method for Polychlorinated Biphenyls in Soil") is not adversely affected by the presence of the aforementioned co-contaminating fuels and oils and was therefore selected to quantify the PCBs. Aroclor 1248 concentrations were determined in over 71 wet samples by extracting the contaminated wet soil samples with a new, two-step, ozone-friendly solvent system followed by analysis using the L2000. An additional, modified aqueous-organic solvent extraction method with a Florisil cleanup column was also used to determine the PCB content of 42 of the same wet samples. The second method was designed to quantify PCBs in the presence of some non-PCB halogenated solvents. A portion of each wet soil sample was then dried and reanalyzed. Ten percent of the nondetected samples along with all samples testing positive (>2 ppm) for PCBs were analyzed using U.S. EPA Method 8080 (gas chromatography with Soxhlet extraction) to establish the extraction efficiencies of each new two-step solvent system. The L2000 results of wet soil samples were adjusted for water content and compared to the values generated by Method 8080 (GC). Both solvent systems demonstrated the ability to efficiently extract (>73%) PCBs from wet and dry lacustrine red clay soil. Data generated by L2000 analysis of PCBs extracted into both new solvent systems exhibit excellent correlation to the data produced by the more sophisticated laboratory (GC) technique. This information lends confidence in PCB field screening data for field engineers.

INTRODUCTION

Under the authority of the Toxic Substances Control Act (TSCA) of 1976, the U.S. Environmental Protection Agency (EPA) regulates the use, storage, and disposal of polychlorinated biphenyls (PCBs)[1]. These rules were imposed because of the health-related concerns and potential environmental impacts associated with PCBs. In July 1979 the U.S. EPA banned the manufacture of PCBs and most uses in which the PCBs were not contained within a closed system (e.g., transformers and capacitors). Latitude was given to certain industries whose PCB usage, such as electrical applications, were not considered to pose unreasonable risk to the environment or human health. In July 1985 the U.S.EPA developed regulations for phasing out the use of PCBs in all enclosed systems.

The U.S. EPA promulgated a PCB Spill Cleanup Policy in April 1987 which is codified in 40 CFR Part 761. The PCB Spill Cleanup Policy requires notification for PCB spills into sensitive areas and for all spills greater than 10 pounds. The PCB Spill Policy also establishes cleanup concentrations for soil and solid surfaces. Clean up of the affected media is required, whether it is soil, groundwater, surface water, infrastructure, equipment, or inventory.

Delineation of the extent of contamination in soil is a critical step in any remediation project. Accurate delineation is more critical in a PCB remediation project due to the limited and expensive disposal alternatives. PCB disposal generally costs an order of magnitude more than disposal of non-PCB containing soil. After the specific type Aroclor, concentration, and extent of PCB contamination are identified in the soil, an appropriate cleanup and disposal plan can be designed. Confirmation of the specific PCB Aroclor and concentration are accomplished using analytical testing procedures performed in accordance with *Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods,* U.S. EPA Publication SW-846. Traditionally, U.S. EPA SW-846 Method 8080, gas chromatography (GC)[2] for the detection of organochlorine pesticides and PCBs is used in conjunction with U.S. EPA SW-846 Method 3540, soxhlet extraction[3] to determine the PCB concentrations in soil.

While laboratory analyses are required to definitively confirm the type and concentration of PCBs, the methods are both time consuming (requiring 2 to 3 days for off-site analyses) and expensive (ca. $100 per analyses). Within the last decade, several field screening techniques have been developed to preliminarily define the extent of PCB contamination in the field and reduce the number of samples requiring laboratory analytical testing. Field screening techniques provide field engineers with on-site, real-time information necessary to make field decisions regarding the investigation or remediation process.

Application of an appropriate field screening method can improve the quality of a site investigation, lessen the time required to characterize a site, decrease the project cost, and increase the efficiency of a remediation process. A reliable field screening technique is selective to the analyte of concern, insensitive to co-constituents, efficient, easy to use, and relatively inexpensive. The field method must also provide good correlation with analytical laboratory data. Inaccurate quantification of PCB concentrations by an ineffective field screening technique could cause responsible parties to make inappropriate and expensive decisions regarding crucial remediation steps.

Accurate quantification of a soil contaminate begins with an efficient extraction of that chemical from the soil media. This report accounts the careful selection and utilization of a total organic halogen (TOX) field screening kit by GZA GeoEnvironmental, Inc. (GZA) to aid in the characterization of a complex, multi-component PCB contaminated site. Furthermore, the results will demonstrate the effectiveness of two novel solvent systems to extract PCBs from clay soil for reliable quantification with a portable field analyzer.

BACKGROUND

GZA was contracted to delineate the extent of PCB containing soil at an industrial property in the midwest. Due to the expense of GC analytical testing, GZA chose to use a PCB field screening method in addition to confirmational GC analysis. A properly chosen field screening method would provide quick, on-site, real-time data at a reduced cost.

Two types of field test methods are currently available for PCB screening. One method relies on immuno-assay chemistry. The immuno-assay chemistry method is based on enzyme-linked immunosorbent assays (ELISA) in which a competitive reaction between PCBs and a PCB conjugate is used to determine the PCB concentration in soil samples. The second method involves chemical dehalogenation of the PCBs followed by analysis with a colorimetric reaction or chloride-specific electrode. Colorimetric analysis yields an estimate of the PCB

concentration greater or less than a fixed concentration. The chloride-specific electrode quantifies PCBs at concentrations ranging from 2 to 2,000 parts per million (ppm).

GZA chose to determine the PCB Aroclor 1248 concentrations in soil using the chemical dehalogenation method followed by analysis with Dexsil Corporation's L2000® PCB/Chloride Analyzer. The L2000 utilizes a chloride specific electrode to quantify the PCBs in accordance with its associated U.S. EPA SW-846 Draft Method 9078 *"Screening Test Method for Polychlorinated Biphenyls in Soil"*[4]. GZA selected the L2000 for two main reasons. First, immuno-assay PCB field screening kits are subject to severe negative interferences in the presence of hydrocarbon co-contaminants as documented by Gaskill, 1993.[5] The study completed by Gaskill also indicated that immuno-assay systems may be susceptible to other non-specific interferences present in organic solutions. Dexsil's L2000 is not adversely affected by the presence of hydrocarbon co-contaminating fuels and oils and was therefore selected to quantify the PCBs.

The second reason GZA chose to use the L2000 is because Dexsil volunteered to research and develop a unique solvent system to extract PCBs from the wet, red lacustrine clay characteristic of the area. Extracting organic contaminates from complex clay (wet and dry) matrices poses a notoriously difficult task. PCBs are especially difficult to extract from wet clays as previously documented by Gauger, *et al,* 1995.[6] Based in part on research and testing completed on soil samples collected by GZA during this study, Dexsil has developed two novel solvent systems specifically designed to achieve high PCB extraction efficiencies from clay soil.

Prior to this study, the L2000 was demonstrated to be an effective PCB field screening tool in a variety of soils including sand and several different clay soil types. The lacustrine red clay soil present in east central Wisconsin posed a new challenge for the L2000 which required modification to the extraction procedure and the need for this study.

One extraction method utilizes a novel aqueous-organic solvent system. The two-step procedure results in a high PCB extraction efficiency from complex soil matrices such as wet clays. For cases in which co-contaminating, non-PCB, halogenated organic compounds are present or suspected, a second extraction method was developed. This method utilizes a similar aqueous-organic solvent followed by a Florisil cleanup column. The Florisil cleanup column removes common non-PCB organic chloride compounds such as pentachlorophenol from the solvent extract. PCBs are not lost on the Florisil cleanup column during the filtration process. Inorganic salts associated with sampled soil cause no interference with PCB quantification by the L2000 after extraction with either solvent system.[5]

The soil samples collected by GZA were extracted as received using the novel solvent systems and analyzed with Dexsil's L2000 to determine the PCB Aroclor 1248 concentration. The data generated using the new solvent systems and L2000 showed an excellent correlation when compared with the results from split samples analyzed in accordance with U.S. EPA SW-846 Method 8080 and Method 3540.

SAMPLING and ANALYSIS

For this study, GZA utilized a Geoprobe to bore approximately 45 soil borings at the industrial property. The objective of the soil boring program was to evaluate PCB contaminated areas and define the extent of PCBs in the soil. Soil samples were collected continuously at 2 foot intervals through the end of boring at average depths of 12 feet below ground surface. A Site Plan showing the layout of the soil borings is presented as Figure No. 1. Precautions were taken during sampling to prevent cross contamination by cleaning the Geoprobe® sampling tools with a Citrisolv detergent solution. The samples were kept on ice and delivered to Dexsil within 48 hours of collection under chain-of-custody documentation. Dexsil homogenized then analyzed the samples using the L2000 in accordance with U.S. EPA SW-846 Draft Method 9078 and GC in accordance with Method 8080 following soxhlet extraction in accordance with U.S. EPA Method 3540.

PCB Aroclor 1248 concentrations were determined in the soil samples using the L2000 with both extraction methods, the aqueous-organic solvent system and the aqueous-organic solvent system followed by filtering through a Florisil cleanup column. The solvents do not contain chlorofluorocarbons and are therefore ozone-friendly. After the chlorinated organic compounds were extracted from the soil samples into the solvent using the aqueous-organic system, or modified solvent followed by the Florisil cleanup, the compounds were reacted with organo-metallic sodium to strip away the chloride. The resulting chloride ions were then quantified using the chloride specific

electrode in the L2000. The L2000 converts the chloride content to the equivalent amount of PCB Aroclor (specified by the user) and displays the value on a digital readout. Samples can also be quantified with the total chloride setting. When the chloride setting is used, the chloride reading is converted to a specific PCB Aroclor concentration using the chloride content in the identified Aroclor.

A portion of each soil sample was weighed into a tray and dried overnight. The calculated water content was used to adjust the L2000 results for the wet soil to the PCB concentration in an equivalent amount of dry soil. The clumps of dried soil samples were broken up then extracted with both solvent systems and re-analyzed using the L2000. Based on the results of the soil samples analyzed using the L2000, 10 percent of the samples where PCBs were not detected and all samples testing positive (>2 ppm) were analyzed using U.S. EPA SW-846 Methods 8080/3540 (GC with Soxhlet extraction).

RESULTS and DISCUSSION

As summarized in Table 1, the average water content of 71 clay samples collected from the soil borings was 13%. Also summarized in Table 1 are the PCB Aroclor 1248 concentrations detected in 71 wet soil samples after extraction using the aqueous-organic solvent extraction method and L2000 analysis, the fraction of solids in the soil, calculated concentrations of PCB Aroclor 1248 in an equivalent amount of dry soil, and the PCB Aroclor 1248 concentration determined by GC after Soxhlet extraction.

Summarized in Table 2 are the PCB Aroclor 1248 concentrations of 16 dry soil samples determined using GC following Soxhlet extraction and the L2000 following extraction with the aqueous-organic system.

Table 3 summarizes the PCB Aroclor 1248 concentrations detected in 42 wet and dry soil samples after extraction using the aqueous-organic solvent extraction method with Florisil cleanup column and L2000 analysis. Included on the Table 2 are the fraction of solids in the soil, calculated concentration of PCB Aroclor 1248 in an equivalent amount of dry soil, and the PCB Aroclor 1248 concentration determined by GC.

The PCB concentration detected in each wet and dry soil sample as quantified by L2000 (with and without the Florisil cleanup column) was plotted against data obtained using U.S. EPA SW-846 Method 8080. Standard residual analyses were done on the data to identify outliers. Linear regression analysis yielded a value for the slope, intercept, and regression coefficient (R^2). The slope of each plot is an indication of the extraction efficiency of the solvent system in wet and dry clay; and a non-zero intercept would indicate any systematic bias. The correlation between the L2000 and GC data was represented by the R^2 value. Data plots for soil samples collected during this study are shown in Graphs 1 through 4.

Graph 1 is a plot of PCB Aroclor 1248 concentrations of the 71 wet soil analyses determined using the L2000 following extraction with the aqueous-organic solvent system versus the PCB Aroclor 1248 concentration quantified by Soxhlet extraction and GC. Four outliers (GP104-S2, GP106-S4, GP130-S4, and GP140-S5) were excluded from the plot based on results from standard residual analysis. Despite the rather high water content, the aqueous-organic solvent system (without the Florisil cleanup column) yielded a 78% PCB extraction efficiency. The correlation between the GC and L2000 field methods was 0.93.

Regression analysis of the data shown in Graph 1 using a 95% confidence level illustrates the intercept of the plot is statistically equivalent to zero. The slope, however, is not statistically equal to one. Since solvent extraction systems generally can not achieve the same efficiency as the laboratory soxhlet extraction method, the "non-one" slope was not surprising. The high correlation with the reference method indicates that the extraction efficiency is repeatable. The L2000 result can, therefore, be corrected using the known extraction efficiency. Furthermore, if an environmental professional establishes that the extraction efficiency of PCBs from their particular soil is significantly different from the value presented here, the L2000 results can be corrected using a site specific correction.

Graph 2 is a plot of the 16 dry soil analyses summarized in Table 2. Plotted in Graph 2 are the Aroclor 1248 concentrations determined using the L2000 following extraction using the aqueous-organic solvent system; versus the Aroclor 1248 concentration quantified by GC following soxhlet extraction. One outlier (GP103-S4) was excluded from the plot based on results from standard residual analysis. The aqueous-organic solvent system (without the Florisil cleanup column) yielded a 77% PCB extraction efficiency.

Regression analysis of the data shown in Graph 2 results in an intercept that is statistically equivalent to zero at a 95% confidence level, indicating insignificant bias. As with the wet soil, and for the same reasons, the slope is not statistically equal to one. The regression coefficient of 0.97 demonstrates an excellent correlation between the data generated using the L2000 and GC.

Graphs 3 and 4 are plots of the 42 wet and dry soil analyses summarized in Table 3. Plotted in Graph 3, for the wet soil samples, and Graph 4, for the dry soil samples, are the Aroclor 1248 concentrations determined using the L2000 following extraction using the aqueous-organic solvent system and Florisil cleanup column versus the Aroclor 1248 concentration quantified by GC following soxhlet extraction. There were no outliers in the resulting data set. The aqueous-organic solvent system with the Florisil cleanup column yielded a 73% PCB extraction efficiency.

Regression analysis of the data shown in Graphs 3 and 4 using a 95% confidence level illustrates that the intercept of the plots are statistically equivalent to zero. As with Graphs 1 and 2, and for the same reasons, the slopes are not statistically equal to one. The regression value of 0.88 for the wet soil samples and 0.91 for the dry soil samples demonstrates a good correlation between the data generated using the L2000 and GC.

CONCLUSION

Although the PCBs present in the soil at the die casting facility were associated with diesel range organics, the PID used to identify hydrocarbon contaminated soil during excavation did not detect the co-contaminating PCBs. Further laboratory analysis, established the presence of Aroclor 1248. Recognizing that the PCBs, in addition to the diesel range organics, would need to be delineated in the excavated soil, GZA researched the feasibility of utilizing different PCB field screening techniques. Few field screening methods were found to have a reputation of being capable of quantifying PCBs in real-time in complex, co-contaminated matrices such as the red clay soil found at the die casting plant.

Prior to this study, the L2000 was demonstrated to be an effective PCB field screening tool in a variety of soil types including sand and several different clay soils. This particular study illustrates the use of two novel solvent systems to extract PCBs from complex clay matrices. Both solvent systems demonstrated highly efficient (>73%) extraction efficiencies of PCBs from lacustrine red clay soil when compared with the soxhlet extraction method. The extraction efficiencies reported using the new solvent systems are greater than the extraction percentages reportedly achievable by other field screening methods.

The L2000, in previous studies, has also been shown to reliably quantify PCBs in the presence of co-contaminating hydrocarbons. With the new modified solvent system and Florisil column, the user can efficiently extract and reliably quantify PCB Aroclors in the presence of some non-PCB halogenated solvents.

The information gained by this study indicates that the L2000 can be used as a valuable PCB field screening tool in a variety of difficult matrices and situations. This should heighten environmental engineers confidence in field data.

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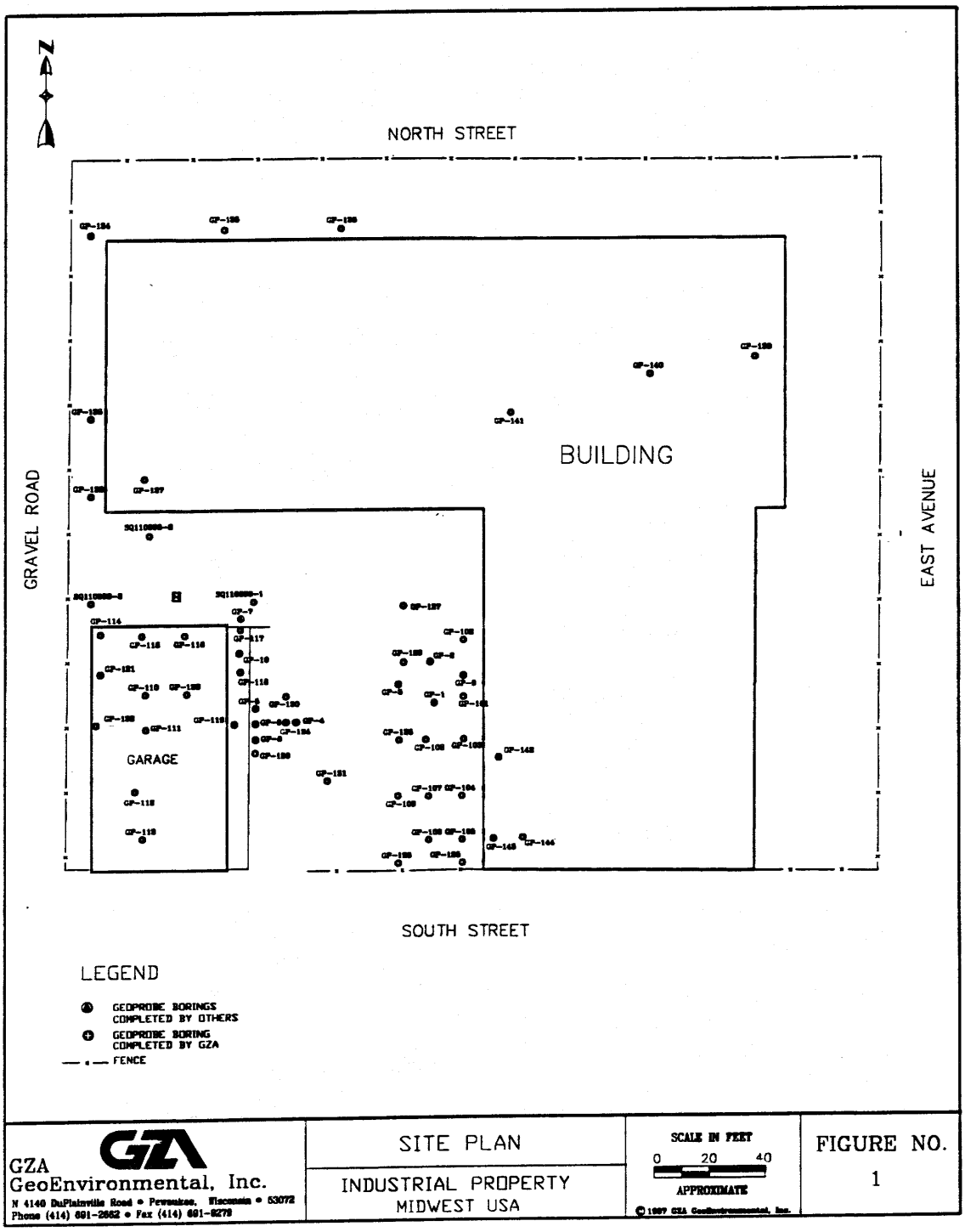
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FENCE
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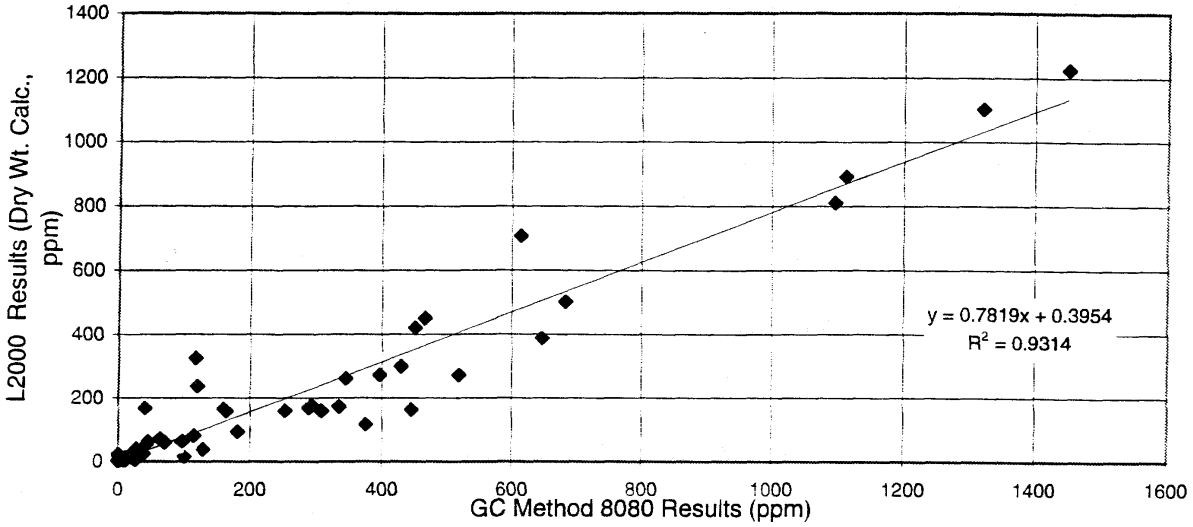
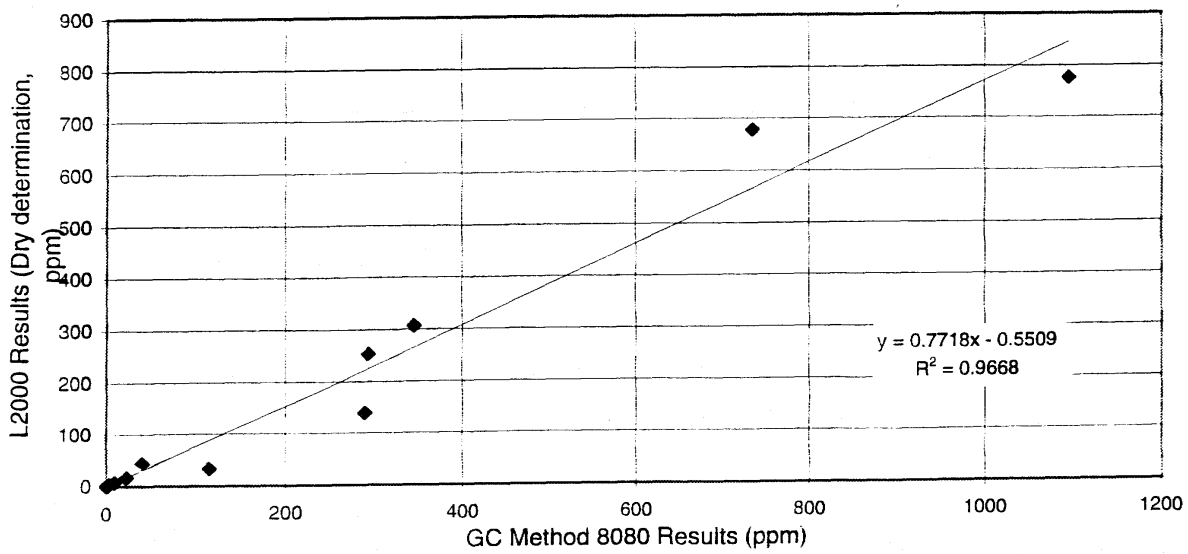
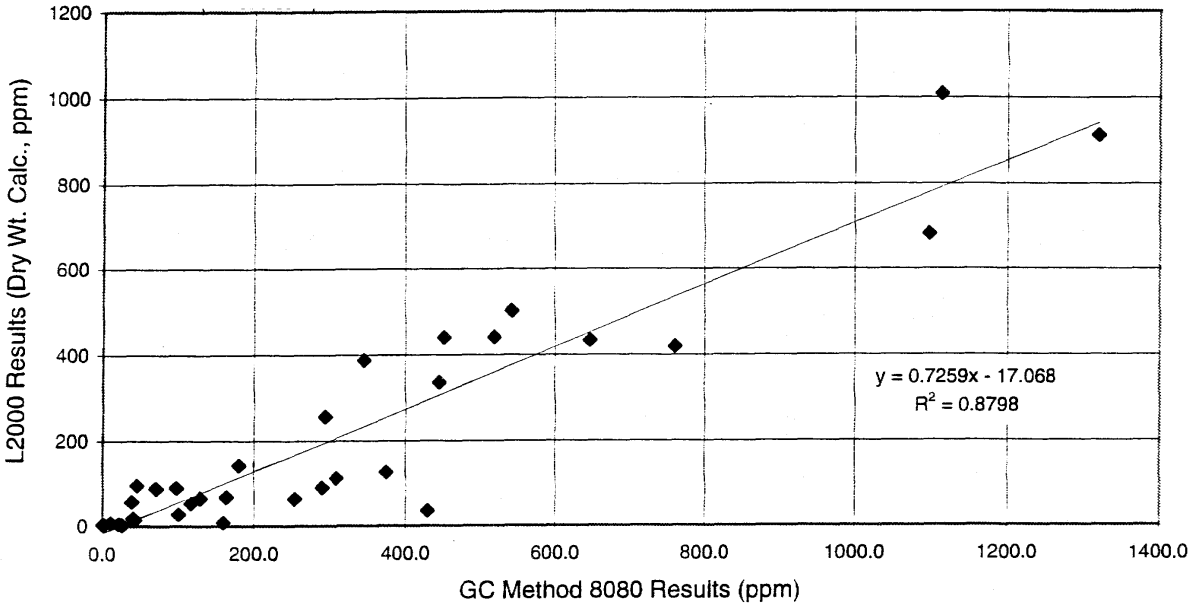
Graph 1. L2000* versus GC Method 8080 Quantification of Aroclor 1248 in Wet Soils
(*These samples were extracted with the aqueous-organic solvent)



Graph 2. L2000* versus GC Method 8080 Quantification of Aroclor 1248 in Dry Soils
(*These samples were extracted with the aqueous-organic solvent)



Graph 3. L2000* versus GC Method 8080 Quantification of Aroclor 1248 in Wet Soils
(*These samples were extracted with the modified solvent and filtered through a florisil column)



Graph 4. L2000* versus GC Method 8080 Quantification of Aroclor 1248 in Dry Soils
(*These samples were extracted with the modified solvent and filtered through a florisil column)

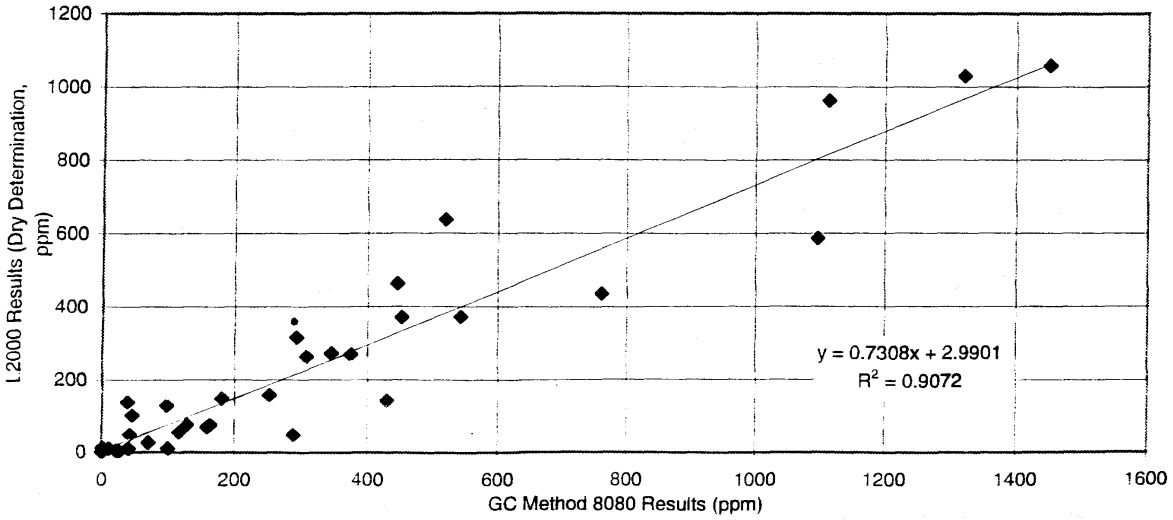


Table 1. Quantification of Aroclor 1248 in Wet Soils by USEPA Methods 8080/3540 compared to Extraction by an Aqueous-Organic System and L2000 Analysis

Sample ID	as 1248 Wet Wght L2000 (ppm)	Fraction Solid	as 1248 Dry Wght (Calc) L2000 (ppm)	Soxhlet GC 8080 1248 (ppm)
GP101-S2	1.05	0.87	1.21	0.19
GP103-S2	1065.75	0.87	1224.50	1450.00
GP103-S3	970.38	0.88	1102.79	1320.00
GP103-S4	437.50	0.87	501.35	682.00
GP103-S5	137.81	0.87	159.31	252.00
GP104-S2	1190.88	0.88	1360.13	3540.00
GP104-S3	712.25	0.88	811.02	1096.00
GP104-S4	615.13	0.87	707.24	613.00
GP104-S6	1.84	0.88	2.09	0.41
GP105-S3	70.79	0.87	81.74	114.00
GP105-S4	389.38	0.87	449.71	466.00
GP105-S6	1.93	0.87	2.21	1.20
GP106-S3	148.75	0.86	172.87	334.00
GP106-S4	93.98	0.88	106.23	734.00
GP106-S5	259.00	0.87	299.10	429.00
GP107-S2	238.88	0.88	271.75	518.00
GP107-S3	156.54	0.88	177.76	292.00
GP107-S4	136.15	0.86	157.48	163.00
GP108-S2	142.36	0.85	168.42	288.00
GP110-S2	1.14	0.86	1.32	0.31
GP113-S1	9.01	0.93	9.66	4.10
GP113-S2	23.54	0.86	27.39	22.00
GP114-S1	15.66	0.96	16.31	1.30
GP114-S2	20.13	0.88	22.97	0.17
GP115-S2	2.01	0.85	2.35	9.20
GP117-S2	2.01	0.86	2.34	3.00
GP117-S3	369.25	0.88	419.71	451.00
GP117-S4	232.75	0.89	261.75	344.00
GP117-S5	138.16	0.87	158.63	307.00
GP118-S3	62.74	0.88	71.21	63.00
GP118-S4	33.08	0.88	37.79	128.00
GP118-S5	147.61	0.88	167.77	40.00
GP119-S2	10.33	0.89	11.55	11.00
GP119-S4	81.81	0.88	93.15	180.00
GP120-S5	12.69	0.87	14.57	100.00
GP121-S2	2.45	0.86	2.86	0.07
GP123-S2	2.36	0.87	2.71	0.40
GP124-S2	5.60	0.88	6.37	11.00
GP124-S4	238.00	0.88	271.99	396.00
GP125-S2	140.35	0.85	165.31	159.00
GP127-S4	1.84	0.88	2.10	0.11
GP128-S6	2.71	0.87	3.11	0.03
GP129-S4	771.75	0.87	891.88	1113.00
GP129-S6	3.85	0.87	4.44	0.67
GP130-S2	4.81	0.87	5.55	26.10

GP130-S4	735.88	0.87	843.94	542.00
GP131-S1	2.63	0.85	3.07	2.40
GP132-S4	0.70	0.87	0.81	0.06
GP134-S2	3.33	0.87	3.81	0.72
GP136-S2	3.06	0.92	3.33	1.30
GP137-S2	4.99	0.85	5.86	2.30
GP138-S3	54.25	0.87	62.37	45.00
GP138-S5	210.88	0.89	236.29	119.00
GP139-S2	1.23	0.87	1.41	0.55
GP139-S5	46.73	0.87	53.52	42.00
GP139-S6	142.45	0.87	163.51	445.00
GP140-S3	4.64	0.87	5.30	12.00
GP140-S4	280.88	0.86	324.76	116.00
GP140-S5	954.63	0.87	1096.68	760.00
GP140-S6	52.68	0.88	60.03	70.00
GP141-S1	35.70	0.88	40.58	27.00
GP141-S3	8.31	0.88	9.43	9.10
GP141-S4	5.86	0.86	6.81	3.10
GP141-S5	12.60	0.87	14.52	20.00
GP142-S4	4.03	0.88	4.60	12.00
GP143-S2	20.56	0.87	23.69	38.00
GP143-S3	102.03	0.87	116.56	374.00
GP143-S4	339.50	0.88	386.57	646.00
GP144-S4	54.43	0.87	62.44	97.00
SQ110896-2	15.93	0.83	19.15	19.20
SQ110896-3	3.24	0.94	3.46	3.40

Table 2. L2000 Analysis (after aqueous-organic extraction) of Aroclor 1248 in Dry Soil compared to GC 8080 Analysis

Sample ID	Aroclor 1248 in Dry Soil L2000 (ppm)	Aroclor 1248 Soxhlet w/ GC 8080 (ppm)
GP101-S2	1.575	0.19
GP103-S4	880.25	682
GP104-S3	776.125	1096
GP104-S6	0.6125	0.41
GP105-S3	32.55	114
GP105-S6	0.9625	1.2
GP106-S4	678.125	734
GP107-S3	253.75	292
GP108-S2	139.65	288
GP110-S2	0.175	0.31
GP113-S2	16.7125	22
GP114-S2	1.6625	0.17
GP115-S2	7.7875	9.2
GP117-S2	4.2	3
GP117-S4	307.125	344
GP118-S5	43.4	40

Table 3. L2000 Analysis (after modified extraction/florisil filter) of Aroclor 1248 compared to GC Method 8080 Analysis in Wet and Dry Soils

Sample ID	Aroclor 1248 Wet Weight L2000 (ppm)	Fraction Solid	Aroclor 1248 Dry Weight (Calc) L2000 (ppm)	Aroclor 1248 in Dry Soil L2000 (ppm)	Aroclor 1248 Soxhlet w/ GC 8080 (ppm)
GP103-S2	407.2	0.86	520.9	1058.8	1450.0
GP103-S3	721.4	0.87	912.1	1029.9	1320.0
GP103-S5	50.2	0.87	63.5	157.9	252.0
GP104-S2	5884.4	0.86	8601.8	>2000	3540.0
GP104-S4	539.4	0.87	681.9	586.2	1096.0
GP106-S5	27.2	0.87	34.4	143.4	429.0
GP107-S2	352.6	0.88	440.8	637.2	518.0
GP107-S3	204.8	0.88	255.9	316.7	292.0
GP107-S4	52.6	0.86	67.3	75.6	163.0
GP108-S2	67.8	0.83	89.9	48.1	288.0
GP113-S2	3.1	0.85	4.0	3.4	22.0
GP117-S3	344.2	0.86	440.3	370.6	451.0
GP117-S4	306.6	0.87	387.7	273.4	344.0
GP117-S5	88.3	0.87	111.6	263.7	307.0
GP118-S4	52.5	0.89	64.9	75.4	128.0
GP118-S5	13.0	0.88	18.5	9.1	40.0
GP119-S4	112.2	0.87	141.8	148.2	180.0
GP120-S5	17.4	0.71	27.0	9.2	100.0
GP124-S2	5.3	0.85	6.8	9.4	11.0
GP125-S2	5.8	0.87	7.3	69.3	159.0
GP127-S2	14.8	0.87	18.7	0.3	
GP128-S2	2.6	0.87	3.3	0.5	
GP129-S4	797.5	0.87	1008.3	962.5	1113.0
GP129-S6	1.9	0.84	2.5	0.1	0.7
GP130-S2	1.5	0.88	1.9	1.7	26.1
GP130-S4	396.7	0.87	501.6	371.5	542.0
GP131-S4	0.3	0.87	0.3	1.4	
GP133-S4	0.3	0.85	0.3	0.4	
GP136-S2	2.1	0.93	2.5	12.5	1.3
GP137-S2	2.3	0.86	2.9	3.4	2.3
GP138-S3	71.8	0.84	94.1	101.7	45.0
GP139-S1	0.4	0.94	0.4	0.8	
GP139-S4	0.3	0.86	0.3	0.5	
GP139-S5	11.2	0.87	14.2	47.4	42.0
GP139-S6	268.6	0.88	335.8	463.0	445.0
GP140-S4	36.2	0.86	53.0	54.9	116.0
GP140-S5	290.5	0.87	419.8	436.0	760.0
GP140-S6	60.2	0.87	87.0	26.6	70.0
GP143-S2	44.0	0.86	56.3	138.6	38.0
GP143-S3	100.1	0.88	125.1	269.5	374.0
GP143-S4	296.6	0.86	433.6	847.0	646.0
GP144-S4	70.3	0.87	88.8	128.5	97.0

SPME PREPARATIVE APPLICATIONS IN ANALYSIS OF ORGANICS IN RADIOACTIVE WASTE

J. Young

ABSTRACT NOT AVAILABLE AT TIME OF PUBLICATION

CONGENER SPECIFIC PCB GC ANALYSIS: A FUNDAMENTAL APPROACH

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The US EPA 846 method which describes Congener Specific Quantitation of PCBs by GC capillary columns is a significant improvement for the quantitation of technical mixtures and environmental samples. However, there are some refinements needed. These refinements reflect new developments in GC stationary phase and a more intense effort by leading laboratories in the United States and in the European Union countries.

We describe separation for key PCBs separated on a 5% phenyl dimethylsiloxane and a 50% phenyl dimethylsiloxane column. Data will be shown to illustrate the resolution of several pairs of PCB congeners, focusing upon the 7 indicator PCBs call out by the BCR protocol of the European Union and neighboring congeners.

Table 1. PCB Retention Indices HP-5 and HP-50+

Indicator Congeners	HP-50+	HP-5
CB 28	2,207	1,892
CB 31	2,168	1,892
CB 52	2,284	1,960
CB 101	2,474	2,110
CB 118	2,617	2,233
CB 149	2,630	2,223
CB 153	2,652	2,280
CB 163	2,748	2,338
CB 138	2,763	2,341
CB 180	2,898	2,485
CB 209	3,333	2,929

Table 1 shows data given for standard retention indices for 11 selected probe PCBs that allow a standard rating of the polarity of the stationary phases. This should be considered in order to compare other columns and other protocols to standard "ground-state" of conditions for the separation and identification of PCBs in technical mixtures and environmental samples.

We show in Table 2 the retention indices on the two different columns for standard probe compounds useful for a generic measure-of-goodness rating fundamentally important before the consideration of how columns separate (or do not separate) the PCBs.

Table 2. Column Checkout Test Mix Retention Indices

	HP-5	HP-5	HP-50+	HP-50+
	Isothermal RI	**Temp Prog RI**	**Isothermal RI**	**Temp Prog RI**
4-Chlorophenol	1,184	1,206	1,424	1,421
1-Decylamine	1,241	1,257	1,333	1,338
Methyl Decanoate	1,320	1,324	1,420	1,329
1-Dodecanol	1,472	1,476	1,600	1,581
Acenaphtylene	1,460	1,476	1,801	1,782

Contrary to conventional opinion, the 5% phenyl phase column has a selectivity for closely eluting PCB pairs allowing resolution of PCB congener pair 128 and 163. It was previously thought that more polarity was needed to obtain this resolution. This may be a result of a difference in commercial stationary phases of the same generic type, or the result of renewed optimization or some combination of both efforts. We will discuss the advantages and disadvantages of each of these columns as well as discussing the tradeoff of the film thickness, column length, use of hydrogen as the carrier and time considerations.

FULL EVALUATION OF A MICROWAVE-ASSISTED PROCESS (MAP™) METHOD FOR THE EXTRACTION OF CONTAMINANTS UNDER CLOSED-VESSELS CONDITIONS

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The Microwave-Assisted Process (MAP™)[1-4] has been the subject of enhanced interest from the environmental sector lately. We report on inter-laboratory "round-robin" work that was just completed with the view to obtain promulgation of an associated method. Other data being presented are the result of previous method development, validation, and certification work aiming at the extraction and subsequent GC/MS determination of mixtures of representative toxic substances. Target analytes under study during the course of this one-year validation process include PAHs, PCBs, base-neutrals, chlorinated and organo-phosphorus pesticides, phenols and substituted phenols, and phenoxy acid herbicides. Spike matrices consisted of soils, marine sediments, harbour sediments, a creosote contaminated soil certified with PAHs and PCBs, sand and air filter media. Recoveries from these matrices were acceptable (>80%); precision was generally in the 10% (RSD) range. The potential problem of degradation of thermally labile pesticides was addressed. Spiked samples before and after the high temperature/high pressure extraction process did not result in additional decomposition products[5].

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OVERVIEW OF RCRA ORGANIC METHODS PROGRAM

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ABSTRACT NOT AVAILABLE AT TIME OF PUBLICATION

SUMMARY OF STABILITY STUDIES FOR VOLATILE ORGANICS IN ENVIRONMENTAL SOIL SAMPLES

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ABSTRACT

Characterizing the distribution of volatile organic compounds (VOCs) in soil is a crucial activity in remediating sites contaminated by chlorinated solvents and/or petroleum hydrocarbons. This typically involves the extraction, speciation and quantification of VOCs from discrete soil samples which are assumed to contain VOC levels that closely reflect in situ values. However, previous studies have shown that analyte concentrations can change during sample collection, pre-analytical holding, and sample preparation prior to analysis. These changes usually result in analyses that underestimate the actual levels of contaminants present at a site. Accurate quantification of VOCs in soil samples can only be achieved if protocols for sample collection, preservation and preparation for analyses can reduce and manage uncertainty of the total process. This includes the potential overwhelming influence of heterogeneity and other sources of uncertainty.

This report summarizes the work at Oak Ridge National Laboratory since 1993 on the stability of VOCs in soil samples. The work described here focuses on the development of preservation methods that can improve the stability of VOCs in soil samples during pre-analytical holding. Alternative sample preservation methods were developed and evaluated for adoption as a standard protocol in environmental sampling. The latter would allow not only improved VOC measurements but also extended holding times for VOC soil samples beyond the currently prescribed maximum of 14 days.

The final goal of this work is to define a standard preservation procedure to maintain the stability of VOCs in environmental soil samples. Work at ORNL and elsewhere has shown acidified water as a promising preservative for soil samples with low concentration levels. ORNL's analyses of containerization suggest that a 40-ml VOA vial with a thick Teflon-lined septum cap minimizes volatilization. Findings and conclusions from the work in the extension of pre-analytical holding times for soils, as well as waters, can contribute to making quality assurance materials that may provide the confidence in the practicality of field analytical methods.

ESTIMATING THE TOTAL CONCENTRATION OF VOLATILE ORGANIC COMPOUNDS IN SOIL SAMPLES

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ABSTRACT

This manuscript describes an on-site method of estimating the total concentration of volatile organic compounds (VOCs) in soil, relative to a site-specific 0.2-mg/kg working standard. The purpose of this decision tool is to allow on-site sampling activities to incorporate the appropriate soil sample collection and handling protocols necessary for high- and low-level gas chromatography/mass spectrometry analysis. Combining rapid on-site analysis with sampling procedures that limit substrate dissaggregation and exposure improves efforts to achieve site-representative estimates for vadose zone contamination.

INTRODUCTION

Gas chromatography/mass spectrometry (GC/MS) Methods 8260 and 8240 are frequently used by laboratories to identify and quantify volatile organic compounds (VOCs) in soil samples. One of the challenges of coupling a sample collection and handling protocol with GC/MS analysis is that it has limited range of detection (two to three orders of magnitude). Of particular concern is that high analyte concentrations may degrade the performance of the MS detector. To cope with this limitation, samples thought to be contaminated with VOCs at levels above 0.2 mg/kg are prepared by extraction with methanol (MeOH)—this is known as the high-level method. In contrast, samples thought to have concentrations below 0.2 mg VOC/kg are analyzed directly (that is, soil is transferred directly to the analysis vessel)—this is referred to as the low-level method.

Also of concern is that VOCs in soil samples fail to maintain their concentration integrity if they are not collected and handled with limited disruption and exposure. To address this issue, two in-vial sample collection and analysis methods (Methods 5035 and 5021) have been proposed for the third update of the Test Methods for Evaluating Solid Waste, SW-846[1]. In-vial methods are most effective at maintaining VOC integrity when samples are transferred directly to a prepared analysis vessel during the field sampling activity[2].

The successful combination of in-vial sample collection and handling and GC/MS analysis requires that either multiple samples be taken for laboratory analysis or a rapid field screening method be used. In the former case, samples would have to be collected in an appropriate fashion for both high- and low-level methods of GC/MS analysis, and at least one additional sample for a laboratory screening analysis needs to be obtained[3]. In the latter case, the use of an on-site screening method would establish which sample handling procedure should be used prior to the collection of a sample for GC/MS analysis.

In this study we investigated the use of a total VOC analyzer to establish which procedure should be used for sample collection. Information concerning the theory of total soil vapor analysis can be found elsewhere[4,5]. The on-site analysis procedure developed here uses a hand-held total VOC analyzer, takes less than 5 seconds for an analysis, and requires only 10 or 20 g of contaminated soil for many chlorinated and gasoline-range organic compounds. Collection and preparation of a soil sample for on-site analysis takes less than 1 minute. Proper preparation and analysis of working standards are important for calibrating the results relative to both sample matrix and the existing meteorological conditions. Furthermore, depending on the objectives of the field investigation, this procedure could be used to estimate a range of concentrations or test whether concentrations are above or below 0.2 mg VOC/kg.

EXPERIMENTAL

Equipment and Materials

A Microtip HL-2000 (Photovac, Inc.) equipped with photo ionization detector (PID) was the total VOC analyzer used. This instrument was modified by replacing the inlet tube with a 3-cm-long Teflon tube (3.17 mm o.d.). Clear, 44-mL VOA vials were selected as the vessels for the working standards and for the analysis of on-site soil vapor samples. These vials were modified by punching a 5-mm hole in the center of the Teflon-lined silicone septum to allow air to easily pass around the PID inlet tube once it is inserted through the hole. To temporarily cover the vials, 3- x 3-cm squares of light-gauge aluminum foil were pressed over the mouth of the glass vial, then secured in place with the septum and screw cap (Figure 1). The collection and transfer of soil to the modified VOA vials was accomplished with a 10-mL disposable plastic syringe, with the tip, rubber plunger cap and holding post removed[3]. Reagent-grade trichloroethene (TCE), fresh unleaded gasoline, and purge-and-trap grade MeOH were used to prepare the stock standards. A 10-µL syringe (Hamilton) was used to transfer volumes of the stock standard when preparing the working standards.

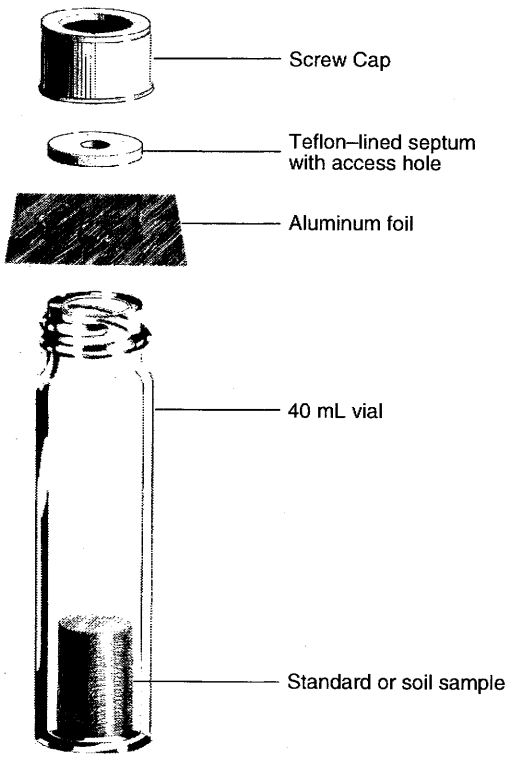

Figure 1. Modified VOA vials for rapid total VOC soil analysis.

Soil Vapor Analysis

In preparation for a soil vapor analysis, the instrument was initially calibrated using zero grade air and standard gas (100 ppm isopropylene) cylinders. To perform an analysis, 10 to 20 g of soil, obtained in 5-g increments (5 g soil ≈ 3 mL) with a 10 mL syringe, were transferred to the VOA vial after the cap, septum and foil liner were removed. Special care was taken when collecting and transferring the soil subsample to minimize disaggregation. Filling the syringe with more than 3 mL of soil is not recommended because larger amounts are often difficult to remove and are easily disaggregated. Once the appropriate volume of soil was obtained and the foil liner, septum and cap tightly secured, the VOA vial was hand shaken for 5-10 seconds to disperse the soil grains. After the foil liner was visually checked for adhering clumps of soil (they were knocked off if present), the sample was analyzed by forcing the inlet tube through the foil liner. The highest reading displayed by the digital meter within a couple of seconds of the foil liner being punctured was the value recorded. The total amount of time between exposing a fresh soil surface and completing this analysis was less than 1 minute.

Working Standards

Separate stock standards of 0.53 mg TCE/mL and 1.1 mg gasoline/mL were prepared by transferring approximately 0.010 and 0.040 mL of these constituents, respectively, into 25 mL of MeOH. Working standards were prepared by transferring 0.004-mL aliquots with a microliter syringe to a clean surface on the inside of the VOA vial. For the site-specific working standards, the VOA vials contained 10 or 20 g of uncontaminated soil so as to achieve the desired VOC concentration of 0.2 mg VOC/kg concentration. Once capped, the vials were hand shaken and allowed to sit for at least 2 hours prior to analysis. The working standards were analyzed using the same procedure as described for soil samples.

Field Samples

Experiments were performed to determine the response of the Microtip HL-2000 to working standards with and without soil present. In addition to evaluating the working standards, a field trial was performed on a site where TCE contamination has been present for 25 years. For the field trial, the on-site rapid total soil vapor measurements were compared with collocated grab sample concentrations. Discrete soil samples were collected and handled using an in-vial procedure that was compatible with an equilibrium headspace gas chromatography (HS/GC) analysis method[6]. The sampling locations for this study were flat 20- x 20-cm surfaces dug with a spade to depths of 10-30 cm. At each location a site-specific TCE working standard was analyzed just before the hole was dug. Immediately after a fresh soil surface was exposed, two 10-mL syringes were used to collect the soil for the total VOC vapor analysis. Once the soil vapor analysis was completed, a single syringe was used to collect a collocated sample for HS/GC analysis. The grab samples taken for HS analysis were transferred with a modified syringe directly to 44-mL VOA vials containing 20 mL of Type 1 water. All three collocated soil subsamples were obtained within 5 cm of one another, and on-site analysis and sample collection was completed within 2 minutes of exposing a fresh surface.

RESULTS

Table 1 shows the response of the Microtip HL-2000 to working standards when the VOA vials were empty or contained 10 or 20 g of soil. These working standards were allowed to sit for 2 to 4 hours, or 1 to 2 days prior to analysis. The results for the working standards, even with soil present, were often more than ten times higher than the laboratory background readings of 0.5-1.0 ppmv. As anticipated, the presence of the soil matrix had a

pronounced effect on the results for the working standards. The near-surface soil used for these site-specific working standards had a moisture content of 15±5% and an organic carbon content of 1±0.5%. For working standards held for more than several hours, the results were lower because of vaporization losses (Table 1), since aluminum foil fails to form a hermetic seal with rigid surfaces[2].

Table 1. Microtip readings of TCE and gasoline standards* in ppmv with and without soil present. Laboratory background Microtip readings ranged between 0.5 to 1.0 ppmv.

TCE ppmv		Gasoline ppmv	
10 g soil**	no soil	20 g soil**	no soil
10.8	18.7	8.9	17.5
11.1	19.0	8.0	20.4
11.6	19.5	9.3	20.8
11.1	17.8	6.9	21.4
11.0	18.7		
12.1	17.4		
11.3	16.7		
11.0	17.9		
11.5	18.3		
	9.8†		
	10.0†		
	4.3††		
	6.9††		

* 0.004 mL of stock standards (0.53 mg TCE/ mL or 1.1 mg gasoline/mL).
** Weight of soil—thus, concentrations were approximately 0.2 mg total VOC/kg.
† Held for 24 hr prior to analysis.
†† Held for 48 hr prior to analysis.

Table 2. Results of rapid total VOC soil vapor and collocated grab sample analyses. In addition, values obtained for the measurement of the site-specific working standards and background are included.

Field trial		
Soil vapor PID (ppmv)	Aqueous HS/GC (mg TCE/kg)	ratio *
32	0.765	42
1.8	0.0064	--
7.9	0.142	--
64	1.62	40
230	6.75	34
1.8	0.0369	--
1.9	0.0518	--
34	1.28	27
42	1.65	25
0.5	0.0239	--
0.2	0.0422	--
20	1.02	20
120	2.25	53
200	3.50	57
590	11.9	50
		39+13†

Response (ppmv) of site-specific TCE working standards and background	
Working Standard	Background
8.2	0.6
8.0	0.3
8.3	0.0
6.0	0.0
7.4	0.0

* Ratio of soil vapor to grab sample concentration, for locations where TCE was >0.2 mg/kg.
† Mean and standard deviation.

Table 2 shows the results obtained during the field trial, including the values obtained for the site-specific TCE working standards. The relationship between the total VOC soil vapor and collocated grab sample analyses was both linear and significant, with a correlation (r^2) of 0.965 (Fig. 2). Indeed, a fairly constant ratio existed between these two analyses for the locations where TCE concentrations were >0.2 mg/kg (Table 2). This relationship is encouraging, since it indicates that concentrations over a range of at least 0.2 to 10 mg VOC/kg could be estimated using the rapid soil vapor measurement technique, provided that an adequate number of confirmation samples were taken. Over the concentration range shown in Figure 2, the response of the PID appears linear; however, at higher concentrations, it is anticipated that this relationship would become nonlinear. It is probable that the linear range could be extended to higher concentrations if a field instrument equipped with a flame ionization detector were used.

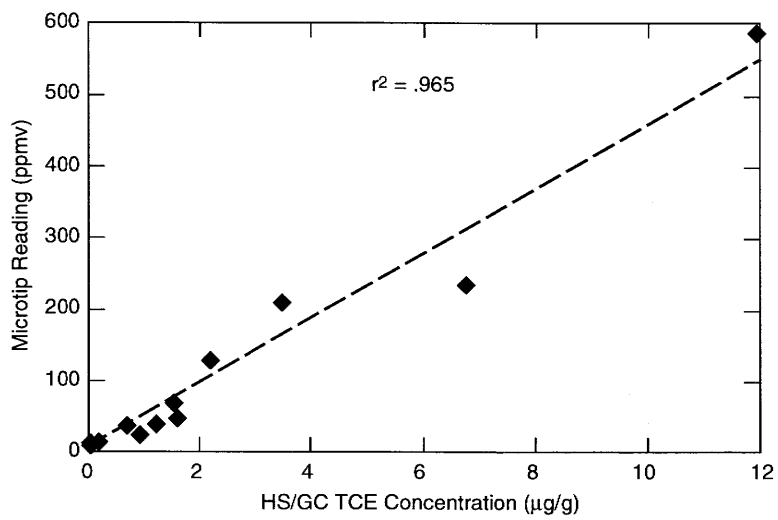

Figure 2. Relationship between rapid total VOC soil vapor analysis and grab samples.

DISCUSSION

Collection of soil for VOC analysis should always be the first operation performed after the surface to be sampled has been exposed to the atmosphere. If a freshly exposed soil surface is not rapidly sampled, analyses existing in a vapor phase diffuse away from an unsaturated porous matrix, thereby disturbing the equilibrium that existed among the vapor, liquid and sorbed phases. Following the depletion of the vapor phase, there are nearly instantaneous shifts in the equilibria between the sorbed and aqueous phases[7]. An example of how quickly this process can occur was observed by tracking the TCE concentrations in a soil subsample collected from the middle (1.2 cm below the surface) of a 3.6-cm-i.d. x 5.1-cm-long split-spoon core liner. Grab samples taken from this subsurface location were shown to have lost more than 90% of this analyte when the core liner was left uncovered in a plastic bag for 40 minutes prior to sampling[2]. In general, as the surface area of exposure increases, the length of time before significant losses occur decreases, even when precautions are taken to limit disruption of the native soil structure. Soil texture also has been shown to be a factor. For instance, sandy soils tend to lose VOC more rapidly than cohesive silts and clays[3].

In addition to exposure concerns, soil samples must be transferred directly to vessels with hermetic seals; these vessels must either contain a solvent or permit an analysis to be performed without them being opened. Proposed Methods 5035 and 5021 both recommend the use of VOA vials with Teflon-faced silicone septa for in-vial sample handling and analysis when VOC concentrations are below 0.2 mg/kg. In general, methods capable of establishing lower VOC concentrations are practical when attempting to establish the full spatial extent of contamination. However, when the primary objective of a site investigation is to locate and remediate source regions where residual

product often exists, methods that maintain the integrity of soil samples contaminated with high VOC concentrations (>0.2 mg/kg) are of paramount concern. For concentrations above 0.2 mg/kg, however, neither method in the initial draft provided guidance on how to handle samples for analysis without incurring large volatilization losses. Losses of VOCs from soil samples with high levels of contamination can be limited to the same extent as the low level samples by transferring them directly to vessels containing MeOH[8, 9].

To avoid placing samples with VOC levels less than 0.2 mg/kg in MeOH, this study has demonstrated a simple method of preparing site-specific working standards and handling field samples so that a rapid on-site analysis can be performed. Tables 1 and 2 show that the performance of working standards was fairly reproducible. Trends in our initial findings suggest that working standards should be prepared within 8 hours (or less) of their use and should be stored in a cool location and out of direct sunlight[10]. The shelf life of a stock standard is on the order of months, if held in a hermetically sealed vessel.

Although this study only considered field samples contaminated with TCE, other compounds are expected to behave similarly because the response of a PID varies by less than a factor of 1.4 for common chlorinated and aromatic hydrocarbons (i.e., TCE, tetrachloroethene, benzene, and toluene). It is possible that this on-site rapid analysis method would also work for fuels heavier than gasoline, although the lower quantity of highly volatile constituents would likely require larger quantities (≈50 g) of soil. We present this on-site systematic and rapid approach to soil vapor analysis so that informed decisions about how to handle and prepare samples for laboratory VOC analysis can be made.

SUMMARY

Failure to maintain site-representative VOC concentrations results in false negative levels, and leads to inadequate remediation and possibly even premature closure. To address these shortfalls, we recommend estimating the total VOC concentration at a sampling location prior to collecting samples for laboratory quantitation. The focus of this method is to inform site investigators when the use of MeOH is justified for on-site sample preparation. Likewise, it also indicates when a low-level in-vial sample handling and analysis procedure (Method 5035 or Method 5021) is appropriate. Additional information concerning this on-site analysis decision tool and how to incorporated it into field sampling plans is available elsewhere[10].

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This publication reflects the views of the author and does not suggest or reflect policy, practices, programs, or doctrine of the U.S. Army or of the Government of the United States.

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AUTOMATED SMALL VOLUME EXTRACTION OF SEMIVOLATILES FOLLOWED BY LARGE VOLUME GC/MS INJECTION

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Historically, semivolatile extractions have been time consuming, labor intensive, and use large amounts of solvents. The EPA has attempted to reduce some of these problems by employing solid phase extractions in some cases which use less solvent than other techniques. Our objective is to take this improvement further by using an automated solid phase and liquid/liquid extractor in conjunction with a large volume injector to reduce sample and solvent size.

This study will consist of extracting water samples spiked with method 8270 analyses. Various sample sizes will be investigated in combination with various injection volumes. Precision, recovery, and MDL data will be presented. The final goal will be to achieve acceptable 8270 method performance.

Extractions will be performed using an automated robotic extractor. Analysis will be by full scan quadrupole GC/MS using a large volume injector. Procedure will follow method 8270 except for sample and injection volumes.

Current work shows 1ml extractions followed by 100ul injections into a GC/MS system are possible to achieve reasonable detection limits.

A COMPARISON OF ASE WITH SOXHLET, SFE AND SONICATION FOR THE EXTRACTION OF EXPLOSIVES FROM CONTAMINATED SOILS

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The ongoing privatization of former military bases and former munitions manufacturing facilities has increased the need to deliver fast results for the determination of explosives in soils. Generally, the chromatographic methods are straight forward and fast. However, there are several ways from which to choose to extract the analyses of interest from the soil samples. Accelerated solvent extraction (ASE) is a relatively new extraction technique. It has been applied to several compound classes ard been shown to be equivalent to standard extraction procedures for the extraction of semivolatiles, polychlorinated biphenyls, organochlorine and organophosphorus pesticides, phenoxy herbicides, hydrocarbon contaminants, and polychlorinated dioxins and furans from solid and semisolid samples. The purpose of this study was to compare ASE to standard extraction procedures for the recovery of explosive

compounds from contaminated soils.

In this study, Soxhlet, sonication, supercritical fluid extraction (SFE) and ASE were used for the extraction of explosive compounds from soils. Soxhlet extraction, while generating accurate and reproducible results, is time-consuming (12-24 hours) and uses large amounts of organic solvents (300-500 mL). Sonication extraction reduces the time required, but the accuracy of the results is questionable. SFE offers a dramatic savings in solvent consumption, but hardware and method development considerations can eliminate potential time savings. ASE was developed to offer extraction laboratories the ruggedness of Soxhlet extraction in a more rapid and efficient manner.

Spiked and incurred soil samples obtained from munitions plants in Germany were extracted using Soxhlet, sonication, SFE and ASE. The extracts were analyzed using HPLC and GC. The data from this study demonstrate that ASE achieves results equivalent to Soxhlet and superior to sonication and SFE, in terms of accuracy and precision, for the extraction of explosive compounds from contaminated soils. Results generated from these techniques will be compared and discussed.

A COMPARISON OF MICROWAVE EXTRACTION SOLVENT SYSTEMS: NON-POLAR VS. NON-POLAR/POLAR, GENERAL DIFFERENCES FROM SOIL SAMPLES

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Microwave assisted organic extraction of environmentally important organic moieties was introduced several years ago by Ganzler and modified by Lopez-Avila[1,2]. Their work demonstrated that microwave extraction is a viable alternative to conventional Soxhlet extractions. The preferred solvent of choice would be a pure hydrocarbon solvent, but these solvents were very poor microwave absorbers. As a result, the solvent systems were altered to incorporate at least 10% polar solvent mixture to absorb microwave radiation. However, the polar solvent extracts many interfering polar compounds. These problems with microwave assisted organic extraction can be overcome through the use of secondary microwave absorbing materials like Weflon[3].

Weflon is a chemically inert Teflon impregnated with silicon carbide, a very strong microwave absorbing material. Weflon can be added externally to microwave vessels through the use of a sleeve for the microwave vessel or internally to the microwave vessel as a disk or other physical designs. During microwave heating, the microwaves are partially or totally absorbed by the Weflon, the Weflon material heats up and transfers the heat to the non-microwave absorbing non-polar solvent. Despite the lagtime in transferring the heat from the Weflon to the solvent, the solvent is rapidly, reproducibly, and controllably heated. Pure dry hexane is only heated to ~70°C in 15 minutes whereas with the addition of Weflon the identical experiment reached an extraction temperature of 115°C in ~1 minute. Similarly, the use of a non-polar/polar solvent combination of 90/10 toluene/methanol heated in ~7 minutes whereas with the addition of Weflon the identical experiment reached the same temperature in ~2 minutes.

The compounds extracted from non-polar and non-polar/polar extraction solvent systems are quite different. A non-polar/polar solvent system extracts many polar compounds in addition to the desired environmentally regulated nonpolar compounds. Many of the polar compounds co-elute during GC-MS analysis resulting in complex or unquantitatable peaks. Omitting the polar solvent eliminates interfering polar compounds from the extraction enhancing analysis of the remaining components. Using an external microwave absorber enables the extraction to be redesigned, allowing the extraction to be based solely on the chemistry of the compounds of interest rather then the microwave absorption capabilities of the solvent. This approach permits a chemically optimized and efficient approach to microwave extraction.

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3. "New Product Announcement; Weflon"; Milestone Corporation, Sorisole, Italy.

ACCIDENTAL CHEMISTRY

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ABSTRACT

There are several procedures which have been incorporated into EPA sample preparation methods which can cause unintended chemical reactions with target analyses. Knowing the potential for these reactions can lead to improved methods which avoid the "accidental chemistry" or aid proper data interpretation. Many analytical tests rely on colorimetric or derivatization steps as integral parts of the analysis process but generally organic sample preparation and analysis attempts quantitate and identify analyses without changing their chemical structure until the actual detector is reached. On some occasions interfering compounds are produced during sample preparation. Three instances have been found where acetone, used in sample preparation, reacted with either target analyses or other compounds present and degraded the analytical results. The methods and analyses affected are: 3520- 8080/81/82 organochlorine pesticides and PCBs, 8150/51 dinoseb and 3540/50 - 8270 primary amines. Some of these problems have only come to light over time as analyte lists have expanded or quality requirements have increased.

ORGANOCHLORINE PESTICIDES AND PCBS

Unknown chromatographic interferents were coeluting with some organochlorine pesticides (OCP) and PCBs when water samples and blanks were prepared using continuous liquid-liquid extractors (Method 3520). These interfering peaks were not present when the extraction was performed by separatory funnel (Method 3510). Typical chromatograms are shown in Figures 1 and 2.

Early attempts to identify the source(s) of the problem examined glassware cleaning, condenser temperature, acetone spike solvent, contamination from lab air and reagent water. These various possibilities were tested one at a time. The most likely sources were examined first. The other possibilities were tested over the months that followed. Some experiments affected contamination levels but the specific process which produced the contaminants was not understood nor did the analysts know how to "make the problem stay away".

After several one-at-a-time attempts to isolate the source(s) of the contaminants a set of factorial design experiments was used to systematically investigate the 8 most likely sources. The sources or variables are called factors in statistical jargon. Each factor was varied between two levels or options. The factors selected were surrogate spike solvent, reagent water source, exposure to laboratory air, glassware cleaning, extraction method, dichloromethane source, condenser temperature and exposure to laboratory light. This set of experiments narrowed down the list of probable sources (factors). The four most likely factors were selected and tested most extensively in another round of factorial design experiments. The results from this study lead to probable sources and a plausible mechanism. Several possible ways to reduce or eliminate the problem were deduced.

The first factorial design experiment set examined the 8 factors listed in Table 1. Each factor was tested at two "levels". A 16 run subset of the 256 possible combinations was selected to provide the best information about the effect of each factor and possible two factor interactions. The factorial design, calculations and data interpretation were carried out with the aid of Design-Ease software from Stat-Ease of Minneapolis, Minnesota. Table 1 shows each factor and the two levels investigated.

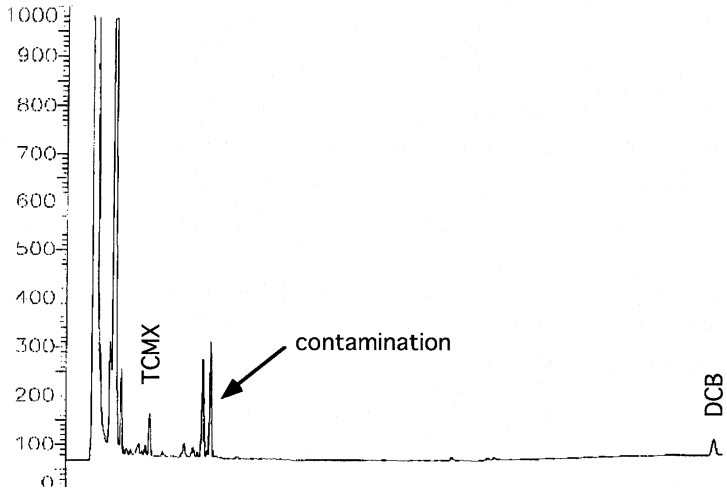


Figure 1. OCP/PCB chromatogram of continuous liquid-liquid extract (acetone)

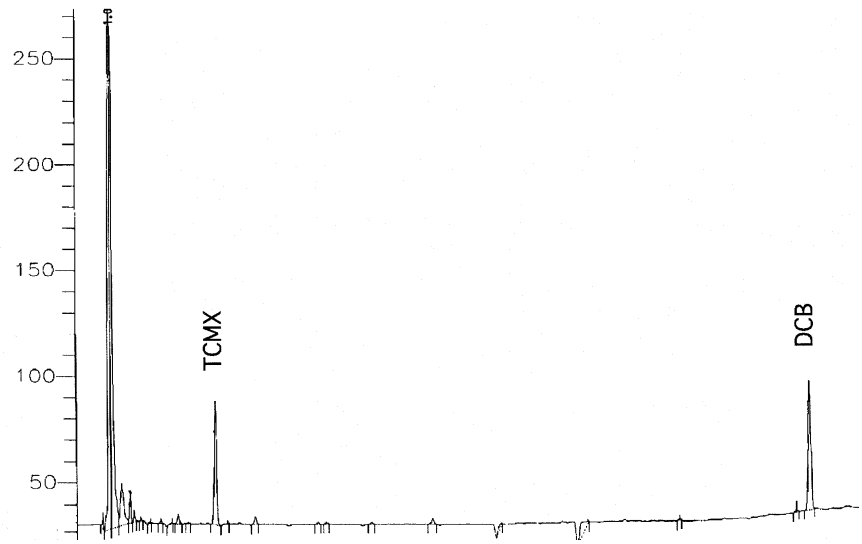


Figure 2. OCP/PCB chromatogram of separatory funnel extract (acetone)

The normal probability plot of the standardized effect calculated from the design shows a group of factors which have an effect larger than the *noise* (or random variability) calculated from the other factors. These factors and factor interactions were extraction method, DCM source, spike solvent & reagent water interaction, spike solvent, spike solvent & extraction method interaction, spike solvent & DCM source interaction and reagent water. Because this was a 1/16th fraction of all possible experimental combinations, all factor interaction effects were aliased with other effects. For example, the calculated effect assigned to spike solvent & reagent water also included the effects from lab air & condenser temperature, cleaning & light exposure and extraction method & DCM source. Also, higher order interactions would be included. Thus, care must be exercised when interpreting the results to avoid misassigning a particular effect to an individual factor or interaction. Previous chemistry knowledge allows some potential interactions to be labeled as very unlikely. For example, potential interactions involving reagent water source and CLLE condenser temperature were unlikely because they do not come in contact with each other.

This factorial design did not completely define all problem factors but eliminated some so focused effort could be directed to the most likely factors. The effects of spike solvent, DCM source, extraction method, reagent water source and light exposure appeared strong enough to warrant further investigation.

A four factor design (16 extractions) with spike solvent (methanol / acetone), DCM source [both sources were residue analysis grade this time] (X/Y'), reagent water (commercial HPLC/DI), light exposure (foil wrap/standard light exposure) was carried out using continuous liquid-liquid extraction exclusively. This was to test the most probable off the shelf fixes and determine whether or not light exposure and reagent water were part of the problem. Since this was a full factorial design, all main factors and interactions would be free of aliases, so no confusion about attributing

effects to particular factors was anticipated. Table 2 shows each factor and the two levels investigated.

Table 1. Factors and Levels from First Factorial Design Set

Factors	+ level	- level
A) use of acetone as spike solvent (**spike solvent**)	standard acetone surrogate spike solution (**acetone**)	special methanol surrogate spike solution (**methanol**)
B) contamination in reagent water (**reagent H_2O**)	standard deionized water (**DI**)	commercial HPLC reagent water (**com H_2O**)
C) contamination in lab air pulled in through CLLE condenser (**lab air**)	standard lab setup (**std cond**)	add carbon trap to condenser top and Teflon tape all joints (**C trap**)
D) contamination on glassware surfaces (**cleaning**)	standard cleaning (**std clean**)	exhaustive cleaning: soap, water, chromic acid, water, NaOH, water, muffle oven (**rig clean**)
E) extraction method, heat input and time may be the key differences (**ext meth**)	continuous liquid-liquid extraction (**CLLE**)	separatory funnel (**sep fun**)
F) contamination in dichloromethane (**DCM**)	brand Y dichloromethane HPLC grade (**Y**)	brand X dichloromethane, residue analysis grade (**X**)
G) CLLE condenser temperature (**cond temp°C**)	tap water cooled condenser (**10°C**)	chiller cooled condenser (**0°C**)
H) exposure to fluorescent light (**light exposure**)	standard exposure to light (**std light**)	wrap extraction glassware in aluminum foil (**foil wrap**)

Table 2. Factors and Levels from Final Factorial Design Set

Factors	+ level	- level
A) contamination in dichloromethane (**DCM**)	brand Y dichloromethane residue analysis grade (**Y'**)	brand X dichloromethane, residue analysis grade (**X**)
B) contamination in reagent water (**reagent H_2O**)	standard deionized water (**DI**)	commercial HPLC reagent water (**com H_2O**)
C) use of acetone as spike solvent (**spike solvent**)	standard acetone surrogate spike solution (**acetone**)	special methanol surrogate spike solution (**methanol**)
D) exposure to fluorescent light (**light exposure**)	standard exposure to light (**std light**)	wrap extraction glassware in aluminum foil (**foil wrap**)

The results of the factorial calculations indicated the most important factors and factor interactions were spike solvent, spike solvent & light exposure interaction, light exposure. A second group of interaction effects were smaller and all related to the DCM source; DCM source & spike solvent, DCM source & light exposure and DCM source & spike solvent & light exposure.

The largest effects were caused by the acetone surrogate spike solvent and exposure to lab light. The large two factor interaction also suggests that the lab light has a *catalytic effect* on the chemical reactions which produce the interfering contaminants. The three factor interaction between acetone, extraction method (CLLE) and dichloromethane postulated in the first factorial was still present, although represented as the two factor interaction between DCM source and spike solvent. This interaction was now clear of the alias with the reagent water factor. Although this interaction was measurable it was small compared to other effects. Also, remember that the DCM grade from supplier Y was changed from HPLC grade to residue analysis grade. This grade appeared to be *cleaner* and probably reduced the measured differences between DCM sources. There was also an interaction between DCM source and lab light exposure. This was consistent with the postulated *catalytic effect* of light exposure. The three factor interaction between spike solvent, light exposure and DCM source had a small but distinguishable effect. Lastly, no effect from the two different reagent water sources was seen, thus lab water contamination was not likely.

There were significant interactions between the 3 main factors (multiplicative effect rather than additive). The spike solvent and light exposure are the main problem sources. DCM source is important but secondary. Since the interactions between spike solvent, DCM source and light exposure appear strong this explains why our early one factor at a time work was confusing.

Spike solvent shows up as the most important factor, which means that either the acetone (or something in the acetone) is reacting (light catalyzed) with other impurities present in the extraction system. These impurities are probably present in the DCM since the final concentration of contaminants correlates with DCM source. Recent experience with a different acetone supplier showed even greater levels of blank contamination. One solvent supplier indicated this was quite likely since different acetone manufacturing process produce different secondary components in the acetone.

The easiest solution to implement was switching from acetone to methanol surrogate and matrix spike solutions. Simply changing the spike solvent made a dramatic difference as illustrated by Figure 3. This extraction, with methanol spiking solutions, was performed under the same conditions as Figure 1.

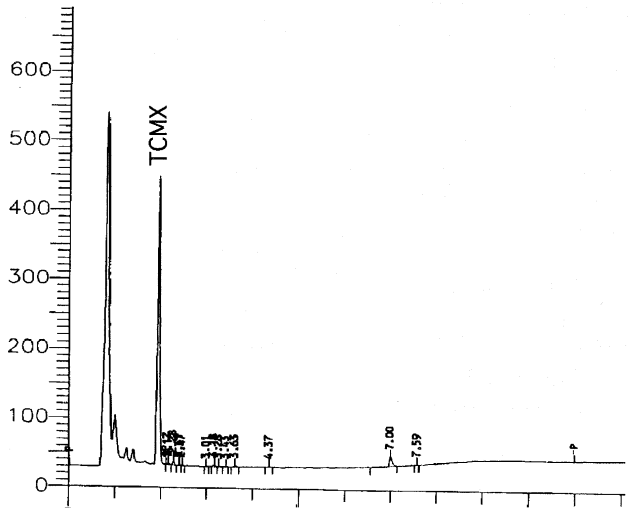


Figure 3. OCP/PCB chromatogram of continuous liquid-liquid extract (methanol)

HERBICIDES

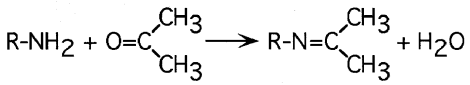
The phenoxy acid herbicide prep and analysis (Method 8150/1) relies on "intentional chemistry" at two key steps, hydrolysis of esters and methylation of acids to esters. The basic method chemistry was developed for carboxylic acids and their esters. Dinoseb, a phenol, is a more recent addition to the method. It appears that dinoseb reacts with acetone during the hydrolysis step for solid samples. The reaction and its products are currently unknown. Shortening the hydrolysis time can improve dinoseb recovery but the hydrolysis of herbicide esters becomes less efficient and results in poor analyte recovery for many other herbicides. However, substitution of methanol for acetone in spiking solutions and eliminating acetone from the extraction improves dinoseb recovery.

SEMIVOLATILE BNAS

A one to one mixture of dichloromethane and acetone (DCM/acetone) is recommended by EPA methods when extracting low level solid samples (Methods 3540 & 3550). Primary amines in the TCL and Appendix IX BNA lists show reduced recovery when concentrated in dichloromethane / acetone compared to dichloromethane alone. Similar trends have been seen in various Method Detection Limit studies. The secondary and tertiary amines do not seem to be affected. If anything the secondary and tertiary amine recoveries are slightly higher when acetone is present.

Similar phenomena were reported by McNally (Anal. Chem. 1993, 65, 596). Primary aromatic amines showed reduced recovery when concentrated by KD when using DCM/acetone. Concentration with a Zymark TurboVap improved analyte recovery. Presumably this is because it used a lower concentration temperature. They suggested that analyte losses may be largely due to ion exchange or hydrogen bonding adsorption on glassware surfaces.

One senior Quanterra chemist suggested that acetone may be reacting with the primary amine to form the imine while being heated in the concentration step. The general equation is



Imine peaks were found in the chromatogram for most amines that showed reduced recovery. Figure 7 shows two of the imine peaks that are visible on the total ion chromatogram. The peaks at 8.0 and 22.6 min. are the imine forms of aniline and 3,3'-dichlorobenzidine. The mass spectra and fragment assignments are shown in Figures 8-10. Since 3,3'-dichlorobenzidine is a diamine it shows both the mono-imine and di-imine forms.

Based on analyte recovery 2-Nitroaniline does not react under these conditions and 4-nitroaniline appears to only react slightly. α,α–Dimethyl phenethylamine has very poor chromatographic peak shape and is a consistent calibration problem, thus it is not surprising that it does not follow the same trend as the remainder of the primary amines.

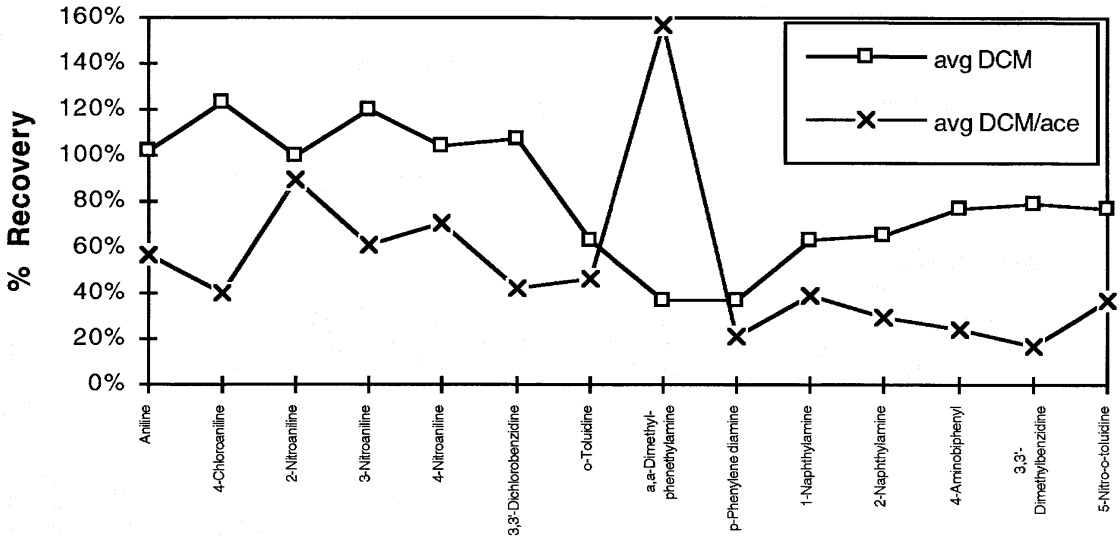


Figure 4. Primary Amine Recovery from DCM and DCM/acetone KD Concentrations

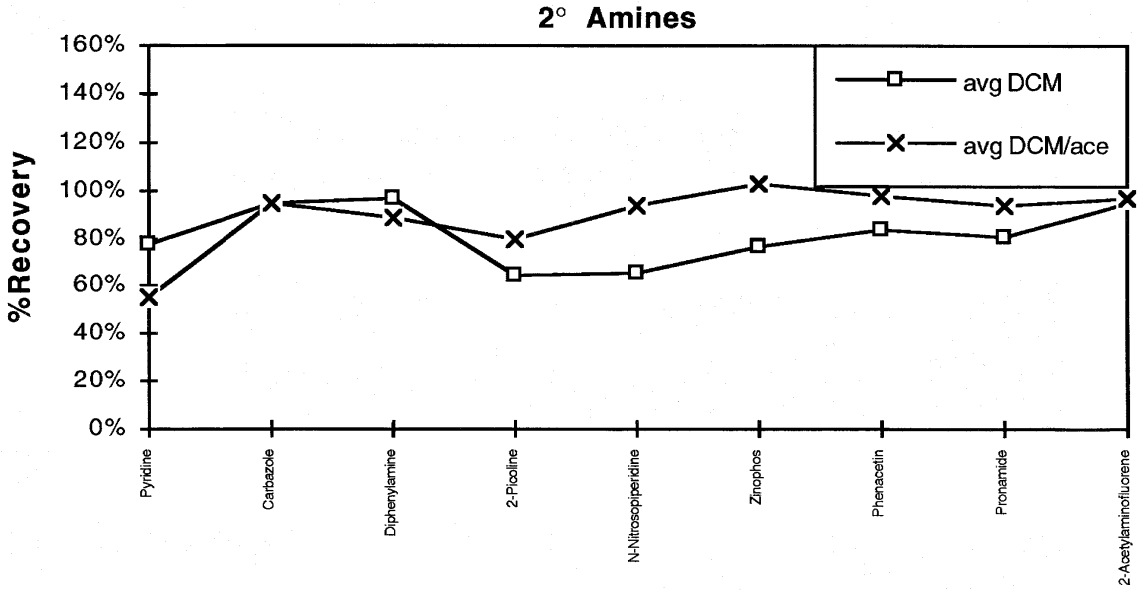


Figure 5. Secondary Amine Recovery from DCM and DCM/acetone KD Concentrations

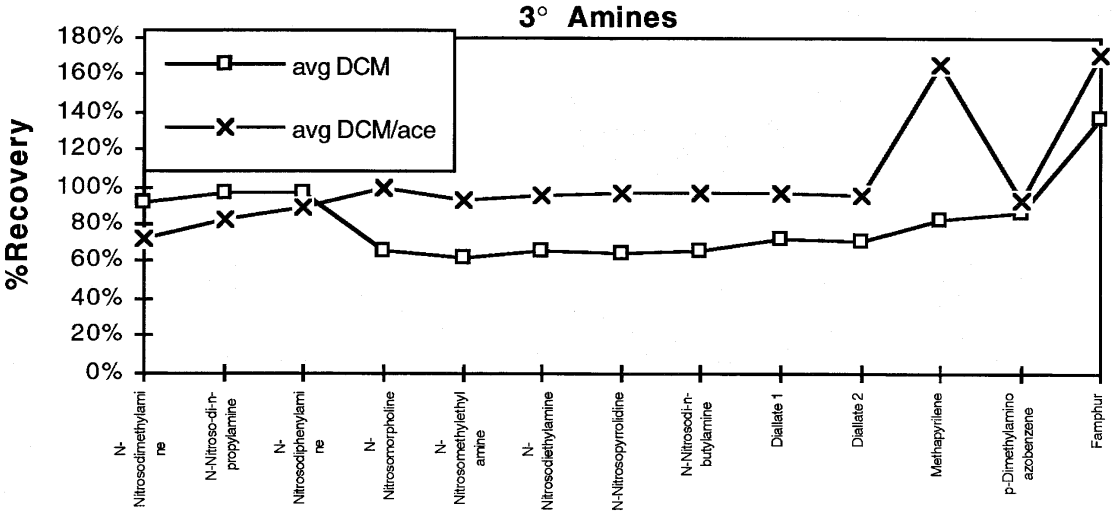


Figure 6. Tertiary Amine Recovery from DCM and DCM/acetone KD Concentrations

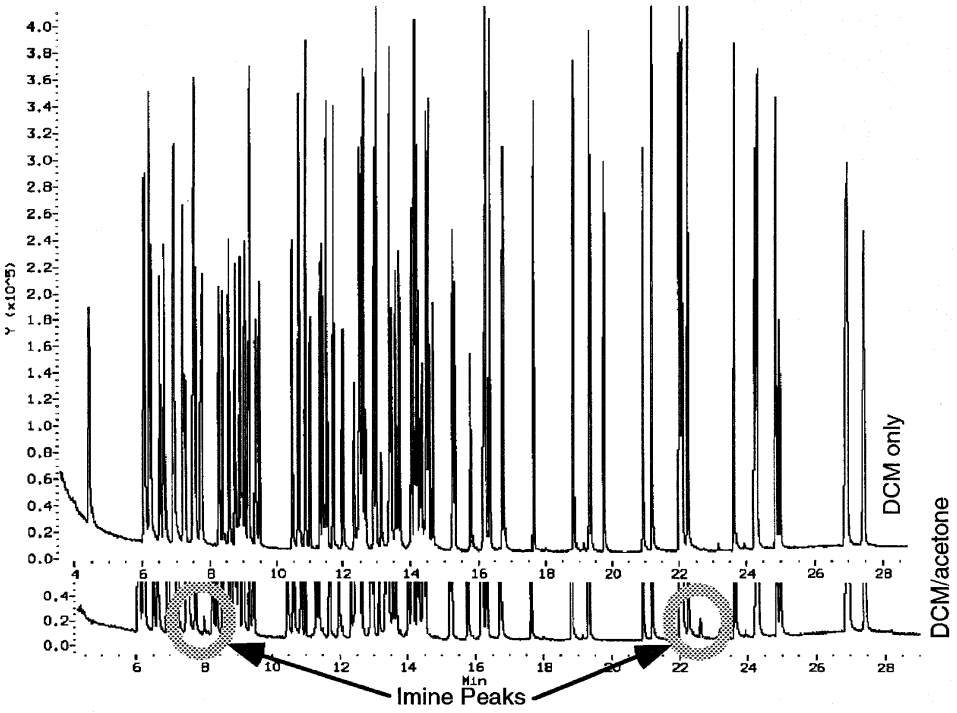


Figure 7. Imine Peaks in BNA Chromatogram

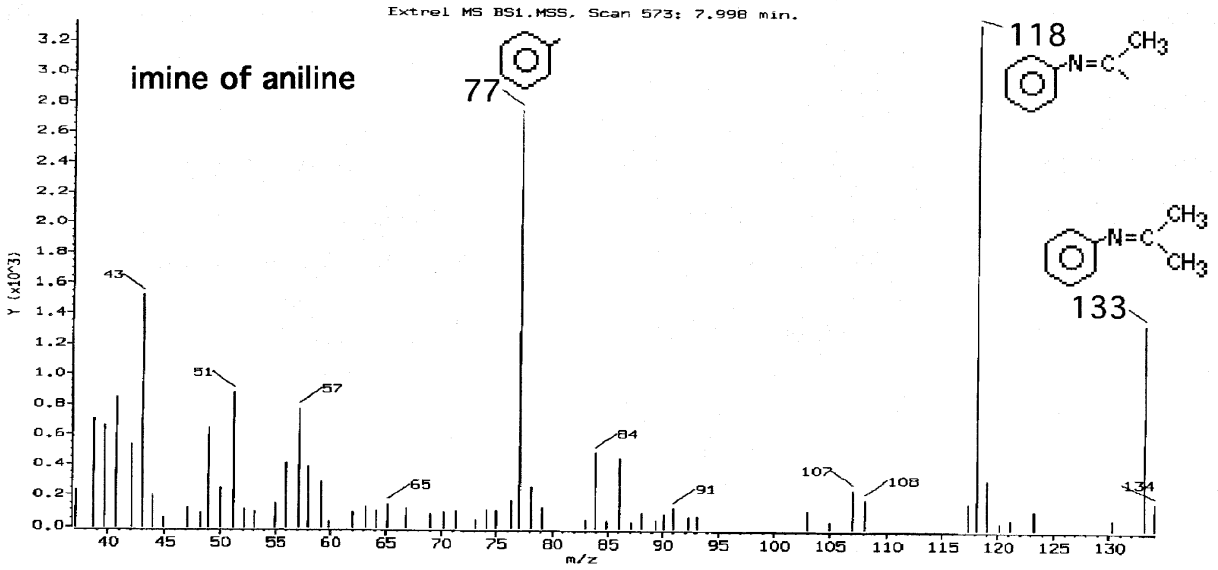


Figure 8. Mass Spectrum of Imine Form of Aniline

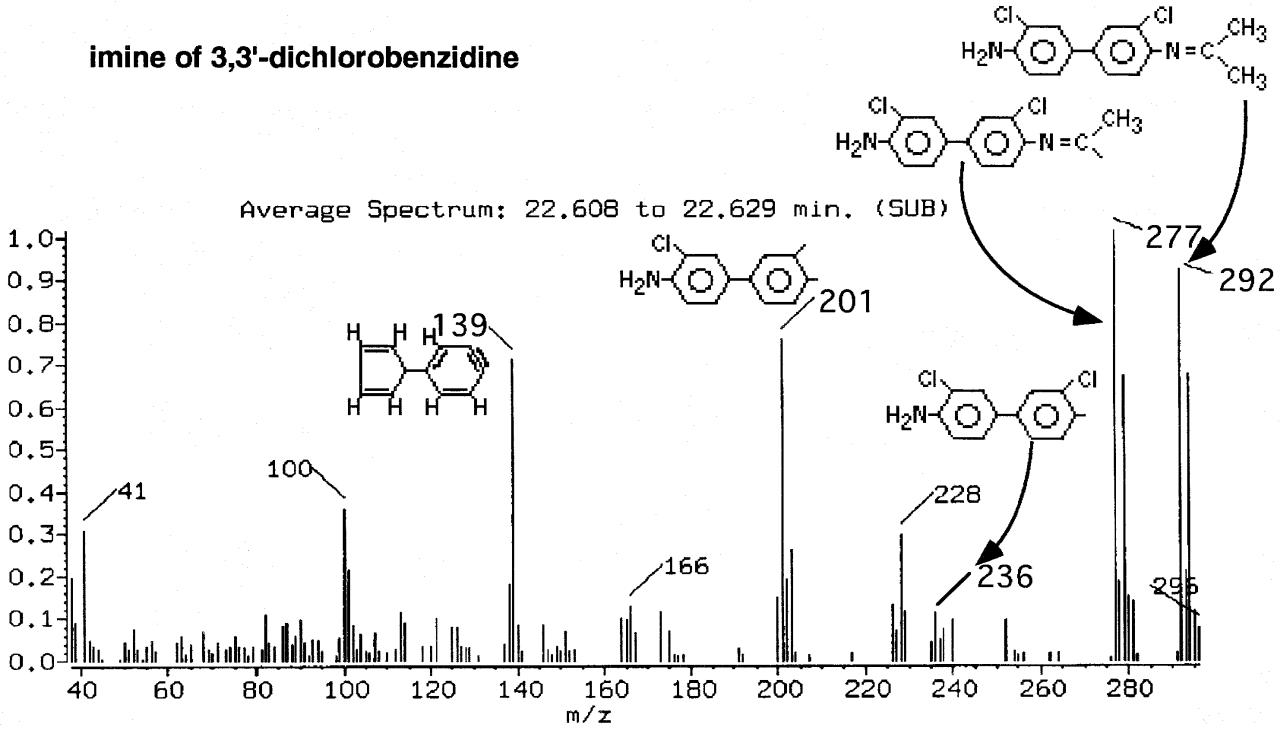


Figure 9. Mass Spectrum of Mono-Imine Form of 3,3'-dichlorobenzidine

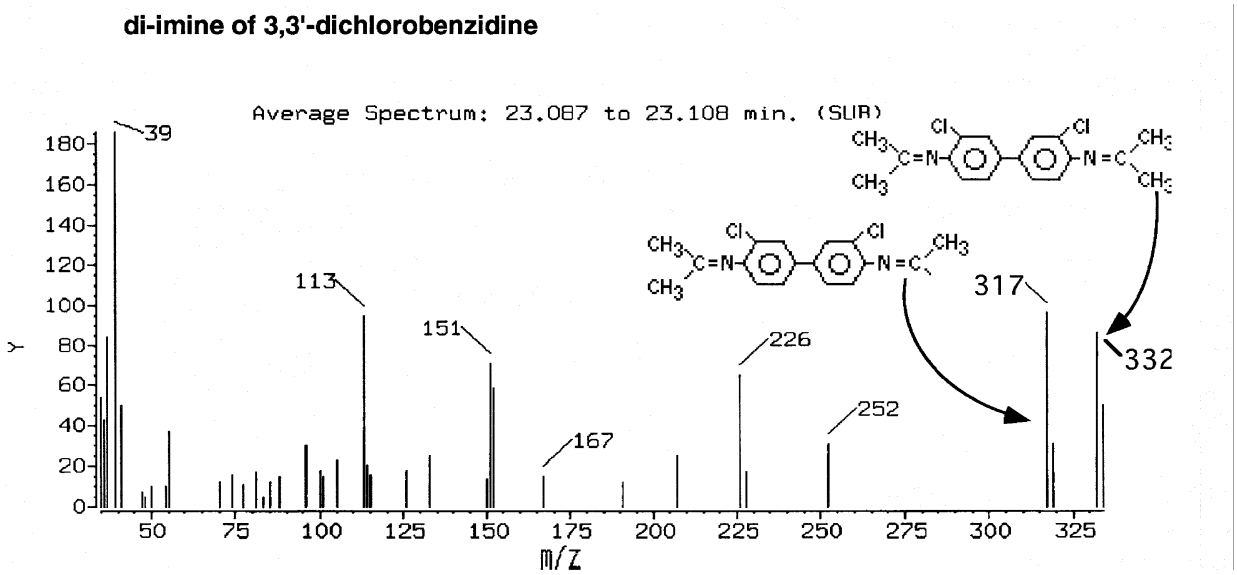


Figure 10. Mass Spectrum of Di-Imine Form of 3,3'-dichlorobenzidine

Imine peaks and spectra were found for: 4-chloroaniline, 3-nitroaniline, o-toluidine, p-phenylene diamine, 1-naphthylamine, 2-naphthylamine, 4-aminobiphenyl, 3,3'-dimethylbenzidine (and di-imine) and N-nitro-o-toluidine.

Since a polar co-solvent is needed to aid extraction from wet samples simply eliminating the acetone may reduce extraction efficiency for all nonpolar analyses. It may be possible to improve amine recovery by reducing the percentage of acetone in the solvent mixture or substituting an alternative polar solvent. Methanol would not be a good substitute since its boiling point is significantly higher than acetone. This would greatly extend KD concentration time and raise the concentration temperature which might cause other unintended reactions. Also, if too much methanol was present in the final concentrated BNA extract, chromatographic performance would be degraded. Using a polar tertiary amine such as dimethylethyl amine instead of acetone should eliminate the conversion of primary amines to their respective imine forms. However, this solvent has practical constraints related to purity, availability and cost at this point in time.

CONCLUSION

The three examples above suggest that acetone is an active participant in "accidental chemistry" reactions which degrade analytical results based on standard EPA sample preparation methods for some target analyses. Substituting methanol produces better results for the organochlorine pesticide and dinoseb examples. A good means of preventing the conversion of primary amines to imines is not available at present. However, knowing that it is a systematic limitation of the preparation methods can aid in data interpretation.

DETERMINATION OF NITROAROMATIC, NITRAMINE, AND NITRATE ESTER EXPLOSIVES IN WATER USING SOLID PHASE EXTRACTION AND GC-ECD

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ABSTRACT

SW-846 Method 8330, the current USEPA method for the analysis of 14 nitroaromatic and nitramine explosives and co-contaminants, uses a liquid chromatograph (LC) equipped with a UV detector. In many environmental laboratories, gas chromatographs (GCs) are the most commonly used instruments because the majority of SW-846 methods for organics are gas chromatographic methods. The desire to make maximum use of gas chromatography naturally leads to attempts to substitute GCs for LCs when analyzing for explosives. However, quantitative analysis of explosives by gas chromatography is complicated by the thermal lability of some of the analyses, particularly the nitramines. We have found, by using high linear carrier gas velocities, deactivated injection port liners, and short wide-bore capillary columns, that the Method 8330 analyses plus nitroglycerin, PETN, and dinitroaniline may be analyzed quantitatively by GC-ECD (gas chromatography-electron capture detector).

Water samples are preconcentrated with solid phase extraction (SPE) and acetonitrile (AcN) elusion using either Waters Porapak RDX Cartridges or Empore Styrene-divinylbenzene membranes. Typically for Method 8330, acetonitrile extracts are mixed with water prior to analysis by LC. The AcN extracts may be analyzed directly by GC-ECD. Method detection limits in the low part per trillion range were obtained for most analyses.

Several SPE-AcN well water extracts from military sites in the US and Canada were analyzed by GC-ECD and LC. Correlation coefficients between the GC-ECD and LC concentration estimates for the analyses most frequently detected—HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), RDX (Hexahydro-1,3,5-trinitro- 1,3,5-triazine), TNT (2,4,6-Trinitrotoluene), and TNB (1,3,5-Trinitrobenzene) were all greater than 0.99.

The GC method provides greater sensitivity than LC, but accurate calibration is more difficult. The UV detector used for the LC analysis has much greater linear range than the ECD used for GC analysis. In addition, the GC instrumentation requires more care than the LC. Specifically, the injection port liner must be changed frequently to maintain accurate determination of the nitramines.

Perhaps the most valuable asset of the GC determination, when used in conjunction with LC, is the ability to confirm analyte presence based on two different physical properties: vapor pressure with GC and polarity with LC. When detection is ambiguous using LC, confirmation by GC will be very useful.

Documentation and performance data will be submitted to the Office of Solid Waste for consideration of this method as a standard for inclusion in SW-846.

INTRODUCTION

The current USEPA method for the analysis of nitroaromatic and nitramine explosives and co-contaminants, SW846 Method 8330[1], involves extraction of water samples using either salting-out or solid phase extraction and analysis of the acetonitrile extract using HPLC-UV. Certified reporting limits[2] range from 0.03 to 0.3 µg/L, and are sufficiently low for determining if water quality criteria are met for most of the analyses for which criteria have been determined.

Because of the prevalence of gas chromatographs in environmental labs, an alternative method for explosives based on GC would provide another option for analysis. Some of the 8330 analyses are already included in current GC SW846 methods[3]. These include the nitroaromatics NB, 2,4-DNT, 2,6-DNT, 1,3-DNB, 1,3,5-TNB, and the isomers of NT (Table 1). The physical properties of some of the other Method 8330 analyses, principally the nitramines, would lead one to believe that GC analysis would be impractical. High melting points, low vapor pressure, and thermal lability are characteristic of the nitramines. For example, the melting point of HMX is 275°C [4], and it is reported to decompose prior to boiling. In addition, the vapor pressure of HMX (10^{-14} torr) is well below what is typical for GC analyses. Nonetheless, explosives, including the nitramines, have been analyzed by GC for several years, primarily for forensic applications[5]. For the most part, quantitative results have been limited to the nitroaromatics.

Hable et al.[6] were the first to report quantitative GC determination of HMX in water. The nitroaromatics 2,4-DNT, 2,6-DNT, and TNT were extracted using toluene, and the more polar nitramines HMX and RDX were extracted from a separate subsample with glass distilled iso-amyl acetate. Successful GC analysis was obtained using deactivated injection port liners, high injection port temperatures, and short, wide-bore capillary columns. Another factor was the elimination of contact between the analyses and metal parts of the injector. Elution of intact HMX, not a thermal

degradation product, from the GC column was confirmed by GC/MS. The certified reporting limits were similar to those obtained using Method 8330[7] for RDX, TNT, and 2,4-DNT, significantly higher for HMX, and lower for 2,6-DNT.

The goal of our work was to develop a GC method that includes all the Method 8330 analyses in a single extraction step, and that uses commercially available and routinely used instrumentation. We included other analyses as well that might be present in explosives-contaminated water. We added 3,5-dinitroanliline, the biotransformation product of TNB, and the nitrate esters NG and PETN. To complement Method 8330, we sought to use a compatible sample preparation method so that a single extract could be subjected to two methods of analysis, thereby allowing direct comparisons of concentration estimates obtained by the two methods and providing another method for analyte confirmation.

Table 1. Current SW-846 gas chromatographic methods[1], which include some of the Method 8330 analyses. Estimated Quantitation Limits, if reported, are listed in parenthesis next to the method number.

	SW-846 Methods (Estimated Quantitation Limit)
NB	8091, 8260, 8270 (10 µg/L)
2,4-DNT	8091, 8270 (10 µg/L)
2,6-DNT	8091, 8270 (10 µg/L)
1,3-DNB	8091, 8270 (20 µg/L)
1,3,5-TNB	8270 (10 µg/L)
o-, m-, p- NT	8091

8091: Nitroaromatics and Cyclic Ketones: Capillary Column Technique.
8260: Volatiles Organic Compounds by GC/MS: Capillary Column Technique.
8270: Semivolatile Organic Compounds by GC/MS: Capillary Column Technique.

EXPERIMENTAL

Calibration Standards. Analytical standards were prepared from SARM (standard analytical reference materials) obtained from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland. Stock solutions were prepared in acetonitrile. Calibration standards were prepared in acetonitrile over the concentration ranges shown in Table 2.

Matrices. Blank matrices used for spike recovery and method detection limit studies were reagent grade (Type 1) water (MilliQ, Milipore) and groundwater from a domestic well in Weathersfield, Vermont. Field-contaminated samples were obtained from Louisiana AAP (Doyline, Louisiana), Kansas AAP (Parsons, Kansas), Umatilla Army Depot (Hermiston, Oregon), and DREV (Defence Research Establishment Valcartier, Quebec),

Sample Preparation. For each sample, up to 1000 mL of water was preconcentrated using Solid Phase Extraction according to manufacture's directions. Both Waters Sep-Pak Vac Porapak RDX Cartridges and Empore SDB-RPS 47-mm Membranes were used. The solid phases were eluted with 4 mL of acetonitrile, and each extract was directly injected into the GC-ECD. When necessary, field sample extracts were diluted with acetonitrile so that peak heights would be bracketed by calibration standards.

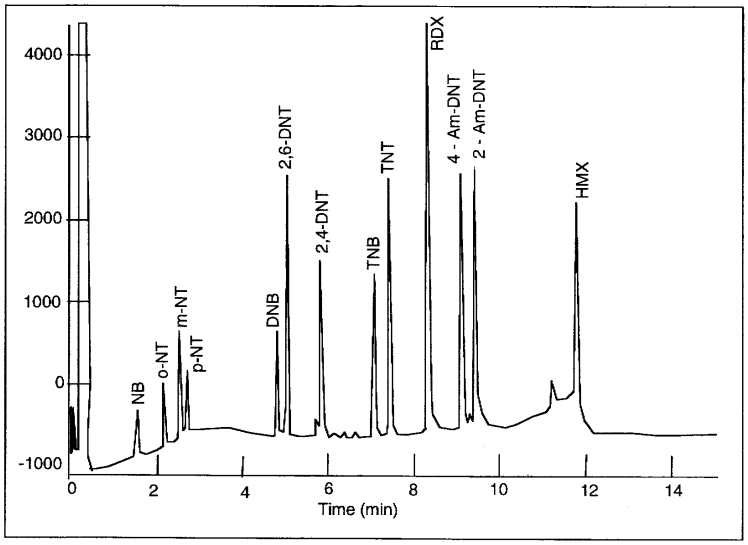
Instrument. We configured the GC based on the work of Hable et al.[6]. The GC parameters were:

GC: HP 5890 with electron capture detector (Ni^{63})
Column: J and W DB-1, 0.53-mm ID, 1.5- or 3.0-µm film (6 m)
Injection Port Liner: Restek Direct Injection Uniliner (deactivated)
Injection Port Temperature: 250°C (varied from 200 to 300°C)
Injection Volume: 1 µL
Carrier: Hydrogen (linear velocity varied from 30 to 185 cm/s)
Makeup: Nitrogen (38 mL/min.)
Oven Program: 100°C for 2 min., 10°C per min. ramp to 200°C, 20°C per min. to 250°C, 5 min. hold.
Detector Temperature: 300°C

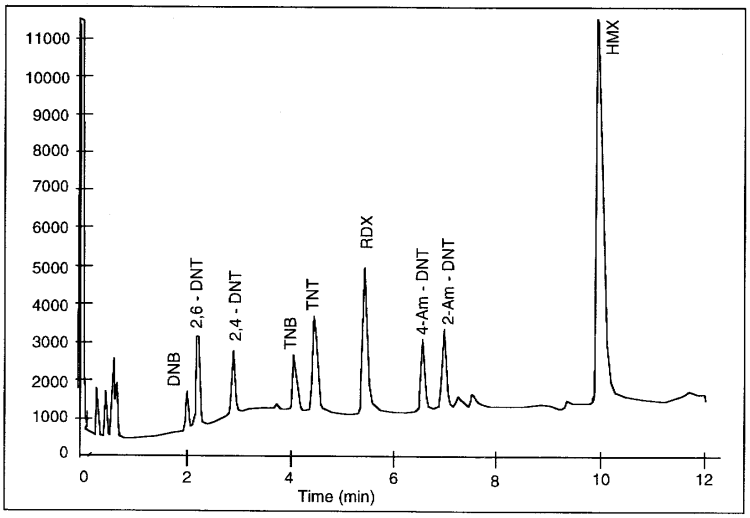
Temperature programs for confirmation columns are given in captions later in this paper.

Table 2. Concentration ranges for calibration standards diluted with acetonitrile.

Conc. (µg/L) Range	
0.5 to 500	Set 1: DNB, 2,6-DNT, 2,4-DNT, TNB, TNT, 4-Am-DNT, 2-Am-DNT
2.5 to 500	Set 2: 3,5-DNA, Tetryl
1.0 to 1000	Set 1: NB, RDX
5 to 5000	Set 1: o-NT, m-NT, p-NT, HMX
25 to 5000	Set 2: PETN
50 to 10,000	Set 2: NG



a. Linear Velocity = 45 cm/s.



b. Linear Velocity = 128 cm/s.

RESULTS AND DISCUSSION

Initial Tests. Initially, we configured the GC based on the work of Hable et al.[6] except that we used a 15-m DB-1 column as provided by the manufacturer. With the exception of HMX, which did not produce a peak, the 8330 analyses eluted as individual peaks, indicating that this column provides adequate resolution for these analyses. However, the additional analyte PETN, which has a vapor pressure almost identical to that of RDX, coeluted with RDX.

We analyzed blanks of commercially available toluene and iso-amyl acetate, the two extraction solvents used by Hable et al.[6]. We also analyzed blanks of acetone and acetonitrile (AcN) after passage through SPE cartridges (Waters Porapak RDX). While the chromatogram of iso-amyl acetate contained numerous large peaks, the chromatograms for the other solvents had no significant peaks. Because acetonitrile produced no background interference and Method 8330 specifies acetonitrile for extraction, we pursued the use of solid phase extraction with acetonitrile elusion as described below.

Figure 1. Calibration standard (from set 1 in Table 2) analyzed using DB-1 at two carrier gas velocities. Higher linear velocities resulted in higher HMX peak heights.

We experimented with different temperature programs and injected a high concentration solution of HMX. With a high temperature isothermal run, HMX eluted as a broad jagged peak. We next shortened the GC column to 6 m and found that HMX now eluted as a sharp peak. This dramatic improvement was not attributable to total time in the GC, but rather to the decreased column length, thus less surface area to which the analyte was exposed. The retention time for HMX was the longest of all the analyses (Fig. 1a). Late elusion was expected because of the extremely low vapor pressure of HMX. As we adjusted the linear velocity, we noticed that the HMX peak height changed significantly with changes in linear velocity (Fig. 1), so we systematically changed the linear velocity to document this effect.

Effect of Carrier Gas Linear Velocity. Optimum linear velocity for peak resolution is 26 cm/s when using hydrogen carrier gas and a 0.53-mm ID column. Restek recommends twice the optimum linear velocity when using the Direct Injection Uniliners. We tested the effect of increasing carrier gas linear velocity over the range 30-185 cm/s and found a significant increase in response from HMX, RDX, NG, and PETN. For example, no peak for HMX was observed at the lowest linear velocity tested. The linear velocity was increased to 55 cm/s and HMX eluted as a sharp peak. Thereafter, the HMX peak height approximately doubled with each doubling of the linear velocity (Fig. 2). Some degradation in peak resolution did occur. With increasing carrier gas linear velocity, the peak for dinitroaniline merged with the peak for 4-amino-DNT.

Effect of Injection Port Temperature. Hable et al.[6] found increased HMX response with increasing injection port temperature, and recommended an injection port temperature of 270°C for the determination of TNT, DNTs, RDX, and HMX. We reexamined the effect of injection port temperature at high linear velocity (133 cm/s) for the 8330 analyses plus NG, PETN, and DNA. We found that the optimum temperatures were different for the different analyses. The lowest temperatures tested (200-220°C) resulted in the highest response for the nitrotoluenes and nitrate esters. Higher temperatures (250-270°C) were best for HMX, RDX, the amino-DNTs, and DNA. However, even at 250°C, responses for the other analyses were at least 90% of the maximum responses. Therefore, we recommend an injection port temperature of 250°C.

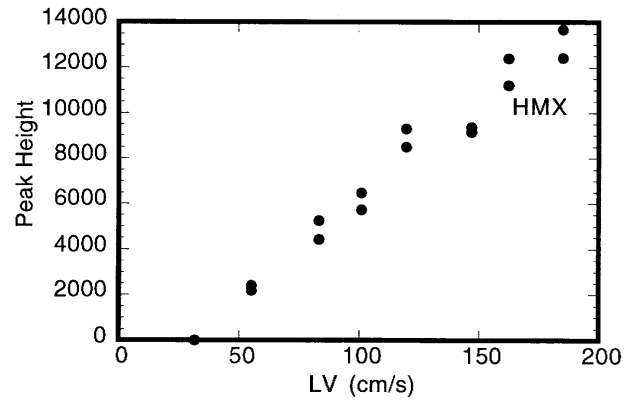


Figure 2. Effect of carrier gas linear velocity on peak height of HMX in a 500-µg/L solution.

Calibration. For all of the analyses, the calibration factors (CF) decreased with increasing analyte concentration (Fig. 3); therefore, a linear model relating response to concentration is not appropriate. SW-846[3] lists four options, in order of increasing difficulty, for non-linear calibration data: adjust the instrument or perform instrument maintenance; narrow the calibration range until response is linear (<20% RSD for CF); use a linear calibration model that does not pass through origin; use a calibration curve or non-linear model.

ECDs typically have a narrow linear range (approximately 40-fold), with a dynamic range of about 1000-fold[8]. The calibration data we observed fell within these specifications. The shape of the curve of peak height data for 2,6-DNT (Fig. 3) over the range 0.508-508 µg/L was representative for other analyses. Increasing the make-up gas was suggested as a potential means to increase the linear range. Therefore, we tried increasing the make-up gas from 37 to 69.5 mL/min. This increase did not improve linearity; it only decreased the GC response. Next, we narrowed the calibration range to the five lowest standards; in general, the calibration factors for most of the analyses were within 20% RSD. This very limited linear range of the ECD is a disadvantage compared to the HPLC-UV, which has a broad linear range. For GC-ECD, sample extracts will need to be diluted within the proper calibration range. For samples with multiple analyses at varying concentrations, a single extract may require several determinations at different dilution factors.

Instability of Low Concentration Standards. The calibration standards for TNB and TNT were unstable when left at room temperature in amber autosampler vials. Previous stability studies had shown that these analyses were stable for several days in acetonitrile[9]. However, the standards in this previous study were much higher in concentration (3 mg/L). We found that analyte loss was most noticeable at the lower concentrations (50 vs. 500 µg/L), and that the loss differed with different brands of acetonitrile and was slowed by refrigeration of the solution (Fig. 4). We were particularly concerned about this instability because samples and standards could potentially sit in an autosampler for several hours close to a heated injection port and GC oven vent. The autosampler we used (HP 6890) was designed so that a coolant could be circulated through the tray containing the sample vials. With this modification, the standards were stable over a typical 12-hour analytical shift.

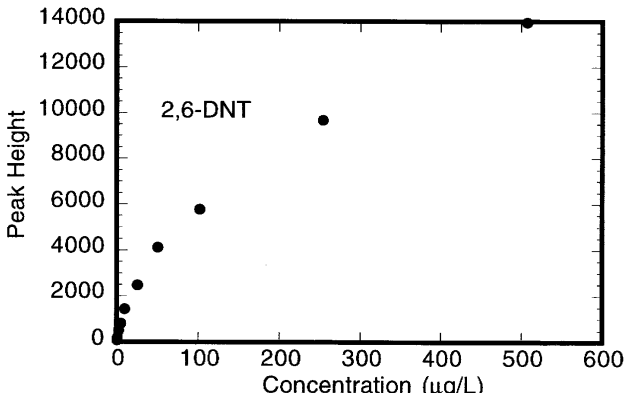


Figure 3. Typical calibration curve obtained by GC-ECD.

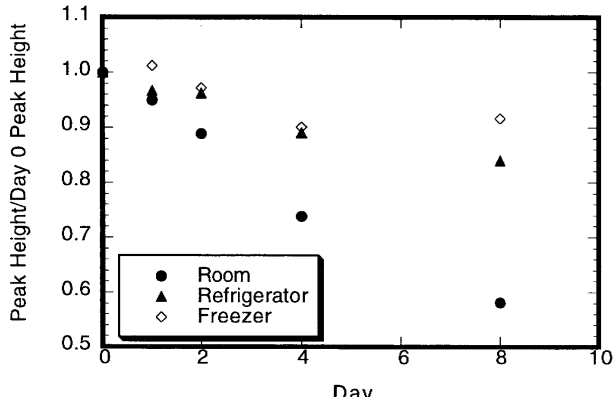


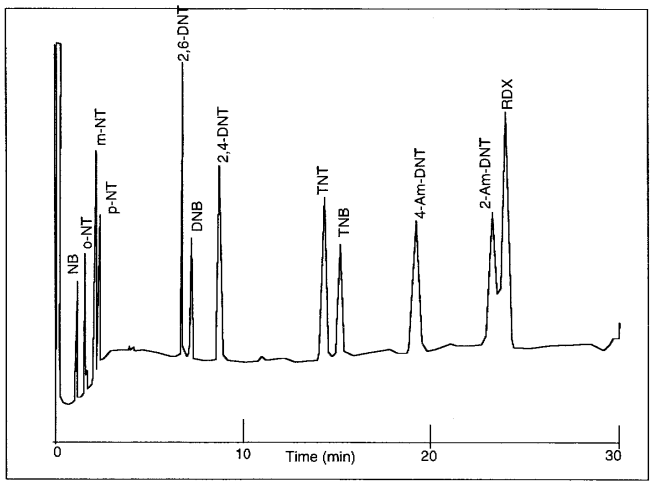
Figure 4. Decrease in peak height observed in 10-µg/L TNT calibration standard stored in vials at three different temperatures.

Confirmation Columns. We have tested four 0.53-mm ID columns for suitability as confirmation columns. In order of increasing polarity, these columns are J and W DB-1301 (6% cyanopropylphenyl methylpolysiloxane), J and W DB-17 (50% phenyl methylpolysiloxane), a Restek RTX-200 (Crossbond trifluoropropyl methylpoly- siloxane), and a Restek RTX-225 (50% cyanopropylmethyl-50% phenyl methylpolysiloxane). The DB-1301 was not acceptable because TNB coeluted with TNT and DNB coeluted with 2,6-DNT. The DB-17 was not suitable because TNB coeluted with TNT. The Restek RTX-200 resolved the 8330 analyses at low linear velocity, but HMX is not detected (Fig. 5a). At high linear velocity, HMX was detected (Fig. 5b), but PETN coeluted with RDX and DNA coeluted with 4-Am-DNT. Finally, on the RTX-225, tetryl co-eluted with RDX and HMX was not detected (Fig. 5c). However, in subsequent analysis of well-water samples from Louisiana AAP, we found this column to be excellent for confirmation of the amino-DNTs. Thus, for confirmation, extracts must be analyzed under the appropriate conditions. Both the RTX-200 and RTX-225 look promising for confirmation because the elution order of several analyses is the reverse of that on the DB-1. For example, 2,6-DNT and DNB reverse order as do TNT and TNB. In addition, RDX elutes after the amino-DNTs, whereas it elutes before the amino-DNTs on the DB-1.

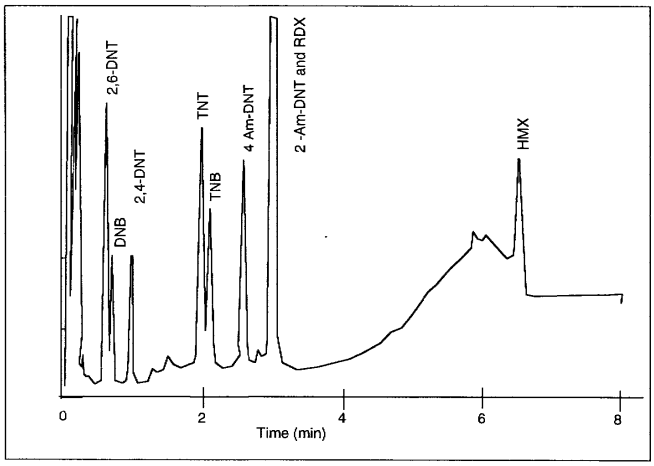
We also tested an Alltech MultiCapillary SE-54 (5% phenyl methylpolysiloxane) column. These columns are only 1 m long and are composed of a bundle of over 900 liquid-phase coated 40-µm capillaries. They provide rapid analysis of pesticides, and accommodate high carrier gas velocities, so we reasoned that they might be suitable for the analysis of explosives. We tested numerous chromatographic conditions and found that the column was suitable for the analysis of NB, the nitrotoluenes, DNB, and the DNTs. Resolution of the other analyses was poor, the peaks for TNB, TNT, and RDX were uncharacteristically small, and HMX did not elute at all. Here again, large column surface area, not total time in the GC, may contribute to HMX loss.

Splitless injection Port. One objective of this method development was to use standard laboratory equipment. We use a packed column injection port that was modified to accept a 0.53-mm ID capillary column. (Conversion kits designed to convert most packed column chromatographs are commercially available from a variety of sources including HP, J and W, Restek.) Some GCs may be equipped solely with Split/Splitless injection ports. Although splitless injection is routinely used for trace analysis, this injection technique is not appropriate for reactive or high boiling compounds such as explosives because of adsorption, condensation, and discrimination in the injection port. However, injection port liners are available that allow direct injections to be made in a splitless capillary port. We tested a Restek Uni-liner that is designed for HP 5890 split/splitless injection port and found no difference in response than that obtained previously. Therefore, either configuration appears to be acceptable for this analysis.

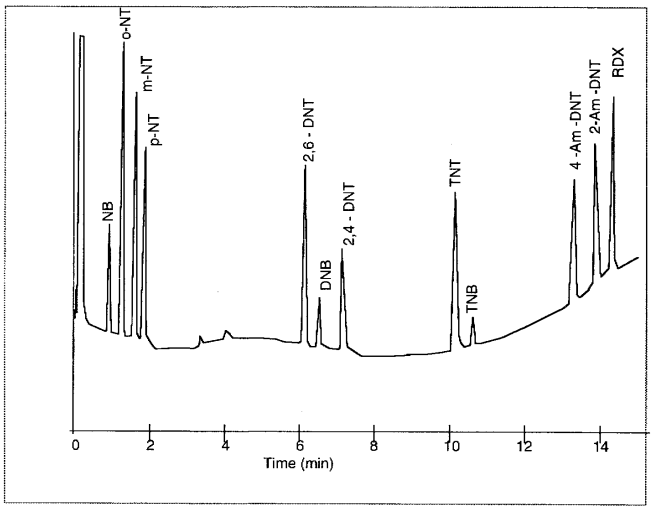
Initial Spike Recovery. A spike recovery study was performed using both the Empore SDB-RPS (47-mm-diameter) disks and the Water Sep-Pak Vac Porapak RDX cartridges. Both of these solid phase extraction devices have been used successfully for the preconcentration of explosives from water prior to HPLC-UV[7]. Also, prior to the availability of commercially packed cartridges, Richard and Junk[10] demonstrated the feasibility of preconcentrating explosives from water onto solid styrene divinylbenzene resins in the field; analyses were eluted in the laboratory and determined by GC-ECD.



a. RTX-200, LV=70 cm/s, oven 100°C for 1.2 min., 5 °C/min. to 140°C, 1 °C/ min. to 160 °C, 20 °C/min. to 250 °C, 5 min. hold. Injector 250 °C. Detector 290 °C.



b. RTX 200, LV = 122 cm/s, oven 150 °C for 1 min., 20 °C/min. to 250 °C, 5 min. hold. Injector 270 °C. Detector 290 °C.



c. RTX 225, LV = 90 cm/s, oven 100 °C 2 min., 10 °C/min. to 220 °C, 8 min. hold. Injector 220 °C (column upper limit). Detector 250 °C.

Figure 5. Chromatograms obtained following injection of a standard (set 1 from Table 2) onto confirmation columns.

Using both membranes and cartridges, we preconcentrated duplicate samples spiked at 5 µg/L for most of the analyses (Table 3). We divided each acetonitrile extract and analyzed each by GC-ECD and HPLC-UV. We found good recovery for all the analyses by both methods. In general, repeatability was better using HPLC-UV.

Field Samples. We analyzed several solid phase extracts of water samples collected from various explosives-contaminated sites. These included extracts from LAAP in which 500-mL samples were preconcentrated using Porapak RDX cartridges and eluted with 5 mL AcN. These extracts were prepared and analyzed by HPLC at the U.S. Army Engineer Waterways Experiment Station (Vicksburg, Mississippi). Water samples from Umatilla

Army Depot and DREV were preconcentrated at CRREL using either cartridges or Empore membranes, with the HPLC analysis performed at CRREL. All GC-ECD analysis was done at CRREL.

Concentration estimates obtained by the two methods of determination for the most commonly found analyses (HMX, RDX, TNT, TNB, and 2,4-DNT) (Table 4) compared favorably for most samples over a wide range of concentrations. Discrepancies between the two methods of analysis, however, do exist. The GC appeared to underestimate the concentration of RDX in some of the low concentration samples. However, the ECD is a more selective detector, so this apparent underestimation may not be real. Secondly, tetryl was detected by GC in some LAAP extracts, but not by HPLC. We suspect that, when we analyze a tetryl standard by GC, the peak we observe actually corresponds to a thermal degradation product of tetryl, possibly n-methyl-picramide[11]. Several LAAP water samples are also contaminated with picric acid, and a co-contaminant of picric acid is potentially the source of the peak we observe on the GC. Finally, 2,6-DNT was detected by GC-ECD in almost every sample that contained 2,4-DNT. These isomers often co-elute on the LC-18 separation specified in Method 8330. However, these isomers can be resolved on other LC columns[12], specifically those with 3-µm phase particles, which are less rugged for routine analysis of large number of samples.

Table 3. Recovery and repeatability of GC and HPLC determinations of analyte concentrations in spiked water samples.

a. Empore SDB-RPS 47-mm diameter

		Found Concentration (µg/L)			
	Spiked Conc (µg/L)	Membrane 1	Membrane 2	Average Recovery (%)	RPD (%)
GC-ECD					
DNB	5.06	4.77	4.35	90%	9.4%
2,6-DNT	5.08	4.88	4.48	92%	8.7%
2,4-DNT	5.12	4.78	4.50	91%	6.1%
TNB	5.04	4.33	4.25	85%	1.7%
TNT	5.01	4.72	4.63	93%	1.9%
RDX	10.0	9.55	9.32	94%	2.4%
4-Am-2,6-DNT	5.06	4.51	4.28	87%	5.1%
2-Am-4,6-DNT	5.02	5.74	5.22	109%	9.5%
HMX	50.1	49.7	47.0	96%	5.6%
HPLC-UV					
DNB	5.06	5.45	5.26	106%	3.7%
2,6-DNT and 2,4-DNT††	10.2	10.6	10.2	102%	3.9%
TNB	5.04	5.62	5.18	107%	8.1%
TNT	5.01	6.04	5.48	115%	9.8%
RDX	10.0	10.3	10.3	103%	0.2%
4-Am-2,6-DNT and 2-Am-4,6-DNT††	10.1	10.8	10.3	105%	5.2%
HMX	50.1	45.9	46.9	93%	2.2%

b. Waters Sep-Pak Vac Porapak RDX Cartridges

	Spiked Conc (µg/L)	Found Concentration (µg/L) Cartridge 1	Cartridge 2	Average Recovery (%)	RPD (%)
GC-ECD					
DNB	5.06	5.20	4.66	98%	11.1%
2,6-DNT	5.08	5.29	4.87	100%	8.3%
2,4-DNT	5.12	5.03	4.80	96%	4.6%
TNB	5.04	4.92	4.73	96%	3.8%
TNT	5.01	5.26	5.07	103%	3.7%
RDX	10.0	10.8	10.6	106%	1.8%
4-Am-2,6-DNT	5.06	5.05	4.58	95%	9.6%
2-Am-4,6-DNT	5.02	5.26	4.85	101%	8.1%
HMX	50.1	68.8	67.7	136%	1.6%
HPLC-UV					
DNB	5.06	5.76	5.70	113%	1.1%
2,6-DNT and 2,4-DNT††	10.16	11.0	11.0	108%	0.3%
TNB	5.04	5.71	5.67	113%	0.7%
TNT	5.01	5.97	5.99	119%	0.4%
RDX	10.0	12.5	12.1	123%	3.3%
4-Am-2,6-DNT and 2-Am-4,6-DNT††	10.1	10.6	10.6	105%	0.4%
HMX	50.1	55.5	56.2	111%	1.3%

††Peak not resolved.

Table 4. Concentration estimates obtained for the most commonly found analyses by HPLC and GC-ECD for water samples collected at explosives-contaminated sites.

a. HMX

Source	SPE Method	HMX Conc. (µg/L) HPLC	GC-ECD
KSS AAP	Cartridge	0.20	0.10
Umatilla	Membrane Membrane	0.29	0.60
Umatilla	Cartridge	0.22	0.59
Umatilla	Cartridge	0.31	0.21
LAAP	Cartridge	19	13
DREV	Cartridge	26	26
DREV	Cartridge	97	110
LAAP	Cartridge	116	109
Umatilla	Cartridge	141	179
LAAP	Cartridge	182	147
LAAP	Cartridge	216	217
DREV	Cartridge	219	280
DREV	Cartridge	250	308
DREV	Cartridge	251	285
LAAP	Cartridge	1300	1378
LAAP		1860	1842

b. RDX

		RDX Conc. (µg/L)	
Source	SPE Method	HPLC	GC-ECD
KSS AAP	Cartridge	0.2	0.2
Umatilla	Membrane	0.27	0.20
KSS AAP	Cartridge	1.6	0.95
DREV	Cartridge	1.7	1.0
DREV	Cartridge	2.0	0.56
LAAP	Cartridge	2.4	0.28
LAAP	Cartridge	3.6	0.6
Umatilla	Membrane	4.9	4.9
Umatilla	Membrane	5.2	5.1
Umatilla	Cartridge	6.5	5.2
Umatilla	Cartridge	6.7	7.0
LAAP	Cartridge	8.9	1.2
DREV	Cartridge	29.8	37.7
DREV	Cartridge	30.9	34.7
DREV	Cartridge	33.8	44.1
LAAP	Cartridge	845	59O
LAAP	Cartridge	1430	1973
LAAP	Cartridge	2060	2241
LAAP	Cartridge	3710	3640
LAAP	Cartridge	11800	8175
LAAP	Cartridge	23400	20833

c. TNT

		TNT Conc. (µg/L)	
Source	SPE Method	HPLC	GC-ECD
LAAP	Cartridge	0.3	0.5
LAAP	Cartridge	0.4	0.1
LAAP	Cartridge	0.5	0.3
LAAP	Cartridge	2.4	1.2
LAAP	Cartridge	152	142
Umatilla	Cartridge	241	233
LAAP	Cartridge	390	405
LAAP	Cartridge	2430	2876
LAAP	Cartridge	2890	3721
LAAP	Cartridge	7500	7781
LAAP	Cartridge	10500	12168

d. TNB

		TNB Conc. (µg/L)	
Source	SPE Method	HPLC	GC-ECD
LAAP	Cartridge	0.1	0.02
LAAP	Cartridge	1.0	0.4
LAAP	Cartridge	1.9	1.0
LAAP	Cartridge	15.6	33.8
LAAP	Cartridge	22.3	34.2
LAAP	Cartridge	649	1128
LAAP	Cartridge	742	782
LAAP	Cartridge	9110	11991
LAAP	Cartridge	9150	10640

e. 2,4-DNT

Source	SPE Method	2,4-DNT† Conc. (µg/L) HPLC	GC-ECD
LAAP	Cartridge	<d	0.07
LAAP	Cartridge	<d	0.06
LAAP	Cartridge	<d	0.05
LAAP	Cartridge	<d	0.15
LAAP	Cartridge	0.69	0.36
LAAP	Cartridge	10.7	11.8
LAAP	Cartridge	24.5	18.7
LAAP	Cartridge	46.8	33.6
LAAP	Cartridge	127	126
LAAP	Cartridge	142	84.8
LAAP	Cartridge	442	341

†In several extracts, 2,6-DNT was detected by GC-ECD, but not by HPLC.

Almost all the extracts from field samples required dilution prior to GC-ECD analysis so that peak heights would fall within the linear calibration range. Dilution actually appeared to improve the accuracy of the GC determination of HMX when several samples were run sequentially. We suspect that dilution served to "cleanup" the extracts and slowed the buildup of non-volatile co-extracted contaminants that deposit in the injection port liner. Accurate determination of HMX required that the injection port liner be changed or cleaned frequently. We changed the liner each time we replaced the injection port septum, at least every 50 injections.

Method Detection Limits. Method Detection Limits (MDLs) were computed from the standard deviation of the mean concentration of 10 replicate spiked water samples and the appropriate Student's t value. The MDLs were lowest for the di- and tri-nitroaromatics, and were all well below current water quality criteria (Table 5). If the analyte of most interest is 2,6-DNT, the MDL could be lowered by preconcentrating a greater volume of water. We limited the volume we preconcentrated to prevent breakthrough of HMX and RDX. 2,6-DNT is well retained on both solid phases, and the volume of water preconcentrated is more likely limited by practical considerations such as time or possible plugging of the solid phase.

Based on the Certified Reporting Limits for Method 8330, and using the generalization that CRLs are similar to MDLs, the GC-ECD appears to have lower detection limits (Table 5). Given that MDLs are highly matrix- and laboratory-specific, more data from multiple labs are needed prior to generalizing about the magnitude of the improvement in detection capability in actual practice.

Table 5. Method detection limits (µg/L) for GC-ECD method, certified reporting limits (µg/L) for the HPLC method[7], and water quality criteria.

	MDLs† by GC-ECD		CRLs by HPLC		Water Quality Criteria (µg/L)
	Cartridge	Membrane	Cartridge	Membrane	
DNB	0.0036	0.0025	0.032	0.036	1.0[a]
2,6-DNT	0.0029	0.0025			40[a], 0.007[b]
2,4-DNT	0.0092	0.0106	0.085	0.044	50[a], 0.1[b]
TNB	0.0066	0.0027	0.042	0.051	
TNT	0.0144	0.0196	0.068	0.13	
RDX	0.0044	0.0174	0.27	0.124	2.0[a]
4-Am-2,6-DNT	0.0026	0.0030			
2-Am-4,6-DNT	0.0030	0.0044	0.046	0.055	
Tetryl	0.0094	0.0100	0.24	0.83	
HMX	0.0041	0.0081	0.21	0.33	400[a]
NB	0.475	0.235			
o-NT	0.352	0.351	0.10	0.20	
m-NT	0.297	0.294	0.13	0.37	
p-NT	0.225	0.325	0.12	0.23	
NG	0.257	0.225			
PETN	0.349	0.275			
DNA	0.046	0.066			

†1L of water preconcentrated to 4.0 mL AcN
[a]EPA Lifetime Health Advisory Number
[b]EPA number for increased cancer risk of 1.0 x 10^{-6}

SUMMARY

A gas chromatographic method for the analysis of explosives in water was developed to serve as an alternative to the current HPLC SW846 Method 8330. Water samples are preconcentrated using solid phase extraction, and the acetonitrile extracts are directly injected onto a short (6-m) DB-1 analytical column. High linear carrier gas velocities resulted in higher peak heights for the nitramines and nitrate-esters, the most thermally labile analyses. Detection limits ranged from 0.003 to 0.5 µg/L.

Analysis of extracts from field samples showed good agreement between the GC-ECD and the standard HPLC method.

Potential advantages over the current HPLC method include lower detection limits, improved chromatographic resolution, and the use of instrumentation most commonly found in environmental labs. Disadvantages of the GC method include non-linear calibration, limited dynamic range of the detector, and increased attention to instrument maintenance (i.e., frequent changes of the injection port liner).

Combined use of GC-ECD and HPLC will provide an improved method for analyte confirmation because chromatographic separations are based on different physical properties (vapor pressure and polarity) and the detectors are based on different principles (electronegativity and UV absorption).

ACKNOWLEDGMENTS

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COMPREHENSIVE, QUANTITATIVE, CONGENER-SPECIFIC PCB ANALYSIS: WHEN IS IT REQUIRED AND WHAT IS NECESSARY TO ACHIEVE IT?

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ABSTRACT

Current regulatory analyses for PCBs specify reporting as equivalents of the commercial mixtures (Aroclors, EPA methods 8081, draft 8082) or short lists of priority congeners (European BCR, 7 priority PCBs, EPA draft method 1668). Studies which must unravel mechanisms which alter congener distributions, such as microbial or photolytic dechlorination, aerobic microbial degradation, or pyrolytic rearrangement and thermal synthesis, require *complete* quantitative characterization of PCB congener distributions by the title procedure (abbreviated CQCS).

CQCS methods such as Mullin's EPA "Green Bay Calibration" and the G.E. "DB-1 118-peak HRGC System" have employed Aroclor mixtures as congener standards, but these are restricted to specific HRGC columns and Aroclor lots. To meet the need for greater flexibility in developing CQCS PCB analysis methods, the author has organized several consortia to obtain retention data for all 209 PCB congeners on 20 different HRGC systems and complete congener distributions for 17 Aroclor mixtures.

From these data this paper evaluates how to best choose systems for CQCS PCB analysis, illustrating with actual data, various HRGC systems employing a variety of detectors, which have been optimized for maximum performance. From the data in these studies, the optimum distribution of all the congeners into a set of 9 mixtures appropriate for calibrating a wide variety of CQCS PCB analyses has been designed and is now marketed by a commercial distributor. A method for the efficient employment of these primary standard sets by combining the 5 mixtures comprising most of the congeners found in Aroclors mixtures is evaluated. The most detailed and comprehensive studies to date of Aroclor congener distributions has been made using these standards and HRGC systems optimized for maximum congener resolution. The pitfalls of using Aroclors as quantitative standards for CQCS PCB analyses become evident upon inspection of the variation in distributions of congeners among different lots of the same-numbered Aroclors.

The initial results of a new collaborative study on the PCB congener detection limits and effective linear ranges of a variety of HRGC detectors (ECD, EI-MS-SIM, medium and high resolution EI-MS-SIM, full-scan ion-trap MS) are described. The results illustrate the tradeoffs in selectivity, sensitivity, cost, ease of use, and accuracy among the different detectors when applied to CQCS PCB analyses. The latest model bench-top instrumentation, exemplified by the new Hewlett-Packard 5973 GC-MS instrument, the Varian Saturn 2000 Ion-Trap MS instrument, and the new Hewlett-Packard micro ECD detector, provide order-of-magnitude improvements in sensitivity and linearity for this application. When these detectors are combined with HRGC capillary columns chosen from the database survey to provide optimal resolution of the most congeners, and the new comprehensive congener calibration sets are used as primary standards, CQCS PCB analysis becomes a practical reality.

INTRODUCTION

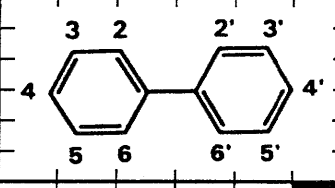
Polychlorinated biphenyls (PCBs) are comprised of 209 chlorine-substituted biphenyl compounds known as congeners. They are often referred to by their IUPAC or Ballschmiter and Zell (BZ) numbers. The matrix diagram of Figure 1 relates the chlorine-substitution pattern on each of the two phenyl rings to the BZ numbering system. It also displays the ring position numbering conventions that underlie the more formal, complete IUPAC nomenclature, and the correlation between the 20 different ring substitution patterns and the 209 different BZ-numbered congeners. Only about 150 of these congeners are observed in the commercial mixtures formerly manufactured by catalytic chlorination of biphenyls (Aroclors (Monsanto) in the USA)). The shading of the cells in the matrix highlights both those congeners which dominate in Aroclors due to highly favored and stable ring substitution patterns as well as those not seen due to ring substitutions which are very difficult to produce and are vulnerable if formed to rapid further chlorination.

The methods of choice for PCB analysis are based on separation of a majority of the congeners by high resolution

gas chromatography (HRGC) on long capillary columns, with detection by sensitive and selective detectors such as the electron capture detector (ECD) or mass spectrometry in the selected-ion-monitoring mode (MS-SIM). Even these powerful techniques are insufficient to measure all the congeners typically found in an Aroclor mixture or the altered patterns resulting from environmental processes such as microbial degradation or dechlorination, photolysis, or degradation or *de novo* synthesis by thermal processes. If an attempt is made to correctly assign all the congeners eluting or coeluting in each resolved HRGC peak and to quantify the total PCB content of each resolved peak, the analysis can be described as "A comprehensive, quantitative, congener-specific PCB analysis". This will be abbreviated in this paper as a CQCS PCB analysis. This most demanding form of PCB analysis should be distinguished from the less complete "congener-specific" methods which specify only a limited list of target congeners, selected both for the ease of resolution, the availability of suitable standards, and relevance to a particular question. Examples of the latter include the 7 European Bureau of Community Reference (BCR) target congeners 28, 52, 101, 118, 138, 153, and 180; or the list of 14 congeners whose abundance and ability to achieve a planar "dioxin-like" conformation qualify them for inclusion in EPA draft method 1668. Several of these lists, and the calibration sets that support the analyses are tabulated in the bottom, unshaded cells of Figure 2. These congener-specific methods contrast with the earlier EPA methods 608, 8080, 8081, and draft method 8082, where the calibration is against the best matching Aroclor mixture(s), and results are reported as equivalents of particular numbered Aroclors. [The Aroclor mixtures are typically numbered from 1221 to 1262, where 12 indicates the 12 carbon atoms in biphenyl, hence a PCB, and the last 2 digits the weight percentage of chlorine in the mixture.]

Figure 1. Matrix of PCB IUPAC No.s vs Phenyl Ring Cl Substitution Positions + Aroclor Abundances

Ring-Cl	none	2	3	4	23	24	25	26	34	35	234	235	236	245	246	345	2345	2346	2356	23456
23456																				209
2356																			202	208
2346																		197	201	207
2345																	194	196	199	206
345																169	189	191	193	205
246															155	168	182	184	188	204
245														153	154	167	180	183	187	203
236													136	149	150	164	174	176	179	200
235												133	135	146	148	162	172	175	178	198
234											128	130	132	138	140	157	170	171	177	195
35										80	107	111	113	120	121	127	159	161	165	192
34									77	79	105	109	110	118	119	126	156	158	163	190
26								54	71	73	89	94	96	102	104	125	143	145	152	186
25							52	53	70	72	87	92	95	101	103	124	141	144	151	185
24						47	49	51	66	68	85	90	91	99	100	123	137	139	147	181
23					40	42	44	46	56	58	82	83	84	97	98	122	129	131	134	173
4				15	22	28	31	32	37	39	60	63	64	74	75	81	114	115	117	166
3			11	13	20	25	26	27	35	36	55	57	59	67	69	78	106	108	112	160
2		4	6	8	16	17	18	19	33	34	41	43	45	48	50	76	86	88	93	142
none	0	1	2	3	5	7	9	10	12	14	21	23	24	29	30	38	61	62	65	116



Peak Wt% Ranges in Aroclors 1221 to 1262

- 5.00-12.5
- 2.00-5.00
- 0.80-2.00
- 0.40-0.80
- 0.20-0.40
- 0.10-0.20
- 0.05-0.10
- 0.02-0.05
- 0.00-0.02

PCB Congener Nomenclature

No.'s 107, 108, 109, 199, 200, 201 (Ref. 8) on this chart equivalent to BZ 108, 109, 107, 201, 199, 200 resp. (7)

e.g., IUPAC name for BZ No. 138 is 2,2',3,4,4',5'-hexachlorobiphenyl, and is often abbreviated to: 234-245 to save space and to clarify the separate phenyl-ring Cl substitutions

2,2',6,6' = *ortho-* 4,4' = *para-*
3,3',5,5' = *meta-* Cl Orientation

Effect of Ring Cl Substitutions on Congener Levels in Aroclors

35- , 246-	= suppressed ring
3-, 26-, 235-, 345-	= unfavored ring
4-, 25-, 34-, 245-	= favored ring
3	# = Region where
3	difference in Cls
3 4	on each ring is
3 3 4 5	equal to 3, 4 or 5

Figure 2.
Top/Down: PCB coeluting in 6 Systems [Shaded Cells]
Bottom/Up: PCB Congener "Short Lists" [Clear Cells]

Three Dual-parallel GC-ECD and Three Single GC-MS-SIM Systems

1	2	3	4	5	6
DB-1 SP-Octyl 2 ECD	Sil C-18 Sil 13 2 ECD	HP5HT5 DB-17 2 ECD	DB-1 MS-SIM	HT8 MS-SIM	DB-XLB MS-SIM
(1)	(1)	(3)	(1)	(1, 4)	(1, 3)
15	20	41	4	5	4
16	24	56	5	20	10
20	41	60	7	33	20
27	44	70	9	41	33
37	53	84	10	47	63
51	76	92	16	48	76
59	99	196	20	71	83
64	114	203	24	75	89
76	115		27	77	109
77	129		32	84	119
84	135		33	92	123
114	163		41	109	129
124	198	209	42	115	163
131		206	43	117	
133		195	56	134	206
138		187	59	193	202
139		180	60	198	194
156		170	64	196	187
157		153	71	203	180
163	189	138	76	99	170
171	180	128	77	97	156
202	170	***126***	84	87	153
	169	118	92	85	151
	167	105	117	74	149
	157	101	124	66	141
180	156	***77***	135	56	138
153	***126***	66	138	52	137
138	123	52	157	49	128
118	118	44	163	47	118
101	114	28	164	44	110
52	105	18	196	31	105
28	***77***	8	203	28	101
BCR 7 Ind.	USEPA 1668	NOAA NS&T		QUASIMEME Q0R02CA	

Specific Congener Short Lists

DISCUSSION

In contrast to the "short-list" congener-specific PCB analytical methods alluded to in the introduction, CQCS analysis will be required to unravel the mechanisms which result in the environmental or human alterations of the initially produced Aroclor-like PCB distributions. These tend to be very congener specific, and distinctive to the particular process(es) which may be acting. For example microbial dechlorination in anaerobic sediments proceeds primarily by selective removal of chlorine atoms from positions *meta*- or *para*- to the other phenyl ring. By contrast photolytic dechlorination is dominated by removal of the *ortho*- chlorines. These processes will often produce some congeners not generally present in the commercial Aroclor mixtures. The disadvantage of this is that the standard congener-specific PCB methods often do not take account of their possible presence and lack suitable calibration standards for them. However, if they can be recognized and quantified, they are distinctive markers for the presence and extent of these processes. Partial degradation processes (e.g. aerobic microbial or thermal) can selectively remove particular congeners, without the appearance of the corresponding product PCBs which are observed in the dechlorination cascades. Highly resolving CQCS analytical procedures are needed to characterize these. Some incomplete thermal degradations or *de novo* syntheses of PCB congeners will display a "scrambling" of the chlorine substitutions around the rings, again resulting in characteristic congener patterns which require CQCS PCB analyses for their full elucidation. It is an often overlooked feature of many industrial processes which involve phenyl rings in the presence of chlorine or chlorinating reagents together with high temperature and catalytic conditions, that

PCBs may form by coupling reactions of chlorinated phenyl rings. The resultant congener distributions will be radically different from those obtained from the catalytic chlorination of biphenyls used to produce Aroclors, and may easily escape detection if one naively attempts to recognize their presence by comparison to Aroclor congener distributions.

A CQCS PCB analytical procedure which is capable of quantifying all 209 congeners or even the subset of about 150 found in the Aroclors in a single pass remains a "Holy Grail", whose attainment is ever more closely approached, but may remain forever beyond the grasp of PCB analytical crusaders. The new technique of comprehensive two-dimensional GC may eventually accomplish this, but the neither necessary pair of columns nor adequate-quantitation software have been demonstrated for this application. Even the development of the present, limited CQCS methods has been a laborious and time consuming task for those laboratories which have attempted it. This accounts for its employment being limited to applications where it is indispensable and researchers have the resources of time and money to support its development. To expedite this process, the author organized in 1994 a consortium of 12 labs to obtain retention order and coelution information on all 209 PCB congeners on 27 HRGC systems spanning 20 different GC stationary phase compositions. The resultant database is due to be published in July 1997 [1,2]. Data from 18 of the systems enabled measurement of levels of all significant congeners in 6 different Aroclors. Knowledge of all congeners present in the Aroclors enabled evaluation of the retention database information to identify GC phases especially suitable for measuring the greatest percentage of the Aroclor congeners. It was possible to process the database information to design the minimum number of mixtures (5 of "Aroclor congeners" and 4 of "non-Aroclor congeners") which could calibrate systems employing the 12 most useful GC phases without significant interference by coelutions. The information in the database was compiled to enable identification of congeners within each-mixture on any of the 12 phases by simply observing their order of elusion. AccuStandard Inc., of New Haven, CT, one of the 2 licensed synthesizers of PCB congeners in the USA, and the only one to offer all 209, immediately prepared the mixtures and marketed them with the requisite tables of elusion orders. This drastically reduced-the costs in materials and time to assign congeners to peaks and calibrate proposed new systems for CQCS PCB analysis.

As a result of the database study, several columns especially suitable for the 2 alternative HRGC methods for CQCS PCB analysis were identified. These methods are:

1. Separation on a single column with detection by GC-MS-SIM, which can provide in many cases independent measurement of coeluting congeners whose chlorination level differs by one chlorine. The method enjoys the high selectivity of MS detection (although isomers (same Cl#) of PCBs in general cannot be distinguished), but it is usually not as sensitive as the ECD detector.

2. A single injection split to a suitably chosen pair of HRGC columns, each measuring the effluent with the generally more sensitive and partially selective (not as much as GC-MS) electron capture detector (ECD).

The congener resolution capability (implied by a listing of Aroclor congeners **not** quantifiable) in three successively more capable systems of category 2 are displayed in the first 3 columns of Figure 2, while the next 3 columns do the same for three improved versions of category 1. The title cells for each column list the GC phases employed, and immediately below are the numbers of the references containing more detailed information on the systems. The IUPAC numbers of PCBs not uniquely measurable due to coelutions are displayed in the shaded cells, where the more heavily shaded ones represent major Aroclor congeners (2 - 12 Wt%) as in Figure 1. Note that System 3 approaches holy grail status most closely, but that the remaining coelutions are almost all major Aroclor components. Its dual-column / ECD technique substitutes confirmation of congener identity by a second column elusion time for System 6's confirmation by characteristic molecular ion masses in the MS-SIM measurement. In the author's experience, the data from a system like that of System 6 are more easily reduced and amenable to automated processing than are the multiple categories of congener measurements in a system like that of System 3. The shorter linear range of older model ECDs makes it harder to cover the 1000-fold range of congener concentrations one may wish to quantify in a complex Aroclor-derived mixture. This disadvantage may be alleviated by the new, even more sensitive and linear micro ECD just introduced by Hewlett-Packard. The dual column ECD systems employ the least expensive instrumentation, but the differential is only on the order of ~30% less.

While HP is keeping the ECD-based systems in the game with its new detector, it is also providing stiff competition for them with the new bench-top 5973 GC-MS system, which lowers the effective measurement range for PCBs nearly an order of magnitude from that of predecessor models. The Varian Saturn 2000 ion-trap, full-scan, bench-top

GC-MS instrument provides comparable data in a similar price range, and provides full scan data and options for MS/MS data acquisition. The more expensive and complex high resolution MS instrumentation which has provided the greatest sensitivity and selectivity for classical methods of trace dioxin, PCDF, and trace coplanar (e.g., PCB 126, *cf* EPA draft method 1668) PCB analyses by isotope dilution MS, may not be as suitable for routine CQCS PCB analysis (if we are willing to consider CQCS as routine!) as the cheaper and easier to-use benchtop instrumentation. Isotope dilution HRGC-HRMS will still continue to be the method of choice for precise quantitation of short lists of critical congeners such as the trace levels of PCBs 77, 126, and 169 whose high toxic equivalency factors (TEFs) sometimes dominate such calculation. This latter, more demanding instrumentation provides much better discrimination against the effects of unsuspected contaminants in complex environmental samples.

Figure 3 displays a comparison of the detection limits and linear response ranges of the old and new HP GC-MS-SIM benchtop instruments and the old HP GC-ECD instrumentation in the context of CQCS PCB analysis. Average values for 3 to 6 congeners at each chlorination level from 1 to 9 chlorines are displayed. The data were obtained from an 18-level serially diluted standard curve vs 2 internal standards at 20 pg each over a range of nearly 6 orders of magnitude. The lower dots represent the signal/noise = 3.3 detection limits and the bars the ± 12% linearity range. The ranges within each chlorination level are numbered and keyed left to right. Note the lower sensitivity of the ECDs to the less chlorinated PCBs. They gradually approach and surpass the performance of the MS instruments as chlorination level increases. Note how the 5973 MS holds its own vs the old ECD over most of the chlorination levels, and performs almost 10-fold better than its predecessor 5972. Note the improvement conferred in extending the linear range to lower levels by performing Gaussian smoothing on noisy low level signals before performing peak integration. This results primarily from the smoother baseline allowing the integration software to more consistently place the start, stop and baseline levels of peaks which are close to the noise limit. This graph represents preliminary results from a more extensive study which will also evaluate the Varian Saturn 2000 ion-trap GC-MS, and several GC-HRMS systems, and the new micro-ECD. A second leg with PCBs at the same levels in 1000 ppm complex petroleum oil contaminated samples will be run to evaluate the advantages of HRMS instrumentation in dealing with highly contaminated samples.

As a follow-up to the Aroclor distribution database study[2] a second collaboration employed one example of dual column/ECD System 3 and 2 examples of single column/MS-SIM System 6 to measure complete congener distributions in 17 Aroclor lots against the 9 AccuStandard calibration mixtures[3]. This demonstrated the effectiveness of these optimized systems and the new calibration mixes in expediting CQCS PCB analysis. The results revealed the pairs of Aroclor 1248 and 1254 lots which were compared differed substantially. This highlights a weakness in CQCS methods which propose calibration against Aroclor mixtures. They are convenient, and represent distributions similar to those often observed, but they are only as good as the tables of distribution provided for them, and they only work if one uses the same or a similar lot. They offer little help if "non-Aroclor" congeners are encountered. One Aroclor 1254 lot was found to have major elevations in the relative amounts of non- and mono-*ortho*-chloro substituted congeners when compared with the more common Aroclor 1254 lots. This has been found to represent a different mode of manufacture for these unusual lots. This finding has important implications both for the employment of such lots as analytical standards and for studies of Aroclor toxicity.

To further simplify analytical calibration with primary standards in CQCS PCB analysis, the 5 AccuStandard "Aroclor congener mixtures" were combined and then serially diluted into a 6 level standard curve covering a 100-fold concentration range. The resultant isomer coelutions in the standard curve when Aroclors were profiled on a GC-MS-SIM system designed on the model of System 6 required correction for the combined response factors present, which were independently measured in a prior experiment. When these and other corrections for fragment ion contributions from coeluting higher homologs (higher chlorination level PCBs) in both the samples and standards were applied to the concentration calculations, results for Aroclor peak distributions comparable to those obtained in reference[3] could be obtained with much less effort. The use of a multilevel inclusive standard curve is believed to improve the quantitation more the uncertainties arising from application of corrections for the presence of isomer or homolog coelutions in the standard mixture and in the samples.

SUMMARY

A comprehensive database of HRGC PCB retention times and Aroclor congener distributions, optimized columns, new ECD or MS detectors, and appropriately designed comprehensive sets of primary congener standards all combine to greatly reduce the effort required to develop and validate CQCS PCB analyses. There is no longer an

excuse for neglecting to perform such analyses when studying processes which give rise to non-standard congener distributions, especially those that give rise to important "process signature" congeners which are minor or missing in the commercial Aroclor products. Designing a PCB analysis to accommodate a CQCS measurement of all resolvable peaks and running the necessary standards to support eventual quantitation of congeners in addition to those specified in many of the short list methods can increase the value of data acquired in a wide range of environmental studies. This should be recommended to researchers who cannot predict whether the levels of some congener initially of no interest may not later on become critical to answering a new question or resolving an as-yet-unidentified problem.

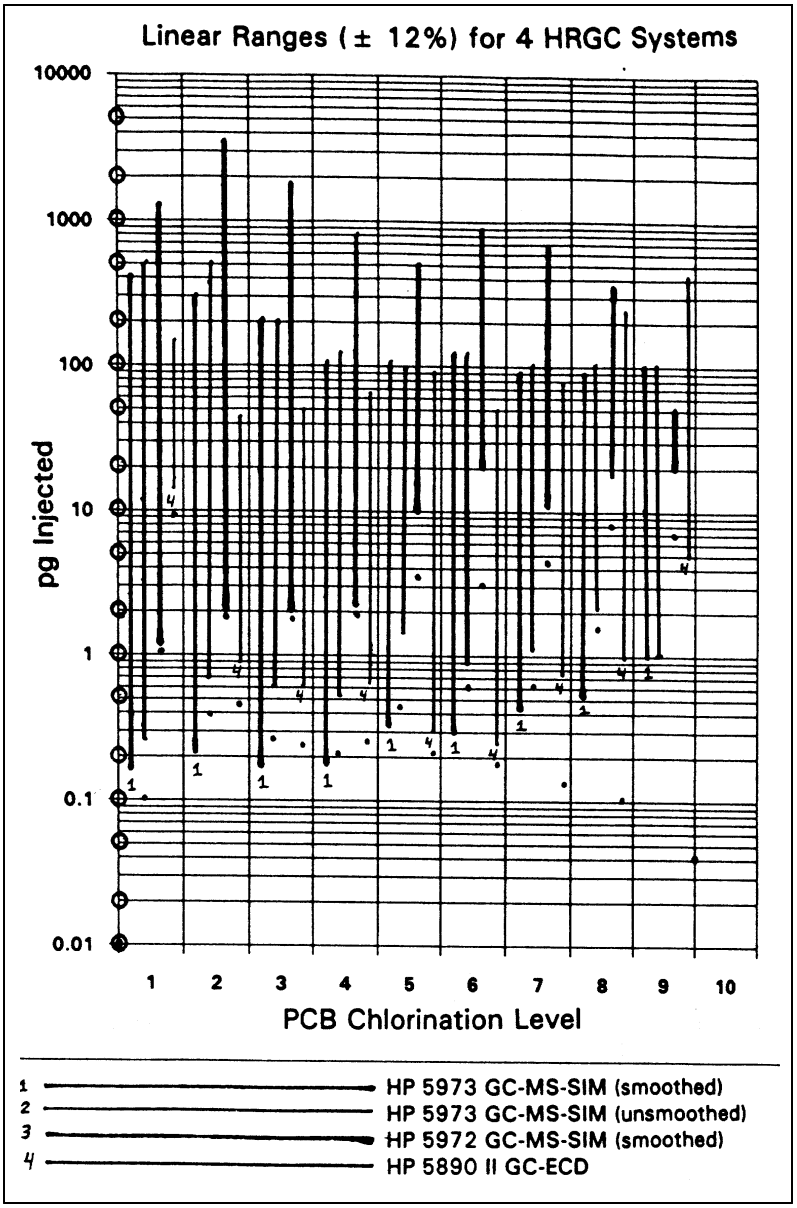


Figure 3. Comparison of PCB Detectors' Sensitivity and Linearity

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PROPOSED US EPA METHOD 8320: A RISK ASSESSMENT METHOD FOR SECONDARY EXPLOSIVES

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Currently, nitramines and nitroaromatics are measured in environmental matrices, such as groundwater and soil, using the standard US EPA Method 8330 (SW-846). This analytical methodology involves a sample extraction step followed by HPLC fractionation using a uV detector for quantification. This approach is useful for monitoring a group of 74 secondary explosives and associated degradation products and precursors at ppm concentrations in samples obtained during the initial characterization phase of a remediation project, as well as for manufacturing, storage, testing, and DOD facilities.

What we are proposing is not an alternate but complementary method for analyzing the same 14 compounds in matrices ranging from groundwater to soil/sediment, and expanded to include biological tissues, air, and ash/residue. The detection limits of this new methodology -- which, once approved, will be given the name of EPA Method 8320 -- are in the ppb range.

Proposed Method 8320 uses isotope-dilution, in which isotopically-labeled compounds are introduced into the sample prior to extraction. These labeled internal standards provide extraction efficiency information, recovery-corrected analyte concentrations and qualitative identification. Isotope-dilution HPLC coupled with negative-ion atmospheric pressure chemical ionization tandem mass spectrometry (MS/MS) of explosives offers lower detection limits than ever before.

The validation data from Method 8320 in a series of matrices will be presented. Also, various aspects and value/benefits of the new methodology will be discussed in terms of precision, accuracy and reliability. A draft version of the method was submitted to the EPA during summer 1996 for consideration and inclusion into the SW-846 compendium of methods. It is concluded that Method 8320 is more valuable for risk assessment studies, with reporting levels two-to-three orders of magnitude lower than for Method 8330.

FAST PRESCREENING OF WATER AND SOIL SAMPLES USING SOLID-PHASE MICROEXTRACTION (SPME)

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The objective of this presentation is to discuss a relatively new prescreening approach for volatile compounds.

Many EPA protocols require purge and trap (PT) analysis coupled with gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) for the analysis of environmental samples. These PT techniques can be costly and inefficient to run due to problems of carry-over and cross contamination. As autosamplers become more prevalent in PT analyses, the inefficiencies due to system contamination become increasingly problematic.

SPME prescreening methods have been developed to determine VOC content in 3 - 4 minutes without the use of cryogens. The SPME methods which have been optimized, require a standard gas chromatograph and flame ionization detector (GC-FID) equipped with an SPME autosampler. The methods developed for both water and soil matrices use SPME headspace sampling. Problems due to carry-over are virtually non-existent. Short lengths of narrow bore capillary columns with relatively high phase ratios yield sufficient chromatographic resolution and loading capacity for these prescreening applications. The use of FID provides stability and a broad linear range. The system is easy to use, maintenance is minimal, and system troubleshooting and repairs are performed easily in-house.

The supporting data for this method includes comparisons between SPME and PT data for a number of water and soil samples. Technique strengths and representative standard and sample chromatograrns will also be presented.

In conclusion, this SPME prescreening technique has been found to be a helpful tool in processing of VOC samples in a time efficient manner.

ON-SITE ANALYSIS OF EXPLOSIVES IN SOIL: EVALUATION OF THIN-LAYER CHROMATOGRAPHY FOR CONFIRMATION OF ANALYTE IDENTITY

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ABSTRACT

Two colorimetric-based methods are commonly used for on-site analysis of explosives in soil. For the TNT method, acetone soil extracts are reacted with base to produce reddish Janowsky anions. For the RDX method, acetone extracts are acidified and reacted with zinc metal to reduce RDX to nitrous acid, which is further reacted with a Griess reagent to produce a reddish product. In both cases, concentrations are estimated using absorbance measurements at 540 or 507 nm, respectively. The limitations on positive analyte identification with these procedures are that the TNT method also reacts with other polynitroaromatics, such as TNB and DNT, and the RDX method reacts with other nitramines (HMX) and nitrate esters (NG and PETN). The ability to qualitatively differentiate among the various analyses that produce positive responses would greatly enhance the usability of these methods.

This study investigated the use of thin-layer chromatography (TLC) as a simple, on-site method to confirm the identity of analyses detected using the colorimetric procedures. Separations using both laboratory-grade and locally available solvents were developed. The combination of petroleum ether:isopropanol (4:1) provided the best separation for the nitroaromatics, and petroleum ether:acetone (1:1) produced the best separation for the nitramines and nitrate esters. Various types of visualization schemes were also investigated. The most sensitive were $TiCl_3$ with dimethylaminocinnamaldehyde (DMACA) for the nitroaromatics, and the Griess reagent with UV exposure for the nitramines. The major limitation of TLC confirmation analysis is that it does not currently provide an analyte detection capability comparable to the colorimetric tests. Using plates with a preconcentration zone and high ratios of soil to solvent, detection levels of about 10 mg/kg seem attainable.

INTRODUCTION

Environmental concerns over explosives contamination in soil have resulted in the determination of the extent of this contamination at numerous Department of Defense installations. Laboratory analytical methods were developed to enable the determination of the most commonly found components of explosives, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and related impurities and environmental transformation products in the soil matrix (Jenkins et al. 1989, U.S. EPA Method 8330). On-site methods for TNT and RDX, the most commonly encountered contaminants (Walsh et al. 1993), were also developed to provide a more expedient means of rapidly characterizing these sites prior to extensive laboratory analyses (Jenkins and Walsh 1992, Teaney and Hudak 1994). Overall, the use of on-site methods has been successful in providing rapid site characterization at explosives-contaminated sites.

The most commonly used pair of on-site methods for TNT and RDX in soil is based on research conducted at the U.S. Army Cold Regions Research and Engineering Laboratory (CRREL). These methods are based on the production of colored products when acetone soil extracts are reacted with the appropriate reagents. In the field

screening methods by Jenkins (1990) and Walsh and Jenkins (1991), TNT and RDX, respectively, are converted to color-specific compounds that are quantified spectrophotometrically. Kits containing the associated reagents and supplies are commercially available from EnSys Corporation (now Strategic Diagnostics, Inc., Newark, Delaware). In the TNT method, acetone soil extracts are reacted with strong base to produce reddish-colored Janowsky anions when TNT is present. Reddish-colored anions are also produced, however, when 1,3,5-trinitrobenzene (TNB) or N-methyl-N-2,4,6-tetranitrobenzenamine (tetryl) is present, and a bluish-colored anion is produced when 2,4-dinitrotoluene (2,4-DNT) is present (Jenkins and Walsh 1991). Thus a positive response on the TNT test does not unequivocally prove that TNT is present, because several other polynitroaromatics can give a similar response.

For the RDX test, soil extracts are first acidified with acetic acid and reacted with zinc to reduce any RDX present to nitrous acid, and the resulting solution is reacted with a Griess reagent to produce a reddish-colored azo dye. Reddish-colored azo dyes are also produced if other nitramines, such as octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) or tetryl, or organonitrate esters such as nitroglycerin (NG), or pentaerythritol tetranitrate (PETN) are present. In addition, nitrate and nitrite ion, if not removed using an anion exchange column prior to reaction with zinc, will also respond. The ion exchanger is specified in the CRREL-developed method, but is not recommended for routine use by EnSys.

For both of these methods, the intensity of the color is directly proportional to the concentration of the analyte of interest, and concentrations are determined by measuring absorbance at 540 nm for TNT and at 507 nm for RDX. Method detection limits for TNT and RDX in soil samples using these methods are 1.1 mg/kg and 1.4 mg/kg, respectively.

Often the capability of the TNT test to detect other polynitroaromatics can be quite useful. For example, in a recent study in Sparks, Nevada, areas contaminated with 2,4-DNT were detected using this test (Jenkins et al. 1996). Likewise the capability of the RDX test to determine HMX concentrations was recently demonstrated at an active anti-tank range at Valcartier, Quebec (Jenkins et al. in press). It is important to be able to discriminate among the various compounds that respond to these tests because cleanup levels for the various explosives can be set at somewhat different concentrations. Therefore it would be quite useful if a simple, inexpensive, on-site method were available to qualitatively determine which of the potentially detectable analyses are giving rise to the colored reaction products from either the TNT or RDX tests.

The objective of this work is to evaluate the use of conventional TLC as an adjunct to current on-site colorimetric methods for TNT, RDX, and related compounds. This work evaluates the ability of TLC to separate TNT and the other common polynitroaromatic compounds, and RDX and the other commonly encountered nitramines and nitrate esters in a cost-effective and timely manner.

MATERIALS AND METHODS

TLC materials

A basic thin-layer chromatography starter kit was purchased from Alltech Associates, Inc. (Deerfield, Illinois). The starter kit included a 20- x 20-cm TLC tank with glass lid, 20- x 20-cm tank liners, microcap (microcapillary) dispensers (for sample spotting), disposable spray box, spotting template, reagent spray unit with glass jar, and 20- x 20-cm Adsorbosil Plus 1 TLC plates. Additional glass-backed plates consisting of EM silica gel 60 F254 (20 x 20 cm, 250 µm), EM silica gel 60 F254 with preconcentration zone (20 x 20 cm, 250 µm), EM silica gel 60 with preconcentration zone (20 x 20 cm, 250 µm), EM HPTLC silica gel 60 F254 with preconcentrated and prechanneled zones (10 x 10 cm, 200 µm), and Adsorbosil HPTLC with phosphor and preconcentrated zone (10 x 10 cm, 150 µm) were purchased from EM Science, Gibbstown, New Jersey. A multiband (254 and 366 nm) UV lamp with a viewing box was obtained from UVP, Inc., in San Gabriel, California.

Standards

Analytical standards for TNT, TNB, tetryl, 2,4-DNT, 4-A-DNT, 2-A-DNT, RDX, HMX, NG, and PETN were prepared from Standard Analytical Reference Materials (SARM) obtained from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland.

Solvents

Commercial-grade solvents were purchased from local paint and hardware stores. These solvents consisted of

Sunnyspec Paint Thinner, Sterling Lynsol, Sterling Thin-X (red), Sterling VM & P Naphtha, Savogram Deglosser, Parks VM & P Naphtha, Recorder Paint Thinner, PPG Industries Duracryl, Sterling Acetone, Ace Paint Thinner, Woolworth brand 70% Isopropanol, Sterling Solvent Alcohol, and 3M General Purpose Adhesive Cleaner.

Visualization

A number of different visualization methods were evaluated as a part of this work. These include use of visual fluorescence while irradiating with UV light, and application of a number of chemical reagents to air-dried TLC plates. A description of the method of preparation for the various chemical visualizing reagents is presented elsewhere (Nam in press).

General thin-layer chromatography procedures

The developing tank was prepared by the addition of the mobile phase (200 mL) and equilibrated for approximately 30 to 40 minutes or until the tank liner had been saturated. The TLC plates were prepared for sample spotting by designating the line of origin (about 2-3 cm above the bottom of the plate) and the solvent front line (10 cm from the line of origin). Using capillary micropipettes, samples were spotted along the line of origin approximately 1 cm apart. Spotting volumes ranged from 0.5 to 30 µL, but in most cases, were spotted 1 µL at a time. The plates were then placed in the developing tank (containing the freshly prepared mobile phase) and developed in an ascending manner until the mobile phase had reached the solvent front line. The plates were removed from the tank and either air-dried or dried with hot air from a heat gun prior to observation. The fluorescence-containing plates were observed under the UV lamp (set at 254 nm) and/or sprayed with visualizing agents. Non-fluorescence-containing plates were sprayed with visualizing agents. The position of the resulting non-fluorescing spots (observed under the UV light) or colored spots were marked and the retention factor (Rf) values were determined by dividing the distance traveled by the compound by the distance traveled by the solvent front. When analyzing soil samples, analyses were identified by comparing the Rf values to the Rf values of the standards, which were spotted on the same plate.

RESULTS AND DISCUSSION

Separation of nitroaromatics using laboratory-grade and commercial-grade solvents

Numerous mobile phase systems were tested to determine the best solvent or combination of solvents that would result in a distinguishable separation of nitroaromatic compounds, such as TNT, TNB, DNT, tetryl, and the isomers of amino-DNTs. The evaluated mobile phase systems using laboratory-grade solvents included hexane:chloroform (4:1), chloroform, petroleum ether:acetone (3:1 and 2:1), petroleum ether:isopropanol (4:1), xylene, and Stoddard solution:isopropanol (2:1 and 1:1). These solvents were chosen according to their eluting strength. The combination of four parts petroleum ether with one part isopropanol resulted in the best distinguishable separation of nitroaromatic compounds compared to the other evaluated mobile phase systems (Table 1).

Commercial-grade paint thinners and alcohols, which are readily available in any local hardware or paint store, were also evaluated for their ability to separate nitroaromatic compounds. However, due to high water content in commercially available alcohols (i.e., Woolworth brand 70% Isopropanol and Sterling Solvent Alcohol), all mobile phases prepared with these alcohols resulted in very little or no movement of the nitroaromatic compounds. However, when commercially available paint thinners were mixed with laboratory-grade isopropanol (4:1), compounds such as TNT, DNT, TNB, and tetryl could be distinguished from each other (Table 2). Commercial brands such as Recorder Paint Thinner, Sterling VM & P Naphtha, Sterling Thin-X Paint Thinner, and Ace Paint Thinner were among the tested brands that have resulted in good-to-fair separation of TNT, DNT, TNB, and tetryl. However, in most cases, the development time took longer than when the mobile phase was composed purely of laboratory-grade solvents.

Separation of nitramines and nitrate esters using laboratory-grade and commercial-grade solvents

The solvent system of petroleum ether and acetone (1:1) was found to be very effective in separating the nitramines, such as RDX and HMX, and in producing a fair separation of the nitrate esters, PETN and NG (Table 3). RDX, PETN, and NG were also effectively separated with petroleum ether:isopropanol (4:1), but HMX failed to move from the line of orlgln.

Mobile phase solvents consisting of commercial-brand acetone and some paint thinners (1:1) were also effective in producing a good separation of RDX and HMX (Table 4). However, the separation of PETN and NG was not as clear, thus resulting in very similar Rf values.

Table 1. Separation of nitroaromatics with laboratory-grade solvents.

Solvent systems	TNT Rf ± S.D.	TNB Rf ± S.D.	2,4 -DNT Rf ± S.D.	Tetryl Rf ± S.D.	2-A-DNT Rf ± S.D.	4-A-DNT Rf ± S.D.
1	0.52	0.36	0.48	0.20	0.16	0.14
2	0.42	0.40	0.40	0.27	0.22	0.23
3	0.47	0.46	0.45	0.33	0.30	0.30
4	0.64 ± 0.02	0.58 ± 0.02	0.52 ± 0.02	0.37 ± 0.02	0.23 ± 0.02	0.27 ± 0.02
5	0.75	0.71	0.63	0.62	0.55	0.55
6	0.74	0.72	0.65	0.67	0.62	0.62
7	0.54	0.50	0.47	0.28	0.1	

Solvent system key:
1) Chloroform
2) Petroleum ether:Acetone (3:1)
3) Petroleum ether:Acetone (2:1)
4) Petroleum ether:Isopropanol (4:1), n = 3
5) Stoddard solution:Isopropanol (2:1)
6) Stoddard solution:Isopropanol (1:1)
7) Xylene

Table 2. Separation of nitroaromatic compounds with commercial-brand paint thinners and laboratory- grade isopropanol (4:1).

Solvent systems	TNT Rf ± S.D.	TNB Rf ± S.D.	2,4-DNT Rf ± S.D.	Tertyl Rf ± S.D.	2,A-DNT Rf ± S.D.	4-A-DNT Rf ± S.D.
1	0.78 ± 0.04	0.76 ± 0.04	0.65	0.68 ± 0.05	0.54 ± 0.01	0.56 ± 0.01
2	0.88 ± 0.02	0.88 ± 0.01	0.86 ± 0.01	0.85 ± 0	0.83 ± 0.01	0.84 ± 0.01
3	0.74 ± 0.01	0.69 ± 0.02	0.71	0.65 ± 0.04	0.59 ± 0.04	0.61 ± 0.04
4	0.70 ± 0.07	0.67 ± 0.05	0.61 ± 0.04	0.52 ± 0.04	0.39 ± 0.01	0.43 ± 0.04
5	0.72 ± 0.02	0.68 ± 0	0.63 ± 0.01	0.57 ± 0.02	0.40 ± 0.01	0.43 ± 0
6	0.65 ± 0.00	0.60 ± 0	0.55 ± 0	0.48	0.35	0.40
7	0.74 ± 0.01	0.68 ± 0	0.64 ± 0.01	0.53 ± 0	0.42 ± 0.02	0.44 ± 0
8	0.79 ± 0.01	0.76 ± 0.01	0.66 ± 0.01	0.62 ± 0.02	0.45 ± 0.01	0.49 ± 0.02

Solvent system key:
1) 3M Adhesive cleaner:Isopropanol, n = 2
2) Duracryl:Isopropanol, n = 2
3) Deglosser:Isopropanol, n = 2
4) Recorder paint thinner:Isopropanol, n = 2
5) Parks VM & P naphtha:Isopropanol, n = 2
6) Sterling VM &P naphtha:Isopropanol, n = 2
7) Sterling Thin-X paint thinner:Isopropanol, n = 2
8) Ace paint thinner:Isopropanol, n = 2

Table 3. Separation of nitramines and nitrate esters with laboratory-grade solvents.

Solvent systems:	n	RDX Rf ± S.D.	HMX Rf ± S.D.	PETN Rf ± S.D.	NG Rf ± S.D.
Petroleum ether:Acetone (1:1)	3	0.72 ± 0.01	0.62 ± 0.01	0.91 ± 0.03	0.88 ± 0.02
Petroleum ether:Isopropanol (4:1)	3	0.30 ± 0.02	No movement	0.84 ± 0.03	0.79 ± 0.02

Table 4. Separation of nitramines and nitrate esters with commercial-brand solvents.

Solvent systems:	RDX Rf ± S.D.	HMX Rf ± S.D.	PETN Rf ± S.D.	NG Rf ± S.D.
3M Adhesive cleaner: Sterling acetone (1:1)*	0.58 ± 0.04	0.53 ± 0.04	0.80 ± 0.05	0.78
Parks VM & P naphtha: Sterling acetone (1:1)*	0.54 ± 0.01	0.45 ± 0.03	0.73 ± 0.05	0.69 ± 0.06
Sterling VM & P naphtha: Sterling acetone (1:1)*	0.53 ± 0.01	0.42 ± 0.02	0.65 ± 0.06	0.63 ± 0.04
Sterling Thin-X: Sterling acetone (1:1)**	0.53 ± 0.03	0.43 ± 0.08	0.59 ± 0.03	0.60 ± 0.03
Ace paint thinner: Sterling acetone (1:1)	0.48 ± 0.07	0.36 ± 0.09	0.61 ± 0.04	0.59 ± 0.05

* n=2
**n = 3

Evaluation of TLC plates

TLC plates used in this study were all glass plates precoated with silica gel. The differing features of these plates included different commercial brands, fluorescence vs. nonfluorescence, and preconcentration zone vs. no preconcentration zone. The preconcentration area, located on the bottom of the plate, is made up of inert material that is meant to absorb sample solvent. The various commercial fluorescent and non-fluorescent plates were found to produce identical separations. However, the plates having preconcentration zones did give better analyte resolution when the spotting volume exceeded 5 µL. This result was in agreement with Rabel and Palmer (1992) and Hauck and Mack (1990), who report enhanced resolution, reproducibility, and recovery of analyses spotted on preconcentration zones.

Evaluation of high-performance thin-layer chromatography (HPTLC) plates

HPTLC plates, like conventional TLC plates, are usually coated with various binders to hold sorbent material together. However, the dimensions of HPTLC plates are approximately half the size of conventional plates. The particle sizes of the sorbent material are much smaller and the size distribution of these particles is much tighter. HPTLC plates are also thinner and the surface is more uniform than conventional plates.

HPTLC plates were evaluated here to determine if the separation and resolution were better than with conventional TLC plates. Two different brands of HPTLC plates were evaluated (EM and Adsorbosil). Both brands had preconcentration zones, with the EM plates also having channeled zones while the Adsorbosil did not. The compounds were spotted along the preconcentration zone using microcapillary dispensers. Due to the size of the plates (10 x 10 cm) and thinner thickness (150-200 µm), the developing time was usually between 10-15 minutes, half the development time of standard TLC plates. The HPTLC plates also required less mobile phase volume compared to standard TLC plates. However, when the compounds were visualized with UV light or with visualizing agents, the separation and resolution of compounds, including nitroaromatics, nitramines, and nitrate esters, were similar to the standard TLC plates whether or not they had channeled zones (Table 5).

Evaluation of visualizing agents

The simplest method for visualizing nitroaromatics and nitramines was viewing the developed plate under shortwave (254 nm) UV light (Grover and Hoffsommer 1973, Malotky and Downes 1983, McCormick et al. 1978, and Zou et al. 1994). We found the fluorescence-containing plates to have bright green backgrounds with light-to-dark spots representing nitroaromatic and nitramine compounds. Following the UV viewing, nitroaromatics could be further distinguished by placing approximately 1 µL of the EnSys TNT developer on the dark spots. The EnSys TNT developer reacts with the nitroaromatic compounds to form Meisenheimer complexes and results in the following color formations: purple for TNT, orange for tetryl, light yellow for 4-A-DNT, orange for TNB, light yellow for 2-ADNT, and light green for 2,4-DNT.

Table 5. Comparison of HPTLC plates and TLC plates.

	Petroleum ether:Isopropanol (4:1)		Petroleum ether:Acetone (1:1)	
Compounds	HPTLC Rf* ± S.D.	TLC Rf* ± S.D.	HPTLC Rf* ± S.D.	TLC Rf* ± S.D.
TNT	0.83 ± 0.03	0.64 ± 0.02		
TNB	0.79 ± 0.02	0.58 ± 0.02		
2,4-DNT	0.73 ± 0.03	0.52 ± 0.02		
Tetryl	0.68 ± 0.01	0.37 ± 0.02		
2-A-DNT	0.57 ± 0	0.23 ± 0.02		
4-A-DNT	0.62 ± 0.02	0.27 ± 0.02		
RDX	0.32 ± 0.02	0.30 ± 0.02	0.65 ± 0.05	0.72 ± 0.01
HMX	No movement	No movement	0.57 ± 0.06	0.62 ± 0.01
PETN	0.82 ± 0.02	0.84 ± 0.03	0.82 ± 0.04	0.91 ± 0.03
NG	0.75 ± 0.02	0.79 ± 0.02	0.79 ± 0.04	0.88 ± 0.02

*n=3

A number of other chemical-based visualization procedures were evaluated and are fully discussed elsewhere (Nam in press). The following are the results for what we consider to be the best candidate visualization systems.

$TiCl_3$ and DMACA

This combination worked well in visualizing nitroaromatic compounds. The developed plate is initially sprayed with the $TiCl_3$ reagent, which reduces the nitroaromatic compounds to amines. When the plate is dried, it is sprayed with DMACA (dimethylaminocinnamaldehyde), which reacts with the amines to form Schiff bases. Compounds sprayed with DMACA became purple. Color development was immediate and was best viewed when the plate was still wet.

Griess reagent and UV exposure

Spraying with Griess reagent followed by UV light exposure for approximately 30 minutes resulted in pink-colored spots for RDX, HMX, PETN, and NG. However, when the plates were dried in a 110°C oven for 20 minutes, RDX and HMX yielded light blue spots while PETN and NG had lime green spots.

Estimation of detection capability

A preliminary estimation of the minimum detectable level for each explosive (Table 6) was determined by spotting different volumes (ranging from 0.5 to 30 µL) of each standard solution. The concentration of the various standard solutions ranged from 5 to 2000 mg/L. The HPTLC plates did not significantly enhance detectability of analysts.

Testing of soil samples collected from the field

Soil samples collected from the field (ranging from ammunition plants to firing ranges) were analyzed using the conventional TLC methods to determine or confirm the accuracy of their separation procedures. The concentration of the analyses in these soil samples was previously determined by standard HPLC methods. In most cases, soil samples were extracted with acetone (1:5) and the filtered extracts were spotted on TLC plates. Soil from Umatilla Army Depot, which contained 716 µg of TNT per gram of soil, was extracted and spotted (10 x 1 µL). This yielded a visible spot under UV light that corresponded to the same Rf value as the spot from a standard solution of TNT. A soil sample from Hawthorne Ammunition Plant containing an array of explosives, including HMX (2.4 mg/g), RDX (8.1 mg/g), TNB (0.088 mg/g), DNB (0.002 mg/g), TNT (13.9 mg/g), and 2,4-DNT (0.007 mg/g), was extracted. When 1 µL of the extract was spotted and developed, two spots were visible under UV light, corresponding to TNT and RDX. Soil collected from Defence Research Establishment, Valcartier (DREV) had tested positive for RDX using the RDX that area was HMX. When 10 µL of the DREV soil extract was spotted and developed using the solvent system of petroleum ether:acetone (1:1), a single pink spot appeared after spraying the plate with Griess reagent and exposing the plate under UV light for 30 minutes. The Rf value corresponded to HMX, and the identity was confirmed by standard HPLC methods.

Table 6. Lowest level of visualization.

Compound	Lowest level (µg)	Visualizing agent	Frequency
Nitroaromatics			
TNT	0.1	UV	4/4
		TiCl3 and DMACA	8/8
		HPTLC*/UV	4/4
		HPTLC*/$TiCl_3$ and DMACA	1/1
Tetryl	0.01	TiCl3 and DMACA	1/1
	0.05	EDA:DMSO (1:1)	1/1
		HPTLC*$TiCl_3$ and DMACA	1/1
TNB	0.1	UV	4/4
		$TiCl_3$ and DMACA	8/8
		HPTLC*/UV	3/3
Nitramines			
RDX	0.1	UV	4/7
		Griess and UV exp.	1/1
		HPTLC*/UV	8/8
HMX	0.1	HPTLC*/UV	3/3
	0.2	Griess and UV exp.	2/2
Nitrate esters			
PETN	0.4	Griess and UV exp.	2/2
NG	0.4	Griess and UV exp.	2/2

* High-performance thin-layer chromatography plates

In most cases, soil samples collected from the field contained a high concentration of TNT with very low levels of other nitroaromatics. When soil extracts from these samples were spotted, due to the high TNT concentration, other nitroaromatics could not clearly be identified. To determine the effectiveness of the TLC method in separating nitroaromatic compounds in extracts from field samples, soil from Savanna Army Depot, which contained similar levels of TNT (14 µg/g) and TNB (9.4 µg/g) were used. Soil samples were extracted in acetone as described above, but to maximize the extract concentration, the soilto-solvent ratio was increased to 1:2 (1 gm of soil to 2 mL of acetone). A volume of 20 µL, which was equivalent to 0.14 µg of TNT and 0.094 µg of TNB, was spotted and developed in the solvent system of Sterling VM & P Naphtha and laboratory-grade isopropanol (4:1). Plates were sprayed with $TiCl_3$, $NaNO_2$, and Bratton-Marshall reagent (Jork et al. 1994). Two spots, light purple in color, were identified as TNT and TNB. These results seemed to indicate that TLC could be used to separate and distinguish different explosive components in actual field samples when appropriate solvent system and visualizing procedures are utilized.

Recommendations for specific separations and visualizing reagents

The solvent system of petroleum ether and isopropanol (4:1) is recommended to separate various species of nitroaromatic compounds, including TNT, TNB, and DNT. The most sensitive visualizing agents tested for nitroaromatics are $TiCl_3$ followed by DMACA spray. For the separation of nitramines such as RDX and HMX, and nitrate esters PETN and NG, the solvent system of petroleum ether and acetone (1:1) is recommended with visualization with Griess reagent followed by UV exposure. Optimal separation occurs with laboratory-grade solvents; however, in cases where laboratory-grade solvents are not readily available, commercially available solvents such as paint thinner and acetone may be substituted. If sensitivity is not an issue, commercially available solvents will be more cost-effective and more readily available in the field.

SUMMARY AND CONCLUSIONS

For the purposes of this evaluation, the conventional TLC approach was used for the following reasons: 1) the conventional TLC techniques involve minimal equipment, thus making them field-portable and inexpensive, and 2) the purpose of this report was to evaluate a method that can be used in conjunction with on-site colorimetric methods. The results indicate that TLC methods could indeed be used to separate various components of explosives such as TNT, TNB, DNT, RDX, HMX, PETN, and NG from soil samples. Using appropriate solvent systems, such as petroleum ether and isopropanol (4:1) and petroleum ether and acetone (1:1), nitroaromatic, nitramine, and nitrate ester compounds could be effectively separated. In most cases, commercial-brand solvents (readily available in hardware stores) were also effective in giving good-to-fair separation of components of explosives. However, as mentioned at the onset of this report, the detection capability of conventional TLC methods is poor and remains the major limitation of this method. The conventional TLC method evaluated in this report is capable of detecting 0.1 µg of TNT or RDX with either UV light, $TiCl_3$ spray followed by DMACA, or Griess reagent followed by UV exposure. This is equivalent to spotting a volume of 1 µL of sample extract containing 100 µg/mL of TNT or RDX, and if the sample extract was prepared using the soil-to-solvent ratio used in the on-site colorimetric methods (20 g of soil to 100 mL of acetone), the concentration of TNT or RDX in soil would correspond to approximately 500 µg/g. This is about 500 times above the minimum detection limit for TNT (1.1 µg/g) and RDX (1.4 µg/g) colorimetric on-site tests. Even if the maximum spotting volume of 30 µL is used, the detection capability remains at about 17 µg/g if the soil-to-solvent ratio is maintained at 20 g and 100 mL. If a larger soil-to-solvent ratio is used to obtain an extract for TLC analysis, the detection capability could be further improved. More experiments aimed at optimizing detection capability by either concentrating sample extracts and/or utilizing higher soil-to-solvent ratios are needed to fully assess the practical limit of detection for the TLC method.

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