

PROCEEDINGS

THIRD ANNUAL SYMPOSIUM



*United States
Environmental Protection Agency
Symposium
on*

SOLID WASTE TESTING and QUALITY ASSURANCE

Volume I

JULY 13-17, 1987

*WASHINGTON, D.C.
WESTIN HOTEL*

Symposium Managed by American Public Works Association

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PROCEEDINGS INTRODUCTION

One of the major environmental problems facing the United States, as well as other nations, is the need for safe handling and disposal of hazardous waste. A fundamental component of all programs relating to waste management is the need to perform measurements. These measurements include waste composition and properties; effectiveness of management processes; engineering properties of materials used in constructing management units; and, last but not least, long term performance of such management units. Thus, the pivotal roles played by the measurement methodology and, its attendant, quality assurance.

The analysis of complex waste matrices presents the environmental community with demanding analytical problems for which solutions are being developed at a rapid rate. This annual symposium series, presented by the EPA's Office of Solid Waste, is designed to focus on recent developments in testing methods and quality assurance of importance to both the RCRA and CERCLA programs.

The symposium highlights developing requirements for quality assurance as well as new analytical procedures intended to be used in EPA's national RCRA and CERCLA hazardous waste management programs. Our purpose in holding these symposia is several fold. First, as a means of communicating what EPA is doing regarding the activities EPA has already initiated to upgrade the state-of-the-art as reflected in the regulations and in SW-846. Second, to describe the direction EPA's program is taking with respect to testing and quality assurance issues. Third, as a forum for discussion between Agency personnel and representatives from public and private laboratories involved in waste sampling and evaluation.

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AIR AND GROUND WATER MONITORING

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COMPARISON OF TOX AND GC/MS DATA
FOR RCRA GROUNDWATER MONITORING WELL SAMPLES

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ABSTRACT

The method for the analysis of total organic halogen (TOX) was developed as a means to trace the products of disinfection in drinking water. It was intended to measure, collectively, compounds such as trihalomethanes and halogenated ethanes. Briefly, the method involves passing a measured amount of sample through a column of activated carbon, followed by pyrolysis of the carbon and analysis of the gases produced for total halogen (as chloride) by microcoulometric titration. It has been shown to produce good recoveries for spiked deionized water and for other simple, fairly clean waters that are free of turbidity. This method is documented as Method 9020 in SW-846 (Test Methods for Evaluating Solid Waste).

RCRA regulations require the monitoring of TOX as an indicator of groundwater contamination at many hazardous waste management facilities (40 CFR 265.92). Method 9020 is recommended as an economical method for TOX analysis. Another way of measuring TOX is to analyze samples for volatile and semivolatile organics by GC/MS (SW-846 Methods 8240 and 8270) and calculating the sum of the halogen contents (as chloride) of all compounds that are found.

Samples from RCRA groundwater monitoring wells are generally more complex than the drinking water samples that were analyzed when Method 9020 was initially evaluated. In the Region II laboratory we have analyzed many groundwater samples by Method 9020 and by Methods 8240 and 8270. This report is a presentation of a preliminary comparison of the results obtained by these two techniques.

BACKGROUND

Dressman, Stevens and co-workers in the Drinking Water Research Division (DWRD) of the Municipal Environment Research Laboratory, US Environmental Protection Agency (USEPA), Cincinnati, Ohio, have been very active in the development of the analysis of Total Organic Halogen (TOX) as a group parameter. They have thoroughly reviewed the history of TOX.¹ Briefly, they reported that the use of TOX began in the early 1970s when Kuhn and Sontheimer² developed a technique for the measurement of total organic chlorine (TOCl) in activated carbon. Their objective was for this test to be used to monitor the operation of activated carbon filters in water treatment plants. These plants were purifying water from the Rhine River for use as drinking water. A second use for the test was for comparison

iodide, making the analysis applicable for TOX, not just for TOCl. This was desirable since organobromides and iodides are also indicators of water contamination.

The next major improvement was made by Takahashi and Moore.⁵ They packed GAC into a microcolumn, passed the sample through the column to adsorb organics onto the GAC, passed a potassium nitrate solution through the column to displace inorganic halides, combusted the sample as described above, and analyzed by microcoulometric titration. They streamlined the analysis step by passing the combustion gases directly into the titration cell, rather than into a collection vessel from which an aliquot was taken and injected into a separate microcoulometric titration cell.

At this point, the DWRD published USEPA Interim Method 450.1, Total Organic Halides.⁶ The same method was also published in SW-846 as Method 9020⁷. This is a method "to be used for the determination of Total Organic Halides as Cl^- ... in drinking and ground waters."^{6,7} Stevens and Dressman reported¹ that the first commercial TOX analyzers were developed by the Dohrmann Division of the Xertex Corporation under contract to the DWRD, and that instruments based on Method 9020 were available from Dohrmann and from Mitsubishi Chemical Industries Ltd.

Method 9020 was published shortly after the promulgation of a regulation requiring many hazardous waste management facilities to monitor the TOX in their groundwater.⁸ In order to monitor the compliance of these facilities with this regulation, we, in the USEPA Region II Laboratory, have analyzed many groundwater monitoring well samples for TOX using Method 9020. Many of these groundwater samples were also analyzed for volatile and extractable organic compound by GC/MS using USEPA Methods 8240 and 8270 respectively.⁹ In this paper, we present a comparison of the results obtained from the Method 9020 TOX analysis with the TOX that would be expected on the basis of a summation of the halogen concentrations in the halogenated organic compounds (as chloride) that were found by GC/MS; this summation will be referred to as TOX by GC/MS. This preliminary comparison is being made for 115 wells at 16 sites for which both Method 9020 TOX and GC/MS analyses were performed.

Previous Studies

Dressman and Stevens¹⁰ reported a comparison of Purgeable Organic Halogen (POX)¹¹ results for spiked deionized water with those obtained by analyzing the same samples for Total Trihalomethanes by GC. They found that the two techniques agreed within 20% for POX values of 40 $\mu\text{g Cl}^-/\text{L}$ or more, but up to 80% differences were found at levels below 40 $\mu\text{g Cl}^-/\text{L}$.

Williams, Coburn and Bancsi¹² compared the results obtained for Method 9020 TOX analyses with TOX by GC/MS analyses of groundwater samples at ten sites in Canada. At nine of these sites (in which a total of 25 wells were analyzed) TOX values were 44 ug/L or less and Method 8240 GC/MS analysis showed volatile organics at 6 ug/L or less. No further analyses were made at these sites. Comparison of the results for the Method 9020 TOX with the TOX by GC/MS from these nine sites showed no correlation between the two sets of values. The authors attributed the apparent lack of relationship to the low values. At the tenth site, six wells were sampled and analyzed by Method 9020 TOX, Volatile Organics by GC/MS (Method 8240) and Extractable Organics by GC/MS (Method 8270). One well was upgradient of the site (well U) and five were downgradient of the site (well D1, D2, D3, D4, and D5). Well U had a method 9020 TOX level of 11 ug/L and TOX by GC/MS of 12 ug/L. Well D3 had lower TOX levels than well U by both methods. The four remaining wells all had substantial levels of TOX by Method 9020 and volatiles by Method 8240. No halogenated compounds were found by Method 8270. In well D4 they found 560 ug/L of TOX by Method 9020 and 849 ug/L of TOX by GC/MS. They explained this 66% recovery by the TOX analyzer of compounds identified by GC/MS as being in agreement with typical recoveries of volatile organics (at concentrations greater than 200 ug/L) previously reported by Dressman and co-workers.¹³ (Dressman and co-workers¹⁰ later reported that their low recoveries were caused by the use of a heavily vitrified tube in their pyrolysis system, and that when a fresh tube was used, nearly complete recoveries were obtained). Wells D1 and D2 had Method 9020 TOX values of 1235 ug/L and 1402 ug/L and TOX by GC/MS values of 559 ug/L and 504 ug/L respectively. In an attempt to account for the difference, Williams and co-workers took the Method 8270 extracts and solvent exchanged them into cyclohexane. These cyclohexane extracts were then analyzed by TOX analyzer. This analysis found 947 ug/L of TOX in D1 and 396 ug/L of TOX in D2 from halogenated compounds that had not been found by GC/MS. Once this is taken into account, the Method 9020 TOX values agree fairly well with the TOX value calculated for the GC/MS samples. Well D5 had a Method 9020 TOX of 122 ug/L and TOX by GC/MS of 56 ug/L. Cyclohexane extracts of this sample were not analyzed because that method was not sensitive enough to account for missing TOX at this low level. When the data from all six wells was analyzed, the TOX values from Method 9020 and the value for TOX by GC/MS augmented by the cyclohexane extract values agreed with each other with a correlation coefficient of 80%. The authors concluded that TOX is a useful technique for screening groundwater for contaminants, and that further study was needed to clarify the relationship between data generated by TOX and GC/MS.

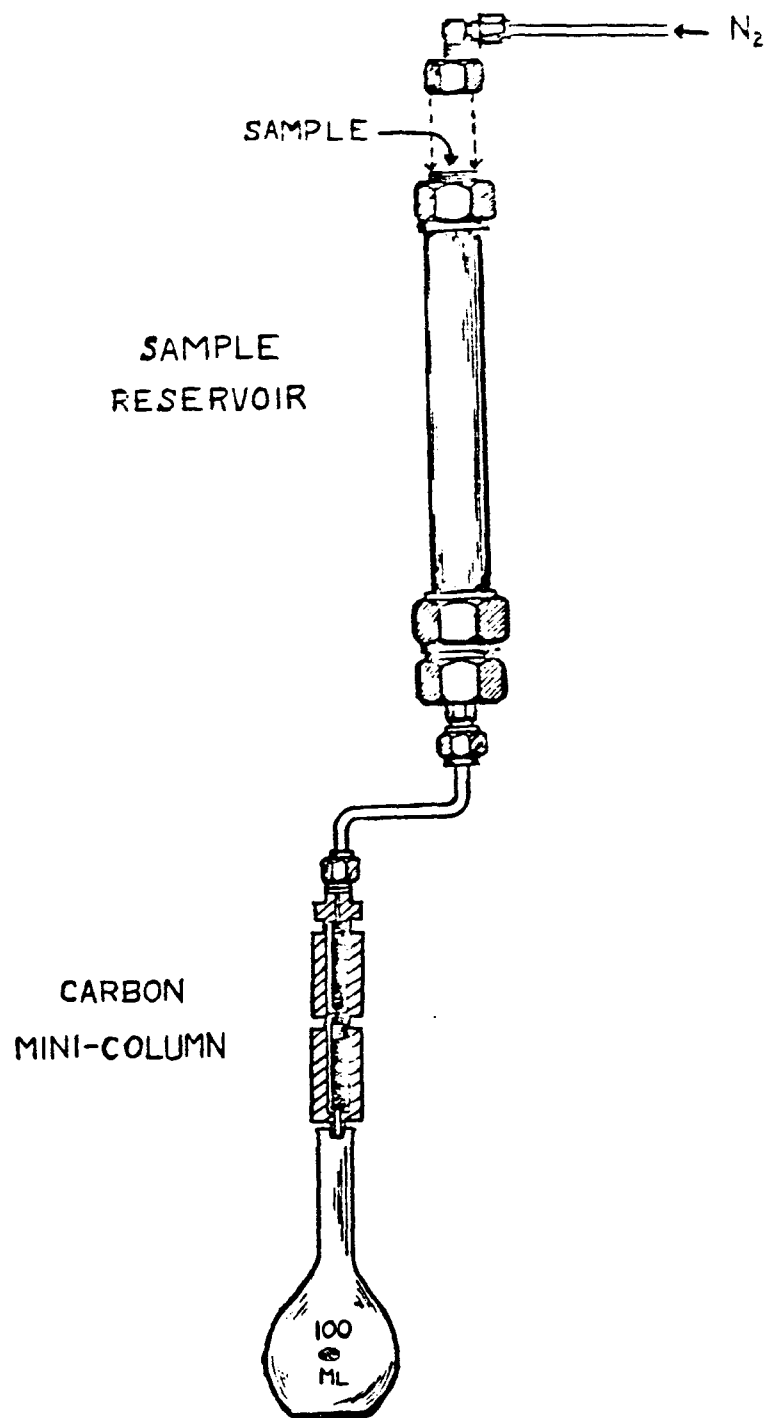
The intent of this study was to continue the work of Williams and co-workers in evaluating a new method, Method 9020, by comparing the results it produces with those obtained using an alternate, more

established procedure, GC/MS. We have added to the database and confirmed the indication by Williams and co-workers that the data are not directly comparable. More work is clearly needed to elucidate and quantify the differences.

METHOD

1. Samples were collected from groundwater monitoring wells at sixteen hazardous waste management facilities throughout EPA Region II. The region consists of New York, New Jersey, Puerto Rico and the US Virgin Islands. Samples were collected by members of the USEPA Region II Environmental Services Division, Surveillance and Monitoring Branch. They followed procedures listed in the RCRA Ground Water Monitoring Technical Enforcement Guidance Document.¹⁴ Samples were collected with bailers except where the facility had some other type of sampling device dedicated to their wells. Samples were then transferred from the bailer to glass sample bottles. Separate bottles were filled for each analysis (volatile organics, then TOX, then extractable organics). All samples from a single well were collected within a three hour period unless the well recharge rate was too slow, in which case they were collected as rapidly as the well allowed. Initially 1000 ml samples, enough for quadruplicate analysis by Method 9020, were collected. It was found that as consecutive samples were decanted from the sample bottle, the measured TOX increased dramatically. This was probably due to organic halides adsorbed on sediment particles, which were more prevalent lower in the bottle. More reproducible results were obtained by using 150 ml sample bottles and only making one determination from each bottle.
2. TOX was determined using USEPA Method 9020 via a Dohrmann DX-20 Total Halogen Analyzer. There are two parts to the analyzer, Figure 1 is a diagram of the microcolumn carbon adsorption system, and Figure 2 shows the pyrolysis and microcoulometric detection system. The method calls for a sample that is free of undissolved solids because the solids will block the sample flow at the top of the carbon column. Most of the groundwater samples that were brought into the lab did not fit this criterion. To avoid excessive loss of volatiles, sample manipulation was kept to a minimum. To permit visible solids to settle, the sample was allowed to sit in its closed container. Then the sample was carefully decanted from the sample container into the adsorption module's sample reservoir. Samples with solids that did not settle after standing were diluted by pipetting an aliquot of the sample into a volumetric flask with some water in it and diluting to volume. This technique was also used for samples that were expected to be high in TOX or in inorganic chloride. Method 9020 requires that duplicate runs agree within 15%. We found that this level of agreement could not be achieved for groundwater

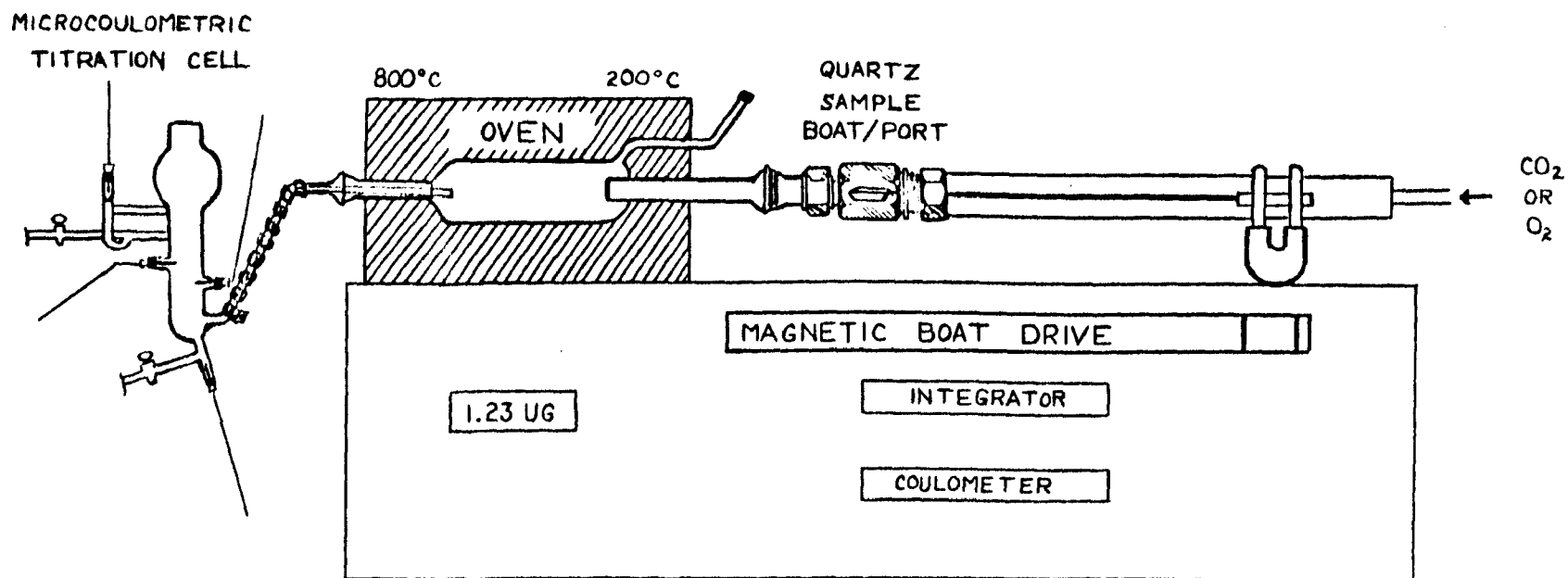
FIGURE 1



MICROCOLUMN CARBON ADSORPTION SYSTEM

FIGURE 2

PYROLYSIS & DETECTION
SYSTEM



samples, so a less rigid standard of 40% difference was adopted. In many cases where even this standard could not be achieved the data was reported with a "J" qualifier to indicate that the value was an estimate.

3. Analysis of Volatile Organic Compounds was performed by USEPA Method 8240 using either a Finningan 3200 GC/MS system with a Hewlett-Packard 7675A Purge and Trap sampler or a Finnigan 3300 GC/MS system with a Tekmar LSC-2 Purge and Trap sampler.
4. Determination of Extractable Organic Compounds was performed by USEPA Method 8270 using a Finnigan 4000 GC/MS system.
5. All analyses reported in this study were performed at the USEPA Region II Laboratory in Edison, New Jersey. These analyses were performed between October 1984 and December 1986. The study includes all wells from which data were reported for TOX (Method 9020), volatile organic compounds (Method 8240), and extractable organic compounds (Method 8270). Although we are reporting all of the Method 9020 TOX analyses which have accompanying GC/MS (8240 and 8270) data, we are not reporting all Method 9020 TOX analyses performed. Many analyses were not reported because they were not accompanied by Method 8240 or Method 8270 analyses. Table 1 shows the number of samples reported at each of the sites in this study.
6. TOX by GC/MS values were calculated as follows. For each analyte found by GC/MS, the concentration of the analyte was multiplied by the weight fraction of halogen, as chloride, in that analyte. The sum of these halogen concentrations was then calculated for all analytes found in the sample. This sum is the TOX. Table 2 shows an example of this calculation for one of the sites that was investigated.

RESULTS AND DISCUSSION

As shown in Table 1, the data fall into four categories; values below the TOX detection limit for both Method 9020 TOX and TOX by GC/MS analyses; values above the detection limit where Method 9020 TOX and TOX by GC/MS agree within a factor of two; samples with Method 9020 TOX values greater than double the corresponding TOX by GC/MS values and samples with TOX by GC/MS values greater than double the corresponding Method 9020 TOX values. Table 1 also shows the number of samples in each category for each site.

In dealing with the first category, samples below the detection limit, it is essential to first decide what that detection limit is. A limit of 10 ug/L is generally used in Method 9020 because that was found in this laboratory to be three times the standard deviation of a nitrate washed column blank pair. When samples have

Table 1

Site #	Number of Samples	Number of Samples From Site in Category			
	From Site	I	II	III	IV
1	11	1	4	6	0
2	6	0	4	2	0
3	4	1	1	2	0
4	3	0	2	0	1
5	7	0	0	7	0
6	7	0	0	7	0
7	6	2	0	4	0
8	5	1	0	4	0
9	13	4	2	7	0
10	9	1	1	7	0
11	8	7	0	0	1
12	6	1	0	4	1
13	4	3	0	1	0
14	5	1	0	4	0
15	4	4	0	0	0
16	17	9	2	5	1
Totals	115	35	16	60	4

Category I Samples with Method 9020 TOX and TOX by GC/MS below detection limits.

Category II Samples with TOX by both methods agreeing within a factor of two.

Category III Samples with Method 9020 TOX more than double TOX by GC/MS.

Category IV Samples with Method 9020 TOX less than half TOX by GC/MS.

been diluted to reduce matrix effects that interfere with the analysis, the detection limit must be adjusted for this dilution. When the detection limit of 10 ug/L is used, 12 of the 115 samples fall into the first category. However, since the summation of detection limits for TOX by GC/MS is greater than 10 ug/L, we have raised the detection limit for this study, arbitrarily, to 25 ug/L. This puts a total of 35 samples into category one.

The second category is where we would hope to find most of the data. This turns out not to be the case. When the criterion for agreement is arbitrarily set at a factor of two

$$\text{i.e.} \quad 0.5 \leq \frac{\text{TOX by GC/MS}}{\text{Method 9020 TOX}} \leq 2$$

it found to contain only sixteen samples.

Category three, where the Method 9020 TOX value is greater than double the TOX by GC/MS results, contains most of the data. This category contains 60 of the 115 samples. Of these 60 samples, 36 had TOX by GC/MS results below 25 ug/L and Method 9020 TOX results above 100 ug/L. There are a number of things that can cause Method 9020 TOX values to be higher than TOX by GC/MS values for a given sample. Some of the most likely causes are:

1. High levels of inorganic chloride in the sample. At least 15 category three samples are from areas where saltwater intrusion into the groundwater is a strong possibility. The potassium nitrate wash can only remove chloride effectively if the inorganic to organic chloride ratio is less than 20,000 to 1.⁶
2. High levels of inorganic sulfides in the sample. Six category three samples had a strong hydrogen sulfide odor. When they were analyzed the silver electrodes in the titration cell turned black.
3. Chlorinated organic compounds not readily detected by GC/MS. Highly polar compounds are not readily purged or extracted from water. Other compounds may not pass through the GC column. These and other compounds should be seen by TOX more readily than by GC/MS.
4. Organic compounds below the GC/MS detection limit. If all of the halogenated organic compounds that are targeted by Methods 8240 and 8270 were present just below their nominal detection limits of 1 to 20 ug/L, the TOX by GC/MS would be about 200ug CL⁻/L. A combination of target and non-target compounds below the detection limit could cause a substantial TOX with no associated GC/MS observations.

5. Halogenated organics adsorbed onto fine particles suspended in the solution. These compounds will not be purged or extracted efficiently for GC/MS analysis, but they will pack at the top of the carbon column and be combusted in the TOX analyzer.
6. Contamination of the carbon columns through contact with inorganic chlorine after the nitrate washing step.
7. Contamination of the activated carbon by halogenated organic compounds as vapors during column preparation.
8. Other sources of laboratory or field contamination such as contaminated sample containers, dilution water or nitrate wash solution.

Only four of the 115 samples fall in category four, where Method 9020 TOX values are less than half of the corresponding GC/MS values. TOX is often used as a screening mechanism to identify contaminated wells. When viewed this way, category three samples could be considered as false positives and category four samples as false negatives. False positives are much less serious than false negative because a positive result will generally be followed by additional, more rigorous analyses. A false negative result could lead to concluding that a hazardous situation is non-hazardous. A closer look at the four samples in category four shows that they would not give false negative results. One sample had over 40,000 ug/L of Method 9020 TOX and over 100,000 ug/L of TOX by GC/MS. This well would certainly receive further attention. The second and third samples in category four were both collected during the same sampling trip and the only compound found by GC/MS was methylene chloride in both samples. The latter sample was labelled as a trip blank. This indicates that the results for these samples are due to contamination of the sample containers. (This may also explain the five category three samples from these sites.) The explanation of the fourth category four sample is not as clear as for the other two. The most likely explanation is that the 58 ug/L of tetrachloroethylene found by GC/MS was introduced into the sample through contamination; another sample from the same site contained 210,000 ug/L of tetrachloroethylene.

CONCLUSION

Comparison of Method 9020 TOX values with calculated TOX by GC/MS values for 115 samples from 16 sites shows that Method 9020 results do not agree with the level of halogenated organic compounds that are found by GC/MS. Most of the samples analyzed were found to have higher TOX levels by Method 9020 than by GC/MS. The work of Williams and co-workers¹² suggests that Method 9020 was detecting halogenated compounds that were not found by GC/MS at the site that

Table 2

TOX calculated from GC/MS Data for Site Three

Compound	%Cl	Sample 1		Sample 2		Sample 3		Sample 4	
		conc ug/L		conc ug/L		conc ug/L		conc ug/L	
Determined by GC/MS		meas	TOX	meas	TOX	meas	TOX	meas	TOX
1,1,1-Trichloroethane	80	U	U	2	1.6	U	U	2	1.6
Chlorobenzene	31	U	U	2	0.6	3	0.9	U	U
trans-1,2-Dichloroethene	73	U	U	U	U	5	3.7	U	U
Dichloromethane	83	24	20	U	U	4	3.3	21	17
Trichloroethene	81	U	U	U	U	2	1.6	U	U
1,3-Dichlorobenzene	48	U	U	2	1.0	U	U	U	U
TOX by GC/MS		20		3.2		9.5		18.6	
TOX by Method 9020 ^a		92 J		264		157 J		24	

meas. - measured by GC/MS.

TOX - calculated from concentration measured by GC/MS.

U - undetected

a. The differences between Method 9020 TOX values and TOX by GC/MS are discussed in the body of this paper.

b. If there had been any brominated compounds in this table, they would have been calculated using the atomic weight of chloride.

they investigated. Our work indicates that the observation of higher values for Method 9020 TOX than for TOX by GC/MS is common for groundwater samples. It is reasonable to assume that the differences between the two would be attributable to the nonvolatile TOX increment observed by Williams and co-workers.

The next step in the evaluation of Method 9020 as a technique for ground water monitoring is to find the major causes of the "false positives." Halogenated organic compounds that are missed by GC/MS should be identified. It is also necessary to determine what the effect of sediments is on Method 9020 TOX results. The filtering of samples must be examined as a sample treatment option. Once these investigations have been performed it will be possible to perform a more thorough analysis of Method 9020.

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NONVOLATILE ORGANICS AS PROBES FOR CONTAMINATED GROUND WATER PLUMES FROM HAZARDOUS WASTE MANAGEMENT FACILITIES

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ABSTRACT

Characterization of ground water quality in the vicinity of hazardous waste disposal facilities is essential in defining site integrity and delineating plume migration. Ground water contamination by organic compounds at such facilities is typically indexed by analysis of volatiles or semi-volatiles, because these parameters can be determined using established EPA analytical protocols.

Ideal probes for these purposes, however, are substances which are: 1) unique constituents of disposed wastes; 2) persistent; 3) nonvolatile; and 4) highly mobile or conservative in ground water. Accordingly, certain non-conventional pollutants may well provide more suitable and accurate tracers than the current target analytes. This report describes studies on the identification and quantification of one such non-conventional pollutant, p-chlorobenzenesulfonic acid (PCBSA), in the BKK landfill ground water plume.

Ground water adjacent to the BKK Landfill, a major hazardous waste disposal site in southern California, was found to contain TOX above 200 mg/L, with less than 4% of the organic halogen accounted for. Subsequent analysis of the ground water samples by liquid chromatography/mass spectrometry and by ion chromatography with conductivity and UV absorbance detectors revealed a plume of PCBSA that accounted for approximately half of the observed TOX. PCBSA, which occurs as an anion above pH 0.5, was associated with DDT manufacturing wastes disposed at the site for many years.

In a further application, PCBSA determinations were instrumental in resolving the contested mechanism of ground water contamination by volatile priority pollutants (e.g., TCE, vinyl chloride). Ground water contamination by these volatile compounds could occur by at least two distinct mechanisms including: 1) direct leaching of the waste prism during infiltration of surface water; 2) dissolution of gaseous components from landfill gas contracting ground water. Engineering controls currently in use at the site focus on landfill gas extraction based on the assumption that the latter mechanism predominates. The occurrence of PCBSA in the plume provides unequivocal evidence that leaching of waste prism does contribute to ground water contamination.

GAS CHROMATOGRAPHY MATRIX ISOLATION FOURIER TRANSFORM
INFRARED (GC/MI-IR) SPECTROSCOPY FOR MONITORING
AIR POLLUTANTS

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ABSTRACT

Procedures will be described for collecting and analyzing ambient air samples on solid adsorbents and in stainless steel canisters from the vicinity of a suspected source of air pollution. Variables involving design of the sampling network, collection of representative samples, sample analyses by GC/MI-IR and other methods, estimations of data quality and the significance of the results will be presented. Infra-red spectra from low nanogram high picogram quantities of matrix isolated pollutants will be shown and related to vapor phase spectra.

INTRODUCTION

Analytical procedures to monitor for the toxic or non-criteria pollutants in ambient air have not been studied nearly as much as have the procedures to monitor for the six criteria pollutants.¹ However, some non-criteria pollutants present in the air are toxic or carcinogenic or both. The media have become more active in recent years in reporting air spills, which is one of the causes of a greater public awareness of both existing and potential air toxic problems. One result of this increasing public awareness in Title III of the Superfund Amendments and Reauthorization Act (SARA) of 1986, which requires the Environmental Protection Agency to develop a more comprehensive air toxics program.²

One of the reasons that more work has not been done on air toxics is that procedures to collect and analyze samples for the extremely low concentrations required have not been available. Another reason is that criteria for evaluating the significance of the concentrations found in the air are also usually available. Any procedure used to monitor ambient air for a toxic material must be specific for that material must be sufficiently sensitive to accurately measure the low concentrations of interest, and must generate data that can be related to the applicable toxicity criteria.

Several kinds of procedures are currently used to monitor for selected toxic pollutants.³ These procedures generally utilize bubblers, solid sorbents, or whole air collection devices. Most of these have one or more deficiencies. Bubblers can be adapted to a wide range of compounds, but they are difficult to use in the field, and therefore, expensive when labor is included in the cost. They also usually have higher detection limits than competing methods.

Solid adsorbents require an extensive amount of work to ensure that the compounds of interest are adsorbed quantitatively, do not break through under field conditions and then can be desorbed in the laboratory in a reproducible manner. Many of these procedures are also less rugged than desired. Grab samples collected in fixed-volume vacuum containers, or variable volume containers, do not always allow the collection of sufficient sample to measure the low concentrations of interest.

We have developed a series of ambient air monitoring procedures and have completed several studies using polyurethane (PUF) and stainless steel spheres for sample collection with gas chromatography and matrix isolated infrared spectroscopy (GC/MI-IR) for sample analyses.⁴ These procedures complement existing procedures described above and appear to be superior to those procedures in some respects. We believe this is the first instance in which GC/MI-IR has been used as a primary analytical tool in a major ambient air study. Based on our experiences there will be many such studies in the future.

PROCEDURES

The monitoring network is designed to provide sufficient data to meet a specific objective. Usually the objective is to determine whether or not the average concentration of a given pollutant at a specific site is above an acute or chronic criteria value, or whether or not the average downwind concentration of a given pollutant is higher than the average upwind concentration at a reasonable confidence level. The procedures we are currently using to determine the number of samples we need to collect at each monitoring location in the network have been described before.⁵ The most common result is that between seven and fourteen samples are needed from each sampling location in the network. This means that a relatively large number of samples are required to satisfy the network completeness criteria. Therefore, selection of an analytical method with a correspondingly high sample volume capability is also required. Once the data are collected, it is important to review all assumptions made during design of the network to be sure the valid data actually collected are sufficiently complete to meet the study objective. Figure 1 is an illustration of the general approach for selecting the number of samples to be collected at each monitor location in the network.

The procedures we use to collect semi-volatile materials have been described by Lewis and Jackson.⁶ Our experience has primarily been with the dioxins and with the polychlorinated biphenyl congeners or PCB's. A large volume of air (350 cubic meters per day) is drawn through a particulate filter and a polyurethane foam plug. The exposed filter and plug are extracted together in a soxhlet. The extract is cleaned using conventional chemistry and analyzed by gas chromatography, gas chromatography-mass spectroscopy and/or by GC/MI-IR as described below.

The procedures we use to site a sampler are those described for locating a Hi-Vol sampler when it is being used to monitor for total suspended particulates in the air.¹ Therefore, samples are collected in the breathing zone, away from physical structures, and are intended to be representative of ambient air.

For volatile pollutants, battery-operated diaphragm pumps are used to meter ambient air into clean 6-liter stainless steel spheres to a pressure of approximately 2 atm.⁷ The spheres are cleaned by evacuating with a vacuum pump, filling with clean air and evacuating again. They can be heated during the cleaning process, but this has not been necessary to date based on the analyses of field blanks. Although the battery packs are capable of operating the pumps for periods of more than 24 hours, our studies to date have been limited to 8 hours so the data could be more directly related to the applicable criteria. Figure 2 is a schematic diagram of the canister sampling apparatus.

For sample analyses, 1,000 milliliter aliquots are taken from each canister at a sampling rate of 50 ml/min. with a mass flow controller and metal bellows pump.⁷ The sample is transferred to a manifold which is open to the atmosphere to insure atmospheric pressure. A 500 ml subsample is simultaneously withdrawn from the manifold at a rate of approximately 25 ml/min. and passed through a dryer to a cold trap which is filled with glass beads and maintained at a temperature of minus 150 degrees centigrade. Once the sample is collected in the cold trap, the gas chromatograph carrier gas (0.6% Argon in Helium) is directed through the cold trap and the cold trap is then quickly (about 30 seconds) heated to 150 C°. This transfers the sample to the capillary column of a Mattson GC/MI-IR Cryolect analytical system. Any stable column that separates the compounds of interest at a flow of less than 3 ml/min. may be used. We usually see a 30M X 0.32 mm dimethylpolysiloxane column with a 1 um film thickness. The column temperature is maintained at 5 C° for 10 minutes, raised to 80 C° at a rate of 5 C°/min. and then raised to 160 C° at a rate of 10 C°/min. A splitter at the end of the column directs approximately 20% of the effluent to a flame ionization detector and the remaining 80% to the Cryolect where the pollutants are trapped in crystalline argon at approximately 13 Kelvin degrees.⁴ Once an entire chromatograph has been trapped on the rotating Cryolect gold-plated cylinder, the cylinder is positioned (via computer) to place each significant peak into the infra-red beam for collection of the infra-red spectra. We typically average 32 spectra per compound at a resolution of 4 reciprocal centimeters. These conditions are sufficient to give a high-quality spectra for approximately one nanogram of each component injected into the chromatograph. However, in extreme cases we have averaged over 10,000 spectra which improves the sensitivity to picogram levels. Figure 3 shows a schematic diagram of the analytical process.

RESULTS

Figure 4 shows the first 25 minutes of a typical gas chromatogram of volatile compounds from an ambient air sample that was collected in stainless steel canisters downwind of a chemical plant that manufactures chlorinated hydrocarbons. Figures 5-7 show three of the resulting infra-red spectra from the ambient samples and compares them to the respective library spectra. As can be seen, the sample spectra match the library spectra. They have good resolution and document the excellent sensitivity that is characteristic of the GC/MI-IR method. Figures 8-13 show spectra of three compounds that are of inferior quality, but which are still considered to be good enough to support a tentative identification in each case. These spectra are shown to illustrate one of the deficiencies still remaining in our procedure for volatile organic compounds. So far, we have been unable to remove all water vapor from the sample without losing some of the compounds of interest. This necessitates a spectral subtraction of the relatively high background and results in lower quality spectra for some compounds at concentrations where we would otherwise expect to obtain higher quality spectra. These spectra also illustrate the practical detection limit using a one-liter sample from a stainless steel canister and a GC/MI-IR analytical method. Quantitative values are obtained by comparing the FID response to a calibration curve obtained by putting calibration standards through the entire analytical process.

Since the library of argon matrix isolated infrared spectra is very limited, we have attempted to compare spectra resulting from field samples with vapor phase library spectra. The results usually provide either an obviously unreasonable match or what appears to be a correct identification of the unknown. Figure 14 is one such example which compares to vapor phase spectra from the library with an argon matrix spectra from an ambient air sample.

In addition to collecting a whole air sample in a sphere at each site, a duplicate TENAX sample is also frequently collected. We collect TENAX samples both to obtain lower detection limits for some compounds and as a method that compliments GC/MI-IR. The TENAX samples are thermally desorbed and analyzed by packed column gas chromatography and low resolution mass spectroscopy. Table 1 shows a summary of those compounds identified by each technique for the study described above.

Figure 15 shows the GC/MI-IR chromatogram for Archlors 1221, 1016 and 1254. Figures 16-19 show infra-red spectra for two of the many congeners (biphenyl and 2-chlorobiphenyl) at different concentrations. Again, the resolution is good and the sensitivity is much better than that of other infra-red procedures. Figures 20 and 21 are comparisons of the linearity of the responses between the flame ionization and infra-red detectors for two of the chlorinated

biphenyls. As can be seen, the linear range of the flame ionization detector is much larger than that of the infra-red detector for both compounds. Not obvious from those figures is the fact that the infra-red detector is more sensitive than the flame ionization detector for these and other polychlorinated biphenyl compounds we have analyzed. Figure 22 demonstrates that the GC/MI-IR absorption versus concentration curve is linear when less than 100 nanograms are injected into the instrument.

Brasch has demonstrated the utility of electronically subtracting GC/MI-IR library spectra from the spectra obtained from unresolved chromatograph peaks and then matching the remaining or "difference" spectrum to a known library spectrum. The technique was used to obtain a positive identification of each of the tetrachlorinated dibenzofuran isomers which could not be resolved by capillary gas chromatography. We have used a similar technique to obtain a tentative identification of a compound where no sample or library spectrum was available and a positive identification could not be made from GC/MS data. Figures 23 and 24 show the library spectra of dimethyl sulfate and of diethyl/sulfate. Figure 25 shows a gas chromatogram of an environmental sample. Figure 26 shows the spectrum of an unknown compound (largest peak in the chromatogram) from that sample. Figure 27 is a spectrum formed by electronically summing the spectra of dimethyl and diethylsulfate. The facts that the retention time of the unknown was between that of dimethyl and diethyl sulfate, the GC/MS appeared consistent and the similarity of the IR spectra shown in Figures 26 and 27 was the basis of our conclusion that the unknown compound was methylethylsulfate.

Figures 28 and 29 show the infra-red spectra of carbon-13 labeled and natural 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD). The matrix isolated spectra of all 22 Carbon 12 tetrachlorinated dibenzodioxins were obtained by Brasch and have been reported by Gurka et. al.⁸ However, the authors did not report detection limits. We are specifically interested in whether or not GC/MI-IR is sufficiently sensitive to measure concentrations of dioxin in ambient air in the one to five picogram per cubic meter range which we find occasionally in ambient air samples from the vicinity of superfund site clean-up operations.⁵ Figure 30 shows an infra-red spectra of one of the less toxic dioxin congeners from approximately 150 picograms of material injected into the gas chromatograph. Since we have demonstrated that at least 1,000 cubic meters of air can be reliably sampled using polyurethane in a PUF sampler without breakthrough,⁵ it appeared that a total method detection limit of 0.1-0.2 picograms per cubic meter was a feasible target. Therefore, a polyurethane foam plug was spiked with 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) and 320 cubic meters dioxin-free urban air were drawn through the plug. The foam plug was extracted and the extract was divided in half to evaluate different clean-up procedures. One microliter (approximately 4 nanograms) of

the extract was injected into the instrument. Figure 31 is the resulting infrared spectrum. It is clearly of sufficient quality to support a qualitative and quantitative analysis of that particular dioxin isomer.

CONCLUSION

It is apparent that the GC/MI-IR analytical procedure, when used as described above, will provide reliable qualitative and quantitative information for many of the toxic pollutants in ambient air. Collection of samples in stainless steel spheres and GC/MI-IR analytical procedures provide several advantages over other available procedures. Probably the most important advantage is greater isomer specificity at much lower concentrations than is available using other procedures. Another major advantage is the additional confirmation of tentative identifications made by other (GC/MS) techniques. There are, however, also many areas needing improvement. Among these are the ability to eliminate the interference from water vapor, improvements in software to improve sample analysis times, and the development of a larger library of matrix isolated spectra. It is the author's opinion that these deficiencies will all be relatively easily solved with time, and that the GC/MI-IR procedure will become another powerful tool for environmental monitoring.

FIGURES

Figure 1 - Graph of the probability that the mean of a given number of measured concentrations will be different from the true mean at the 95% confidence level. Typical curves for different kinds of data are shown and assume a normal distribution.

Figure 2 - Schematic diagram of the apparatus used to collect ambient air samples in stainless steel canisters.

Figure 3 - Schematic diagram of the apparatus used to transfer a measured volume of air from a stainless steel canister into the GC/MI-IR.

Figure 4 - Gas chromatogram from the GC/MI-IR of an ambient air sample collected downwind from a plant that synthesizes chlorinated hydrocarbons.

Figure 5 - Argon matrix isolated infrared spectrum of approximately 630 ng of methylene chloride from an ambient air sample relative to the library spectrum.

Figure 6 - Matrix isolated spectrum of approximately 650 nanograms of chloroform from an ambient air sample.

Figure 7 - Matrix isolated spectrum of approximately 400 nanograms of carbon tetrachloride from an ambient air sample.

Figure 8 - Matrix isolated infrared spectrum of 29 nanograms of 1,2-dichloroethane from an ambient air sample. The noise is caused by water vapor.

Figure 9 - Comparison of the infrared spectrum of 1,2-dichloroethane from an ambient air sample with the library spectrum.

Figure 10 - Infrared spectrum from a compound believed to be chloroethane in an ambient air sample.

Figure 11 - Comparison of the infrared spectrum of chloroethane found in an ambient air sample with the library spectrum.

Figure 12 - Infrared spectrum of 1,1,1-trichloroethane found in an ambient air sample.

Figure 13 - Comparison of the infrared spectrum of 1,1,1-trichloroethane found in an ambient air sample to the library spectrum.

Figure 14 - Comparison of the matrix isolated infrared spectrum of tetrachloroethylene found in an ambient air sample with a vapor phase spectrum from the library. Note the better resolution of the matrix isolated spectrum resulting from less translational and rotational broadening of the vibrational modes.

Figure 15 - Gas chromatograms from the GC/MI-IR of three aroclor mixtures. Those peaks caused by the commercially available polychlorinated biphenyl congeners have been identified using the GC/MI-IR library search routines. Work is in progress to obtain spectra of all 209 congeners.

Figure 16 - No legend.

Figure 17 - No legend.

Figure 18 - No legend.

Figure 19 - No legend.

Figure 20 - Graph showing the responses of both the flame ionization (FID) and infrared detectors on the GC/MI-IR as a function of the amount of H-chlorobiphenyl injected into the instrument. Note that the infrared detector becomes nonlinear at approximately 150 nanograms.

Figure 21 - Graph showing the responses of both the FID and IR detectors as a function of the amount of 2-chlorobiphenyl injected into the GC/MI-IR.

Figure 22 - Graph showing that the infrared detector is linear with concentration at low concentrations. The infrared detector is more sensitive to those polychlorinated biphenyl congeners we have analyzed than the FID detector on the GC/MI-IR. However, the IR detector is less sensitive for the PCB congeners than it is for most other compounds we have run.

Figure 23 - No legend.

Figure 24 - No legend.

Figure 25 - No legend.

Figure 26 - No legend.

Figure 27 - No legend.

Figure 28 - An infrared spectrum of carbon-13 labeled 2,3,7,0-tetrachlorodibenzodioxin (2,3,7,8-TCDD). There are no peaks above 2,000 reciprocal centimeters.

Figure 29 - Infrared spectrum of 2,3,7,8-TCDD. There are no peaks above 2,000 reciprocal centimeters.

Figure 30 - A comparison of the spectrum obtained from 156 picograms of 2,3,7,8-TCDD with the library spectrum.

Figure 31 - An infrared spectrum of 2,3,7,8-TCDD from a PUF sampler. Approximately 350 cubic meters of urban air were drawn through the PUF prior to extraction, cleanup and analyses.

FIGURE 1

Probability of Error

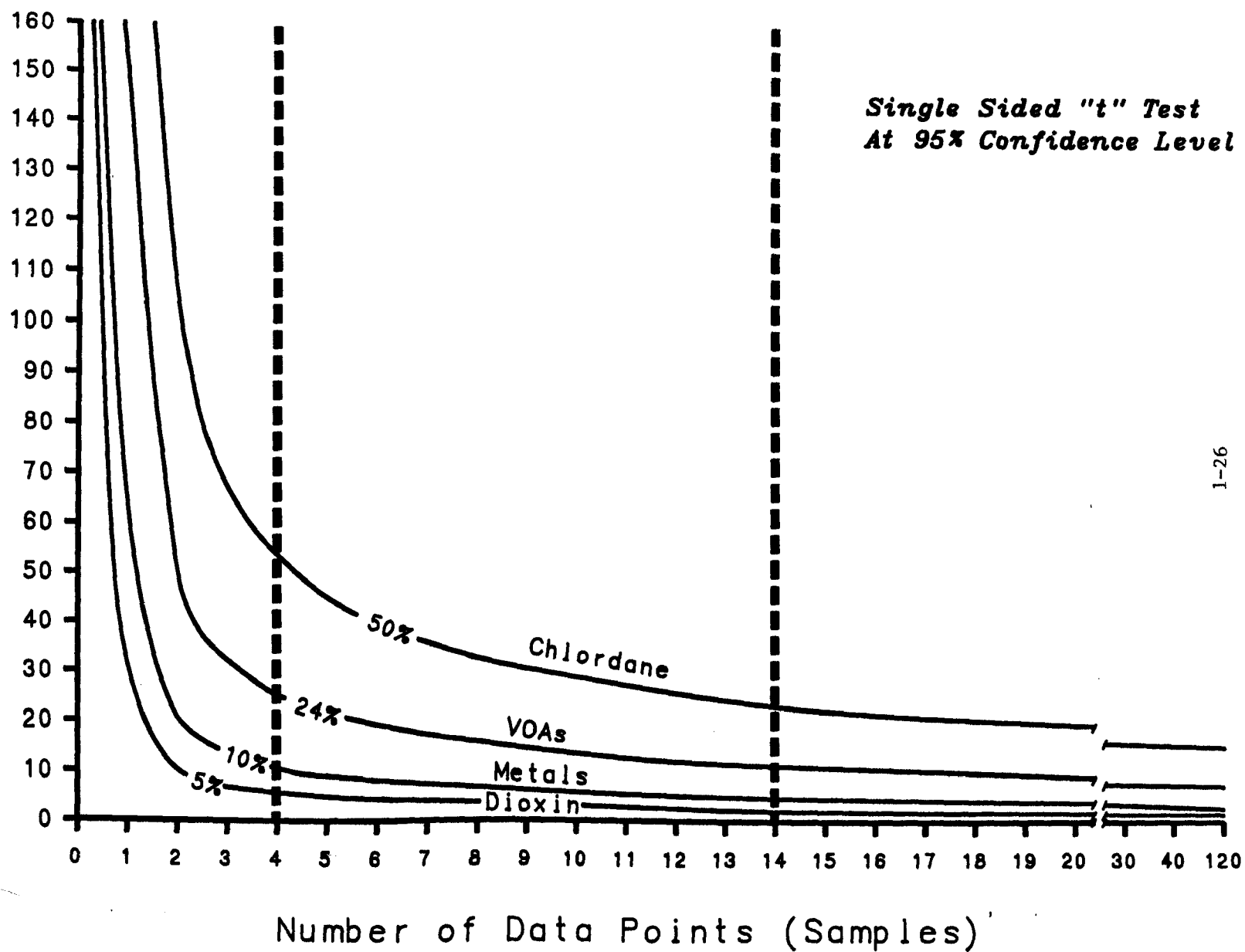


FIGURE 2

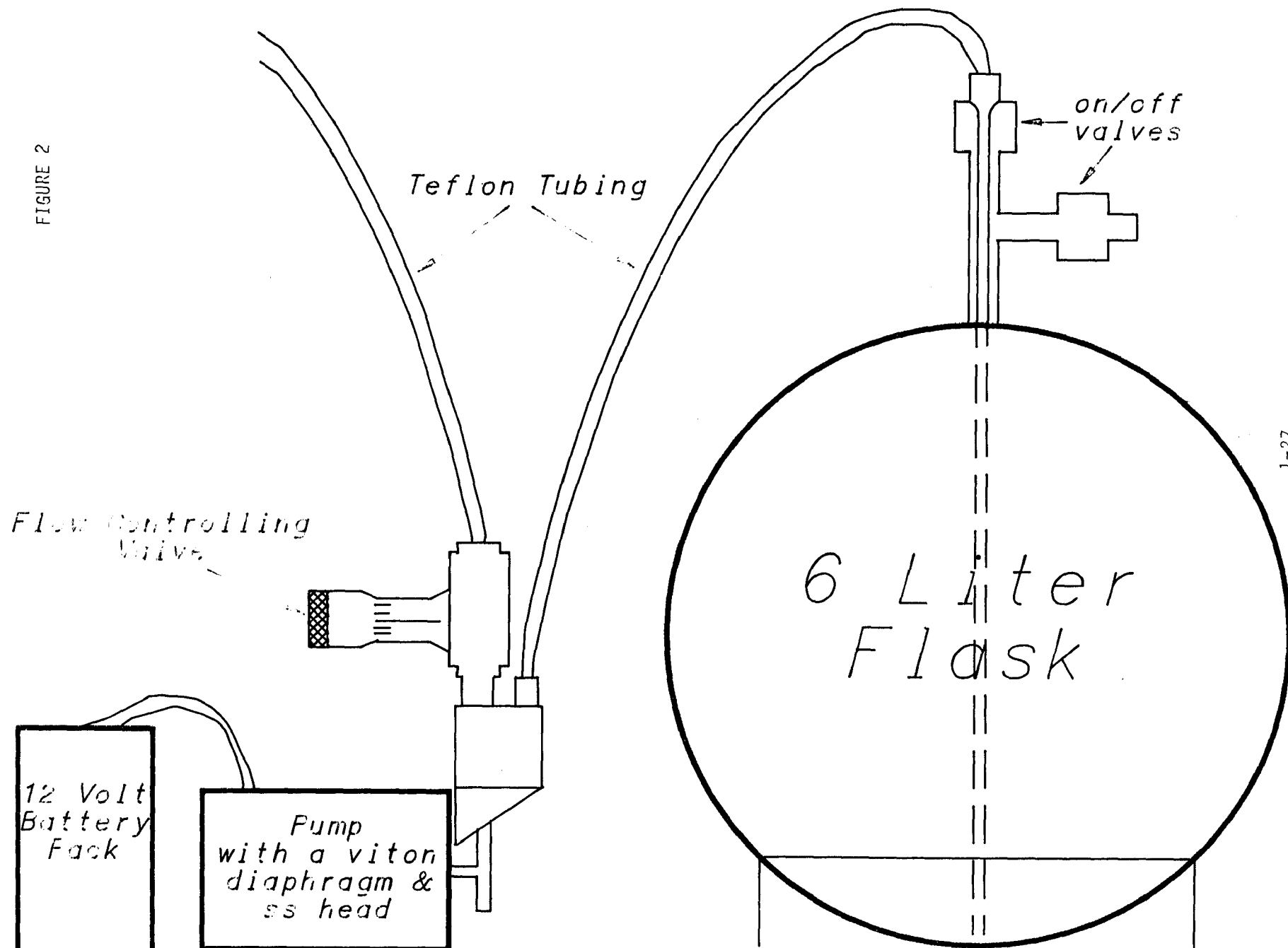
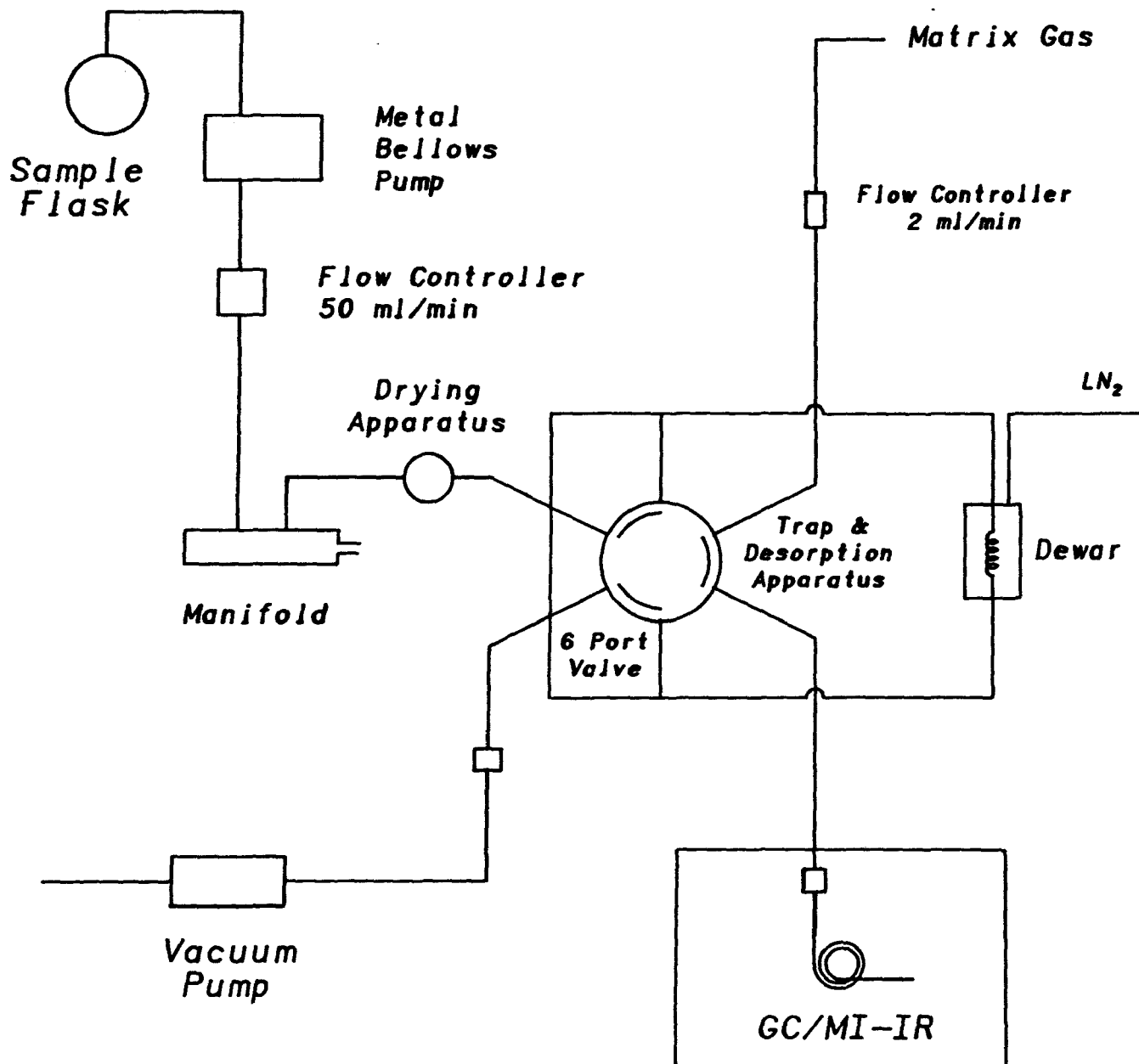


FIGURE 3



*Chromatogram from Ambient Air Sample
collected in stainless steel cannister
downwind of a Chemical Plant*

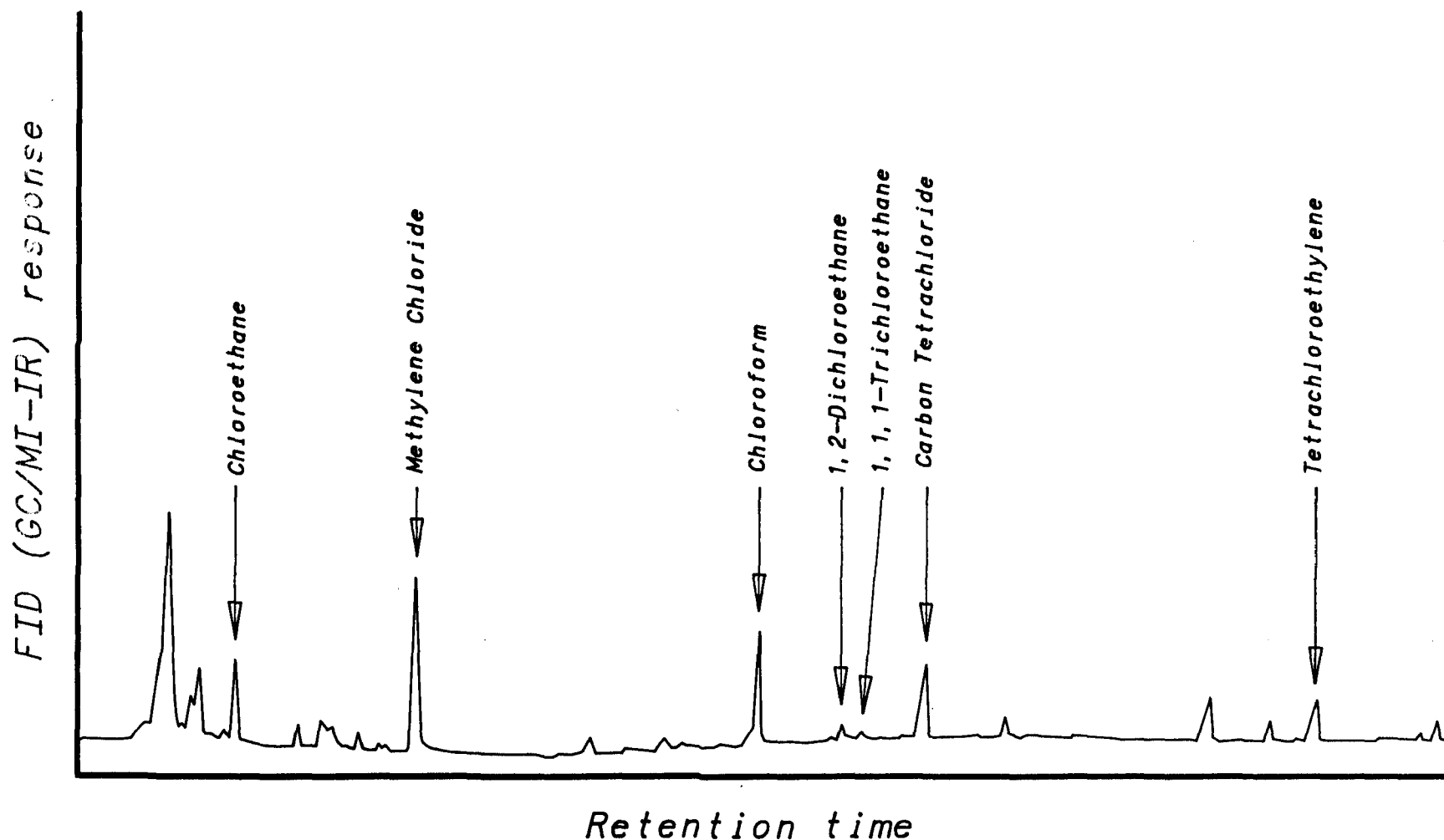


FIGURE 4

Methylene Chloride
Sample Spectrum vs Library Spectrum

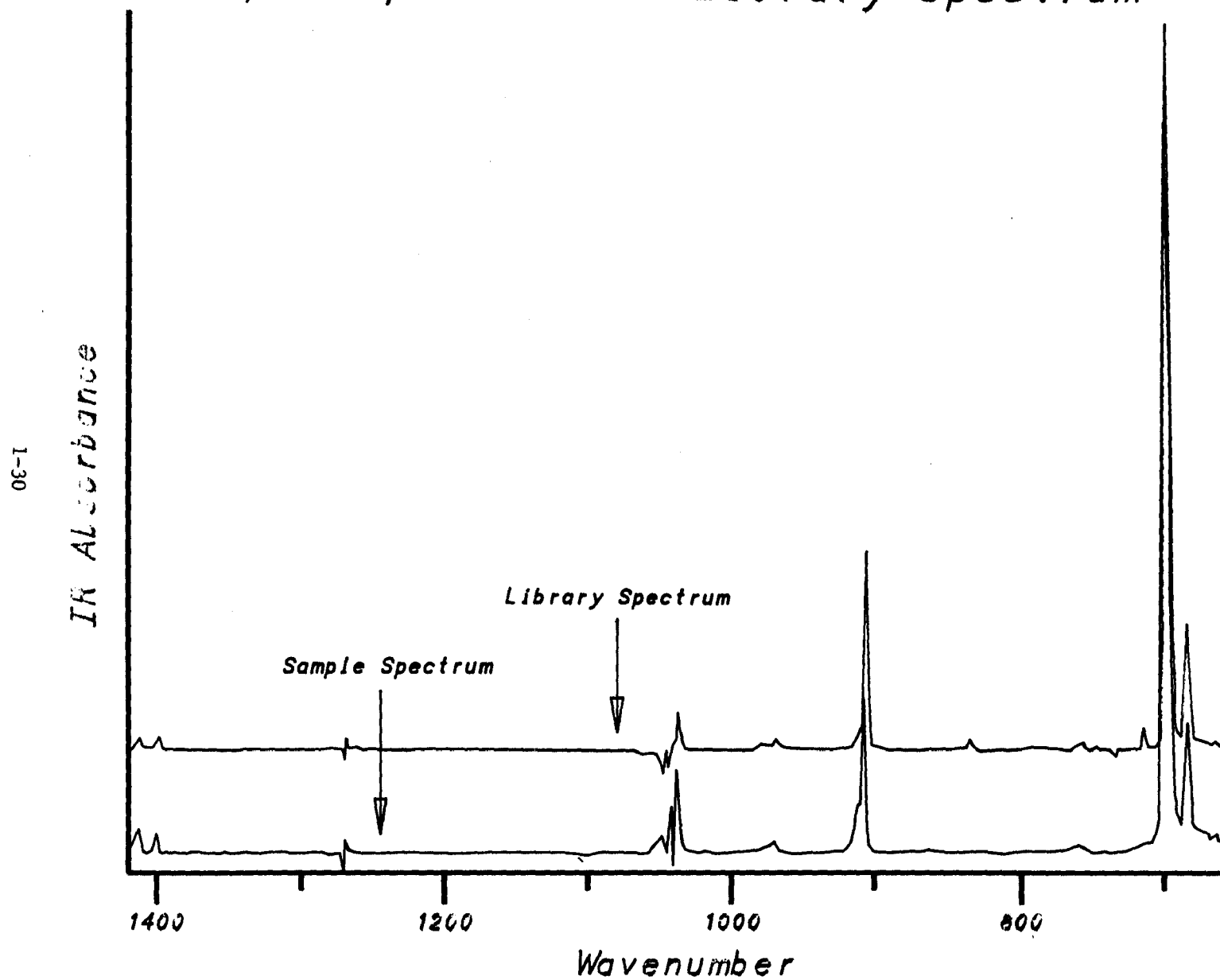


FIGURE 5

Chloroform
Sample Spectrum vs Library Spectrum

1-31

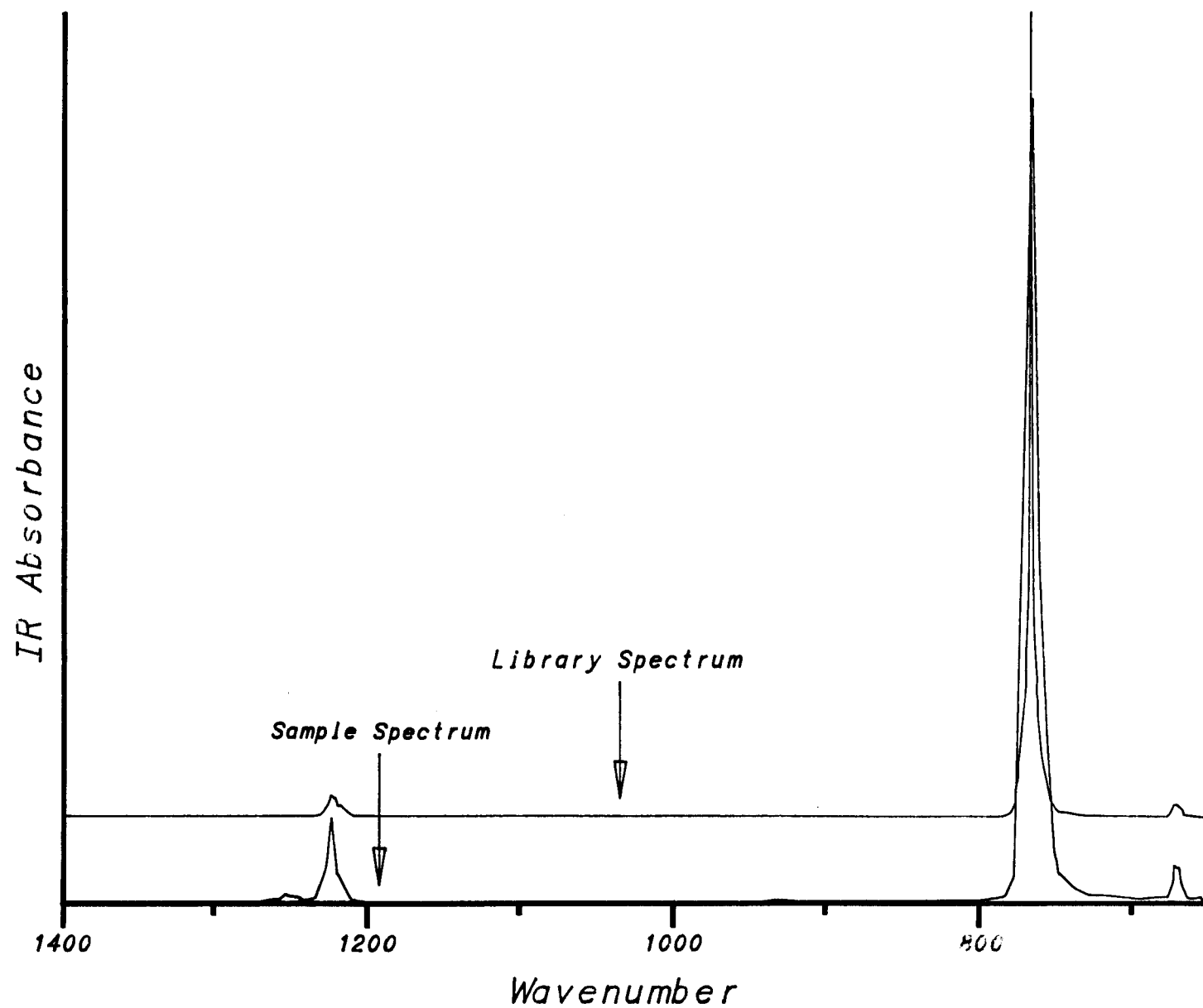


FIGURE 6

Carbon Tetrachloride Sample Spectrum vs Library Spectrum

IR Absorbance
1-32

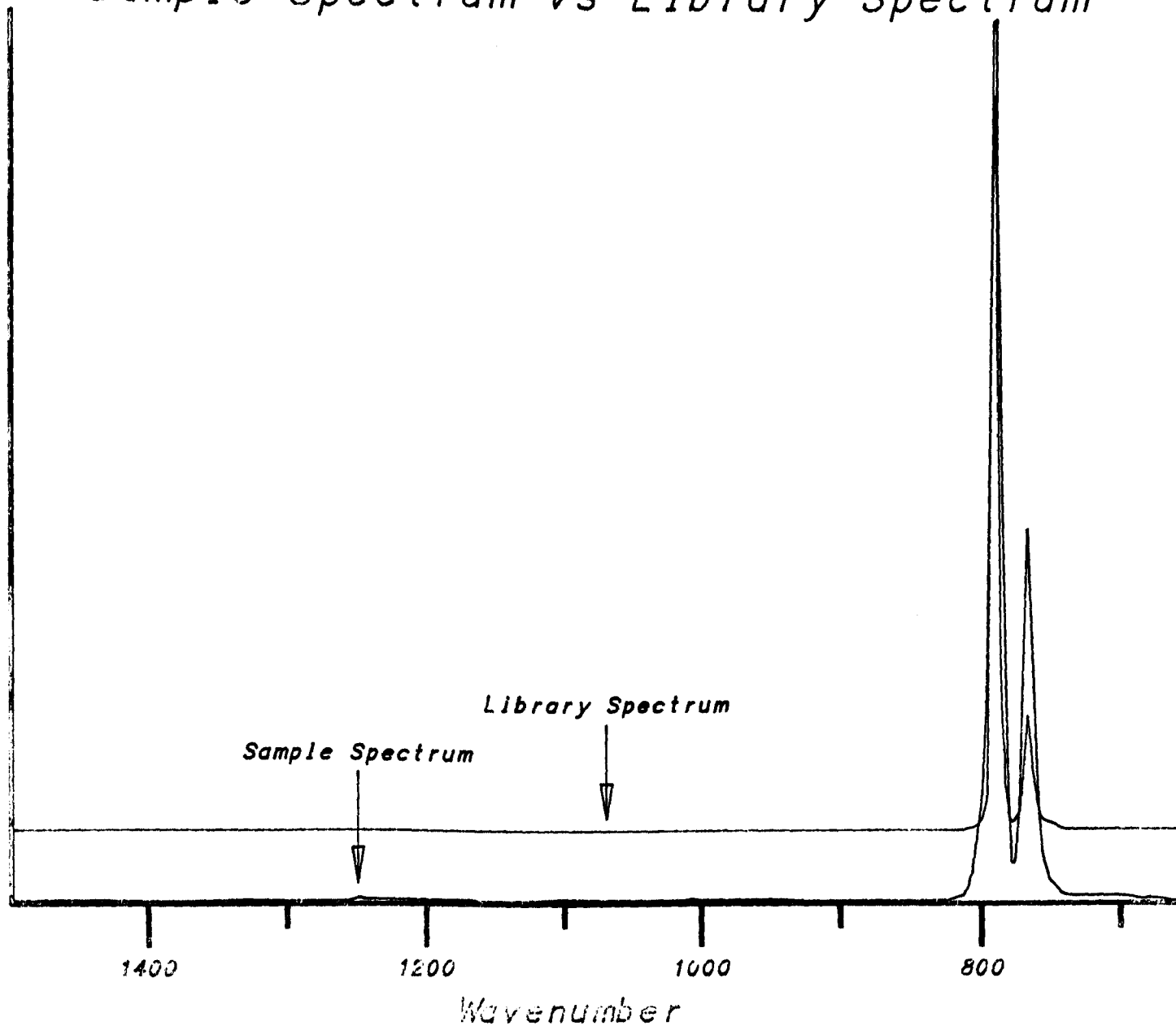


FIGURE 7

Sample EJ28126 * (29 $\mu\text{g}/\text{M}^3$) - GC/MI-IR Spectrum

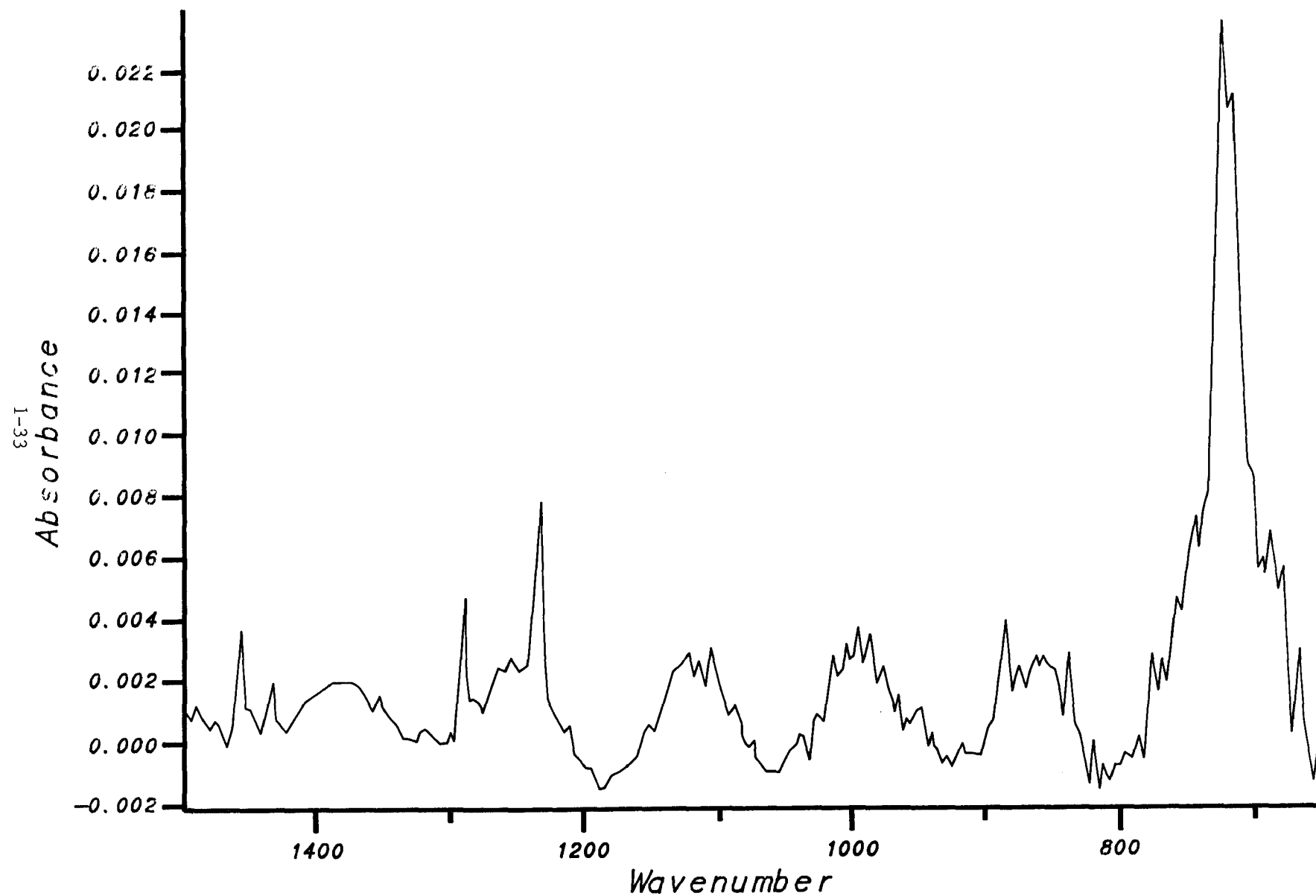


FIGURE 8

1,2-Dichloroethane
Sample Spectrum vs Library Spectrum

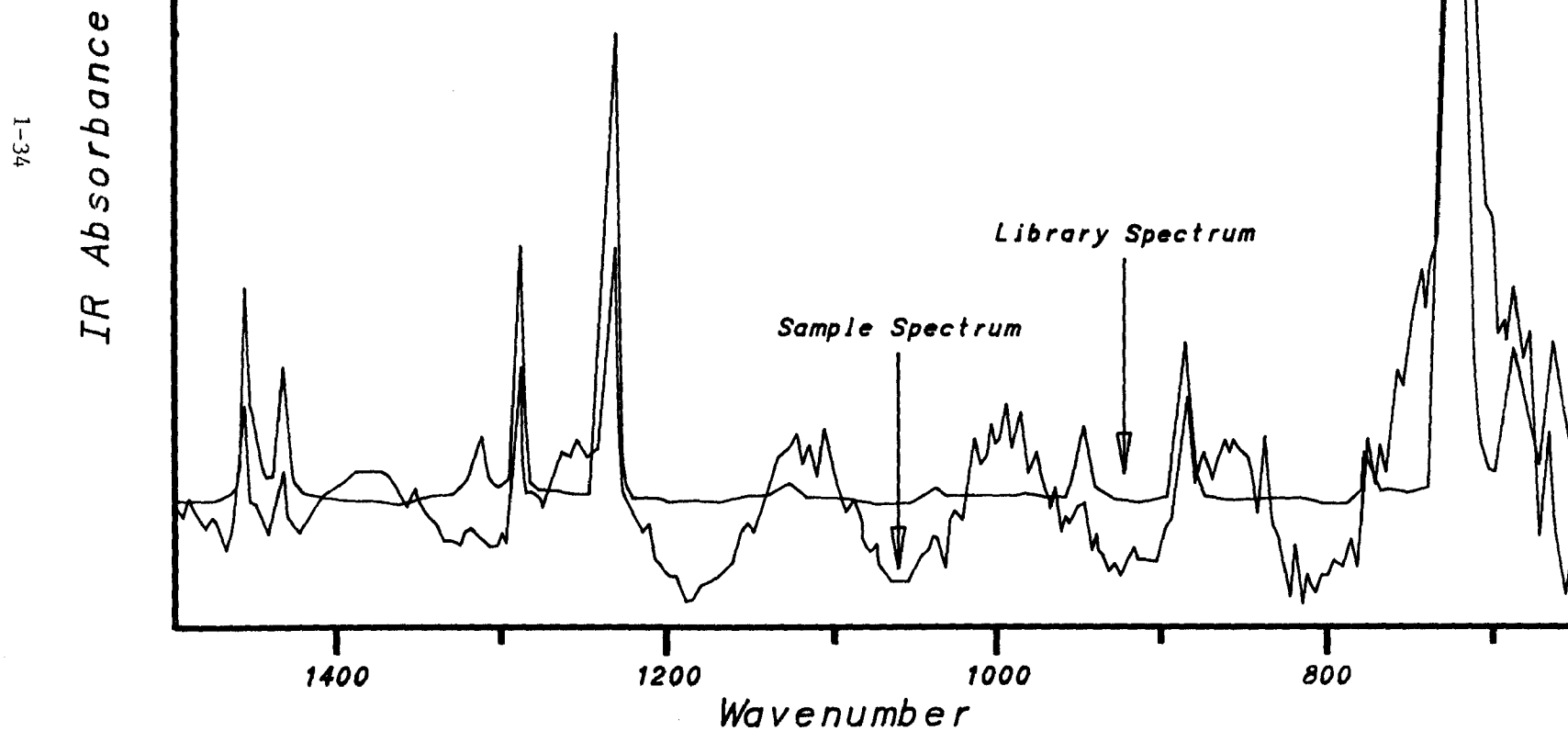
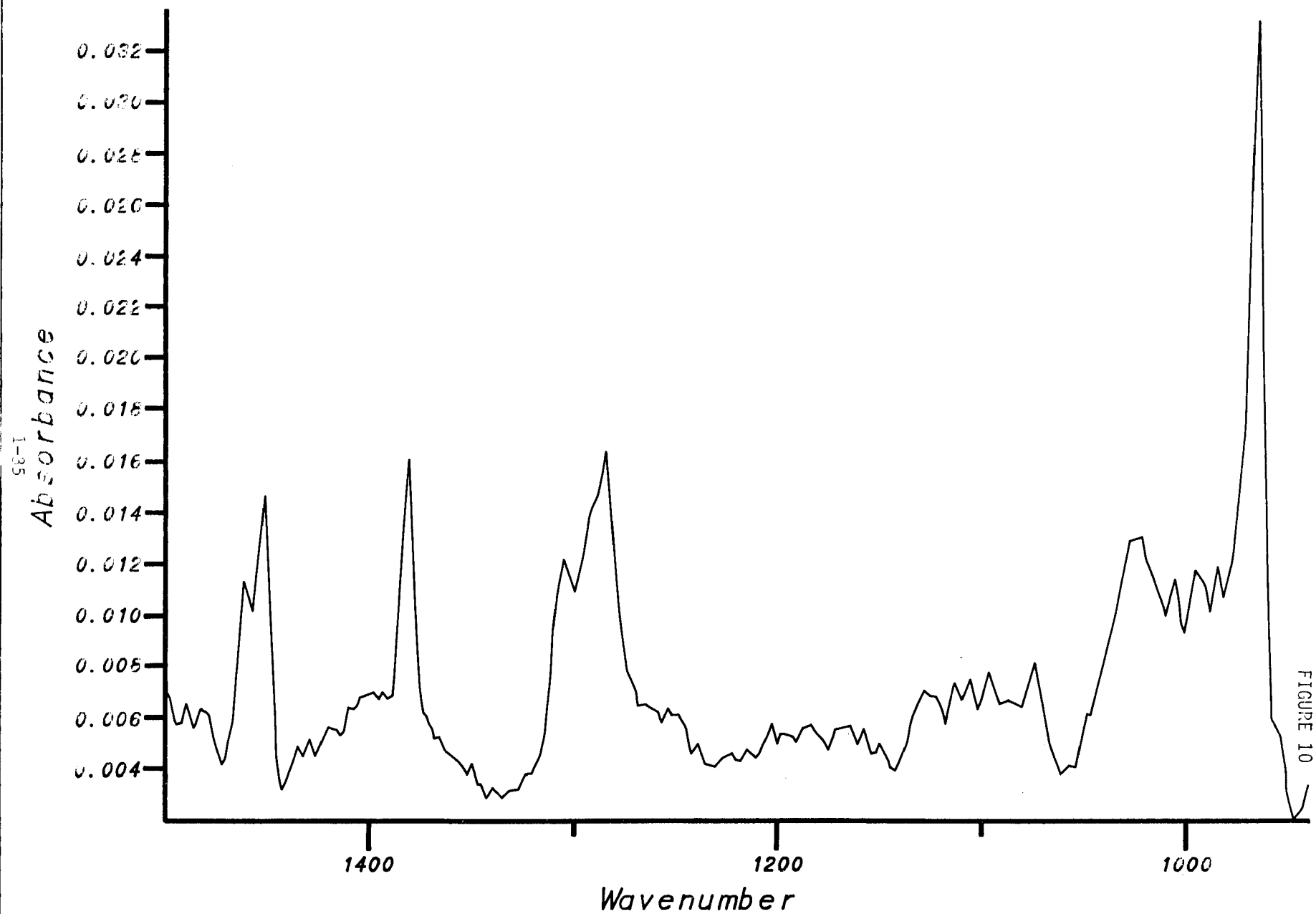
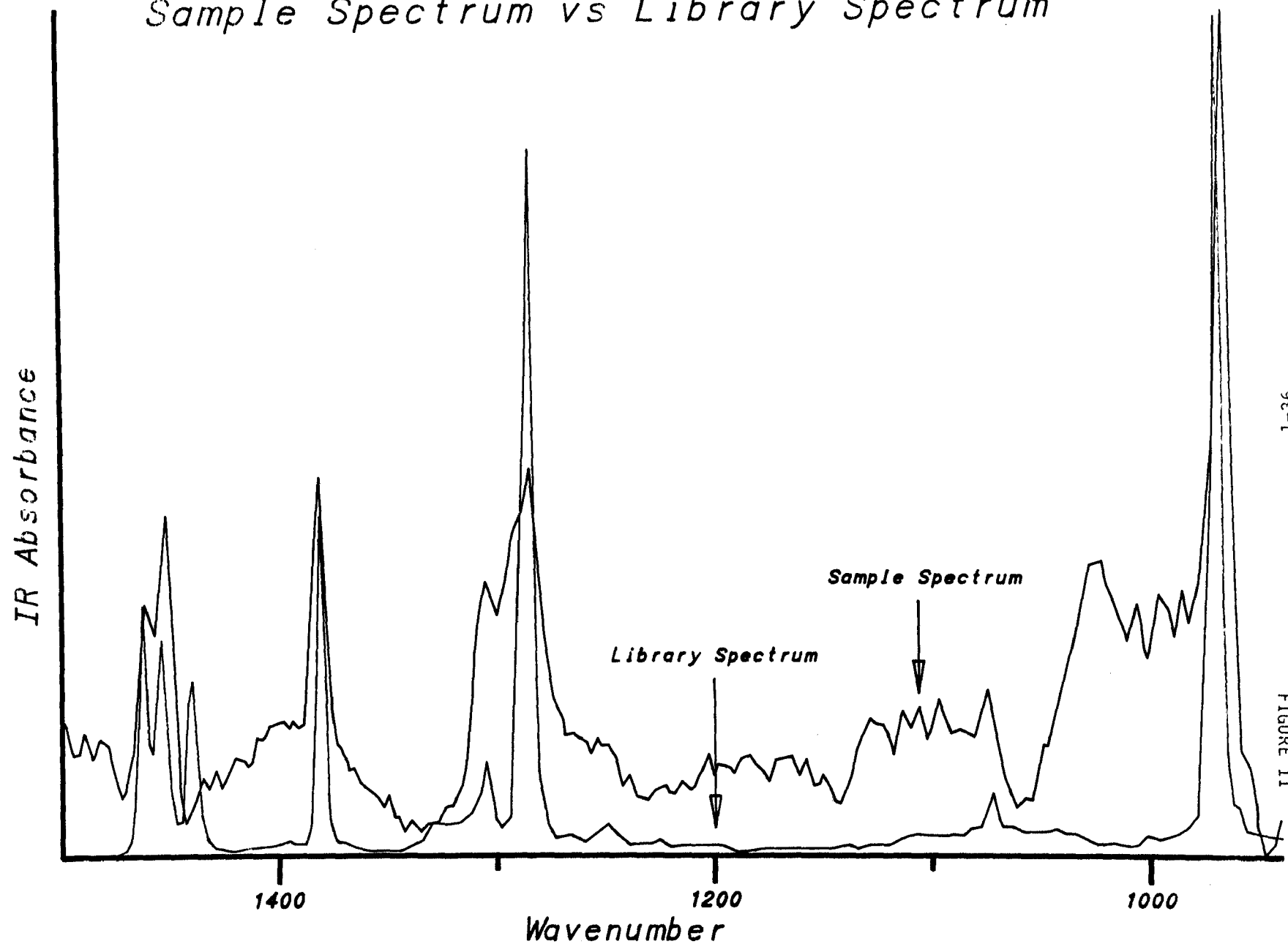


FIGURE 9

Sample EJ28126 * (64 $\mu\text{g}/\text{M}^3$) - GC/MI-IR Spectrum



Chloroethane
Sample Spectrum vs Library Spectrum



*Sample EJ28126 * (39 $\mu\text{g}/\text{M}^3$) - GC/MI-IR Spectrum*

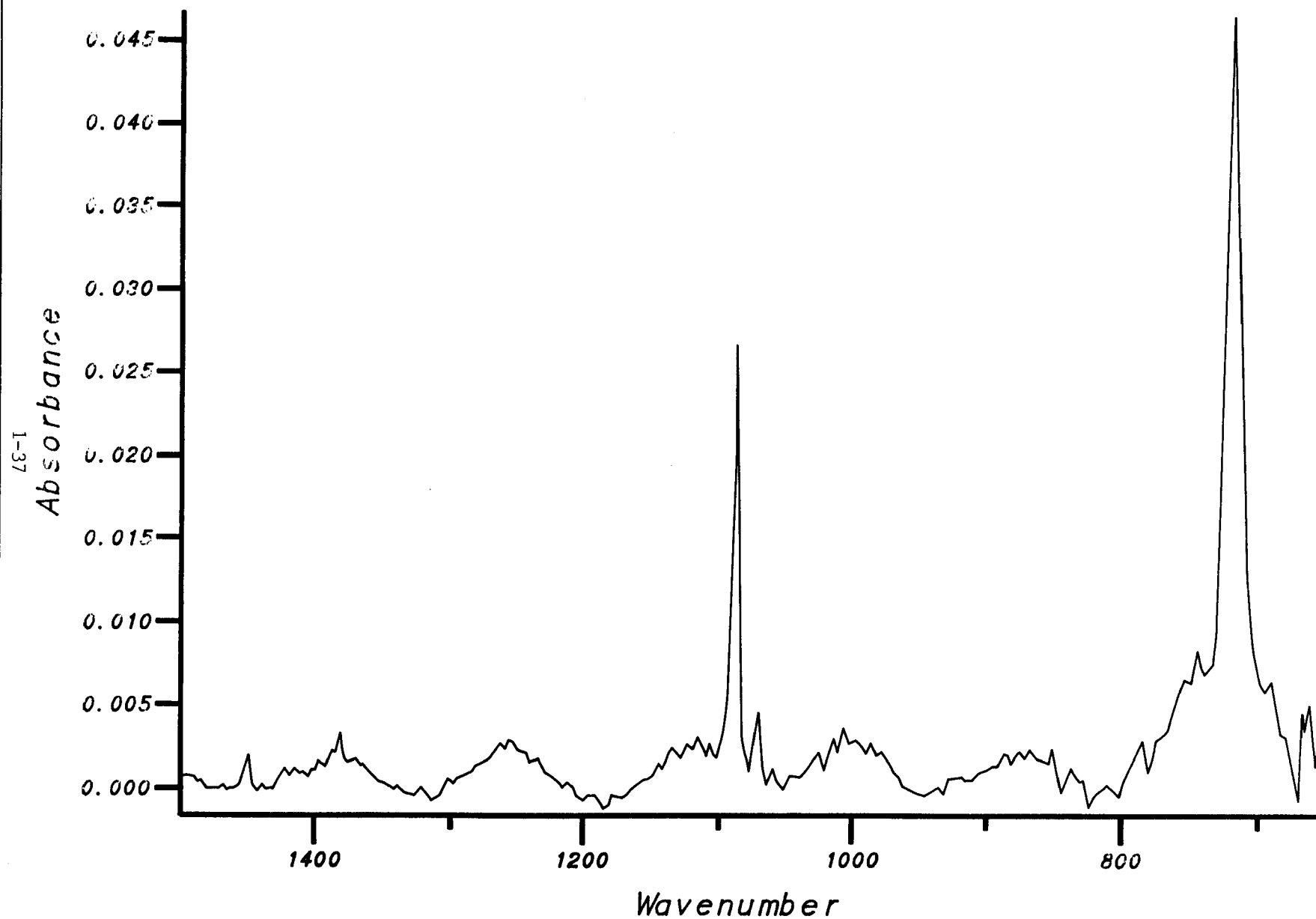


FIGURE 12

1, 1, 1-Trichloroethane
Sample Spectrum vs Library Spectrum

IR Absorbance

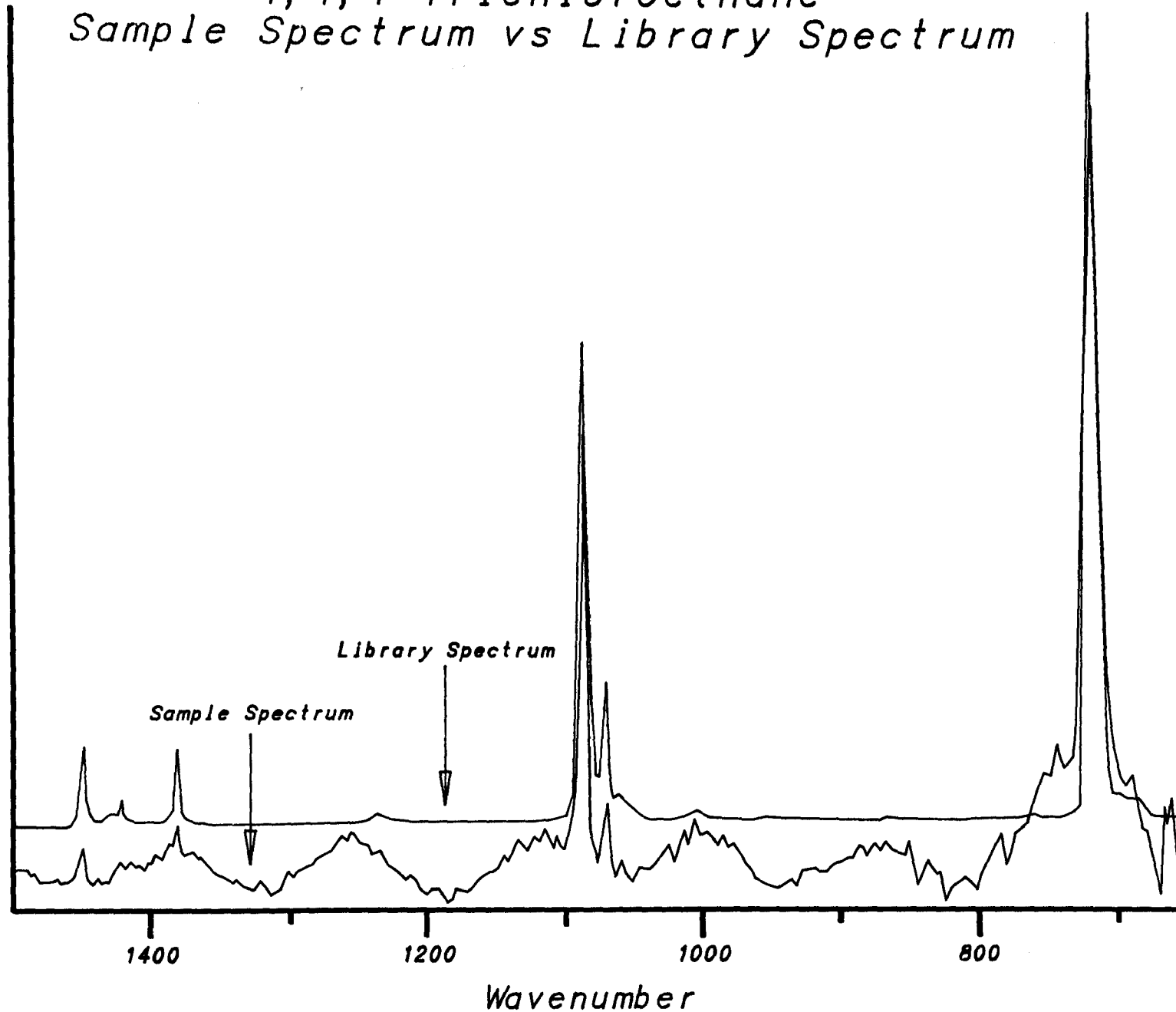
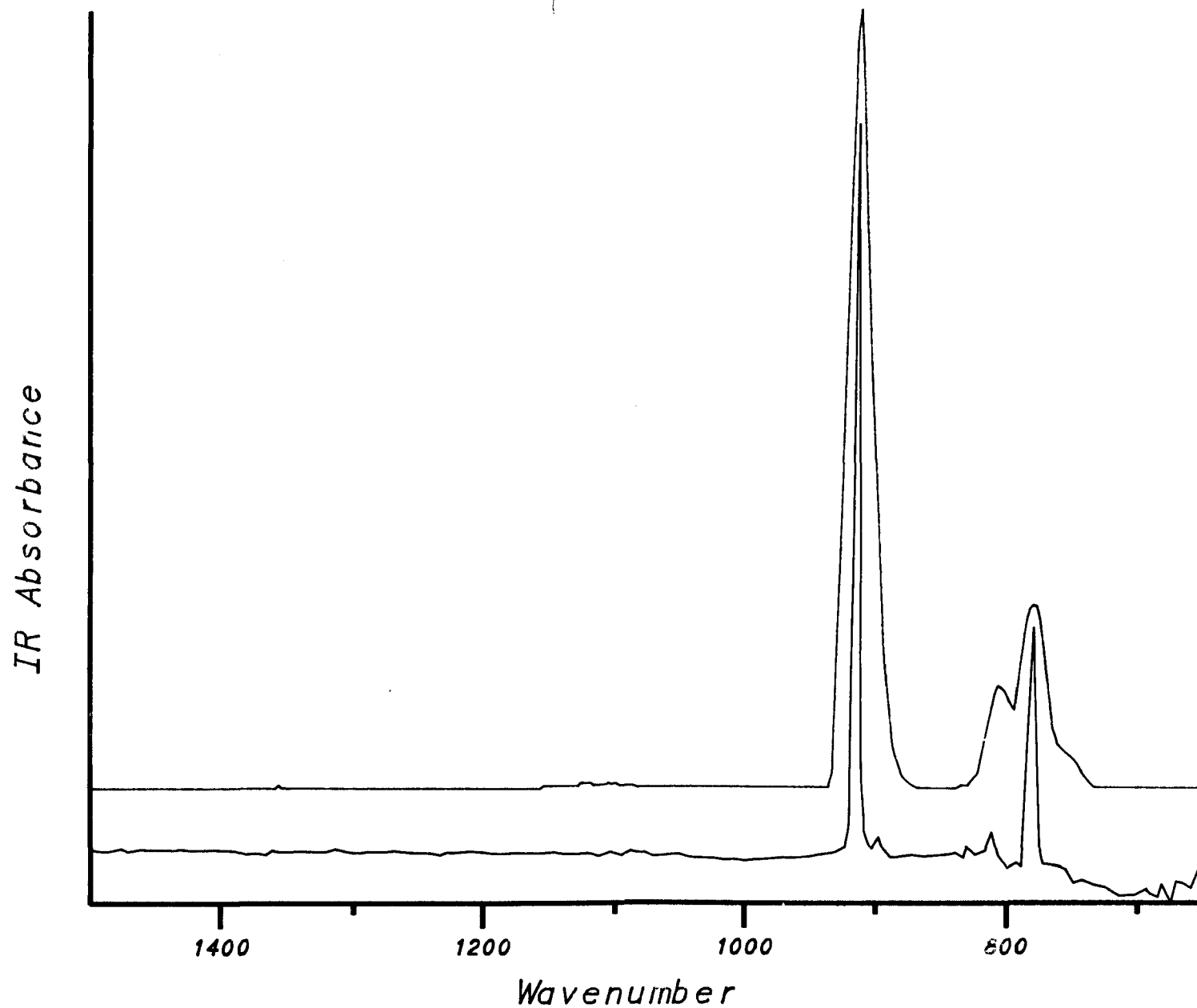


FIGURE 13

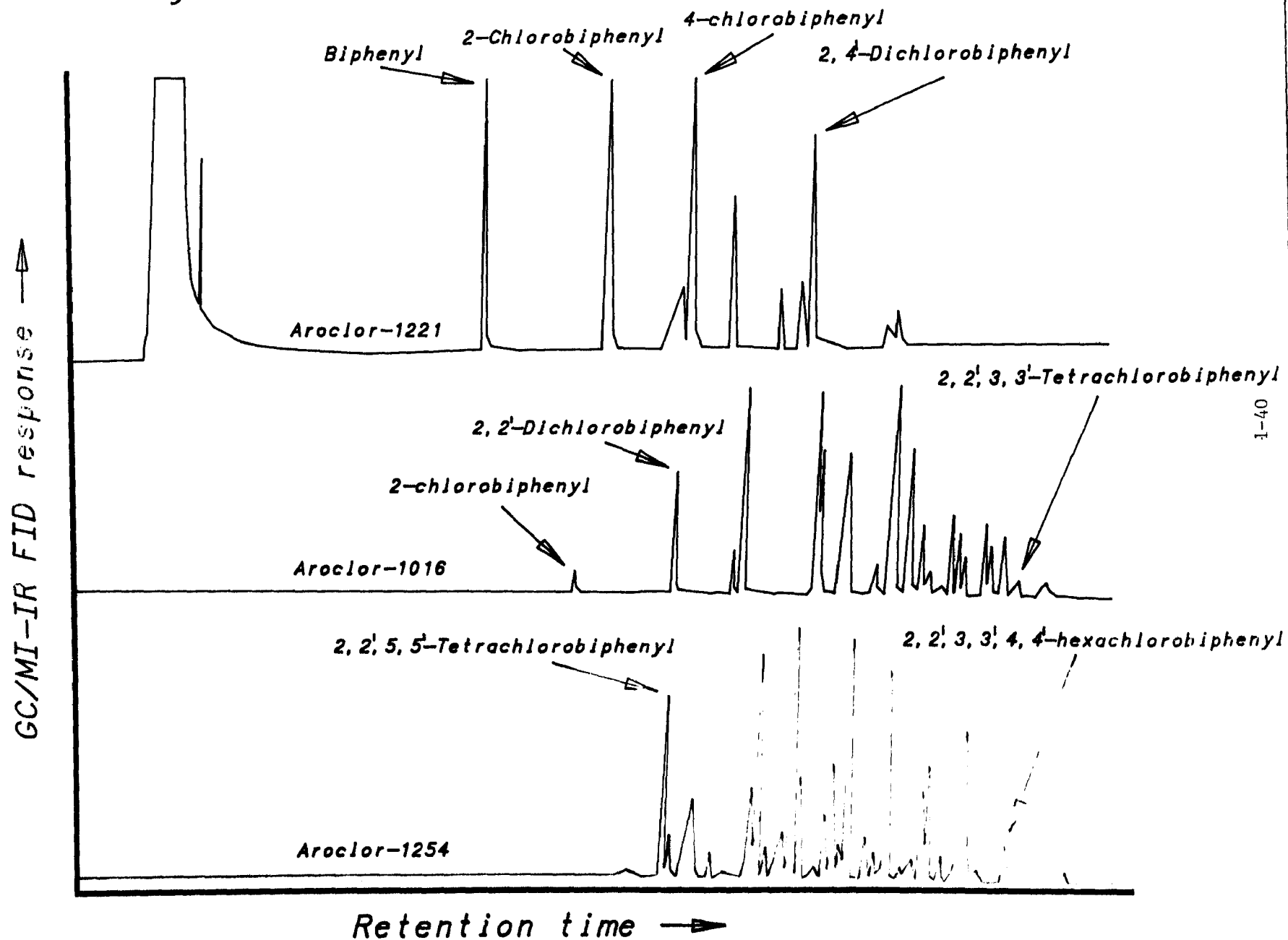
Tetrachloroethylene
Sample Spectrum vs Vapor Phase Library Spectrum

FIGURE 14



Polychlorinated Biphenyl Mixtures

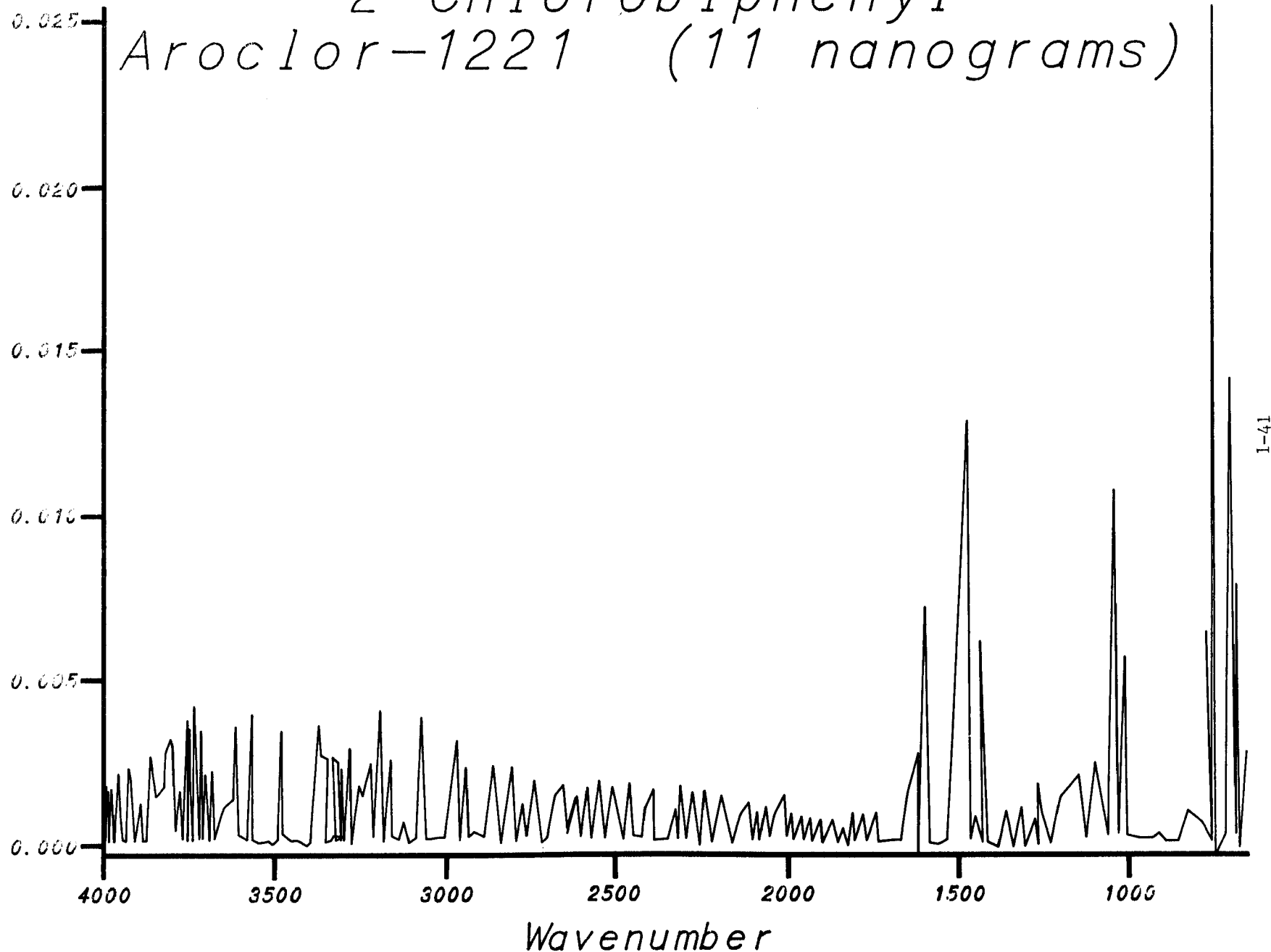
FIGURE 15



2-Chlorobiphenyl
Aroclor-1221 (11 nanograms)

FIGURE 16

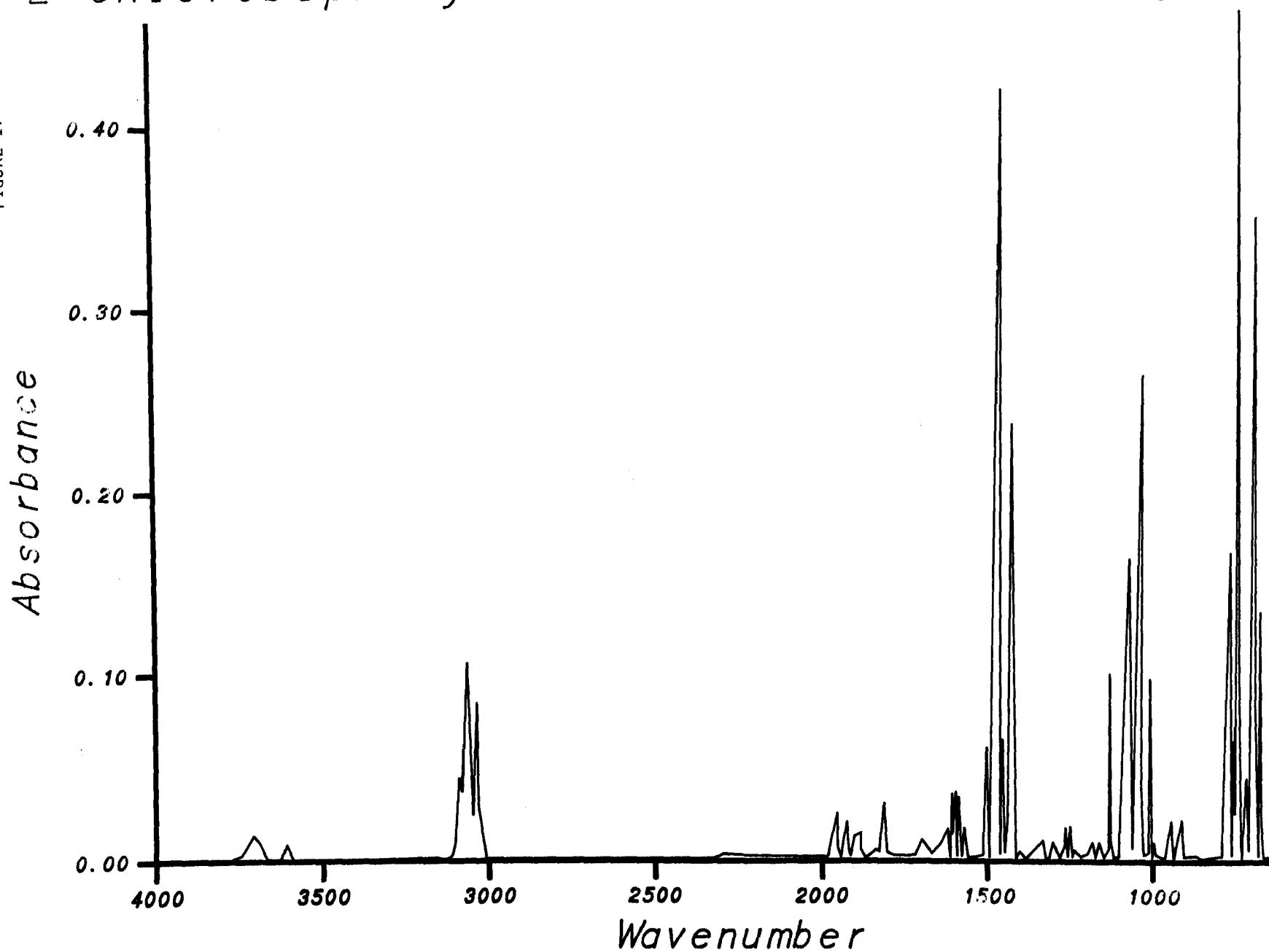
Absorbance



1-41

2-Chlorobiphenyl - Aroclor 1221 2300 ng/ul

FIGURE 17



Biphenyl – Aroclor 1221 2300 ng/ul

FIGURE 18

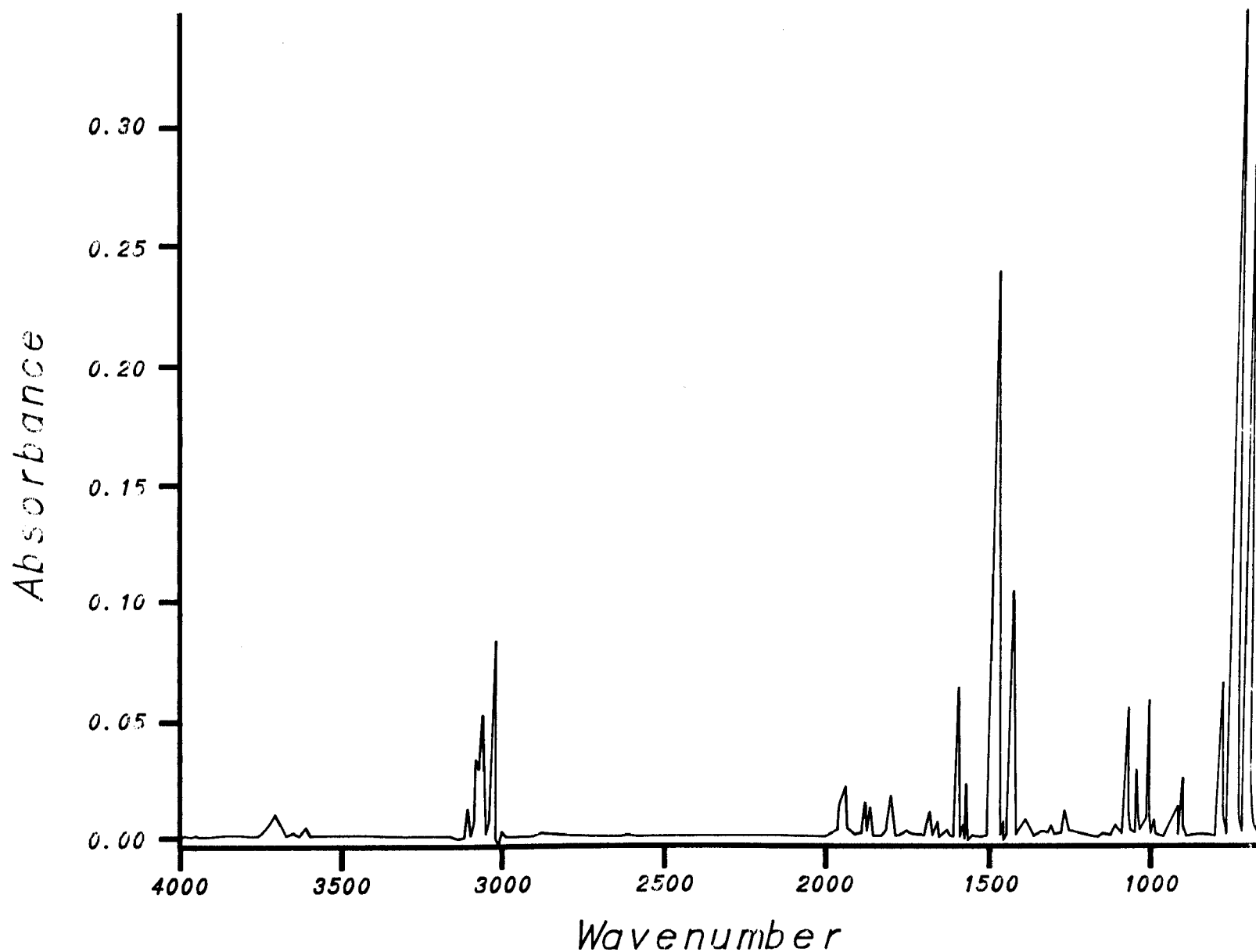


FIGURE 19

Absorbance

Biphenyl
Aroclor-1221 (13 Nanograms)

0.025

0.020

0.015

0.010

0.005

0.000

4000

3500

3000

2500

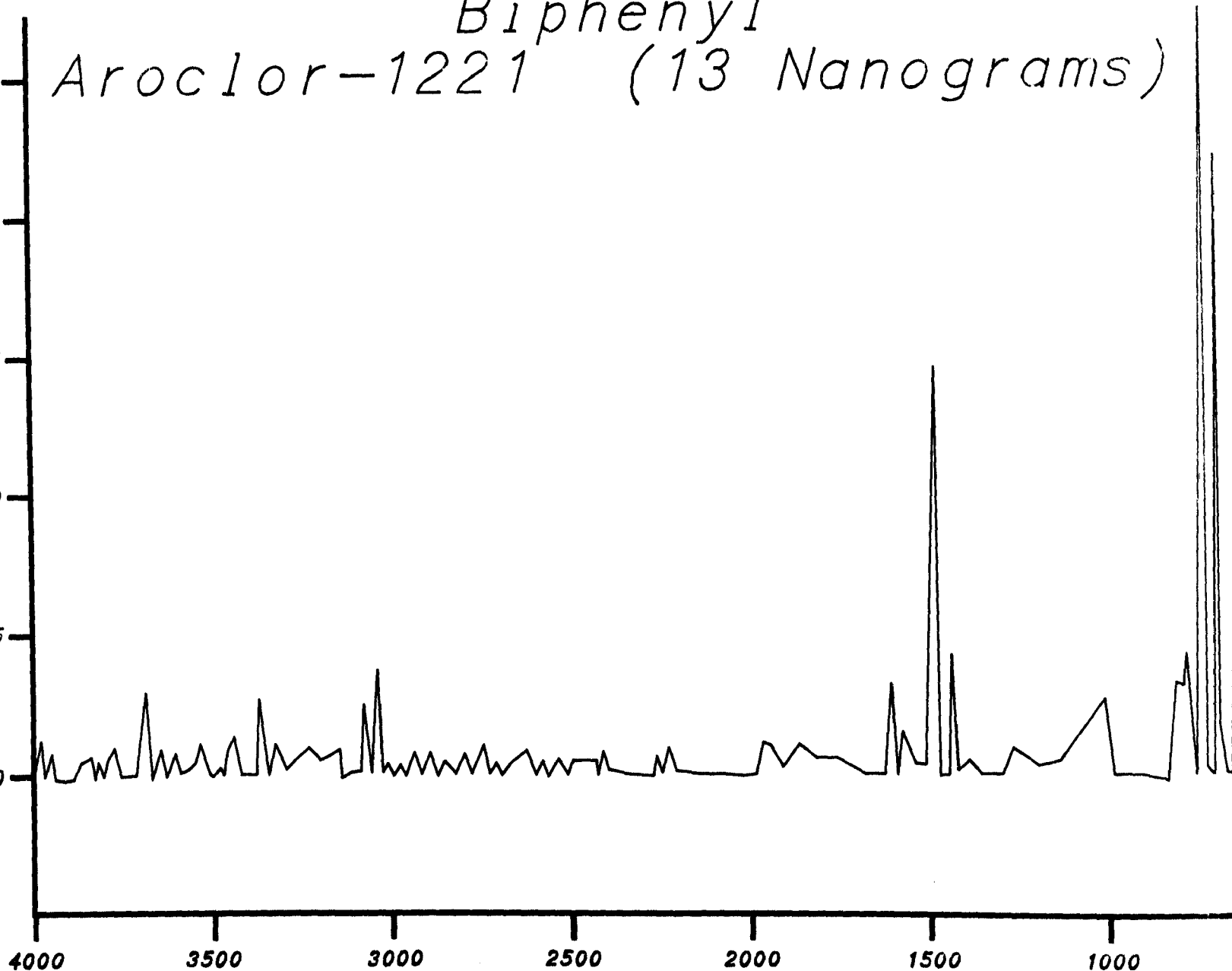
2000

1500

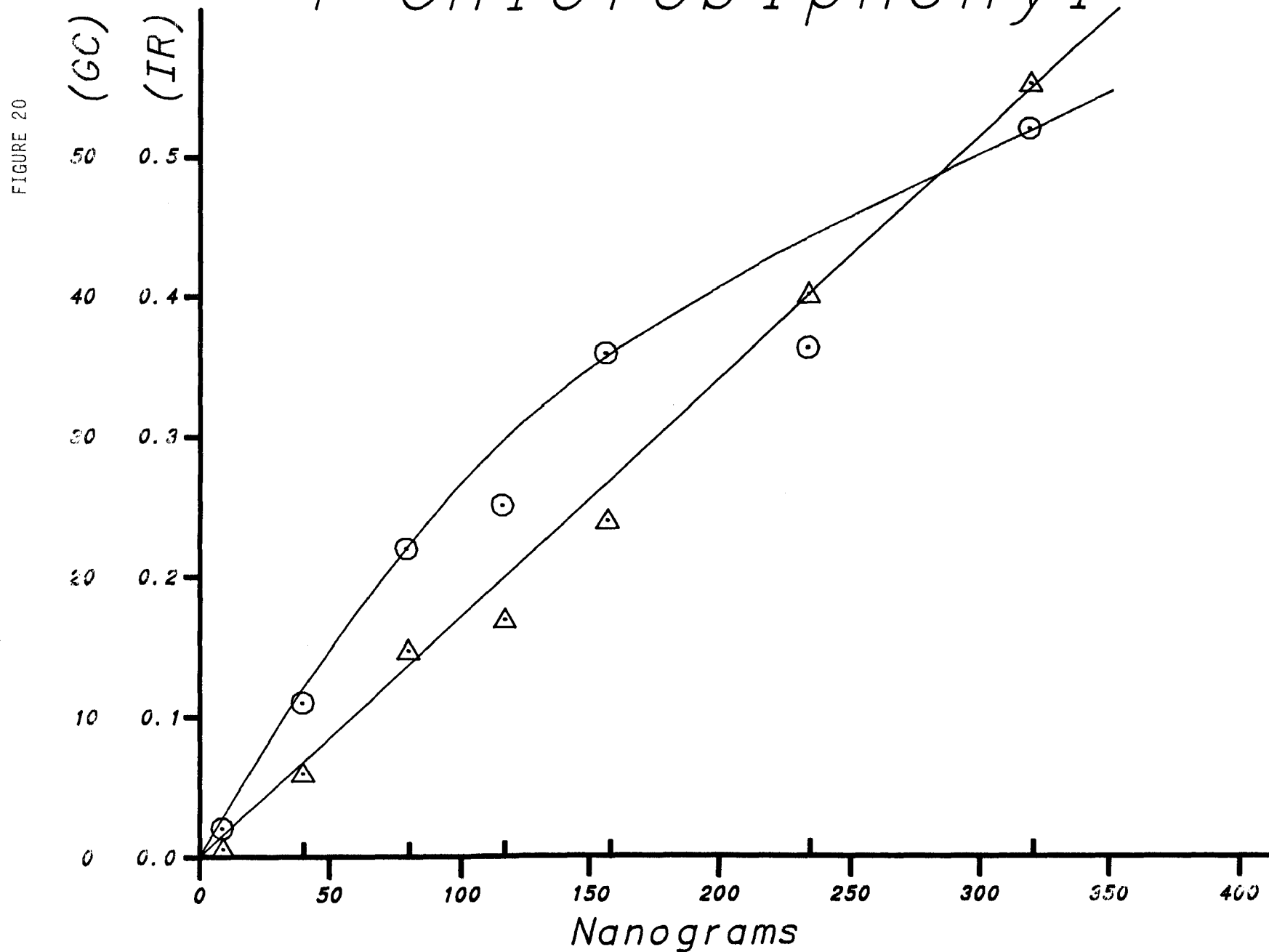
1000

Wavenumber

1-44

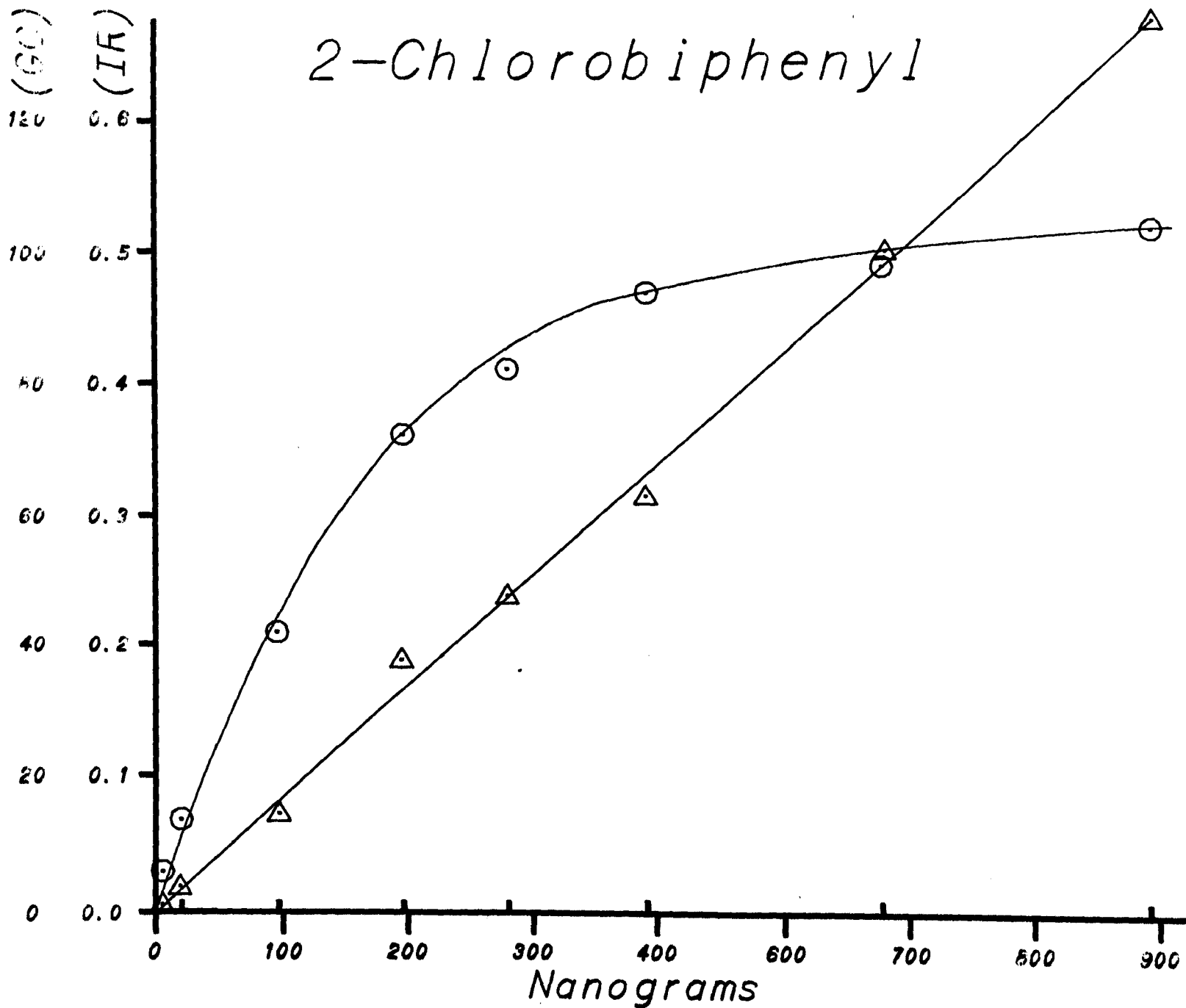


4-Chlorobiphenyl



2-Chlorobiphenyl

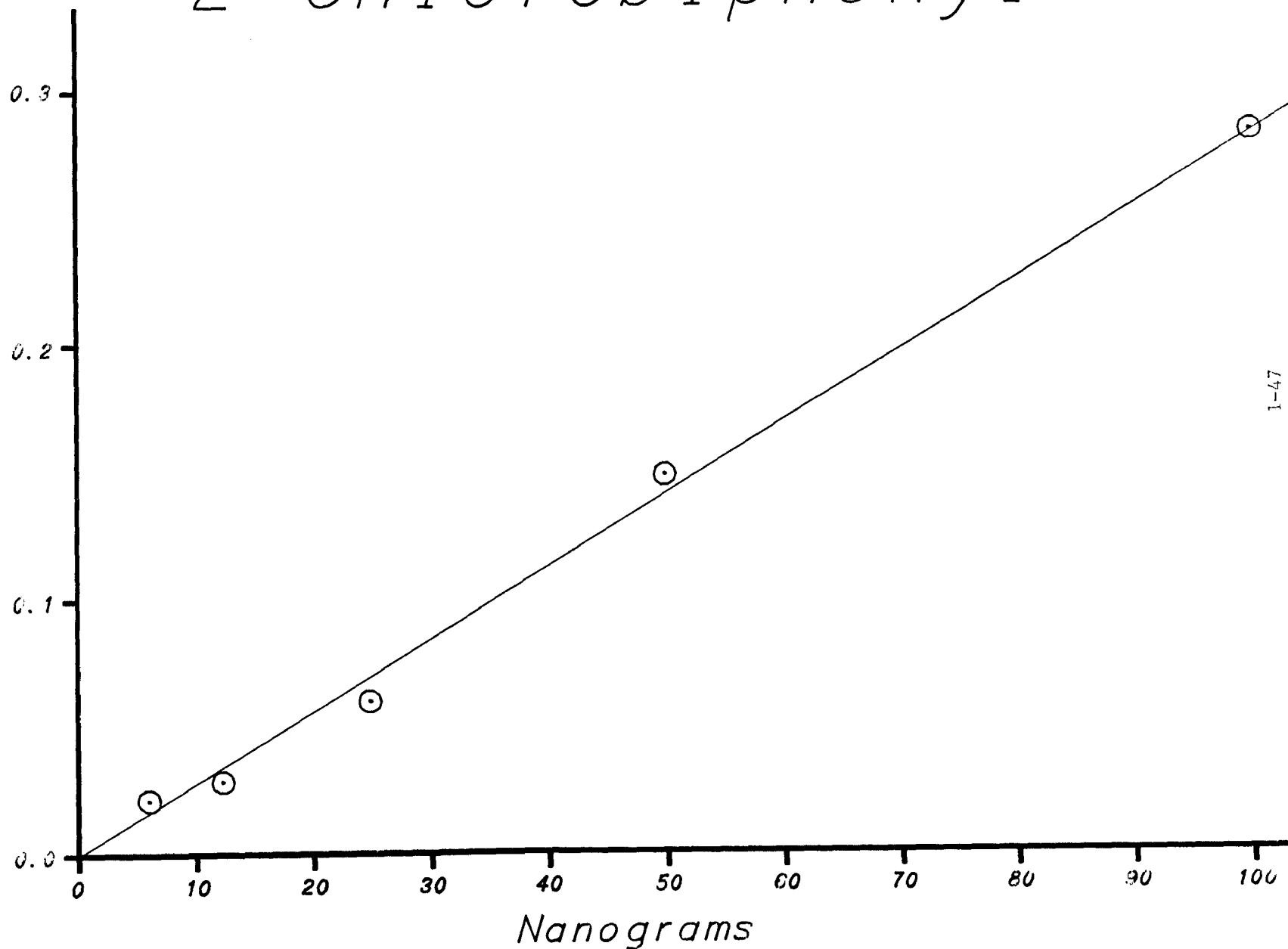
FIGURE 21



2-Chlorobiphenyl

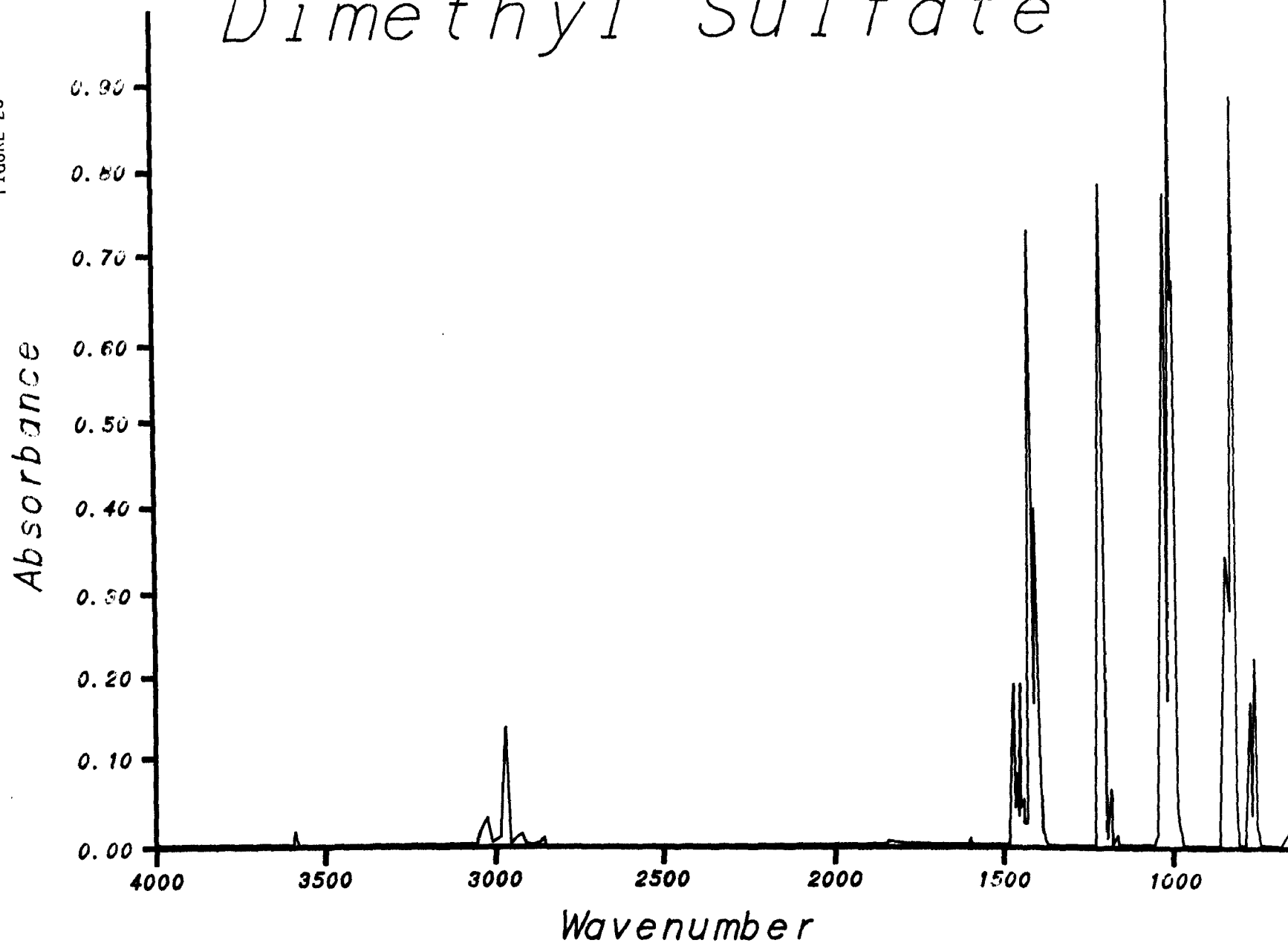
FIGURE 22

Absorbance (GC/MI-IR)



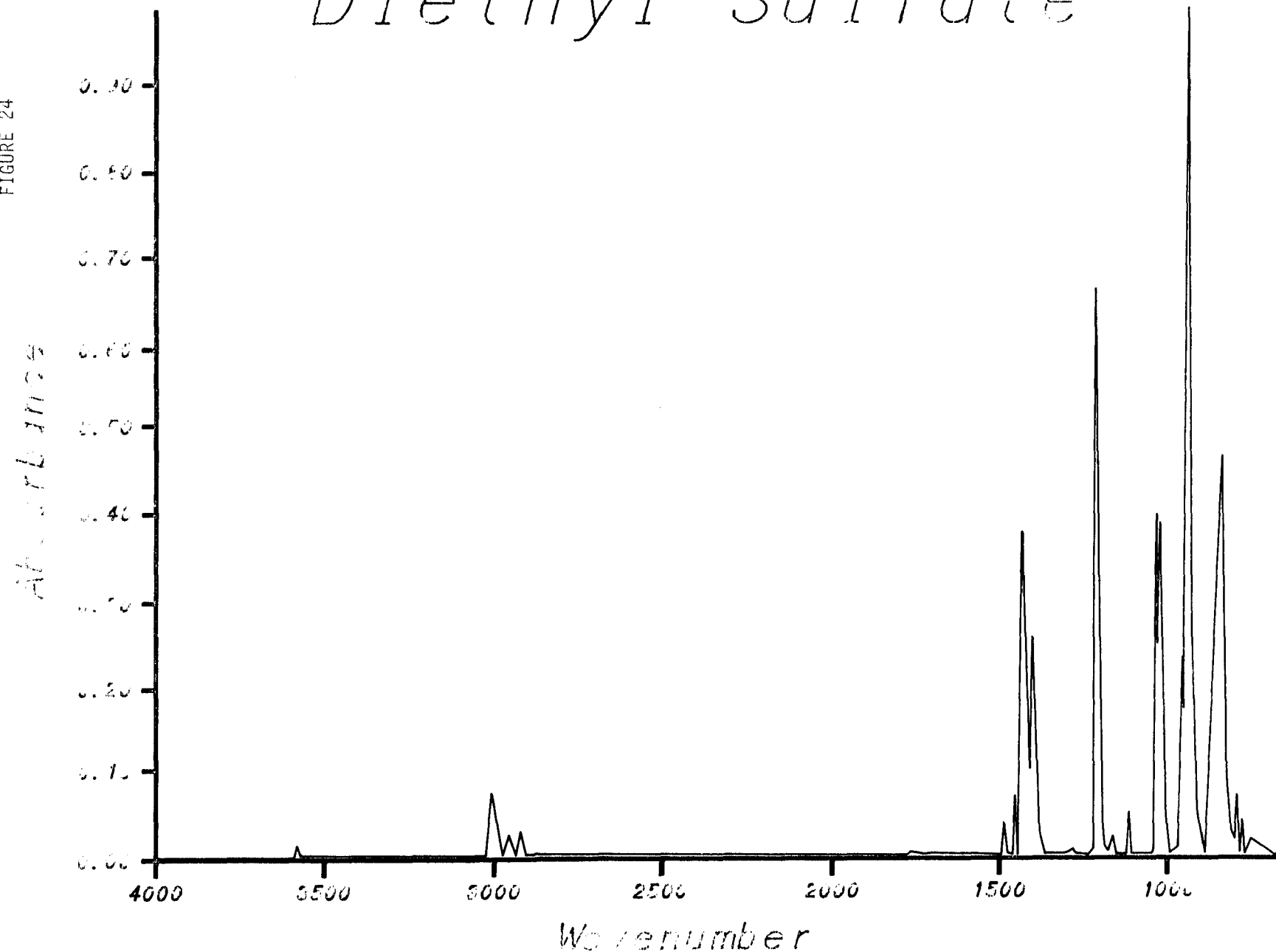
Dimethyl Sulfate

FIGURE 23



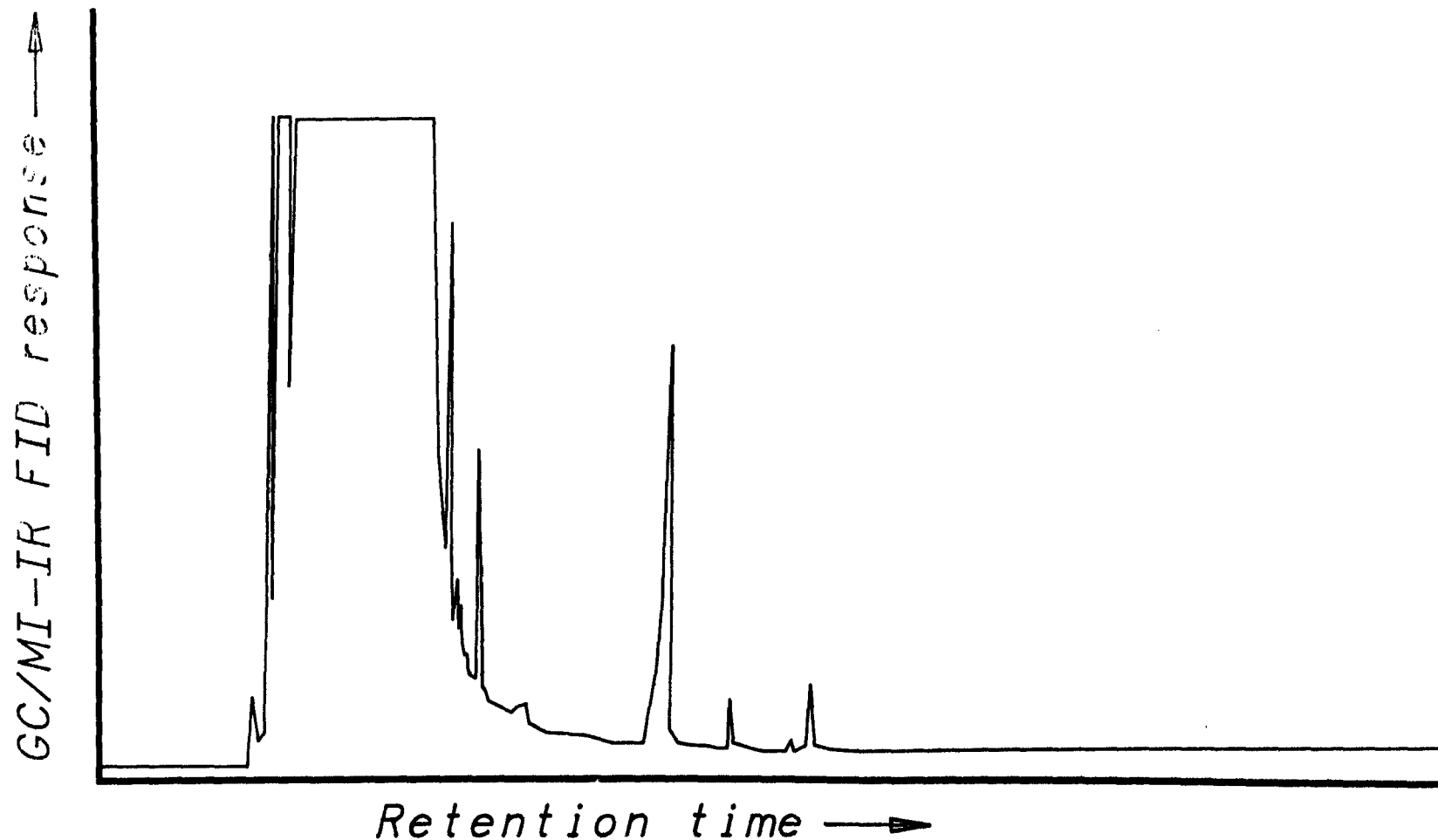
Diethyl Sulfate

FIGURE 24

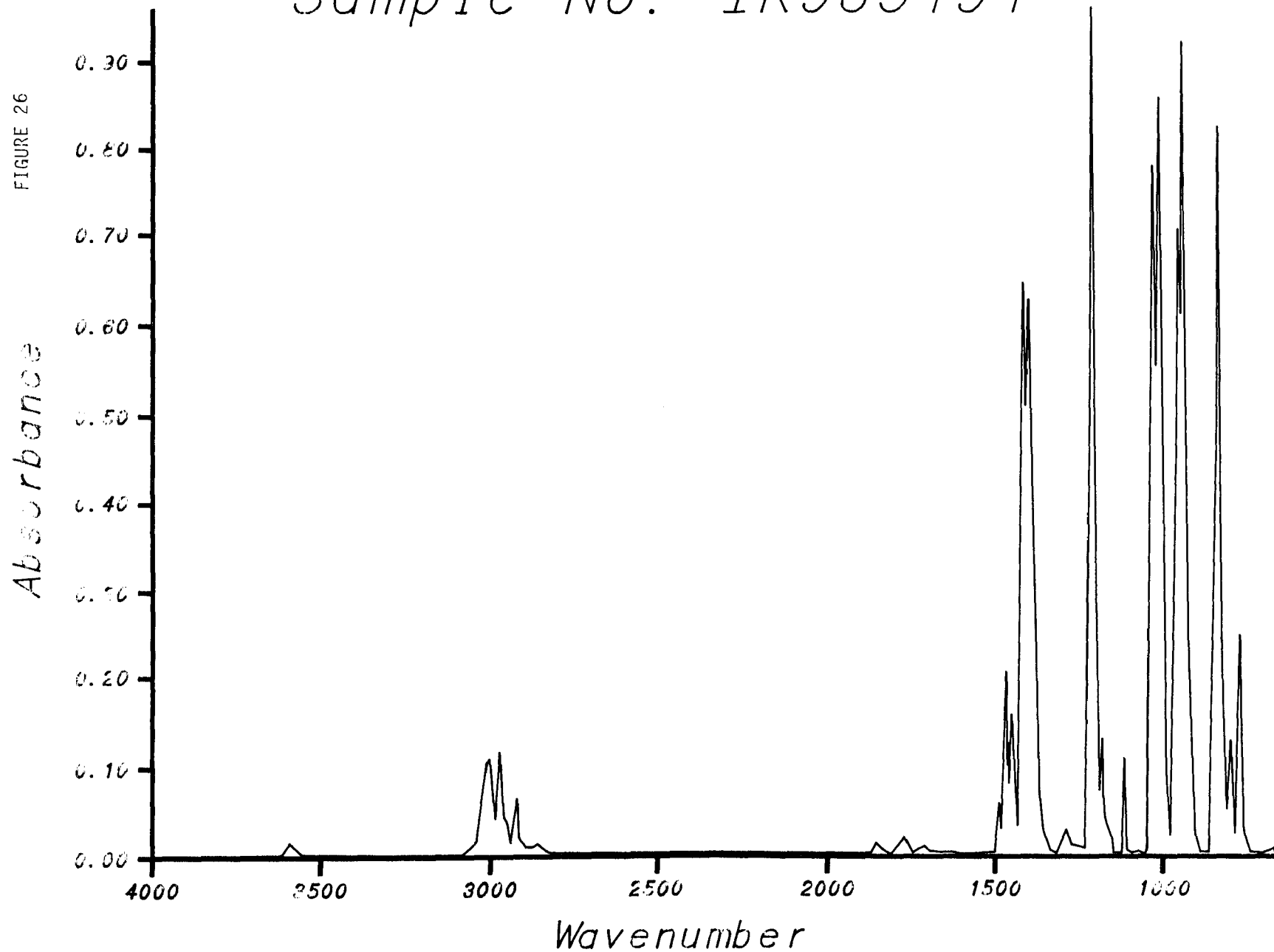


Sample IK935194
Methylethyl Sulfate

FIGURE 25

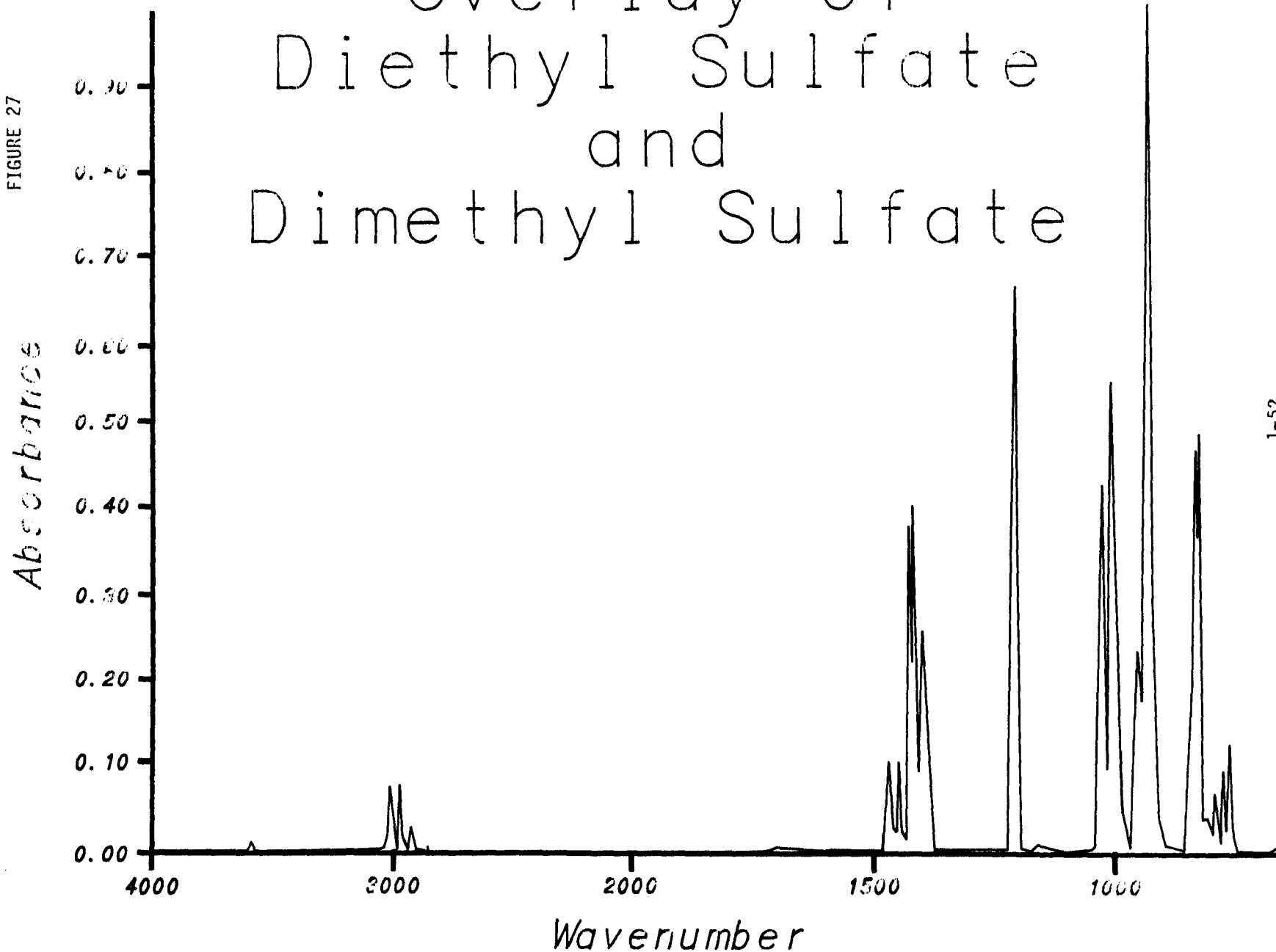


Sample No. IK935194



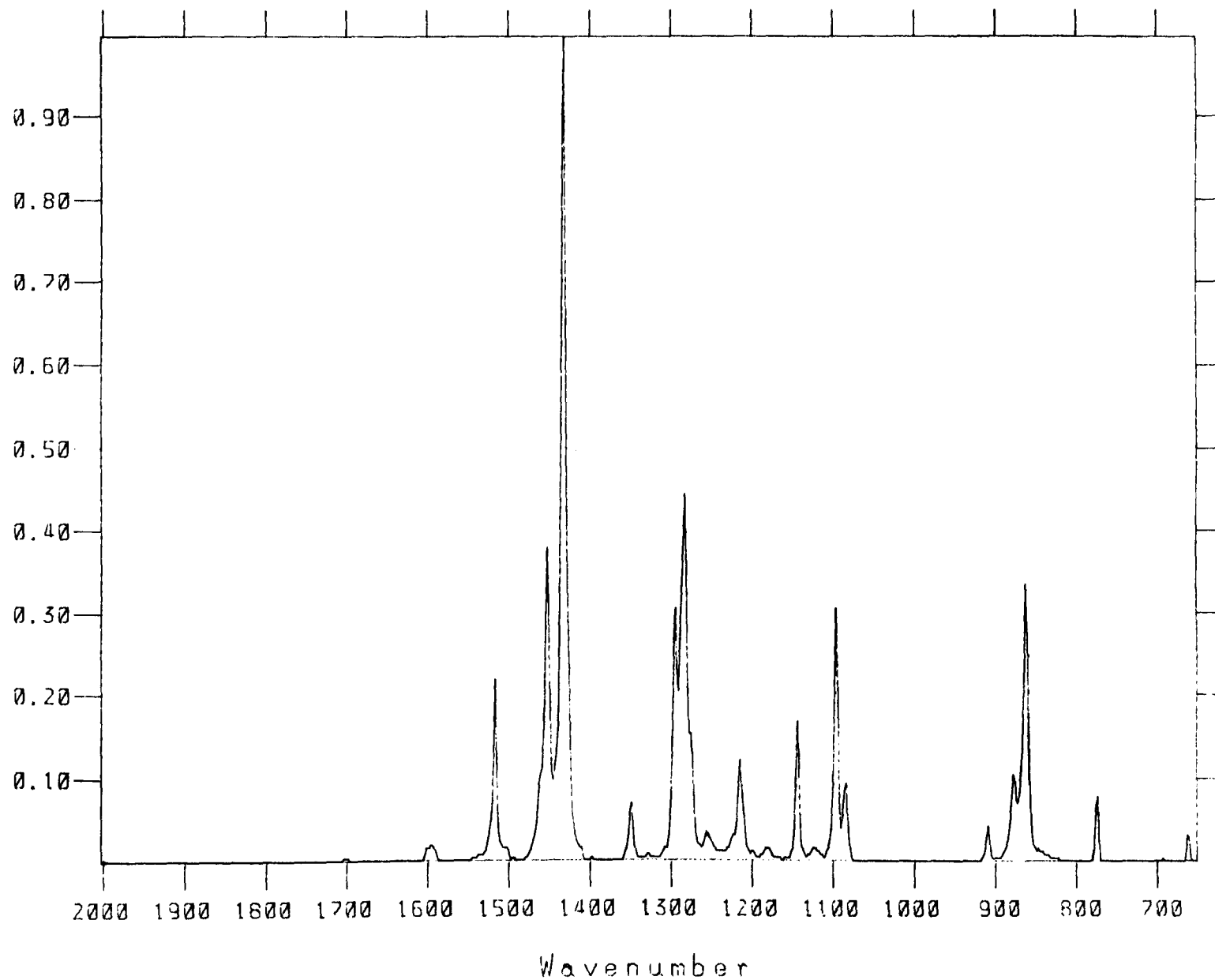
Overlay of Diethyl Sulfate and Dimethyl Sulfate

FIGURE 27



dxn.9 2,3,7,8-TCDD Carbon 13 labeled

FIGURE 28
A
b
s
o
r
b
a
n
c
e



1-53

dxn.9

FIGURE 29
Absorbance

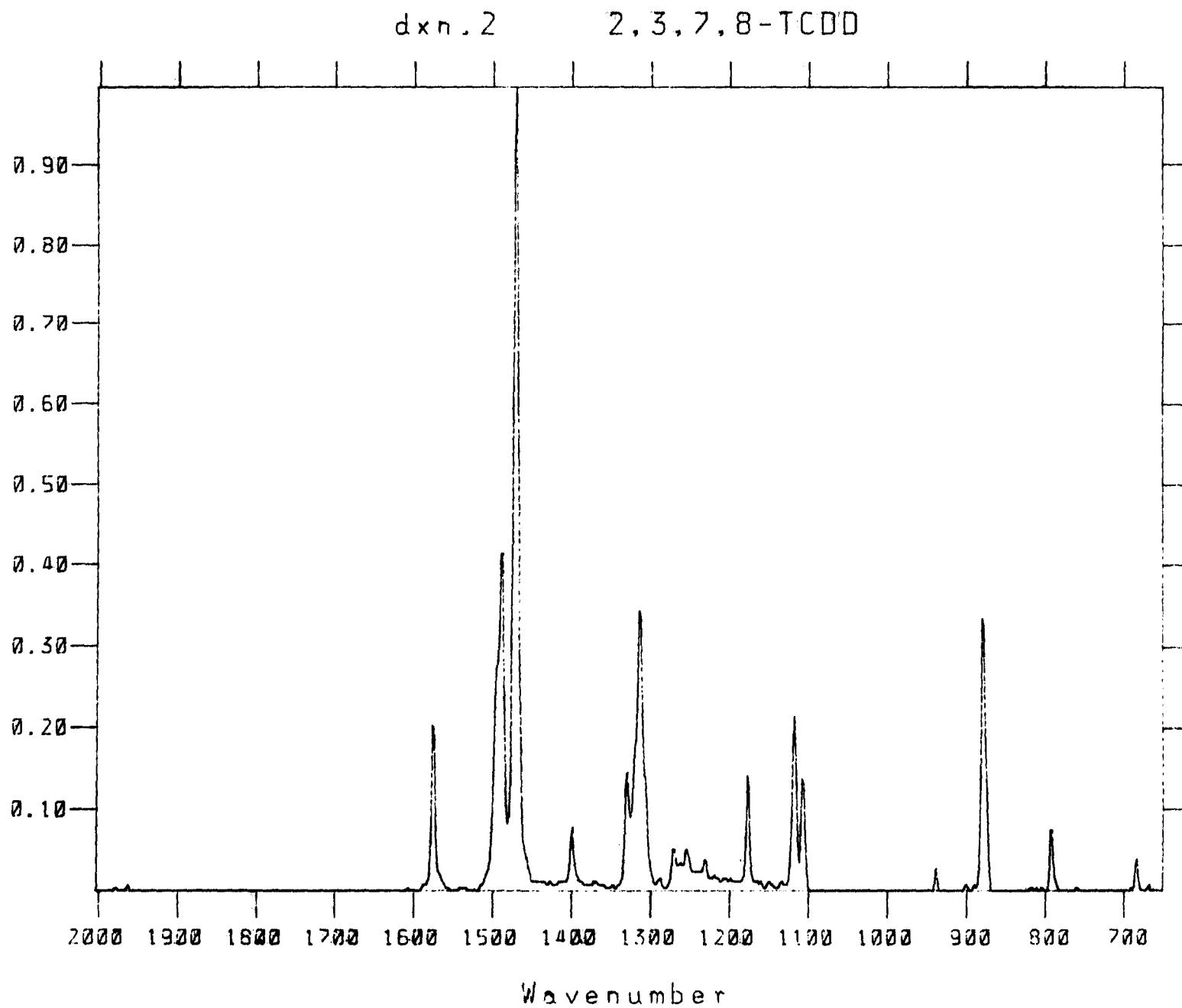


FIGURE 30

dio. 4

1,2,3,4-TCDD

A
b
s
o
r
b
a
n
c
e

Library Spectrum

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10

A
b
s
o
r
b
a
n
c
e

156 Picograms

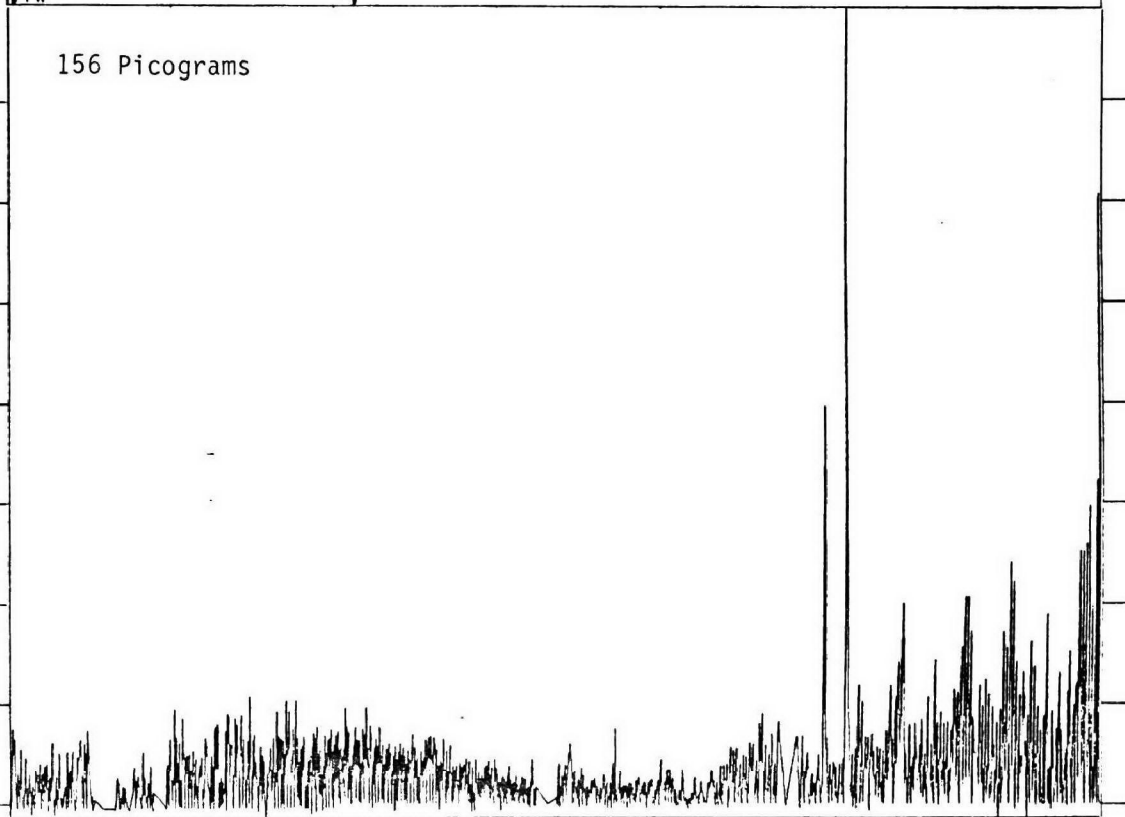
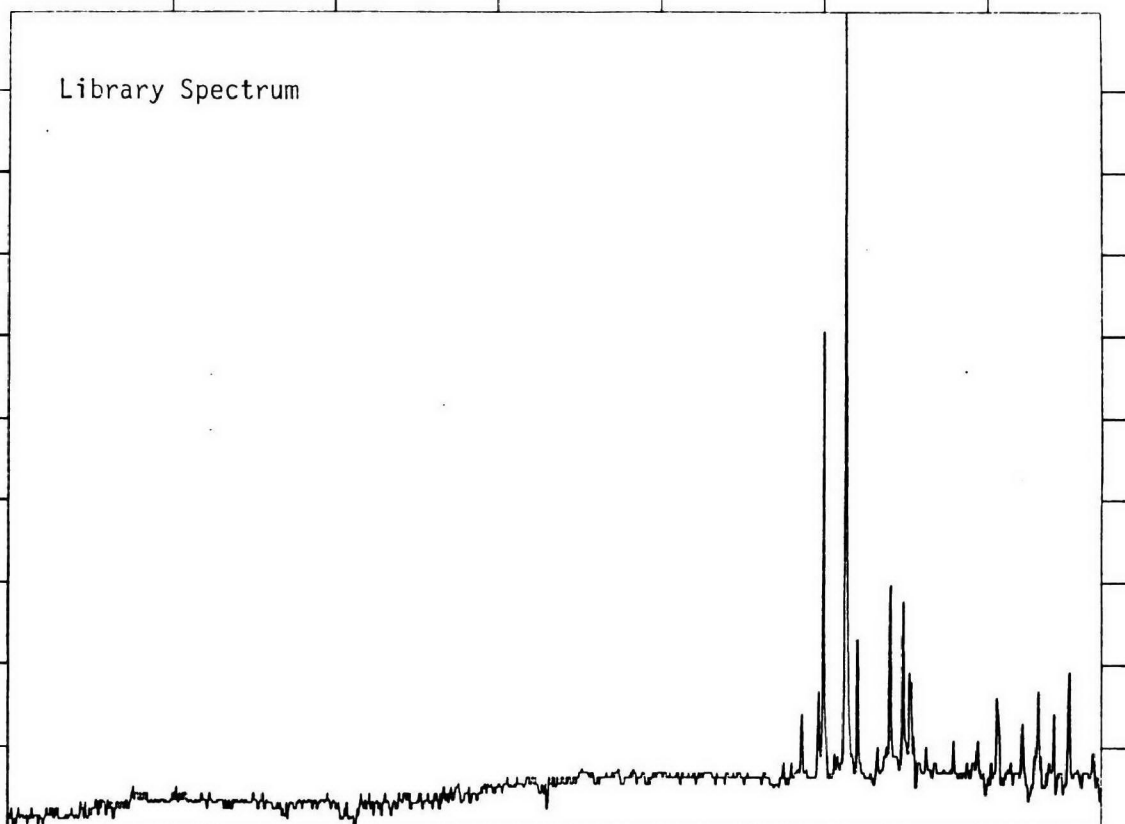
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000

4000 3500 3000 2500 2000 1500 1000

Wavenumber

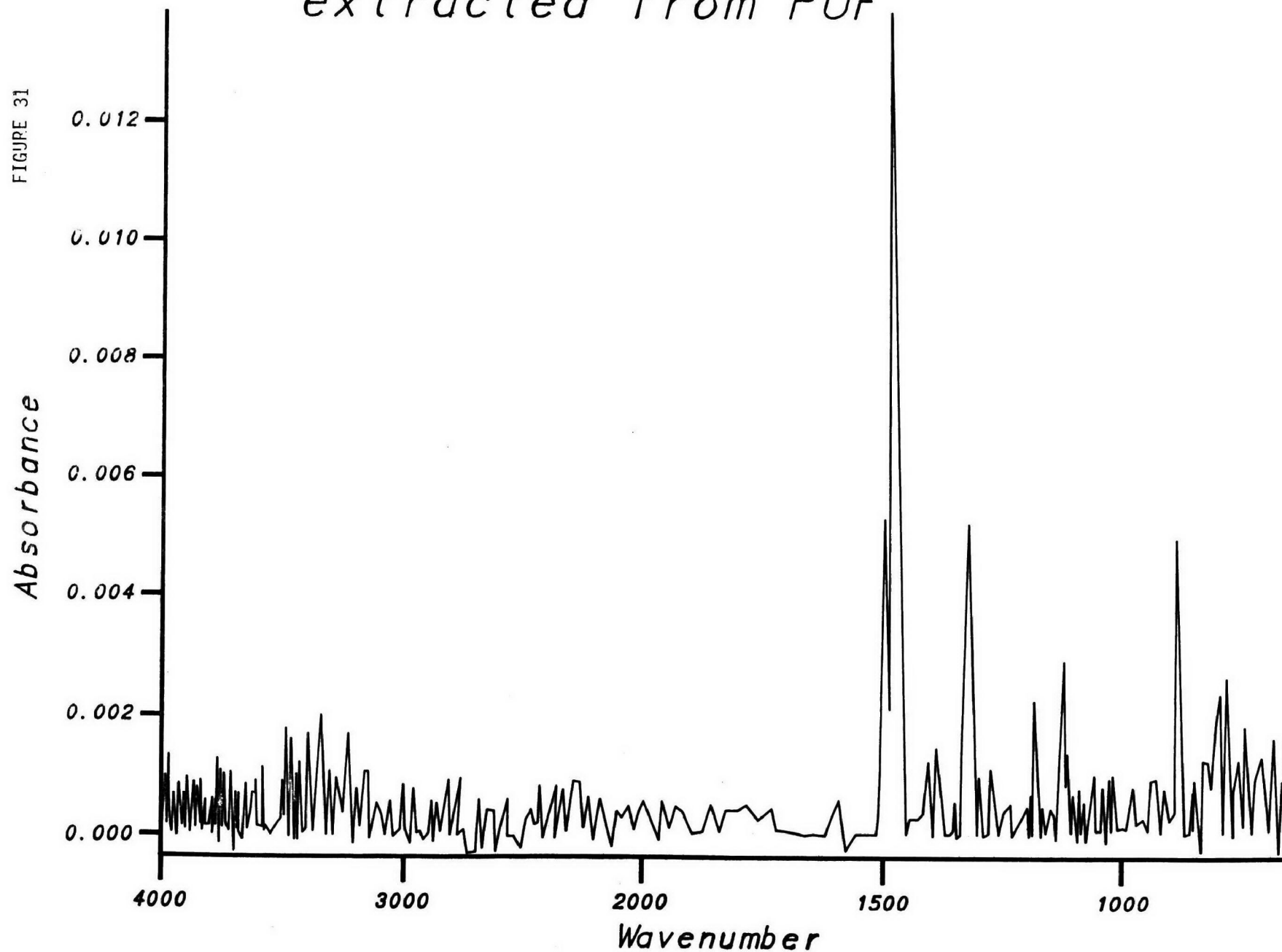
1-55

c17.40.abs



2, 3, 7, 8-TCDD (4 Nanograms)
extracted from PUF

FIGURE 31



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EVALUATION OF THE FLUX CHAMBER METHOD FOR MEASURING
AIR EMISSIONS OF VOLATILE ORGANIC COMPOUNDS
FROM SURFACE IMPOUNDMENTS

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ABSTRACT

Enclosure methods have been used to measure air emissions of a variety of compounds from soils, water, sediments, and living organisms. A flux chamber method, which employs the enclosure method, recently has been used to measure organic air emissions from hazardous waste treatment, storage, and disposal facilities. Using a simulated surface impoundment facility (SIS), this flux chamber method was evaluated. The liquid surface of the SIS was enclosed so that the total emission rate from a liquid surface could be determined experimentally. Emission measurements using the flux chamber method made at several points on the surface were compared with the emission rate measured for the total surface inside the enclosure. Both the accuracy and precision of the flux chamber method were predicted from these measurements. The influence of sweep flowrate, emission rate, and different organic compounds on precision and accuracy were investigated.

The results of this study show that a consistent negative bias exists for all the flux chamber measurements. This bias became significantly more negative at a low sweep flowrate (2 L/min). The bias also was found to be compound dependent. Precision was less than 5 percent under all conditions for the single component studies and between 6 and 13 percent for the three component study.

INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) has been instructed to set air emission standards for hazardous waste treatment, storage, and disposal facilities (TSDF) by the 1984 Resource Conservation and Recovery Act amendments. In order to determine the potential health and environmental effects of these air emissions, methods to measure or predict them are required. The flux chamber method has been developed and is being used to measure air emission from TSDF to provide a data base for regulatory decisionmaking and to validate proposed models used to predict air emissions^{1,2,3,4}.

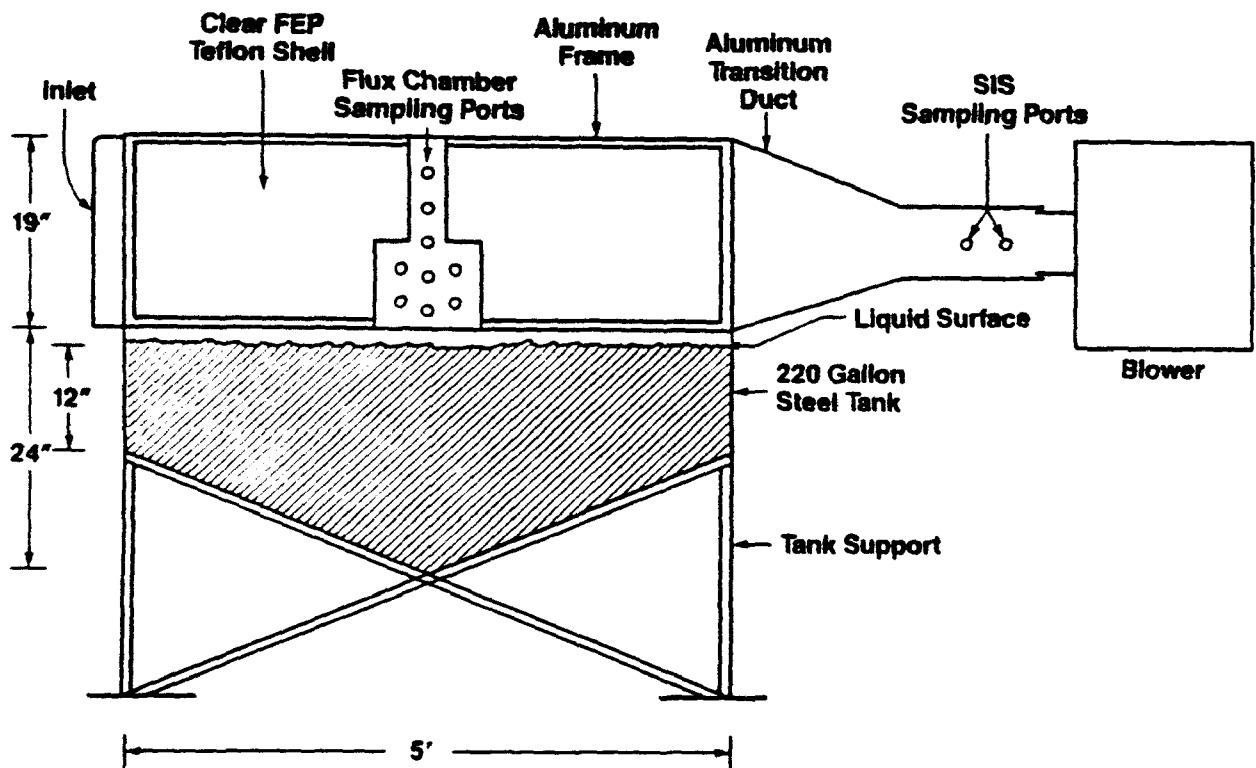


Figure 1. Side view of surface impoundment simulator (SIS).

In an attempt to define the quality of the data being produced by the flux chamber method, this study was made to determine the accuracy and precision of the flux chamber method for use on surface impoundment facilities. The influence of the experimental parameter of sweep flowrate and the environmental parameters of emission rate and volatile organic composition on accuracy and precision was investigated.

EXPERIMENTAL METHODS

The flux chamber design used for this evaluation was developed for the USEPA by Radian Corporation.⁵ It consists of a stainless steel cylinder with an enclosure area of 0.13 m². The top is enclosed with a clear acrylic dome fitted with ports for sweep flow inlet, sample outlet, temperature probes, and a gas exit. The volume of the enclosure with a 1 in. insertion depth is approximately 30 L.

The design for the surface impoundment simulator (SIS) is shown in Figure 1. The surface area of the liquid is 1.86 m² with an average depth of 0.46 m. The surface is enclosed in a shell covered with Teflon with one end opened and the other end attached to the inlet of a blower. Sampling ports are provided for two flux chambers, flow monitoring, and sampling the air before the blower.

The volatile organic compounds (VOC) measured for this study were 1,1,1-trichloroethane for single component studies and a mixture of 1,1,1-trichloroethane, toluene, and 2-butanone for the three component study. The organic components were added to the bottom of the tank. The density of the compounds or mixtures was always greater than 1.0 g/mL to prevent forming an organic layer on the surface. Two immersion heaters were located just above the organic layer to control the tank temperature and increase the convective mixing inside the tank. A pump was used periodically to increase the aqueous concentrations of the organic components.

All analyses were performed using a gas chromatograph (GC) with a flame ionization detector (FID). Flux chamber samples were collected in a syringe and injected into a gas sampling valve with a 2-mL loop. SIS air samples were collected in syringes or aluminum gas cylinders and preconcentrated on Tenax that was thermally desorbed. SIS water samples were collected in glass vials with no headspace and analyzed by syringe injection.

RESULTS

Single Component Study

Accuracy and precision of the flux chamber method were calculated from a series of colocated flux chamber measurements made inside

the SIS containing a single VOC, 1,1,1-trichloroethane. A series of nine initial emission measurements were made under similar emission conditions and the same flux chamber conditions. The sweep flowrate was set at the 5 L/min recommended by Radian's study. Table 1 shows the variance found between each duplicate measurement. The coefficient of variance (CV) was less than 5 percent for all the measurements except for two. Using a pooled standard deviation, the precision was predicted from these data to be 3.0 percent. This value was chosen to be the control condition precision.

Colocated measurements, conducted at night, were made at an emission rate approximately one tenth of the control condition measurements. Table II compares the precision calculated for these conditions with the earlier conditions. No change in the precision was found for the low emission rate conditions. The variance found at night was half that found for the control conditions. The improved precision at night could be due to an effect of sunlight on the performance of the flux chamber or the effect of the sunlight on the real emission rate. Emission rates made in full sunlight were found to be highly variable both from flux chamber measurements and SIS measurements. All measurements reported here were made in the shade or on overcast days.

The precision and accuracy of the method also were determined at a sweep flowrate of 2 L/min and 10 L/min. Table II shows the results for three colocated tests at each flowrate. A decrease in the precision value (improved precision) was found at the higher flowrate, and a slight increase in the precision value (poorer precision) was found for the lower flowrate. These results indicate that precision is improved by increasing the sweep flowrate possibly due to improved mixing at the higher flowrate.

The average emission rate calculated from a colocated flux chamber study was compared with the average emission rate calculated before and after from the total SIS measurement to predict the accuracy of the method. Table III lists the average percent bias found between the flux chamber and SIS values for the control conditions and the other flux chamber conditions studied. The bias was consistently negative (the flux chamber values lower than the SIS values). The average bias values were not significantly different within the 90 percent confidence limit (CL) for all the measurements except the low sweep flow study, which had a significantly more negative bias. This suggests that the accuracy of the method decreases at the lower sweep flowrate.

Three Component Study

The precision and accuracy study was repeated with three VOC in the tank. These included 1,1,1-trichloroethane, 2-butanone, and

TABLE I. FLUX CHAMBER PRECISION AT 5 L/MIN FOR
SINGLE COMPONENT SOURCE

Sample number	Average surface temperature	Surface liquid concentration	Average emission rate, (g/min/m ²)	% CV
1	23	130	14,300	2.1
2	23	130	12,100	0.14
3	22	130	13,200	1.5
4	21	16.2	9,730	4.6
5	21	16.2	9,750	6.0
6	23	16.2	8,910	0.46
7	23	93.6	7,470	4.8
8	24	83.6	7,350	11
9	24	93.6	7,350	4.8

CV = Coefficient of variance.

TABLE II. RESULTS OF FLUX CHAMBER PRECISION
STUDY FOR SINGLE COMPONENT

Variable parameter	Number of replicates	Precision ^a
Control	9	3.0
Low emission rate	3	2.9
Nighttime	3	1.5
2 L/min sweep flow	3	4.1
10 L/min sweep flow	3	1.4

$$^a\text{Precision} = \sqrt{\frac{(\overline{X_1 - X_2})^2}{2n}}$$

toluene. Comparing the results of nine colocated duplicate flux chamber measurements with the control of the single component study reveals several apparent differences. Table IV shows the precision estimated for each compound. The values are greater than for the single component control and vary by almost a factor of two between themselves. The increased variance may be due to the large difference in emission rates being pooled.

The accuracy of the three component results showed similar differences when compared with the single component study. Table V shows the results of bias calculations between the flux chamber and the SIS emission results. Three significantly different average bias values were found, and each one except toluene varied significantly from the single component results. Of special interest is that 1,1,1-trichloroethane in the mixture had an average bias of less than one-half of 1,1,1-trichloroethane in the single component mixture. The total emission measurement bias showed no significant difference from the single component results.

CONCLUSIONS

The results of the precision and accuracy study indicate that precise emission measurements can be made using the flux chamber method. The consistent negative bias found indicates that the flux chamber method may underestimate the emission rate from a surface impoundment. Either the flux chamber depresses the emission rate over the area it covers or the total emission rate may not be equally distributed over the surface with higher emission at the sides.

Of the experimental parameters investigated, only daylight and sweep flowrate was found to affect the accuracy or precision significantly. Results suggest that sunlight may affect the variance between two colocated flux chambers and lower sweep flow rate (2 L/min) increases the variance between measurement and increases the bias. Both precision and accuracy appear to be compound dependent and are dependent on the matrix.

Studies are recommended to determine the effect of solar intensity on both the emission rate and the flux chamber method, the cause of the compound dependency of the flux chamber accuracy, and the reason for the consistent negative bias found in the results. Plans for determining the flux chamber precision in the field currently are being made.

TABLE III. RESULTS OF FLUX CHAMBER ACCURACY
STUDY FOR SINGLE COMPONENT

Variable parameter	Number of replicates	Average bias, % + 90% CL
Control	9	-45.1 + 6.4
Low emission rate	3	-67.1 + 16.6
2 L/min sweep flow	3	-81.5 + 9.6
10 L/min sweep flow	3	-49.3 + 8.3
Nighttime	3	-57.2 + 21.3

CL = Confidence limit

TABLE IV. RESULTS OF PRECISION STUDY FOR THREE COMPONENT MIXTURE

Compound	Number of duplicate measurements	Range of emission rates		Precision, %
2-Butanone	9	11,000	42,000	6.7
1,1,1-Trichloroethane	9	46,000	100,000	10.6
Toluene	9	5,100	14,000	13.1
Total	9	65,000	160,000	8.6

TABLE V. RESULTS OF ACCURACY STUDY FOR THREE COMPONENT MIXTURE

Compound	Number of measurements	Average bias, % + 90% CL
2-Butanone	9	-68.1 + 3.1
1,1,1-Trichloroethane	9	-21.0 + 4.0
Toluene	9	-38.2 + 4.4
Total	9	-40.7 + 3.3

CL = Confidence limit

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FIELD EVALUATION OF THREE METHODS OF SOIL-GAS
MEASUREMENT FOR DELINEATION OF GROUND WATER CONTAMINATION

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ABSTRACT

Three techniques for analysis of soil gases for detection and delineation of ground-water contamination by volatile organic compounds were evaluated above two distinct VOC ground-water plumes (chloroform and benzene/chlorobenzene) in Henderson, Nevada. The objectives of the studies were to evaluate the correlation of results with the results of ground-water analyses and to assess the variability among results from locations separated by short distances. A driven probe was used to obtain grab samples for on-site analysis by field gas chromatography, the PETREX SST-Py/MS passive charcoal sampling/remote analysis system was evaluated, and the Lockheed passive-sampling system (LPSS) using industrial-hygiene charcoal passive samplers and remote analysis was tested. Results from the grab-sample/on-site analysis technique and the LPSS method showed good correlation with ground-water concentrations above the chloroform plume. Results from the PETREX SST-Py/MS technique did not correlate with chloroform concentrations. The short-range (6-foot) precision of the grab-sampling/on-site analysis method was characterized by relative standard deviations of 12 to 40 percent while that of the LPSS method ranged from 10 to 20 percent. Relative standard deviations of SST-Py/MS results over short distances were very high. Above the benzene/chlorobenzene plume none of the three techniques detected those compounds in the overlying soil gases. The only successful technique for detection of contamination there was measurement of CO₂ soil-gas concentrations on the ground-water organic carbon concentrations in monitoring wells there gave a correlation coefficient indicating a greater than 95 percent significant correlation. Further studies on soil-gas network design and data analysis are underway.

Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency through contract 68-03-3249 to Lockheed Engineering and Management Services Company, Inc., it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of commercial products or trade names does not constitute endorsement of their use.

INTRODUCTION

Protection of the nation's ground water is a national priority. Contamination from past waste disposal and leaking underground tanks can destroy aquifers for decades. Some of the worst cases of such contamination are from organic compounds because of their long lifetime in the subsurface. For this reason, environmental scientists have increasingly studied the fate, transport, and detection of these contaminants.

In order to effectively respond to subsurface organic contamination, it is necessary to establish the extent of the problem. Because of the high cost of obtaining and analyzing ground-water or soil samples, attention has recently turned to indirect methods for preliminary site characterization. By utilizing a preliminary reconnaissance-type technique, more efficient sampling networks can be planned for further work. Soil-gas surveying is an emerging technology applicable to detection and delineation of subsurface contamination by volatile organic compounds (VOC's). Table 1 lists the twenty-five most frequently encountered substances at Superfund sites; of these, fifteen are VOC's amenable to detection by soil-gas analysis. Because of the potential applications of this technology, the U.S. EPA has funded an evaluation of soil-gas surveying techniques.

In this paper, a field evaluation of three different soil-gas surveying methods is discussed. The three methods were: a grab sample/on-site analysis technique, the PETREX SST-Py/MS method, and a passive-sampling/remote-analysis system. The evaluation was above a contaminated aquifer in Henderson, Nevada.

EXPERIMENTAL

The field evaluations were performed at the Pittman Lateral in Henderson, Nevada (Figure 1). At the site unconsolidated gravel alluvium with discontinuous caliche cement forms an unconfined aquifer above a clay aquiclude and there is little or no soil development. The ground surface, water table, and upper surface of the aquiclude all dip gently towards the Las Vegas Wash to the north and groundwater flow is to the north. Unconfined groundwater occurs at 6 to 20 feet and contains two separate contaminant plumes believed to originate at the industrial complex to the south. Groundwater is monitored by analysis of samples from an east-west line of wells separated by 200-foot intervals. Figure 2 is a hydrogeologic cross section of the site. The groundwater at the Pittman Lateral contains a wide variety of organic and inorganic substances. However, for the purposes of this study, the chloroform plume on the eastern side (up to 900 $\mu\text{g/L}$) and the benzene/chlorobenzene plume on the western side (up to 5,000 $\mu\text{g/L}$)

TABLE 1. MOST FREQUENTLY IDENTIFIED SUBSTANCES AT
546 SUPERFUND NATIONAL PRIORITY LIST SITES^a

Rank	Substance	Henry's Law Constant ^b (ppbv•L/μg)	Percent of Sites
1	Trichloroethylene	72	33*
2	Lead and compounds	NA	30
3	Toluene	56	28*
4	Benzene	71	26*
5	Polychlorinated biphenyls (PCBs)	<<1	22
6	Chloroform	40	20*
7	Tetrachloroethylene	123	16*
8	Phenol	<<1	15
9	Arsenic and compounds	NA	15
10	Cadmium and compounds	NA	15
11	Chromium and compounds	NA	15
12	1,1,1-Trichloroethane	30	14*
13	Zinc and compounds	NA	14
14	Ethylbenzene	59	13*
15	Xylene	43	13*
16	Methylene chloride	23	12*
17	<u>trans</u> -1,2-dichloroethylene	570	11*
18	Mercury	NA	10
19	Copper and compounds	NA	9
20	Cyanides (soluble salts)	<<1	8
21	Vinyl chloride	5100	8*
22	1,2-Dichloroethane	88	8*
23	Chlorobenzene	32	8*
24	1,1-Dichloroethane	420	8*
25	Carbon tetrachloride	3600	7*

^a Source: Kerfoot, 1987.

^b Henry's Law constant is the equilibrium gas-phase VOC concentration (ppbv) divided by the concentration in water (μg/L).

* Compounds amenable to detection by soil-gas analysis.

are of interest because these compounds are VOC's amenable to soil-gas surveying.

The three techniques have been described in detail elsewhere (Kerfoot, 1987; Voorhees et al., 1984; Kerfoot and Mayer, 1986). The grab-sample method uses a steel pipe with an internal stainless steel tube connected to sampling ports in the probe tip. The probe is hammered in to a prescribed depth, a sampling manifold is attached, soil gas is withdrawn through the manifold, and subsamples are taken from the manifold with a gastight syringe. The syringes are then carried to a nearby mobile laboratory for analysis by gas chromatography. Real-time results for the VOC concentration at the sampling location are obtained. The PETREX[®] SST-Py/MS method uses a wire with a charcoal-coated tip placed in a glass screwcap tube. The tube is opened and placed with the open end down in a shallow (ca. 1 foot) hole and buried. The sampler is retrieved after a measured exposure time, sealed, and shipped to a laboratory for analysis by pyrolysis/mass spectrometry (Py/MS). Results are reported in ion counts and are said to represent the "integrated vertical flux" of VOC's at the sampling location. The Lockheed passive-sampling system (LPSS) uses diffusional charcoal samplers. The samplers are buried in a manifold at a shallow depth (ca. 1 foot) for a prescribed time, are retrieved and sealed, and are transported to the laboratory for analysis by gas chromatography. Results are in an average concentration of VOC's at the sampling locations. Carbon dioxide measurements were made using the probe described above and a carbon dioxide detector tube (Draeger). Samples were from a depth of 5 feet. Organic carbon analyses were performed with a Dohrman carbon analyzer.

Sampling above the chloroform-contaminated ground water was performed at the locations shown in Figure 3. Sampling above the ground water contaminated with benzene and chlorobenzene was performed at the locations shown in Figure 4. Grab samples were taken from a depth of 4 feet except where otherwise noted. Passive samplers and PETREX[®] SST-Py/MS samplers were buried at a 1-foot depth; passive samplers were left in place for 14 days above the chloroform plume and for 3 months above the benzene/chlorobenzene plume. All PETREX[®] SST-Py/MS samplers were left in place for 10 days.

Samples were taken at locations 20 feet from monitoring wells to allow evaluation of the correlation of the soil-gas results with the results of ground-water analyses. At some sampling locations, closely spaced (ca. 3 feet) groups of two, three or four samples were placed to allow evaluation of the short-range variability of results from each technique. Because of the results obtained above the benzene/chlorobenzene contamination, the passive samplers that measure concentration were deployed near only one well and were left in place for a very long time (3 months) and grab samples were taken

within 1 foot of highly contaminated ground water, in an effort to detect those compounds in soil gas there. PETREX[®] samplers were left in place for ten days.

RESULTS AND DISCUSSION

Chloroform Plume

Table 2 lists the ground-water chloroform concentrations, the mean soil-gas chloroform concentrations of grab samples from a 4-foot depth as measured by on-site gas chromatographic analysis, the results from the Lockheed passive sampling system (LPSS) at a 1-foot depth, and the PETREX[®] SST-Py/MS results. The 4-foot grab-sample and LPSS data correlate well with the ground-water data, and the two data sets correlate very strongly with each other. The SST-Py/MS results (ion counts) do not correlate with the ground-water data; the highest results (chloroform ion counts) were obtained from samples 800 feet away from the closest location where chloroform was detected in the underlying ground water. Figure 5 shows the mean results from each technique and ground-water analyses as a function of the east-west coordinate.

Table 3 lists the mean values and associated standard deviations for the closely spaced samples analyzed by each method. The precision shown among these measurements helps indicate how much of a variation in results can be attributed to inherent variability of the method. This variability is a combination of sampling and analysis imprecision along with real variations in the chloroform concentrations. The precision of the grab-sample/on-site analysis method, as indicated by the relative standard deviation (RSD; $100 \times \text{standard deviation} / \text{mean}$), was 7 percent and 43 percent; the passive-sampling method gave RSD values between 12 and 25 percent; the SST-Py/MS results had RSD values of 98 percent (3 samples) and 140 percent (2 samples).

On the basis of the results of evaluation above the chloroform plume, both the grab-sample/on-site analysis technique (Kerfoot and Barrows, 1987) and the passive-sample/remote-analysis method (Kerfoot and Mayer, 1986) gave results that correlated well with ground-water concentrations. In addition, acceptable precision was obtained with both methods. The PETREX[®] SST-Py/MS method gave results that did not correlate with ground-water chloroform concentrations and the precision of results from closely spaced samplers was unacceptable.

BENZENE/CHLOROBENZENE PLUME

Results of grab sampling with on-site analysis above the benzene and chlorobenzene contaminant plume did not indicate the presence of these compounds anywhere in soil gases above the contaminated ground

TABLE 2. GROUND-WATER AND MEAN SOIL-GAS ANALYTICAL RESULTS ABOVE THE CHLOROFORM PLUME

Well	Ground-Water Concentration ($\mu\text{g/L}$)	Mean Soil-Gas Concentration (ppb) ^a		SST-Py/MS Results (ion counts) ^a
		Grab Sample (4 ft) ^a	Passive Sampling (1 ft)	
635	ND ^b	--d	--d	5482
631	ND ^b	5 ^c	ND ^b	--d
629	11	23	2.0	--d
627	175	68	7.8	--d
625	866	370	19.2	3121
624 ^e	---	150	14.3	--d
623	555	40	2.4	2835
621	ND ^b	10.5 ^c	11 ^c	--d

- ^a Mean of 4 locations around the well (see Figure 2) unless otherwise noted; source: Kerfoot and Barrows, 1987.
- ^b ND = not detected; ground-water detection limit = 5 $\mu\text{g/L}$, passive sampling detection limit = 0.02 ppb.
- ^c Only one location.
- ^d Not sampled
- ^e Location 624 is halfway between 623 and 625.

variability is a combination of sampling and analysis imprecision along with real variations in the chloroform concentrations. The precision of the grab-sample/on-site analysis method, as indicated by the relative standard deviation (RSD; $100 \times \text{standard deviation} \div \text{mean}$), was 7 percent and 43 percent; the passive-sampling method gave RSD values between 12 and 25 percent; the SST-Py/MS results had RSD values of 98 percent (3 samples) and 140 percent (2 samples).

TABLE 3. MEAN AND RELATIVE STANDARD DEVIATIONS OF CHLOROFORM MEASUREMENTS FOR CLOSELY SPACED SAMPLES^a

Location	Passive-Sample (ppb;1-ft depth)	Grab-Sample (ppb;4-ft depth)	SST-Py/MS (ion counts; 1-ft depth)
635	--	--	4593 (140) ^b
631	N.D.	--	--
629	2.6 (14) ^c	--	--
627	6.7 (14) ^c	124 (43) ^d	--
625	15 (20) ^c	--	--
624	14.3 (12) ^e	189 (7) ^f	--
623	2.6 (25) ^c	--	9092 (98) ^f
621	N.D.	--	--

^a Relative standard deviation (%) in parentheses

^b Two samples separated by 3 feet

^c Four samples in a square pattern 3 feet on a side

^d Four samples in a trapezoidal pattern 3 ft x 3 ft x 6 ft x 4.2 ft

^e Ten samples in a north-south line at 3-foot intervals

^f Three samples in a line at 3-foot intervals

water. Although grab samples were taken 1/2 to 1 foot above the water table adjacent to well 641, neither benzene nor chlorobenzene was detected in them. LPSS samplers exposed for three months did not show any benzene or chlorobenzene, either. Table 4 lists the concentrations measured in ground-water samples from wells in that plume as well as soil-gas results from nearby sampling locations. Because PETREX[®] SST-Py/MS results were the only ones to detect benzene or chlorobenzene in soil gases above that contaminant plume, an evaluation of the precision of results among closely spaced samplers was performed. At the two locations with multiple samplers the RSD values were 81 percent (two samplers) and 170 percent (three samplers) for benzene. Chlorobenzene ion counts had an RSD value of 58 percent (2 samples at 641), with all non-detect results at the other multi-sampler location (649).

In consideration of the contrasting results obtained above the chloroform plume and the benzene/chlorobenzene plume, under the same hydrogeologic circumstances it was postulated that compound-specific properties must be responsible for the differences observed. Chloroform has Henry's Law and diffusion constants intermediate to those of benzene and chlorobenzene and the maximum concentrations of the aromatic compounds are nearly an order of magnitude higher than chloroform, so that differences in partitioning into soil gases or diffusing through the vadose zone are not the causes of the situation encountered. It has been noted that hydrocarbons biodegrade readily in shallow soil gases (Evans and Thompson, 1986), and chlorobenzene has also been shown to biodegrade aerobically while chloroform is resistant to that process (Bouwer, 1984). Therefore, the disappearance of the two aromatic compounds from the soil gases could be due to this process.

As a test of this hypothesis, carbon dioxide concentrations in soil gases were measured at a 5-foot depth several locations at the site and dissolved organic carbon concentrations of ground-water samples were measured. Table 5 shows the two data sets. Linear regression shows that there is a 90 percent significant correlation between the two variables. This is in agreement with, but does not prove, the hypothesis that aerobic biodegradation is occurring at a rate limited by the concentration of organic carbon present.

CONCLUSIONS

Soil-gas surveying can be a valuable field reconnaissance technique to delineate VOC contamination. Both active sampling with on-site analysis and passive sampling with remote analysis methods gave results that adequately delineated ground-water VOC contamination in this study, although not all the passive sampling methods used gave the same quality of data. However, the methods that were used successfully were not able to detect nearby subsurface contamination at the same site. Although evidence indicates that aerobic

TABLE 5. GROUNDWATER DISSOLVED CARBON AND SOIL-GAS CARBON DIOXIDE CONCENTRATIONS

Well	Organic Carbon (mg/L)	Inorganic Carbon (mg/L)	Soil-Gas Carbon Dioxide (%)
631	2.52	40.80	0.089
641	13.01	75.99	0.452
643	12.27	73.20	0.318
645	14.79	76.80	0.352
647	12.54	73.65	0.410
649	9.20	57.86	0.096

TABLE 4. GROUNDWATER AND SOIL-GAS MEASUREMENTS FOR BENZENE/CHLOROBENZENE PLUME

Well	Ground-Water Concentration (µg/L)		Soil-Gas Concentration (ppbv)				Mean ^a SST-Py/MS Results (ion counts)	
			Grab Sample		Passive Sample			
	C ₆ H ₆	C ₆ H ₅ Cl	C ₆ H ₆	C ₆ H ₅ Cl	C ₆ H ₆	C ₆ H ₅ Cl	C ₆ H ₆	C ₆ H ₅ Cl
623	<10	<10	ND	ND	ND	ND	218	103
635	340	520	---b	---b	---b	---b	793	575
639	4700	3200	ND	ND	ND	ND	529	139
641	3200	4520	ND	ND	---b	---b	380	121
645	3100	4880	ND	ND	---b	---b	218	133
649	1300	2400	ND	ND	---b	---b	189	148
653	<10	<10	ND	ND	---b	---b	353	133
Blank	--	--	ND	ND	ND	ND	ND	ND

^a Mean of samplers around a well.

^b Not sampled.

biodegradation may be responsible for that, further work would be required to prove the hypothesis. However, these results indicate that workers using the technology should be careful to demonstrate its correlations with subsurface contamination and its short-range precision at each site as routine quality assurance precautions.

ACKNOWLEDGEMENTS

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Figure 1
Study Site Location.

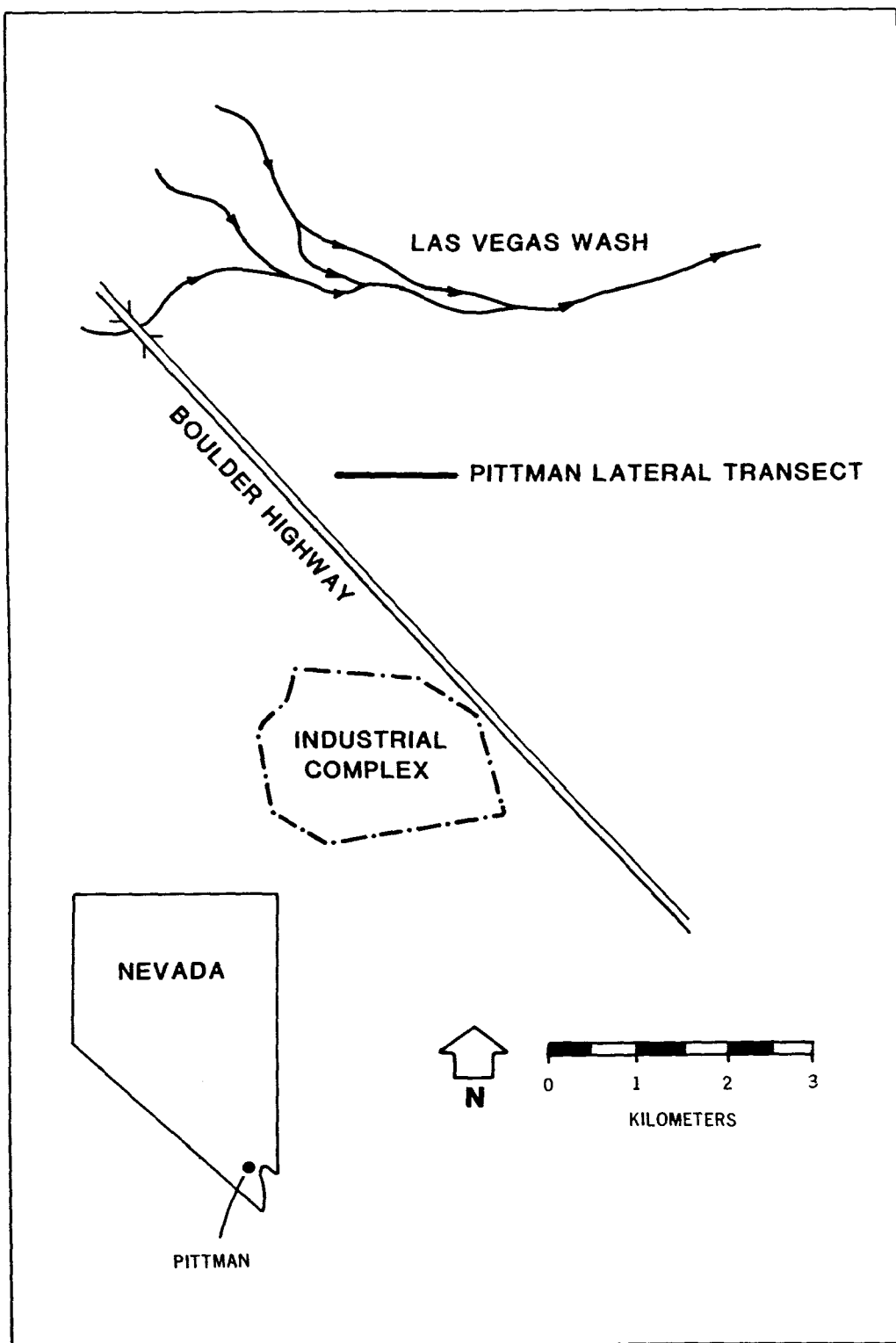


Figure 2

Hydrogeologic Cross Section of the Study Site.

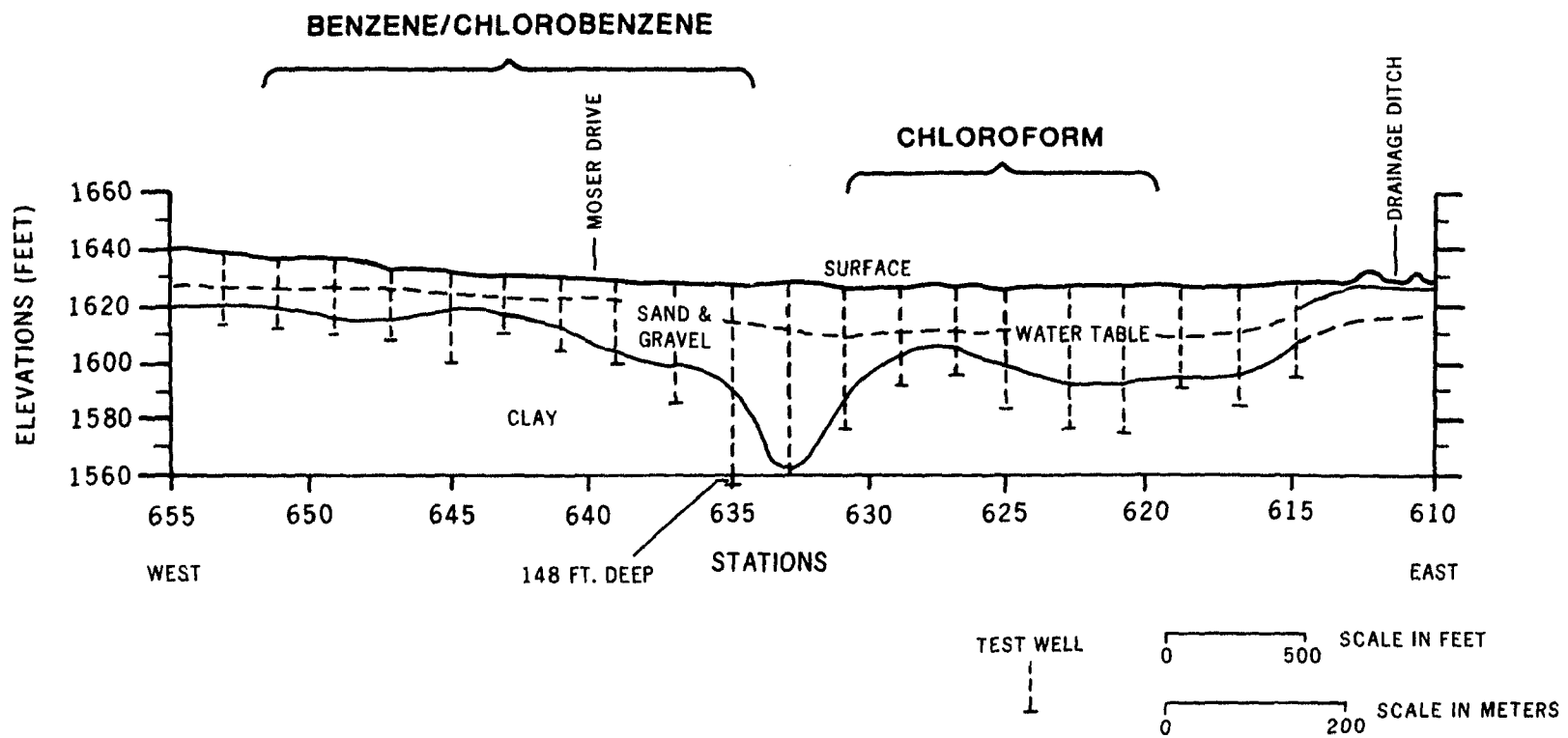


Figure 3

Sampling Locations Above the Chloroform Plume.

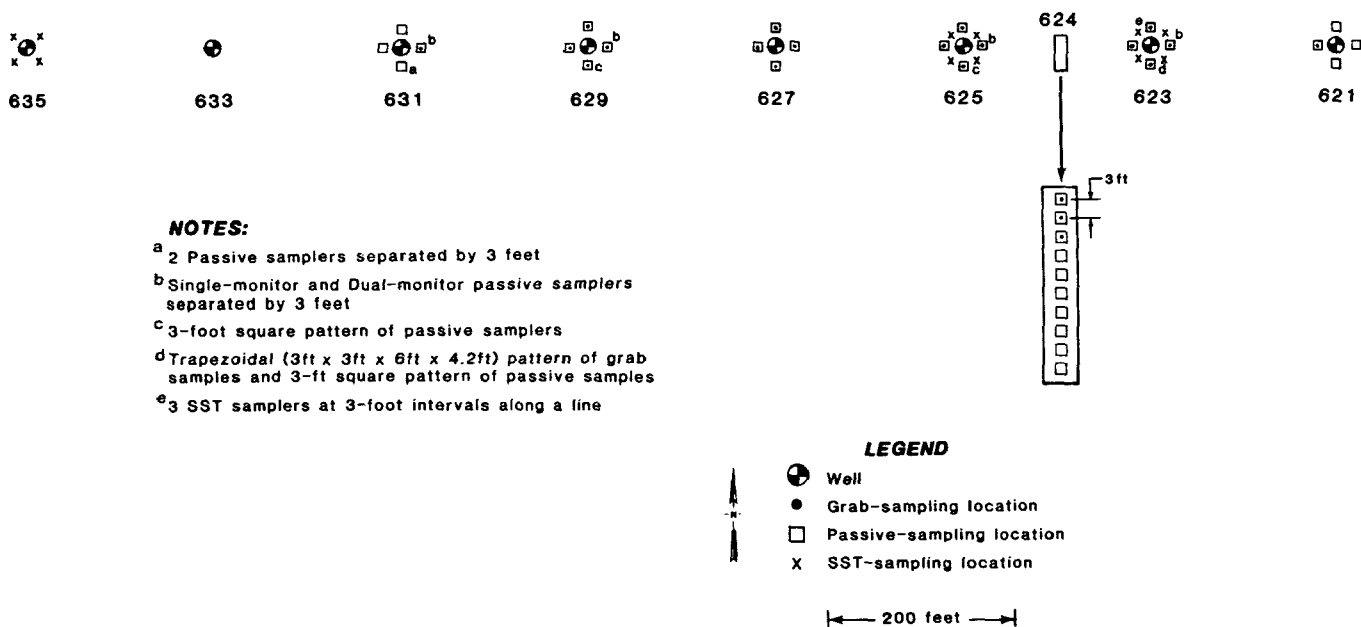


Figure 4

Sampling Locations Above the Benzene/Chlorobenzene Plume.

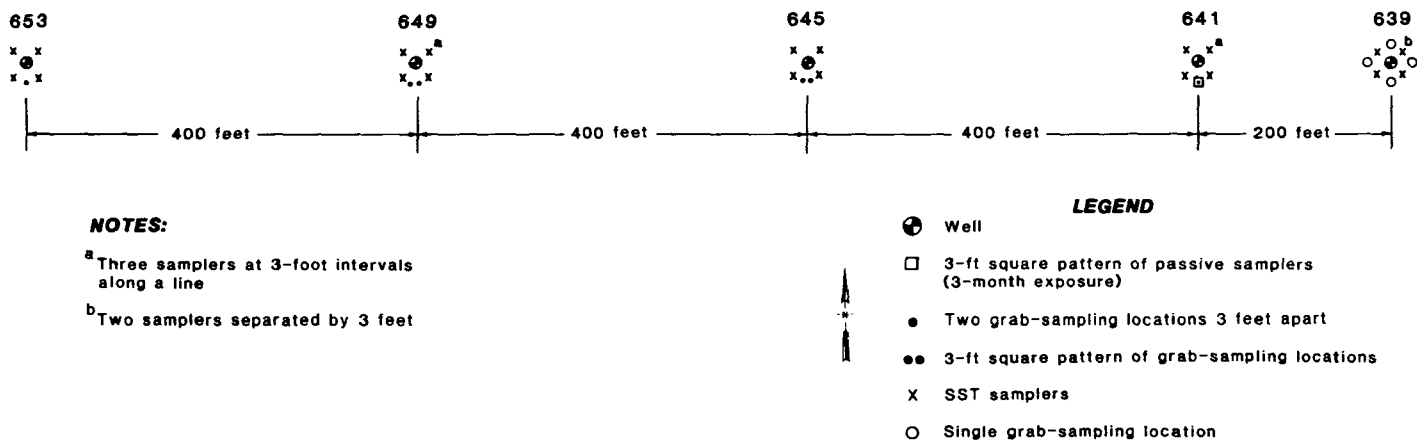
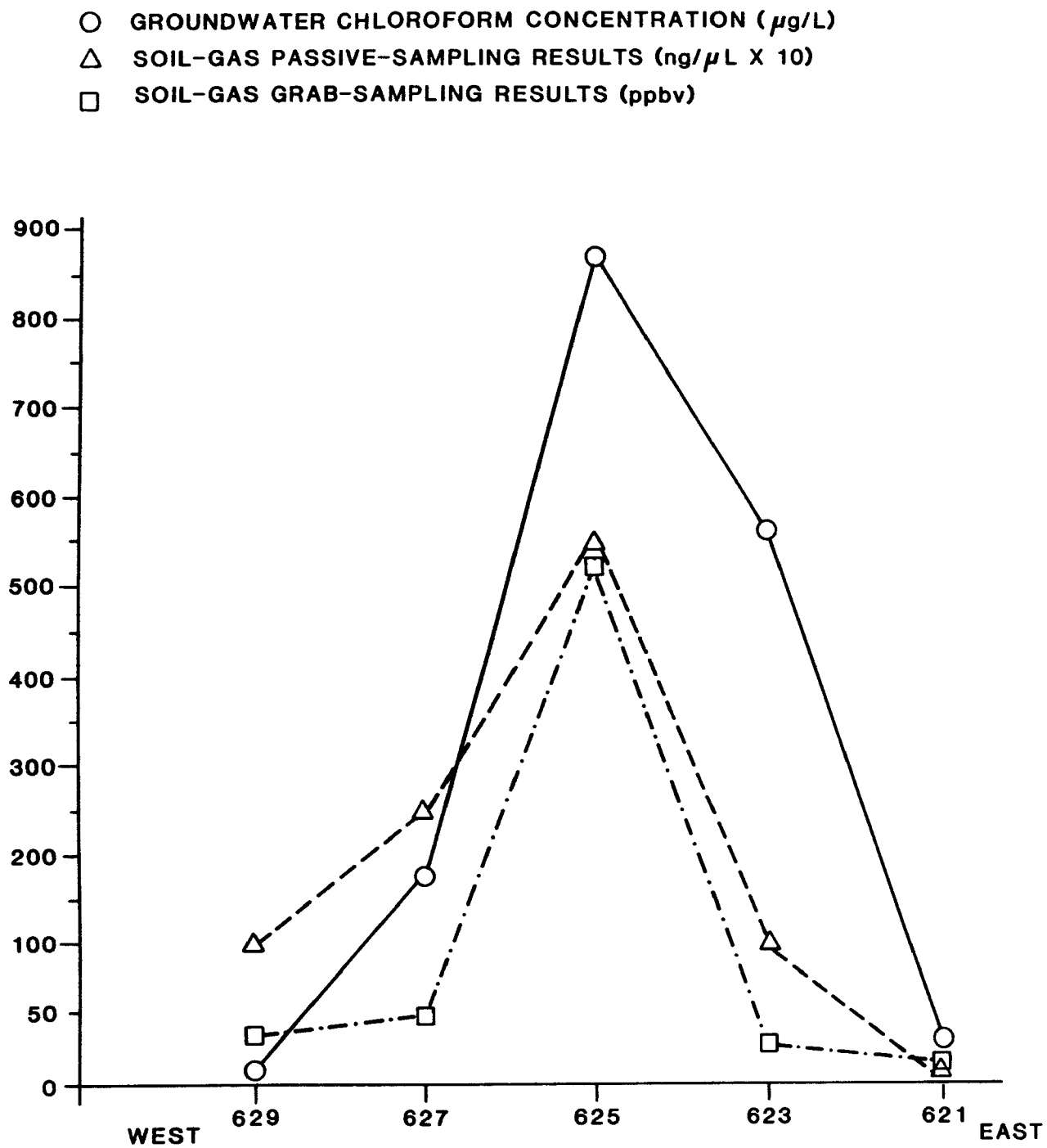


Figure 5

Soil-Gas and Ground-Water Measurement Results.



PROCEDURES USED TO MEASURE THE AMOUNT OF
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
(2,3,7,8-TCDD) IN THE AMBIENT AIR
NEAR A SUPERFUND SITE CLEAN-UP OPERATION

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ABSTRACT

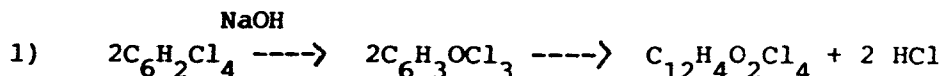
Sampling and analytical procedures are described that were successfully used to monitor for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in air samples collected near a superfund site clean-up operation. Measured concentration of 2,3,7,8-TCDD in air samples are related to both an action level (3.0 picograms per standard cubic meter of air) and to a calculated no observed effect level (5.5. pg/M³). The study concluded that it is possible to collect reliable data for 2,3,7,8-TCDD in air at concentrations that are below the action level specified by the Centers for Disease Control. Data quality was defined relative to the quality control procedures described in the study. There was no apparent relationship between particulate matter in the air and 2,3,7,8-TCDD in the air.

INTRODUCTION

For some animal species, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is one of the most toxic synthetic compounds known.¹ The LD-50 has been reported to be 0.6 ug per Kg of body weight for the guinea pig, 115 ug per Kg for the rabbit and 22 ug per Kg for the rat.¹ However, 2,3,7,8-TCDD does not appear to be nearly as toxic to humans as it is to other animal species.² Some of the chlorinated dioxin isomers are, for all practical purposes, not toxic at all. The LD-50 for 2,7-dichlorodibenzo-p-dioxin is approximately 2.0 g/Kg of body weight for the rat.¹ These facts require that any analytical procedure used to measure for the concentration of 2,3,7,8-TCDD must be isomer specific.

In addition to being toxic, 2,3,7,8-TCDD is also a suspected carcinogen. Recently, Dr. R. Kimbrough and others suggested the extremely low number of 2.8×10^{-14} g/Kg body weight per day as being the dose responsible for a cancer risk of one in one million.³ Previous studies have detected concentrations of labeled dioxin in the air in the range of 10^{-15} grams per cubic meter.¹ Several studies have shown the presence of dioxins in emissions from incinerators at similarly low concentrations. Attempts to measure dioxin concentrations in ambient air at these levels require very sensitive analytical procedures.

In the early 1970's, 2,3,7,8-TCDD was formed as a byproduct in the production of hexachlorophene and/or Agent Orange in a small facility in southwest Missouri. It is known that, unlike some other sources of dioxin, this process resulted in a very high fraction of the 2,3,7,8-TCDD isomer being formed. It is now believed that the 2,3,7,8-TCDD was produced by condensation of 2,4,5-trichlorophenol. The phenol resulted from alkaline treatment of 1,2,4,5-tetrachlorobenzene as shown below in equation 1.



Waste materials containing the dioxin byproduct were mixed with used oil and subsequently applied to roads and other surfaces for dust control.

One of the places where the waste material from southwest Missouri was applied was a trailer park near St. Louis, Missouri. The site covers an area of approximately 11 acres. It has an irregular shape as shown by the site map (Figure 1). The surface and subsurface soils at the site were tested and were found to contain 2,3,7,8-TCDD at concentrations above 1.0 ug per Kg of soil (1.0 ppb) at most locations within the site. Therefore, a mitigation plan was prepared to control exposure to the contaminated soil.⁵ The plan called for the removal of the contaminated soil from the surface and for storage of the removed material in a safe location on site until detoxification procedures are available.

The EPA has a responsibility under the comprehensive Environmental Response, Compensation and Liability Act (CERCLA or Superfund) to clean up hazardous waste sites known to be contaminated with toxic or hazardous materials. A second responsibility is that agency clean-up activities must not constitute a source of pollution for members of the general public who are in the immediate vicinity of the clean-up operation. At the beginning of this work, it was not apparent that it would be possible with available technology to meet both of these responsibilities for the site in question. This paper describes considerations which went into the design and operation of the network and our findings during the first five months of operation. Some of the specific questions we had to address and hoped to answer are listed below:

Can the concentration of 2,3,7,8-TCDD in ambient air be measured at levels that cause an insignificant risk to the public?

Can a cost-effective network which measures the average amount of dioxin in the air be implemented over time, with a high degree of confidence? If not, are other options available that will provide acceptable answers?

If dioxin is found in the air, will it be absorbed to particulate matter that is trapped on a filter or will it be in the vapor phase or both?

Relative to local weather conditions and the normal scatter in any measurement, how many sampling sites should the network contain? How many samples should we collect at each sampling site each day? How long should the network be operated.

What are the best available procedures to collect a sample? What are the best analytical procedures? What reliability can be expected from these procedures?

Is there any relationship between the amount of particulate matter in the air and 2,3,7,8-TCDD in the air?

Is there a measurable cause-effect relationship between clean-up operations and measured concentrations of 2,3,7,8-TCDD in the air? Is any of the 2,3,7,8-TCDD in the air coming from background sources?

If significant concentrations of 2,3,7,8-TCDD are found in the air, can models be used to predict the path of any plume that might be caused by the clean-up operations?

Are any of the pollution abatement actions more effective in controlling the release of dioxin than others?

PROCEDURES

1. Design of the Monitoring Network

When designing the monitoring network, we assumed that we needed a measurement detection limit in the range of 0.1 to 1.0 picogram/M³ in order to obtain reliable measurements at the 5 picogram/M³ level. The response from the Centers for Disease Control (CDC) contained their recommendation of 5.5 picogram/M³ as an estimated no observed effect level (NOEL) and their concurrence with our recommendation of 3.0 picogram/M³ as a "warning" or action level.⁶ Realizing that the total cost of sample collection and analyses would probably exceed \$1,000 per sample, our objective was to collect the minimum number of samples that would have a good (95% confidence level) probability of showing an exceedance of the action level if one occurred and of showing that no exceedance occurred if indeed one did not occur. Based on prior experience, we estimated that we could monitor for the concentration of 2,3,7,8-TCDD in ambient air at these levels with an accuracy of 25% relative standard deviation based on the recovery of field spikes. We assumed that the upwind and downwind site data would each have a normal distribution. The number of measured concentrations (samples) needed at each sampling location was then calculated as a function of the

difference between the action level (true mean) and the mean of a given number of measured values.

The statistical calculation is outlined as follows. The average concentration of all samples collected at a site is used as an approximation of the true average concentration (which would be obtained from continuous monitoring of the air over a long period of time). The uncertainty of that approximation is indicated by the 95% confidence limits as calculated by the following formula.

$$95\% \text{ confidence limits} = \bar{Y} \pm t_{.975, n-1} \frac{s}{\sqrt{n}}$$

where \bar{Y} = the sample average

n = the number of samples at each sampling location

s = the standard deviation of samples collected at that sampling location

$t_{.975, n-1}$ = the tabulated student's t value for $n-1$ degrees of freedom

The width of the 95% confidence interval for a site depends on the number of samples collected and the scatter among the data from those samples, as is shown in Table 1.

A comparison of the experimental data with the action level will result in a conclusion that no exceedance occurred if the 95% confidence interval is completely below the action level. The data will demonstrate that an exceedance did occur if the 95% confidence interval is completely above the action level. The data will demonstrate that more samples would be needed in order to show conclusively whether or not the true average concentration exceeded the action level if the action level is within the 95% confidence level.

We elected to initially collect 14 samples at each monitoring site. Using 14 data values and the assumptions described above, a definitive conclusion would then be reached if the data were either 14% above or below the action level.

In order to obtain sufficient data to be able to assess the effects of on-site activities on the off-site ambient air under variable wind conditions, the monitoring network had to be designed to provide long-term monitoring of the air at or near the property boundaries of the site. Based on the physical configuration of the site (see Figure 1), which is long relative to its width, we decided that a minimum of six fixed monitoring locations would be needed to insure consistency throughout the study and to have one upwind

on the appropriate number of monitoring sites in the network, was to minimize the probability that contaminated air would pass between the monitors without being detected. The three largest variables effecting this probability were the size of the area within the site that might be releasing dioxin, the local variation in the wind direction during a sample collection time period and the duration of the study.

Considering that dioxin might be released from approximately one-fifth of the site at any given time from truck traffic and construction work, that the wind direction was very seldom in one direction for 80% of a 24-hour period and that the study would require approximately 200 working days, we concluded that six monitoring sites would be sufficient. An on-site meteorological monitoring station was incorporated into the network design for the purpose of obtaining adequate wind speed and direction data.

A site visit was made prior to finalizing the network design to obtain detailed information on the topography, and to choose the specific sampling locations relative to anticipated activities at the site. The specific locations for the air samplers were selected so they would be near but just inside the perimeter fence for security purposes, would be consistent with accepted siting guidance for criteria pollutant monitoring, would provide permanent placement throughout the life of the project (i.e., the samplers would not have to be relocated during the course of the excavation activities at the site), and would provide adequate coverage for most wind directions. The specific sampling locations which were selected are shown in Figure 1.

2. Collection of Representative Samples

To collect representative samples, we used commercially available modified high-volume air samplers that employ both a filter for collecting particulate matter and a solid adsorbent for collecting vapors. A diagram of the air sampler (General Metal Works, Inc., Model PS-1) is shown in Figure 2. In operation, a known volume of air (calculated from the flow rate and time of sampling) is drawn through a dual-chambered sampling module (see Figure 3) and exhausted to the air via a 10-foot exhaust duct. The upper portion of the sampling module holds a 4-inch diameter glass fiber filter which collects the particulate matter and the lower portion consists of a cylindrical glass cartridge (65 mm x 125 mm) containing a 3-inch-long solid adsorbent which entraps selected vapor phase compounds. Polyether-type polyurethane foam (PUF) plugs were utilized as the solid adsorbent material.

To measure the very low concentrations of dioxin required in this project, a large sample volume of air was required. Since the air samplers are only capable of providing a flow rate of approximately 0.280 cubic meters/minute, it was determined that the samples should be collected on a 24-hour basis ($\pm 15\%$). This time and flow will give a sampled air volume of 300-400 cubic meters of air.

The samplers were placed on 1-meter high platforms to obtain samples of the ambient air in the breathing zone. The samplers were operated daily except on Sunday. If excavation occurred on Sunday, the samplers were operated on that day as well.

We assumed the wind direction could not be predicted for the 24-hour sampling period, nor would it be constant over that period of time. Therefore, all of the samplers were operated to collect samples each day.

During the first 14 days of sampling, all of the samples were analyzed for both particulate matter and 2,3,7,8-TCDD to obtain baseline data for the site. Pollution abatement activities were occurring at the site during this time period. Subsequent to this initial sampling period, only one upwind and one downwind sample was submitted for 2,3,7,8-TCDD analysis each day. The selection was based on the prevailing wind direction and the amount of particulate matter collected on each filter for the sampling period.

3. Comparability

For a risk assessment, we estimated the maximum amount of dioxin a person just off-site would experience during the time of the clean-up activities. We decided to use a daily averaging process because an average is more comparable to an action level that is based on chronic effects than is a single value. We decided to average the data values from each monitor separately (rather than average data from different monitors) because that would be more representative of exposure for someone living near that monitor. Since we wanted to be able to take pollution abatement actions as quickly as possible after the data were available, we decided to use a 14-day running average (average concentration of the most recent 14 days) which would be calculated daily. In the interest of safety, we elected to use the detection limit as a measured value when calculating the running averages for all samples that did not contain a measurable concentration of 2,3,7,8-TCDD. Finally, since none of the analyses of non-downwind samples showed any measurable 2,3,7,8-TCDD, we decided to use an average of these numbers for those days when data were not available. A daily data point might be missed for a specific monitor if that monitor was neither upwind nor downwind of the site, or if no work was occurring at the site due to bad weather or for a non-working Sunday.

4. General Procedures

Particulate Matter (PM) and 2,3,7,8-TCDD sampling utilized samplers as previously described. Polyurethane foam (PUF) cartridges were precleaned by the laboratory and shipped to the on-site sample coordinator in sealed, glass sample jars. Filters were obtained from the PUF sampler manufacturer in lots of 100. Five percent of the filters were run as blanks to ensure acceptable detection limits. Sample modules were collected and new samples started each morning prior to the start of any remedial activity on site. Surgical gloves

and Teflon tipped forceps were used to remove the glass fiber filter and the PUF cartridge from the sample module. The glass fiber filter was placed on a calibrated balance and weighed to the nearest 0.1 milligram. The glass fiber filter was then folded in half twice (sample side inward) and placed in the glass cartridge on top of the PUF plug. The sample cartridge was then wrapped in aluminum foil (to shield it from sunlight) and placed in the sample jar from which it came.

The concentration of the particulate matter (PM) for each sample was calculated immediately and used in the selection of samples for 2,3,7,8-TCDD analyses.

5. Analytical Procedures

All samples were analyzed by an EPA contractor in accordance with a Region VII standard method titled "Determination of 2,3,7,8-TCDD in Air Samples Using Gas Chromatography-Mass Spectrometry."

The samples were spiked with internal ($^{13}\text{C}_{12}$ -2,3,7,8-TCDD) and surrogate ($^{37}\text{Cl}_4$ -2,3,7,8-TCDD) standards of isotopically labeled 2,3,7,8-TCDD. The samples (filter, PUF and glass cylinder) were then extracted with methylene chloride in a Soxhlet apparatus. The extracts were cleaned using silica gel, modified silica gel, alumina and carbon prior to the analyses by high resolution gas chromatography and low resolution mass spectrometry.

The gas chromatography column was a 30m x 0.32 mm I.D. fused silica capillary DB-5 with a 0.25 μ film thickness. Calibration was done by tabulating the peak heights or peak areas from triplicate injections of 2,3,7,8-TCDD versus the internal standard. Quantification is based on the response of native TCDD relative to the isotopically labeled TCDD internal standard. Performance is assessed based on extensive quality assurance requirements. These include a requirement for accuracy of a surrogate analyses on each sample.

6. Quality Assurance

To minimize sample handling and/or contamination in the field, two sampling modules equipped with quick release connectors were acquired for each air sampler. The availability of two complete sampling modules, which were numbered for ready identification, made it possible to simply exchange a clean sampling module for the module containing the sample in the field. The modules were transported to and from the sampling sites individually wrapped in plastic bags and stored in a closed container (an ice chest customized to hold the modules upright). This practice enabled all sample handling (disassembly of module and placement of the filter, PUF and glass cartridge in a sample container) and sampling module preparation (cleaning and assembly) to occur in a controlled environment.

A five-point calibration curve (equation) for each sampler was obtained initially using a magnehelic gauge and a calibrated orifice. The equation for each sampler was then used to calculate sample volumes. The calibration was repeated monthly and a one-point check was performed every other week. A flow audit on 25% of the air samplers in use was performed monthly by an individual other than the normal operator and with a different calibrated orifice. The maximum acceptable difference between the reported (sampler) and actual (calibrated) flows was established as $\pm 7\%$.

Laboratory GC/MS instrument calibration consisted of an initial 3-point calibration conducted in triplicate. The mean, standard deviation, and % Relative Standard Deviation (RSD) of the Relative Response Factor (RRF) for 2,3,7,8-TCDD was calculated at each of the three concentration levels, as well as for the overall. Acceptable calibration required an RSD of 10% or less at each individual level, as well as for the overall. Every 8 hours, the calibration was to be verified through the analysis of a low level standard solution. The percent difference of the RRF for the continuing calibration from that of the overall mean RRF of the initial calibration could not be more than 10%.

Both system and performance audits were included in the air monitoring plan to ensure that the established procedures were actually being followed. The audit process provided the means for continually evaluating the quality of the data being generated, identifying apparent problems quickly, and making in-process changes to correct apparent problems.

The use of quality control samples was included as a routine means of tracking the precision and accuracy of the data generated and detecting problems relating to the quality of the data reported. Throughout the sampling effort, one field blank, one blank to be spiked by the laboratory, and one field-spiked sample were submitted for analysis with every 17 actual samples. In addition, during the initial 14-day sampling period, a second air sampler was collocated with one of the perimeter samplers to collect duplicate field samples.

RESULTS, DISCUSSION AND CONCLUSIONS

Data Quality

We have grouped the data quality information into qualitative information and quantitative data.¹⁰ Based on the results of our field and laboratory audits, we concluded that the procedures described above were being followed as written, and that, with the exception of two data points, all data were acceptable relative to the qualitative variables.

Two concentration levels were utilized in the performance evaluation (PE) samples. A high level PE of 11.6 ng was used to monitor bias above the exposure limit. The three data points generated at this level gave a mean value of 10.99 ng or 95% recovery. Control limits at

the 95% confidence interval were 9.65-13.6 ng. Twenty-six medium level performance audit samples containing 5.8 ng were analyzed. Mean recovery was 5.29 ng or 91% recovery. A 95% confidence interval of 4.35 - 6.09 ng was determined.

The field blanks consisted of a filter, PUF and glass cylinder to which only surrogate and internal standard solutions were added. The 19 field blank analyses resulted in no detectable quantities of 2,3,7,8-TCDD.

The laboratory fortified 19 samples with 5 ng of 2,3,7,8-TCDD. The mean recovery of these was 4.75 ng or 95% recovery.

Nine audits of the sampler flows were performed during the study and gave a standard deviation of flow difference of 1.72%.

Considering the variances in the measurements of both the volume of air sampled and the amount of dioxin found in the sample, we estimate the data are accurate to within $\pm 12\%$ of the reported values at the 95% confidence level.

Is 2,3,7,8-TCDD in the Vapor Phase or on Particulate Material?

Two experiments were conducted to try to determine what fraction of the 2,3,7,8-TCDD and 2,3,7,8-TCDF would pass through the filter and what fraction would remain on the filter under the sampling procedures described above. We assume that the materials were in the vapor phase when they passed through the filter.

The first experiment was designed to evaluate the potential for analyte breakthrough of 2,3,7,8-TCDD, 1,2,3,4-TCDD and 2,3,7,8-TCDF. The experiment consisted of spiking clean filters with 10 ng each of 2,3,7,8-TCDD and 2,3,7,8-TCDF and 13.8 ng of 1,2,3,4-TCDD in 200 μ l hexane. Ambient air was then drawn through the samplers for varying lengths of time using identical flow rates. At least three samples were collected for each time period. The PUF and filter were analyzed together as described above. The results of the experiment are shown in Table 2.

From these data we conclude that the sampling procedures are effectively collecting all of the 2,3,7,8-TCDD in the sampled air and that our field spikes can be used for an accurate estimate of method bias. These conclusions are supported by work recently reported by F.L. DeRoos, J.E. Tabor, S.E. Miller, S.C. Watson and J.A. Hatchel.

In the second experiment, a solution from a standard containing both 2,3,7,8-TCDD and 2,3,7,8-TCDF (tetrachloro-p-dibenzofuran) was placed on the glass fiber filter. Uncontaminated air was then passed through the sampler as described above for 17.5 minutes, 2 hours and 24 minutes, and 24 hours. The filters and PUF's were then analyzed separately. The average total (filter and PUF) percent recovery for

dioxin was 112% with a standard deviation of 1.3%. The average percent recovery of Furan was 90% with a standard deviation of 11%. The resulting data are shown in Table III. It was obvious from this experiment that the dioxin was very slowly migrating from the filter to the PUF. We conclude that an analyses of only the particulate matter or only the vapor phase in the sample would both give erroneous results. It also appears that the Furans are more easily transferred from the filter to the PUF than are the dioxins.

Effectiveness of Sampling Methodology

The data described above provides a basis for concluding that the sampling equipment and procedures were adequate for this study. Clearly, the sampler must capture both particulate and vapor phase materials if the data are to be representative of the air sampled. The volume of air sampled was just large enough to give adequate data precision (the maximum RSD at the monitor showing highest concentrations of 2,3,7,8-TCDD was approximately 22%) in the 3-5 picogram/M³ concentration range. The samplers were reliable (no down time) and they maintained the initial flow characteristics very well (maximum change in measured flow was approximately 2%). The ability to very easily change the sampling modules made field work much more convenient and probably improved data precision considerably.

Are there any Relationships between Weather Conditions and 2,3,7,8-TCDD in the Air?

At no time during this study did we observe a measurable concentration of 2,3,7,8-TCDD at a non-downwind monitor. Approximately 200 samples were collected and analyzed for 2,3,7,8-TCDD. Ten of those samples contained concentrations of 2,3,7,8-TCDD above the detection limit. Each of the positive concentrations occurred on days when clean-up activities were occurring in the immediate vicinity of the monitor and when the clean-up activities were upwind of the monitor for most of the day. From these data we conclude that there is a measurable relationship between wind direction and concentrations of 2,3,7,8-TCDD in the air. We also conclude that the 2,3,7,8-TCDD we found in the air was originating at the location of the clean-up work and that the contamination remains in the air only for a short time period. Any background of 2,3,7,8-TCDD is below the method detection limit.

We were unable to find a model that would, in our hands, accurately give the experimental results we obtained.

Can Particulate Matter Concentrations be Used to Predict 2,3,7,8-TCDD Concentrations?

Figure 4 is a graph showing the relationship between particulate matter concentrations and 2,3,7,8-TCDD concentrations. Although there may be a very general relationship, it is apparent that one could not reliably predict 2,3,7,8-TCDD from measured particulate matter

concentrations. It is also obvious from the data in the graph that there is no particulate matter concentration that could be used as a control limit for 2,3,7,8-TCDD. The particulate matter concentrations referred to were obtained by the procedures described in this paper. They are not equivalent to the TSP values usually obtained with a Hi-Vol sampler. However, a good relationship between TSP by the EPA-approved procedure and the measured PM concentrations has been demonstrated for this study.

Since dioxin analyses are so expensive relative to TSP analyses, it may be beneficial in future studies to look for a 2,3,7,8-TCDD/TSP relationship under different conditions.

Was the General Public Exposed to Significant Concentrations of 2,3,7,8-TCDD as a Result of this Action?

Figure 5 is a graph showing the 14-day running average and the associated 95% confidence levels of those averages for the monitoring site with the highest concentrations. As can be seen, all of the 14-day averages were well below the warning level and the NOEL at all times. We would emphasize that the averages were calculated using the assumption that the detection limit was a measured concentration for all samples that did not have a measurable concentration of 2,3,7,8-TCDD. Therefore, the actual exposure was probably even less than the maximum possible indicated by the graph. From this data, we conclude that concentrations of 2,3,7,8-TCDD that cause an insignificant risk to the public can be measured in ambient air using the procedures described above. We also conclude that during this study, the public was not exposed to a significant concentration (5.5 picogram/M³ for a "few months") of 2,3,7,8-TCDD at any time.

Can Monitoring Costs be Reduced in Future Studies?

We estimate the total cost of the study described above was approximately \$295,000 or approximately \$1,500 per sample. After reviewing the data from this study, we see no way to substantially reduce the costs without additional data. It does not appear to be possible to use particulate matter as an indicator parameter for dioxin, thus, reducing the analytical costs. We would not recommend reducing the size of the monitoring network for a site of this size. We believe the network should be in operation any time pollution abatement activities are occurring at the site. We doubt that efforts to improve data quality would be cost-effective. The authors are investigating sample breakthrough for longer sampling times both for the PUF and other solid absorbents. An option that might result in a reduced cost would be to reduce the time spent to obtain baseline data from 14 days to a shorter time period. This kind of decision is properly a role of management since costs must be balanced against the increased risk of obtaining a false positive and of unknowingly exposing the public to a significant amount of 2,3,7,8-TCDD. The results of this work should make that decision easier to reach.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Morris Kay and John Wicklund for providing resources and encouragement, and to the many EPA and EPA contractor employees who have participated in this effort by doing their assigned work in an efficient and professional manner.

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TABLE I

Estimation of Number of Samples Needed
to Meet Study Objective

Number of Samples per Site	t.975, n-1	RSD = 12%	RSD = 24%	RSD = 36%
		95% confidence limits = sample average \pm		
7	2.447	11%	22%	33%
14	2.160	7%	14%	21%
28	2.052	5%	9%	14%
56	1.96	3%	6%	9%

TABLE II
Breakthrough Study Results

		Sampling Time (hours)/Air volume + 5% M ³				
		0/0	12/180	24/360	48/720	72/1080
2,3,7,8-TCDD	MPR	89.6%	64.7%	81.2%	93.7%	88.9%
	RSD	9.1%	2.95%	1.91%	5.71%	3.90%
1,2,3,4-TCDD	MPR	92.8%	70.8%	93.6%	100%	92.9%
	RSD	5.47%	5.92%	7.91%	2.59%	11.2%
2,3,7,8-TCDF	MPR	92.4%	74.4%	99.1%	112%	99.4%
	RSD	6.20%	6.86%	5.43%	9.20%	8.95%

MPR = Mean Percent Recovery

RSD = Relative Standard Deviation

TABLE III

Distribution of TCDD and TCDF between the Filter and PUF

Percent Found on the Filter and PUF				
Sampling Time (Minutes)		17.5	144	1440
TCDD	Filter	100	97	82
	PUF	0	4	18
TCDF	Filter	63	10	0
	PUF	37	90	100

Figure 1

A diagram of the site. During pollution abatement activities, approximately 6" of soil would be removed from a section (5,000 square feet) with a backhoe and placed in plastic bags. The new surface would then be tested for dioxin and be either declared clean or another 6" of soil would be removed. Air pollution could originate either from the soil handling operations or truck traffic within the site. The six monitoring locations relative to the contaminated area are shown in the figure (M, O, D, K, L & E). The prevailing wind is from Monitor E to Monitor O.

Figure 2

Modified Hi-Vol Sampler (General Metal Works, Inc., Model PS-1) used to collect 2,3,7,8-TCDD samples.

Figure 3

A detailed illustration of the sampling head module (see Figure 2) showing the fiberglass filter used to collect particulates, and the glass cylinder/PUF used to collect vapors.

Figure 4

Data from samples collected from a single monitor with both valid PM and 2,3,7,8-TCDD concentrations above the respective detection limits are shown. There is no reliable correlation.

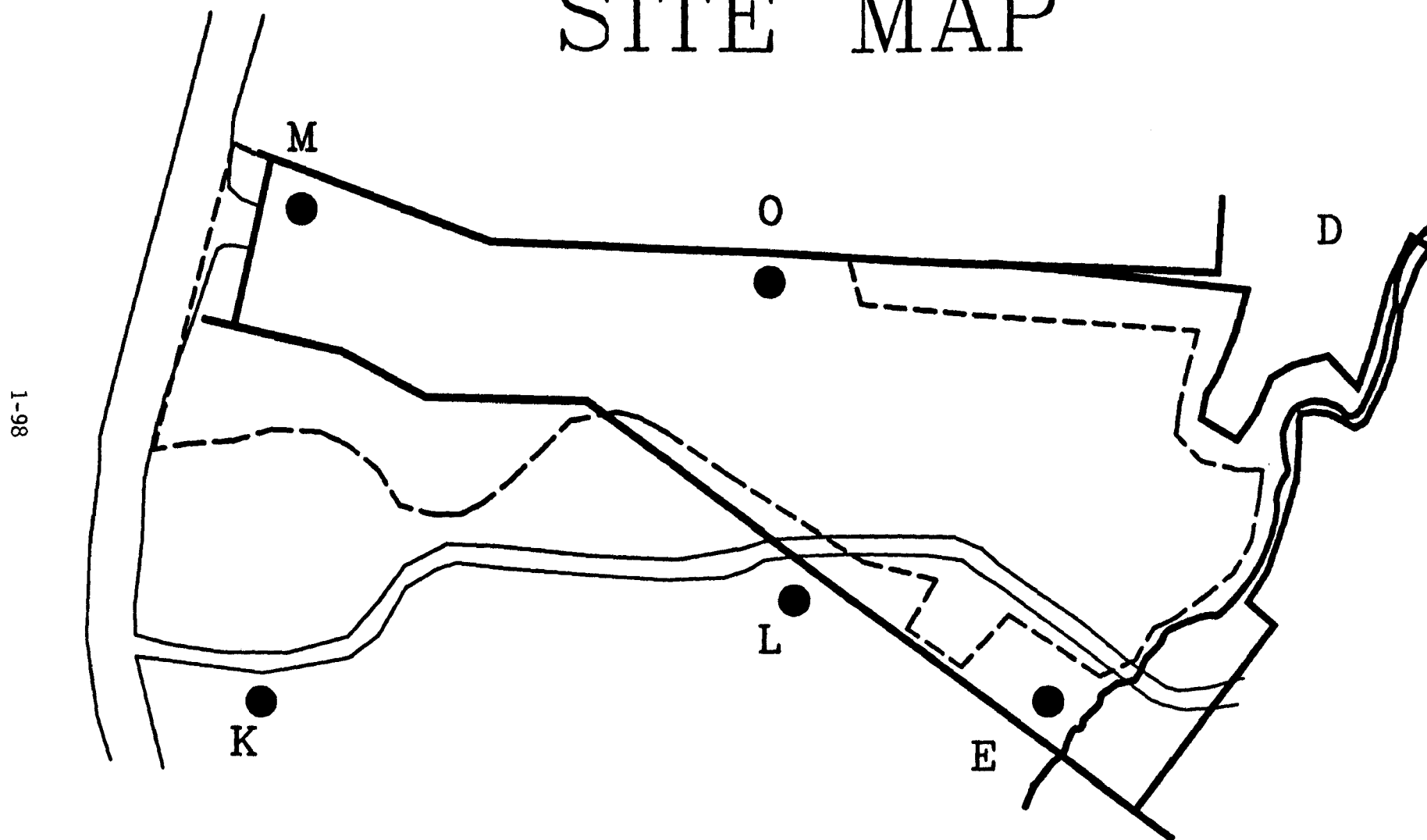
Figure 5

The data points are representative (we only show every 5th day for clarity) averages of measured or estimated (see text) concentrations for the most recent 14 days. A downwind sample is one in which the monitor (O in Figure 1) was downwind of the clean-up activity at the site during the last day of the 14-day averaging period. A non-sampling value is shown for those days when the concentrations at site "O" were estimated rather than measured on the last day of the averaging period.

The average of all measured (or estimated) concentrations for 14 consecutive days is plotted on the Y axis against the last day of the 14 day averaging period on the X axis for monitor "O" (see Figure 1). Detection limits were taken as measured concentrations. If a measurement was not taken for a given day because the monitor was not downwind or upwind on that day, an estimate of the concentration was made by averaging all of the non-downwind values to date. Since concentrations above the detection limit (usually in the range 0.4-0.8 picogram/M³) were obtained only when a monitor was downwind of the site, all estimated values were averages of detection limits. The data are presented in this manner to illustrate our best estimate of the maximum exposure of any off-site population that might have resulted from this clean-up activity.

Figure 1

SITE MAP



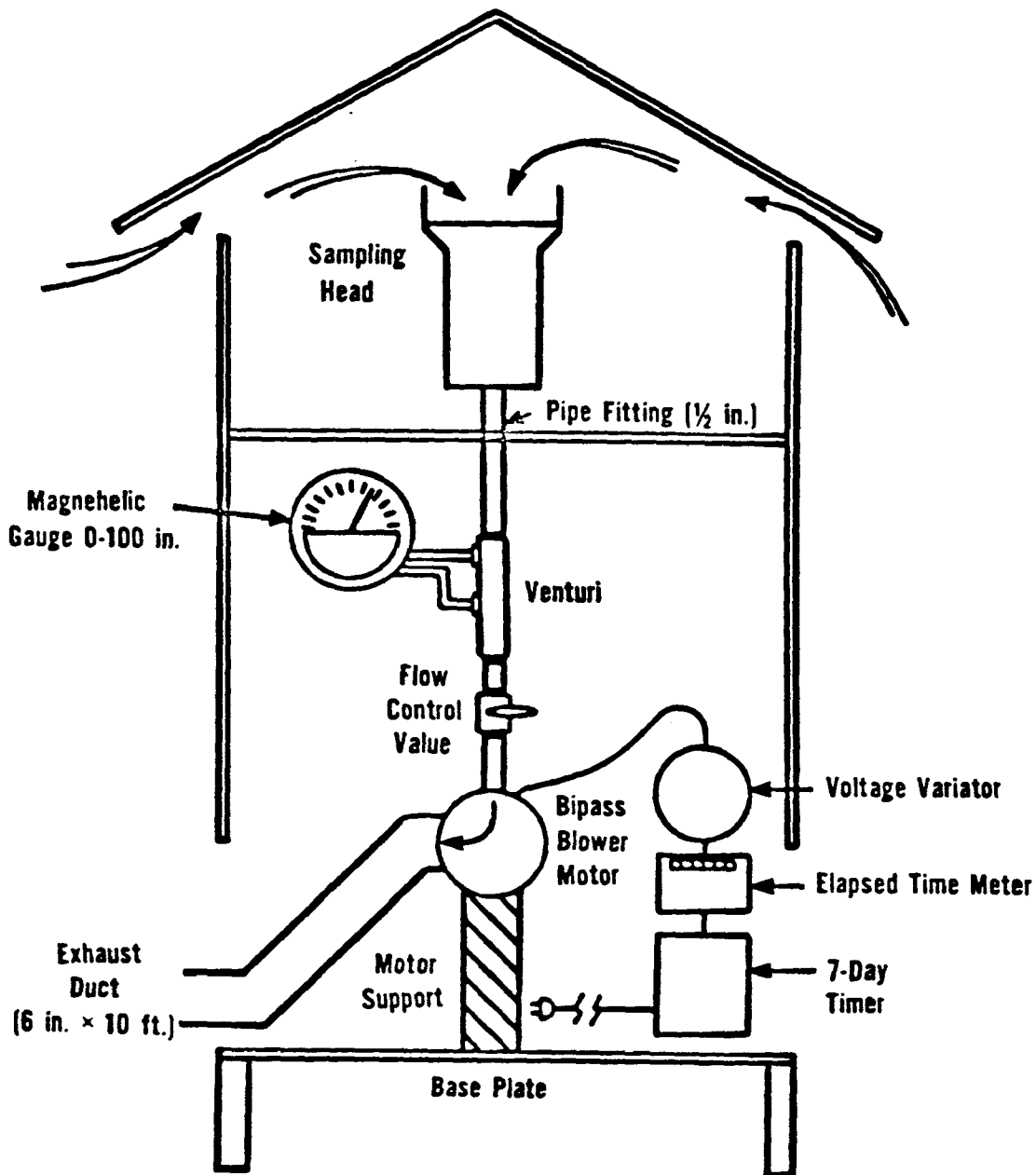
1-98

M - E MONITORS

SAMPLE
LOCATION

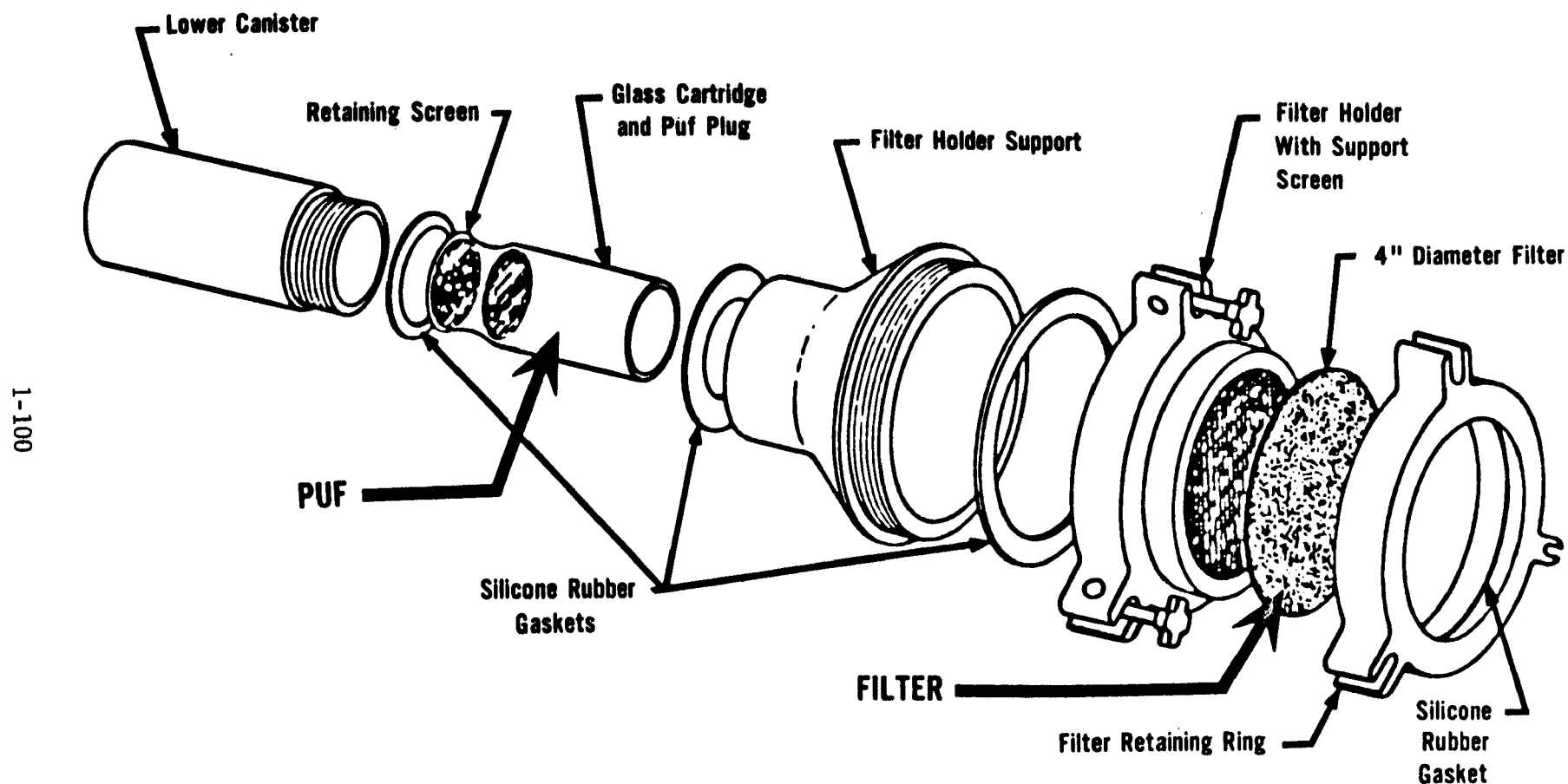
----- CONTAMINATED
AREA

Figure 2



Modified Hi-Vol Sampler
(General Metal Works, Inc., Model PS-1)
used to Collect 2,3,7,8,-TCDD Samples.

Figure 3



A Detailed Illustration of the Sampling Head Module Showing the Fiberglass Filter Used to Collect Particulates and the Glass Cylinder PUF Used to Collect Vapors.

SAMPLES FROM QUAIL RUN WITH 2,3,7,8-TCDD VALUES ABOVE THE DETECTION LIMIT

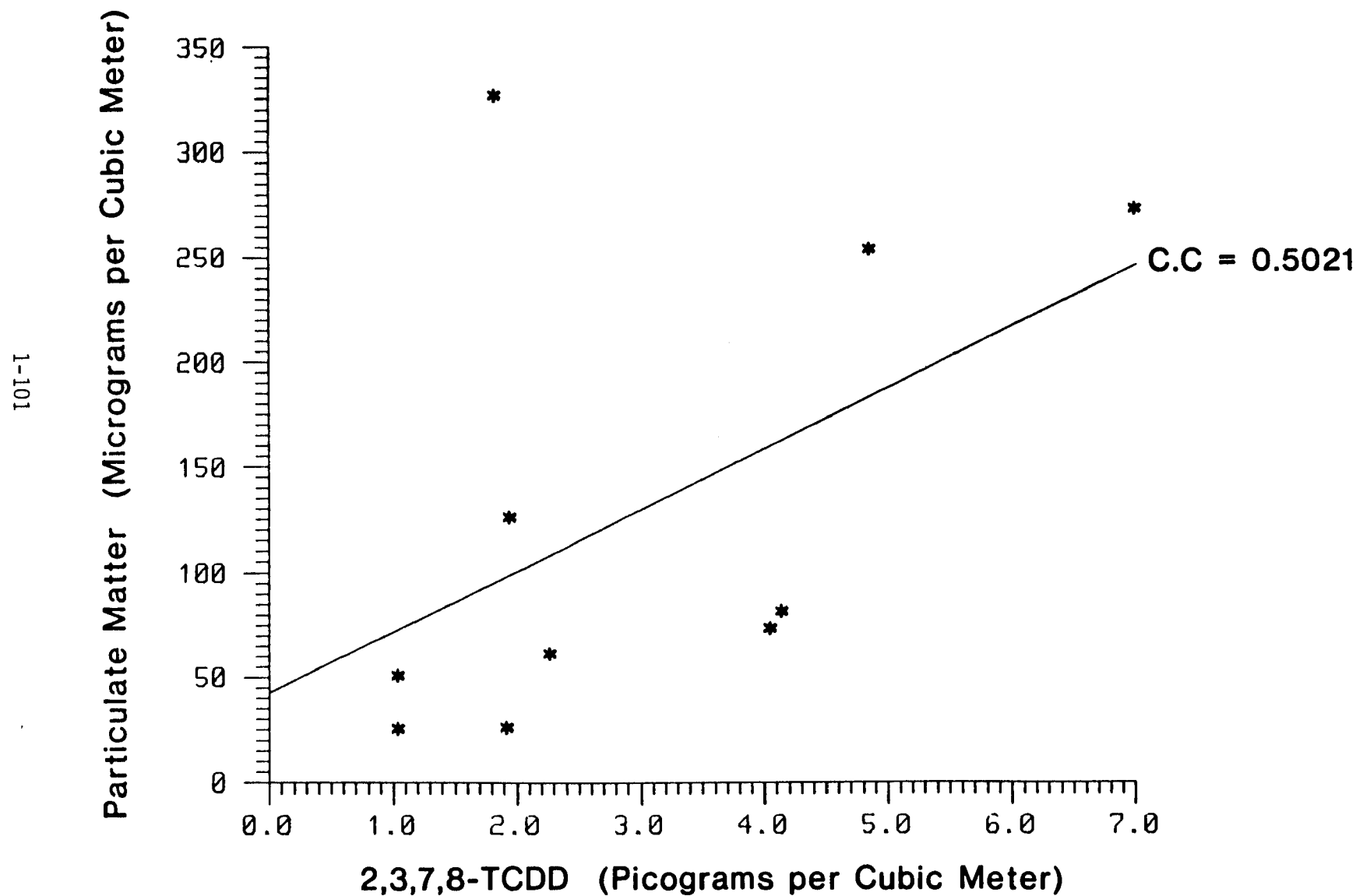
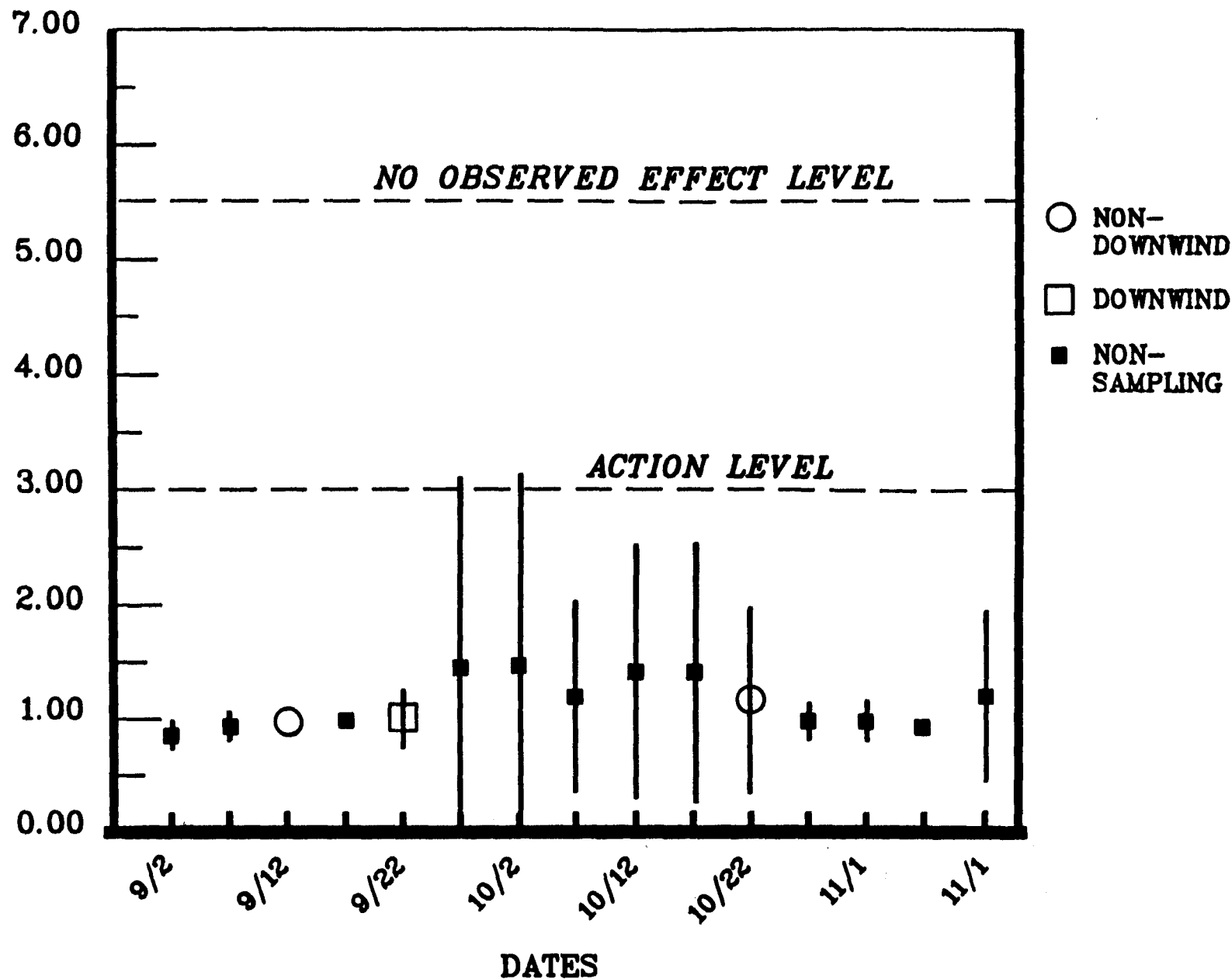


Figure 5

14 DAY RUNNING AVERAGE

1-102
PICOGRAMS OF 2,3,7,8-TCDD PER
STANDARD CUBIC METER OF AIR



VOC EMISSION RATES FROM SOLID WASTE LANDFILLS

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ABSTRACT

Regulatory agencies and the public have directed increased attention at the health risks associated with exposure to trace constituents present in landfill gases. Direct field measurements of surface emissions are difficult and often inconclusive, indicating a need for quantitation by alternative means. To this end, a study was conducted to estimate the maximum surface and subsurface emissions for volatile organic compounds (VOCs) from RCRA Subtitle D disposal sites. The purpose of these estimates was to provide baseline data for assessing potential air quality impacts.

Estimates were based on measurements from pump test programs common to the landfill Gas industry. Field pump tests performed from 1982 through 1986 at 20 selected landfill sites provided gas generation rates for wet and dry climates per volume of landfilled solid waste. The median LFG generation rates for wet and dry geographic regions were 207 cubic feet of gas per cubic yard of refuse per year (cf/cy/yr) and 81 cf/cy/yr, respectively. In addition, VOC concentrations were derived from a compilation of laboratory analyses from sites between 1982 and 1986. Various landfill configurations were evaluated on the basis of surface area, waste depth, location of disposed wastes (above ground, below ground, and above and below ground), and region.

Results from the study indicated that the solid waste landfill emissions are greater for sites in wet climate regions due to the amount of available moisture required for biological activity. However, VOC concentrations do not change considerably with climate. Total gas emissions in wet climates ranged from 124 million to 2,960 million cubic feet per year for the various landfill configurations. VOC mass surface emissions for the same landfills ranged from 1 to 22 tons/year.

APPROACH

Regulatory agencies and the public have directed increased attention at the health risks associated with exposure to trace constituents present in landfill gases. Correlations of landfilled waste types, landfill gas (LFG) characteristics, and direct field measurements of surface emissions are difficult and often inconclusive, indicating a need for quantitation by alternative means. To this end, a study was conducted to estimate the maximum surface and subsurface emissions for volatile organic compounds (VOCs) from RCRA Subtitle D

disposal sites. The purpose of these estimates was to provide baseline data for assessing potential air quality impacts.

Estimates were based on measurements from pump test programs common to the landfill gas industry. Field pump tests performed from 1982 through 1986 at 20 selected landfill sites provided gas generation rates for wet and dry geographic regions per volume of landfilled solid waste. In addition, VOC concentrations were derived from a compilation of laboratory analyses from sites between 1982 and 1986. A range of landfill configurations was assigned and evaluated on the basis of surface area, waste depth, location of disposed wastes (above ground, below ground, and above and below ground), and region. Estimates could then be made to calculate VOC emission rates from all landfill configurations and for wet versus dry climatic areas. Our approach for determining these landfill emission rates is depicted in Table 1.

COMPILE LANDFILL GAS GENERATION RATES

Landfill test data were compiled to determine typical LFG generation rates and VOC emission rates per volume of in-place refuse. Pump test programs, which provide empirical data for sustained LFG production rates over a period of several days or weeks, were surveyed from field studies conducted from 1982 through 1986 at municipal solid waste landfills nationwide. On the basis of refuse moisture contents, methane content, estimated landfill life (for LFG production), and refuse density, the gas generation rate was determined, expressed in units of cubic feet (cf) of raw landfill gas per cubic yard (cy) of in-place waste per year (cf/cy/yr).

Available moisture within the landfill is required for biological activity to occur and hence, the moisture content of the in-place wastes directly affects LFG generation rates. As a result, geographical regions having different net precipitation/infiltration rates will produce LFG at different rates. The field studies were separated into wet and dry regions based on geographic location and precipitation rates. For instance, southern California is a relatively dry region with an average rainfall of about 20 inches per year. By comparison, the Midwest and East Coast are relatively wet regions with an average annual rainfall over 35 inches. Wet regions have generally higher gas generation rates and refuse moisture contents, but have estimated production "lives" lower than those for dry regions. A general relationship is that the greater the net precipitation/infiltration rate, the greater the gas generation rate, and the shorter the overall duration of LFG production (or landfill life).

Results from the compilation provided median gas generation rates for wet geographic regions (207 cf/cy/yr) and dry geographic regions (81 cf/cy/yr).

TABLE 1
HOW VOC EMISSION RATES WERE DETERMINED

1. Compile LFG Generation Rates from Field Test Data
 - o RCRA Subtitle D disposal sites
 - o Nationwide pump test programs
 - o Wet versus dry geographic regions
2. Compile Laboratory Analyses for VOCs Present in LFG
 - o RCRA Subtitle D disposal sites
 - o Major VOCs found in landfill gas
 - o Median concentrations
3. Select Range of Landfill Configurations
 - o Surface Area
 - o Waste Depth
 - o Location of disposed wastes (above or below ground, or both)
 - o Emissions pathway (surface or subsurface, or both)
4. Calculate VOC Emission Rates
 - o All landfill configurations
 - o Total gas and total VOCs
 - o Wet versus dry geographic regions

COMPILE LFG ANALYSES FOR VOCs

A literature search was conducted to compile laboratory analyses for VOCs present in landfill gas. While published reports provided some pertinent data, most of the data were collected directly from laboratory analyses. Sources for many of these analyses were engineering firms which had conducted nationwide landfill gas test programs, monitoring of gas extraction systems, or general environmental investigations. Various references were assembled and reviewed with regard to site descriptions, landfill gas collection systems, sampling and analytical methods, detection limits, and other information. These provided an initial compilation of all trace constituents sampled in landfill gas.

Selection criteria were established for references and specific sampling events in order to derive a reliable data base; i.e., one representative of solid waste landfills with active biological gas production. The analytical results included in the data base met the following criteria:

- o Landfill gas samples were taken from landfills that reportedly received only non-hazardous solid wastes and essentially were regulated as RCRA Subtitle D disposal sites. However, hazardous wastes from small quantity, generators or within household hazardous wastes may have been disposed at these landfills.
- o Landfill gas samples were taken after 1982. Sampling and analytical results obtained prior to that time were not included in the data base due to questionable or inadequate sampling methods and limitations of available analytical techniques.

This data base identified 87 VOCs present in LFG on the basis of samples taken and frequency of detection. However, many of these compounds were sampled and detected at less than five of the 35 surveyed sites. Consequently, a list of the "typical" LFG constituents was derived for those compounds found in at least 50 percent of the total samples analyzed and in at least 20 percent of the sites surveyed. This list includes the 16 VOCs presented in Table 2. As shown, the range, median, and mean concentrations are provided for comparison purposes. Note that the median values are always less than the mean values, indicating that the reported data are skewed, presumably due to one or more high readings for each compound.

The permissible exposure level (PEL) established by OSHA is included in Table 2 for each listed compound. Vinyl chloride has the lowest PEL at 1 volume part-per-million (Vppm) and it is the only compound where the PEL was exceeded by most of the samples.

TABLE 2

TYPICAL TRACE CONSTITUENTS IN LANDFILL GAS

Compound	Range of Concentration (Vppm)	Median Concentration (Vppm)	Mean Concentration (Vppm)	Standard Deviation (Vppm)	PEL* (Vppm)
Methylcyclohexane	0.02 - 19	3.3	7.2	7.7	500
Acetone	0.05 - 12	4.2	4.8	3.5	1000
Trichlorofluoromethane	0.26 - 8.8	1.4	2.5	2.8	1000
n-Hexane	0.001- 31	4.9	7.8	9.4	500
1,1-Dichloroethane	0.06 - 20	0.65	4.2	6.4	100
1,2-Dichloroethene	0.17 - 95	1.7	9.1	21	200
1,1-Dichloroethene	0.03 - 4.2	0.21	0.8	1.2	5
Methylene Chloride	0.079-380	3.8	32	74	500
Xylenes	0.001-110	5.8	16	25	100
1,1,1-Trichloroethane	0.003- 31	0.057	1.8	5.8	350
Ethylbenzene	0.012- 91	1.7	6.2	16	100
Trichloroethylene	0.011- 44	0.51	3.1	6.8	100
Vinyl Chloride	0.03 - 50	4.7	6.9	8.9	1
Tetrachloroethylene	0.006-190	0.79	11	27	100
Benzene	0.016- 24	0.54	2.0	3.5	10
Toluene	0.004-360	7.4	27	53	200

* Permissible Exposure Level prescribed by OSHA for workplace exposure.

In addition to compound-specific identifications and concentrations from the analytical data base, values for total non-methane VOCs were obtained from the surveyed sites. The median concentration of non-methane VOCs was found to be 58.5 Vppm, which includes compounds in addition to those listed in Table 2. VOC emission rates are typically reported in units of mass per volume of air (or gas). Consequently, the median value of 58.5 ppm was converted to a mass/volume basis for VOC emissions calculations. A value of 58.5 ppm is equivalent to 0.000015 pounds (lbs) of total VOCs per cubic foot of landfill gas, assuming an average VOC molecular weight of 100 grams/mole.

SELECT LANDFILL CONFIGURATIONS

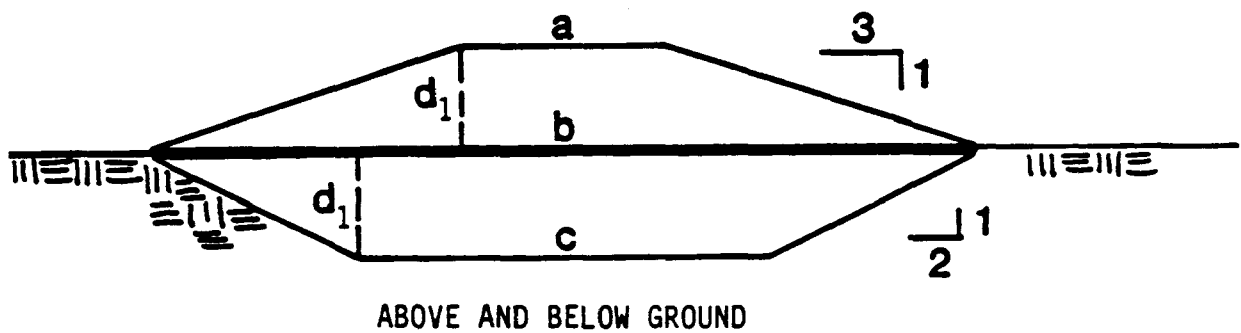
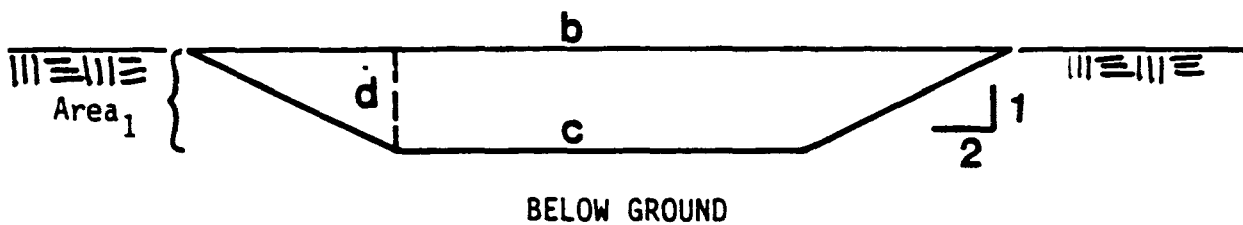
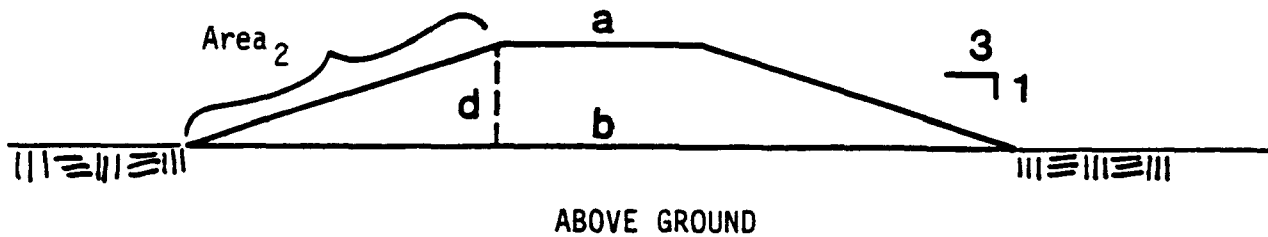
The next step for the determination of VOC emission rates was to select representative landfill configurations. Twelve landfill scenarios were selected to provide a general range of landfill sites that typify existing disposal sites. The configurations differed on the basis of surface areas (20 and 100 acres), total waste depth (20, 40, 50 and 100 feet), and location of disposed wastes (above and below ground, all below ground, and all above ground). The configurations were consistent on the basis of shape (each landfill was square, with length equal to width) and side slopes (above ground side slopes were 3:1, and below ground side slopes were 2:1).

Figure 1 illustrates the generic landfill configurations, including side slopes and locations of wastes. Note that subsurface areas with regard to emissions are based on the vertical projection of each subsurface side slope. This is because subsurface emissions were assumed to travel horizontally and generally not in a downward direction. The landfill volumes range from 592,000 to 14,320,000 cy of refuse.

Except for the above ground landfills, surface areas greatly exceed subsurface areas, principally because gas emissions through the bottom of subsurface portions of the landfills were not considered. For the above ground landfills, however, the subsurface gas emission pathway did consider migration through the bottom of the landfill and thus, the surface and subsurface areas for emissions were about the same.

CALCULATE EMISSION RATES

A series of algebraic calculations was performed in order to determine total emissions (raw landfill gas and VOCs) from the 12 landfill configurations. After a surface area (in acres) and a depth were assigned for a particular landfill configuration, areas and volumes relative to gas emissions were calculated. Total gas emissions (cf/yr) were determined for each configuration by the



WHERE:

a = length of top surface

b = length of landfill at natural ground level

c = length of bottom of landfill

d, d_1 = depth of disposed waste where $d_1 + d_1 = d$

$Area_1$ = subsurface area of vertical projection of each side slope (below ground)

$Area_2$ = surface area of each side slope (above ground)

Figure 1. Landfill Configurations

product of the in-place waste volume (cy) and the gas generation rates (cf/cy/yr) for both wet and dry regions. Because there is a direct relationship between total LFG emissions and total VOC emissions, the total VOC emission (lbs/yr) were determined simply as the product of the total LFG gas emissions (cf/yr) and the median VOC generation rate (lbs/cf).

Algebraic calculations were also performed to determine surface and subsurface flux rates. Flux rates are volumes of landfill gas emitted per surface (or subsurface) area of the emissions pathway per year (cf/sf/yr) for the VOCs, flux rates are in units of lbs/sf/yr. Surface flux rates were defined as the maximum potential emission rates through surface routes or pathways. When determining surface flux rates, we assumed that all of the gases generated were emitted through the surface pathway. That is, no portion (zero percent) of the landfill gases or VOCs are emitted through subsurface pathways. Similarly, the determination of subsurface flux rates assumed that all the landfill gas and VOCs are emitted only through subsurface pathways, with zero percent surface emissions.

Tables 3 and 4 present estimated surface and subsurface flux rates for landfill gas emissions and for the non-methane VOCs. At most landfills, a portion of the gases are emitted through the surface, and a portion are emitted through subsurface pathways. Furthermore, a percentage of the VOCs are absorbed by the soil on the landfill cap and/or the subsoil (for subsurface migration). Accordingly, the numbers presented in Tables 3 and 4 should be considered "worst case" values for typical solid waste landfills.

RESULTS

Table 3 presents estimated LFG emissions and potential flux rates for the 12 landfill configurations. In addition, emissions were calculated for both wet and dry climates. Based on in-place waste volumes, total gas emissions ranged from 49 million to 1,160 million cy/yr for the dry climates. Total gas emissions for the same landfill configurations in wet climates increased by a factor of about 2.6 (range = 124 million to 2,960 million cf/yr). The greatest gas emissions were generated in the largest landfills (100 acres with 100 feet of total waste depth).

These total gas emissions are derived for raw landfill gas, which consists of about 50 percent methane and 50 percent carbon dioxide. Thus, a reliable estimate for methane emissions is simply half the value shown in Exhibit 3. As an example, the landfill configuration with wastes disposed 20 feet below ground, 20 acres in a wet climate, would emit approximately 62,000 million cf of methane gas per year.

TABLE 3

ESTIMATED GAS EMISSIONS FROM SELECTED LANDFILL CONFIGURATIONS

Dry Climate						Wet Climate		
Surface Area (ac)	Waste Depth (ft)	Waste Volume (MM cy)	Total Gas Emissions (MM cf/yr)	Maximum Potential Flux		Total Gas Emissions (MM cf/yr)	Maximum Potential Flux	
				Surface Only* (cf/sf/yr)	Subsurface Only** (cf/sf/yr)		Surface Only* (cf/sf/yr)	Subsurface Only** (cf/sf/yr)
I. Wastes Disposed Below Ground								
20	20	0.6	49	56*	685	124	142*	1,730
20	50	1.3	105	121	629	269	309	1,610
100	20	3.1	251	58	1,530	642	147	3,910
100	50	7.3	591	136	1,490	1,500	344	3,780
II. Wastes Disposed Above Ground								
20	20	0.6	49	56*	56***	124	141*	142***
20	50	1.2	97	108	111	248	277	285
100	20	3.0	243	55	56	621	142	142
100	50	7.0	567	128	130	1,450	328	333
III. Wastes Disposed Above and Below Ground								
20	40	1.2	97	110*	1,360**	248	281*	3,470**
20	100	2.5	203	227	1,220	518	578	3,100
100	40	6.2	502	115	3,060	1,280	292	7,800
100	100	14.3	1,160	262	2,920	2,960	670	7,500

* Assumes all emissions are through landfill surface.

** Assumes all emissions are through sides of landfill.

*** Assumes all emissions are through bottom of landfill.

TABLE 4

ESTIMATED VOC EMISSIONS FROM SELECTED LANDFILL CONFIGURATIONS

Dry Climate						Wet Climate		
Surface Area (ac)	Waste Depth (ft)	Waste Volume (MM cy)	Total VOC Emissions (lbs/yr)	Maximum Potential Flux		Total VOC Emissions (lbs/yr)	Maximum Potential Flux	
				Surface Only (lbs/sf/yr)	Subsurface Only (lbs/sf/yr)		Surface Only (lbs/sf/yr)	Subsurface Only (lbs/sf/yr)
I. Wastes Disposed Below Ground								
20	20	0.6	740	0.00085*	0.010**	1,860	0.0021*	0.026**
20	50	1.3	1,600	0.0018	0.010	4,040	0.0046	0.024
100	20	3.1	3,800	0.00087	0.023	9,600	0.0022	0.059
100	50	7.3	8,700	0.0020	0.022	22,500	0.0052	0.057
II. Wastes Disposed Above Ground								
20	20	0.6	740	0.00084*	0.00085***	1,860	0.0021*	0.0021***
20	50	1.2	1,500	0.00167	0.00172	3,720	0.0042	0.0043
100	20	3.0	3,600	0.00082	0.00083	9,320	0.0022	0.0021
100	50	7.0	8,500	0.00192	0.00195	21,750	0.0049	0.0050
III. Wastes Disposed Above and Below Ground								
20	40	1.2	1,370	0.0016*	0.019**	3,720	0.0042*	0.052**
20	100	2.5	3,050	0.0034	0.018	7,770	0.0087	0.047
100	40	6.2	7,530	0.0017	0.046	19,200	0.0044	0.117
100	100	14.3	17,400	0.0039	0.044	44,400	0.0100	0.112

* Assumes all emissions are through landfill surface.

** Assumes all emissions are through sides of landfill.

*** Assumes all emissions are through bottom of landfill.

Flux rates shown in Exhibit 3 differed significantly relative to emissions pathways, differed to a lesser degree relative to landfill configuration, and differed by a factor of about 2.6 for wet versus dry climates. In all cases, the subsurface emissions pathway yields the higher potential flux rate. Except for the above ground landfills, the subsurface flux rate greatly exceeded the surface flux rate, generally by factors from 5 to 26. For the above ground landfills, however, the surface and subsurface flux rates were similar.

Landfill configuration affects the resultant emissions flux rate but not to a large extent. That is, if waste depth is increased, there is a direct proportional increase in surface flux rates and, for above ground landfills, in subsurface flux rates. If surface area is increased as much as five-fold, the surface flux rate remains about the same. However, this same five-fold increase in landfill surface area resulted in a more than two-fold increase in the subsurface emissions flux rate. The exceptions to this relationship were the above ground landfill configurations, where the subsurface flux rate did not change significantly with surface area.

Exhibit 4 presents estimated VOC emissions and potential flux rates for the same landfill configurations. Because there is a direct proportional relationship between estimated LFG emissions and total VOC emissions, the impacts of variables such as surface area, waste depth, and climate are the same as those discussed above. The VOC emissions were calculated on the basis of mass emitted annually (lbs/yr), and ranged from 740 to 17,400 lbs/yr for the landfill configurations located in dry climates. Total VOC emissions for the same landfills in wet climates increased by a factor of about 2.6 (range = 1,860 to 44,400 lbs/yr).

CONCLUSIONS

Compilation of literature sources and empirical data from landfill test programs provided estimates of landfill gas and VOC emission quantities for a variety of landfill configurations. Subsequent calculations provided corresponding surface and subsurface flux rates. Our conclusions are as follows:

1. Total landfill gas emissions ranged from 49 to 2,960 million cf of gas per year. Since landfill gas consists of 50 percent methane, annual methane emissions can be estimated to range from 24 to 1,480 million cf of methane per year.
2. Total VOC emissions ranged from 740 to 44,400 pounds per year (0.37 to 22.2 tons per year), assuming a molecular weight of 100 for the non-methane VOCs present in landfill gas.

3. Landfill emissions are greater for sites located in wet climate regions due to the amount of available moisture required for biological activity. In general, gas and VOC total emissions were estimated to be about 2.6 times higher in wet climates versus dry climates.

Our emission calculations were based on several simplifying assumptions which were applied to a limited number of landfill configurations. For example, a single gas emission rate for VOCs provides a rough measure of actual emissions at landfill sources. Similarly, the use of only two gas generation rates (for a wet and dry climate) is a generalization. In addition, surface and subsurface flux rates normally do not occur in the absence of the other.

A CONTROL CHART STRATEGY FOR GROUND WATER MONITORING

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ABSTRACT

Monitoring the groundwater around a waste impoundment site presents a statistical problem in testing compliance or leaking. Two types of errors are present in statistical tests, and both are undesirable. If the test is designed for early detection, there may be too many false positives. This means an out-of-control state is declared when it does not exist and unnecessary increased sampling is started. In contrast, if the test is designed for conservative detections, there may be too many false negatives. That means an in-control state is declared when an out-of-control state exists and pollution takes place undetected.

One commonly used statistical procedure that suggests an answer for this type of problem is control chart methodology. This poster session explains control chart decision logic and applies two of the more promising types to actual data. The Shewhart chart is designed to detect a large, binary leak from a seasonal or random fluctuation. The Cusum chart is designed to detect a growing leak. A monitoring strategy that uses a combination of these two charts is discussed.

INTRODUCTION

Under the Resource Conservation and Recovery Act of 1976 (RCRA), the U.S. Environmental Protection Agency has developed regulations for landfills, surface impoundments, waste piles, and land treatment units that are used to treat, store, or dispose of hazardous wastes. These regulations include requirements for the monitoring of ground water in the top aquifer below the hazardous waste site (HWS). This monitoring involves the drilling of wells into the aquifer up-gradient and down-gradient of the HWS, and the sampling and analysis of well water at regular time intervals to help determine whether leachate from the HWS has entered the aquifer. There are several as yet unsolved problems in this monitoring program. These problems include determination of appropriate methods for obtaining accurate measurements of some constituents such as volatile organics, specifications for well construction, and detection and accommodation of shifting direction and rate of aquifer flow. However, this paper discusses the problem of developing good decision rules for determining when additional regulatory action may be required and recommends that the

development be based on a realistic model for the ground-water measurements. Industrial quality control schemes are considered in terms of their possible application to the ground-water monitoring decision problem.

GROUND-WATER CHARACTERISTICS

To develop a reasonable model for the measurements of GQP, some discussion of ground-water characteristics is required. The horizontal velocity of water in an aquifer is slow (a few meters per day is considered a high velocity), and lateral dispersion of a contaminant is considerably slower than fluid flow; this means that plumes do not widen to any great extent as they extend through the aquifer (Freeze and Cherry, 1979). At a particular sampling time, the water at different wells near a HWS will have entered the aquifer at different times and therefore will carry different concentrations of various monitored constituents. Hence, the value of a monitored parameter may be increasing at one well while it is decreasing at another. Concentrations of contaminants in samples of ground-water may also change because of changes in water table. A high water table resulting from recent rains or flooding may cause a reduction in measured concentrations of a pollutant because of dilution of the contaminant or because the contaminant is floating at the top of the aquifer which is now above the well screen. Therefore, changes in the water table may have similar and virtually simultaneous effects on all wells near a HWS.

PROBLEMS ASSOCIATED WITH THE ANALYSIS OF GROUND-WATER DATA

There are several characteristics of ground-water monitoring data that severely complicate the development of statistical decision procedures. They are as follows:

1. Chemical analysis of water samples is expensive (sometimes on the order of \$1,000 per sample);
2. Several water quality parameters are monitored;
3. Changes in aquifer water quality and flow characteristics are effected by human intervention off the HWS such as intermittent pumping of water from the aquifer or accidental spills of pollutants into the aquifer;
4. For some monitored substances such as volatile organic compounds, measurement error variance and bias tend to be large;
5. For some monitored substances, the chemical analyses will result in "below instrument detection limit" or "not detected" for most of the water samples submitted; and

6. It is difficult to obtain consecutive samples from a well at a particular sampling time that are true replicates because of a tendency for such observations to follow trends.

High costs of analyses and large measurement variances make it difficult to obtain an adequate number of background measurements to characterize the system. Similarly, it is costly to obtain a sufficient number of samples and aliquots at a monitoring well to estimate current GQP values with high precision or to provide tests with good power characteristics.

The fact that the values of several water quality parameters are being monitored at each of several down-gradient wells implies that in each sampling period, many decisions must be made as to whether parameter values at down-gradient wells have increased sufficiently to justify some form of regulatory action. If a separate test of hypotheses is performed for each of these decisions, the probability of falsely rejecting at least one null hypothesis is likely to be quite high even though the probability of such a false-positive is low for each test. While the simplest and most typical reaction to a large concentration that signals the occurrence of an unusual event is to take another sample to confirm the measurement, one cannot be complacent about false alarms since the cost of taking unnecessary additional samples can be a heavy burden for the HWS owner. To reduce the number of tests, one might think of using a multivariate procedure such as Hotelling's T^2 -test (Anderson, 1984), but such tests usually require estimation of the covariance matrix of the vector of measurements. Unfortunately, the number of sampling periods required to obtain the data to estimate the covariance matrix is greater than the dimension of the observation vector. Costs and start-up time restrictions make it unlikely that the covariance matrix could be adequately estimated from measurements taken prior to start-up of the HWS. In addition, human intervention is likely to change the covariance matrix of the observations during monitoring. Beyond these problems with the covariance matrix, a significant large T^2 -value does not necessarily imply an unusual increase in any of the monitored chemical concentrations.

Another and probably better approach to the multiple tests problem is to use the Bonferonni simultaneous inference technique (Miller, 1966) which prescribes the use of a significance level of α/m for each of the m tests to be performed at a given time of sampling so as to keep the overall probability of making at least one false positive (Type 1) error at or below α . Naturally, the reduction of the significance levels of the individual tests from α to α/m also reduces the power of the tests.

The "below instrument detection limits" measurements of a water quality parameter are impossible to employ in the types of statistical analyses and decision rules mentioned above. While in many cases these qualitative statements can be replaced by instrument values obtained in the chemical analysis, these numbers are likely to have a considerably different error distribution than will observations that are above the instrument detection limits. The decision as to whether an above-detection-limit measurement represents an increase in the mean concentration of the chemical over previous "not detected" readings is often a question which must involve the analytical chemist because of his knowledge of the quality of the current measurement. Statistical methods proposed by Aitchison (1955) for positive continuous random variables with positive probability mass at zero may be useful here; these same statistical methods were considered in terms of air monitoring by Owen and DeRouen (1980).

An inability to obtain true replicate samples makes it impossible to estimate sampling variance and difficult to obtain desired levels of precision for quality estimates.

CONTROL CHART SCHEMES

If the slow flow of water and the narrowness of plumes in the aquifer make the tests of means and interactions inappropriate and if outlier tests are lacking in power, what other decision procedures might one apply? An approach suggested by Vaughan and Russell (1983) for monitoring effluent from waste treatment plants is to use industrial quality control schemes. Such schemes compare an observation with the observations that came before it at that location.

A prime consideration in using industrial quality control methods to monitor ground-water quality at a HWS is that it separates effects of location and well construction from the decision process. Instead of comparing the concentration of a monitored chemical at a particular well with measurements at other wells, one compares the current measured concentration with the past history of measured concentrations of the chemical in water from this well. Some other advantages and also some disadvantages related to the earlier list of problems with ground-water measurements, will become evident as the nature of quality control schemes is presented.

Discussion in this section is restricted to one-sided control schemes because the common concern in monitoring is to detect an increase in pollutant concentration. The extension to two-sided schemes is straightforward if they are needed for an indicator such as pH.

SHEWHART CHARTS

The Shewhart (1931) quality control chart is one of the oldest and simplest of the industrial quality control procedures. The chart is simply a graph of time of sampling, or sample number if samples are equally spaced in time, versus the sample mean value for the quality parameter being monitored. Time, or sample number, is the abscissa and sample mean value is the ordinate of a point on the graph. Typically the horizontal axis is positioned so as to intersect the vertical axis at the steady-state mean value, μ for the quality parameter. A horizontal line is also drawn to intersect the vertical axis at $\mu + Z\sigma$ where Z is the upper α quantile of the standard normal distribution and σ is the long-run standard deviation of the sample means. This line is called the upper control limit, and when a point falls above the line, the process is declared out of control. The average in-control run length (i.e., the average number of samples between declarations that the system is out of control, when in fact it is in control) is $1/\alpha$ if the sample means have a normal sampling distribution. The commonly used value of Z is 3, for which the corresponding value of α is 0.0013. In industrial quality control, the sample sizes are usually somewhere between 5 and 10 depending on cost and internal variability between members of a sample.

Lorenzen and Vance (1986) give a procedure for determining on an economic basis the sample size n , Z , and the time between samples. It would appear that their approach could be generalized to other control schemes and to ground-water monitoring situations.

A second control chart is often kept for the variability of the product. It is similar to the Shewhart chart for the sample means, only now the ordinate is the sample range, or standard deviation, and the horizontal lines representing upper and lower control limits are located on the basis of the distribution of the statistic (sample range or sample standard deviation) under the assumptions of a normal distribution for the quality parameter measurements. In practice the lower limit is seldom used (Guttman et al., 1982). This chart is not nearly as robust with respect to the assumption of normality as is the chart for the sample means, and so out-of-control situations for variability must be viewed with more skepticism than similar results on the means chart. If the variability of measurements of the quality parameter changes, the height of the upper control line on the Shewhart chart for means is adjusted accordingly, or action is required to bring the variability back to its previous level.

THE CUSUM QUALITY CONTROL SCHEME

The CUSUM (for cumulative summation) control scheme derives from a paper by Page (1954) and is somewhat more complicated than the

Shewhart chart. (A review article by Lucas (1958a) gives the current state of development of this procedure). The CUSUM control scheme makes use of information in the present sample and in the previous samples in reaching decisions as to whether the process is in control, whereas the Shewhart chart makes decisions on only the current observation (i.e., the Shewhart chart is a graphical representation of a sequence of individual tests of the mean, whereas the CUSUM scheme is a sequential probability ratio test of the mean). The one-sided CUSUM scheme involves the computation of a cumulative sum S which for the i th sample is given by the formula.

$$S_i = \max \{ 0, z_i - k + s_{i-1} \}$$

where z_i is the standardized i th sample mean (i.e., $z_i = \{\bar{x}_i - u\}/\sigma$) and k is a parameter of the control scheme. When S_i exceeds a specified value h , the process is declared out of control (i.e., in pollution monitoring a decision is made to begin additional monitoring activity). The values of h and k are chosen to obtain desired average run lengths (ARL) under in-control and specified out-of-control situations. For a scheme designed to be sensitive to changes in mean quality of size D , k is usually chosen to be $D/2$, and h is selected to give the largest in-control ARL consistent with an adequately small out-of-control ARL. Typically S_0 is taken to be 0; however, Lucas and Crosier (1982a) have suggested that by employing a slightly higher value of h and starting with $S_0 = h/2$, one can decrease out-of-control ARL while maintaining in-control ARL. They also give tables of ARL for in-control and out-of-control situations and various values for h and k . However, if one is reasonably confident that there is no contamination coming from the HWS at the start, then one can start with $S_0 = 0$ and can somewhat decrease the chance of an early false positive.

Example 2:

To illustrate the Shewhart and CUSUM schemes, random normal ($u=10$, $\sigma^2=4$) deviates were drawn from a table of such numbers. To illustrate an out-of-control situation, 2 was added to each of the numbers drawn after the fourth ($i=4$). These numbers are to represent the sample means of a process that went out of control between the fourth and fifth samplings. The in-control situation has sample means distributed $N(10,4)$, so $z_i = (\bar{X}_i - u)/\sigma = (\bar{X}_i - 10)/2$. The CUSUM scheme (Table 2) indicates that a decision to take action (process is out of control) should be made at $i=10$. The CUSUM chart (Figure 1) gives a visual impression of when the process went out of control. The corresponding Shewhart chart with an upper control limit of $(u + 3\sigma)=16$ is given in Figure 2.

This example illustrates the weakness of the standard Shewhart chart in detecting small changes in the value of the mean. One way to

TABLE 2. CUSUM QUALITY CONTROL SCHEME

($k=0.5$, $h=5$)

i	In-Control			Out-of-Control at $i=5$		
	\bar{X}	\bar{Z}	\bar{S}	\bar{X}	\bar{Z}	\bar{S}
0			0			0
1	14.504	2.252	1.752	14.504	2.252	1.752
2	11.108	0.554	1.806	11.108	0.554	1.806
3	7.594	-1.203	0.103	7.594	-1.203	0.103
4	7.580	-1.210	0.000	7.580	-1.210	0.000
5	11.588	0.794	0.294	13.588	1.794	1.294
6	12.002	1.001	0.795	14.002	2.001	2.595
7	10.434	0.217	0.512	12.434	1.217	3.312
8	9.378	-0.311	0.000	11.378	0.689	3.501
9	10.708	0.354	0.000	12.708	1.354	4.355
10	11.278	0.639	0.139	13.278	1.639	5.494

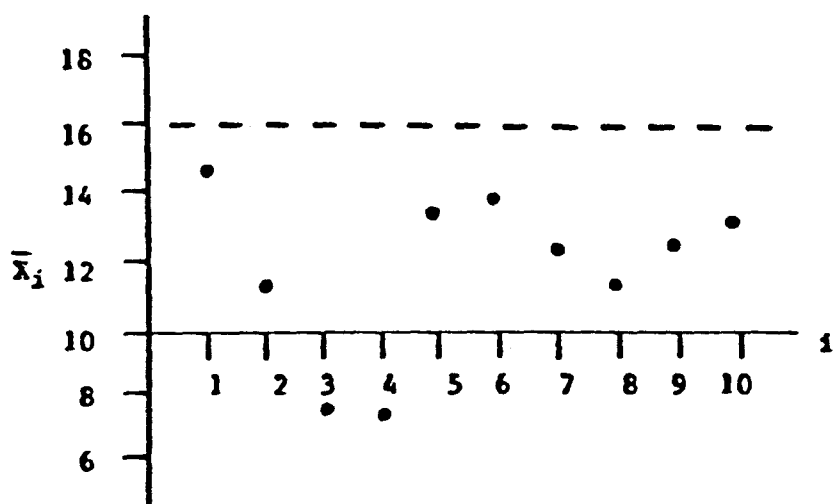


Figure 2. Shewhart quality control chart
($\mu=10$, $\sigma=2$)

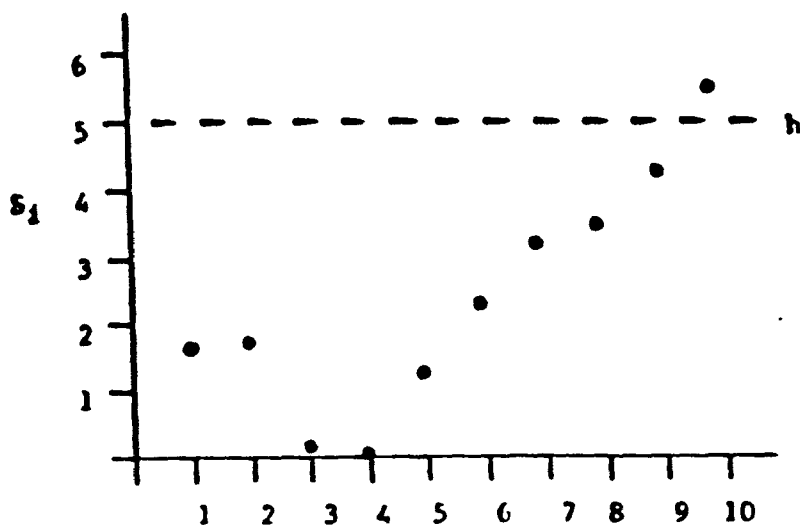


Figure 1. The CUSUM control chart
($k=0.5$, $h=5$)

reduce this weakness in the Shewhart chart is to declare the process out of control whenever there are r successive sample means with value above u . The value of r is usually chosen to be 7 or 8. However, this procedure also reduces the in-control ARL.

THE COMBINED SHEWHART CUSUM SCHEME

The Shewhart scheme is better than the CUSUM scheme in quickly detecting large ($>3\sigma$) shifts in the mean μ , whereas the CUSUM scheme is usually faster in detecting a small change in that persists. Bissell (1984) has also shown that the CUSUM scheme is to be preferred when the mean is increasing in a linear time trend. To take advantage of the good properties of both tests, Lucas (1982) suggested combining the two procedures. This is accomplished by declaring the process out of control if the CUSUM S_i is above specified upper Shewhart limit or if the CUSUM S_i is above a specified limit h . To keep a reasonable in-control ARL, Lucas suggests using an upper Shewhart control level of $(\mu + 4\sigma)$. Lucas calculated that if this upper Shewhart control limit is used with CUSUM parameter values $k=0.5$ and $h=5$, the in-control ARL is 459 while the out-of-control ARL is 10.4 if the true mean shifted upward by σ and only 1.6 if the mean shifted upward by 4σ . If the nature of the data is such that there are occasional outliers (perhaps because of contamination of samples during handling and processing), Lucas and Crosier (1982b) suggest the use of two-in-a-row rule. That is, require values in two successive samplings above the upper Shewhart control limit before declaring an out-of-control situation based on the Shewhart control limit being exceeded.

For measurements that are typically near or below the instrument minimum detection limits, it may be possible in some cases to treat the measurements, or some transformation of the measurements, as Poisson count data (Ingamells and Switzer, 1973). Lucas (1985b) has discussed how the CUSUM quality control approach can be employed with Poisson count data.

MULTIVARIATE QUALITY CONTROL SCHEMES

In monitoring ground water at a HWS, the concentrations of a several GQP may be measured at each of several monitored wells. If a quality control chart is kept for each parameter at each well, then one has the problem that while the chance of a false alarm is kept small for each chart, the overall probability of a false alarm becomes large (i.e., the in-control ARL for the whole set of charts may be quite small in spite of large in-control ARL's for each individual chart). This problem is addressed in survey papers by Jackson (1985) and Alt (1985). One approach is to use a control scheme that in effect performs a Hotelling's T^2 test on each sample. This is essentially a two-sided procedure in that abnormally low concentrations or unusual combinations of moderate

concentrations as well as abnormally large concentrations of some pollutants may trigger the alarm. The statistic calculated at each time of sampling is

$$T^2 = (X - u_0)^T \Sigma^{-1} (X - u_0)$$

where X is the vector of (transformed) parameter measurements at the monitored wells, u_0 is the steady state mean vector and Σ is the (positive definite) covariance matrix for the vector of the random variables corresponding to X . Under assumptions of multivariate normality for the measurement vector, T^2 will have a chi-square distribution with degrees of freedom equal to the dimension of the X vector when the system is in steady state. Hence the control chart is similar to the Shewhart chart in that each observed T^2 -value is plotted against the number of its sampling period, and the system is declared out of control if T^2 exceeds an upper control limit which is the upper 100 -percent point of the appropriate chi-square distribution. Jackson and Mudholkar (1979) have suggested additional statistics that might be monitored to determine whether outliers or changes in have occurred. Montgomery and Klatt (1972) have determined optimum sample size and interval between sampling times for the T^2 control procedure.

A procedure, call MCUSUM for multivariate CUSUM, proposed by Woodall and Ncube (1985) advocates running individual CUSUM charts on the different GQP or on principal components of the GQP values and choosing h and k so that the combined ARL under in-control conditions will be acceptable. Unfortunately, when running CUSUM's on measurements of individual GQP where measurements on different parameters or on the same parameter from different wells are correlated, the calculation of appropriate values for h and k is extremely difficult. If principal components are used, computation of appropriate h and k values is easier to perform, but the control charts for principal components will have to be two sided in most cases, and when action levels are exceeded, it is difficult to interpret the cause, and it may not be due to excessively high concentrations of a monitored parameter. (This is the same problem encountered with the T^2 procedure.)

A DECISION PROCEDURE USING A CONTROL CHART SCHEME

A decision procedure for ground-water monitoring must be adaptive and pragmatic. It must be in accord with available knowledge about the aquifer being sampled. At the beginning of sampling when little information about the system is available, simple procedures such as outlier tests should be employed. As more information about the mean and variance of measurements taken at the various wells becomes available, changes to more powerful procedures should be possible.

At the end of a year of sampling (at least once per quarter at each up- and down-gradient well), the temporal variance for each GQP may be estimated by using the analysis of variance procedure discussed earlier. With this variance estimate and the sample mean for the GQP at a well, one can initiate a combined Shewhart-CUSUM scheme with possible inclusion of the robust two-in-a-row rule of Lucas and Crosier (1982b) that was mentioned above. At this stage it would be well to choose a larger than normal CUSUM threshold value h , because of the lack of precision in the estimation u and σ . At the end of each year, provided a basic change in the system (i.e., the aquifer) has not occurred, the year's data should be combined with the data from previous years to obtain improved estimates u and σ . The quality control scheme should then be updated by using these new estimates. If experience indicates a fairly steady system and that the estimates of u and σ are reasonably accurate, the value of the threshold h in the CUSUM may be reduced to increase the power of the scheme in detecting changes in u that may be due to leakage from the HWS.

When the control scheme for a GQP at a down-gradient well indicates an out-of-control situation, the pattern of change in measurements of the GQP at this well should be compared to the patterns observed at the up-gradient wells. If a similar pattern was observed earlier or is currently at one of more up-gradient wells, it would indicate that the out-of-control situation is being caused by something other than the HWS and that increased monitoring activity would not be required. The importance of monitoring up-gradient wells and keeping corresponding control charts on them cannot be overemphasized. They are the principal means of determining when out-of-control situations down-gradient are caused by off-HWS activities. In addition, when similar patterns cannot be found up-gradient, they give greater confidence to a call for additional monitoring action in down-gradient out-of-control situations.

The combined Shewhart-CUSUM procedure was selected here because different types of leakage (e.g., small or massive) are possible at a HWS. The robust two-in-a-row rule should be considered because gross errors in GQP measurements are a problem which is usually due to contamination of some stage of the measurement system. It would be useful in situations where the slow flow of water in the aquifer allows the wait for a decision till the next sampling period without significantly increasing health risks or remedial costs if a leak has occurred. However, in most cases, it will be necessary to follow the current procedure of checking for measurement error by taking a second sample at the well as soon as possible after an unusually high sample measurement is obtained. Multivariate control schemes do not appear appropriate to ground-water monitoring because of the amount of information required to accurately estimate the covariance matrix.

PROBLEMS ASSOCIATED WITH THE QUALITY CONTROL APPROACH

There are some difficulties to be overcome in the application of quality control methods to the monitoring of HWS. Before a quality control scheme can be instituted, the system must be sampled in a steady state situation over a long enough period to obtain good estimates of its first and second moments. Intervention by humans and nature may make it difficult to study a system in steady state condition for a long enough period to establish a control scheme.

The control schemes are based on the assumption that measurements are approximately normal in distribution. In industrial quality control, several samples are taken and their measurements are averaged so as to obtain a measurement for the sampling period that is approximately normal and has reasonable small variance. The high cost of sample analysis and possible redundancy of measurements of successive ground-water samples make it difficult to employ this procedure here. Data transformations may provide near normality of the data, but they cannot help in considerations of power of the test implicit in the quality control scheme.

Cyclic trends in GQP values are hard to detect, measure, and remove. However, failure to remove such trend can have serious effects on the quality control scheme.

SUMMARY

It is essential that a realistic workable model for the measurements be formulated and used both in construction and evaluation of decision procedures. Tests based on unrealistic models will not succeed in providing an answer to the ground-water monitoring decision problem no matter how simple and elegant the test may be. The formulation of good decision procedures (i.e., procedures that give few false positives and few false negatives and that are based on an affordable data base) for determining when increased monitoring activity is needed at HWS is extremely difficult because of the high cost, low precision, and multivariate nature of ground-water monitoring data along with system instability which is due to intrusions of the aquifer caused by man outside the HWS.

The application of industrial quality control schemes to each type of water quality measurement from each well or to a vector of the measurements from each well or from all monitored wells was discussed. The advantages of the quality control approach are that it provides a method for looking at what is happening to water quality over time and that it gives users a better feel for the nature of the data that they have obtained than a simple test of means does. A sequence of decision rules starting with outlier tests and ending with combined Shewhart CUSUM control schemes is suggested as a reasonable approach to monitoring ground-water at a

HWS. Unfortunately, quality control schemes do not solve all the problems involved in determining a statistical decision rule for when an alarm should be sounded. Problems involving cost and precision of measurements will ultimately have to be solved by improvements in measurement technology.

NOTICE

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THE USE OF GEOSTATISTICS FOR CONTOUR MAPS

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ABSTRACT

Monitoring data are spatial variables, called regional variable in geostatistical text books. This means they have locations as well as amount of pollutant, and they are correlated in space. Structural correlation means that samples that have locations close together usually have similar amounts of the pollutant, and samples with locations farther apart have less similar amounts of the pollutant. Experience in monitoring confirms that monitoring data have these characteristics and need spatial analysis or geostatistics.

Spatial variables are not adequately described by a mean and a standard deviation, but rather they must be described by a control map of means and a contour map of interpolation errors or kriging errors. This poster session will show contouring of actual pollution plumes with discussion of problems such as outliers.

MONITORING ACTIVITIES BASED ON GEOSTATISTICS

The number and location of sampling sites and the frequency of sampling are essential decisions in all monitoring activities. To improve the statistical basis for monitoring activities, researchers at EPA's Environmental Monitoring Systems Laboratory in Las Vegas are adapting geostatistical methods and times series analysis to the space and time problems of monitoring, with particular attention to soil and groundwater pollution. These approaches will improve the design of sampling programs and the interpretation of monitoring data.

Pollution plumes generally spread in a contiguous manner. Thus, samples taken close to one another in space or time should be more alike than samples taken further apart. Such samples are called "correlated" or regional variables. The contaminant levels in samples of such closely associated variable obey different statistical laws than "random" variables.

Sampling of random variables assumes that each sample value is unrelated to the proximity in time or space of other sample values. Thus, the best sampling schemes for this kind of variable are random sampling designs. The variability of the pollution concentrations at the site and the uncertainty in the data that is acceptable determine the sample size. The larger the variation at the site or the smaller the acceptable uncertainty, the larger the number of samples that must be taken.

For correlated variables, the best sampling schemes are systematic approaches, such as sampling on grid, instead of random sampling. The number of samples required for correlated variables as for random

variables is determined by the variation in pollutant levels at the site and the acceptable uncertainty. But analysis of correlated variables can use the relationships between samples to reduce the number of samples needed.

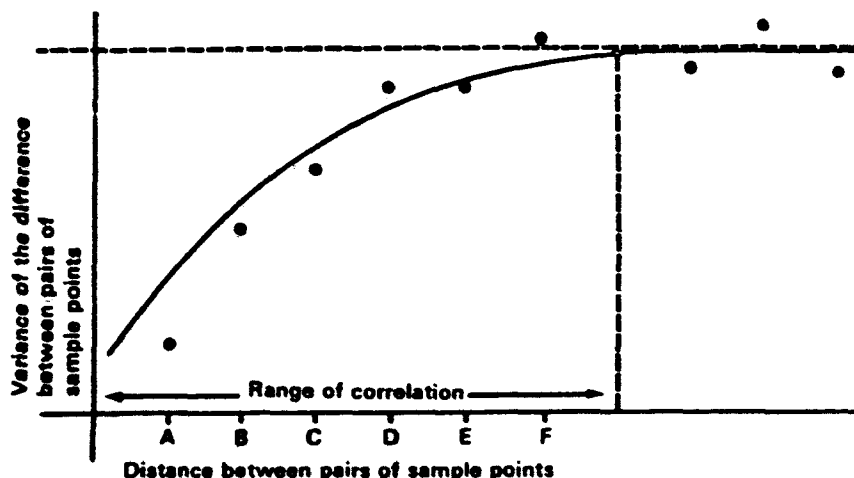


Figure 1. A semi-variogram

THE SEMI-VARIOGRAM

The semi-variogram is a geostatistical tool based on information about time or space relationships between sample observations. The semi-variogram in Figure 1 graphically shows the relationships between observations in terms of the distance from each sample to each of the other samples. The x-axis represents the distance between sample points, and the y-axis represents the "variance" of the difference in pollutant levels between pairs of samples which are equal distances apart.

Since both the distance and the difference between two samples of correlated variables taken at the same point are zero, their point of intersection on the graph corresponds to the intersection of the x and y axis. When the distance between two samples increases and their correlation weakens, the difference in their values also increases, resulting in a rising curve or semi-variogram. As the difference between samples becomes sufficiently great, the sample values become independent of each other. Their difference becomes more nearly constant, and the curve becomes a horizontal line. The distance along the x-axis where the semi-variogram is rising represents the "range of correlation," the distance within which samples are related. This range is used for determining the grid design for sampling, for contouring pollutant concentrations on a map, and for calculating the uncertainties in contouring.

SAMPLING STRATEGY

In practice an initial semi-variogram can be constructed on the basis of preliminary sampling undertaken in the area of suspected contamination. A semi-variogram can be computed from the sample data by analyzing all possible pairs of the data. For example, in Figure 2

point 1 through 5 might represent soil samples. There are four pairs (1 and 2, 2 and 3, 3 and 4, 4 and 5) of these samples separated by a distance equal to A. Thus, for the x-axis value of A, the y-axis value would be the variance of the differences of these four pairs. In practice, even for a small preliminary sample, the calculations of all possible pairs can be so long that a computer program must be used. If the correlation among samples is weak—that is, samples close together or far apart have similar differences—the semi-variogram approaches a horizontal line. A horizontal semi-variogram implies that the variable is random and can be represented simply by a mean and a variance. If the correlation is strong, then the differences between samples taken close together are smaller than differences between samples taken further apart, and the semi-variogram is a rising curve as shown in Figure 1.

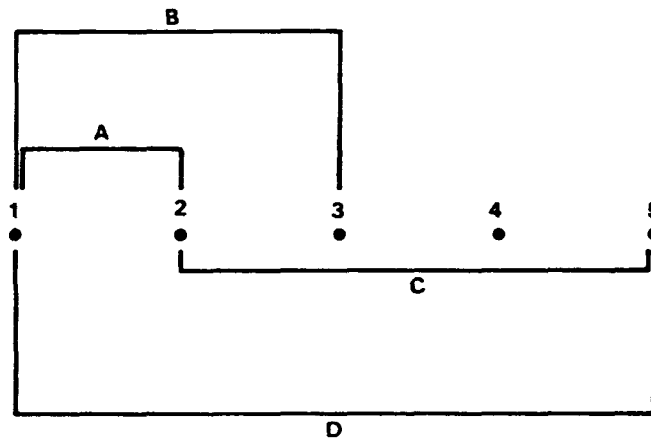


Figure 2. Possible pairs of sampling points.

The spacing of sampling points for intensive sampling can be determined using the semi-variogram as a guide. A grid designed to cover the area to be monitored provides a tool for determining sample sites, with samples taken at each grid intersection. Usually a grid spacing of about two-thirds the range of correlation insures that the sampling points in the intensive sampling phase are close enough to each other to have correlated values. To sample at closer distances would provide little new information while greater spacing could miss a change in pollution levels. The results of the intensive sampling can be used to refine the semi-variogram and confirm the appropriateness of the sampling distances.

KRIGING

A technique called "kriging" interpolates pollution levels at points between the sampling sites so that "isopleths" of pollution levels can be drawn on a map. The kriging estimate of the pollutant level at any particular point is the weighted average of the nearest neighboring sample values. The size of the neighborhood is determined by the range of correlation. The result is an isomap, a contour map with isopleths tracing the lines of equal values. Figure 3 presents pollutant levels for a study area in Southeast Ohio. Isomaps can be used as overlays for

aerial photographs or maps of the study area. The three-dimensional map show in Figure 4 is another way of presenting data shown in Figure 3 and clearly distinguishes the same high and low pollutant levels.

Kriging also can compute the standard errors of estimation for the estimated samples values using the range of correlation. These error estimates can also be mapped. Figure 5 contours the standard errors of estimates or the concentration levels shown in Figures 3 and 4.

By using these three types of geostatistical outputs, a decision maker can identify areas requiring cleanup, more sampling, or no action. A cleanup area would be delineated by pollution averages which are above a chosen action level and standard errors which are below a chosen acceptable level. An area for more investigation would have high values for both pollution averages and standard errors, and an area for no action would have low values for both.

To date, the Laboratory's efforts have been directed toward applying geostatistical techniques to two-dimensional problems, such as contamination of surface soil. Future efforts will investigate the feasibility of using similar techniques in addressing three-dimensional problems, such as those encountered in groundwater contamination.

NOTICE

Although the research described in this article has been supported by the United States Environmental Protection Agency, it has not been subjected to Agency review, and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

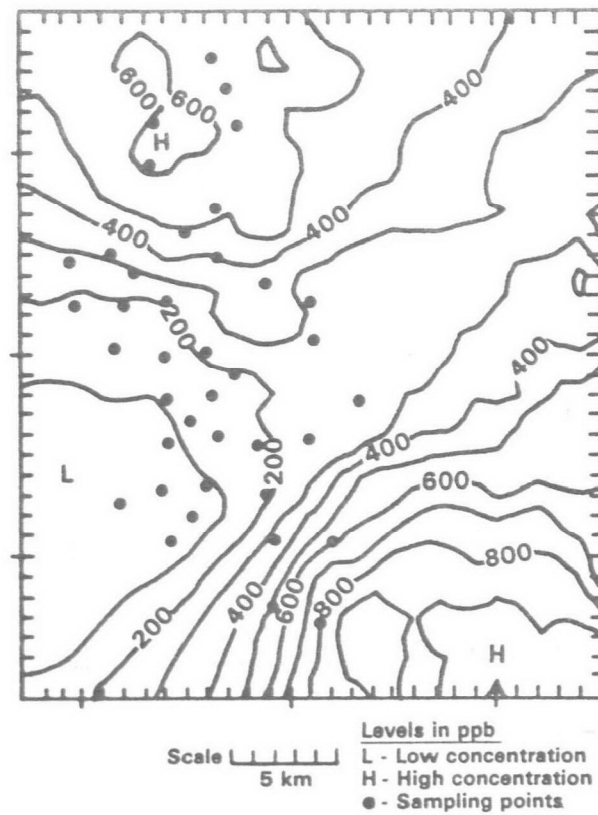


Figure 3. Kriging of benzo-a-pyrene concentration in soil.

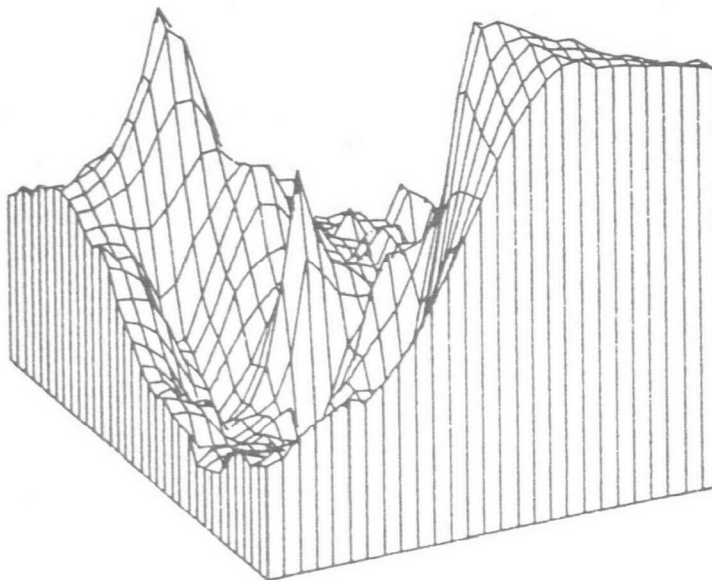


Figure 4. Three dimensional representation of data used for Figure 3.

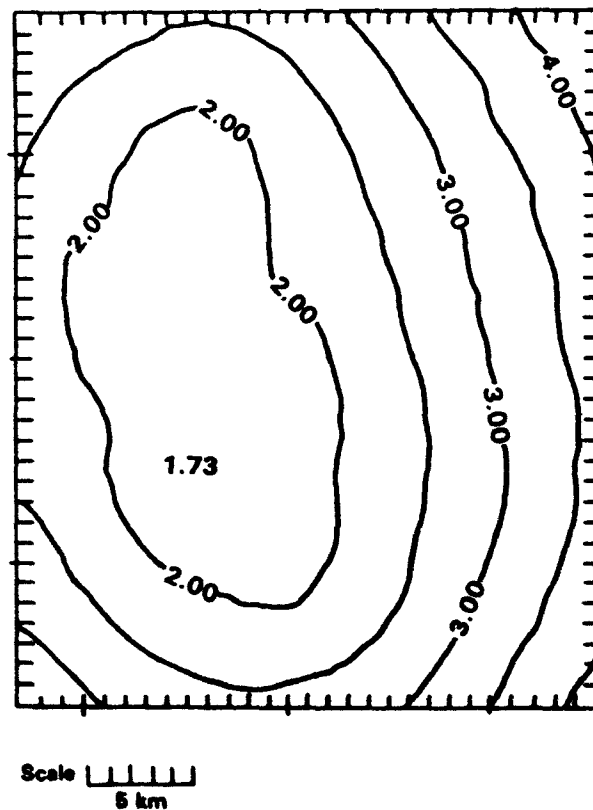


Figure 5. Standard errors of estimates for benzo-a-pyrene concentration in soil.

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To date, the Laboratory's efforts have been directed toward applying geostatistical techniques to two-dimensional problems, such as contamination of surface soil. Future efforts will investigate the feasibility of using similar techniques in addressing three-dimensional problems, such as those encountered in groundwater contamination.

BIOLOGICAL TEST METHODS

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UTILITY OF IMMUNOASSAY FOR TRACE ANALYSIS OF ENVIRONMENTAL CONTAMINANTS

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ABSTRACT

Immunochemical technology is being rapidly applied to solve problems in environmental chemistry. Immunoassays such as the enzyme-linked immunosorbent assay (ELISA) are sensitive bioanalytical procedures applicable to many classes of compounds. Immunoassay development must be guided by the basic principles of analytical chemistry. Sample collection and preparation, and data acquisition and handling all need to be optimized.

INTRODUCTION

Improvements in selectivity, sensitivity, speed, and simplicity are important goals in analytical chemistry (Milby and Zare, 1984). The evolution and application of new analytical methods will help meet these requirements. Since their introduction, immunoassays have become widely used for the analysis of proteins, hormones, and drugs, and are rapidly being extended to pesticides, and environmental contaminants (Table I). Immunoassays utilize specific antibodies and a detectable label on either antibody or antigen. The high selectivity of immunoassays is assured by the inherent selectivity of immunological reactions, their sensitivity is determined by antibody affinity and by the detection limit of the label.

Immunochemical methods of analysis offer advantages of sensitivity, specificity, and speed of analysis (Hammock and Mumma, 1980). Compounds which are most difficult to analyze by classical procedures are frequently amenable to analysis by immunochemistry. Immunoassays are especially applicable to compounds that: 1) must undergo extensive derivatization for gas chromatography or high-pressure liquid chromatography; 2) are water soluble; 3) require a multi-step sample preparation, and 4) are chemically complex. In several of these instances, immunoassays not only complement other analytical techniques but may be the most practical method. Products of biotechnology such as genetically engineered microorganisms, plant gene manipulations, and fermentation products are particularly amenable to immunochemical detection.

ASSAY FORMATS

Several different immunoassay formats are currently in use. As the technology becomes more commercialized many other assay permutations can be expected. Some of these new assay designs will be developed

to solve a particular need, while others will be introduced as slight modifications to existing procedures. Whatever the assay design, each is dependent on the highly specific antigen-antibody reaction. Although antibodies are biologically derived reagents, immunoassays must not be confused with bioassays. These two techniques are fundamentally distinct and are based on different principles.

ANTIBODIES

Polyclonal antibodies are adequate for most immunoassays, however, monoclonal antibodies could be developed against pesticide and environmental contaminants yielding an analytical reagent that is physically, chemically, and immunologically homogeneous. The large supply of monoclonal antibodies could facilitate the standardization of immunoassays as several laboratories would be using the same antibody clone. Although a very small amount of antibody is needed per assay, having a large supply of monoclonals could alleviate fears of eventually exhausting the supply. Yet, it is important to consider that, in some cases, polyclonals will be superior to monoclonal antibodies (Hammock and Mumma, 1980). However, as immunoassay moves into the private sector, there will be compelling administrative and legal pressures to employ monoclonal antibodies (Van Emon et. al., 1985).

ADVANTAGES OF IMMUNOASSAYS

For a chromatographic analysis, unless a large number of expensive instruments are employed, usually just one analysis can be done at a time by one person (Chait and Ebersole, 1981). With immunoassays, many analyses can be performed at once by a single individual without elaborate apparatus, and the results can be monitored visually in some cases, eliminating an instrumental detector.

Immunochemical methods, traditionally unfamiliar to the residue chemist, offer exciting possibilities for newer cost-effective approaches for residue analysis (Hemingway, 1984). Affinity columns loaded with antibody could selectively remove and clean-up an analyte from solution before analysis by a conventional method. Immunoassays could also be used as selective detectors for chromatographic separation techniques.

The analytical use of antibodies enables substances to be quantitated at low levels in crude extracts. The costly and time-consuming steps of extraction and isolation required of many other analytical methods can often be avoided. This elimination of steps reduces the amount of error in the procedure. Due to the minimal sample preparation and speed of analysis, immunoassays are excellent screening methods for the more expensive analytical techniques such as gas chromatography/mass spectrometry.

Since the assay is performed in an aqueous media, slightly different methods of extraction and sample preparation should be expected. Such changes should result in an overall simpler procedure. However, extraction and procedural efficiencies must be determined for each assay.

Antibodies which will detect parent compound plus toxic metabolites could be used in combination with one or more highly specific antibodies to quantitate several compounds of interest. If the antibody is of moderate specificity, one can develop a general screening assay for the presence of a class of compounds or the presence of a specific functionality. However, if one needed to analyze several different compounds simultaneously in one matrix, immunoassay may not be the method of choice, due to the large amount of controls and standards needed. Immunoassays could be successfully used for the rapid screening of a large number of samples for the presence of specific types of compounds and for confirmatory tests (Ercegovich, 1971).

Immunoassays can be optimized for speed, portability, or sensitivity. Highly sensitive assays can be developed for research purposes, but by using the same reagents one can develop an assay that will give a quick qualitative answer in real time. Such rapid assays can be performed on site. Immunoassays can be adapted as simple field screening procedures, since reagents are stable, have long shelf-lives, and present no health hazards (Anonymous, 1979; Voller et al., 1976). Assays that are optimized for speed will be done so at the expense of sensitivity. However, the sensitivity of the antigen-antibody reaction is so great that a small loss in detection limit will still afford a usable assay. Qualitative immunoassays could be used to determine sources of contamination, direct the collection of samples, and monitor cleanup operations.

CONCLUSIONS

An immunoassay consists of the specificity of an immunological reaction with a sensitive indicator system. Improvements in all the components of immunoassay are certain, and will yield improved assays. These improvements include new enzyme labels; sensitive methods of enzyme determination such as cofactor cycling; and better cross-linking reagents, especially of the hetero-bifunctional type (Wisdom, 1976). Many modifications can be expected in the delivery systems for these assays. For instance, one can envision ELISA systems coupled to photon counters or luminometers if enzymes such as luciferin were used or to silicon chip if redox enzymes are used. Such assays could provide a dynamic output of concentration of analyte in real time.

The field of immunoassay is very broad with new techniques constantly being introduced. The various types of immunoassays are

complimentary and there is no one ideal label for use in all immunoassays. The choice of assay format and label should be based on the analyte to be measured and the purpose for which the measurement will be made. There is a wide variety of circumstances and environments in which immunoassay and other similar ligand binding assay techniques could be employed. Although there appears to be an overindulgence in pursuing novel methods, alternative methodologies based on the antigen-antibody reaction will continually emerge, many designed for a particular circumstance.

Table 1

IMMUNOASSAYS WITH ENVIRONMENTAL APPLICATIONS

<u>Compound</u>	<u>References</u>
1. Aldrin and Dieldrin	Langone and Van Vunakis, 1975
2. <u>Bacillus thuringiensis</u>	Wie et al., 1982
3. Benomyl	Lukens et al., 1977
4. Chlorsulfuron	Kelley et al., 1985
5. 2,4-D and 2,4,5-T	Rinder and Fleeker, 1981
6. Paraoxon	Hunter and Lenz, 1982
7. Paraquat	Van Emon et al., 1986
8. Parathion	Ercegovich et al., 1981
9. S-Bioallethrin	Wing and Hammock, 1979

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DERIVATION AND USE OF MONOCLONAL ANTIBODIES FOR ENVIRONMENTAL MONITORING

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ABSTRACT

Over the past 12 years, monoclonal antibody technology has proven valuable in numerous biomedical applications. This talk will summarize unique advantages and difficulties of extending this technology to environmental toxicology, chemical quality assurance, and risk assessment, where it has evolved more slowly.

Most analytes of environmental interest are of low molecular weight, somewhat nonpolar, sparingly soluble in aqueous media, and at low concentration in the test sample. Monoclonal antibodies (MAbs) for detecting these substances must have the highest possible affinities. The analytes (haptens) are made immunogenic by covalent coupling to "carrier" molecules. Conjugates must be designed with regard to sterism, linkage chemistry, linker length, hapten density, and choice of carrier. Immunization and screening procedures should make use of different conjugates. Immunization protocols should promote T-helper cell responses and minimize T-suppressor cell responses to the hapten. Different mouse strains should be immunized, as they can differ significantly in their antibody responses. Because any set of MAbs will have a broad spectrum of affinities and specificities, it is highly desirable to clone hybridomas during initial selection, and to screen a great many. Robotic sampling systems and computer-assisted screening are essential for this purpose. Newer techniques such as splenocyte transplantation and antigen-focused cell fusion may improve the yield of useful hybridomas, but these methods have not yet been widely tested.

Enzyme and radioimmune assays are the most common analytical methods in current use, but several others, ranging from immunoelectrodes and solid-state immunosensors to disposable cards and "dipsticks," are rapidly being developed. MAbs are also potentially useful agents for extracting and concentrating the substances of interest from complex mixtures or from solid surfaces. Problems of analyte concentration and elimination of interfering substances are different for immunoassays than for standard analytical procedures. Techniques to allow immunoassay of substances that are nearly insoluble or chemically unstable in aqueous solvents remain to be developed.

Immunoassays are ideal for rapid screening of large numbers of samples, e.g., for on-site monitoring of the degradation and dispersal of toxic materials. For the near future, most applications will involve monitoring of air, water, and soil pollution and residue levels in foods. Other potential uses include quality analysis in chemical manufacturing, determination of worker exposure and safe field re-entry intervals, and tracking of organisms and recombinant gene products used in biological and other conventional analytical methods.

MAB-based environmental monitoring presents new regulatory problems. Agencies must develop criteria for standardizing diverse immunochemical assays and relating them to instrumental analysis. Policy makers will have to factor immunoassay results into their decision-making processes. Immunoassays for adducts or metabolites of genotoxic substances have great potential for dosimetry and risk assessment, but their implementation will raise legal and ethical issues.

IMMUNOASSAY FOR THE DETERMINATION OF PENTACHLOROPHENOL
AND RELATED COMPOUNDS IN WATER SAMPLES

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ABSTRACT

The use of immunochemical methods has been wide spread in clinical laboratories, and the techniques have also been applied to the determination of a variety of low molecular weight compounds such as drug residues, mycotoxins, pesticides and plant hormones. Immunochemical analytical methods are generally based on the principle of competition between analyte and labeled form of the analyte for a specific receptor. In a cooperative effort between Westinghouse Bio-Analytical Systems, EPA, and Lockheed-EMSCO, a new monoclonal antibody-based immunochemical analytical procedure for the determination of pentachlorophenol (PCP) levels in water has been evaluated.

The method is a competitive inhibition enzyme immunoassay using anti-PCP antibodies prepared by the monoclonal antibody technique. The free PCP in solution binds to the anti-PCP antibodies and inhibits their subsequent binding to the solid-phase adsorbed PCP protein conjugate. After an incubation, the anti-PCP antibodies not bound to the surface of the microtiter wells are removed by washing. The number of antibodies bound to the solid-phase is determined by adding an enzyme-labeled second antibody with binding specificity for the anti-PCP antibody. After an incubation and final washing, the substrate of the enzyme is added and the amount of anti-PCP antibodies bound to the solid-phase is directly proportional to the intensity of color produced by the enzymatic reaction. The intensity of this color is therefore inversely proportional to the concentration of PCP in the sample. Quantitation is achieved by comparing the mean optical density obtained with the sample to a standard curve generated from known concentrations of PCP on the same plate.

Thirty-six water samples from various sources (tap, ground and surface water) were spiked with different levels of PCP and analyzed by two independent laboratories. The samples were also analyzed using an EPA approved GC/MS method. In addition, aliquots of the sample extracts prepared for GC/MS analysis were also analyzed by the immunoassay. For water samples that were analyzed directly, the method detection limit was 88.8 ppb (parts per billion). The method also provides for the use of an internal standard addition to determine possible interferences. During the course of this study several hardware problems associated with this type of assay were encountered (mainly, the plate effect and the reader effect). These unexpected effects have resulted in considerable delay in finishing this study. Nevertheless, the results indicate that this immunoassay can be used to detect PCP in selected water samples, and that once the hardware problems are resolved, the precision, accuracy and the speed of this procedure will be much improved. This study is the first of several

planned to evaluate methods based upon immunochemical techniques that offer potential for low-cost, sensitive and selective detection of target chemicals of Agency interest. The study was developed in coordination with the OSW staffs to demonstrate the equivalency of proposed alternatives to Agency-accepted methods.

ULTRA SENSITIVE BIOASSAY FOR DIOXIN

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ABSTRACT

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to induce ethoxyresorufin-O-deethylase (EROD) activity or other biological responses in a number of cell cultures. Using a new cell line, WBAS 86-163B, an ultra sensitive bioassay for the detection of TCDD and related compounds has been devised. The target cells can detect TCDD concentrations as low as $2 \times 10^{-13}M$, which is at least 1.5 logs more sensitive than other bioassay systems. With the WBAS cell line, an induction response can be obtained after treatment of the target cells for only 18-24 hours, four to 10 days less than required by other bioassays. These cells have been used successfully to detect TCDD in soil and water samples.

Several dibenzo-p-dioxins, chlorinated phenols and polychlorinated biphenyls were tested for their abilities to induce EROD in the target cells. TCDD was at least several thousand fold more potent in eliciting a response than were the other chemicals. TCDD was 8 times more active as an EROD inducer than the next most effective chemical, 2,3,7,8-tetrachlorodibenzo-furan.

INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) contamination in the environment has become a major public health issue. TCDD and related compounds such as polychlorinated dibenzofurans are produced as by-products of the manufacture of industrial organic compounds (eg. 2,4,5-T) and also as the result of several combustion procedures (1-3). TCDD is an extremely stable compound which has been shown to cause liver and kidney damage, chloracne, mutagenesis of bacterial and mammalian cells as well as immunosuppression in mice (1,4-7). Thus, rapid and reproducible assays are needed to meet regulatory requirements for the detection of TCDD.

TCDD analysis is conventionally done by gas chromatography/mass spectrometry (GC/MS) analysis. To detect low concentrations of dioxin usually requires using the mass spectrometer in its most sensitive mode. However, under these conditions, other compounds of interest (eg. chlorinated dibenzofurans) may not be detected. An alternative method for detection of TCDD and related compounds is the use of an assay based upon the biological response of cells to the chemical.

Several bioassays from the detection of TCDD have been described, including in vitro systems based upon inhibition of cell division,

altered cellular morphology and enhanced differentiation of specific cell types, as well as induction of enzymes in vivo and in vitro (2, 8-10). In this paper we describe a detection method for TCDD which is quicker and more sensitive than other bioassays. The assay has a dynamic range of 0.060 pg/ml to 1.0 pg/ml and has been used to detect TCDD in water samples, sediment from water samples, and benzene extracts of soil samples.

METHODS

Target Cells: WBAS cell culture 86-163B (patent pending) was used in all experiments. The parent cell line was derived from mouse liver cells. After mutagenesis and cloning, cells were isolated based upon the inducibility of the enzyme ethoxyresorufin-O-deethylase (EROD) by TCDD. The cells were cultured in William Medium E (WME) with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 10 mM Hepes buffer (pH 7.4).

Chemicals and Sample Preparation: TCDD was obtained from the National Bureau of Standards (Gaithersburg, MD) or Cambridge Isotope Laboratories (CIL; Woburn, MA). Other dibenzo-p-dioxins, chlorinated phenols and polychlorinated biphenyls were purchased from Chem Services (West Chester, PA) or were synthesized at WBAS. 2,3,7,8-tetrachlorodibenzofuran (TCBDF) was obtained from CIL.

For routine use in the assay, benzene extracts of TCDD-containing soils are reduced to near dryness under a flow of nitrogen gas. The samples are then brought to volume (1 ml) in dimethylsulfoxide (DMSO). Sediment is removed from water samples by centrifugation, then dried, weighed and resuspended in 250 ul of DMSO. The sediment-free water is then passed over a C18 reverse-phase column. The resulting water fraction ("stripped" water) is retained for testing. Organic material on the column is eluted with acetone and taken to near dryness as described above and brought to volume in DMSO.

Assay: WBAS 86-163B cells are planted into 35 mm tissue culture dishes 20-24 hours before treatment. The medium is then removed from the cells and they are refed with 3 ml WME as described above, except that the FBS concentration is reduced to 1% (1% WME). For comparison of induction of EROD, the cells are treated with stock solutions of dibenzo-p-dioxin and 12 selected chlorinated compounds (Table 1) prepared in DMSO. Duplicate wells are treated with 10 ul of sample and an additional 10 ul of DMSO, resulting in final DMSO concentration of 0.67%. A standard curve is generated by treating duplicate wells with appropriate concentrations of TCDD to yield final doses of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0 pg/ml, all in a final concentration of 0.67% DMSO.

For benzene extracts which have been exchanged into DMSO, duplicate wells of target cells are treated as described above. An additional set of duplicate wells also treated with 10 ul of each sample and 10 ul of TCDD spiking solution (150 pg/ml). The final concentration of the TCDD spike in these wells is 0.5 pg/ml. If further dilutions of a sample are required, they are made in DMSO, so that the solvent concentration remains constant.

Stripped water samples are initially mixed with 5X WME at a ratio of 4 parts sample: 1 part 5X WME and this mixture is used to treat the cells. However, if significant toxicity is induced by the samples under these conditions then they are tested at lower concentrations, usually at dilutions of 1:10 through 1:50.

Approximately 20 hours after treatment the cells are analyzed for EROD activity induced by the TCDD standards or by inducers in the samples. The general details of the enzyme assay have been described elsewhere (14).

RESULTS

Comparison of Inducers: Thirteen compounds have been tested for their abilities to induce EROD in target cells (Table 1). The concentration of each chemical needed to induce an EROD response is compared to the amount of TCDD needed to induce a similar response (Table 1). For example, 20,000 pg/ml of 2,4,5-trichlorophenol (2,4,5-TCP) induces a response equivalent to that induced by 0.063 pg/ml of TCDD. By this method, TCDD is estimated to be 3.2×10^5 more potent (20,000/0.63) than 2,4,5-TCP as an inducer of EROD in the WBAS target cells. Table 1 shows that none of the dibenzo-p-dioxins, polychlorinated biphenyls or chlorinated phenols are nearly as effective as TCDD in provoking a response in the target cells. Only TCDBF is within a log of TCDD in its ability to induce the enzyme in the target cells.

Sample Toxicity: Biological response systems for the detection of chemicals such as TCDD are particularly susceptible to toxicity-related phenomenon. The toxicity is sometimes overt, leading to altered morphology in the target cells; more subtle effects are also noted. For example, a sample which causes no overt cellular toxicity might induce a calculated TCDD equivalent value of 0.127 at a dilution of 1:20 and a calculated value of 0.164 at 1:50. The higher EROD response at the higher dilution indicates some residual toxicity associated with the sample that prevents full expression of the induced enzyme. In such cases the calculated value for the TCDD spike usually falls below the acceptable limit of 0.375 pg/ml. Further dilutions are required to bring the toxic substances down to an acceptable level and allows complete induction of the enzyme.

TABLE 1

Induction of EROD in WBAS Target Cells by
Chlorinated Compounds

Test Chemical	Inducing Dose (pg/ml)	Equivalent TCDD Dose (pg/ml) ^a	Relative TCDD Effect ^b
Dibenzo-p-dioxin (DD)	40,000	0.20	2.0 X 10 ⁵
7-Cl-DD	40,000	0.32	1.3 X 10 ⁵
2,7-dichloro-DD	40,000	0.20	2.0 X 10 ⁵
1,2,3,4-tetrachloro-DD	20,000	2.00	1.0 X 10 ⁴
1,2,3,4,7,8-hexachloro-DD	4,000	1.00	4.0 X 10 ³
Pentachlorophenol	40,000	0.25	1.6 X 10 ⁵
2,4,5 trichlorophenol	20,000	0.063	3.2 X 10 ⁵
2,4 dichlorophenol	20,000	0.063	3.2 X 10 ⁵
2,2',3,3',6,6' Poly-chlorinated biphenyl (PCB)	40,000	0.125	3.2 X 10 ⁵
2,3,3',4,4',5 PCB	10,000	2.00	5.0 X 10 ³
2,2',4,5,6' PCB	40,000	0.125	3.2 X 10 ⁵
2,2',4,5,5' PCB	40,000	0.125	3.2 X 10 ⁵
2,3,7,8-tetrachloro-dibenzofuran	8	1.00	8

^a Dose of TCDD required to give EROD response equivalent to that of the test chemical

^b Dose of the test chemical divided by the dose of TCDD required to give an equivalent EROD response.

Another problem area, although not necessarily related to toxicity, is high concentrations of inducers in a sample. Thus, the results obtained from an unknown sample might be 2.03 pg/ml at a dilution of 1:21600 with a similar value at a dilution of 1:43200. This lack of dilution effect indicates that such high concentrations of inducing substances are present that they are beyond the range of analysis.

Performance of the Assay: Figure 1 shows the results of a typical TCDD bioassay. Each point is the average of two replicates. The r^2 for this set of data is 0.997. In general, r^2 below 0.950 are rare, but for comparative purposes the assays are normalized with respect to the luminescence response of the 1.0 pg/ml TCDD control sample, setting this value to 100%. All other values are determined by dividing the luminescence at each dose by the luminescence at 1.0. (e.g., if the luminescence at 1.0 pg/ml is 400 and at 0.25 pg/ml is 130, the relative response at 0.25 pg/ml is 0.325). A summary plot of seven assays is presented in Figure 2. The r^2 of the data is 0.985; the midpoint of the curve is approximately 0.5 pg/ml (approximately $1.6 \times 10^{-12}M$). Inclusion of values for 2.0 pg/ml (which is outside the dynamic range of the system) lowers the r^2 to approximately 0.900. However, if the 2.0 pg/ml point is included, 4-parameter logistic analysis can be used and an r^2 of 0.990 can be obtained (not shown). Generally, the lowest concentration of TCDD used as a standard is 0.0625 pg/ml. However, the inclusion of 0.03125 pg/ml does not adversely affect the r^2 of the standard curve, as demonstrated in Figure 1. This lack of effect on the r^2 indicates that the 0.03125 pg/ml standard is within the dynamic range of the curve. However, at present, 0.0625 pg/ml (approximately $5 \times 10^{-13}M$) is used as the limit of detection. Samples falling below that level are retested at higher concentrations.

DISCUSSION

In this paper we describe a rapid, sensitive and reproducible bioassay for the quantification of TCDD and other EROD inducers in environmental samples. The target cells, WBAS 86-163B was selected for its hyper inducibility by TCDD. Like other cell culture-based assays for TCDD (2,10,11), related compounds also induce a response in the target cells. Except for TCDD, TCDBF was the most potent inducer of EROD in the WBAS cells. Approximately an 8-fold increase in TCDBF, compared to TCDD, was required to obtain the same response. These results are similar to those obtained by Gierthy and Crane (2) using the "flat cell assay" (FCA) and by Safe (10) using H4IIE cells, both of whom found that approximately 10-fold more TCDBF than TCDD was required to produce the same response. However, the sensitivity of the WBAS target cells is greater than reported for other systems. The midpoint of the dynamic range for our cells is approximately $1.6 \times 10^{-12}M$, as compared to $1.9 \times 10^{-10}M$ reported by Safe (10). The limit of detection in the FCA is $10^{-11}M$;

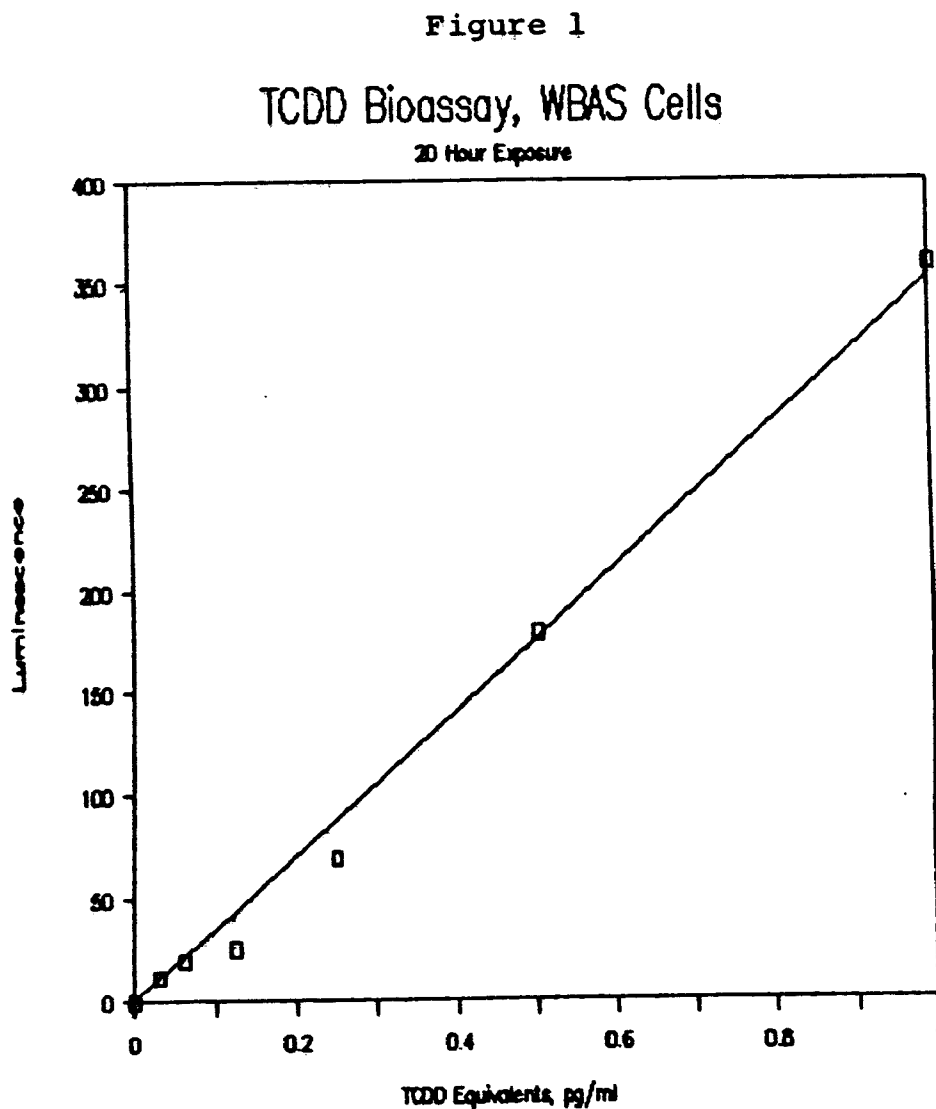


Figure 1: A standard curve from a typical TCDD bioassay. Each point is the average of two replicates. Standards were tested in two-fold dilutions from 1.0 down to 0.03125 pg/ml. The r^2 of this line was 0.997.

Figure 2

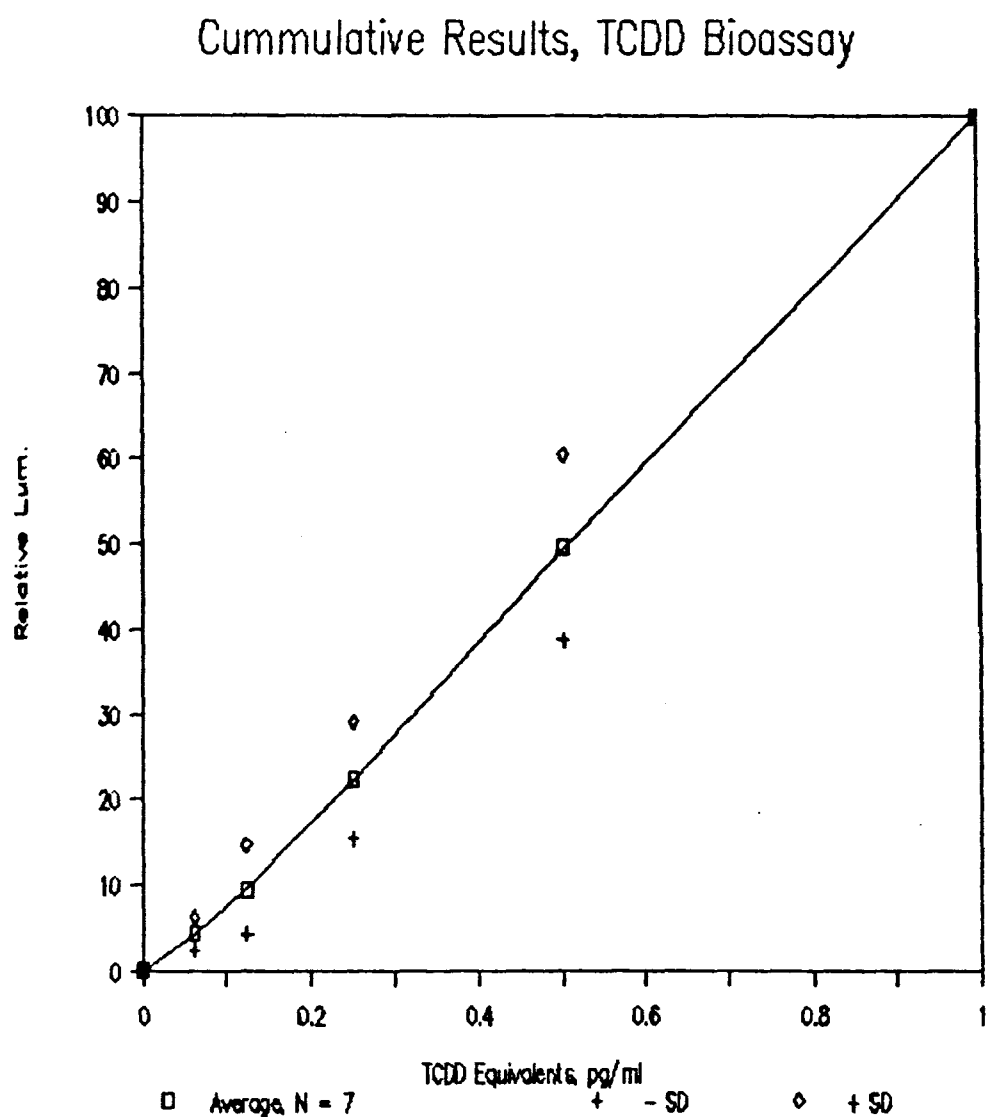


Figure 2: A standard curve generated from the normalized data of 7 TCDD bioassays. Standards were tested in two-fold dilutions from 1.0 to 0.0625 pg/ml. The r^2 of the line is 0.985.

with the WBAS cells it is below $2.0 \times 10^{-13}M$. Another advantage of the WBAS bioassay is the rapid turnover rate. The bioassay data presented here are for 20-24 hour exposures. The H4IIE system used by Safe includes a 4-5 day incubation of cells with inducer; the FCA assay requires 14 days.

TCDD and related compounds are currently detected by the use of GC/MS. The major advantage to GC/MS analysis is its ability to detect different isomers within a single sample. However, under conditions required for maximum sensitivity, multiple analyses may be required to detect similar compounds within a single sample.

The bioassay, while not able to distinguish between similar isomers, provides many advantages not available with the GC/MS. In general, the assay does not require highly skilled personnel or complex equipment. The assay is inexpensive and rapid, allowing for multiple testing and the generation of statistical data for samples. Although isomer specificity is not provided by the bioassay in a single test, the presence or absence of toxic chemicals which induce EROD, can be determined and expressed as "TCDD equivalents." Since many such compounds are subject to regulatory cleanup processes, the data obtained from bioassays can be used to establish priorities in toxic chemical decontamination programs within industry and government.

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STRATEGIES FOR USING BIOASSAY METHODS FOR THE IDENTIFICATION
OF HAZARDOUS COMPONENTS AND COMPARATIVE RISK ASSESSMENT
OF COMPLEX MIXTURES

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ABSTRACT

Two strategies particularly useful for approaching the toxicology of complex mixtures are: (1) bioassay-directed chemical characterization and (2) comparative bioassay studies. Bioassay-directed fractionation and chemical characterization is a strategy for identifying biologically active compounds or compound classes in complex mixtures. The identification and assessment of mutagens and carcinogens in complex mixtures has been significantly advanced by the use of short-term genetic bioassays. Bioassay-directed fractionation coupled with new organic characterization methods has provided the tools needed to more efficiently identify potential carcinogens in complex mixtures. A comparative potency strategy for evaluating the relative toxicity, mutagenicity and carcinogenicity of a series of different mixtures has been used to provide comparative potency data for risk assessment. The comparative mutagenicity and carcinogenicity of a series of combustion emissions has been assessed using dose-response studies in bacteria, mammalian cells and rodents. This data base has been used to develop a comparative risk assessment methodology for combustion emissions which is being extended to the evaluation of hazardous waste incinerator emissions and other complex mixtures.

INTRODUCTION

Combustion emissions consist of a very complex mixture of thousands of chemicals resulting from incomplete combustion of either vegetative sources (e. g., tobacco, wood, etc.), fossilized fuel (e. g., oil, coal, gasoline, diesel fuel) or synthetic sources (e.g., syn-fuels, other chemical products). This complex mixture generally contains a high percentage of organic chemicals including aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), substituted PAHs and other polar organic compounds. The emission mixture itself is heterogeneous and consists of very volatile gases (e.g., CO, NO_x, SO₂, hydrocarbons and aldehydes), semi-volatile material (e.g., 2-3 ring aromatics), condensed organic and inorganic matter adsorbed onto very small (usually submicron) carbonaceous particles often called soot.

These very complex mixtures cannot now or in the foreseeable future be completely characterized chemically since they contain thousands of components. Therefore, toxicological studies on the individual components is an unsatisfactory means of assessing the toxicology of

the total mixture. These mixtures, however, can in most cases be reproducibly generated by the emission source (e.g., automobile on a dynamometer) to generate the mixture to which humans are exposed. Considerable engineering effort has been devoted to standardizing combustion conditions and in some cases even the fuels, so that these emissions can be reproducibly generated. This has been done in order to chemically and physically characterize the emissions, however, these same techniques may be applied to the generation of a representative emission source for toxicological studies.

The toxicological data base on combustion emissions varies from being very extensive and good for some sources such as cigarette smoke and certain automotive emissions (e.g., diesel emissions) to very limited for wood combustion and synfuels. The more extensive data base for certain sources, including human data, should make it possible to draw some conclusions as to the utility of various methods and approaches which may be generalized to other combustion emissions.

The soot from combustion emissions has been judged to be carcinogenic in humans (IARC, 1985) and many soots have also been found to be carcinogenic in animals. These emissions are also mutagenic in bacteria, mammalian cells, and certain rodent assays. Several of the available animal models for carcinogenesis studies, however, do not always produce results which agree with the human data. In vitro methods are therefore extremely useful for: (1) quantitative comparisons of emissions, (2) mechanistic studies, (3) bioavailability studies, and (4) identification of the active components in mixtures.

BIOASSAY DIRECTED FRACTIONATION AND CHARACTERIZATION

The objective of this strategy is to identify the biologically active (e.g., toxic, mutagenic) components in complex mixtures. This and other approaches to identifying genotoxic compounds have been reviewed by Claxton 1982. The two principle considerations in applying this approach are the bioassay to be employed and the fractionation method, both of which are discussed below.

The bioassay(s) chosen to be employed will depend on the complex mixture and what is known about either the chemical composition or toxicological effects of the mixture. Bioassays which have been used include assays for: mutagenicity (e.g., Ames test), tumor initiation, cytotoxicity, etc.

The methods which have been used to fractionate complex mixtures prior to bioassay include nearly all the methods which have been used for separations prior to chemical analysis. Often several of these methods are combined sequentially as shown in Figure 1. One of the most widely used approaches to separate mixtures is to use

solvent partitioning to separate a mixture into the organics and inorganics. These will each be bioassayed. If most of the activity of interest is in the organic fraction, it will be further solvent partitioned into organic acids, organic bases and neutral compounds. If bioassay of these three fractions shows that the activity is largely isolated into the organic neutral fraction, it may be further fractionated by silica gel chromatography or high pressure liquid chromatography (HPLC) into the aliphatics, aromatics, moderately polar and highly polar neutral compounds. This reiterative approach, diagrammed in Figure 1, is repeatedly used until the active compounds have been sufficiently separated from the nonactive components to allow chemical characterization of the biologically active compounds.

At each point in the fractionation scheme where activity is measured, recovery of mass and activity should also be determined. A reconstituted mixture is bioassayed and compared to the unfractionated (neat) mixture to determine the recovery of bioactivity. If the sum of the activity of the mass weighted fractions equals the unfractionated (neat) mixture then the mutagenicity is considered to be additive. One should be cautious to consider that simultaneous chemical changes as a result of the fractionation procedure could both increase the activity of some components and decrease the activity of others resulting in the appearance of no change and therefore that biological activity is recovered.

In several cases this approach has resulted in the identification of biologically active components of mixtures which were very minor components by mass but were extremely potent. Examples of such components are the potent mutagenic dinitropyrenes found in Xerographic toners (Rosenkranz et al., 1980) and diesel exhaust (Schuetzle, 1983); the potent toxin, aflatoxin, found in peanuts; and the potent polychlorinated biphenyls (e.g., TCDD) found in emissions from burning transformers.

Once a highly active fraction is obtained and chemical analysis provides a list of the confirmed or suspected components, one critical requirement must be met before biologically active compounds are identified. Either bioassay data must be available for each of the compounds identified, and/or pure standards need to be obtained to bioassay the identified compounds in order to determine their contribution to the total activity observed.

CHARACTERIZATION AND IDENTIFICATION OF THE MUTAGENS AND CARCINOGENS EMITTED FROM COMBUSTION SOURCES

Bioassay-directed fractionation closely coupled to chemical characterization has been shown to be the most efficient and effective approach to identifying the mutagenic and turorigenic

compounds in combustion emissions. This approach has been used to identify tumor initiators and tumor promoters in cigarette-smoke condensates (Hoffman and Wynder, 1976), automotive exhaust emissions (Grimmer et al., 1982), and urban-air particles (Hueper et al., 1982). More recently, this approach has been coupled with short-term genetic bioassays, including both microbial and mammalian-cell mutation assays, to identify mutagens and potential carcinogens in complex mixtures (Epler et al., 1978). We first employed this method to identify the chemical classes and specific components associated with diesel particulate emissions that were mutagenic in the Ames Salmonella typhimurium mutagenesis assay (Huisinigh et al., 1979).

Diesel particles collected by the dilution-tunnel method (Bradow, 1982) were Soxhlet extracted with dichloromethane and solvent-partitioned into organic acids, bases, and neutral components. The neutral components were further fractionated into paraffins, aromatics, transitional moderately polar (TRN) compounds, and oxygenated highly polar (OXY) compounds. The mutagenic activity of each fraction was determined using the Ames Salmonella typhimurium/microsome assay in TA1535, TA1537, TA98, and TA100 (Huisinigh et al. 1979). The distributions of the mass of each fraction and of its mutagenic activity in TA98 are shown in Table 1 for a four-stroke V-8 Caterpillar 3208 engine used in urban service vehicles. The moderately and highly polar neutral compounds in the TRN and OXY fractions account for 89-94% of the mutagenic activity of the extractable organics and only 32% of the mass. Conventional gas chromatography/mass spectroscopy identified many fluorenones and methylated fluorenones as major constituents of these fractions which we found to be non-mutagenic. None of these or other identified constituents accounted for the direct-acting frameshift mutagenic activity observed. Studies with nitroreductase-deficient strains of Salmonella typhimurium showed in a reduction in the mutagenicity of these organics, suggesting that nitrated compounds contributed to this direct-acting mutagenicity (Claxton and Huisinigh, 1980). Nitrated polycyclic aromatic hydrocarbons (NO₂-PAHs) are potent direct-acting frameshift mutagens detected in xerographic toners (Rosenkranz et al., 1980). A series of NO₂-PAHs in diesel extracts were later identified and quantitated in order to estimate their contribution to the mutagenic activity of diesel particulate emissions (Nishioka et al., 1982) as described in the next section. Similar studies have also been conducted by Schuetzle, 1983. These studies show that NO₂-PAHs, di-NO₂-PAHs, and hydroxy-NO₂-PAHs together account for much of the mutagenicity observed in Salmonella typhimurium. Particulate emissions from catalyst-equipped gasoline-engine vehicles using unleaded fuel contain significantly less of these NO₂-PAHs (Nishioka et al., 1982). The mutagenic activity of both leaded- and unleaded-gasoline emissions is substantially increased with the addition of an exogenous metabolic activation (MA) system, suggesting that the

unsubstituted PAHs may play a more important role than do NO₂-PAHs in the mutagenicity and carcinogenicity of gasoline emissions (Lewtas, 1982, 1983).

Characterization of the mutagenicity of emissions from both vegetative and fossil fuel show general similarities. Comparison of the distribution of mutagenic activity in different organic fractions extracted from a light-duty diesel vehicle and urban air particle extracts in (Lewtas, 1985) Table 2. In both wood and diesel combustion, 82-99% of the mutagenicity was in the neutral fraction. Very little mass or mutagenic activity was observed in the organic bases. Differences in these sources were observed in the distribution of mass and mutagenicity in the neutral subfractions.

Bioassay-directed fractionation and chemical characterization has also been used to characterize and compare the complex organic emissions from roofing tar pots, coke ovens, and cigarette smoke. To obtain a gross characterization of the chemical classes present in the samples, the chemical class distribution was determined by solvent partitioning the organics into acidic, basic, neutral, and cyclohexane insoluble (a highly polar fraction) fractions. The neutral fractions were further separated into the nonpolar neutrals, aromatics (nitromethane soluble) and polar neutrals. Characterization of the distribution of mutagenic activity (Ames Salmonella typhimurium bioassay) in each of these fractions (Austin et al., 1985; Williams et al., 1986) showed significant differences between the diesel, coke oven main, roofing tar, and cigarette smoke condensate samples as shown in Fig. 2. In the diesel samples, over 90% of the mutagenic activity is located in the aromatic and polar-neutral fractions, and a significant portion of this activity can be accounted for by NO₂-PAHs. The cigarette smoke condensate, coke oven main, and roofing tar samples did not contain detectable amounts of NO₂-PAHs (Williams et al., 1986). Most of the mutagenicity of coke oven main sample was found in the basic fraction (37%) and polar neutral fraction (39%). The cigarette smoke condensate sample also had significant activity in the basic fraction (66%), but chemical analysis indicated that the components differed significantly from those of the coke oven main sample. The roofing tar sample contained aromatic (14%) and polar (75%) mutagenic constituents that were not NO₂-PAHs. The PAH subfraction of each of these samples accounted for only a small portion of the mutagenicity (e.g., diesel (0.2%), cigarette smoke condensate (0.1%), roofing tar (5%) and coke oven main (8%).

Although the specific mutagens in these different sources are not identical, they all cause frameshift mutations and appear to be compounds that could be classified as polycyclic organic matter (POM). Chemical characterization suggests that in addition to nitrated NO₂-PAHs found in the slightly and moderately polar

neutrals, hydroxylated and carboxylated polycyclic organics are found in the organic acid fraction, aromatic amines and nitrogen heterocycles are found in the organic bases and highly oxygenated quinones, diones, and nitro-oxygenated compounds are found in the polar neutral fractions.

CONTRIBUTION OF NO₂-PAHs TO THE MUTAGENIC ACTIVITY OF AUTOMOTIVE EMISSIONS AND URBAN AIR PARTICLES

Although NO₂-PAHs were qualitatively identified in extract of diesel particles and urban air particulate matter as described earlier, the detection and quantification of these compounds at low levels has posed problems for analytical chemists because the conventional analytical techniques for quantifying PAHs [e.g., high performance liquid chromatography (HPLC) with fluorescence detection and gas chromatography (GC)/mass spectrometry (MS) with electron impact ionization (EI)] are relatively insensitive to nitro-substituted PAHs. The 1, 3-; 1,6-; and 1, 8-dinitropyrene isomers are so highly mutagenic in the Ames (TA98 -S9) assay that trace concentrations of these compounds, if present, could account for a major proportion of the observed mutagenic activity. A capillary column GC/MS analytical technique using on column injection and negative chemical ionization (NCI) detection was therefore developed to detect these and other nitro-PAHs at very low concentrations (Nishioka et al., 1982). This technique was applied to extracts of soot particles from diesel and gasoline vehicles and urban air particles. Twenty-three different NO₂-PAHs were identified in the diesel engine extracts; five NO₂-PAHs in the urban air particle extracts; and only one, 1-nitropyrene was detected in the unleaded gasoline engine extract. 1-Nitropyrene was the NO₂-PAH detected in greatest quantity in the diesel extracts (107-1590 ppm, relative to the weight of the extract), followed by the nitrophenanthrene/anthracene isomers. The only dinitro-PAHs for which analytical standards were available, the dinitropyrene isomers, were detected in one diesel extract sample at sub-ppm concentrations (0.4-0.6 ppm).

Quantification of the concentration of these NO₂-PAHs and determination of their contribution to the direct-acting mutagenicity in Salmonella typhimurium TA 98 (Table 3) shows that the mono-NO₂-PAHs make relatively minor contributions to the mutagenicity of the urban air particles (2%). 1-Nitropyrene was present at the highest concentration (107-1590 ppm) in the diesel particle extracts, and yet it accounted for only 3-13% of the mutagenicity. The concentration of several of the NO₂-PAHs in a series of extracts of diesel and gasoline exhaust particles were however highly correlated with their mutagenicity both in S. typhimurium and L5178Y mouse lymphoma cells and skin tumor initiating activity in Sencar mice. 3-Nitrofluoranthene present at 1 ppm to 7 ppm in the diesel samples accounted for 0.8% to 1.4% of the mutagenicity. By using the mutagenicity values determined in

separate experiments for 2-nitrofluorene and those reported in the literature for 1-nitroaphthalene, these compounds were estimated to account for less than 0.01% of the mutagenic activity. Although the dinitropyrene isomers: 1,3; 1,6; and 1,8 were detected only in diesel sample C at 0.4-0.6 ppm (sum of 1.6 ppm), their mutagenic activity (496,000; 629,000; and 870,000 rev/ug, respectively) was high enough to account for 26% of the mutagenicity of this sample. The total "direct-acting" mutagenic activity in S. typhimurium TA98 that can be accounted for by the 23 nitro-PAHs quantified in Diesel C is 40%. This estimation is supported by the loss of 50% the mutagenic activity of this extract it was assayed in TA98FRI (renamed TA98NRD), a classical nitroreductase-deficient S. typhimurium tester strain obtained from H. Rosenkranz (Rosenkranz et al., 1981).

The fact that dinitropyrenes at concentrations below the ppm level can account for nearly one-third of the mutagenic activity (-S9) suggests that the presence of other highly potent dinitro-PAHs may account for even more of the mutagenic activity (-S9) in the moderately polar neutral fraction. A recent application of S. typhimurium tester strains developed to exhibit resistance to dinitropyrenes (e.g., TA98/1,8DNP₆) has led to even larger estimations of the contributions of dinitropyrenes to the mutagenicity of diesel particle extracts. The lack of quantitative data on the concentrations of the dinitropyrene isomers in such samples has previously made it impossible to confirm whether the concentrations of the dinitropyrenes are indeed sufficient to account for the contribution predicted by the resistant tester strains. Pederson, 1983, has reported that in several light-duty diesel particle extracts, 50% to 90% of the TA98 (-S9) mutagenicity is lost when the extracts are tested in TA98/1,8DNP₆. Since these strains may show a resistance to other dinitro-PAHs, it is possible that highly potent dinitro-substituted isomers of other parent PAHs present in the particle extracts may also contribute to the mutagenicity of the moderately polar neutral fraction.

CHARACTERIZATION AND IDENTIFICATION OF MUTAGENIC METABOLITES

When toxicological studies are conducted on an individual chemical (e.g., benzo[a]pyrene) which is metabolized to form a number of metabolites, we don't generally consider the resulting mixture of metabolites to be a "complex mixture." The strategy which is described above for identifying mutagens in environmental mixtures can also be applied to the identification of mutagens in a mixture of metabolites.

The identification of the bacterial mutagen, 1-nitropyrene (NP), in diesel emissions and ambient air as described above, led us to conduct a series of studies on the mammalian metabolism of NP. Ball et al., 1984 reported that rats treated with NP excreted a mixture

of mutagenic metabolites. This mixture was separated by HPLC into fractions which were bioassayed in the Ames *S. typhimurium* plate incorporation assay and characterized using high resolution GC/MS. Among the principal metabolite fractions identified were 3-hydroxy-1-nitropyrene, 8-hydroxy-1-nitropyrene, 6-hydroxy-N-acetyl-1-aminopyrene, and 8-hydroxy-N-acetyl-1-aminopyrene. We used a similar approach to identify the mutagenic metabolites of NP after metabolism by lung S9 (King et al., 1984) and lung and tracheal cells (King et al., 1986). These studies led to the identification of a whole series of mutagenic hydroxylated nitropyrenes where the OH group was in the 3, 6, 8 and 10 position. An example of the use of the Ames assay to identify the mutagenic metabolites of 1-nitropyrene formed by lung S9 is shown in Table 4. This approach led to further efforts to characterize the highly mutagenic fractions (e.g., β) and the various isomers of OH-nitropyrene.

IDENTIFICATION OF HYDROXYLATED NITRO-PAHs IN URBAN AIR

We have recently identified and quantified hydroxy-NO₂-PAHs in both a polar neutral fraction and acidic fraction of an ambient air particulate extract using bioassay-directed fractionation (Nishioka et al., in press). Figure shows the overall fractionation and bioassay data. The three fractions of greatest interest were the acid, methylene chloride and methanol fractions which contained 38%, 23%, and 29%, respectively, of the recovered mutagenicity. Since the methylene chloride fraction was more amenable to subfractionation by existing HPLC methods, this fraction was chosen for further fractionation. Normal phase HPLC was used to fractionate the methylene chloride fraction (24). The first HPLC sub-fractionation resulted in one fraction, methylene chloride-C, which contained nearly 50% of the mutagenicity in 25% of the mass. Analysis of this fraction by both EI and NCI HRGC/MS techniques indicated that the complexity of the sample was not reduced sufficiently to identify individual components. Sub-fraction C was further fractionated by normal-phase HPLC into 12 fractions for bioassay. As we proceeded to higher levels of sub-fractionation, less and less mass was available for bioassay. In this study, the first level fractions obtained from the preparative techniques were bioassayed in triplicate at seven doses with and without activation in order to be able to quantitate the distribution and recovery of mutagens. As we proceeded to the first and second level of fractionation, fewer doses and plates were used for the bioassay until nearly the entire fraction was used for a single plate. Therefore, our ability to quantitate the recovery of both mass and mutagenicity decreases as we subfractionate. The bioassay chromatogram showed peaks of relatively higher mutagenicity in fractions 4 and 11 with 29 and 37 rev/ug, respectively. Mass spectral analysis of peak 4 demonstrated that the goal of sequential fractionation had been achieved in that individual components could be resolved and quantified using HRGC/MS. The major mutagenic

compounds identified in the methylene chloride-C-4 fraction were hydroxy-nitro-substituted fluoranthenes (OH-NO₂-PAH) and work is continuing to synthesize and identify other isomers.

MICRO-BIOASSAY METHODS COUPLED TO ANALYTICAL SEPARATION TECHNIQUES

The quantity of material (mixture or fraction) needed for many of the established bioassay methods ranges from milligram quantities (e.g., Ames assay) to gram quantities (e.g., tumor initiation). Recently a number of micro-bioassay methods have been reported for measuring forward mutation (Thilly et al., 1983; Goto et al., Submitted), reverse mutation (Kado et al., 1983), in Salmonella typhimurium and phage induction in E. coli (Rossman et al., 1985). Such micro-bioassay methods will facilitate the coupling of bioassay methods to analytical scale separation techniques such as reverse-phase HPLC. Thilly demonstrated the possibilities of this approach by applying the forward mutation micro-assay to particle emission extracts from HPLC fractions of a residential oil burner. Figure 4 demonstrates the utility of this approach when this assay is used to bioassay the fractions from a mixture of metabolites of 1-nitropyrene incubated with lung S9. Such a bioassay chromatogram facilitates the immediate visualization of the most mutagenic fractions for further characterization. This approach should eliminate the need for large-scale fractionation techniques which are often fraught with problems of the spillover of chemicals from one fraction to another and with poor resolution. High resolution fractionation techniques used sequentially may make it possible to result in fractions which contain either one chemical or a very small number of chemicals which will greatly facilitate characterization of the fractions.

COMPARATIVE POTENCY EVALUATION OF COMPLEX MIXTURES

Comparative potency studies of a series of substances in one or more bioassay is a particularly useful approach to the toxicological evaluation of complex mixtures. Many complex mixture problems involve the assessment of a series of different mixtures, such as, fuels derived from various sources, soots emitted from different combustion engines, or air particles from various exposure environments. Since all soots from incomplete combustion are expected to contain carcinogenic polycyclic organics, the question often posed is whether a new combustion source will produce soot which is more carcinogenic than the existing sources.

The comparative potency approach has been used in comparing the acute toxicity of a series of petroleum hydrocarbons using as range of products from light oils and gasoline to heavy fuel oils (Beck et al., 1982). The toxicity of shale derived fuels and other syn-fuels have been determined primarily in comparative studies in general toxicity tests, target organ studies, behavioral studies, muta-

genesis, carcinogenesis, teratology and neurotoxicity tests. Many of these studies are reviewed by MacFarland et al., 1982.

The design considerations for comparative potency studies include a number of factors which may not always be considered when using other toxicology methods. These include: 1) simultaneous evaluation of all comparisons in one experiment, where possible; 2) exposure doses (or concentrations) needed for statistical analysis and potency measure; and 3) fractional factorial design for mixtures strategy for evaluating the toxicity of mixtures of gasoline blends using fractional factorial designs, multiple bioassays and a standardized reference fuel as a center point.

DEVELOPMENT OF A COMPARATIVE POTENCY METHOD FOR CANCER RISK ASSESSMENT OF COMBUSTION EMISSIONS

A comparative potency method for cancer risk assessment has been developed based upon a constant relative potency hypothesis. This method was developed and tested using data from a battery of short-term mutagenesis bioassays, animal tumorigenicity data and human lung cancer risk estimations (Lewtas, 1981; Nesnow et al., 1982a,b; Albert et al., 1983; Lewtas et al., 1983). This data base was developed from a series of complex mixtures including emissions from coke ovens, roofing tar pots, cigarette smoke, and automotive engines.

The comparative potency method for cancer risk assessment is based on the hypothesis that there is a constant relative potency between two different carcinogens (C_1 and C_2) across different bioassay systems (B_1 and B_2). The mathematical expression for the constant relative potency model is the following;

$$\text{Relative Potency } \frac{C_1}{C_2} \text{ in Bioassay(1)} = (k) \text{ Relative Potency } \frac{C_1}{C_2} \text{ in Bioassay(2)}$$

This assumption is implicit in any comparison which utilizes the relative toxicity of two substances in animals to determine which substance would most likely be more or less toxic to man. This constant relative potency assumption is a testable hypothesis, if the relative potency of two mixtures or components in one bioassay (e.g., humans) can be determined and compared to the relative potency in a second bioassay. The test of this model is whether there is a constant relationship (k) between the relative potencies in the two bioassays being compared such that:

$$\frac{\text{Relative Potency } (C_1 \div C_2) \text{ in Bioassay(1)}}{\text{Relative Potency } (C_1 \div C_2) \text{ in Bioassay(2)}} = \text{constant } (k)$$

This hypothesis was tested for three complex organic emissions from a coke oven, roofing tar pot, and cigarettes by using human lung cancer data from epidemiological studies of humans exposed to these emissions and testing these emissions in a series of short-term mutagenesis bioassays and animal tumorigenesis assays (Lewtas, 1985).

COMPARATIVE ASSESSMENT OF THE MUTAGENICITY, TUMORIGENICITY AND NITRO-PAH CONTENT OF DIESEL AND GASOLINE EMISSIONS

The mutagenicity of the extractable organic material from diesel and gasoline emissions has been reported for a battery of in vitro bioassays detecting gene mutations, DNA damage, chromosomal effects, and oncogenic transformations (Lestas, 1983). Skin tumor initiation and carcinogenic activity of these samples has also been reported in SENCAR mice (Nesnow, 1982). Very good correlations (r^2 0.90) were observed when the slope of the dose-response for the mutagenic activity in S. typhimurium strain TA98(-MA) was plotted versus the mutagenic activity in the mammalian cell assays and the skin tumor initiating activity.

The correlation of mutagenic and skin tumor initiating activity with the concentration of selected nitro-PAHS was examined for these diesel and gasoline samples. Table 5 shows the high correlations observed between concentrations of 1-nitropyrene and 3-nitrofluoranthene in these samples, and their mutagenic activity in S. typhimurium (-S9), L5178Y mouse lymphoma cells (-S9), and skin tumor initiating activity in SENCAR mice. The r^2 correlation coefficient in the presence of S9 (not shown) was somewhat lower. The mutagenicity and tumor initiation activity (-S9) also correlated well ($r^2 > 0.9$) with the concentrations of the nitro-252 isomer, the nitro-228 isomers, nitromethylpyrenes and nitrofluorenes while no correlation was observed with several of the other less mutagenic, lower molecular weight nitro-PAHs (nitrophenanthrenes and nitronaphthalenes).

The quantified mono-NO₂-PAHS account for less than 20% of the direct-acting bacterial mutagenicity of these samples. The high correlations observed between the concentration of these compounds and the mutagenic activity of the total extract, therefore, suggest that the concentrations of the unidentified mutagens responsible for the remainder of the mutagenic activity and possibly the mutagenic and carcinogenic activity in other bioassays is directly related to the relative concentrations of these mono-NO₂-PAHs. Because the remaining unidentified mutagens appear to be primarily located in the chemical fractions that are more polar than the fraction which contains the unsubstituted and mono-NO₂-PAHs, it is possible that other di-NO₂-PAHs (e.g., dinitrofluoranthenes) or other oxygenated NO₂-PAH's (e.g., hydroxy-nitro-PAHs) species are primarily responsible for the major portion of the unidentified mutagenic activity.

SUMMARY

Toxicology studies of complex mixtures nearly always employ the same general methods, models and bioassays that have been developed and validated using single agents. The strategies for combining and applying these methods however may differ due to the complexity of the mixture and the questions being addressed. In initial studies to determine the toxicological effects of an unknown mixture, the approach is generally to administer the mixture as you would a single agent in standard in vivo and in vitro bioassays. Attempts to approach the toxicology of a complex mixture from an analysis of its components are generally successful only if the number of components is limited (2-5 components) and well defined. In approaching a very complex mixture where both the toxicological effects, components and active agents are unknown, the two strategies described above can be very useful. Prior to undertaking either of these two strategies, however, the toxicological endpoint of concern must be identified.

The two strategies described here have been successfully applied to the evaluation of different complex mixtures which cause a variety of effects. Wastewaters which are acutely toxic to fish have been comparatively evaluated to determine which wastewaters, industrial effluents, or sites are the most toxic using acute toxicity assays in fish. Bioassay-directed fractionation techniques have also been used to identify the class of compounds (e.g., organic vs. inorganic etc.) responsible for this toxicity.

Recent inhalation studies of unleaded gasoline demonstrated renal toxicity and carcinomas in rodents. Toxicology studies of the renal toxicity of different fractions of the gasoline led to the identification of isoparaffins as the active agents.

It is important to keep in mind that the two strategies described here are limited by the limitations of the bioassay methods employed. If, for example, you wish to identify carcinogens in a complex mixture which you know contains chlorinated hydrocarbons, then the Ames Salmonella typhimurium bioassay would not be a wise choice since it is well documented that this bioassay does not detect many carcinogens of this class. In some cases several bioassay methods may have to be applied in using these strategies to comparatively evaluate mixtures and elucidate the active toxicological agents.

DISCLAIMER

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency

nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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TABLE 1

Distribution of the Mass and Mutagenic Activity of Fractionated Diesel Particle Organics^a

Fraction ^b	Mass (%)	Specific Mutagenic Activity ^c (rev/mg)		Weighted Mutagenic Activity ^d (rev/mg)		Distribution of Mutagenic Activity ^e (%)	
		-S9	+S9	-S9	+S9	-S9	+S9
Organic acids (ACID)	14.9	193	248	28.8	37.0	4.9	9.5
Organic bases (BASE)	0.3	43.8	132	0.13	0.40	0.02	0.10
Ether insolubles (INS)	3.9	53.9	80.9	2.1	3.2	0.36	0.80
Paraffins (PRF)	36.7	Neg.	Neg.	0.0	0.0	0.0	0.0
Aromatics (ARM)	6.9	49.5	30.1	3.42	2.1	0.60	0.54
Transitionals (TRN)	5.0	7520	2620	376	131	64.9	33.5
Oxygenates (OXY)	26.9	629	798	169	215	29.2	55.4

^aDiesel particles obtained by the dilution tunnel method from a four-stroke V-8 Caterpillar 3208 engine operated on the standard 13 mode series of steady states.

^bDichloromethane extractable organics were solvent-partitioned into acids, bases, and neutrals as previously described. The neutral fraction was further fractionated on silica gel into four fractions by the following solvents: hexane (paraffins), 1% ether in hexane (aromatics and transitionals), and 50% acetone in methanol (oxygenates).

^c*Salmonella typhimurium* mutagenesis assay performed with and without metabolic activation (S9). Slope determined from linear regression analysis of the initial portion of the dose-response curve.

^dWeighted mutagenic activity of each fraction relative to the total extract is the product of distribution of mass in each fraction (% mass) and the specific mutagenic activity (rev/mg). The sum of these weighted mutagenic activities is used to determine the distribution of mutagenicity (%) as described below.

^eDistribution of mutagenic activity is the percentage of weighted mutagenic activity in each fraction compared to the sum of the weighted mutagenic activities. This distribution assumes that any mutagenicity not recovered through the fractionation is equally distributed across all fractions.

TABLE 2

Characterization of Mutagenicity from Combustion Sources and Urban Air

Fraction ^a	Diesel Auto ^b		Wood Stove ^c		Urban Air ^d	
	mass %	Ames TA98 +S9 mutagenicity ^e %	mass %	Ames TA98 +S9 mutagenicity %	mass %	Ames TA98 +S9 mutagenicity %
1. organic acids	7.6	17.9	44.0	0.0	7.3	21.0
2. organic bases	0.2	<0.1	2.2	4.7	0.8	1.0
3. neutrals						
A. aliphatics (hexane)	56.1	0.0	14.2	27.9	21.5	10.9
B. aromatics (hexane/benzene)	12.4	37.0	18.1	23.3	13.6	12.1
C. moderately polar (di- chloromethane)	3.1	24.7	29.0	11.6	8.6	45.0
D. highly polar (methanol)	20.6	20.4	38.7	32.6	34.0	21.0

^aThe acids and bases were first separated by liquid-liquid partitioning to separate the acids and bases. The neutrals are partitioned into four fractions by open column chromatography on 5% water deactivated silica gel utilizing the solvents designated in parenthesis.

^bDiesel particles collected by the dilution tunnel technique from a 1973 Datsun Nissan 220C diesel automobile operated on a chassis dynamometer using the highway fuel economy test cycle (HWFET). The total exhaust was diluted (10:1) with filtered air prior to collection on 20 x 20 inch Teflon coated Pallflex T60-A20 filters. The particles were Soxhlet extracted with dichloromethane (DCM) for 48 hrs prior to fractionation.

^cWood combustion particles collected by the dilution tunnel technique from an airtight woodstove burning oak (Johnson Energy Converter) during the constant burn phase. The emissions were diluted, collected, and Soxhlet extracted by the same techniques employed for the diesel particles.

^dUrban air particles (<1.7 μ m) collected by a massive air volume sampler in an industrial/residential area of Philadelphia, PA. The particles were Soxhlet extracted with DCM for 24 hrs prior to fractionation.

^eData from the Salmonella typhimurium plate incorporation assay in TA98 with metabolic activation was analyzed by the model slope method to determine the specific mutagenic activity in revertants/ μ g of fraction tested. Distribution of mutagenic activity between each fraction (% mutagenicity) is the percentage of weighted mutagenic activity [mass (%) x specific mutagenic activity (rev/ μ g) of each fraction] of each fraction compared to the sum of the weighted mutagenic activities.

TABLE 3

Contribution of NO₂-PAHs to the Mutagenic Activity of
Particle Extracts in Salmonella typhimurium TA98 (-S9)

Extract Sample	Mutagenic Activity (rev/μg) TA98 -S9	Concentration and Contribution to Mutagenic Activity (%)					
		<u>1-nitropyrene</u>		<u>nitrofluoranthene</u>		<u>dinitropyrene isomers</u>	
		Conc. (ppm)	Contrib. (%)	Conc. (ppm)	Contrib. (%)	Conc. (ppm)	Contrib. (%)
Diesel Auto 1	13.	1590	11.	7.0	1.4	--	--
Diesel Auto 2	3.9	589	13.	1.2	0.8	1.6	26.
Diesel Auto 3	3.5	107	2.7	0.9	0.8	--	--
Gasoline Auto 4	1.6	2.5	0.1	--	--	--	--
Urban Air A	1.8	1.0	0.05	0.8	1.8	--	--
Urban Air B	4.4	0.2	0.004	0.4	0.4	--	--

TABLE 4

Mutagenicity of Lung S9 Metabolite Fractions Isolated by HPLC^a

HPLC Metabolite Fraction	Specific Mutagenicity (revertants/nmol) ^b	
	-S9	+S9
Solvent Front	55	14
α	554	70
β	1250	113
δ	168	44
K-DHD	373	94
δ	201	94
NAAP	62	347
10-OH-1NP	77	215
1-AMP	157	151
Phenols	84	168
3-OH-1NP	584	222
1-NP	315	227
W	126	142

^a14C-1-NP (2773 nmol) incubated with rabbit lung S9 (2.8 g protein) for 45 min as described in King et al., 1984.

^bMutagenicity determined in the Ames Salmonella typhimurium plate incorporation assay in TA98.

TABLE 5

Correlation Analysis of Nitropyrene and Nitrofluoranthene
Concentrations with Mutagenic and Tumorigenic Activity

<u>Particle Extract</u>	<u>1-NP^a</u> ppm	<u>3-NF^b</u> ppm	<u>Ames</u> <u>TA98 -S9</u> rev/ μ g ^c	<u>Mouse</u> <u>Lymphoma -S9</u> MF/ μ g/ml ^d	<u>Tumor</u> <u>Initiation</u> pap/mouse/ μ g ^e
Diesel Auto 1	1590	7.0	13.0	4.2	590
Diesel Auto 2	589	2.9	3.9	0.98	240
Diesel Auto 3	107	1.2	3.5	1.2	310
Gasoline Auto 4	2.5	0.9	1.6	0.38	170
<u>Correlation Coef.</u>					
r ² with 1-NP			0.91	0.98	0.82
r ² with 3-NF			>0.99	0.99	0.95

^a1-Nitropyrene^b3-Nitrofluoranthene^cRevertants per μ g^dMutation frequency (mutants per 10⁶ survivors) per μ g per ml^ePapillomas per mouse per μ g in SENCAR mice

FIGURE LEGEND

1. General Schematic Diagram of Bioassay Directed Fractionation and Characterization
2. Distribution of Mutagenicity in Coke Oven Emissions (Coke), Cigarette Smoke Condensate (CSC), Diesel Emissions, and Roofing Tar Emissions
3. Bioassay Directed Fractionation of Urban Air Particulate
4. HPLC Bioassay Chromatogram of the 1-Nitropyrene Metabolites from Lung S9

BIOASSAY DIRECTED FRACTIONATION AND CHARACTERIZATION

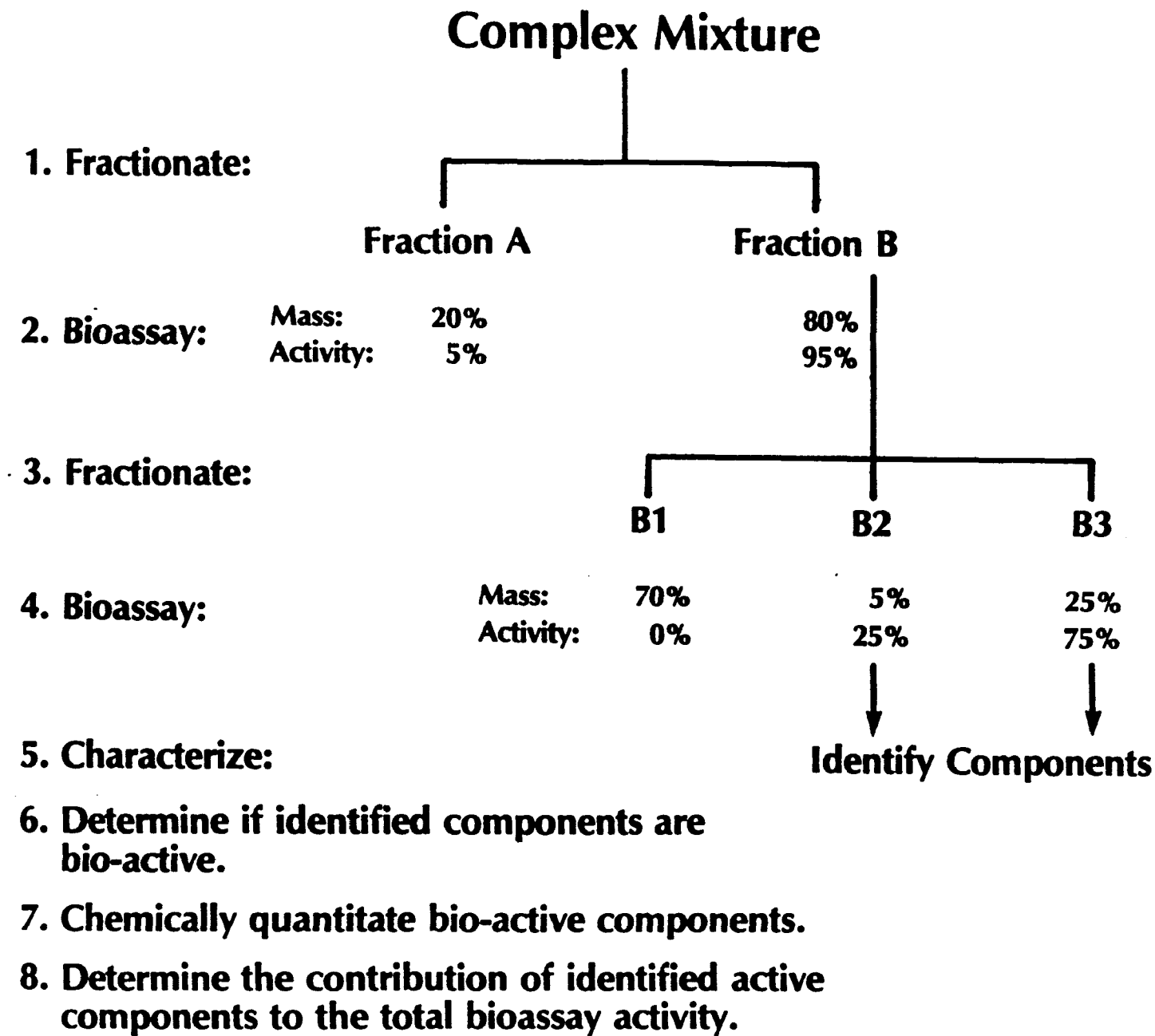


FIGURE 1

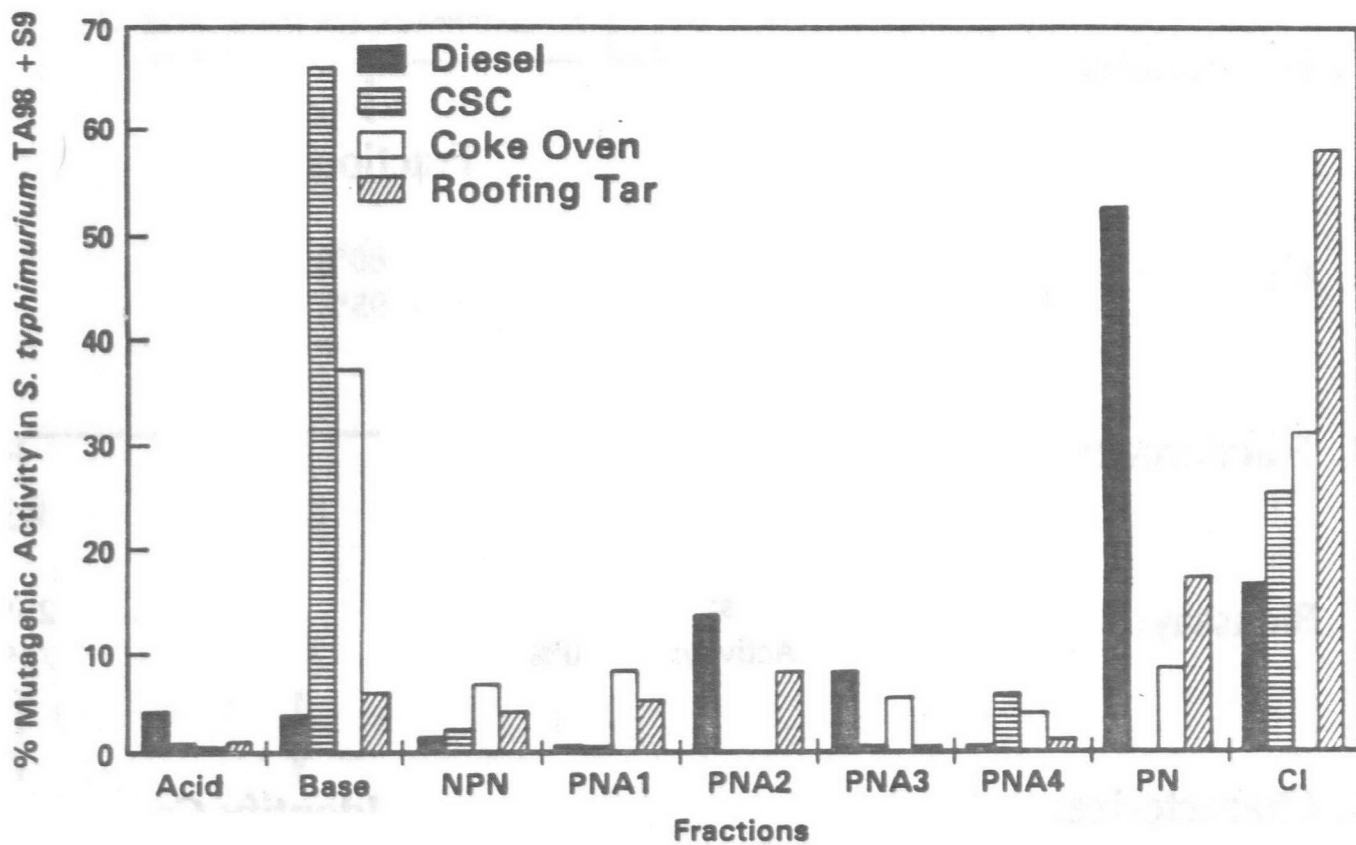


FIGURE 2

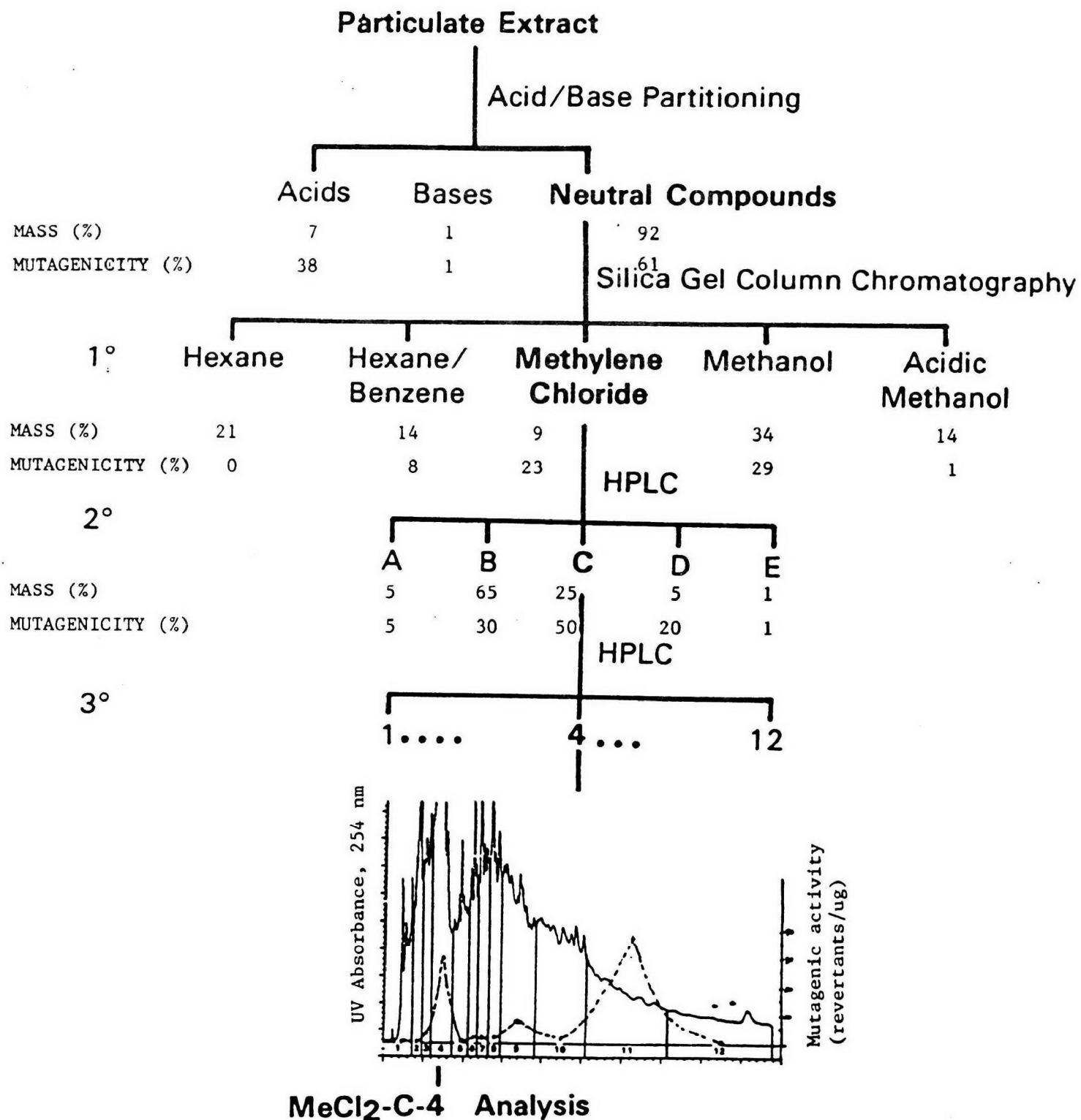
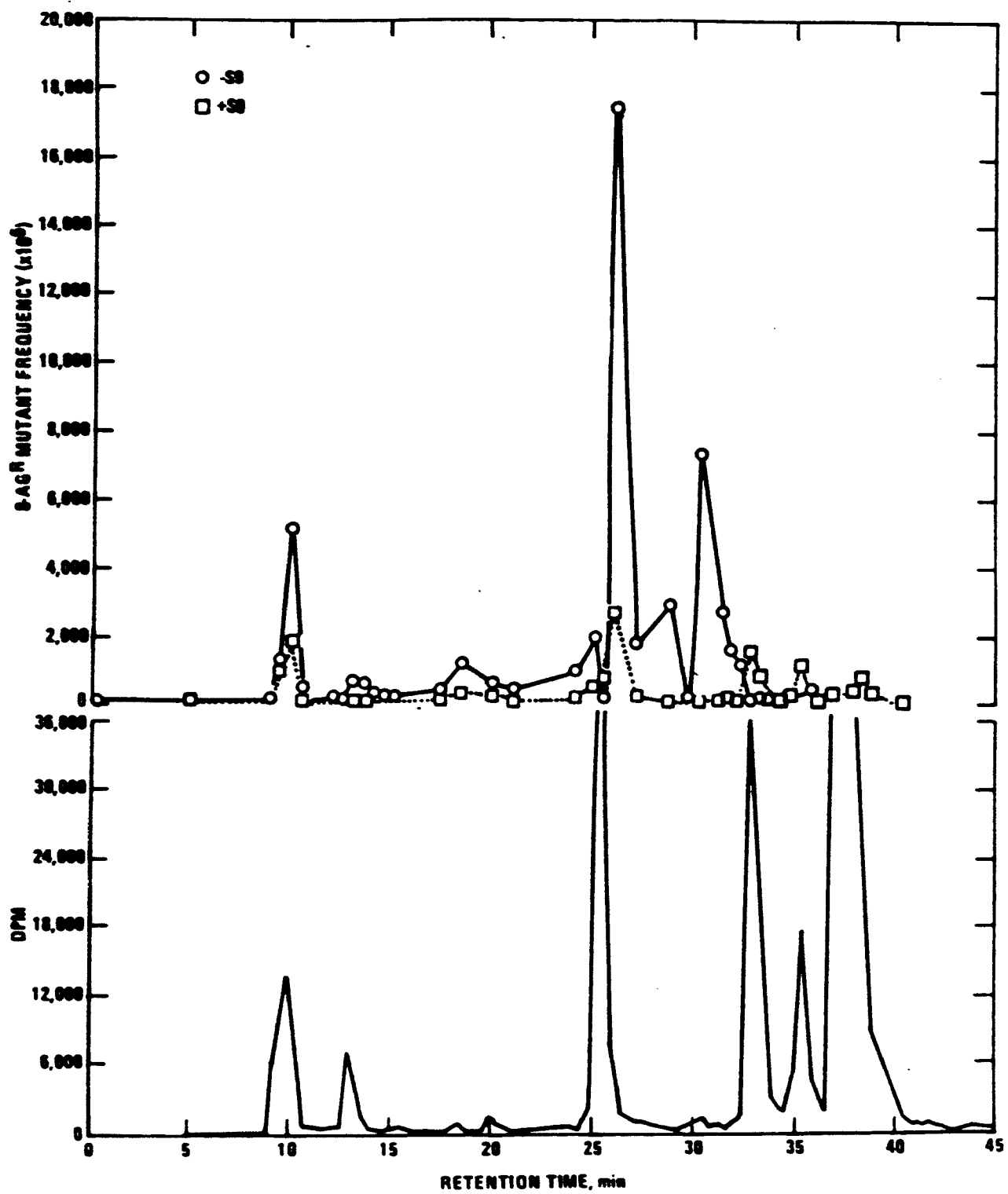


FIGURE 3



2-44
FIGURE 4

MUTAGENICITY IN SALMONELLA OF HAZARDOUS WASTES AND URINE FROM RATS FED THESE WASTES

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ABSTRACT

Fifteen hazardous industrial waste samples were evaluated for mutagenicity in the Salmonella plate-incorporation assay using strains TA98 and TA100 in the presence and absence of Aroclor 1254-induced rat liver S9. Dichloromethane/methanol extracts of the crude wastes were also evaluated. Seven of the crude wastes were mutagenic, but only 2 of the extracts of these 7 wastes were mutagenic; extracts of 2 additional wastes also were mutagenic. In addition, 10 of the crude wastes were administered by gavage to F-344 rats, and 24-h urine samples were collected. Of the 10 raw urines evaluated, 3 were mutagenic in strain TA98 in the presence of S9 and β -glucuronidase. The 3 crude wastes that produced these 3 mutagenic urines were, themselves, mutagenic. Adequate volumes of 6 of the 10 raw urines were available for extraction/concentration. These 6 urines were incubated with β -glucuronidase and eluted through Sep-Pak[®] C₁₈/columns; the methanol eluates of 3 of the urines were mutagenic, and these were the same 3 whose raw urines also were mutagenic. In general, the C₁₈/methanol extraction procedure reduced the cytotoxicity and increased the mutagenic potency of the urines. To our knowledge, this is the first report of the mutagenicity of urine from rodents exposed to hazardous wastes. Based on the present results, the use of only TA98 in the presence of S9 might be adequate for general screening of hazardous wastes or waste extracts for genotoxicity. The urinary mutagenesis assay does not appear to be a useful adjunct to the Salmonella assay for screening hazardous wastes. The problems associated with chemically fractionating diverse types of hazardous wastes for bioassay are also discussed.

INTRODUCTION

The potential health effects of exposure to hazardous wastes may include chromosomal damage and cancer (Maugh, 1979; Vianna and Polan, 1984). Thus, knowledge of the mutagenic potential of hazardous wastes would aid greatly in evaluating the hazardous nature of and/or potential health effects from exposure to certain wastes. Most studies on the genotoxicity of hazardous wastes have used the Salmonella assay (Houk and Claxton, 1986; Nestmann et al.,

1980); however, eukaryotic and/or mammalian cell assays been used to a limited extent (DeMarini et al., 1984; Donnelly et al., 1985; Hopke et al., 1982). Combinations of short-term genotoxicity assays have been proposed as possible screens for hazardous wastes (Barfknecht and Naismith, 1984).

We have begun a series of studies to identify short-term assays and chemical fractionation schemes that might be useful for screening hazardous wastes for genotoxic activity. Our initial studies have indicated that the use of mammalian cell assays might not improve significantly the ability to detect the genotoxicity of hazardous wastes beyond that afforded by the Salmonella assay alone (DeMarini et al., 1987a). Because in vivo mammalian metabolism may be a critical factor in the generation of mutagenic metabolites from complex hazardous wastes, we have investigated the utility of a rodent urinary mutagenicity assay as a possible adjunct to testing the wastes themselves in the Salmonella assay.

Most previous studies with rodent urinary mutagenicity assays have involved the use of pure compounds--frequently potent mutagens known to require metabolic activation (Legator et al., 1982). Consequently, the raw urine from such studies was mutagenic and did not require concentration. However, B-glucuronidase was usually required to observe rodent urinary mutagenicity in these studies. This is in contrast to studies of mutagenic activity of human urine in which the urines must be concentrated (usually with XAD resins), and B-glucuronidase is usually not required (Legator et al., 1982).

We are aware of only 3 studies on the mutagenicity of urine from rodents exposed to complex mixtures. Belisario et al. (1984, 1985) demonstrated the mutagenicity of urine from rats exposed by gavage to diesel emission particles (DEP), whereas Ong et al. (1985) did not find mutagenic urine from rats exposed by inhalation to DEP and/or coal dust. Concentration of the urine by XAD-2/acetone did not reveal any mutagenicity in one study (Ong et al., 1985), and it reduced the mutagenic potency compared to raw urine in the other studies (Belisario et al., 1984, 1985). Solid-phase extraction using Sephadex LH-20 resin or liquid extractions using chloroform, toluene, or dichloromethane (DCM) also reduced the mutagenic potency compared to raw urine (Belisario et al., 1985).

However, Ball et al. (1984) found that the mutagenic activity of urine from rats administered 1-nitropyrene was enhanced by extracting and concentrating the urine on Sep-Pak® C₁₈ cartridges eluted with methanol. Consequently, we have studied the mutagenicity of urine from rats exposed by gavage to crude hazardous wastes by testing both the raw urine as well as C₁₈/methanol concentrate of the urine in strain TA98 in the presence of Aroclor 1254-induced rat liver S9 and B-glucuronidase.

Previous studies have shown that DCM may extract mutagenic organics from hazardous wastes (Andon et al., 1985; DeMarini et al., 1987a). Thus, we have explored the applicability and utility of preparing and bioassaying DCM (as well as methanol) extracts of the 15 hazardous waste samples used in the present study. We selected samples that were representative of wastes from diverse manufacturing plants, including petrochemical, pharmaceutical, and plastics manufacturing plants. We also tested combinations of wastes from a variety of sources. Some of the samples were aqueous wastes, some were organic wastes, and others were a mixture of both types. Some wastes were from a single manufacturing process; others were composite wastes from a variety of different manufacturing processes. All of the wastes were liquid wastes, although many contained solids. The fact that these 15 waste samples had been partially chemically characterized (U.S. EPA, 1984), which is information not normally available for hazardous wastes or other complex mixtures, provided an additional reason for using these wastes to explore bioassay procedures appropriate for hazardous wastes.

MATERIALS AND METHODS

Wastes and Waste Extracts

Fifteen samples of hazardous industrial wastes were obtained from Edward L. Katz, Hazardous Waste Engineering Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH (Table 1). Three of the waste samples (A, B, and C) were from 3 different manufacturing plants (petrochemical, pharmaceutical, and plastics). The remaining samples were from 4 commercial hazardous waste incineration facilities that burn a mixture of hazardous wastes from a variety of industrial sources.

Table 1 shows the results of a partial chemical characterization that was performed on these samples (U.S. EPA, 1984). For the purposes of a previous study on the performance of hazardous waste incinerators (U.S. EPA, 1984), 8 of the waste samples (B, E, F, G, J, K, L, and M) were spiked with carbon tetrachloride and trichloroethylene, which are not mutagenic in *Salmonella* (Kier et al., 1986). Consequently, the concentrations of these 2 chemicals in these 8 waste samples reflect this addition (Table 1). The wastes were analyzed for the presence of a limited number of priority organics and/or metals identified in the EPA Appendix VIII list of priority pollutants (U.S. EPA, 1984). Thus, the chemical characterizations shown in Table 1 should not be viewed as indicative of the overall chemical composition of the wastes. The procedures used to extract the wastes and to evaluate the wastes and waste extracts in the *Salmonella* plate-incorporation assay have been described previously (DeMarini et al., 1987b).

Urine and Urine Extracts

Ten of the 15 waste samples were evaluated for their ability to produce mutagenic urine based on the chemical diversity that they represented. We had planned to dose animals with 4 different doses for 10 days before collecting 24-h urine from 3 rats per dose. However, the available amounts of waste samples permitted the use of this protocol for only waste C. For the other 9 waste samples, a single dose of the crude waste samples was administered by gavage to 70-day-old male F-344 rats. Doses were selected based on lethality and the maximum volume that could be administered to the animals. After dosing, rats were placed in metabolic cages (Nalgene), and urines were collected on dry ice for 24 h from 2-3 rats receiving the same dose of a particular waste. All of the urines were centrifuged (10,000 rpm, 10 min.), filter sterilized (0.45-um filter), and frozen at -20°C.

Portions of some of the urines were processed further as follows. One ml of B-glucuronidase (Sigma Type VII from *Escherichia coli*) at concentration of 1000 units/ml of potassium phosphate buffer (0.15 M, pH 7.4) was added to 2-10 ml of thawed urine, and the mixtures were incubated with shaking for 1 h at 37°C. Two serially connected Sep-Pak[®] C18 cartridges (Waters Associates, Milford, MA) containing 1 g of adsorbent were prepared for use by eluting 40 ml of methanol followed by 50 ml of double-distilled water. Then, each B-glucuronidase-urine incubate was poured through the columns, followed by 40 ml of double-distilled water. Water was aspirated from the columns until dry. Concentrates were then eluted with 2, 4-ml aliquots of methanol. The methanol concentrates were evaporated under a stream of nitrogen and solvent exchanged into a volume of DMSO to produce 5-X concentrates. These were stored at -20°C until they were bioassayed. Thus, there were two types of urine for bioassay: (1) raw urine and (2) urine that had been incubated with B-glucuronidase and extracted and concentrated 5X by means of C18/methanol elution. The Salmonella plate-incorporation assay was performed as described (Maron and Ames, 1983; DeMarini et al., 1987b).

RESULTS

Of the 15 crude wastes evaluated for mutagenic activity in Salmonella, 7 were mutagenic (Table 3). The mutagenic potencies (rev/ug) ranged from 1 for waste G and O, to over 1000 for waste A. Four of the wastes (L, M, G, and O) were positive only in strain TA98, and all but waste G required S9. Three wastes (C, A, and F) were positive only in strain TA100, and they were positive in the presence and absence of S9. Dose-response curves for the mutagenic wastes are shown in Fig. 1 (circles).

Extract (Table 2) were prepared for all of the wastes except for waste B, which reacted violently with methanol--leading to the loss of the sample. Of the 14 waste extracts evaluated, 4 were mutagenic, and their mutagenic potencies ranged from 2 for extract H, to over 5000 for extract C (Table 3). Two extracts (H and J) were mutagenic only in strain TA98; extract A was mutagenic only in strain TA100; and extract C was mutagenic in both strains. Two extracts (C and J) required S9, and 2 (H and A) were positive in the presence and absence of S9. Dose-response curves for the mutagenic extracts are shown in Fig. 1 (squares).

Raw urine from untreated control rats (up to 1 ml per plate) was not mutagenic in strain TA98 plus S9 plus B-glucuronidase (Fig. 2). Of the 10 wastes evaluated for urinary mutagenic 3 (C, L, and M) were mutagenic (Table 4). Sufficient volumes of urine were available for C₁₈/methanol concentration for only 6 of the 10 urines studied. The concentration procedure did not reveal mutagenic activity that was not already evident from raw urine for these 6 urines. Thus, the 3 mutagenic raw urines, (C, L, and M) were also the only mutagenic urine extracts (Table 4).

The C₁₈/methanol concentration procedure appeared to eliminate cytotoxic components from the urines and to enhance the mutagenic potencies of the mutagenic urines (Table 4, Fig. 2). In addition to dose responses based on the amount of urine per plate, we used waste C, which had been administered for 10 consecutive days, to generate dose responses based on the amount of waste/rat (Fig. 2). A qualitative summary of all of the data is shown in Table 5.

DISCUSSION

Mutagenicity Of Urine And Urine Extracts

Based on the wastes used here, the urinary mutagenesis assay does not appear to provide any additional useful information beyond that obtained from testing the wastes or waste extracts directly in the Salmonella assay. Only 3 of the 10 wastes tested produced mutagenic raw urine (Table 4), and all 3 of these wastes were mutagenic when tested directly in Salmonella (Table 3). Combined with the additional expense and time required to perform the urinary mutagenesis assay, this assay does not appear to be a useful adjunct to testing the wastes or waste extracts directly for mutagenicity.

Although the urinary mutagenesis assay appears to be of limited usefulness in screening hazardous wastes, the data presented here are, to our knowledge the first report of the mutagenicity of urine from rodents fed hazardous wastes. The results raise the issue of whether the mutagenic component(s) of the hazardous wastes were excreted unmetabolized or were converted to mutagenic metabolites. Table 3 shows that, in general, the 3 wastes that produced mutagenic

urine (wastes C, L, and M or their extracts) required S9 in order to be mutagenic in strain TA98. Our preliminary studies (data not shown) indicate that β -glucuronidase was required to detect mutagenic activity in the urine or urine extracts. This is consistent with findings for many pure compounds studied in rodent urinary mutagenesis assays (Legator et al., 1982) and suggests that certain components of the wastes were metabolized and conjugated to glucuronide.

Fractionating and concentrating the urines by means of the C₁₈/methanol procedure did not identify a urine to be mutagenic that was not identified as mutagenic from the studies with raw urine (Table 4). Thus, this additional procedure did not enhance overall detection capabilities. However, the C₁₈/methanol concentration procedure did appear to enhance the mutagenic potency of the three mutagenic urines (Table 4, Fig. 2). The procedure either concentrated mutagenic components of the urine and/or reduced the concentration of cytotoxic components in the urines, permitting better detection of the mutagenic activity. The procedure did not increase to the same extent the mutagenic potency of the three urines. As shown in Fig. 2, it enhanced considerably the detection of the mutagenicity of urine from rats fed waste L, but it did not increase the potency of the urine from rats fed waste C (at least up to 0.5 ml or ml equivalents/plate).

Mutagenicity of Wastes and Waste Extracts

The additional time and expense required to prepare the extracts did not produce extracts that yielded much additional information that was not obtainable from the crude wastes. Table 3 shows that 7 crude wastes were mutagenic, but for only 2 of these (A and C) were the extracts mutagenic. However, 2 wastes (H and J) were identified as mutagenic based solely on the mutagenicity of their extracts. Thus, out of a total of 9 mutagenic wastes, only 2 (22%) would have been missed if only the crude wastes had been tested. It is important to note that all wastes can be tested directly due to microbial contamination or physical state, e.g., viscosity, pH, etc. Thus, extraction/fractionation procedures will be necessary for some, if not most, hazardous wastes in order to examine their biological activity.

Design of Testing Schemes

Consideration also must be given to how complex a testing matrix needs to be in order to have an acceptable detection capability and still be cost-effective. For the 15 wastes tested, the matrix shown in Table 3 requires the production of 120 dose-response curves, each with at least 3 or 4 points, each in duplicate, and each repeated. (Inclusion of the urine assay for all 15 wastes would add an additional 30 dose-response curves.) Although only 2 strains and 2

activation conditions were used, would a reduced test matrix have produced an acceptable level of detection for these waste samples?

Judicious selection of a test matrix (and test battery) are required to screen hazardous wastes for biological activity in a cost-effective manner without an unacceptable loss of detection capability. Considering the effort required to produce a test matrix as large as the one in Table 3, the use of only strain TA98 + S9 would have reduced the time and expense considerably, with the concomitant loss of only $\sim 20\%$ of the detection capability provided by the test matrix in Table 3. Consistent with this proposal is the finding by the National Toxicology Program that the use of strain TA98 or TA100 in the presence of S9 detects approximately 80% of the mutagenic pure compounds in their data base that were identified using 4 strains with and without S9 (Zeiger et al., 1985).

Chemical Analysis and Biological Activity

Although extremely limited, the partial chemical characterization of the waste sample (Table 1) is more extensive than would be available for most waste samples--the vast majority of which would be of completely unknown chemical composition. Because of the limitations of the chemical analysis, a comprehensive picture of the composition of each waste sample is lacking. Consequently, the chemistry does not provide a ready explanation of the biological effects. For example, a comparison of the mutagenicity of the aqueous to the organic phase of the waste samples shows that the organic phase is more mutagenic than the aqueous phase for 3 out of 4 sets of waste samples (Table 3). The wastes were analyzed for the presence of only a few chemicals, representing a few chemical classes (organic solvents, chlorinated organics, and metals). However, few of these agents are mutagenic in *Salmonella* (Kier et al., 1986); thus, the compounds and their concentrations shown in Table 1 do not predict or even suggest the mutagenic activity exhibited by some of the wastes.

This raises the issue of the relevance of this type of chemical analysis, which is expensive and time-consuming to obtain, for predicting the potential mutagenicity (or other biological activities) of hazardous wastes. In order to explore this problem further, Simons et al., (1987) have examined the lethality and hepatic toxicity of 10 of these waste samples in the rat. The presence of known hepatotoxins in these wastes suggested that some of the wastes might be hepatotoxic. However, the chemistry did not predict the lethality of the waste samples.

In our search of a suitable short-term test to either complement or replace the *Salmonella* assay for screening hazardous wastes, we also have examined the ability of these wastes to induce bacteriophage lambda (Houk and DeMarini, 1987). The results indicated that this

assay was more sensitive than the Salmonella assay, and it was comparable to the Salmonella assay in terms of time and expense.

Current schemes used by the U.S. EPA and other agencies to identify wastes as hazardous rely primarily on physical characteristics and chemical composition of the wastes (Friedman, 1985). Greer (1984) has discussed the limitations of the current definition of hazardous waste, and the U.S. EPA has published possible guidelines to correct this situation by adding health effects, including mutagenicity data, to the evaluation of hazardous wastes (Federal Register, 1983; 1984a, b).

Both government (Federal Register, 1983; 1984 a, b) and industry (Barfknecht and Naismith, 1984; Guiney, 1985) have recognized the important role that short-term tests could play in the toxicological assessment of hazardous wastes. This consensus has prompted us to investigate the feasibility of such a proposal. Based on our present results, short-term bioassays may offer an important insight into the potential health effects of wastes and, thus, may aid in the identifications of some wastes as hazardous.

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TABLE 1

Concentration of Chemicals and Metals Identified in Hazardous Wastes ($\mu\text{g/g}$)

Chemical / Metal ^a	Petro- Chemical	Pharma- ceutical	Plastics	Hazardous Waste Incineration Facilities											
				1		2				3		4			
				Aqueous	Organic	Organic		Aqueous		Organic		Organic		Aqueous	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Aniline		14000	550000												
Benzyl Chloride	3000														
Bis-(2-ethyl- hexyl)-phthalate		500		< 100	3800							200	230	< 10	< 10
Butylbenzyl-phthalate				< 100	320	120	450	< 5	8	160	140				
Chlordane												< 60	< 60	19000	19000
Chlorophenylisocyanate		21000								2000	2500				
Cresol(s)						620	1300	240	240						
Diethylphthalate															
m-Dichlorobenzene		23000													
o-Dichlorobenzene		46000													
p-Dichlorobenzene		59000													
m-Dinitrobenzene			< 100												
Diphenylamine			6200												
2,4-Dimethylphenol										500	2000				
Hexachlorobutadiene												< 10	< 10	< 10	< 10
Hexachloroethane	560											< 10	< 10	230	260
Hexachlorocyclo- pentadiene															
Isophorone				< 100	110										
Mononitrobenzene			< 100												
Naphthalene	< 100			< 100	350	250	450	44	49	450	490	38	33	< 10	< 10
Phenol				34000	1000	1500	1700	1800	2900						
Phenylenediamine			2300												
Phenylisocyanate		160000													
Trans-1,4-dichloro- 2-butene	59000														
1,2,4-Trichlorobenzene		290													
Benzene				< 3	260							46000	58000	< 10	< 10
Carbon tetrachloride	68000	44000		< 2	6000	3700	4400	< 10	< 10	5900	5700	9100	11300	< 10	< 10
Chlorobenzene		4100										390	500	< 10	< 10
Chloromethane		1200													
Chloroform	2900					170	270	21	22			110	60	< 10	< 10
Cis-1,4-dichloro- 2-butene	18000														
Methylene Bromide												< 10	< 10	3100	4700
Methylene Chloride	21000											100	340	44	60
Methyl Ethyl Ketone				27	9700					18000	38000				
Tetrachloroethylene	11000			< 1	28	7100	9800	1600	1300	7900	8100	87	150	< 10	< 10
Toluene	240000			110	2400	32000	45300	2900	2700	56000	48000	160000	390000	22	20
1,1,1-Trichloroethane	< 100									24000	16000	330	230	< 10	< 10
Trichloroethylene	4000	40000		< 1	5500	3700	4400	90	85	8100	7800	8300	10300	< 10	< 10
Ag	< 3					< 1	< 1	< 1	< 1			< 1	< 1		
As	< 24					< 20	< 20	< 20	< 20	< 14	< 14	< 23	< 23		
Ba	< 7					110	140	6	7	1160	1150	990	1100		
Be	< 2					< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1		
Cd	< 5					6	6	< 1	< 1	153	15	49	55		
Cr	< 5					50	57	3	3	431	425	250	290		
Hg	< 22					< 10	< 10	< 10	< 10	< 4	< 4	< 50	< 50		
Ni	< 68					7	7	2	2	26	27	< 4	< 4		
Pb	< 19					140	150	< 10	11	1830	1800	1200	1300		
Sb	< 12					58	61	< 10	< 10	437	373	< 24	< 24		
Se	< 470					340	< 100	< 100	< 100	< 21	< 21	< 160	< 160		
Ti	< 23					< 20	< 20	< 20	< 20	< 9	< 9	< 22	< 22		
Si										< 1	< 1				
pH		2		8		7	7	6	7				7	7	7
Water (%)	2		5	94	3	38	48	95	67	21	23	5	5	94	96

^a Data from EPA (1984). Waste samples B, E, F, G, J, K, L, and M were spiked with carbon tetrachloride and trichloroethylene; therefore, the concentrations of these 2 chemicals in these wastes reflect this addition.

TABLE 2

SOLVENT EXTRACTIONS OF HAZARDOUS WASTES

Waste	Phase separation ^a		Final volume ^b (ml)
	DCM	Methanol	
A	No	No	4
B	No	-- ^c	
C	No	No	5
D	Yes		2
E	No	No	2
F	No	Yes	2.5
G	Yes		2.5
H	No ^d	Yes	2
I	Yes		2
J	No	No	5
K	No	Yes	2
L	No	No	7
M	No	No	5
N	Yes		2
O	Yes		2

^aTen ml of each waste was subjected to extraction as described in Materials and Methods.

^bFinal volumes vary because variable amounts of DMSO were required to reconstitute each sample due to the different solubilities of the samples.

^cExplosive reaction occurred upon the addition of methanol; no extract was available for bioassay.

^dPhase separation occurred when both solvents were added; DCM phase was used for bioassay.

TABLE 3

MUTAGENIC POTENCIES OF WASTES AND WASTE EXTRACTS IN SALMONELLA

Waste	Revertants per μg^a							
	Crude Wastes				Waste Extracts			
	TA98		TA100		TA98		TA100	
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
A			1870	1326			79	37
B					NT ^b	NT	NT	NT
C			18	329	5581		168	
F			3	4				
G	1	1						
H					6	2		
J					3177			
L	58							
M	31							
O	1							

^aValues are the model slopes calculated from the dose-response curves as described in the Materials and Methods. Blank areas represent nonmutagenic responses. Wastes or extracts of samples D, E, I, K, and N were not mutagenic. The average DMSO control values (rev/plate \pm S.D.) were: 52 ± 8 (TA98 + S9), 33 ± 5 (TA98 - S9), 156 ± 19 (TA100 + S9), 151 ± 12 (TA100 - S9).

^bNT, not tested.

TABLE 4

MUTAGENIC POTENCIES OF URINES AND
URINE EXTRACTS IN STRAIN TA98

Waste	Amount of waste per rat (g/kg)	Revertants per ml or ml equivalents ^a	
		Raw	extracts
C	0.2	395	455
L	2.5	259	1586
M	5.0	63	205
G	5.0		
O	5.0		
E	5.0		
H	5.0		NT
J	2.5		NT
K	2.5		NT
B	5.0		NT

^aValues were calculated from the linear portion of the dose-response curves as described in the Materials and Methods. Urines were tested in the presence of S9 and β -glucuronidase. Blank areas represent nonmutagenic responses. The average control (no urine) value \pm S.D. was 41 ± 6 .

TABLE 5

SUMMARY OF RESPONSES

Waste	Wastes ^a		Urines	
	Crude	extracts	Raw	extracts
C	+	+	+	+
L	+	-	+	+
M	+	-	+	+
G	+	-	-	-
O	+	-	-	-
E	-	-	-	-
H	-	+	-	NT
J	-	+	-	NT
K	-	-	-	NT
B	-	NT ^b	-	NT
A	+	+	NT	NT
F	+	-	NT	NT
D	-	-	NT	NT
I	-	-	NT	NT
N	-	-	NT	NT

^aFour responses (2 strains +/- S9) were merged into one response for both the crude wastes and the waste extracts (a total of 8 responses) using the following criterion: If a positive response was obtained under at least 1 of the 4 test conditions, the summary response was positive.

^bNT, not tested.

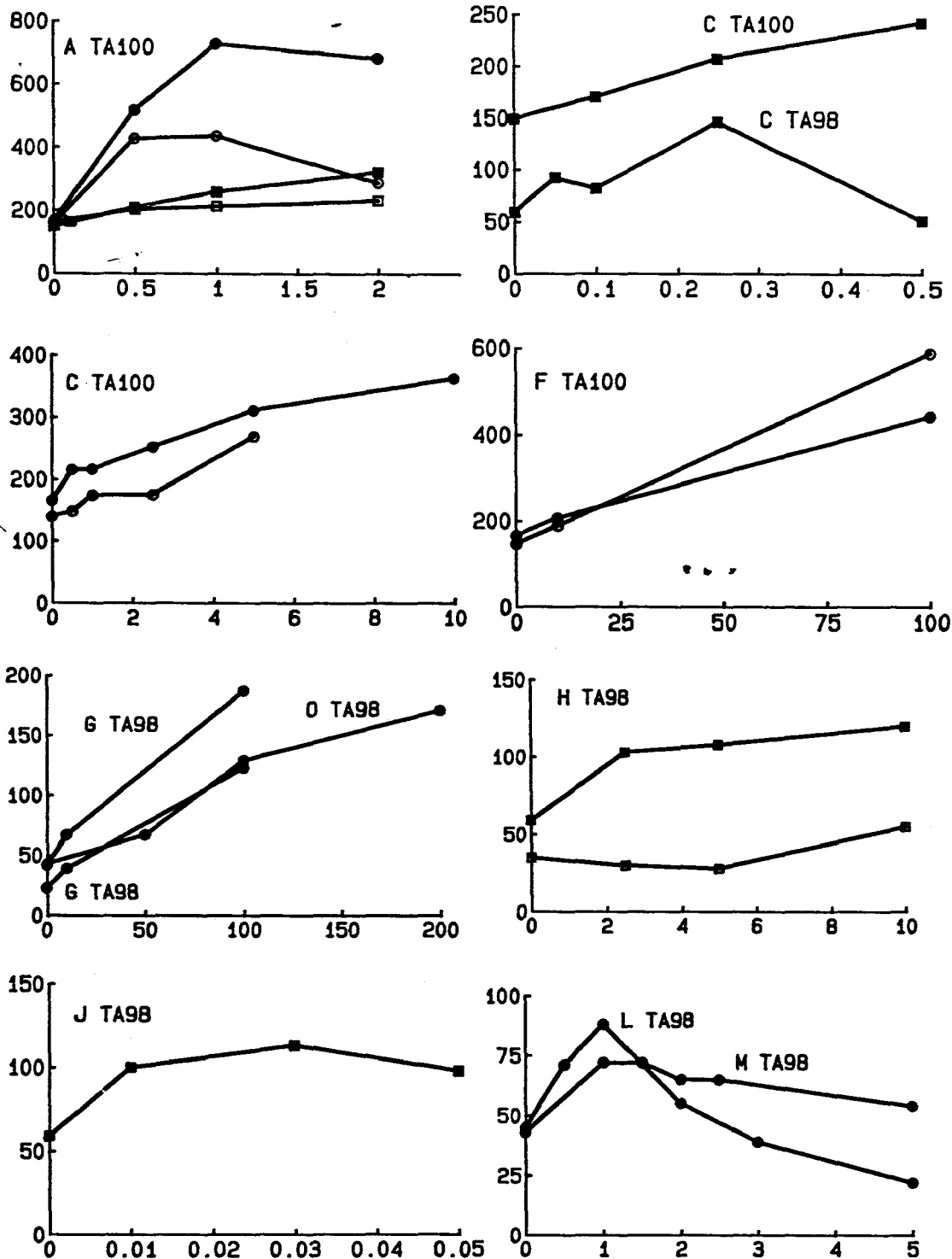
LEGENDS TO FIGURES

Fig. 1. Dose-response curves of crude wastes (circles) and waste extracts (squares) in the presence (open markers) or absence (solid markers) of Aroclor 1254-induced rat liver S9. Experiments were performed as described in the Materials and Methods. Results are from representative experiments.

Fig. 2. Dose-response curves from strain TA98 of raw urines or urine extracts from F-344 rats administered crude wastes. Dosing of animals, preparation of urines and extracts, and bioassay protocols are described in Materials and Methods. Waste C urines were collected after 10 days of dosing; waste L and M urines were collected after a single dose performed as described in Materials and Methods. Results are from representative experiments.

Fig. 1

Revertants/plate

Dose ($\mu\text{g}/\text{plate}$)

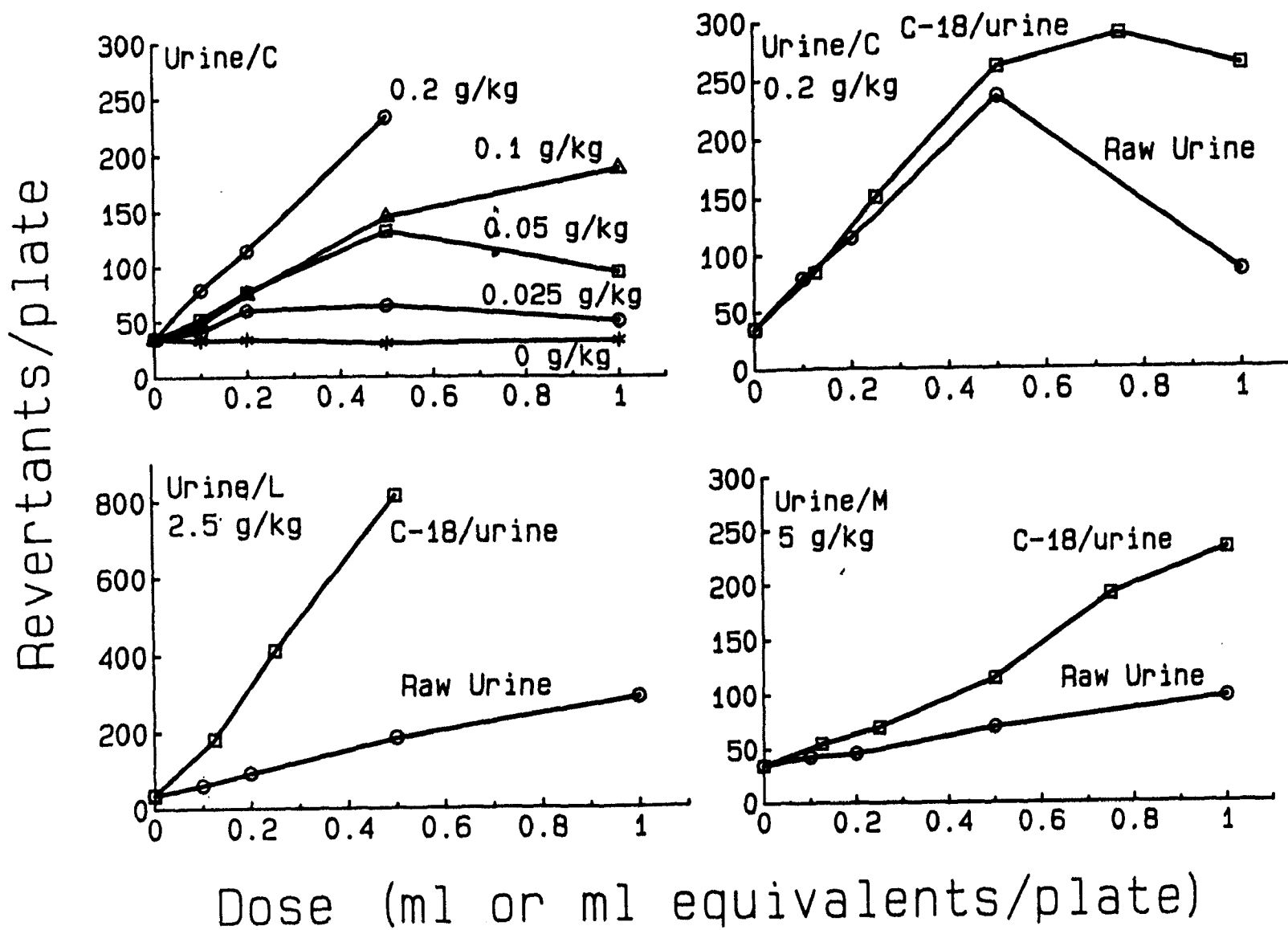


Fig. 2

APPLICATION OF A SIMPLE SHORT-TERM BIOASSAY FOR THE IDENTIFICATION OF GENOTOXINS FROM HAZARDOUS WASTES

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ABSTRACT

The proper disposal of hazardous wastes currently generated and the clean up of the waste disposal sites of the past is a challenge facing regulatory agencies in the industrialized nations. The estimation of the levels of toxicity is an essential step in prioritizing the industrial effluents and solid wastes for treatment and disposal. A number of short-term bioassays has been developed to supplement information from chemical analysis for evaluating the potential of chemical complex mixtures to induce adverse human health effects and environmental contamination. Among these bioassays, plant test systems provide simple, inexpensive, and rapid means of evaluating the toxic effects of industrial wastes based on multimedia exposure. Two such assays, tradescantia thaliana and Zea mays, have been used for monitoring the genotoxic effects of ambient air, municipal wastes, industrial effluents, solid wastes, water sediments, and pesticides. We applied the Arabidopsis embryo assay to evaluate the mutagenicity of complex environmental mixtures including coal and wood combustion extracts (from the People's Republic of China), industrial effluents, and sludges. The coal and wood smoke condensate samples were extracted with methylene chloride and solvent exchanged to DMSO. The industrial waste samples were tested either unextracted or as dichloromethane extracts. The comparative analysis of the data on these samples in Arabidopsis and in other commonly used bioassays will be presented. The significance of short-term plant bioassays for use in environmental assessment will be discussed.*

INTRODUCTION

Approximately 200 short-term in vitro and in vivo test systems (STT) are currently available to evaluate the genotoxic potential of environmental chemicals (Waters et al., 1984). These bioassays were developed in the early 70's after a discovery by Dr. Bruce Ames (1973) of the University of California, Berkeley, CA, that most of the carcinogens can be identified by using simple microbial mutagenicity bioassays. Most of these STT employ either in vitro procaryotic or eucaryotic cells or in vivo animal test systems. Substantial progress has been made in utilizing these assays for

*This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

identifying mutagens and carcinogens in a variety of products such as agricultural chemicals, drugs, consumer products, industrial chemicals, etc. Since humans are rarely exposed to individual chemicals, but exposure occurs mostly to a mixture of chemicals, the STT technology has been applied for evaluating the genotoxicity of complex chemical mixtures such as industrial emissions, effluents, and hazardous waste samples, and for evaluating the level of mutagenicity in the ambient environment.

One of the STT that appears very useful for identifying mutagens and presumptive carcinogens in the environment is the Arabidopsis embryo assay (AEA). Originally developed by Andreas Muller (1963, 1965), this assay has been used to assess the mutagenicity of over 120 chemicals (Gichner and Veleminsky, 1967; Redei et al., 1980; Veleminsky and Gichner, 1968; Gichner et al., 1982), and complex mixtures (Sandhu and Acedo, 1986; Acedo et al., 1987). One of the easily detectable genetic endpoints in the AEA is the loss of chlorophyll pigmentation in the developing embryos of the mutant population. As an in vivo bioassay, Arabidopsis offers certain unique advantages which include: (1) short study period (4 weeks), (2) economy of space (200 plants can be grown in a petri dish), (3) high fecundity, (4) easy to grow and score for mutation, (5) multiple genetic endpoints, and (6) induced mutations are observed in the progeny of the exposed population. Although data are limited, it appears that this small crucifer has the capability to biotransform promutagens into ultimate mutagens (Redei et al., 1984).

Since the usefulness of AEA for evaluating the mutagenicity of individual compounds has been established, we have attempted to extend its utility for assessing the genotoxic hazard of complex environmental mixtures. In the study reported here we have applied AEA for analysis of the genotoxicity of five hazardous waste samples obtained from a phenyl mercuric acetate plant.

MATERIALS AND METHODS

Hazardous Waste Samples

Two liquid effluent and three solid waste samples from a mercuric acetate plant were received through Versar, Inc., New Jersey. The liquid samples were Cosan Scrubber water and Cosan Separated water. The Cosan Scrubber water sample was very alkaline (pH 11.0), whereas the Cosan Separated water sample was extremely acidic. The three solid samples were identified as Noudex Presscake No. 2 (NP2), Noudex Presscake No. 3 (NP3), and Noudex-Hg (N-Hg) recovery sludge. These samples were evaluated for their genotoxic effects either as crude samples or after extraction with methylene chloride or water. The pH of the liquid samples was adjusted to 7.0 for Cosan Scrubber and to 5.0 for Cosan Separated to allow the growth of Arabidopsis. Concentrations of the sample ranging for 0.1-1,000 mg/ml were made

with buffered mineral medium (Redei, 1965). A total volume of 1 ml of the test solution contained in scintillation vials was used for treatment.

Sample Preparation

The solid samples were tested in their crude form, as received, and as aqueous or methylene chloride extracts. For testing the crude samples, seeds were exposed by planting them directly in mixtures containing various proportions of the test material and PromixTM medium (Premier Brands, New Rochelle, NY 10804). The mixtures were prepared by kneading the desired amount of PromixTM and sample together in a plastic bag and putting the mixture in petri dishes where the *Arabidopsis* is grown.

Aqueous extracts of the solid samples were obtained by preparing 1:1 (v/v) mixtures of sample and deionized water. These mixtures were allowed to stand for 1 h, centrifuged at 4,500 rpm for 10 min, and the supernatants were collected. Dilutions of these extracts were tested for evaluating the the genotoxicity of these samples.

Methylene chloride extracts were solvent exchanged to DMSO to a concentration equivalent to 1 mg/ml, and further dilutions ranging from 0.01-0.50 mg/ml were made and evaluated for their genotoxicity.

Arabidopsis Assay Protocol

For testing liquid samples and sample extracts, approximately 300 seeds of *Arabidopsis thaliana* (Columbia wild type), contained in a small cloth bag, were immersed in each vial of test solution for about 12-15 h at room temperature. At the end of the treatment period, the seed bags were rinsed under running tap water for 1 h. The washed seeds were dispersed in water and planted in glass Petri dishes (85x75 mm) containing pasteurized Promix medium. The dishes were subsequently placed in a growth chamber set to provide a 16-h photoperiod and a temperature of 24 C.

Crude samples (nonextracted solid samples) were tested by growing the *Arabidopsis* seeds in Petri dishes containing sample/PromixTM mixtures, then placing the dishes in the growth chamber.

After 3 weeks of growth (Fig. 1), when the seeds were mature but not yet brown, three fruits from each of the 100 plants selected at random for each concentration were opened with sharp forceps. The embryos within these fruits were scored for white (mutant) embryos among the normal green embryos (Fig. 2). The extent of sterility induced by each concentration was also determined. Sterility is manifested as empty spaces within a fruit or in severe cases no fruits are formed at all along the stem.

Each experiment contained a concurrent positive control (0.2% EMS) and a solvent negative control. The data presented are an average of the two experiments except for the methylene chloride extracts for which the results of a single experiment are given. Confidence intervals (CI) were determined using the statistical methods of Ehrenberg (1977).

In evaluating the experimental data, a sample was considered mutagenic to *Arabidopsis* if it induced a twofold or greater increase in the number of chlorophyll mutants at two or more doses as compared to solvent control or if it induced dose-related increases at three or more doses.

RESULTS

The data on the mutagenicity of the liquid samples, Cosan Scrubber water and Cosan Separated water, presented in Table 1 indicate that both samples are mutagenic for *Arabidopsis*. A slight dose response in mutagenicity is indicated by Cosan Scrubber water, and although Cosan Separated water shows a similar trend, there was no further increase in mutagenicity beyond the concentration of 1 mg/ml (Fig. 3). In addition to mutagenicity, a high degree of sterility was induced by 10 mg/ml of Cosan Scrubber water and the undiluted (1,000 mg/ml) Cosan Separated water (Table 1). Germination of the treated seeds was considerably reduced at higher concentrations. These results must, however, be viewed with caution since the pH of both samples had to be drastically adjusted prior to testing to allow growth of *Arabidopsis*. The alkaline Cosan Scrubber sample was adjusted using whole pellets of KOH. Although these pH changes were necessary to allow testing, it is quite probable that some components of these complex mixtures were substantially altered in the process. The nature of these alterations and their effect on the overall mutagenicity of the samples is not known.

The solid waste samples, Noudex NP2, NP3, and N-Hg recovery sludge were tested as unextracted crude samples as well as after extraction with methylene chloride and water. The toxicity range-finding experiments showed that the unextracted crude samples were very toxic for *Arabidopsis*. Therefore, these materials were tested for mutagenicity after various dilutions with Promix medium. The mutagenicity of these samples for *Arabidopsis* is shown in Table 2. NP2 was very toxic to *Arabidopsis*. Even after mixing 1 part of the sample with 9,000 parts of PromixTM the survival of the treated plants was only 50%. At this low concentration, NP2, however, induced about four times more chlorophyll mutations than the solvent control (Table 2). It was interesting to note the relatively low degree of sterility (7.7%) induced in the surviving plants, indicating that the observed toxicity may be primarily due to inhibition of growth rather than interference with genetic mechanisms. Proportions of sample lower than 1:9,000 were not tested due to difficulties

encountered in accurately measuring and mixing with Promix such a small amount of the sample.

NP3 and N-Hg recovery sludge were relatively less toxic to Arabidopsis as compared to NP2. Nevertheless, both samples were mutagenic for Arabidopsis (Table 2).

The results of the treatment of Arabidopsis with the samples extracted with methylene chloride are shown in Table 3. All three samples were positive for mutagenicity; however, dose-related response was not observed for these extracts. For lack of sample availability, testing at doses lower than 0.1 mg/ml and a repeat experiment could not be performed.

Table 4 shows the mutagenicity for Arabidopsis after treatment with the aqueous extracts from each of the three solid samples. NP3 and N-Hg recovery sludge samples showed dose-related mutagenic response although the mutagenicity values are much lower than that of NP2. A high degree of sterility was induced by N-Hg especially at 100% concentration of the extract.

DISCUSSION

The identification and proper disposal of hazardous wastes are challenges facing regulatory agencies in the industrialized nations. At present, most of the regulatory agencies, including the U.S. EPA, use chemical components of the industrial wastes as a basis for hazard evaluation (Friedman, 1985). However, chemical analysis is currently limited to the detection of only a few known toxic compounds, whereas numerous other potentially harmful genotoxins may be undetected. Even if all the chemicals present in hazardous wastes were identified (which is an improbable task), chemical analysis alone will not provide information on the synergistic and antagonistic effect that may result from the interaction of these complex chemical mixtures. Therefore a biological evaluation along with their chemical analysis has been repeatedly emphasized for assessing the health hazards of complex chemical mixtures (Waters et al., 1978; Sandhu et al., 1987).

A large assortment of STT may be utilized for the evaluation of hazardous industrial wastes, and plant bioassays are uniquely suitable for this purpose. Certain bioassays such as Zea mays (Plewa, 1984), Tradescantia micronucleus assay (Ma et al., 1983), and Tradescantia gene mutation assay (Schairer et al., 1983) have been very useful for evaluating the levels of genotoxins in the terrestrial and aquatic environment (see review by Ma and Harris, 1985). The primary advantages of the plant systems are their simplicity, cost effectiveness, and their utility in assessment of simultaneous multimedia exposure. In contrast to cell culture bioassay, one does not have to worry about microbial contamination.

The purpose of analyzing the hazardous waste is twofold: (1) to assess its overall health hazards for its proper disposal and (2) to identify the specific components of the waste responsible for the particular effects so that control technology can be applied to minimize the production of toxic chemicals. In the study reported here, we have applied a simple bioassay in an attempt to accomplish the first objective. Our choice of test samples, obtained from a phenyl mercuric acetate plant, was based on their availability. The data presented in this study are based only on results from crude samples and on methylene chloride and aqueous extracts. Although extraction reduced cellular toxicity, it did not alter the qualitative mutagenicity results of the samples. Therefore, if samples are not very toxic, testing complex mixtures directly without elaborate sample preparations may be a convenient way to make a preliminary assessment of their genotoxic potential.

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Figure 1.--A dish containing 200-250 Arabidopsis thaliana plants 3 weeks after planting.

Figure 2.--A Arabidopsis thaliana plant with segregating white and green embryos.

Figure 3.--Mutagenicity induced in Arabidopsis by the Cosan liquid samples.

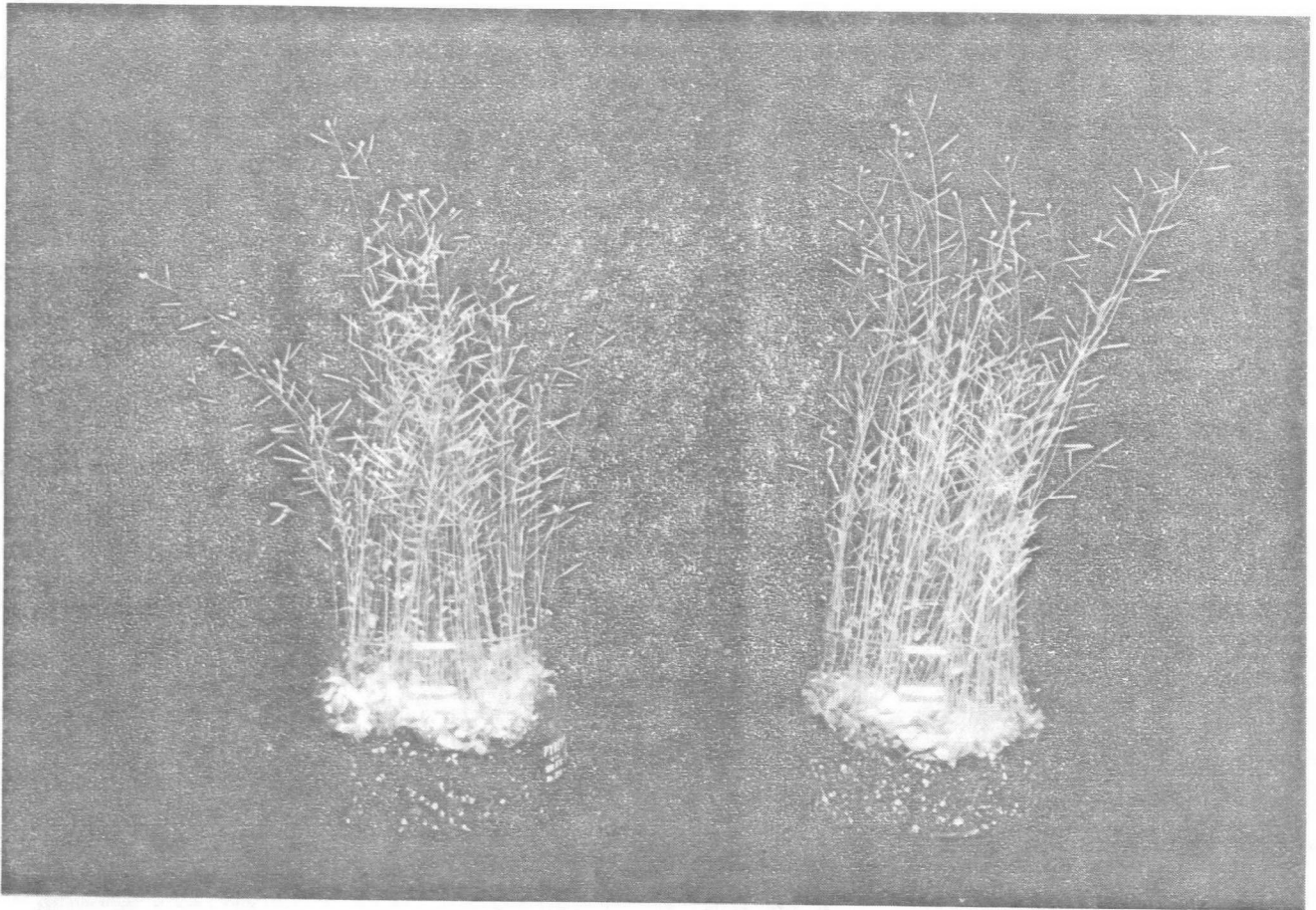


Figure 1

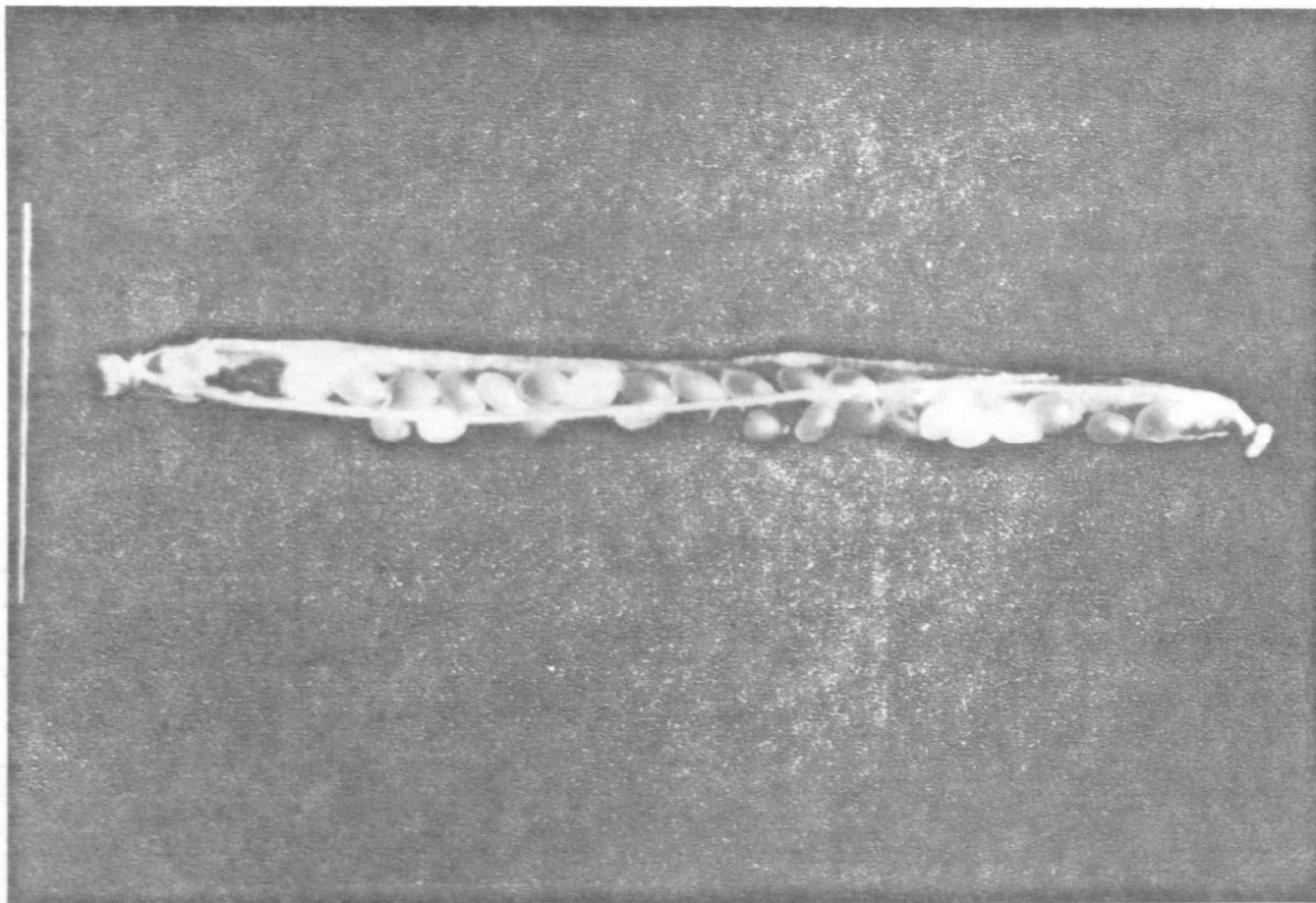


Figure 2

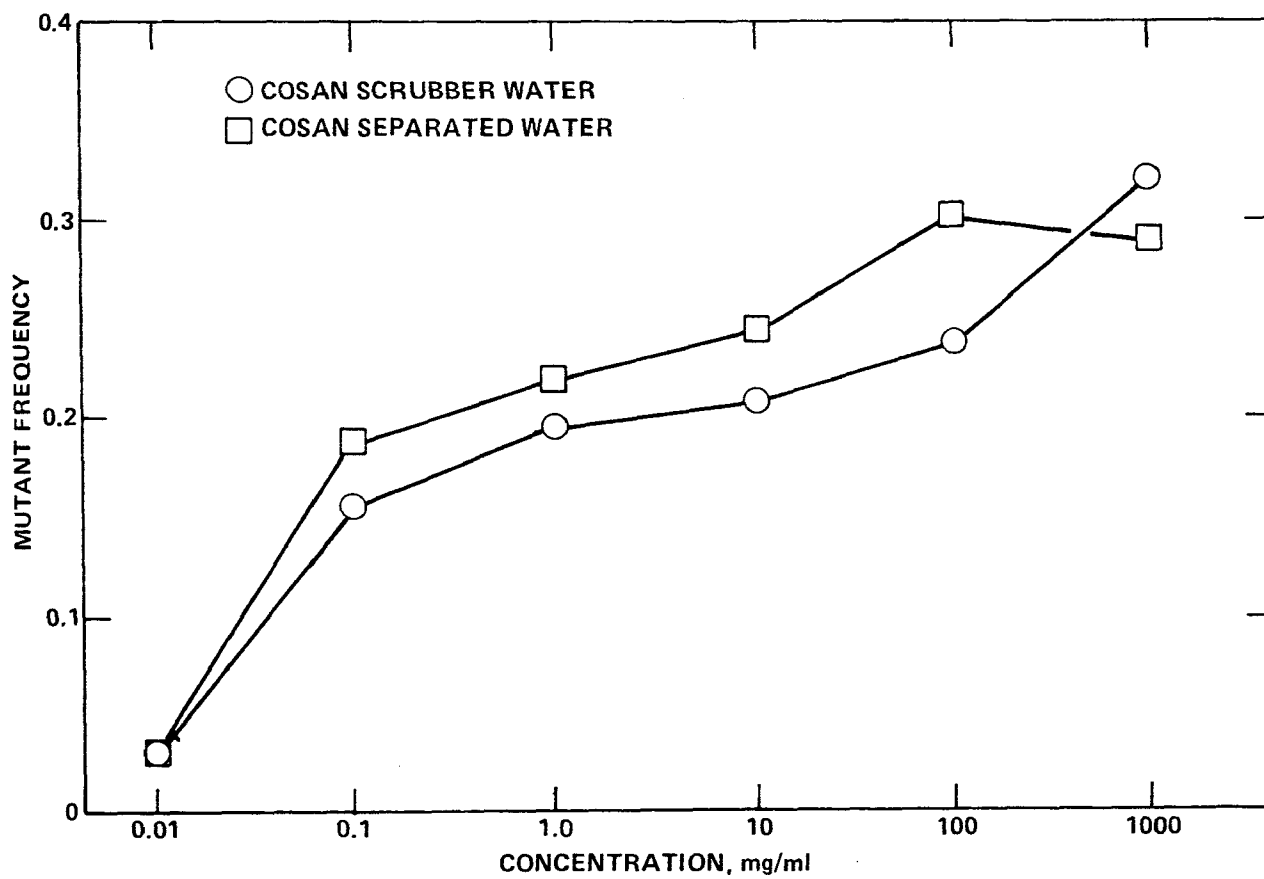


Figure 3. Mutagenicity induced in Arabidopsis by the Cosan liquid samples.

Table 1. Mutagenicity of Cosan Liquid Samples in the Arabidopsis Embryo Assay

Sample	Concentration (mg/ml)	Germination (%)	% of Fruits Containing Mutants \pm CI	Sterility (%)
Cosan Scrubber Water	0.1	95.5	15.5 \pm 4.94	1.5
	1.0	97.5	19.5 \pm 8.90	8.5
	10.0	99.0	20.8 \pm 13.77	14.0
	100.0	73.0	23.6 \pm 7.75	2.0
	1000.0	50.0	32.0 \pm 4.76	3.5
Cosan Separated Water	0.1	97.5	18.0 \pm 1.96	4.5
	1.0	89.0	22.6 \pm 8.64	7.8
	10.0	96.0	24.4 \pm 13.95	4.6
	100.0	55.0	28.9 \pm 16.60	2.0
	1000.0	52.0	28.6 \pm 9.29	40.0
Solvent Control (mineral medium)	0	100.0	4.0 \pm 2.78	0
Positive Control (EMS)	0.20	97.0	80.0 \pm 1.96	64.0

Table 2. Mutagenicity of Three Solid Waste Samples in Arabidopsis

Sample	Proportion (Sample: Promix™ v/v)	Germination (%)	% of Fruits Containing Chlorophyll Mutants ± CI	Degree of Sterility (%)
Noudex Presscake No. 2	1:9000	50	19.2 ± 5.50	7.7
Noudex Presscake No. 3	1:4000	85	15.0 ± 5.90	8.3
	1:2000	95	19.3 ± 2.70	13.8
	1:1000	94	20.5 ± 8.44	17.9
Noudex Hg	1:4000	80	19.0 ± 7.86	6.5
	1:2000	83	16.5 ± 4.91	8.5
Promix™ only		98	5.0 ± 1.96	3.0

Table 3. Mutagenicity of Methylene Chloride Extracts in Arabidopsis (data from one experiment)

Sample	Concentration (mg/ml)	Germination (%)	% of Fruits Containing Chlorophyll Mutants	Degree of Sterility (%)
Noudex Presscake No. 2	0.01	90.0	25.6	15.0
	0.10	91.0	22.0	4.0
	0.25	95.0	22.6	1.0
	0.50	88.0	22.0	13.0
Noudex Presscake No. 3	0.01	98.0	12.0	10.0
	0.10	86.4	13.0	12.0
	0.25	85.0	18.0	0
	0.50	82.7	13.0	12.0
Noudex Hg	0.01	100.0	8.6	1.0
	0.10	100.0	14.0	10.0
	0.25	89.0	18.0	3.0
	0.50	87.3	14.0	13.0
Solvent Control (DMSO)	100.00	86.5	7.0	7.0

Table 4. Mutagenicity of Aqueous Extracts in Arabidopsis

Sample	Concentration (%)	Germination (%)	% of Fruits Containing Chlorophyll Mutants \pm CI	Degree of Sterility (%)
Noudex Presscake	1.0	85.0	18.9 \pm 5.70	0
No. 2	10.0	67.0	26.5 \pm 0.98	1.0
	100.0	50.0	21.5 \pm 2.95	9.0
Noudex Presscake	1.0	100.0	5.0 \pm 3.93	0
No. 3	10.0	100.0	8.0 \pm 5.90	0
	100.0	65.0	13.7 \pm 6.55	2.8
Noudex Hg	1.0	100.0	6.0 \pm 3.93	9.0
	10.0	70.0	8.3 \pm 7.27	6.0
	100.0	63.0	11.5 \pm 6.88	18.0
Solvent Control (water)	0	100.0	5.0 \pm 1.96	0
Mutagen Control (EMS)	0.20	100.0	81.5 \pm 0.88	80.0

APPLICATION OF BATTERY OF AQUATIC TOXICITY TESTS TO SOLID WASTE
LEACHATE CHARACTERIZATION AND ENVIRONMENTAL EFFECTS PREDICTION

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ABSTRACT

Most regulatory concerns about chemicals in effluents and leachates are based on toxicity to some kind of organisms. Only organisms can sense toxicity; analytical instruments cannot. Minimization and control of toxicity, as a characteristic of complex water is becoming commonplace. Tests to measure toxicity of effluents are well established and are performed by many laboratories. The ability of these tests to predict impact in receiving water has been extensively tested. Methods are now becoming available to identify, fractionate, and reduce cause of toxicity to permit source control, if practicable. The use of toxicity tests is a cost-effective method to identify where toxicants need to be controlled and what type and degree of treatment may be needed to limit emissions to acceptable levels.

SCREENING OF COMPLEX SOLID WASTES FOR CHEMICALS WHICH BIOACCUMULATE
AND CAUSE ENVIRONMENTAL HAZARDS

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ABSTRACT

The complex waste research program of this laboratory is developing rational hazard ranking protocols to assist in option selection. If the waste is uncharacterized chemically, the low-cost toxicity assessment and fractionation protocol presented by Donald Mount in this conference will enable toxic waste streams to be minimized. If the waste has been characterized chemically, the relative hazards of the chemical as well as the toxicity of the mixture of chemicals can be adequately estimated from the list of chemicals using the QSAR system.

The QSAR system is an integrated chemical information and modeling system based on structure-activity relationship. The system integrates the environmental toxicity database, AQUIRE, the chemical properties database, CHEMPROF, with a variety of other toxicity databases. Unavailable data for 14 key chemical properties, bioaccumulation potential, acute and chronic toxicity, persistence, and carcinogenicity are estimated for each chemical from the structure. Finally, the toxicity data are incorporated into the ERL-Duluth joint toxic action models to estimate the toxicity of the complex mixture. We have found these estimates limited only by the accuracy of the chemical analysis.

BIOACTIVITY DIFFERENCES OF WATER AND SODIUM ACETATE ELUATE FROM MUNICIPAL AND INDUSTRIAL WASTES

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ABSTRACT

Aqueous and sodium acetate eluates prepared from municipal and industrial waste products, and the sodium acetate extraction fluid (eluent) recommended in the EPA Toxicity Characteristic Extraction Procedure (TCLP) were assayed for their toxicity potential. The bioassays included algae, Selenastrum capricornutum; macroinvertebrates, Daphnia magna; lettuce root elongation, Lactuca sativa L.; and Microtox, Photobacterium phosphoreum. The pH 5.0 TCLP sodium acetate eluent was highly toxic to each of the organisms. Adjustment of its pH to 7.0 decreased toxicity approximately 2.5-fold for algae and lettuce, and 6-fold for D. magna. This reduction, while statistically significant, did not change the toxic classification of the TCLP eluent. Photobacterium phosphoreum was unaffected by pH 7 sodium acetate after 30-minutes exposure. Toxicity of the industrial waste TCLP eluates to S. capricornutum and D. magna was similar to that obtained with water extraction. The response of D. magna to the TCLP eluates from sewage sludge (POTW #2), municipal ash, paint sludge, Midco volatile soil, and First Chemical indicated that these samples contained acetate soluble contaminants which were more toxic than the acetate eluent itself. Bioassays detected toxicity in either TCLP or water eluted samples. Toxicity of water eluted samples can be attributed only to materials leached from the waste. Toxicity of the TCLP eluted samples is complicated by the uncertainty of how much toxicity is attributable to the leachate itself vs. the materials leached from the samples.

INTRODUCTION

The Environmental Protection Agency (EPA) has developed a Toxicity Characteristic Leaching Procedure (Friedman, 1985), designed to simulate waste leachate from a sanitary landfill. The Toxicity Characteristic Leaching Procedure (TCLP) replaces the Extraction Procedure (EP) toxicity test recommended in the 1976 Resource Conservation and Recovery Act. The EP toxicity test is designed to extract and screen for heavy metals, whereas the TCLP is designed to extract and screen for heavy metals and organic contaminants.

The TCLP involves mixing site samples with sodium acetate in a specially designed extractor vessel capable of handling volatile and

nonvolatile organic compounds. The extracted contaminants are chemically analyzed to determine the composition of the eluates from samples collected at hazardous waste sites and sanitary landfills. Sodium acetate was chosen after comparing the chemical analysis of waste mixture eluates extracted with carbonic acid, deionized water, or pH 2.9 and 5.0 buffered solutions of sodium acetate. No attempt was made to determine toxicity of the various eluents. It is assumed that toxicity of a sodium acetate TCLP waste eluate is represented by the sum of the toxicities of its individual, chemically analyzed constituents. This assumption is flawed because the authors of chemical criteria did not intend for them to be used additively (they specifically advised against it). Furthermore, chemical criteria values were developed in aqueous solutions or with water soluble organic solvents known as toxicity. The toxicity of an eluent must be established if it is used to prepare eluates in which living organisms will be used to assess toxicity.

Daniels (1981) cited acetic acid as an example of an organic solvent that has toxicity characteristics in and of itself. The concern for this problem led us to evaluate the feasibility of using TCLP acetic acid derived eluates during biological assessment of the toxicity of solid waste leachates. The evaluation was performed by testing both aqueous and TCLP eluates and comparing the resultant EC₅₀ or LC₅₀ concentrations.

Jackson et al. (1984) and Porcella (1983) recommend deionized water as the eluent for determining the potential fate and environmental hazard of chemicals at hazardous waste sites and landfills. The advantages of deionized water are that: (1) it is nontoxic to test organisms; (2) it is a clean, readily partitioned solvent for inorganic and organic analysis; (3) it is a realistic solvent, given that soil leachate mobility is driven primarily by precipitation events; and (4) its use permits prediction of the amount of water soluble contaminants that might be mobilized from a specific waste site soil, sludge, or landfill sample.

The U.S. EPA Office of Solid Waste (OSW) wanted to determine the feasibility of using TCLP eluates for biological toxicity characterization of wastes in addition to the chemical characterization. The objectives of this study were to: (1) define toxicity of the recommended TCLP sodium acetate eluent; and (2) determine the suitability of aqueous vs TCLP eluates for assessing toxicity of leached substances.

METHODS

Selected industrial wastes, sewage sludges, municipal ash, a dimethylphenol Fullers earth positive toxicant control, and the TCLP eluent solutions (pH 5 and pH 5 adjusted to 7) were bioassayed. TCLP eluates from the wastes were prepared by ENSECO,¹ Inc.,

Cambridge, Massachusetts, in accordance with the conditions set forth in the proposed TCLP and sent to CERL under the direction of Gail Hanson, EPA, OSW, Washington, D.C.

Bioassays performed on the eluates were a 96-hr algal test (Selenastrum capricornutum) and a 48-hr macroinvertebrate test (Daphnia magna) according to the methods and data quality assurance requirements prescribed by Porcella (1983). They were bioassayed with the Microtox (Photobacterium phosphoreum) test conducted according to Beckman (1982) and the lettuce (Lactuca sativa L.) root elongation (RE) test.

The RE tests were conducted in 100-mm diameter glass petri dishes. Each dish contained 4.0 ml of test solution dispensed onto a sheet of 90-mm diameter #3 Whatman filter paper. Five seeds were placed in each of three replicate control and treated petri dishes and incubated at $24 \pm 2^\circ\text{C}$ in the dark for 120-hours. At the end of the incubation period, percent germination was recorded and individual root length was measured.

The pH tolerance limits for each bioassay test organism has been established (Miller et al., 1978; Greene, 1984) as follows: S. capricornutum, 5.5-10.0; D. magna, 6.5-10.05; L. sativa, 3.5-10.0; P. phosphoreum, 6.0-9.0. Therefore, the pH of TCLP eluates (3.8 to 5.8) were adjusted, prior to assay, to ensure against pH shock. The pH of all water eluates ranged from 6.9 to 8.9 and were not adjusted.

Water eluates were prepared by mixing 1.0 kg of solid waste sample with 4.0 liters of deionized water. The mixtures, each contained in a 10-liter cubitainer, were shaken at 100 rpm in a constant temperature room held at $24 \pm 2^\circ\text{C}$. The waste/water mixture was centrifuged in 300 ml polycarbonate bottles for 10 minutes at 10,000 rpm. The centrifugate was filtered through a 0.45 μm membrane filter prior to assay.

The pH of each TCLP eluate was adjusted to fall within the range of 6.5 to 7.5 with 0.1 N NaOH prior to assay with S. capricornutum, D. magna, and L. sativa. The neutral pH (7.0 ± 0.5) of the 2% NaCl Microtox osmotic adjustment solution was unaffected by the small volume (usually 1.5 ml) of the TCLP eluates added to the Microtox test medium, thus no pH adjustments were necessary.

EC₅₀ eluate concentrations for S. capricornutum and the results of the root elongation (RE) test were calculated by linear regression analysis of the control and treatment measurements. The trimmed Spearman-Kärber method of probit analysis (Hamilton et al., 1977) was used to calculate D. magna LC₅₀ values.

RESULTS AND DISCUSSION

Sodium Acetate Toxicity

The first objective of this study was to define the toxicity of the TCLP sodium acetate eluent. A volume to volume dilution series of the pH 5 and pH 5 adjusted to pH 7 eluents was prepared and assayed with P. phosphoreum, D. magna, S. capricornutum, and L. sativa. The mean LC₅₀ or EC₅₀ responses are reported in Table 1. Tests conducted at pH 5 demonstrated very high "toxicity" responses, i.e., very toxic at low concentrations of eluent. It took only 0.5% eluent to produce an EC₅₀ response with S. capricornutum while 7.6% eluent was required to produce an LC₅₀ response from D. magna.

Assays with P. phosphoreum, D. magna, S. capricornutum, and L. sativa are normally run within the tolerance limits of pH 6 to pH 10. Therefore, the pH 5.0 sodium acetate bioassay results are unreliable relative to toxicity per se vs pH shock. Because of this, the assays were also conducted on the eluent with the pH adjusted to 7 (well within the tolerance limits of all the test organisms). The same order of sensitivity was demonstrated with TCLP eluent adjusted to pH 7 as was seen in tests conducted at pH 5.0. Adjustment of pH from 5 to 7 eliminated pH shock. Therefore, the pH 7 column reflects toxicity of the TCLP sodium acetate eluent in and of itself (Table 1). These data demonstrate that the eluent is: highly toxic to S. capricornutum and L. sativa; moderately toxic to D. magna; and nontoxic to P. phosphoreum, according to the rankings of Porcella (1983).

Water Eluate Toxicity

EC₅₀ values, and their respective 95% confidence limits, for the water eluates of waste samples, are presented in Table 2. The municipal ash samples, including the algal response observed in the #2 sample, were nontoxic. Chemical analysis of the #2 municipal ash eluate measured 1.5 mg/l aluminum. Aluminum is a phosphorous removal agent (Cooke et al. 1986). Addition of phosphorus to #2 municipal ash eluate, in excess of its reactive aluminum content, restored the yield of S. capricornutum to that obtained in the control. The highly toxic algal response to the #2 ash eluate was due to inactivation of phosphorus and not to toxicity. D. magna was the only test organism to identify the toxicity of the water extracted Fullers soil #1 and #2 (positive toxicant control) samples spiked with 50 and 150 mg/l dimethylphenol, respectively. Each of the aquatic tests identified the First Chemical and paint sludge industrial waste eluates as being highly toxic (Table 3). Approximately 78% of the water eluates were toxic to D. magna, 67% to S. capricornutum, and 22% to P. phosphoreum.

Table 1. Response of test organisms to TCLP sodium acetate extraction eluents at pH 5 and at pH 5 adjusted to pH 7. Results are reported as v:v (%) EC₅₀ or LC₅₀ (Daphnia) eluent concentrations.

Bioassay	pH 7		pH 5	
	Mean	95% CI	Mean	95% CI
<u>S. capricornutum</u>	1.2	0.9 - 1.7	0.5	0.2- 0.8
Microtox 30-min	NO EFFECT		2.1	0.3- 3.7
Lettuce Root Elong.	15.0	6.4 -26.8	5.7	0.0-18.3
<u>D. magna</u>	45.8	43.7 -48.0	7.6	7.1- 8.0

Table 2. LC_{50} ¹ or EC_{50} response (as % of eluates) of test organisms to water eluates from selected sewage sludge, municipal ash, and industrial waste samples.

Sample ID	Algae	D. magna	Microtox	Lettuce RE ²
POTW SLUDGE #1	13.840	34.280		
lower 95% CI	8.350	29.02	NE ³	NE
upper 95% CI	18.340	40.490		
POTW SLUDGE #2	21.760	43.43		
lower 95% CI	5.200	42.430	NE	NE
upper 95% CI	48.900	43.43		
MUNICIPAL ASH #1				
lower 95% CI	NE	NE	NE	NE
upper 95% CI				
MUNICIPAL ASH #2	14.560 ⁴			
lower 95% CI	9.140	NE	NE	NE
upper 95% CI	21.400			
PAINT SLUDGE	0.270	3.930	0.940	1.4
lower 95% CI	0.038	3.490	0.830	0.6
upper 95% CI	0.580	4.420	1.050	2.9
MIDCO VOLATILE SOIL	26.560	86.260		83.5
lower 95% CI	11.330	77.050	NE	32.4
upper 95% CI	43.740	95.850		100.0
DMP FULLERS SOIL #1		84.570		
lower 95% CI	NE	80.430	NE	NE
upper 95% CI		100.000		
DMP FULLERS SOIL #2		62.300		
lower 95% CI	NE	48.500	NE	NE
upper 95% CI		80.010		
FIRST CHEMICAL	0.005	0.001	0.054	3.1
lower 95% CI	0.001	0.001	0.046	2.5
upper 95% CI	0.009	0.002	0.062	3.9

¹ LC_{50} is for D. magna.

² RE = root elongation.

³ NE = no observable toxic effect.

⁴ Results were caused by inactivation of phosphorus by aluminum. This sample did not contain toxic constituents.

Table 3. Toxicity classification of OSW water and TCLP eluates.¹

Waste Sample	Algae	Daphnia	30 Min Microtox
	Water/TCLP	Water/TCLP	Water/TCLP
First Chemical	H / H	H / H	H / H
Paint Sludge	H / H	H / H	H / H
POTW #1	H /NSD	M /NSD	NE / H
Municipal Ash #2	H / H	NE / H	NE / H
POTW #2	M /NSD	M /NSD	NE / NE
Midco Soil	M / H	L / H	NE / H
DMP Fullers Soil #1	NE ³ /NSD	L /NSD	NE / H
Municipal Ash #1	NE / H	NE / H	NE / H
DMP Fullers Soil #2	NE /NSD	M /NSD	NE / NE
	67 / 56 ⁴	78 / 56	22 / 0

¹ Toxicity of the eluates ranked as high (H = < 20%), moderate (M = 20-75%), and low (L = > 75%) according to Porcella (1983).

² NSD = Not significantly different from that obtained for pH 7.0 TCLP eluent (Table 1).

³ NE = no observable toxic effect in 100% water eluate.

⁴ Percent of OSW water and TCLP eluates, respectively, which exhibited toxicity.

TCLP Eluate vs. Water Eluate Toxicity

The second objective of this study was to evaluate TCLP and water eluate toxicity of the same samples (Table 2 and 4). Interpretation of these results is confounded by the fact that the TCLP eluent may have introduced a toxic effect into the overall TCLP toxicity value. If the toxic effect of the eluent was predictable, one could theoretically account for it by calculation of the leached substance toxicity value, i.e., total toxicity - eluent toxicity = eluate toxicity. However, as pointed out by Daniels (1981), there are too many uncertainties associated with chemical mixture toxicity (synergism, antagonism, degradation compound effects, etc.) to make this simple assumption. For example, toxicity of four TCLP eluates for S. capricornutum and D. Magna (Table 4) fall within the confidence limits developed for the pH 7 TCLP eluent (Table 1). However, interpretation of these results is impossible, relative to eluent vs. eluate toxicity given the test organisms sensitivities and the range of response exemplified by the confidence limits for the test, i.e., all of the toxicity response could be due either to the eluent or to a combination of eluent and eluate components.

S. capricornutum and D. Magna response to the municipal ash #1 and #2, paint sludge, Midco volatile soil, and First Chemical TCLP eluates (Table 4) all produced EC₅₀s or LC₅₀s, lower than, and outside of, the confidence intervals shown for the pH 7 TCLP eluent in Table 1. The highly toxic response for these samples would suggest that something in these eluates produced a toxic effect in addition to the toxic effects of the eluent.

D. Magna responses for POTW sludge #1, and DMP Fullers soil #1 and #2 eluates are within the levels of toxicity associated with the TCLP eluent. The S. capricornutum response to the POTW sludge #2 eluate was the same as that measured for the TCLP eluent. These observations are of particular interest when compared with D. magna and S. capricornutum water eluted toxicities for the same samples. Water eluate toxicity for POTW #1 was moderate for D. magna to high for S. capricornutum; that for DMP Fullers soil #1 and #2 were low to moderate for D. Magna and nontoxic to S. capricornutum. The TCLP eluate D. magna responses for the POTW sludge and Fullers soil samples fall within the range of confidence limits expected for the pH 7 TCLP eluent.

First Chemical and paint sludge TCLP and water eluates was highly toxic to all four of the test organisms. Comparison of the toxicity classification of the water vs TCLP eluates in Table 3 shows that the First Chemical, paint sludge, Midco soil and municipal ash TCLP eluates are highly toxic to S. capricornutum, D. magna and P. phosphoreum. A shift in toxicity from low or moderate to high (Midco soil) and no observable effect highly toxic (Municipal Ash #1) was obtained by TCLP extraction using D. magna and S.

Table 4. LC₅₀¹ or EC₅₀ response (as % of eluates) of test organisms to pH adjusted TCLP eluates from selected sewage sludge, municipal ash, and industrial waste samples.

Sample ID	Algae	D. magna	Microtox	Lettuce RE ²
POTW SLUDGE #1	0.630	50.580	12.23	4.28
lower 95% CI	0.520	44.400	7.30	2.82
upper 95% CI	0.740	57.400	17.12	6.09
POTW SLUDGE #2	1.250	39.360		56.77
lower 95% CI	0.990	30.940	NE ³	45.84
upper 95% CI	1.730	50.070		70.29
MUNICIPAL ASH #1	0.390	8.300	11.38	41.29
lower 95% CI	0.280	6.970	10.38	34.41
upper 95% CI	0.520	9.880	12.37	48.44
MUNICIPAL ASH #2	0.240	5.140	5.10	19.22
lower 95% CI	0.040	3.960	3.69	13.21
upper 95% CI	0.450	6.670	5.61	26.51
PAINT SLUDGE	0.360	1.210	0.55	1.61
lower 95% CI	0.180	1.040	0.42	1.10
upper 95% CI	0.570	1.400	0.60	2.28
MIDCO VOLATILE SOIL	0.200	0.270	7.15	14.18
lower 95% CI	0.190	0.210	5.08	6.24
upper 95% CI	0.200	0.350	10.62	22.54
DMP FULLERS SOIL #1	0.520	45.440	16.80	24.00
lower 95% CI	0.150	41.380	11.43	12.34
upper 95% CI	1.400	49.890	21.83	38.56
DMP FULLERS SOIL #2	0.590	54.330		23.00
lower 95% CI	0.210	47.520	NE	9.35
upper 95% CI	1.060	62.100		38.34
FIRST CHEMICAL	0.005	0.004	0.13	0.85
lower 95% CI	0.002	0.003	0.12	0.53
upper 95% CI	0.008	0.006	0.15	1.32

¹ LC₅₀ is for D. magna.

² RE = root elongation.

³ NE = no observable toxic effect.

capricornutum, respectively. We are, however, confident that the toxicity classifications of the water eluates were due to water extractable contaminants since no artificial toxicants were added. Highly toxic samples, such as paint sludge and First Chemical, will be detected by either the TCLP or water elution process. However, the use of water eluates will greatly reduce the potential for false positive responses while using an environmentally realistic extractant.

SUMMARY AND CONCLUSION

Toxicity of the Office of Solid Waste proposed TCLP (sodium acetate) extraction fluid was characterized using four bioassay tests (algae, S. capricornutum; macroinvertebrates, D. magna; Microtox, P. phosphoreum, and lettuce L. sativa L.). The same bioassay tests were used to compare toxicity responses for TCLP and water eluted samples from seven wastes and two Fullers soil positive controls. Conclusions from the study are as follows:

1. The TCLP (sodium acetate) eluent was highly toxic to S. capricornutum, and L. sativa, and moderately toxic to D. magna despite adjustments of its pH to 7 prevent pH shock to the test organisms. It was nontoxic to P. phosphoreum exposed for 30 minutes.
2. Toxicity of the TCLP eluent may confound interpretation of toxicity in some of the TCLP eluates, since it introduces an unpredictable toxic factor that cannot be corrected for in any simple manner.
3. Water elution is suggested as an environmentally relevant and meaningful procedure for measurements of leachable toxicity from soil and solid waste.

FOOTNOTES

¹Mention of trade names or commercial products does not constitute recommendation or endorsement by the EPA.

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THE USE OF MOSSES AS INDICATORS OF AIR POLLUTION

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ABSTRACT

Epiphytic moss possess certain properties that make them ideal specimens for assessing air pollution; they obtain their nutrients from the air and concentrate these pollutants. Among the pollutants they are known to collect are heavy metals, polyaromatic hydrocarbons, and chlorinated hydrocarbons. Recent studies have shown that pollutant concentrations in moss correlate with the atmospheric deposition of the pollutant over a time period, demonstrating that mosses are integrators of air pollutants. The measurement of pollutant concentrations in moss biomonitors is therefore a very convenient, simple, and inexpensive means of quantitating air pollution over a period of time. Some recent studies in the use of mosses to quantify levels of pollutants in air and map pollutant distributions will be reviewed with an eye to their utility in assessing air pollution from waste incinerators.

INTRODUCTION

Coupled with the increased reliance on resource recovery plants or incinerations to solve the solid waste crisis, is the need to insure the safety of these facilities. This requires knowing the nature and quantity of the pollutants they emit into the environment. Investigators have reported on various classes of these chemicals including polyaromatic hydrocarbons and their derivatives, polyhalogenated aromatics, and metals which may impact on health and the environment¹. If one is to insure the safety of waste incinerators, it is prudent to perform a certain amount of monitoring of both the ambient air and deposition in the vicinity of the plants.

Many of the pollutants of greatest concern emanating from waste-burning facilities are associated with particulate matter¹⁰. These chemicals may be deposited on soil or leaves and taken up by plants or may simply be adsorbed on the surface of the plant. The contaminated plants may then be ingested by grazing animals. Ingestion has been found to be the most important route of intake in humans for many of these substances²⁰ and dioxins bioconcentrated into mother's milk was the cause of a recent moratorium on resource recovery plants in Sweden. Traditional air monitoring is extremely expensive and time consuming to perform on a meaningful scale. In addition, air concentrations of pollutants such as trace metals, for example, do not correlate well with amounts deposited on land and taken up into the food chain³. The reason for this is apparent when one notes the importance of factors such as wind speed and surface

characteristics of the receptor on the amount of deposition³. While modeling stack emissions is a useful way of estimating environmental concentrations, these calculations must be validated and the results supplemented with actual measurements. Results of this modeling is often highly inaccurate for simple gaseous emissions² and modeling is far more difficult when chemicals adsorbed or partially adsorbed on particulates are included.

Mosses possess properties that make them ideal for monitoring concentrations of air pollutants emitted from waste incinerators, particularly heavy metals, polyaromatic hydrocarbons, and higher molecular weight halogenated chemicals. Accumulations of pollutants in moss better reflect the amount of the chemical deposited on vegetation and take up in the food chain than traditional air monitoring³. The development of moss bags has produced an element of flexibility and an enhanced ability for standardization that make the use of bryophytes extremely attractive for site monitoring⁴⁻⁶. Other recent experiments which show that the bioaccumulation of heavy metals and polyaromatic hydrocarbons in moss is highly correlated with dry and wet deposition^{7,8}, makes the use of these biomonitors even more attractive. It is our intention to review some of the key work and recent developments concerning the use of mosses for assessing aerial burdens of pollutants.

Mosses have been used as biomonitors of airborne metal and organic pollutants, primarily in Europe, for close to 20 years^{9,14}. The early studies primarily used indigenous mosses and showed that mosses were efficient indicators and integrators of aerial metal burdens. In one of these studies, Goodman and Roberts⁹ demonstrated that concentrations of the metals Zn, Pb, Cd, Ni, Cu, and Mg in the epiphytic moss, *Hypnum cupressiforme*, around Swansea in South Wales were indicative of local sources and wind direction and that the moss was much more efficient at accumulating metals than grass or soil. They also pioneered the use of transplants, moss on logs taken from uncontaminated sites, and moss bags, moss hung out in nylon mesh bags, as bioaccumulators. The results of the first study employing moss bags showed that elevated metals levels appeared 10 km upwind of Swansea, rose to about 4-20 times background in the city, and declined to background levels 25-30 km downwind of Swansea. It was also observed that mosses kept accumulating metals by a passive process after they died.

There are a number of properties that make moss so useful for monitoring aerial burdens of pollutants. Many species obtain all their nutrients from the air. Moss has an extremely high adsorptivity due to its extraordinary surface area and roughness and high cation exchange capacity. The bryophytes have no epidermis and cuticle¹¹. This makes the cell walls easily penetrable. Transport of nutrients between segments of new growth is poor due to lack of vascular tissue¹¹. In addition mosses have wide geographic

distribution and are extremely drought resistant. The strong adsorptivity for metals combined with lack of connectivity between new and old growth has enabled Lee and Tallis to show the historical development of the lead industry in England by plotting peat profiles¹².

As with all biomonitors, one has problems resulting from differences in accumulation between different species, individual plants depending on age and environmental factors, and parts of the plant^{11,17,21}. Problems may arise with indigenous bryophytes from lack of knowledge of the period of accumulation, from having mixed species, and from not having flexibility in placing samples. Accumulation of chemicals will depend on how exposed the moss is to the wind and rain whether it is beneath the leaf canopy where it collects throughfall of rain. Therefore it is best to standardize the procedure carefully and use composite samples of a single species for regional studies employing indigenous samples^{15,16}. This was done in Grodzinska's survey of heavy metal pollution in Polish National Parks¹⁵. She even separated the moss into brown and green parts, the green parts usually representing the latest 2 years, and the brown the previous three. Finally at very high exposure levels to more than one metal, the more strongly adsorbed metal may displace a less strongly bound one¹⁷. Fewer regional surveys of polyaromatic hydrocarbons and chlorinated hydrocarbons in mosses have been reported. Thomas reported on accumulations of some of these chemicals in *Hypnum cupressiforme* in Bavaria⁷ and Bacci et al. measured accumulations of chlorinated hydrocarbon insecticides and PCBs in moss in Antarctica²³.

By taking moss covered logs from unpolluted areas, one can overcome many of the drawbacks inherent in the use of indigenous moss. The transplants can be placed at desired locations around a site of interest and variability of the monitor due to environmental factors is reduced because they can be taken from the same location or even the same log. While mapping the PAH concentrations of mosses from 9 sites in Western Europe, Thomas⁸ noted that the ratio of fluoranthene to benzoperylene is 1.4 in an industrial area and rises to 6-10 in remote areas since a higher proportion of the fluoranthene is in the vapor phase and therefore transported further. Analogous regional differences in the ratios of α -BHC to γ -BHC were also noted and explained by the greater presence of B-BHC in agricultural areas. In using transplants, rather than indigenous moss, exposure time can be controlled. Pilegaard used transplants of *Dicranoweisia cirrata* to study the relation between the accumulation of nine heavy metals with exposure time and fallout from the atmosphere¹⁹. Accumulations in moss and fallout, both wet deposition and dry fallout, were measured at 12 stations around a steelworks and regression equations and correlation coefficients determined after seven months of exposure. Good correlations were obtained (correlation coefficients between 0.816 and 0.985) for all

metals but Vanadium. Thomas was able to establish a good multiple regression equations relating the concentrations of the metals Zn, Cd, and Pb and the polyaromatic hydrocarbons, fluoranthrene, benzo(a)pyrene, and benzoperylene in *Hypnum cupressiforme* with their respective concentrations in rainwater, atmospheric particulate matter and the amount of precipitation^{7,8}. On the other hand, chlorinated hydrocarbons did not give good correlations and it was suggested that a high exchange rate between the plant and air prevented long-term accumulation by the moss. The results clearly show that the accumulation of PAHs is most strongly correlated with the concentrations in atmospheric dust, while concentrations in dust and bulk precipitation contribute equally to the accumulation of metals.

While the use of transplants has enabled researchers to control sample variability to a large extent and have flexibility in sample placement, moss bags allow for even greater standardization and improvement in technique. Sphagnum moss soon became the bryophyte of choice for use in moss bags. Sphagnum moss is abundant and its high absorbancy and cation exchange capacity are well known and have long been put to practical use. One can be selective and choose similar specimens for an entire project since even the height above the water table affects (increases) the cation exchange capacity of the moss²². When used to monitor aerial metal pollution, the plants can be soaked in acid to replace any bound metal and assure a low background level⁴. An additional benefit is that there is no need to separate the moss from a log or rock and remove attached debris before analysis. Gailey and Lloyd⁴ performed a detailed study at Armadale, a town in central Scotland containing a foundry. Spherical nylon net bags containing sphagnum moss were hung at 47 locations around the town for 8 two month periods after which the bags were analyzed for seven heavy metals. Results revealed two main areas of metal deposition in the town, areas where clusters of lung cancer cases had been found. One area was north of the foundry at which the high concentrations of metal were due to local topography and wind. Mapping enabled gradients to be seen and correlation between concentration gave testimony to a common source. This type of study would enable good validation of modeling studies. The cost of materials for the 17 month survey was less than \$500.

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USE OF TRADESCANTIA FOR TOXICITY TESTING OF HAZARDOUS WASTE

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INTRODUCTION

The current output of solid waste increases the need for a variety of rapid, cost-effective and accurate bioassays for toxicity. Traditionally, animals have been used for most bioassay, although bacteria, such as the Ames test, have also become important. By contrast, plant bioassay, particularly higher plants, are lamentably underdeveloped and underexploited. There are several plant systems, e.g., seed germination and early seedling growth tests, and duck weed and fresh water and marine algal tests, but compared to animal test the number of systems is small. Several higher plant bioassays show potential and one in particular, the spiderwort Tradescantia, is immediately applicable. Tradescantia can be used as a system capable of the integrative monitoring one at a time or any combination of air, water and soil contamination associated with solid waste as well as the gaseous, liquid and solid phases of solid waste. It is readily used both in the laboratory and in situ in the field under the complexity of real world conditions and for acute and chronic testing area for periods of exposure of minutes to months. A variety of biological effects including genotoxicity are measurable: five aspects of perturbations of electron transport in photosynthesis; chromosome breakage in pollen mother cells and the production of micronuclei; somatic stamen hair mutations; sister chromated exchange in root tips; and growth as flower production. Stomatal conductance and CO₂ production in photosynthesis and pollen abortion are under investigation. Currently, the main assays are the stamen hair test and micronucleus test.

Tradescantia may qualify as a sentinel species, that is, a species which can be used as an indicator of ecosystem health. Even though it is not a normal resident species its wide applicability to assessing air, water and soil contamination, its applicability in situ to a large variety of ecological situations as well as its use in the laboratory and the variety of biological end points qualify it for consideration.

The stamen hair mutation system of Tradescantia was initially used in radiation studies (Sparrow et al., 1972; Underbrink et al., 1973; Nauman et al., 1975) and exposure to conditions in Biosatellite II (Sparrow et al., 1971). Subsequently, the stamen hair system was used for the testing of chemicals (Sparrow et al., 1974; Underbrink et al., 1973; Sparrow et al., 1976; Nauman et al., 1976). The pollen mother cell micronucleus assay was developed by Ma (1979) and

then applied to the testing of over 140 agents (Ma et al., 1976; Ma et al., 1978; Ma et al., 1984). Both systems have also been used for in situ field testing for integration of the effects of pollution variously ranging in time for hours to over five months to assess ambient air pollution at truck stops around cities and petrochemical complexes, soil and air contaminants of a lead smelter and an oil refinery complex, military obscurant smokes, water pollution from drinking water reservoirs, water from shallow and deep water wells, sewage sludges, cooling tower water, bottom sediments from a drinking water reservoir, ambient environment at a nuclear facility, airports, coal-burning residential district in the Peoples Republic of China and a foundry complex (Ma et al., 1983a; Ma et al., 1983b; Lower et al., 1978; Lower et al., 1983a; Lower et al., 1983b; Lower et al., 1985; Schaeffer et al., 1986; Sandhu and Lower, 1987).

DESCRIPTION OF THE TRADESCANTIA BIOASSAY SYSTEM

The Tradescantia tests differ in their sensitivity and one or more of these tests may differentially recognized particular physiologically hazardous or mutagenic agents that might go unregistered in the others. This aspect is currently under consideration. The use of the combined test may also permit accurate monitoring over a wide range of concentrations and various effects of toxic substances, particularly complex mixtures.

Tradescantia is particularly useful because of its versatility. Either cuttings or entire rooted plants may be exposed to gaseous agents in air or liquids, or grown in contaminated soils, and grown hydroponically in or watered with aqueous solutions of single chemicals or complex pollutants. Unrooted cuttings may be exposed for periods of minutes and hours or up to 2 weeks and rooted plants may be exposed for months if provision is made for suitable light and temperature conditions.

STAMEN HAIR TEST

Tradescantia has been used for radiobiological cytogenetic studies for over twenty-five years. However, in the mid 1960's it was found that its array of long filamentous chains of contiguous, single cells growing from the stamens (stamen hairs) presented a plant structure which could be used as a sensitive and cost efficient test of chemical mutagens (Underbrink et al., 1973).

The average number of stamen hairs per flower, the denominator of mutation frequency, is routinely determined at the beginning of the scoring period. A total of 36 stamens from six randomly picked flowers are counted for each experiment. The average number of stamen hairs per flower ranges between 300-400. Each inflorescence (flower cluster) produces about 18-20 flowers; one blooms about

every other day and the mutations appear as a phenotypic color change from the dominant blue to the recessive pink in cells of the stamen hairs of flowers of clones heterozygous for flower color. The mutation frequency is expressed as the number of mutant events per thousand stamen hairs.

In short term experiments scoring of stamen hairs for pink mutant events usually begins 5 to 7 days after treatment and continues daily until approximately 15 days after treatment, or until the number of mutations declines from any evident peak. The time from treatment to scoring constitutes time for development of buds from sensitive stages at which they are exposed to maturity when they exhibit the mutation events in the stamen hairs. If the plants are allowed to remain in a contaminated area for long periods of time, the scoring is done daily until the mutation frequency reaches a stable plateau or as long as the experiment dictates.

The sample size can be pre-determined and depends on the anticipated strength of the mutagen and percent standard error deemed acceptable (Underbrink et al., 1973; Underbrink and Sparrow, 1974). Mutagenicity studies using this test have been carried out routinely in situ in the field and in the laboratory with agents either in the gaseous or liquid state (Lower et al., 1978; 1983a; 1983b; 1984; Nauman et al., 1979; Schairer et al., 1979).

MICRONUCLEUS TEST

The micronucleus test of Tradescantia was developed in 1976 and is 6 to 8 times more sensitive than the stamen hair test (Ma. 1983). The mutagenic end point is the induction of chromosome breaks or lagging chromosomes which appear as micronuclei and are scored during the tetrad stage of meiosis in pollen development. After acetocarmine staining, the micronuclei appear as small dark red bodies in the pink cytoplasm and are easily distinguished from the much larger, darker red nucleus.

The preparation of inflorescences for scoring of micronuclei involves the following: After termination of the treatment period, cuttings are placed in aerated 1/2 strength Hoagland's nutrient solution in a controlled-environment chamber under a light regimen of 16 hrs light/8 hrs dark for 24-32 hours, which is the time necessary for the chromosomes damaged during early prophase of meiosis to progress to the tetrad stage of pollen formation (Ma. 1983). Flower buds are then fixed in ethanolacetic acid (3:1 ratio) for 4 to 30 hours, and transferred to 70 percent ethanol for storage.

For scoring, individual flower buds are removed from the inflorescences which have been stored in alcohol and the buds likely to contain the tetrad stage cells are selected based on bud size and position on the inflorescence. The anthers are dissected out of the

bud, macerated, and stained with acetocarmine. Tetrads are scored at 400 X magnification. Approximately 300 tetrads are scored from each bud, and from 5 to 10 buds are scored for each experimental and control point.

The micronucleus test has been used as a method of estimating mutagenesis resulting from chromosome breakage in investigations of the mutagenic effects of a wide variety of environmental pollutants in water reservoirs, parking garages, truck stops, diesel exhaust, sewage sludge, cooling tower water, etc. (Clevenger, et al., 1983; Ma, 1983; Ma et al., 1983; Lower et al., 1984; Schaeffer et al., 1986).

PERTURBATIONS OF THE ELECTRON TRANSPORT OF PHOTOSYNTHESIS

Room temperature chlorophyll fluorescence in vivo is a measure of oxidation-reduction state of the photosystem II primary acceptor (Q). Segments of leaves which are illuminated with a specific wavelength of light will fluoresce a range of wavelengths of light in response. Rapid changes in the yield of chlorophyll fluorescence occur within the first 15-30 sec. of illumination in all photosynthetic plants (Kautsky effect). This chlorophyll fluorescence induction can be used as an indicator of photosynthetic energy conversion and has been important in the characterization of photosynthetic reaction mechanisms.

The fluorescence induction curve (a plot of fluorescence against time for the period immediately following onset of illumination) is modified by many factors which affect photosynthesis. Present understanding of the various fluorescence transients allows the fluorescence induction curve to be used as an immediate and reliable test of photosynthetic activity. Variable fluorescence yield can be increased if electron transport is stimulated, or decreased if electron transport is inhibited depending upon where a given chemical produces a lesion in the complex chain of biochemical events prior to and including the photolysis of water in the Hill reaction.

Perturbations in electron flow are measured with a plant fluorometer, a portable AC/DC operated unit suitable for measurements in the field as well as the laboratory. It is connected to an AC/DC strip chart recorder. At the end of an exposure or treatment, samples of leaf material are collected and placed in plastic bags with moist cotton and dark-adapted for at least 15 minutes. A 2 cm segment of leaf is placed in a leaf holder. The fluorometer probe is inserted on top of the leaf segment, the dark adapted leaf segment is illuminated at 670 nm and simultaneous fluorescence at wavelengths greater than 710 nm is detected and recorded on the strip chart over 10 or more seconds (Schreiber et al., 1978). For each data point at least 10 strip

chart recordings are made from 10 samples of leaf material and measurements are averaged to determine percent increase or decrease in variable fluorescence.

Changes in the electron transport system have been observed in studies of cooling tower water (Lower et al., 1985) and military obscurant smokes used as smoke screen (Schaeffer et al., 1986).

GAS EXCHANGE IN PHOTOSYNTHESIS

The measurement of photosynthetic rate as CO₂ evolution and stomatal conductance is commonly applied to obtain information on growth of plants and plant gas exchange research. The same technique can be applied to plants exposed to toxic materials in both the laboratory and in situ in the field as an adjunct to the measurement of the electron transport systems of photosynthesis. No known systematic investigation has to date been done with this in reference to solid waste.

Both net photosynthetic rate calculated from net CO₂ exchange between a leaf and the atmosphere and stomatal resistance calculated from measured transpiration rate and leaf and air temperature can be determined in both the laboratory and field using a portable instrument developed for that purpose.

GROWTH AS FLOWER PRODUCTION

An inflorescence of Tradescantia contains 18 or more flower buds under continuous development which produce, on the average, a flower every second day. This continuous production of flowers can be used as a measurement of growth. Flower production has been used as an indicator with exposure of Tradescantia to obscurant smoke (Schaeffer et al., 1986).

Pollen Abortion

Pollen abortion normally occurs to some degree in plants, has both physiological and genetic causes often difficult to differentiate under many test conditions, but has potential as a measurement of toxicity. Anthers are placed in a drop of lacto phenol cotton blue stain and mascerated with forceps. The debris is removed, a cover slip is placed over the stained pollen and the non-aborted blue pollen and aborted empty pollen cells are counted under high dry 400X magnification with a compound microscope.

Examples of Use of Tradescantia

Tradescantia may be used in a variety of ways. With chemicals assayed in the laboratory the cut flower stalks are placed in an aqueous solution of the test material or exposed in a chamber if a

gas or an aerosol is tested. Examples are presented on the use of Tradescantia in a variety of situations involving complex mixtures.

FREQUENCY OF STAMEN HAIR MUTATIONS OF TRADESCANTIA GROWN IN A GREEN HOUSE IN BOTTOM SEDIMENT OF A DRINKING WATER RESERVOIR (FREQUENCY \pm S.E.) $\times 10^{-3}$

<u>Time Period</u>	<u>Sampling Period</u>	<u>Control</u>	<u>Sediment</u>	Ratio of Mutation Frequency of Sediment/Control	<u>p Value*</u>
1	09/11-11/19	3.2 \pm 0.3	5.6 \pm 0.3	1.7	0.005
2	11/20-12/03	2.2 \pm 0.1	4.2 \pm 0.2	1.9	0.005
3	12/04-12/17	2.8 \pm 0.2	3.6 \pm 0.2	1.3	0.05
4	12/18-01/07	2.1 \pm 0.1	3.3 \pm 0.1	1.6	0.005
5	01/08-02/05	2.5 \pm 0.3	3.1 \pm 0.2	1.2	0.05

*p Value of Two Tailed Approximation to Binomial

Bottom sediment from a water reservoir was removed and placed in untreated wooden boxes in a greenhouse. Tradescantia were rooted in the sediment and the stamen hair mutation frequencies were determined beginning approximately one month after planting. Controls were grown in soil. The mutation frequencies were continuously determined over the time of 149 days. The data are grouped for presentation.

The mutation frequency of Tradescantia grown in sediment is consistently higher than the controls during the five month time period. There is also a continual decrease in the mutation frequency of the Tradescantia in the sediment with $r^2=0.589$ and $b=0.59$ as the mutagenicity of the sediment diminishes.

FREQUENCY OF STAMEN MUTATIONS OF TRADESCANTIA FROM SEPTEMBER 30 TO OCTOBER 10 AT LOCATIONS MEASURED FROM A LEAD SMELTER (FREQUENCY \pm SE) $\times 10^{-2}$

<u>Location (km)</u>	<u>Mutation Frequency</u>
0.3	4.9 ± 0.4
1.7	4.8 ± 0.4
3.2	4.4 ± 0.3
11.4 (Local Control)	3.5 ± 0.3
Laboratory Control	2.8 ± 0.3

Regression with distance of 0.3, 1.7, 3.2 and 11.4 km

$$b = -0.125$$

$$r^2 = 0.974$$

Tradescantia were planted as whole rooted plants in situ in the native soil at the various distances down wind from the lead smelter. All old flower stalks were removed and the new flower stalks and inflorescences developed. The plants were in place May 29 to October 10. These data are part of a larger study, but illustrate the in situ use of Tradescantia with significant changes in stamen hair mutations with distance from the smelter.

FREQUENCIES OF MICRONUCLEI OF TRADESCANTIA EXPOSED TO SEWAGE SLUDGES FROM FIVE DIFFERENT COMMUNITIES ($\times 10^{-2}$)

<u>Source</u>	<u>0% Sludge</u>	<u>25% Sludge</u>	<u>50% Sludge</u>	<u>p Value of Regression</u>
1	3.0	3.6	7.6	0.005
2	3.0	4.1	11.5	0.01
3	3.0	8.3	13.5	0.005
4	3.0	8.9	32.0	0.001
5	3.0	14.0	15.0	0.005

Sewage sludges from five different municipalities were diluted and mixed with reverse osmosed-deionized water to concentrations of 25% and 50% sludge. Control was reverse osmosis-deionized water. Tradescantia flower stalks were cut and the flower stalks were

placed upright in the control water and sewage sludges for 24 hours in a growth chamber under 16h light:8h dark. At the end of the exposure the distal tips of the flower stalks were trimmed off and the stalks were placed in reverse osmosis-deionized water for another 24 hours under 16h light:8h dark. The inflorescences were then removed, pickled and processed for the determination of micronuclei. The cut flower stalks negate the root barrier and may allow any materials that can be translocated by the vascular system of the plant to be transported to the target tissues or cells of interest.

The sludges all show mutagenicity and exhibit regression coefficient significantly different from zero.

TRADESCANTIA EXPOSED TO DIESEL OBSCURANT SMOKE
GENERATED BY ARMY TANK

<u>Distance (M)</u>	<u>MCN</u>	<u>SH</u>	<u>VF</u>	<u>EP</u>	<u>FP</u>
15	40.2	3.6	33.2	10.4	NS
25	29.8	3.6	33.2	9.6	126.9
50	NS	2.7	34.1	10.2	68.4
Control	30.7	0.9	26.8	6.8	100.0

MCN = micronuclei $\times 10^{-2}$
 SH = stamen hair pink enents $\times 10^{-3}$
 VF = variable fluorescence
 EP = electron pool
 FP = flower production
 NS = not statistically significant at $p \geq 0.10$

Cut flower stalks with attached leaves were exposed in the Southern California desert to diesel oil obscurant smoke used in military maneuvers. The diesel is aerosolized into screening-smoke by an attachment on the combat tank's engine manifold. The flower stalks were exposed for 30 min. at distances of 15, 25, 50 meters - and further distances not reported on here - and measurements taken of five biological end points. All end points showed some response to the obscurant smoke, but only the frequencies of micronuclei (MCN) and stamen hair mutations (SH) showed a gradient with distance.

CONCLUSION

Tradescantia has many appealing properties that qualify it as an organism as part of a repertoire systems for wide use in the toxicity assessment of solid waste. Other plants with a relatively

modest amount of additional development and appropriate standardization offer great potential for solid waste toxicity assessment both in the laboratory and in the field. Examples are: Selenastrum, which has been developed for water pollution, but is being developed and tested for soil contamination; Arabidopsis, a small mustard, with a data base of over 200 chemicals tested; seed germination/root elongation and early seedling growth of a number of species of plants including tomato rye, oats, cucumber, etc.; corn, used for both laboratory and field testing; barley, laboratory tested for more than 60 agents. To date, however, Tradescantia offers the most versatility for bioassay.

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STATISTICAL APPROACHES TO SCREENING HAZARDOUS WASTE SITES FOR TOXICITY

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ABSTRACT

The kinds of questions bioassays can answer, as well as their advantages, are given. The ability to answer questions, such as "where is the waste?" or "is it toxic?" results from field studies based on twelve procedural steps for conducting remedial action field bioassay studies. Simple or stratified random, systematic, and judgment sampling are presented as three possible paradigms for field sampling.

Bioassay results from two field studies are used to illustrate how maps of toxicity can be prepared based on systematic sampling and to show how cleanup decisions can be made using bioassay results based on few samples.

In the first study, logarithmically spaced soil samples (0-15 and 15-30 cm depths) were obtained along four parallel transects (90 m long and 15 m apart) at the Rocky Mountain Arsenal. A total of 72 soil samples (36 at each of two depths) were subjected to phytoassay using lettuce seeds; most samples were also subjected to Daphnia, Microtox, algal, earthworm and lettuce root elongation bioassays. These latter bioassay results (exception earthworms) were inconclusive regarding toxicity, but allowed us to ignore several classes of compounds (i.e., water-soluble heavy metals, herbicides, and insecticides) since our prior results using pure chemicals showed depressed algal growth in the presence of these contaminants.

In order to depict the spatial pattern of observed seed mortality at each depth, we used kriging (a statistical technique developed for use in the mining industry) to produce contour maps. The results clearly showed that lettuce seed mortality was higher in the 15-30 cm fraction, that waste-trench soil was highly phytotoxic, and that toxicity decreased as a function of distance from the trench. In addition, we found that mortality contours produced by kriging could be useful in site cleanup decisions.

The second study was conducted using a series of water and sediment samples collected from a narrow stream adjacent to a wood treatment plant in Canton, Mississippi. Both creosote and pentachlorophenol were used for wood treatment. Sediment samples (15 cm) were collected every 20 m in the visibly contaminated zones. Based on sample linear interpolation of bioassay results, we found that different bioassays led to different conclusions regarding the

toxicity of different areas, suggesting that contaminants other than creosote may have caused the observed toxicity. Moreover, chemical analysis was an inaccurate predictor of toxicity.

INTRODUCTION

In the context of hazardous chemical waste site management, bioassays may be defined as the exposure of biological indicators to field-collected environmental samples in order to detect the presence of toxicity and/or to identify potential for toxic effects on resident species. Typically, a hazardous waste site bioassay involves laboratory testing of soil, soil leachates, water, or sediment samples, using a standard array of test organisms under controlled laboratory conditions.

Bioassay studies are appropriate before, during, and after remedial action as a cost-effective way to detect the presence of toxic wastes and/or to determine their biological availability at known or suspected hazardous chemical waste sites.

Bioassay studies may be used before remedial action to detect the presence of hazardous materials and to determine if immediate remedial action is needed. Because bioassays directly evaluate the effect of chemical wastes on biota, they are powerful and efficient tools for ranking sites requiring remedial attention. Bioassay data are also useful for gauging the areal extent of needed remedial action, evaluating remedial action alternatives, and assessing and characterizing waste sites.

During remedial action, bioassay studies may be used both to monitor the cleanup process and to evaluate cleanup impacts on the site. Bioassays are also useful after remedial action has been taken to evaluate the efficacy of the cleanup activities.

The questions that often need to be addressed in support of remedial action decisions may be grouped into four major areas: 1) Where are the contaminants? 2) Are the contaminants toxic? 3) What quantities of the contaminants are present? and 4) What are the contaminants?

Bioassay studies are a cost-effective method for evaluating "where" questions because they can detect contamination, determine contaminant distributions, and define contaminant migration beyond site boundaries. Bioassays are also currently the only means of evaluating the bioactivity of chemical wastes. However, bioassay studies are not generally useful in answering "how much" and "what" questions regarding hazardous chemical waste sites. Further, bioassay tests usually cannot identify the specific chemical contaminants present. However, they may give some indication of the class of chemicals to which the contaminants belong (e.g., organics,

metals). Some examples in which the identification of specific chemicals have been attempted are given Thomas et al. (1986) and Miller et al. (1985).

DESIGNING BIOASSAY STUDIES

Design of bioassay studies includes planning the collection of field samples and the laboratory analyses of those samples. It is imperative that the entire project, from objectives to expected results, be thoroughly planned before the actual fieldwork is started. Without proper planning, the study will waste both time and resources. Further, all individuals who will contribute to the project should be involved as early as possible in project planning. Personnel who should be involved at the initial stages of the project include the project manager, a statistician, a field biologist, a chemist, the scientist who will oversee the laboratory work, possibly a hydrologist, meteorologist, or modeler when appropriate, and risk assessment and Quality Assurance/Quality Control experts.

The steps to be used in designing and executing a bioassay study are listed below.

1. Assemble information relevant to the problem.
2. Prepare a statement of the study objectives.
3. Define the evaluation criteria and reliability requirements for the results.
4. Determine what is to be sampled in the field.
5. Choose test organisms for the bioassays.
6. Define the data analysis techniques.
7. Design the field sampling and laboratory studies.
8. Determine the sample collection methods.
9. Define the operational procedures.
10. Review the design.
11. Periodically evaluate progress in the field and laboratory.
12. Analyze and evaluate the results.

These steps are listed in the order in which they should be applied by the planning team. However, study design is an iterative

process, and decisions made at later steps in the process may require the review and revision of decisions made at previous steps.

These 12 steps should be considered whether the study is an initial site investigation, a feasibility study, or a full remedial action. The amount of effort and degree of professionalism required for each step will vary depending on the objectives and cost of the study and reliability required of the results. For example, in a full and potentially expensive remedial action investigation, the entire team should be involved from the beginning. During preliminary investigations, the objectives, uses of the results, and methods should be carefully planned before sampling; however, the advice of team members from each area of expertise may not be necessary. Depending on the quality of information gathered during the preliminary investigations, the results of the initial studies may be used to design the remedial action program.

SAMPLING STRATEGIES FOR COLLECTING FIELD SAMPLES

The three basic design strategies for collecting field samples are simple or stratified random, systematic, and judgment sampling. These strategies are described in Ford and Turina (1985) and are discussed here only as they apply to bioassay studies at hazardous chemical waste sites.

All remedial action decisions for the hazardous waste site will be based on the results obtained from samples collected on the site. Therefore, it is important that the samples obtained accurately represent the conditions on the site. The traditional approach to collecting a representative sample is to randomly select the sampling locations over the entire site with the aid of a random number table or similar device. This procedure is called simple random sampling and is an appropriate strategy when no prior information is available on the likely location or distribution of the contaminant.

With stratified random sampling, sampling sites are randomly chosen within several defined site areas or strata. Appropriate strata for a hazardous chemical waste site might be any division of areas in which it is anticipated that toxicity will differ; for example, areas at increasing distance from the known or suspected source of chemical contamination. Stratified random sampling is often a useful technique even if there is insufficient information available to identify distinct strata a priori, and is likely to produce a more widespread distribution of sampling locations on the site than will a simple random sampling strategy. The construction of arbitrary strata can allow the variability in toxicity between different areas of the site to be estimated and may help identify actual strata. For these reasons, stratified random sampling is a particularly useful approach for preliminary studies.

Systematic sampling involves the collection of samples at regular intervals over the site. This method is sometimes preferred to random sampling strategies because it ensures even coverage of the site. However, as Eberhardt and Thomas (1986) warn, a systematically distributed contaminant may not be detected when using this strategy. Systematic sampling (often in grids) is often the preferred method to provide input data for kriging and other mapping techniques.

Judgment sampling relies on the sampler's judgment of what constitutes a representative site sample. The purpose of these samples might be to assess the presence or absence of contaminants in obvious places (e.g., a streambed if transport is a concern), or for use in special studies of a preliminary nature (e.g., are toxic chemicals nearer the spill source?). However, judgment sampling is biased and such results should not be used in a statistical analysis. In addition, judgment samples can be collected along with samples from the designed study in the event that unusual or interesting circumstances arise or are discovered during sampling. A statistical evaluation of the data from the judgment samples can be used to suggest additional sampling or to make statements without accompanying error statements or probabilistic assertions. In cases where prior information about a possible spill location becomes available during sampling, that information should be used to advantage in the survey design. In fact, design modifications can be made onsite (e.g., an extra grid, transect, or stratum).

Locating field sampling sites in order to implement any of the sampling designs discussed is not a trivial matter. At least one-third of the field effort should be devoted to locating and marking the sampling sites. Each sample location should be accurately recorded to aid in the interpretation of the bioassay results, to accurately define areas in which remedial action may be necessary, and to permit return to any sampling site to collect necessary additional material.

FIELD STUDIES: ILLUSTRATIONS OF BIOASSAY METHODS AND DESIGN DECISIONS

The two field studies in this paper were selected to illustrate the steps in conducting a bioassay study, and the statistical principles involved. The studies were designed to evaluate toxicity and define areal extent, if toxicity was detected. At two New Jersey sites not discussed in this paper, extra samples were collected and some composited, [see Skalski and Thomas (1984) for a discussion of compositing]. Field sampling and the use of bioassay results from a systematically sampled grid at the Rocky Mountain Arsenal (Commerce City, Colorado) are illustrated in site 1, while the more usual case where inferences are based on a few bioassayed samples from the

basis for a discussion of results from site 2 [a wood treatment site (Canton, Mississippi)].

SAMPLING AT THE ROCKY MOUNTAIN ARSENAL

The Rocky Mountain Arsenal was used principally by the U.S. Army and the Shell Chemical Company to manufacture, test, and dispose of toxic chemicals. Certain areas of the 26-mile² (67.6-km²) arsenal have been contaminated by various spills during waste disposal operations. The site is surrounded by homes, farms, and businesses (e.g., Stapleton Airport, Commerce City, and Denver). In 1974 diisopropylmethylphosphonate (DIMP) and dicyclopentadiene (DCPD) were detected in the surface water draining from a manmade bog at the northern boundary of the arsenal.

Study objective 1 at the arsenal was to assess the toxicity of a trench site in Basin A (Figure 1). If one or more bioassays identified to devise a contour map useful for cleanup decisions (objectives 2). Finally, an assessment of contaminant mobility was needed (objective 3). To meet those objectives, a toxic site at the Rocky Mountain Arsenal had to be located and the most sensitive bioassay selected.

SITE CONTAMINATION

Decontamination wastes and process waste streams containing toxic materials consisting of salts, heavy metals, and pesticides were deposited in defined areas on the Rocky Mountain Arsenal. Two waste basins (A and F), were the major sites of waste material storage.

Certain portions of the arsenal were leased to private industry for chemical manufacturing. A major chemical company leased a considerable portion of the manufacturing facilities at the Rocky Mountain Arsenal since 1952. Alterations and additions were made to the facilities for the manufacture and disposal of waste residuals of GB, a chemical warfare agent, TX, a biological anticrop agent, and cyanogen chloride and phosgene. Since 1970, several major chemical demilitarization actions have been conducted. These actions include the incineration of both the anticrop agent TX and a mustard agent.

Because of the foregoing manufacturing and disposal actions, various analyses of chemicals in air, water, soil, and certain organisms have been conducted over the years. Table 1 contains a partial list of some of the chemicals identified.

DESIGN OF THE FIELD SAMPLING AND LABORATORY STUDIES

Previous results indicated that an area in Basin A was toxic (objective 1) and would be useful for addressing the second study

objective. Soils from the Basin A location caused a major reduction in lettuce seed germination (insoluble compounds were likely causative) and had a variable effect on other bioassays (little likelihood of soluble compounds; objective 3). Because of these results and, in part, because it appeared that plant growth diminished with distance from the trench, a sampling location was established near the trench in Basin A (to meet objective 2).

The results also suggested a possible gradient of contamination on the west side of Section 36, extending north-south from a trench that drains Basin A and runs to the west. This possibility of a toxicity gradient offered good prospects for the kriging method of preparing contour maps because it appeared that a required, physical

TABLE 1. Examples of Some Chemicals Found In Soils, Air, Water, Animals, and Plants at the Rocky Mountain Arsenal

Aldrin	Methylphosphonic acid
Arsenic Compounds	Isopropyl methylphosphonate
Benzene	Diisopropyl methylphosphonate
Chlordane	Dicyclopentadiene
Chloroform	p-Chlorophenyl methyl sulfone
Dieldrin	Hexachloronorboradiene
Endrin	Tetrachloroethylene
Lewisite	1,4-Thioxane
Lewisite oxide	Methylene chloride
Mercury salts	Toluene
Mustard gas (HD)	Xylene
Thiodiglycol	

mechanism of toxicant dispersal (Journal 1984) for valid error predictions was a reasonable assumption. Thus, four parallel transects were established on the west side of Basin A, each beginning on the north bank of the trench and running south for 90 m (Figure 2). A logarithmic scale was used beyond the south trench edge because it was assumed that contamination might have been moved by some physical means (e.g., wind or water). The transects were 15 m apart and labeled L, M, N, and P. The first three sample points of each transect fell within the trench and the fourth was on top of the south bank. Sample numbers 5 through 9 were 15, 20, 30, 50, and 90 m, respectively, south of the north trench edge (Figure 2). Each of the 36 sampling points was marked with a stake.

A drilling company was hired to do most of the soil sampling. At each sampling point a split spoon was used to take two 7.6-cm-diameter soil cores. One core was taken from a 0- to 15-cm depth, and the second was taken from a 15- to 30-cm depth. Together, these cores weighed approximately 4 kg. Between sampling points the split spoon and drill bit were decontaminated by washing

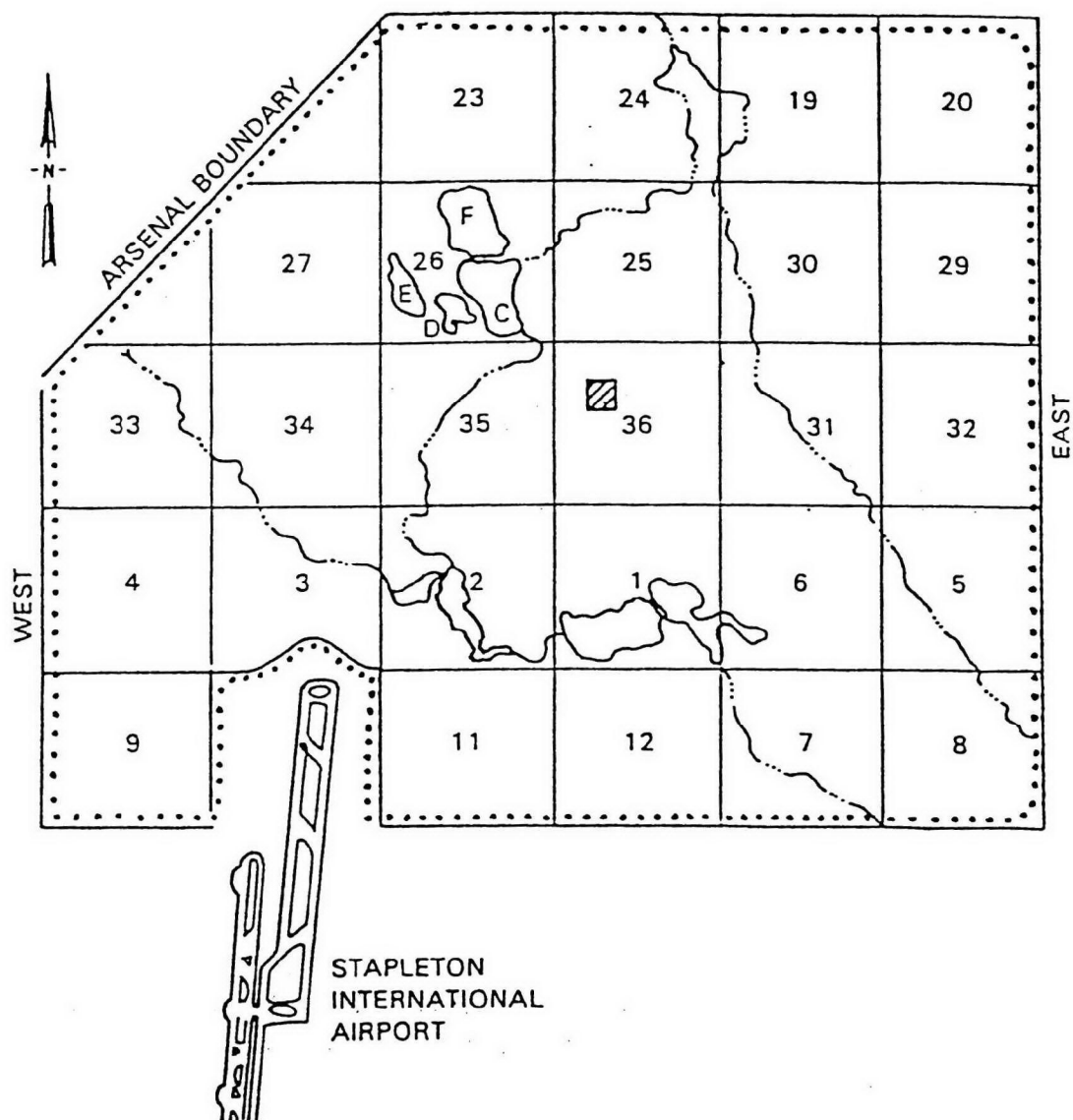


FIGURE 1. Location of the Study Site in Basin A (which includes most of Section 36) at the Rocky Mountain Arsenal. The areas in Section 26 labeled C, D, E, and F are or were waste ponds.

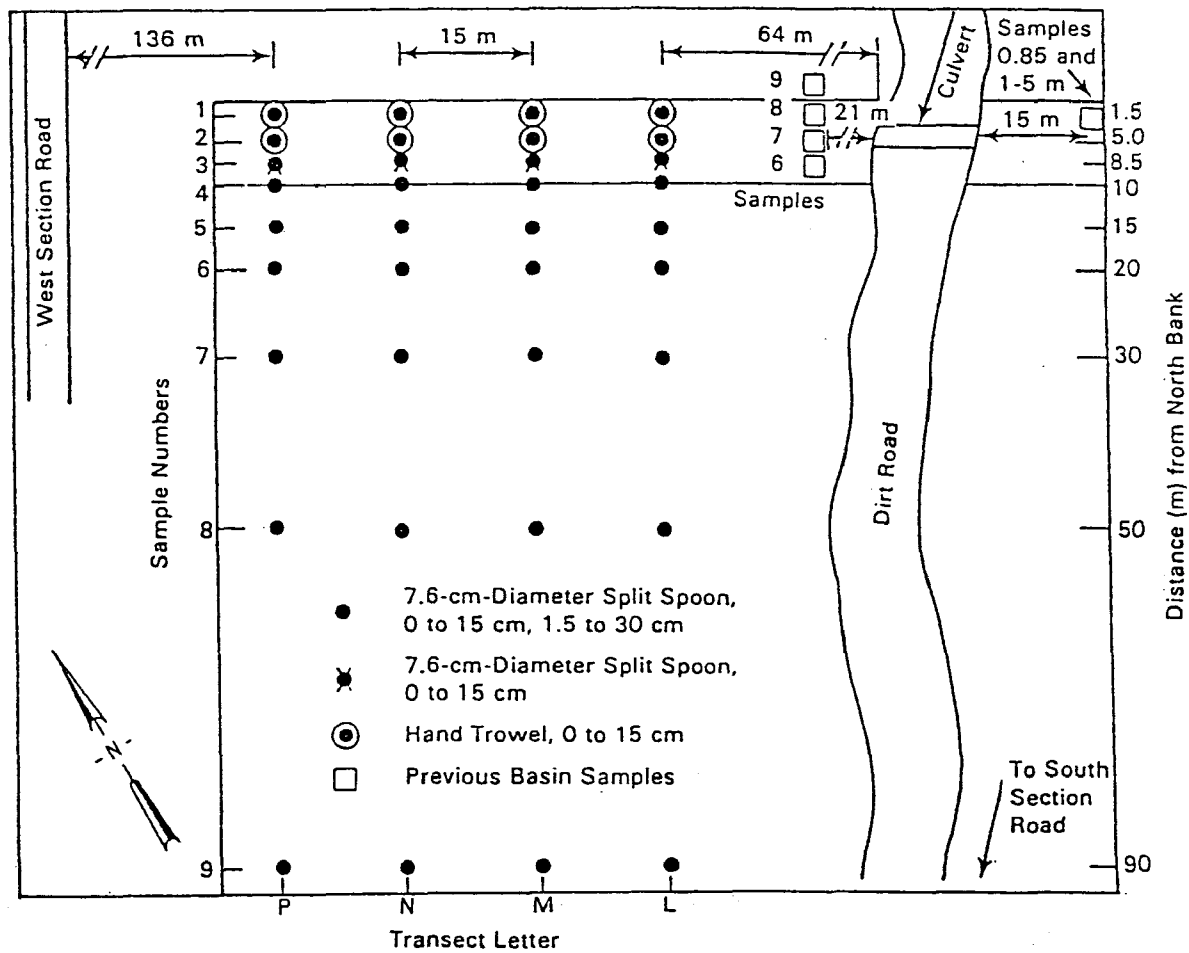


FIGURE 2. Location of Logarithmic Sampling Points in Basin A (Section 36) at the Rocky Mountain Arsenal

with methanol and rinsing with distilled water. All samples were put in plastic bags, sealed, and labeled. The area being sampled and any problems encountered (e.g., mud, accessibility) dictated exactly how the cores were taken and the variations on the basic sampling scheme (see below).

In Basin A, the first two points in each transect were in the trench, which was very wet and soft. The samples from these points were difficult to obtain. It was impossible to sample by depth, so a hand trowel was used to take two surface samples (to approximately 15 cm deep) from these points. The split spoon was used to take most of the other samples in Basin A (points 4 through 9 in transects L, M, N, and P). Sample points L, N, and P-3 were just over the south bank of the trench and could not be reached from the drill rig. At these points, the split spoon was hammered into the ground and extracted by hand. Only a 0- to 15-cm sample was obtained from each of these points; the soil from 15 to 30 cm deep was too wet to stay in the split spoon. A surface sample of undefined depth was taken from the M-3 transect because the entire profile was very wet.

Analysis and Evaluation of the Results

Lettuce seeds were used in the bioassays of the Rocky Mountain Arsenal field study soils, because they are more sensitive than other seeds (Thomas et al. 1986) and our previous work has shown these soils to be phytotoxic. The mortalities (mean from three subsamples from each core fraction; Figures 3 and 4) indicate that four samples in transect M and three in transect P showed large differences as a function of depth, suggesting that the contaminants had either migrated below 15 cm or were purposely placed there. We found no record to support the latter argument and concluded that the toxic material had migrated.

The results for all other bioassays (Porcella 1983) conducted using Basin A field study soils were inconclusive. No mortality was observed using the *Daphnia* or *Microtox* bioassays. Results from the algal bioassay revealed that small quantities of elutriate from all but four samples were stimulatory. The four elutriate samples that inhibited algae were obtained on or very near the waste trench on transects L and N. According to the criteria outlined in Porcella (1983), these sites would be classified as moderately toxic. Interestingly, only a 1% to 14% elutriate from transect M samples was needed to stimulate algal growth. Because there was little effect on the algal bioassay, it appears that the toxic components detected using lettuce seeds (see Figures 3 and 4) were not water-soluble heavy metals, herbicides, or insecticides (except perhaps at sites L-2, L-4, and N-2), since results using pure chemicals (Miller et al. 1985) showed depressed algal growth in the presence of these contaminants. This evidence suggests the presence

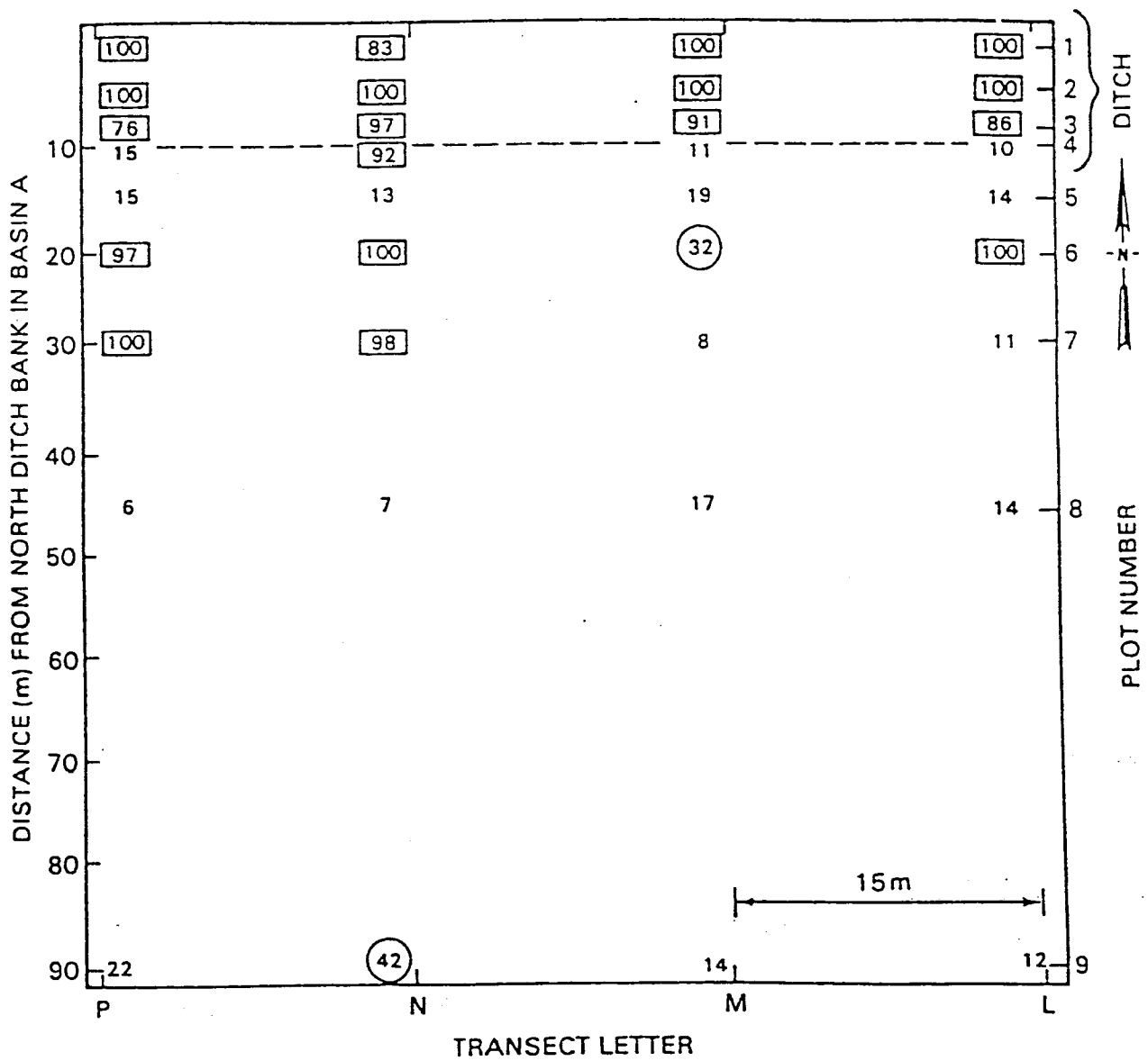


FIGURE 3. Observed Mean Lettuce Seed Mortality at Each Basin A Plot, 0- to 15-cm Soil Fraction. Means enclosed with a box are >75%, an enclosing circle indicates means from 30% to 75%, and numbers with no symbol are means <30%.

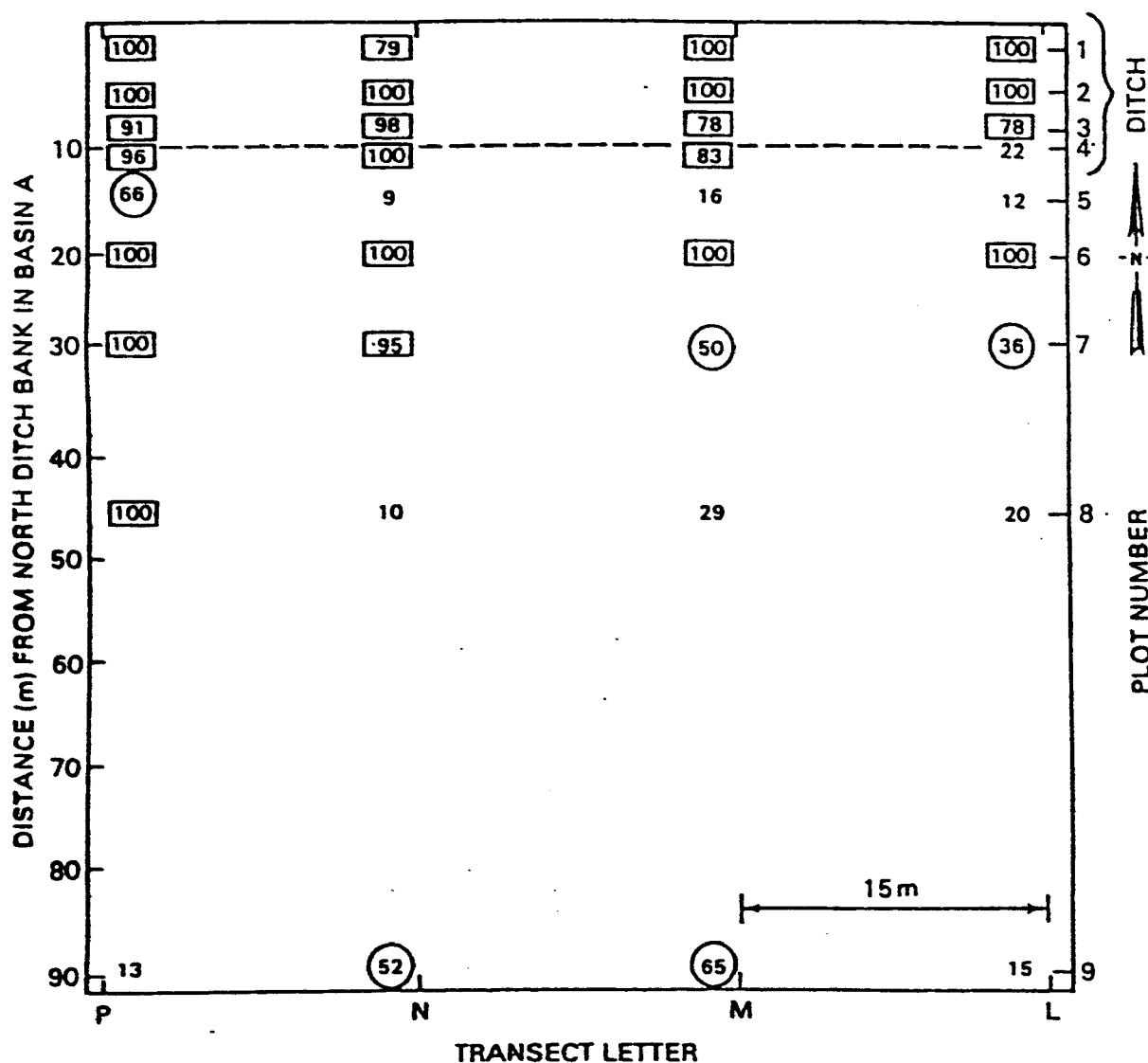


FIGURE 4. Observed Mean Lettuce Seed Mortality at Each Basin A Plot, 15- to 30-cm Soil Fraction. Means enclosed with a box are >75%, an enclosing circle indicates means from 30% to 75%, and numbers with no symbol are means <30%.

of water-insoluble contaminants that are not likely to migrate (objective 3).

Except for plot P-3, earthworms were only affected by Basin A soil when lettuce seed mortalities were greater than 70%. In contrast, the five soil samples that caused 20% to 70% lettuce seed mortality resulted in no earthworm deaths. Thus, for these Basin A samples, it appears that lettuce seeds are more sensitive to lower levels of an insoluble toxic component than are earthworms (objective 3).

Results from the lettuce root elongation (based on elutriates) and the lettuce seed mortality (based on intact soil) bioassays show only a slight correspondence for samples from transect L. It appears that the phytotoxic component that impairs lettuce seed germination may not be water soluble (objective 3) or does not affect root elongation.

Cleanup Decisions Based on Bioassays and Kriging

One way to depict the lettuce seed mortality patterns observed at each depth (see Figures 3 and 4) is to prepare a contour map based on the observations (objective 2). We estimated contours for a map of lettuce seed mortality using a relatively new statistical technique called kriging, which was developed for use in the mining industry and is used principally in Europe and South Africa (Clarke 1979). Kriging provides a variance estimate that can be used to construct a confidence interval for the true value. Results based on block kriging are presented in Figures 5 and 6. The results clearly show the lettuce seed mortality differences at the two depths. Estimated contamination is greater from 15 to 30 cm deep than from 0 to 15 cm deep. This contamination difference was also indicated by the qualitative analyses of results (see Figures 3 and 4)

Contour maps are useful for making site cleanup decisions. As a scenario, we selected 30% lettuce mortality as a criterion for cleanup of the Basin A site (see Figures 5 and 6). In the absence of any other guidance, the cleanup criterion was selected as two standard deviations above the mean control mortality (i.e., 16.7 ± 14.0 , $n = 6$). The shaded areas below the heavy solid black lines would be targeted for cleanup. The cleanup decision would be different for the 0- to 15-cm-deep (Figure 5) and the 15- to 30-cm-deep fractions (Figure 6). While this difference complicates decision making, the available data and the kriging maps show that the field situation is complex, and cleanup decisions based solely on either soil fraction would not result in a "clean" site. Cleanup using the 30% mortality contour of the 15- to 30-cm samples would remove all known contamination, but additional samples taken below this depth would be needed to ensure that the site meets the 30% mortality cleanup criterion.

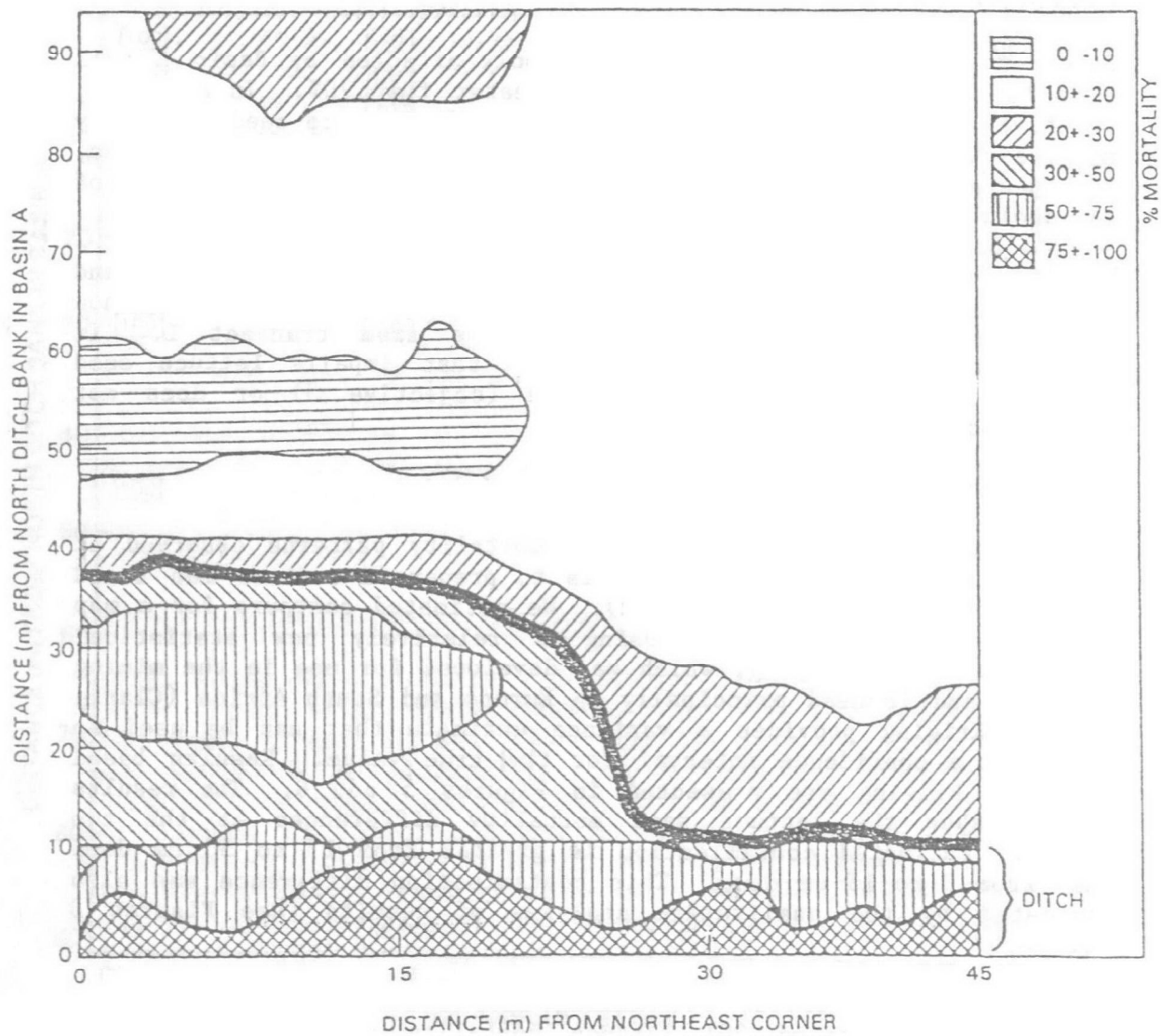


FIGURE 5. Estimated Lettuce Seed Mortality (based on kriging) for the 0- to 15-cm Soil Fraction from the Rocky Mountain Arsenal

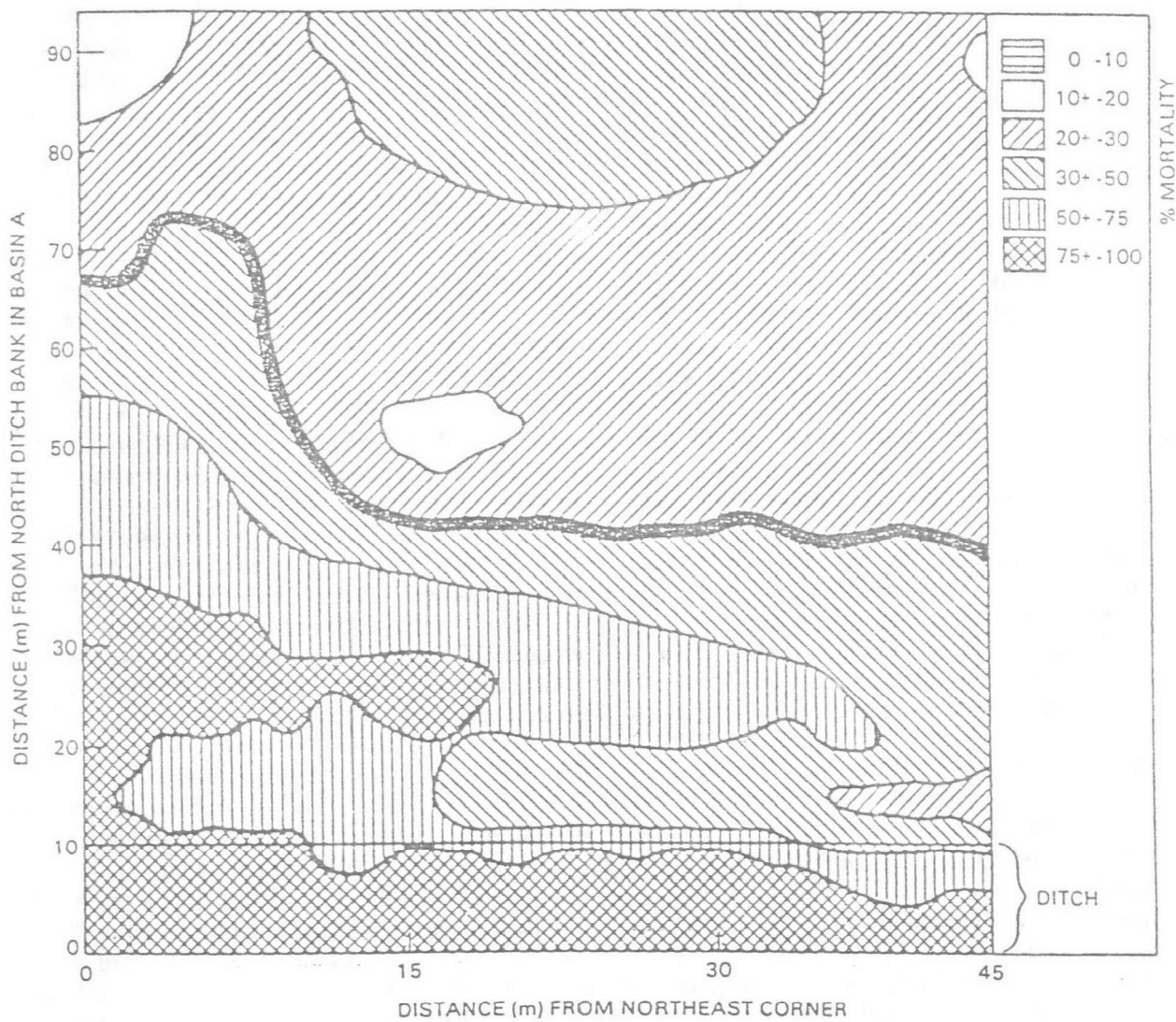


FIGURE 6. Estimated Lettuce Seed Mortality (based on kriging) for the 15- to 30-cm Soil Fraction from the Rocky Mountain Arsenal

It appears that bioassays of field samples and subsequent kriging analyses (objective 2) offer a practical method to aid in cleanup decisions based on environmental toxicity, especially when accompanied by error estimates for the mortality isopleths. We did not present confidence limits here because of some questions about the possibility that the observed toxicity was caused by pollutants that were "dumped" rather than spread from the trench by wind or water. Journal (1984) argues that the confidence limits may be invalid unless movement is caused by physical forces. The limits in our study average between $\pm 10\%$ and 25%, and depended on data density, whether block or point kriging was used, and the contour of concern.

SAMPLING AT A WOOD TREATMENT SITE

The wood treatment site in Mississippi ceased operation in 1979 and was known to be contaminated with creosote and other wood-preserving materials. The results discussed below are from an exploratory study to determine whether bioassays can be used to detect creosote contamination in stream sediments and water, and if feasible, to define the boundaries of creosote-contaminated zones on the site. Samples were also subjected to chemical analyses. The objectives of the study were to 1) determine if standard bioassays could detect creosote contamination in water and sediments, and, if so, 2) map the distribution of creosote contamination in the creek.

Site Description

A preliminary site visit was made to obtain background information on the history of creosote disposal, to determine the dimensions of the site, and to define any special sampling problems. The site is bounded on the south by a city street (Covington Avenue) and on the east and north by a creek. The creek is approximately 2 m wide at the widest point, with 2-m-high banks on either side. The creek flows northwest (on the site), enters an open concrete channel at the western site boundary, and then flows into a nearby city.

Records obtained from the Mississippi Department of Natural Resources (Bureau of Pollution Control) indicate that creosote and occasionally pentachlorophenol were used for wood treatment on the site. From 1965 to 1979, the owner of the site permitted wastes from the treatment process to flow overland to the creek, in violation of state pollution laws. Little cleanup had been done when the site closed in 1979. However, it was clear from the site visit that the site had been covered with fill material. Creosote was still being emitted from the bank into the water along some parts of the stream. Piles of creosote-contaminated material, as well as pools of black sludge, were located immediately adjacent to some old tanks on the south side of the site.

The primary interest in this study was creosote contamination of creek sediments. Therefore, stream sediment samples comprised the bulk of the samples taken. A small number of water samples were taken, as well as some samples from the bank where creosote appeared to be entering the stream. In addition, samples were taken from an upstream site (negative control) and from the pile of creosote-contaminated sludge (positive control).

A description of the spatial distribution of the contamination was one objective of the study. Kriging was the first choice as a data analysis technique because it permits generation of confidence intervals about estimates of areal distribution. However, kriging generally requires a large number of data points, and in the absence of a sufficient amount of data to perform kriging, a simple linear interpolation could be substituted (principally because the area of concern was a narrow stream channel).

Design of the Field Sampling and Laboratory Studies

The field sampling scheme is diagrammed in Figure 7. The girder bridge was used as the staging area and the starting point from which distances to each sampling location was measured. One sediment sample was collected at the point just before the stream entered the concrete channel at the western end of the site, 660 m west of the initial sampling location at the girder bridge. The next sample was collected at 420 m west of the initial location, then every 40 m to the east until the visibly contaminated zone of the stream was reached. Samples were collected every 20 m in the visibly contaminated zone, and beyond until a location 220 m east of the starting point was reached. Three tributaries (Eastern, Western, and Northern on Figure 7) drain into the creek. The last sample (220 m east of the initial point) was collected upstream of the three tributaries. In addition, one composite sample was collected from each of the three tributaries. The negative control sediment sample was taken from the creek south of Covington Avenue, upstream from the site. The positive control sample was taken from the piles of creosote-contaminated sludge near the storage tanks. This sludge was believed to be the same material that was visibly contaminating the stream.

Water samples were collected at 660 m west of the initial point (farthest downstream location), 380 m west of the initial point, 220 m east of the initial point (farthest upstream location), and south of Covington Avenue, where the negative control sediment sample was taken (Figure 7). All samples were taken on the same day to maximize the comparability of bioassay results and to minimize the sampling costs [samples were collected from west to east (downstream to upstream) to minimize cross-contamination of samples]. The laboratory analyses were completed in two phases. In phase 1, only sediment samples from 660, 380, and 20 m west of the

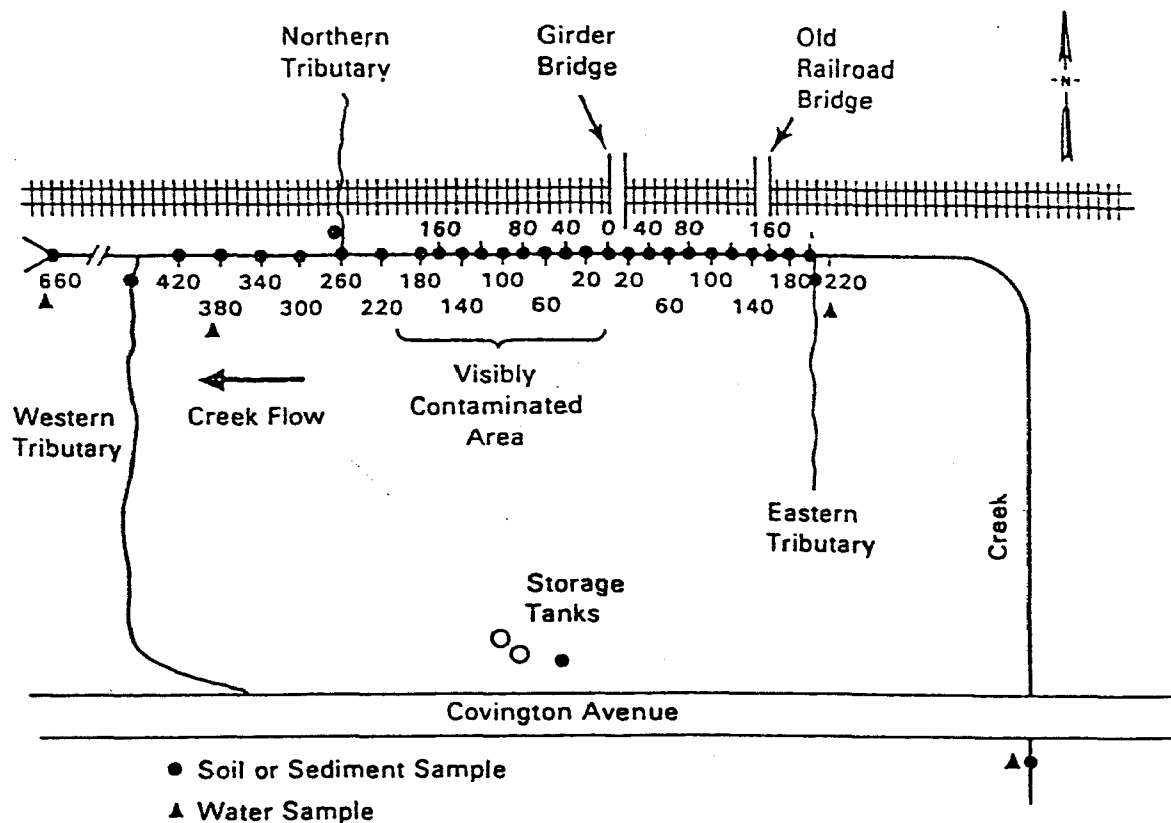


FIGURE 7. Location of Samples Collected from the Wood Treatment Site in Mississippi (distances are in meters). Soil or sediment samples are indicated by solid circles and water samples with solid triangles.

initial point, 120 and 220 m east of the initial point, the positive and negative controls, the composite sample from the eastern tributary, and the water samples were analyzed. The results of these bioassay tests were used to bracket the contaminated zone. In phase 2, samples from 300, 140, and 60 m west of the initial point were analyzed to aid in defining the contaminant boundaries.

Because the clay sediments lining the creek were very hard, they were sampled using a hand coring device. Where possible, surface sediment samples were collected to a depth of 15 cm with hand trowels.

Analysis and Evaluation of the Results

The results of bioassay analyses of phase 1 samples are shown in Table 2. The only locations where appreciable toxicity occurred were the positive control and locations 660 and 380 m west of the initial sample location. The water sample from the 380-m-west location was highly toxic, while the sediments from that location were less toxic. At 660 m west, however, the sediments were highly toxic to some organisms while the water was not toxic.

Figure 8 contains toxicity maps of creek sediment elutriates from 660 m west to 220 m east of the initial point. These maps were based on bioassay and chemical analyses from both phase 1 and phase 2 samples (distances to the west of the initial point on Figure 8 are indicated by negative numbers). The respective creosote concentrations determined for each sample by IR (infrared spectroscopy) are expressed as a percentage of the highest creosote value measured (9500 and 25 ppm for sediments and sediment elutriates, respectively). We note that creosote is a complex mixture of organic compounds. Since there were insufficient data to use the kriging technique to devise maps, the maps were prepared using simple linear interpolation of the results between the sampling points. Therefore, the precision of the division locations between different zones of contaminant concentrations cannot be estimated (as in kriging).

The top four bars on Figure 8 illustrate EC50s from the algal, Daphnia, Microtox, and root elongation bioassays in which the test materials were sediment elutriates. The result from different elutriate bioassays led to different conclusions regarding the relative toxicities of different areas of stream sediments. Such variable biological responses could result from different organic compounds in creosote, which may bind differentially in each area of stream sediments, or from in-stream seeps from the waste site. IR analyses for creosote indicated that the most severe contamination occurred in the extreme downstream portion of the creek study area. In contrast, the algae, Daphnia, and Microtox bioassays indicated that the most extreme toxicities actually occur about 300 m west of the initial point. The Microtox bioassay was most sensitive to the chemical contaminants in the downstream sediment elutriates. The results from root elongation tests show a complete absence of a detectable phytotoxic component. A comparison between relative creosote amounts and algae, Daphnia, and Microtox response to sediment elutriates collected between -140 and -400 m (Figure 8) suggests that contaminants other than creosote caused the toxicity.

CONCLUSIONS

Several conclusions are possible based on results from this study. First, standard bioassay organisms are sensitive to contaminants resulting from wood treatment operations, and different bioassay organisms have different sensitivities to the mixture of creosote resulting from wood treatment operations. Second, infrared measurements of sediment contaminants resulting from wood treatment operations are inaccurate predictors of biotoxicity. Finally, bioassay results can be mapped using kriging and these maps offer good prospects for aiding in regulatory decisions.

TABLE 2. Bioassay Results from Phase 1 Samples Collected from the Wood Treatment Plant in Mississippi

Sample Location	Sample Type	EC50					
		Algae ^(a)	Daphnia ^(a)	Microtox ^(a)	Root Elongation ^(a)	Neubauer ^(b)	Earthworm ^(b)
660 m west	Sediment	63.7	73.6	4.0	100	70.3	27.9
	Water	NE ^(c)	NE	NE	NE	NE	NE
380 m west	Sediment	73.7	NE	29.9	100	100	27.9
	Water	6.6	0.2	9.6	100	NR ^(d)	NR
20 m west	Sediment	NE	NE	NE	NE	NE	NE
120 m east	Sediment	NE	NE	NE	NE	NE	NE
220 m east	Sediment	NE	NE	NE	NE	NE	NE
	Water	NE	NE	NE	NE	NE	NE
Negative Control	Sediment	NE	NE	NE	NE	NE	NE
	Water	NE	NE	NE	NE	NE	NE
Positive Control	Sediment	0.6	6.9	8.5	8.1	0.9	3.9
Eastern Tributary	Sediment	NE	NE	NE	NE	NE	NE

(a) Tests conducted with sediment elutriates.

(b) Tests conducted with sediment samples.

(c) NE = No effect.

(d) NR = Bioassay not required.

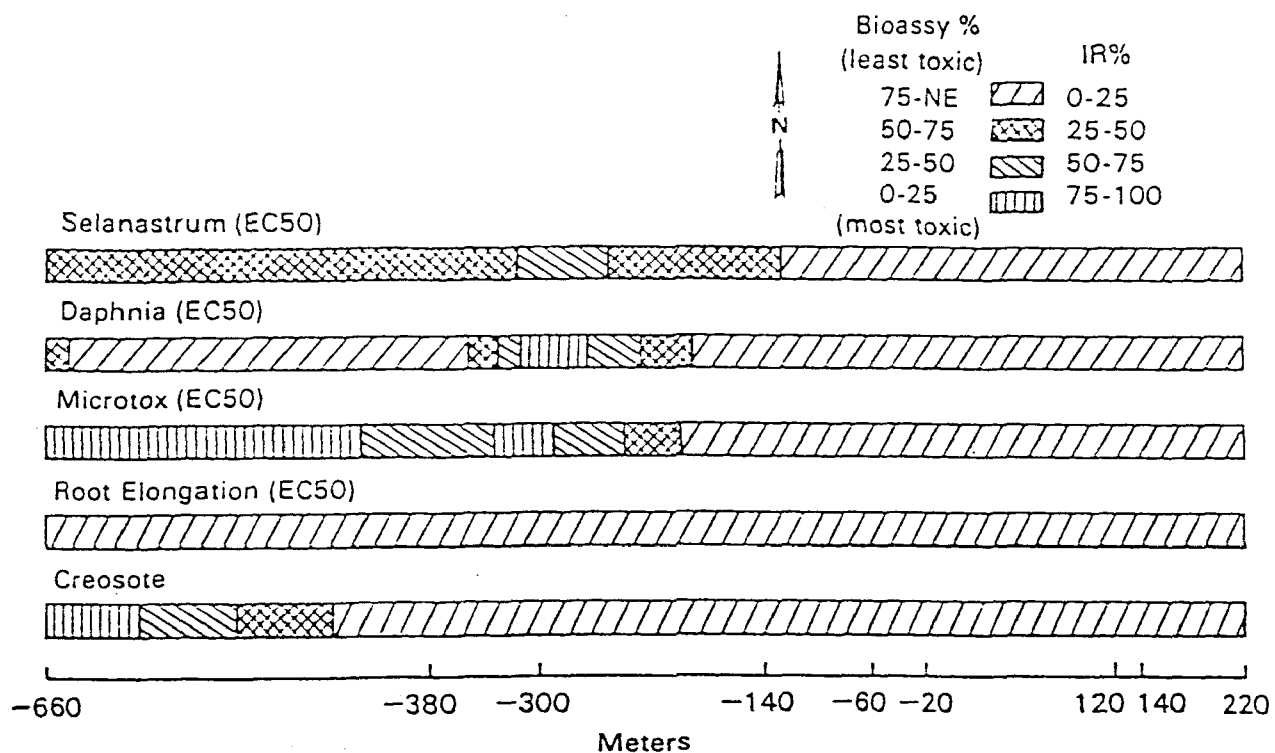


FIGURE 8. Bioassay Results from Sediment Elutriates at the Wood Treatment Site in Mississippi. Negative numbers represent samples collected downstream from the site.

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APPLICATION OF A BIOMARKERS-WASTE CHARACTERIZATION APPROACH
TO THE PREDICTION OF ORGANISM RESPONSES FOLLOWING EXPOSURE TO
CONTAMINATED MARINE SEDIMENTS

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ABSTRACT

This paper reports on the application of short-term biomarkers to predict organism responses induced by contaminated marine sediments. Laboratory results were confirmed by observing the same responses to the same sediments in both laboratory- and field-exposed animals. Identification of causative agents is being investigated by a combination of sediment chemical analysis and short-term testing of fractionated solvent extracts of sediments. Whole extracts of a contaminated sediment induced concentration-dependent responses in the Salmonella/microsome (Ames) test with strain TA100 in the presence of an exogenous (S-9) metabolizing system. Increased frequencies of sister chromatid exchange (SCE) were observed in a marine worm, *Nephtys incisa*, exposed to whole sediment in laboratory tests. Comparable increases in SCE frequency were observed in the same species sampled from feral populations at a field disposal site exposed to this same sediment. These responses suggest a tumorigenic potential for the sediment. This potential has been realized by induced tumor development in American oysters, *Crassostrea virginica*, and winter flounder, *Pseudopleuronectes americanus*, exposed to this sediment in the laboratory and the field. Partial chemical characterization of this sediment has identified many organic and inorganic compounds reported to be carcinogenic, cocarcinogenic, genotoxic and tumor-promoting. Preliminary tests with fractionated solvent extracts indicate mutagenic activity (Ames test) to be primarily associated with fraction 2, a PAH fraction. Effects on cell-to-cell communication between cultured mammalian cells, a potential biomarker for tumor promoters, suggest the presence of tumor-promoting agents in fraction 4 (a highly polar fraction). Other biomarkers (enzyme inhibition, fertilization impairment, and macrophage repression) are being explored as short-term tests for characterizing complex wastes and predicting organism responses.

ENVIRONMENTAL MONITORING

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ABSTRACT

Throughout the world are field scientists who are in a unique position to see subtle changes in their object of study and their environment. Field scientists keep extensive field notebooks, but seldom communicate findings such as these, unless and until they have relevance to one of their projects. The object of this discussion is the compilations of these findings into a database related to putative man-made environmental degradation. Some of the uses of such a database are obvious and I will discuss others. A shotgun collection of data would be unproductive. Therefore initial studies will be concentrated in coastal areas.

When proximity of hazardous waste sites was investigated it was found that many sites on the National Priority List are contaminating marine waters and sediments via contaminated surface runoff or ground water. However, many more sites posing severe environmental hazards are not listed as Superfund sites because they have not been shown to represent direct threats to human health, thus have low priority for corrective action. Toxic agents of concern at these sites include pesticides, metals, hydrocarbons, PCB's and others.

Possible questions that may arise will be discussed, among them: 1) Relative sensitivity to toxic substances of bottom dwelling and pelagic organisms; 2) normal and pathological concentrations of metals in fish and sea mammals; 3) quantitative studies of the relationship between sewage outfalls and plankton blooms; and 4) normal cycles of plant or animal populations.

The ideal computer for this would be an IBM PC-AT or PC compatible, because of the available generic software. Data collection could be implemented, using existing electronic mail and "bulletin board" packages. Access to these media is available to scientists across the country. The proposed database should be either a relational database, such as Oracle or a collection of related databases such as Database III.

ASSESSMENT OF THE MICROSCREEN PHAGE-INDUCTION
ASSAY FOR SCREENING HAZARDOUS WASTES

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ABSTRACT

The Microscreen phage-induction assay, which quantitatively measures the induction of prophage in *Escherichia coli* WP2 (λ), was used to test 14 crude (unfractionated hazardous industrial^s waste samples for genotoxic activity in the presence and absence of metabolic activation. Eleven of the 14 wastes induced prophage, and induction was observed at concentrations as low as 0.4 picograms per ml. Comparisons between the mutagenicity of these waste samples in *Salmonella* and their ability to induce prophage λ indicate that the Microscreen phage-induction assay detected genotoxic activity in all but one of the wastes that were mutagenic in *Salmonella*. Moreover, the Microscreen assay detected as genotoxic 5 additional wastes that were not detected in the *Salmonella* assay. Partial chemical characterizations of the wastes showed high concentrations of carcinogenic metals, solvents, and chlorinated compounds, most of which are detected poorly by the *Salmonella* assay. However, recent studies of the induction of prophage by these chemical classes have suggested that phage induction may be a sensitive endpoint for these groups of chemicals. This may explain the enhanced ability of the Microscreen phage-induction assay to detect genotoxic activity in 5 additional wastes compared to the *Salmonella* assay. The applicability of the Microscreen phage-induction assay for screening hazardous wastes for genotoxic activity is discussed along with some of the problems associated with screening highly toxic wastes containing toxic volatile compounds.

INTRODUCTION

According to estimates by the U.S. Environmental Protection Agency (US EPA), more than 260 million metric tons of hazardous wastes are generated annually, a quantity equal to more than 70 billion gallons (Dietz et al, 1984). Some of the acute health effects linked to hazardous waste exposures include lung and skin irritations (US EPA, 1980), eye irritations and menstrual problems (Grisham, 1986), and transitory liver damage (Clark et al, 1982; Meyer, 1983). Included among the chronic health effects associated with hazardous waste exposure are chromosomal damage, cancer, and reproductive anomalies (Maugh, 1979; Vianna and Polan, 1984; Warren, 1981).

One of the ways by which wastes are classified as hazardous is on the basis of specific chemical constituents that may be present in the waste (Federal Register, 1980; Greer, 1984). However, analyzing a waste for a limited set of compounds may not provide data that reflect the overall chemical composition of the waste, nor does it take into account the possibility of chemical interactions (antagonisms, synergisms, etc.) or the production of metabolites resulting from degradative pathways. In contrast to chemical analysis, the identification of potential biological effects elicited by a waste may provide more useful information for determining human health effects. Because of the presence of known mutagens and carcinogens in many wastes, genetic toxicity is an important endpoint that should be determined for wastes.

The Salmonella assay developed by Dr. Bruce Ames and coworkers (Ames et al, 1975) has been used more than any other short-term assay for determining the genotoxicity of complex mixtures and hazardous wastes. However, hazardous wastes can contain high concentrations of carcinogenic metals, chlorinated compounds, and solvents (US EPA, 1984) that are detected poorly by the Salmonella assay (Kier et al, 1986; Zeiger and Tennant, 1986; Claxton et al, 1987). Thus, the genotoxic potential of some wastes may escape detection when evaluated by the Salmonella assay.

The Microscreen phage-induction assay developed by Rossman et al (1984), has been shown to detect some carcinogenic metals (Rossman et al, 1984), chlorinated pesticides (Houk and DeMarini, 1987), and solvents (Houk and DeMarini, in prep.) that are not mutagenic in Salmonella. It is a rapid and inexpensive assay that quantitatively measures the induction of prophage λ in *Escherichia coli* WP2 (λ). The induction of prophage is one of several events that may occur upon induction of the SOS response, which is a cellular reaction to DNA damage. Other manifestations of the SOS response include DNA repair and mutagenesis, and many agents that induce the SOS response in bacteria are also genotoxic in a variety of other organisms (D'Ari, 1985).

MATERIALS AND METHODS

In the present study, we have used the Microscreen phage-induction assay to evaluate the genotoxicity of 14 crude hazardous waste samples obtained from Edward L. Katz, Hazardous Waste Engineering Laboratory, US EPA, Cincinnati, OH (Table 1). Three of the waste samples were from petrochemical, pharmaceutical, and plastics manufacturers (A, B, and C, respectively). The remaining wastes (D through O) were composites of wastes from a variety of industrial sources that were collected by 4 commercial hazardous waste incineration facilities and classified according to their aqueous or organic properties. Table I provides a cursory physical description of the wastes, all of which were liquids or semi-solids.

Table II shows the results of a partial chemical analysis that was performed on these samples (US EPA, 1984). These characterizations should not be viewed as indicative of the overall chemical composition of the wastes because the analysis was limited to specific organics and/or metals identified in the EPA Appendix VIII list of priority pollutants (US EPA, 1984). In addition, for purposes of a previous study on the performance of hazardous waste incinerators (US EPA, 1984), 7 of the waste samples (B, F, G, J, K, L, and M) were spiked with carbon tetrachloride and trichloroethylene. Thus, the concentrations of these 2 chemicals in the 7 waste samples reflect this addition.

The phage-induction assay was performed as described by Rossman et al. (1984) with modifications (Houk and DeMarini, 1987). For a more detailed explanation of materials and methods, see Houk and DeMarini (1987, and submitted). The bacterial strains are derivatives of E. coli B/r. WP2 (λ) is a lambda lysogen of WP2s (trpE, uvrA), and SR714 (trpE, uvrD₃)^S is the indicator strain.

The crude (unfractionated) waste samples were serially diluted in supplemented minimal medium in sterile 96-well microtiter dishes (Corning). The lysogenic strain of E. coli was then exposed overnight (20 h, 37°C) to the diluted wastes in the presence and absence of an exogenous metabolic activation system (S9). Following exposure, wells of the microtiter dishes were scored for turbidity (cell survival and growth) or clarity (cytotoxicity and/or inhibition of cell growth). Turbidity in the wells of microtiter dishes was often difficult to discern due to the physical nature of these crude complex mixtures. Many of the waste samples produced a precipitate (noted by a "P" in Table III) or an oily film (noted by an "F") that complicated the determination of whether the wells were clear or turbid. The cytotoxic responses of the waste samples are noted by a "T" for clear wells and a "t" for slightly turbid wells in Table III.

Wells of the microtiter dishes were then sampled for the presence of prophage by exposing diluted contents of selected wells to the indicator strain (SR714) and enumerating the resulting plaque-forming units (PFU). The appearance of a dose-related increase of induced (observed minus background) PFU that reached or exceeded the upper limits of the 99% confidence interval for the control plates indicated a positive--or genotoxic--response. The background PFU/plate were calculated by averaging results from eight medium control wells (with and without S9) for each experiment. The upper limits of the 99% confidence intervals were 102 PFU/plate in the presence of S9 and 30 PFU/plate in the absence of S9.

RESULTS

The response for each waste sample based on the criterion described above is shown in Table III, which also presents raw data from a representative experiment for each of the wastes. Doses were selected to illustrate the response range for each waste tested. Eleven of the 14 crude hazardous waste samples induced prophage; only 2 of the wastes (D and J) required S9 for induction. In addition to showing the induced PFU/plate, Table III also indicates the fold increase over the background. For most of the positive waste samples, the dose that produced either 102 induced PFU/plate (+S9) or 30 induced PFU/plate (-S9) generally resulted in a 3- or 4-fold increase over the background, which is consistent with the fold increase observed in a previous study of pure compounds (Houk and DeMarini, 1987). This is also consistent with the 3-fold increase recommended by Rossman et al (1985) for a positive response.

Dose-response curves for the waste samples are illustrated in Figs. 1-3. Except for waste samples A and C in the presence of S9 (Fig. 3), each waste sample produced a dose-response curve exhibiting a linear portion that spanned ~ 1 order of magnitude of dose (Figs. 1 and 2). Based on the linear portions of these dose-response curves, we calculated the concentration required by each waste to produce an induced PFU/plate equal to the upper limits of the 99% confidence intervals of the medium controls (Table IV). Each of these values represent the minimum concentration of the waste required to produce a positive response based on our criterion described previously. The waste samples were then ranked from the most to the least potent (Table IV).

Although waste samples A and C were positive in the presence of S9 (Table III), they did not product simple linear dose responses. Instead, they produced reproducible, multimodal dose-response curves that ranged over 4 orders of magnitude (Fig. 3). In addition, waste sample G produced a nonlinear dose response at the doses tested in the presence of S9 (Table III; data not plotted).

DISCUSSION

The partial chemical characterization available for these wastes (Table II) is more extensive than would ordinarily be available for most waste samples. However, even this level of chemical analysis is inadequate to distinguish a genotoxic from a nongenotoxic waste or to indicate which waste might be more genotoxic than another. For example, the chemical analysis of the petrochemical and pharmaceutical waste samples (A and B, Table II) does not necessarily suggest the remarkable cytotoxic and genotoxic potencies exhibited by these two samples compared to the other waste samples (Tables III and IV). Likewise, the finding that waste samples K, N, and O were not genotoxic is not readily apparent from their chemical profiles, which are relatively

indistinguishable from those of the genotoxic waste samples (Table II). The limitations of the chemical analysis for predicting the biological effects of these wastes argue in favor of bioassays to characterize the possible hazardous nature of wastes.

The similar genotoxic potencies exhibited by most of the waste samples (Fig. 1, Table IV) may have been due to the fact that most of these wastes were composites of wastes from a variety of sources. Thus, mixtures of potent and weak wastes may result in samples with similar average potencies. Consistent with this interpretation is the fact that the two samples that have vastly different potencies from the rest (samples A and B) are not composite wastes but are from two distinct industrial sources, the petrochemical and pharmaceutical industries.

Table V compares the mutagenicity of these waste samples in *Salmonella* to the ability of these waste samples to induce prophage. All but one of the wastes that were mutagenic in *Salmonella* also induced prophage. This is consistent with studies of pure compounds which show that for those compounds tested, most chemicals that are mutagenic in *Salmonella* also induce prophage and/or the SOS response (Elespuru, 1984; Oda et al, 1985; Ohta et al, 1984; Quillardet et al, 1985; Rossman et al, 1986). Thus, at least some concordance between the assays is not surprising. The inability to detect waste sample O in the phage-induction assay may have been due to a toxic or inhibitory effect of the sample on prophage production as evidenced by a reduction in PFU/plate at concentrations above 100,000 µg/ml (Table III).

The phage-induction assay detected 5 additional waste samples as genotoxic that were not mutagenic in *Salmonella* (Table V). As mentioned previously, the Microscreen phage-induction assay has been shown to detect some carcinogenic metals (Rossman et al, 1984), chlorinated organics (Houk and DeMarini, 1987), and solvents (Houk and DeMarini, in prep.) that are not mutagenic in *Salmonella* (Keir et al, 1986; Zeiger and Tennant, 1986). Metals and compounds of these types are present in most of the waste samples studied here (Table II), and the ability of some of these compounds to induce prophage may account for the detection by the phage-induction assay of the 5 additional waste samples that were not detected by the *Salmonella* assay. In addition, accumulating evidence indicates that phage-induction (and the SOS response in general) is a broader genetic endpoint than reverse mutation in bacteria, occurring by a variety of mechanisms (Elespuru, 1984; Hofnung and Quillardet, 1986) and involving a number of classes of genetic damage (Rossman et al, 1985; Elespuru, 1984).

For most assays, complex mixtures must be extracted or fractionated prior to bioassay to make them compatible with the test system. However, with the Microscreen assay we were able to test the crude wastes directly, saving considerable time, effort, and expense. The unfractionated wastes posed no sterility problems, probably due to their extreme toxicity. Other wastes, however, may contain microbial contaminants that would necessitate the preparation of organic extracts.

Currently, the US EPA identifies wastes as hazardous based primarily on physical characteristics and chemical composition of the wastes (Federal Register, 1980; Friedman, 1985; Greer, 1984). However, the deficiencies of this definition have led EPA to consider adding health effects, including mutagenicity, to their definition of hazardous waste (Federal Register, 1983; 1984a,b). In addition to government, industry also has recognized the important role that short-term assays could play in the toxicological assessment of hazardous wastes (Barfknecht and Naismith, 1984; Guiney, 1985). In combination with chemical analysis, assessments of the biological effects of wastes could contribute valuable information regarding the hazardous nature and risk associated with exposure to industrial wastes and effluents. Our data suggest that the Microscreen phage-induction assay may provide a simple, sensitive, and inexpensive way to screen hazardous wastes for an important biological endpoint, genotoxicity.

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TABLE I PHYSICAL DESCRIPTIONS OF HAZARDOUS WASTES

Waste	Physical State	Description
A	Liquid	Black, very thin oil from a petrochemical manufacturing plant
B	Liquid	Black, oily liquid from a pharmaceutical manufacturer
C	Tar	Black, pourable tar produced during the production of plastics
D	Liquid	Composite of aqueous wastes; watery liquid with red oil droplets
F and G	Semi-solid	Composite of organic wastes; biphasic gray sludge with reddish-brown liquid
H and I	Suspension	Composite of aqueous wastes; thin, gray slurry
J	Liquid	Composite of organic wastes; gray, thick liquid with suspended solids
K	Liquid	Similar to K, but lighter in color and thinner
L and M	Tar	Composite of organic wastes; black, thin, pourable tar
N and O	Liquid	Composite of aqueous wastes; clear, watery liquid

TABLE II

CONCENTRATIONS OF CHEMICALS AND METALS IDENTIFIED IN HAZARDOUS WASTES (ug/g)

Chemical/Metal ^a	Petro-chemical	Pharmaceutical	Plastics	Hazardous waste incineration facility											
				1		2		3		4					
				Aqueous	Organic	Organic	Aqueous	Organic	Organic	Organic	Aqueous				
											D	E	F	G	H
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Aniline		14000	550000												
Benzyl chloride	3000														
Bis-(2-ethyl-hexyl)-phthalate		500		<100	3800							200	230	<10	<10
Butylbenzyl-phthalate				<100	320	120	450	<5	8	160	140				
Chlordane												<60	<60	19000	19000
Chlorophenylisocyanate		21000													
Cresol(s)										2000	2500				
Diethylphthalate						620	1300	240	240						
m-Dichlorobenzene		2300													
o-Dichlorobenzene		46000													
p-Dichlorobenzene		59000													
m-Dinitrobenzene			<100												
Diphenylamine			6200												
2,4-Dimethylphenol										500	2000				
Hexachlorobutadiene												<10	<10	<10	<10
Hexachloroethane	560														
Hexachlorocyclopentadiene												<10	<10	230	260
Isophorone				<100	110										
Mononitrobenzene			<100												
Naphthalene	<100			<100	350	250	450	44	49	450	490	38	33	<10	<10
Phenol				34000	1000	1500	1700	1800	2900						
Phenylenediamine			2300												
Phenylisocyanate		160000													
Trans-1,4-dichloro-2-butene	59000														
1,2,4-Trichlorobenzene		290													
Benzene				<3	260							46000	58000	<10	<10
Carbon tetrachloride ^b	68000	44000		<2	6000	3700	4400	<10	<10	5900	5700	9100	11300	<10	<10
Chlorobenzene		4100										390	500	<10	<10
Chloromethane		1200													
Chloroform	2900					170	270	21	22			110	60	<10	<10
cis-1,4-dichloro-2-butene	18000														
Methylene bromide												<10	<10	3100	4700
Methylene chloride	21000											100	340	44	60
Methyl ethyl ketone				27	9700					18000	38000				
Tetrachloroethylene	11000			<1	28	7100	9800	1600	1300	7900	8100	87	150	<10	<10
Toluene	240000			110	2400	32000	45300	2900	2700	54000	48000	160000	390000	22	20
1,1,1-Trichloroethane	<100									24000	16000	330	230	<10	<10
Trichloroethylene ^b	4000	40000		<1	5500	3700	4400	90	85	8100	7800	8300	10300	<10	<10
Ag	<3					<1	<1	<1	<1			<1	<1		
As	<24					<20	<20	<20	<20	<14	<14	<23	<23		
Ba	<7					110	140	6	7	1160	1150	990	1100		
Be	<2					<1	<1	<1	<1	<1	<1	<1	<1		
Cd	<5					6	6	<1	<1	153	15	49	55		
Cr	<5					50	57	3	3	431	425	250	290		
Hg	<22					<10	<10	<10	<10	<4	<4	<50	<50		
Mn	<68					7	7	2	2	26	27	<4	<4		
Pb	<19					140	150	<10	11	1830	1800	1200	1300		
Sb	<12					58	61	<10	<10	437	373	<24	<24		
Se	<470					340	<100	<100	<100	<21	<21	<160	<160		
Tl	<23					<20	<20	<20	<20	<9	<9	<22	<22		
Si										<1	<1				
pH		2			8		7	7	6	7			7	7	7
Water (%)	2		5	94	3	38	48	95	67	21	23	5	5	94	96

^aData from EPA (1984)^bWaste samples B, E, F, G, J, K, L, and M were spiked with these 2 chemicals.

TABLE III INDUCTION OF PROPHAGE LAMBDA BY HAZARDOUS WASTES

Waste	+S9				-S9			
	Dose ($\mu\text{g/ml}$)	PFU per Plate ^a	Induced PFU per Plate ^b	Fold in- crease	Dose ($\mu\text{g/ml}$)	PFU per Plate	Induced PFU per Plate	Fold in- crease
A	0	21	0	--	0	12	0	--
	5.0×10^{-5}	82	61	3.9	2.0×10^{-7}	30	18	2.5
	2.0×10^{-4}	160	139	7.6	4.0×10^{-7}	49	37	4.1
	1.2×10^{-2}	320	299	15.2	8.0×10^{-7}	60	48	5.0
	2.5×10^{-2}	388	367	18.5	1.5×10^{-6}	2(T)	0	--
	1.5	552	531	26.3				
	50	241	220	11.5				
	200	182	161	8.7				
	400	0(T)	0	0				
	Response		+				+	
B	0	21	0	--	0	12	0	--
	0.05	249	228	11.9	2.0×10^{-7}	24(P)	12	2.0
	0.1	421	400	20.0	4.0×10^{-7}	49(P)	37	4.1
	0.2	582	561	27.2	8.0×10^{-7}	106(P)	94	8.8
	0.4	957	936	45.6	1.5×10^{-6}	136(P)	124	11.8
	0.8	561(T,P)	540	26.7	3.0×10^{-6}	572(t,P)	560	47.8
	1.6	2(T,P)	0	--	6.0×10^{-6}	24(P)	12	2.0
	Response		+				+	
C	0	21	0	--	0	26	0	--
	1.0×10^{-2}	29	8	1.4	200	24	0	--
	5.0×10^{-2}	56	35	2.7	400	30	4	1.2
	0.2	125	104	6.0	800	57	31	2.2
	0.8	81	60	3.9	1,600	68	42	2.6
	3	53	32	2.5	3,100	0(T)	0	--
	12	48	27	2.3	6,300	0(T)	0	--
	50	136	115	6.5				
	200	9(t)	0	--				
	Response		+				+	
D	0	52	0	--	0	12	0	--
	800	76	24	1.5	800	19	7	1.6
	1,600	138	86	2.7	1,600	12	0	1.0
	3,100	131	79	2.5	3,100	12	0	1.0
	6,300	148	96	2.8	6,300	11	0	--
	12,500	218	166	4.2	12,500	12	0	1.0
	25,000	23(t)	0	--	25,000	0(T)	0	--
	Response		+				-	
F	0	53	0	--	0	9	0	--
	100	63	10	1.2	200	15	4	1.7
	200	78	25	1.5	800	33	24	3.7
	400	103	50	1.9	1,600	75	66	8.3
	3,100	115	62	2.2	3,100	235	226	26.1
	12,500	259	206	4.9	6,300	721	712	80.1
	25,000	3,755(P)	3,702	70.8	12,500	1,960	1,951	217.8
	50,000	416(P)	363	7.8	25,000	962	953	106.9
	100,000	0(P)	0	--	50,000	1(P)	0	--
	Response		+				+	

Waste	+S9				-S9			
	Dose (µg/ml)	PFU per Plate	Induced PFU per Plate	Fold in- crease	Dose (µg/ml)	PFU per Plate	Induced PFU per Plate	Fold in- crease
G	0	89	0	--	0	19	0	--
	1,250	353	264	4.0	780	20	1	1.0
	2,500	346	257	3.9	3,100	100(t)	81	5.3
	5,000	402	313	4.5	5,000	950(t)	931	50.0
	6,300	400	311	4.5	6,250	5,430(t)	5,411	285.8
	12,500	466	377	5.2	10,000	3,450(t)	3,431	181.6
	25,000	4,200(P)	4,111	47.2	20,000	2,300(T)	2,281	121.1
	40,000	1,800(P)	1,711	20.2	25,000	71(T,P)	52	3.7
	50,000	17(P)	0	--	50,000	0(T,P)	0	--
Response			+				+	
H	0	53	0	--	0	9	0	--
	400	33	0	--	400	10	1	1.1
	800	76	23	1.4	800	14	5	1.6
	1,600	202	149	3.8	1,600	19	10	2.1
	3,100	373	320	7.0	3,100	42	33	4.7
	6,300	591	538	11.2	6,300	64	55	7.1
	12,500	792	739	14.9	12,500	138	129	15.3
	25,000	0(P)	0	--	25,000	0(P)	0	--
Response			+				+	
I	0	53	0	--	0	9	0	--
	800	88	35	1.7	800	11	2	1.2
	1,600	361	308	6.8	1,600	34	25	3.8
	3,100	768	715	14.5	3,100	68	59	7.6
	6,300	740	687	14.0	6,300	44	35	4.9
	12,500	936	883	17.7	12,500	27	18	3.0
	25,000	0(P)	0	--	25,000	0(P)	0	--
Response			+				+	
J	0	62	0	--	0	12	0	--
	12	56	0	--	6	10	0	--
	50	76	14	1.2	12	13	1	1.1
	100	111	49	1.8	25	15	3	1.3
	200	173	111	2.8	50	26	14	2.2
	400	133	71	2.1	100	28(t)	16	2.3
	800	12(t)	0	--	200	10(t)	0	--
Response			+				-	
K	0	62	0	--	0	26	0	--
	50	69	7	1.1	200	27	1	1.0
	100	60	0	--	400	29	3	1.1
	200	66	4	1.1	800	3(t)	0	--
	400	87	25	1.4	1,600	3(t)	0	--
	800	16(t)	0	--	3,100	1(t)	0	--
Response			-				-	

Waste	+S9				-S9			
	Dose ($\mu\text{g/ml}$)	PFU per Plate	Induced PFU per Plate	Fold in- crease	Dose ($\mu\text{g/ml}$)	PFU per Plate	Induced PFU per Plate	Fold in- crease
L	0	62	0	--	0	12	0	--
	12	69	7	1.1	800	29	17	2.4
	400	116	54	1.9	1,600	69	57	5.8
	6,300	204	142	3.3	3,100	101	89	8.4
	12,500	464(F)	402	7.5	6,300	266	254	22.2
	25,000	2,350(F)	2,288	37.9	12,500	1,692	1,680	141.0
	50,000	0(F)	0	--	25,000	5(F)	0	--
Response		+					+	
M	0	62	0	--	0	12	0	--
	3,100	132	70	2.1	1,600	53	41	4.4
	6,300	131	69	2.1	3,100	79	67	6.6
	12,500	184(F)	122	3.0	6,300	224	212	18.7
	25,000	406(F)	344	6.5	12,500	2,085	2,073	173.8
	50,000	287(F)	225	4.6	25,000	7,590(F)	7,578	632.6
	100,000	0(F)	0	--	50,000	0(F)	0	--
Response		+					+	
N	0	87	0	--	0	26	0	--
	37,500	107	20	1.2	37,500	28	2	1.1
	50,000	139	52	1.6	50,000	23	0	--
	75,000	135	48	1.6	75,000	23	0	--
	100,000	142	55	1.6	100,000	24	0	--
	200,000	23	0	--	200,000	16	0	--
	300,000	18	0	--	300,000	10	0	--
Response		-					-	
O	0	87	0	--	0	26	0	--
	37,500	77	0	--	37,500	12	0	--
	50,000	84	0	--	50,000	11	0	--
	75,000	94	7	1.1	75,000	12	0	--
	100,000	100	13	1.1	100,000	16	0	--
	200,000	31	0	--	200,000	10	0	--
	300,000	18	0	--	300,000	6	0	--
Response		-					-	

^aEach value is the average of two plates. T, toxic (clear well); t, slightly toxic (slightly turbid well); P, precipitate; F, oily film on surface of medium. The positive control results were: 2-aminoanthracene (0.3 $\mu\text{g/ml}$) 716 ± 288 PFU/plate; 2-nitrofluorene (150 $\mu\text{g/ml}$) 400 ± 264 PFU/plate.

TABLE IV RELATIVE POTENCIES OF WASTES

Concentration ($\mu\text{g/ml}$) required to produce an induced PFU/plate equal to the upper limit of the 99% confidence interval based on the medium controls ^a			
Waste	+S9	(rank)	-S9 (rank)
B	0.03	(1)	3×10^{-7} (1)
A	+b		4×10^{-7} (2)
C	+		1082 (3)
J	185	(2)	-
I	976	(3)	1888 (7)
H	1346	(4)	3355 (8)
L	3029	(5)	1200 (4)
D	6076	(6)	-
F	6444	(7)	1490 (5)
M	9561	(8)	1638 (6)
G	+		3456 (9)

^aThis is the concentration ($\mu\text{g/ml}$) required to produce 102 induced PFU/plate (+S9) or 30 induced PFU/plate (-S9).

^bPotency value incalculable due to nonlinear dose response (see text).

TABLE V COMPARISON OF PROPHAGE INDUCTION IN Escherichia coli AND
MUTAGENESIS IN Salmonella typhimurium

Waste	Prophage Induction in <u>E. coli</u>	Mutagenicity in <u>S. typhimurium</u> ^a
A	+	+
B	+	+
C	+	+
D	+	-
F	+	+
G	+	+
H	+	+
I	+	+
J	+	-
K	-	+
L	+	+
M	+	+
N	+	+
O	-	+

^a From DeMarini et al (1987b). Wastes were tested in strains TA98 and TA100. A positive response in either strain, with or without S9, was sufficient for a positive summary response reported here.

Fig. 1. Dose-response curves of prophage induction by hazardous waste samples in the presence and absence of S9. Only the linear portions of the dose-response curves are shown, and the correlation coefficients (" r^2 " values) are 0.99 for all of the curves except for L (0.97) and F (0.92) in the presence of S9; and for C (0.91), M (0.98), and G (0.88) in the absence of S9.

Fig. 2. Linear portions of the dose-response curves of prophage induction by hazardous waste samples A and B in the absence of S9. Correlation coefficients are 0.94 and 0.99 for A and B, respectively.

Fig. 3. Dose-response curves for waste samples A and C in the presence of S9.

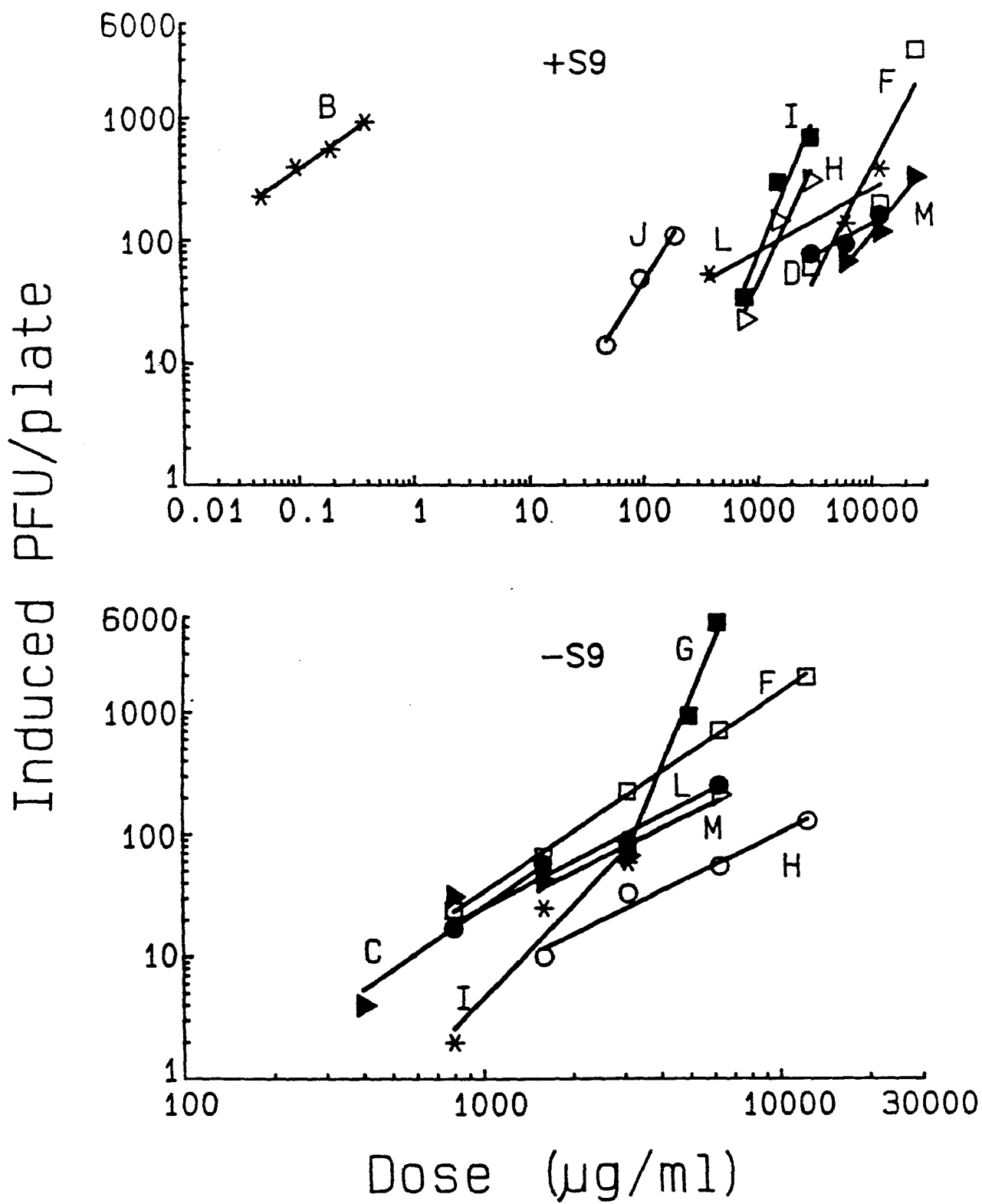


FIGURE 1

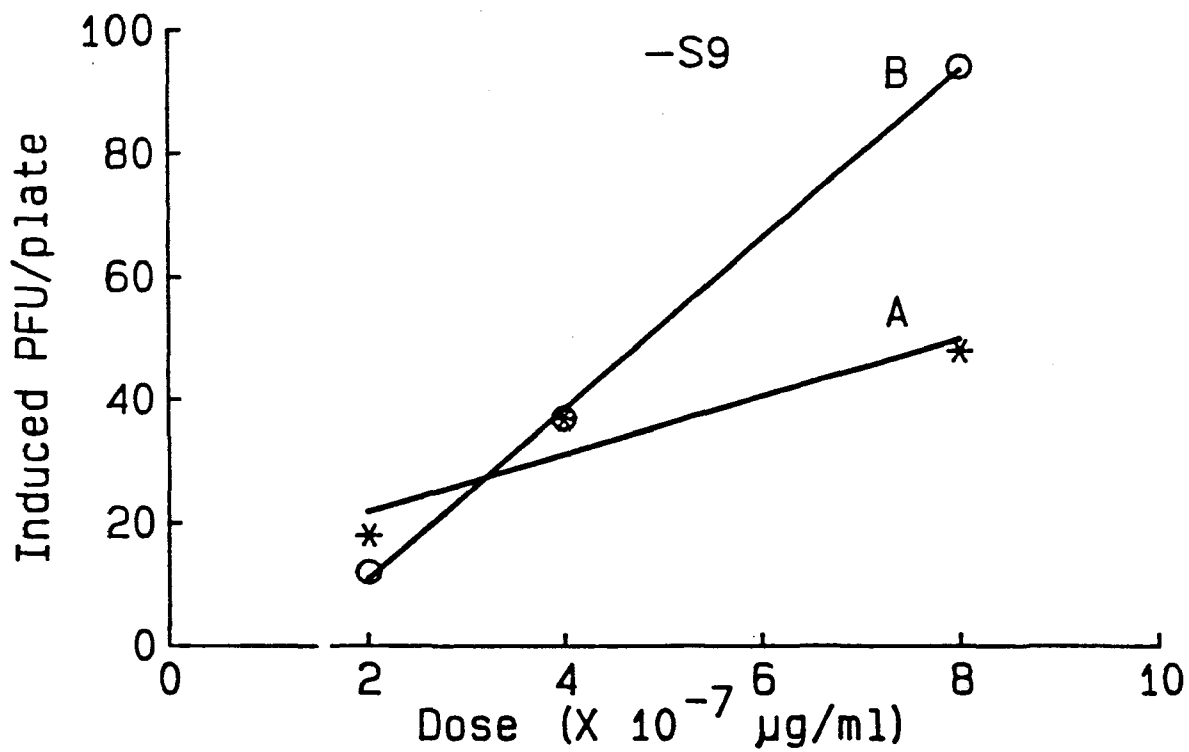


FIGURE 2

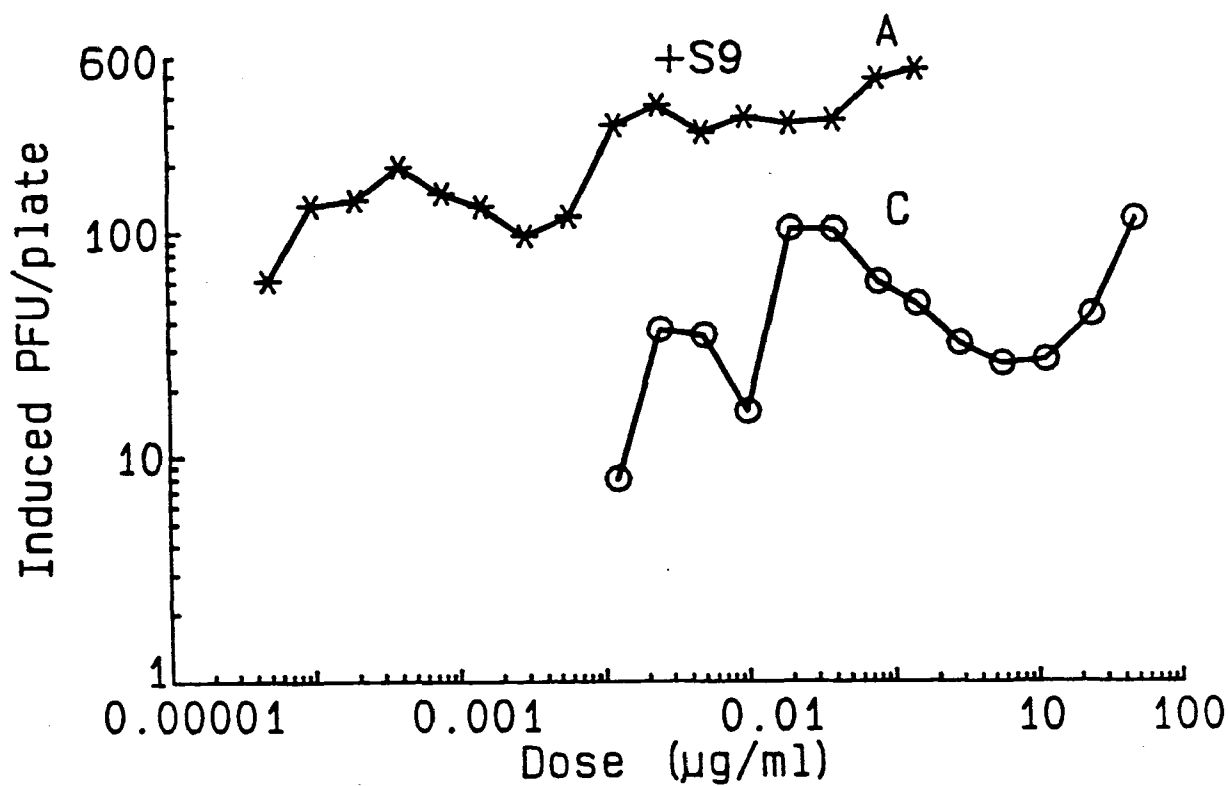


FIGURE 3

ASSESSMENT OF THE TLC/SALMONELLA ASSAY FOR SCREENING HAZARDOUS WASTES

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ABSTRACT

Using a modified version of the TLC/Salmonella assay developed by Bjorseth et al. (1982), ten complex hazardous wastes were tested for mutagenic activity. This method couples thin layer chromatography (TLC) with the Salmonella/mammalian-microsome (Ames) assay for the detection of mutagenic constituents in complex mixtures. Crude hazardous wastes and selected hazardous waste extracts were fractionated on commercially available cellulose TLC plates. Mutagenicity testing was performed by applying a single overlay of minimal growth agar containing a tester strain of Salmonella and the optional metabolic activation system directly onto the developed chromatogram. The appearance of localized clusters of revertant colonies or an increase in total revertant growth vis-a-vis control plates indicated a mutagenic response. Seven of ten hazardous wastes demonstrated mutagenic activity when tested by this method.

To assess the sensitivity of the modified TLC/Salmonella assay, fourteen Salmonella mutagens from a wide range of chemical classes and polarities were tested. The selected compounds included heterocyclics, aromatic amines, alkylating agents, antitumor agents, a nitrosamine, and a nitroaromatic. Eleven of the fourteen mutagens were positive in this test system. The three compounds refractory to analysis included a polycyclic aromatic hydrocarbon and two volatile compounds.

INTRODUCTION

Complex hazardous wastes are composed of compounds that manifest diverse chemical, physical, and toxicological properties. Human exposures to hazardous wastes have been associated with a number of adverse health effects, including chromosomal damage, cancer, and reproductive anomalies (Maugh, 1979; Vianna and Polan, 1984; Warren, 1981). Adequate characterization of the genotoxic potential of hazardous wastes is therefore crucial to the assessment of risk to human health.

Assessments of genotoxic risk are based in part upon results from short-term in vitro assays. One such test, the Salmonella/mammalian-microsome assay (Ames et al., 1975; Maron and Ames, 1983) has been used for over a decade to predict the mutagenic potential of pure compounds and complex mixtures. To enhance detection capabilities, in vitro assays are often coupled with chemical or physical fractionation techniques to separate or remove toxic components from the complex

sample prior to testing and to reduce the effects of interactive mechanisms. For example, in a study performed by Bjorseth et al. (1982), thin layer chromatography was used to segregate potential mutagens in a complex environmental mixture, and the Salmonella assay was then applied directly onto the developed chromatogram. The subsequent appearance of localized clusters of revertant colonies suggested the presence of a mutagenic constituent. This assay has been successfully applied to the analysis of air samples (Bjorseth et al., 1982), extracts of typewriter ribbons and carbon paper (Bjorseth et al., 1982; Moller et al., 1983), and emission samples from coal- and oil-fired boilers (Alfheim et al., 1983).

We modified the TLC/Salmonella assay described by Bjorseth and his colleagues by replacing the double-agar system with a single overlay composed of agar, tester strain, and the metabolic activation system. This modification simplified the technique, reduced preparation time, and, additionally, provided direct contact between the bacteria and partitioned components on the chromatogram, ostensibly enhancing detection of nonpolar and intermediately polar compounds. A complete description of this work has been published elsewhere (Houk and Claxton, 1986).

Ten hazardous wastes were screened for mutagenic activity using this modified assay. Samples were selected from a variety of dissimilar waste types, including acids, caustics, tars, emulsions, and sludges. Solids, semi-solids, and liquids were represented, as were inorganic and organic wastes.

The detection capabilities of this modified TLC/Salmonella assay were challenged with fourteen compounds known to be mutagenic in the Salmonella plate incorporation assay. Mutagens were selected from a wide range of chemical classes and polarities, and included alkylating agents, aromatic amines, antitumor agents, a nitrosamine, and a nitroaromatic.

MATERIALS AND METHODS

The hazardous waste samples evaluated in this study were obtained from Battelle Columbus Laboratories, Columbus, Ohio, courtesy of Dr. M. McKown. Table 1 provides gross chemical and physical characterizations and a cursory description of each waste tested. Results from gas chromatography/mass spectrometry (GC/MS), emission spectroscopy, and analysis for purgeable and semi-volatile organic compounds have been published elsewhere (Battelle, 1981; Houk, 1984; Miller et al., 1981; Warner et al. 1981).

Most samples arrived in physical states that favored direct application to TLC plates (liquids, emulsions, sludges, and a tar). The organic still bottoms and the dewatered municipal sludge, however, were solids and could not be applied to plates in their unadulterated states.

Ethanol (ETOH) extracts of these wastes were tested. Ethanol and dimethyl sulfoxide (DMSO) extracts of the coke plant waste were also obtained and were tested in addition to the crude sample. Extractions were performed as previously described (Andon et al., 1985).

The TLC/Salmonella technique was a modification of the procedure developed by Bjorseth et al. (1982) and is described in detail in Houk and Claxton (1986). The TLC separations were performed on commercially available glass-backed cellulose plates (100 mm x 100 mm). Neat samples or extracts were spotted on the plates at an origin 15 mm from the bottom edge and 20 mm apart. Doses ranged from approximately 0.1 percent to 16 ul per application point. Plates were developed at room temperature in chloroform for 15 to 20 min, at which time the solvent front had ascended to approximately 10 mm from the top edge of the plate. When chloroform proved ineffective in fractionating the sample, other carrier systems were utilized (see Results and Discussion).

After the plates were removed from the developing chamber and the mobile phase had evaporated from the plate, developed plates were inspected for chromatographic patterns using ambient and ultraviolet light sources. Chromatograms were placed sorbent side up into oversized Petri dishes (150 mm by 15 mm) for mutagenicity testing. Genotoxic analyses were performed in situ. A mixture containing agar, minimal growth medium, the bacterial strain, and the optional metabolic activation system was poured over the TLC plates. The Petri dishes were incubated for 72 h at 37°C.

Salmonella typhimurium strains TA98 and TA100 were obtained from Dr. Bruce Ames. Rat liver homogenate (S9) was prepared from rats injected with Aroclor 1254 as previously described (Ames et al., 1975). Developed blank TLC plates were used as negative controls. Undeveloped plates spotted with 2-anthramine, 2-nitrofluorene, or sodium azide were used as positive controls.

After incubation, the plates were examined for toxicity, as evidenced by (1) a reduction in the overall number of revertants when compared to spontaneous values or (2) a discrete toxic zone devoid of bacterial growth. A mutagenic event was indicated by the appearance of one or more localized clusters of histidine revertants or as a reproducible twofold or greater increase in revertant colonies (for the total plate) over spontaneous background values. Dose-response relationships were examined by applying four different volumes per plate. Independent experiments were performed to guarantee reproducibility and to confirm positive or negative findings. Results were documented by tracing observed chromatographic patterns and revertant colony locations onto transparent sheets of cellulose acetate.

To examine the predictive capabilities of the assay, fourteen Salmonella mutagens of various classes and polarities were selected for testing. Increasing volumes of the mutagen solution were spotted in clockwise fashion at the four corners of a cellulose plate. Plates were not chromatographed, but were placed undeveloped into Petri dishes and tested for mutagenic response using the single overlay previously described.

RESULTS AND DISCUSSION

Analysis of Hazardous Wastes

Results from the mutagenicity testing of the hazardous waste samples are given in Table 2.

ORGANIC SLUDGE

A relatively nonpolar mutagenic component migrated to a high Rf value following chromatography of the organic sludge sample. Figure 1a illustrates the observed mutagenic response when the chromatogram was tested with TA98 in the absence of metabolic activation.

The diluted counterpart of this sludge, spiked with eleven organic compounds, failed to demonstrate similar activity (Table 2). Two explanations are plausible. The organic compounds which were added to the original sludge could have interfered with the mutagenic expression of the resulting sample. On the other hand, diluting the parent sludge may have masked the original activity, permitting mutagenic expression of the added compounds.

EDC SPENT CAUSTIC

The EDC spent caustic contained a mutagen(s) that induced a dose-dependent cluster of revertant colonies in both strains without metabolic activation. Activity was localized around fluorescent orange bands that migrated with the solvent front (Figure 1b). The migration of this direct-acting mutagen suggests that the compound is relatively nonpolar.

HERBICIDE MANUFACTURING ACID

With activation, the herbicide manufacturing acid produced intense clustering of revertant colonies around the points of sample application (both strains). Clustering was localized within circular fluorescent zones (Figure 1c). At doses greater than 1 ul, a zone of toxicity surrounded by a ring of revertant colonies was evident. These results indicate that indirect-acting mutagen(s) are present that are toxic to Salmonella when administered at sample doses greater than 1 ul.

The mutagenic constituent present in this herbicide waste was not desorbed by chloroform and consequently did not ascend the TLC plate. Thus, two nonpolar mobile phases described for pesticide separations were selected to try to enhance migration. The first was a solvent system for separation of chlorinated pesticides (heptane-acetone, 98:2) described by Stephens and Chan (1980). The second was a system that has proven useful in the separation of organophosphorus pesticides (hexane-acetone, 5:1) (Stephens and deVera, 1979). Both solvent systems failed to partition the herbicide waste. A polar mobile phase (dichloromethane/ethanol/water, 10:20:1) described by Tomingas et al. (1977) subsequently proved successful in desorbing the mutagenic compound(s). Most revertant activity remained localized midway up the plate within the fluorescent zone (Figure 2).

The initial localization of revertant activity around the points of sample application suggests that the mutagens are very polar. Changes in the mobile phase system from nonpolar to polar have supported this assumption. These findings demonstrate the usefulness of testing different solvent systems to more clearly define chemical properties.

COKE PLANT WASTE

The neat coke plant waste produced approximately a two-fold increase in the number of spontaneous His⁺ revertants when tested with TA98 in the absence of exogenous activation. Ethanol and DMSO extracts of this sample produced clusters of colonies at points of sample application (figures not included). Independent analysis using the standard plate incorporation assay supports our findings of mutagenic activity in the extracts (Andon et al., 1985). Additional verification of the mutagenic potential of this coke plant waste comes from GC/MS analysis, which revealed the presence of the mutagens/carcinogens benzo(a) pyrene (1.12ug/g), benzo(b) and benzo(k)fluoranthene (0.69 ug/g), and chrysene and benzo(a)anthracene (0.5 ug/g) (Miller et al., 1981).

INK PIGMENT SLUDGE

No mutagenic activity was evident when ink pigment sludge was tested with TA98. With TA100, results proved inconclusive. Three independent experiments were performed with increasing dosages, but convincing results could not be obtained.

LATEX PAINT WASTE

Results from the TLC/Salmonella assay of the latex paint waste were negative. However, elemental analysis of this waste indicated the presence of several carcinogenic and mutagenic metals, including cadmium, arsenic, and selenium (Miller et al., 1981). In general, the Salmonella reversion assay does not detect mutagenic metals without bioassay modifications, so it follows that the TLC/Salmonella assay would also prove unsuitable for the routine screening of metal-containing wastes.

COAL GASIFICATION TAR

The coal gasification tar was too viscous to be drawn into a pipette tip. Instead, the tip was touched onto the surface of the sticky substance and a small amount of tar was transferred to the TLC plate. Consequently, applied sample volumes are unknown.

When chromatographed, this sample streaked the length of the plate with no definition of component parts. Mutagenicity testing suggests that this complex sample contains indirect-acting frameshift mutagens that are not particularly water-soluble. This conclusion is based upon the fact the TA98 (+S9) revertants are localized along the length of the visualized streak and are not profuse across the plate (Figure 1d). There also appear to be direct-acting frameshift mutagens that are more water-soluble, as evidenced by the diffuse response observed on plates without metabolic activation (figure not shown).

ORGANIC STILL BOTTOMS, ETOH EXTRACT

There was no evidence of mutagenic or toxic activity in either strain.

DEWATERED MUNICIPAL SLUDGE, ETOH EXTRACT

Revertant clustering was observed in the area of the origin when the chromatogram of the municipal sludge was tested with metabolic activation (both strains). These results suggest the presence of indirect-acting mutagens that are polar and probably water-soluble.

OIL REFINING WASTE

In independent duplicate experiments there appeared a slight dose-response clustering around the origin in TA98 with activation. This effect was evident only at low doses (from 0.5 to 2 ul) and disappeared at volumes of 4 ul and greater. The explanation for this response may be linked to the influence of a fluorescent spot that became larger as sample volumes increased. This separated component appeared to be toxic to the bacteria. An overall reduction of colony counts vis-a-vis control plates was also noted.

Supportive evidence of this sample's toxic potential comes from a study using the CHO cytotoxicity test (Andon et al., 1985). This sample produced a greater than 50% reduction both in the cellular ATP concentration and in the viability index in Chinese hamster ovary cells. Because oil refining wastes have been shown to contain significant levels of lead and chromium (U.S. Environmental Protection Agency, 1980), the observed cytotoxic response may ostensibly be linked to these toxic metals.

Analysis of Model Compounds

Results from the testing of the Salmonella mutagens are given in Table 3. Eleven of the fourteen test compounds were positive. Three compounds --benzo(a)pyrene (BaP), epichlorohydrin, and dimethylcarbamy chloride--registered negative responses, even at very high dose levels (Table 3). Previous TLC/Salmonella research on BaP also resulted in negative findings (Bjorseth et al., 1982). As reported by Alfheim et al., (1983) and Moller et al. (1983), this assay system appears unsuitable for the detection of non-polar organics such as unsubstituted PAHs.

The lack of demonstrable mutagenic activity shown by epichlorohydrin and dimethylcarbamy chloride is presumably linked to their high volatility. These unstable compounds probably volatilized from the chromatography plate prior to testing, thereby precluding detection by the mutagenesis assay.

A nonmutagen (1-naphthylamine, 2-80 ug) was also tested and did not register a mutagenic response.

CONCLUSION

Our findings demonstrate that the TLC/Salmonella assay described in this paper can detect mutagens in complex hazardous wastes. Of ten wastes tested, seven were found to contain mutagenically active compounds. The assay proved to be applicable to a broad range of chemically and physically dissimilar waste types. Liquid or near-liquid samples could be applied to the TLC plates without preparatory workup. Solid wastes, however, had to be converted to a form suitable for TLC application and development. The system proved sensitive to microliter volumes of crude test sample.

Additionally, our investigation has shown that detection capabilities cover a wide range of mutagen classes and polarities. Mutagenic activity was detectable in the microgram range. Compounds refractory to analysis by the TLC/Salmonella assay included two mutagens that were volatile and an unsubstituted PAH, a class that does not ordinarily evoke a mutagenic response in a spot test.

Chemical and physical properties of detected mutagens may be inferred from parameters such as localization on the TLC plate (expressed as an R_f value), polarity of the mobile phase, and response to visualization techniques (fluorescence, color, etc.). Fluorescent mutagens were present in the organic sludge, the EDC spent caustic, the herbicide manufacturing acid, and the coal gasification tar. Polar mutagens (those with low R_f values) were detected in the herbicide manufacturing acid, the municipal sludge, and the oil refining waste, while nonpolar mutagens were detected in the organic sludge and the EDC spent caustic.

The TLC/Salmonella assay offers several important advantages over other coupled fractionation/bioassay schemes. The procedure is very rapid. Fractionation of the samples by chromatography can be completed within 10 to 20 minutes. Mutagenicity results are documented after 72 hours. Samples can be applied to the system in their neat state; thus, extraction procedures are obviated except for samples that cannot be chromatographed in their crude form (e.g., solids). Compounds that may exert synergistic or antagonistic effects are separated from the complex sample during chromatography and are detected independently. Problems with sample toxicity are minimized.

In summary, the TLC/Salmonella assay represents a simple screening tool for the qualitative detection of mutagens in complex environmental samples. The speed and economy with which results can be obtained make it useful for routine testing and for processing large numbers of samples. The presumptive identification of mutagenic constituents in a sample can lead to a systematic selection of that sample for more conclusive chemical or biological evaluation.

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FIGURE 1: SEPARATION OF MUTAGENIC COMPOUNDS BY THE TLC/SALMONELLA ASSAY.

Samples were spotted at increasing volumes across the plate, chromatographed with chloroform, and tested for mutagenic activity. Sample application points and chromatographed spots are indicated (see text for discussion). Negative control plates (spontaneous revertants) are also illustrated. o, application point, -----, solvent front.

- (a) Organic Sludge (0.25, 0.5, 1.0, 2.0 ul) tested with TA98, -S9.
- (b) EDC Spent Caustic (2, 4, 6, 8, ul) tested TA100, -S9.
Plate was broken during excision of top corners.
- (c) Herbicide Manufacturing Acid (0.5, 1.0, 2.0, 4.0 ul) tested with TA100, +S9.
- (d) Coal Gasification Tar (unknown volumes) tested with TA98, +S9.

TABLE 1
DESCRIPTION OF HAZARDOUS WASTE SAMPLES

Waste	Physical state	pH ^a	Total solids ^a (%)	Description
Organic sludge	Liquid	NM	NM	Sludge from an industrial solvent recovery process
Organic sludge, spiked	Liquid	NM	NM	Sludge described above, diluted and fortified with 11 organics
EDC spent caustic (water portion)	Liquid	5.2	<1.0	Clear, tan liquid waste from ethylene dichloride production
Herbicide mfg. acid	Liquid	0.5	42	Thin, gray aqueous suspension from an herbicide production process
Coke plant waste	Liquid	8.0	4	Thin, brown fluid with suspended solids, 95% DMSO-soluble, water-insoluble
Ink pigment sludge	Semi-solid	8.7	14	Pourable black jelly from ink production
Latex paint mfg. waste	Suspension	7.5	41	Thick polymeric emulsion of pigment, 50% soluble in DMSO
Coal gasification tar	Tar	8.1	79	Black, viscous tar produced during coal gasification
Organic still bottoms	Solid	3.8	83	Thick, black slurry from tank bottoms
Dewatered municipal sludge	Solid	7.5	44	Dark, wet cake of chemically treated municipal sludge
Oil-refining waste	Semi-solid	7.0	59	Dark liquid with black flocculent and oil droplets, DMSO- and water-soluble

^a Analysis performed by Research Triangle Institute (Sparacino, 1983).
NM, not measured.

TABLE 2

RESULTS FROM THE TLC/SALMONELLA ANALYSIS OF HAZARDOUS WASTES

Waste	Extract ^a	TLC plate dose range (μ l/application)	Mutagenicity			
			TA98		TA100	
			+ S9	- S9	+ S9	- S9
Organic sludge	NEAT	0.25-2	+ ^b	+ ^c	+ ^d	-
Organic sludge, spiked	NEAT	0.25-16	-	+ ^d	-	-
EDC spent caustic	NEAT	0.5-8	-	+ ^c	-	+ ^{b,c}
Herbicide mfg. acid	NEAT	0.1-4	+ ^{b,c}	-	+ ^{b,c}	-
Coke plant waste	NEAT	0.5-10	-	+ ^b	-	-
	EtOH	0.5-6	+ ^c	-	-	-
	DMSO	0.5-8	-	-	-	+ ^d
Ink pigment sludge	NEAT	0.5-4	-	-	incl	incl
Latex paint mfg. waste	NEAT	0.5-12	-	-	-	-
Coal gasification tar	NEAT	unknown	+ ^{b,c}	+ ^b	+ ^d	-
Organic still bottoms	EtOH	0.1-8	-	-	-	-
Dewatered municipal sludge	EtOH	1-16	+ ^d	-	+ ^d	-
Oil-refining waste	NEAT	0.5-4	+ ^d	T	-	-

^a Extract: NEAT, unadulterated; EtOH, ethanol; DMSO, dimethyl sulfoxide.

^b 2-fold or greater increase in revertant counts (for the total plate) over spontaneous background values.

^c Clustering of colonies; distinct localization.

^d Clustering of colonies; localization detected by grid analysis.

incl, inconclusive findings.

T, toxic.

TABLE 3

RESULTS FROM TLC/SALMONELLA ANALYSIS OF MODEL COMPOUNDS

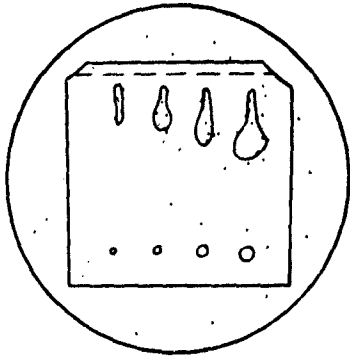
Compound	Responsive dose range ^a (μ g/application)	Mutagenic activity		
		Result	Strain	Activation
Sodium azide	2-16	+ ^b	TA100	- S9
MMS	2-16	+ ^b	TA100	- S9
β -Propiolactone	100-400	+ ^b	TA100	- S9
2-Nitrofluorene	2-16	+ ^c	TA98	- S9
Cyclophosphamide	2-16	weak + ^c	TA100	+ S9
	20-80	+ ^c	TA100	+ S9
2-AAF	2-16	+ ^c	TA98	+ S9
	20-80	+ ^c	TA100	+ S9
Daunomycin	0.125-1	+ ^c	TA98	- S9
	2-16	toxic	TA98	- S9
Streptozotocin	2-16	+ ^d	TA100	- S9
4-NQO	0.5-16	+ ^d	TA98	- S9
	0.5-16	+ ^d	TA100	- S9
MNNG	2-16	+ ^d	TA100	- S9
2-Anthramine	2-16	+ ^d	TA98	+ S9
	2-16	+ ^d	TA100	+ S9
Benzo[a]pyrene	2-80	-	TA98	+ S9
	2-80	-	TA100	+ S9
Epichlorohydrin	2-200	-	TA100	- S9
Dimethylcarbamyl chloride	2-2000	-	TA100	- S9

^a Responsive dose range does not necessarily reflect the lowest possible or highest achievable dose. It merely indicates those doses tested.

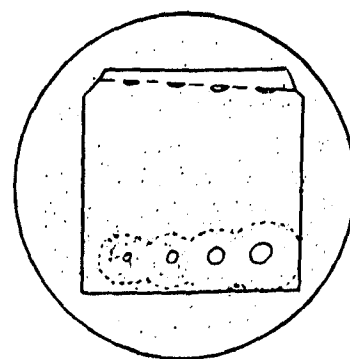
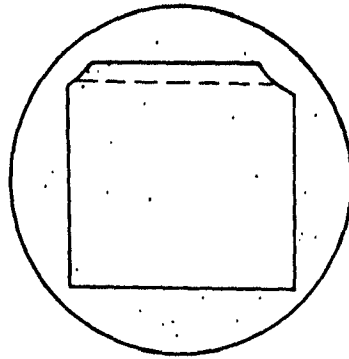
^b Profusion of revertant colonies across the plate.

^c Localized clusters of revertant colonies at points of sample application.

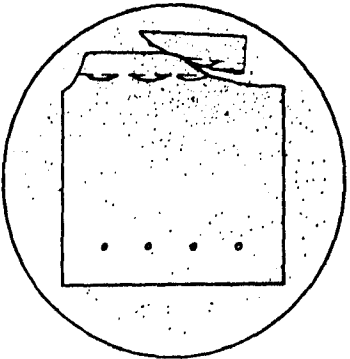
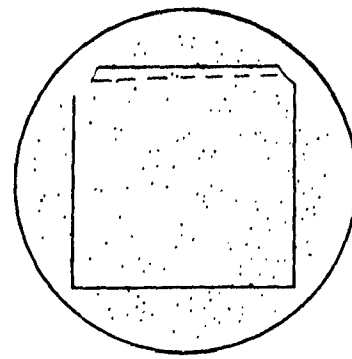
^d Dose-dependent ring of revertant colonies around a central toxic zone.



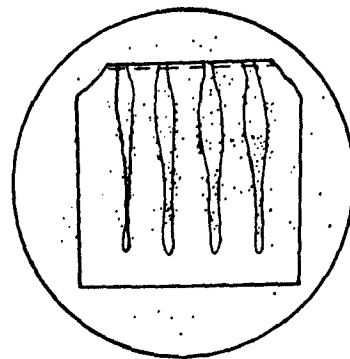
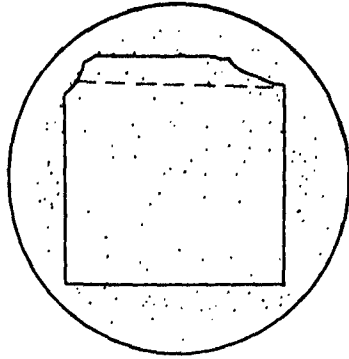
(a)



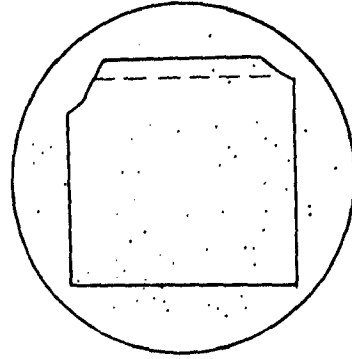
(c)



(b)



(d)



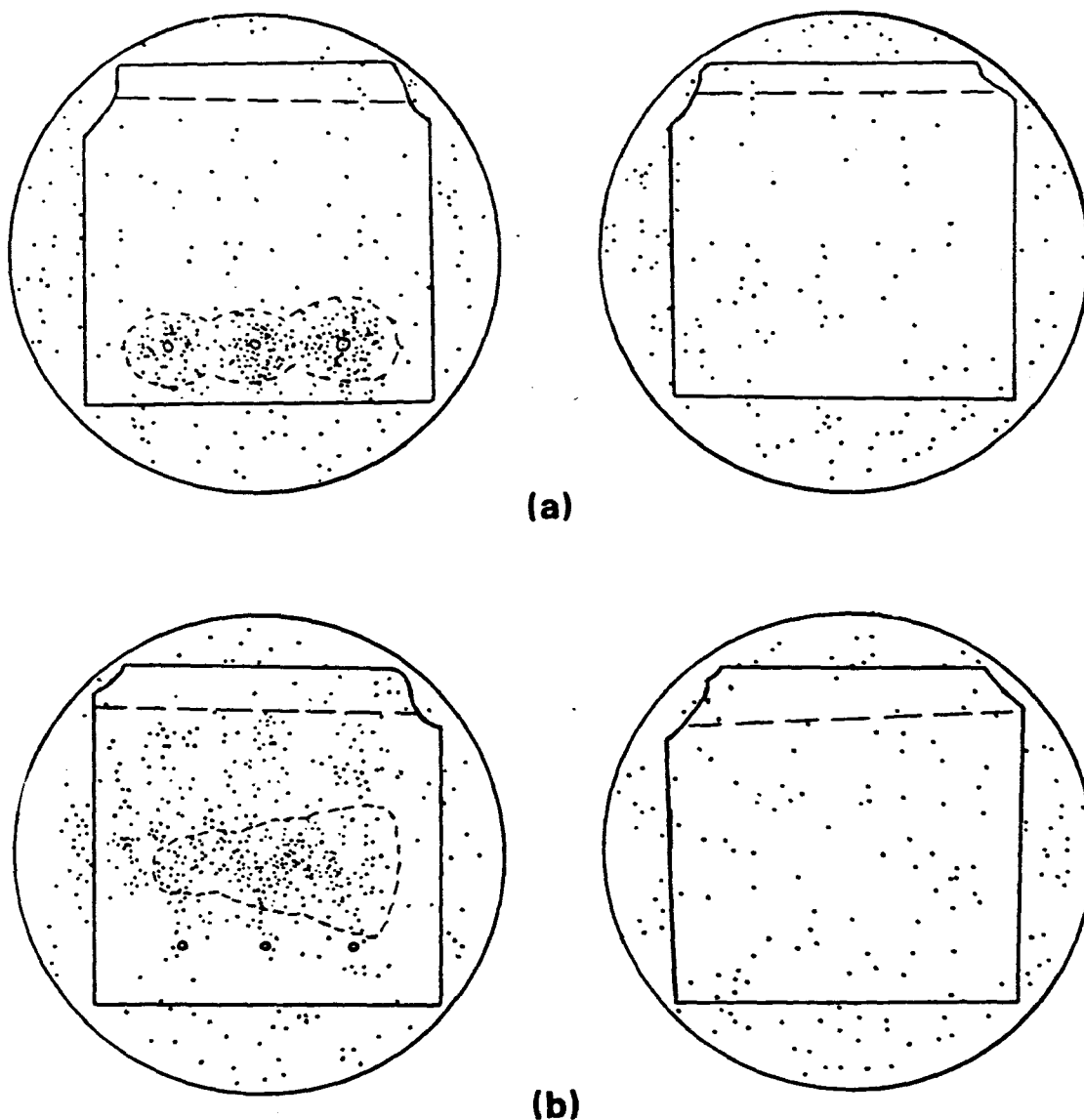


FIGURE 2: TLC/SALMONELLA ANALYSIS OF THE HERBICIDE MANUFACTURING ACID
CHROMATOGRAPHED WITH DIFFERENT SOLVENT SYSTEMS

Fluorescent zones (denoted by a dashed line) are located around sample application points in (a) and midway up the plate in (b). Negative control plates (spontaneous revertants) are also illustrated.

(a) Sample Volumes: 0.1, 0.25, 0.5 ul
Mobile Phase: Chloroform
Mutagenicity Testing: TA100 with activation

(b) Sample Volumes: 0.1, 0.25, 0.5 ul
Mobile Phase: DCM/ETOH/Water, 10:20:1
Mutagenicity Testing: TA100 with activation

o, application point; - - - -, solvent front.

METHODOLOGY FOR EVALUATING POTENTIAL HUMAN HEALTH EFFECTS OF MICROORGANISMS THAT DEGRADE HAZARDOUS WASTES

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ABSTRACT

Microorganisms are being developed to environmentally degrade hazardous wastes. Before such organisms are deployed, methods need to be designed to monitor the organisms and waste by-products for potentially adverse human health and environmental effects. Initial work in our laboratory involved the study of the biological effects of a mutant microorganism that biodegrades polychlorinated biphenyl compounds (PCB's). We have designed several mouse model systems to examine the ability of these organisms to colonize the intestines of the mouse and compete with the resident intestinal flora. These include two 48 hour studies with and without ampicillin pretreatment, a 14 day study in conventional and metabolism cages, and examination of the fecal material. Mice were dosed by gavage with 10^3 , 10^6 , or 10^9 organisms and sacrificed at time intervals up to 48 hours following exposure. The hazardous waste degrading microorganisms, Pseudomonas aeruginosa, was recoverable on a selective medium in all methods evaluated. The normal intestinal flora was also monitored. This included enumeration of the lactose positive enteric microorganisms, total aerobic and anaerobic counts, and the obligately anaerobic, predominantly Gram-negative rod populations. Depending on the method utilized, an alteration in one or more of the normal flora was observed in a dose related manner.

INTRODUCTION

Microorganisms are being isolated and developed to biodegrade hazardous wastes such as polychlorobiphenyls (PCB) (18, 24), formaldehyde (18), chlorobenzoates (10, 4) and pesticides (21, 20). The ability of microorganisms to detoxify the target compounds is being enhanced through mutagenicity or recombinant DNA techniques. The ultimate goal is to apply these organisms to a site contaminated with an environmentally hazardous compound and metabolize it to a nonhazardous state, in situ. The need to transport the compound to a central location such as a landfill or incinerator would be unnecessary, thus eliminating the associated risks.

Very little research has been reported on the potential human health effects associated with these biodegradative microorganisms. The proposed release of these bacteria constructed through the use of recombinant DNA techniques into the environment has received national attention (6, 7, 9). Federal and state agencies now regulate the

environmental application of these bacterial strains (17, 19). The purpose of this study is to propose and evaluate methods that will examine the potential human health effects of these recombinant, mutant, and naturally occurring microorganisms used to detoxify hazardous environmental chemicals. The methods presented in this study examine the potential of these biodegradative microbes to colonize the intestines of mice and determine how they compete with the normal flora. An alteration of the normal flora may indicate a change in the intestinal metabolism that could have an effect on digestion, absorption, and the overall physiology of the host. The results reported depict the competition and colonization potential of a PCB degrading microorganisms, P. aeruginosa, that was isolated from a commercially available product for PCB degradation. Manuscripts submitted and in preparation describe this research in greater detail.

MATERIALS AND METHODS

Chemicals. All chemicals utilized in this study were obtained commercially and of reagent grade. Vancomycin HCl, kanamycin sulfate, ampicillin, hemin, and vitamin K₁ were purchased from Sigma Chemical Company, St. Louis, MO. Mercuric chloride was obtained from Mallinckrodt, Paris, KY.

Media. P. aeruginosa stain BC16 was enumerated on Pseudomonas isolation agar (Difco, Detroit, MI) containing mercuric chloride (HgCl₂) (30 ug/ml). MacConkey agar (Difco) was prepared according to the manufacturer's direction. Brucella blood agar (BBA) was prepared as follows: Brucella agar (BBL Microbiology Systems, Cockeysville, MD) as directed, 5% (v/v) laked, defibrinated sheep blood (Environmental Diagnostics, Burlington, NC), vitamin K₁ (1.0 ug/ml) and hemin (5 ug/ml^{30 3926, 11}). Where indicated, vancomycin HCL (7.5 ug/ml) and kanamycin (75 ug/ml) were added to BBA after autoclaving. Yeast extract-tryptone (YT) (15) medium without added NaCl was used as culture medium where indicated.

Bacterial strains. P. aeruginosa was isolated from BI-Chem 1006 PB (Sybron Chemicals, Inc., Salem, VA) as directed by the manufacturer. Bacterial strains in this product were designed to biodegrade polychlorinated biphenyl compounds. Identification was confirmed by Gram staining and using the API20E method (Analtab Products, Plainview, NY).

Culture conditions. All incubations for bacterial growth were done at 37°C. Inocula for dosing was prepared by sedimenting an overnight 25 ml YT culture of P. aeruginosa. The cells were resuspended in 5 ml Dulbeccos phosphate buffered saline (PBS) (GIBCO Laboratories, Chagrin Falls, OH) prior to animal dosing. All other incubations were done aerobically for 48 h or anaerobically for 72 h.

Animal studies. Sixty-day old strain CD-1 male mice (Charles River Laboratories, Raleigh, NC or Kingston, NY) were used in the studies. Animals were given sterilized food (Sterilizable Rodent Blox, Continental Grain Company, Chicago, IL) and water ad libitum. Animals were housed in cages with wood shaving bedding unless otherwise indicated. When metabolism cages (Nalge Company, Rochester, NY) were employed, animals were fed sterile pelletized chow (Dustless Pellets-45 mg. R.C., Bioserv Inc., Frenchtown, NJ) and water ad libitum. Animals were administered 0, 10^3 , 10^6 , or 10^9 CFU/ml of P. aeruginosa by gavage. At time intervals (0, 3, 6, 12, 24, 48 for the 48 h study, 14 days for the metabolism cage study, and 3, 12, 24, and 48 h for the ampicillin pretreatment 48 h study) the animals were sacrificed and the intestines surgically removed and immediately placed into 5 ml reduced PBS. The intestines were homogenized using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY) under a constant stream of nitrogen and then placed into an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) containing an atmosphere of 5% CO₂, 10% H₂, and 85% N₂. Serial dilutions of the homogenate were made in reduced PBS and all anaerobic plating and incubation was done in the chamber. Aerobic plating was done aerobically on selective media (Figure 1.)

Where indicated, feces were collected from regular cages on a daily basis (1, 2, and 3 days) or metabolism cages at 0, 1, 2, 4, 6, 8, 10, 12, and 14 days after dosing. Approximately 1.0 g of fecal material was placed into 5 ml PBS, vortexed, and the dosed microorganisms selected on Pseudomonas isolation agar containing HgCl₂ (30 ug/ml).

Three independent animal studies were performed using the above protocol. The first examined the survival and competition potential of the dosed microorganism over a period of 48 h. Results are an average of values enumerated from four animals per dose-time combination. The second study compared the flora from animals housed in regular (five animals per dose-time combination) cages for 14 days after dosing. In the third study, animals were pretreated with 5 mg of ampicillin on days 1 and 2 then 1 mg of ampicillin on day 3 prior to dosing with the microorganism (four animals per dose-time combination). A general outline for the animal studies is shown in Figure 1.

Statistical analysis. Analyses of variance and T-tests were performed using the RS/1 statistical software package (BBN Software Products Corporation, Irving, Texas) on an IBM-AT personal computer.

RESULTS

Recovery of P. aeruginosa in the feces. Fecal material was collected from the cages over a three day period. Recovery of the microorganism was examined by selection on Pseudomonas isolation agar containing HgCl₂. The results are shown in Table 1. P. aeruginosa was recovered only from animals at the higher dose.

A more in depth study was done in metabolism cages, using only the high dose, 10^9 CFU, where the fecal material was collected and the organisms enumerated. P. aeruginosa was recovered from the fecal material for only two days after dosing (Table 1).

Survival of P. aeruginosa in the gastrointestinal tract. A more quantitative assay for the survival of the dosed microorganism was needed so the intestines were analyzed for recovery and competition. Three separate studies were performed to look at the competition of the PCB degrading microorganism in the mouse intestine: 1) enumeration over 48 hours, 2) ampicillin pretreatment followed by enumeration over 48 hours, and 3) enumeration after 14 days in regular and metabolism cages. In both 48 hour studies, the survival of the organism was similar with counts in the 10^1 CFU/g intestines range for animals administered 10^9 CFU (Table 2). Animals dosed with 10^3 CFU yielded less than 10^1 CFU/g intestines after 48 hours in both ampicillin treated and untreated animals.

In the 14 day study, P. aeruginosa appeared to colonize the gastrointestinal tract in animals dosed with 10^9 CFU yielding 4.0×10^4 CFU/g intestines (Table 2). Animals housed in metabolism cages, where fecal material was not in contact with the animals, yielded less than 10^1 CFU/g intestines.

Effect of P. aeruginosa on the normal intestinal flora. Four populations of normal flora were monitored in the 48 hour and 14 day studies. They included 1) lactose positive enteric organisms, 2) total aerobic counts, 3) total obligately anaerobic predominantly Gram-negative rods, and 4) total anaerobic counts.

In the 48 hour study, the lactose positive counts ranged from 5.0×10^3 to 3.8×10^5 CFU/g intestines. Results from the 14 day study gave number of similar magnitude. Ampicillin pretreatment markedly increased these counts yielding a range from 5.4×10^7 to 4.5×10^9 CFU/g intestines. The results are tabulated in Table 3.

The total aerobic count, which included aerobes and facultative anaerobes was also increased in animals pretreated with ampicillin. The counts are shown in Table 4. Ampicillin pretreatment selected for several different species including Enterobacter cloacae (predominant), Proteus vulgaris, and Lactobacillus spp. Though numbers were lower in the animals not pretreated with ampicillin, a more diverse group of organisms was observed and rarely were E. cloacae or P. vulgaris observed. Values in the 10^7 - 10^8 CFU/g intestines range were observed in the animals without ampicillin treatment whereas those that received ampicillin had counts in the 10^7 - 10^9 CFU/g intestines range.

Ampicillin pretreatment greatly reduced the obligately anaerobic Gram-negative rod counts (Table 3). These organisms were selected for by resistance to the antibiotics kanamycin and vancomycin. Included in

this group were Bacteroides spp. and Fusobacterium spp. (8). Ampicillin treatment reduced the counts from 10^7 - 10^8 CFU/g intestines in the 48 hour and 14 day studies to 10^1 - 10^4 CFU/g intestines in the ampicillin pretreatment study. Results are shown in Table 5.

The total anaerobic counts from mice dosed with P. aeruginosa are tabulated in Table 6. Included in this count were facultative and strict anaerobes. Facultative organisms included Streptococcus spp., Staphylococcus spp., Lactobacillus spp., and organisms from the family Enterobacteriaceae (2). Strict anaerobes included the Clostridia, Eubacteria, Bacteroides spp., and the Fusobacteria as well as others (8, 2, 16).

In the 48 hour study, counts range from 2.2×10^8 to 8.7×10^8 CFU/g intestines. The 14 day study in regular and metabolism cages showed similar values. Again, ampicillin increased the observed counts. Values ranged from a low of 2.7×10^4 to a high of 2.2×10^9 CFU/g intestines. The flora enumerated were similar to that observed in the total aerobic counts.

In order to determine whether the presence of P. aeruginosa caused an alteration in the normal murine intestinal flora, statistical analyses were performed on the data collected in Tables 3-6. P-values are reported in Table 7.

DISCUSSION

This study was designed to develop and evaluate methods to determine some of the indirect adverse human health effects potentially associated with microorganisms that degrade hazardous wastes. Four different studies were proposed, three of which involved enumeration of the biodegrading microorganisms from the gastrointestinal tract and one study that examined the fecal material for the organism's presence. Further effects on the normal flora were investigated. They included enumeration of 1) the enteric lactose positive microorganisms, 2) the total aerobic counts, 3) obligately anaerobic Gram-negative rod counts, and 4) the total anaerobic counts.

The initial study involved the examination of the mouse fecal material for the presence of the dosed biodegrading microorganism. This method was utilized by Levy and Marshall (13) and Levy et al. (14) to determine colonization of recombinant Escherichia coli strain in mice. Similar studies were done in human volunteer (1, 14, 22, 23). The results were not very promising with recovery being observed in regular cages only at the highest dose, 10^9 CFU (Table 1). Further studies in metabolism cages extended the recovery time to two days at the same dose. Several questions arose such as whether oxygen or dessication had an effect on the microbial content. In order to avoid these potential problems, the mouse intestines were examined.

None of the mice dosed by gavage with P. aeruginosa appeared unhealthy during these studies. No diarrhea was observed and animal weights did not decrease. No abnormal pathology was observed. Therefore, all of these methods were able to evaluate the direct health effects on mice such as morbidity or mortality.

In the 48 hour study, P. aeruginosa was recoverable from the mouse intestines at all three doses. By the end of 48 hours, organisms were countable on the selective medium.

Ampicillin pretreatment was incorporated into the study in order to alter the normal flora and enhance the survival of the biodegradative microbe, which was resistant to ampicillin. Cohen et al. (5) and Laux et al. (12) used streptomycin to select for colonization by E. coli derivatives. Ampicillin pretreatment did not appear to have a selective effect on the survival of strain BC16 at 48 hours. Survival counts were similar for both the experiments (Table 2). An increase in survival at the other time points was noted in ampicillin treatment animals.

A difference in normal flora counts was observed in the two 48 hour experiments. An increase in total counts was observed for the ampicillin treated animals in all populations monitored with the exception of the obligately anaerobic Gram-negative rods (Table 3-6). Antibiotics have been reported to alter the normal flora in the gastrointestinal tract populations (3, 25). Ampicillin treatment decreased those counts by at least four orders of magnitude (Table 5). The increase in the other counts was due to an increase in the E. cloacae population. This organism is facultative so therefore can grow on MacConkey agar and Brucella blood agar both anaerobically and aerobically.

The resulting alterations in the normal flora between the two 48 hour studies differed. A dose effect (p less than 0.05) was observed for the lactose positive and obligately anaerobic Gram-negative rod counts in animals without ampicillin pretreatment (Table 7). The total anaerobic population counts were affected (p less than 0.1) in the ampicillin treatment experiment (Table 7).

Survival and alteration of the intestinal flora was also examined at the end of 14 days in animals housed in regular and metabolism cages. This provided results from a longer term of study. P. aeruginosa was recovered (4.0×10^4 CFU/g intestines) 14 days after dosing in animals housed in regular cages (Table 2). The results varied from those observed in animals housed in metabolism cages. Mice are coprophagic and the observed results were probably due to this phenomenon. Again the alterations in the normal flora were different. The obligately anaerobic Gram-negative rod and total anaerobic populations showed a significant difference (p less than 0.1) upon exposure to P. aeruginosa (Table 7). Only the total aerobic populations were affected (p less than 0.1) when mice were housed in regular cages.

The comparison of intestinal flora from animals housed in regular or metabolism cages provided a good model to compare repeated exposure versus a single dose. The organisms that were excreted were re-administered through this coprophagic behavior. Because all fecal material was separated from the animals in the metabolism cages, no re-dosing could occur through this route.

The 48 hour study provided a short term method for examining the fate of the biodegrading microorganism and its effect on the normal flora in both conventional mice as well as ones whose normal flora had been altered. The ampicillin treatment may give researchers insight into how compromised humans (those on antibiotic therapy or immunosuppressant drugs) may be effected upon exposure to this class of microorganisms.

These studies provide methods to examine the colonization and competition potential of microorganisms that degrade hazardous wastes. If the normal flora are altered due to the presence of these strains, then the effect on intestinal flora metabolism, absorption, and the host's physiology need to be considered. In vitro methods are currently being developed to investigate colonization, competition and metabolic changes associated with exposure to the biodegraders using both mouse and human intestinal flora.

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Table 1. Recovery of P. aeruginosa from mouse fecal material.^a

Time(days)		0	10 ³	10 ⁶	10 ⁹
Regular Cages	1	- ^b	-	-	+
	2	-	-	-	-
	3	-	-	-	-
Metabolism Cages	0	-	nd ^c	nd	-
	1	-	nd	nd	+
	2	-	nd	nd	+
	3	-	nd	nd	-
	4	-	nd	nd	-
	6	-	nd	nd	-
	8	-	nd	nd	-
	10	-	nd	nd	-
	12	-	nd	nd	-
	14	-	nd	nd	-

^a Mice were dosed with P. aeruginosa and at designated time intervals, approximately 1.0 gram of fecal material was collected and placed into 5.0 ml PBS. A 0.1 ml aliquot was plated onto Pseudomonas isolation agar containing HgCl₂, incubated 24 hours, and the organism enumerated.

^b (-), no organism detected; (+), growth of dosed strain was evident.

^c not determined

Table 2. Survival of P. aeruginosa in mouse intestines.^a

		Dose (CFU/animal)				
		Time(h)	0	10 ³	10 ⁶	10 ⁹
48 hour study	0	<10 ^{1b}	6.1 x 10 ⁰	1.1 x 10 ²	1.1 x 10 ⁶	
	3	<10 ¹	2.4 x 10 ⁰	1.3 x 10 ³	5.0 x 10 ⁵	
	6	<10 ¹	7.5 x 10 ⁰	2.0 x 10 ²	6.0 x 10 ⁵	
	12	<10 ¹	<10 ¹	2.9 x 10 ⁰	1.7 x 10 ⁴	
	24	<10 ¹	<10 ¹	4.6 x 10 ⁻¹	1.8 x 10 ¹	
	48	<10 ¹	2.6 x 10 ⁰	2.0 x 10 ¹	1.6 x 10 ¹	
48 hour study ampicillin pretreatment	3	<10 ¹	1.1 x 10 ¹	n.d. ^d	4.6 x 10 ⁵	
	12	<10 ¹	<10 ¹	n.d.	3.9 x 10 ⁶	
	24	<10 ¹	<10 ¹	n.d.	2.0 x 10 ⁵	
	48	<10 ¹	<10 ¹	n.d.	2.1 x 10 ¹	
		Time(d)				
14 Day study ^e :						
regular cages	14	<10 ¹	n.d.	n.d.	4.0 x 10 ⁴	
metabolism cages	14	<10 ¹	n.d.	n.d.	<10 ¹	

^a Mice were dosed with P. aeruginosa. At designated time intervals, animals were sacrificed, the intestines removed and homogenized under N₂ gas in 5 ml reduced PBS. Homogenates were then placed into an anaerobic chamber and dilutions were made in PBS. A 0.1 ml. aliquot of the diluted material was used to inoculate Pseudomonas isolation agar containing HgCl₂. Plates were incubated 24-48 hours at 37°C. Counts are an average from 4 animals unless stated otherwise.

^b No colonies were detectable on the enumeration medium.

^c Animals were pretreated with 5 mg ampicillin (2 days) followed by 1 mg ampicillin (2 days) prior to dosing with P. aeruginosa.

^d Not determined

^e Counts are an average from 5 animals.

Table 3. Total lactose positive counts in P. aeruginosa dosed mice: enumeration from an intestinal homogenate.^a

		Dose (CFU) x 10 ⁴			
Time		0	10 ³	10 ⁶	10 ⁹
(h)		CFU x 10 ⁴			
48 hour study	0	37.7 ^b	1.1 ^b	1.8 ^b	3.7 ^b
	3	6.1	2.4	0.5	2.4
	6	1.2	10.1	5.5	12.8
	12	1.9	0.5	0.8	1.4
	24	5.9	3.6	4.1	2.3
	48	0.5	1.5	0.6	7.1
(h)		CFU x 10 ⁷			
48 hour study ampicillin pretreatment	3	156.1	67.9	n.d. ^c	184.3
	12	110.8	86.7	n.d.	95.9
	24	449.7	5.4	n.d.	202.3
	48	273.1	189.8	n.d.	337.9
(days)		CFU x 10 ³			
14 day study					
metabolism	14	23.0 ^d	n.d.	n.d.	14.0 ^d
regular	14	1.0 ^e	n.d.	n.d.	0.3 ^e

^a Mice were dosed with 0, 10³, 10⁶, or 10⁹ CFU and sacrificed at indicated time intervals. The intestines were removed, homogenized, and dilutions of the homogenate prepared in PBS. A 0.1 ml. aliquot of the appropriate dilution was spread plated onto MacConkey Agar and plates were incubated 24-48 hours. Counts are an average of values from 4 animals unless otherwise indicated.

^b Counts are an average of values from 2 animals.

^c not determined

^d Counts are an average of values from 10 animals.

^e Counts are an average of values from 5 animals.

Table 4. Total aerobic counts in *P.aeruginosa* dosed mice: enumeration from an intestinal homogenate.^a

		Dose				
		Time	0	10 ³	10 ⁶	10 ⁹
		(h)	CFU x 10 ⁷			
48 hour study	0	4.8 ^b	6.0 ^b	2.6 ^b	15.0 ^b	
	3	5.8	23.4 ^b	9.1	12.9	
	6	2.9	4.5 ^b	14.7	7.1	
	12	1.5	2.2 ^b	1.7	1.5	
	24	9.8	12.9 ^b	13.1	10.8	
	48	7.3	17.2 ^b	12.6	22.5	
		(h)	CFU x 10 ⁷			
48 hour study ampicillin pretreatment	3	200.1 ^b	87.6 ^b	n.d.	247.8 ^b	
	12	116.2 ^b	96.4 ^b	n.d.	107.0 ^b	
	24	786.1 ^b	8.0 ^b	n.d.	311.5 ^b	
	48	411.9 ^b	240.8 ^b	n.d.	529.1 ^b	
		(days)	CFU x 10 ⁷			
14 day study						
metabolism	14	0.22 ^d	n.d.	n.d.	0.23 ^d	
regular	14	0.78 ^e	n.d.	n.d.	2.65 ^e	

^a Mice were dosed with 0, 10³, 10⁶, or 10⁹ CFU and sacrificed at indicated time intervals. The intestines were removed, homogenized, and dilutions of the homogenate prepared in PBS. A 0.1 ml. aliquot of the appropriate dilution was spread plated onto Brucella Blood Agar and plates were incubated 48 hours. Counts are an average of values from 4 animals unless otherwise indicated.

^b Counts are an average of values from 2 animals.

^c not determined

^d Counts are an average of values from 10 animals.

^e Counts are an average of a values from 5 animals.

Table 5. Total obligately anaerobic predominantly Gram negative rod counts in P.aeruginosa dosed mice: enumeration from an intestinal homogenate.^a

		Dose (CFU)				
		Time	0	10 ³	10 ⁶	10 ⁹
		(h)	CFU x 10 ⁸			
48 hour study	0	1.0 ^b	3.3 ^b	1.8 ^b	2.0 ^b	
	3	4.7 ^b	2.9 ^b	1.8 ^b	3.1 ^b	
	6	3.6 ^b	3.7 ^b	3.8 ^b	4.5 ^b	
	12	0.9 ^b	1.3 ^b	1.7 ^b	2.6	
	24	2.0 ^b	1.3 ^b	2.0	2.4	
	48	1.1 ^b	1.1 ^b	2.3	2.4	
		(h)	CFU x 10 ²			
48 hour study ampicillin pretreatment	3	>10 ⁴	16.3	n.d. ^c	1.0	
	12	35.8	0.5	n.d.	3.4	
	24	180.2	10.4	n.d.	683.9	
	48	>10 ⁴	672.3	n.d.	663.6	
		(d)	CFU x 10 ⁷			
14 day study						
metabolism	14	11.08 ^d	n.d.	n.d.	6.54 ^d	
regular	14	8.80 ^d	n.d.	n.d.	13.88 ^d	

^a Mice were dosed with 0, 10³, 10⁶, or 10⁹ CFU and sacrificed at indicated time intervals. The intestines were removed, homogenized, and dilutions of the homogenate prepared in PBS. A 0.1 ml. aliquot of the appropriate dilution was spread plated onto Brucella Blood Agar supplemented with vancomycin and kanamycin. Counts are an average of values from 4 animals unless otherwise indicated.

^b Counts are an average of values from 2 animals.

^c not determined

^d Counts are an average of values from 5 animals.

Table 6. Total anaerobic counts in P.aeruginosa dosed mice: enumeration from an intestinal homogenate.^a

	Time		Dose		
	(h)	0	10 ³	10 ⁶	10 ⁹
48 hour study	0	2.3 ^b	7.9 ^b	4.8 ^b	4.0 ^b
	3	6.9	6.0 ^b	4.2	6.4
	6	6.2	8.7 ^b	7.6	6.2
	12	2.2	2.4 ^b	2.4	2.6
	24	2.6	2.7 ^b	3.5	4.1
	48	3.4	3.5 ^b	5.9	6.3
	(h)	CFU x 10 ⁷			
48 hour study ampicillin pretreatment	3	131.8	2.7	n.d. ^c	203.4
	12	145.6	42.1	n.d.	117.6
	24	145.7	9.4	n.d.	105.9
	48	203.8	223.5	n.d.	192.6
	(d)	CFU x10 ⁸			
14 day study					
metabolism	14	8.45 ^d	n.d.	n.d.	3.25 ⁵
regular	14	5.14 ^d	n.d.	n.d.	3.84 ⁵

^a Mice were dosed with 0, 10³, 10⁶, or 10⁹ CFU and sacrificed at indicated time intervals. The intestines were removed, homogenized, and dilutions of the homogenate prepared in PBS. A 0.1 ml. aliquot of the appropriate dilution was spread plated onto Brucella Blood Agar. Counts are an average of values from 4 animals unless otherwise indicated.

^b Counts are an average of values from 2 animals.

^c not determined

^d Counts are an average of values from 5 animals.

Table 7. P-values for the effects of P.aeruginosa dose on the normal flora.

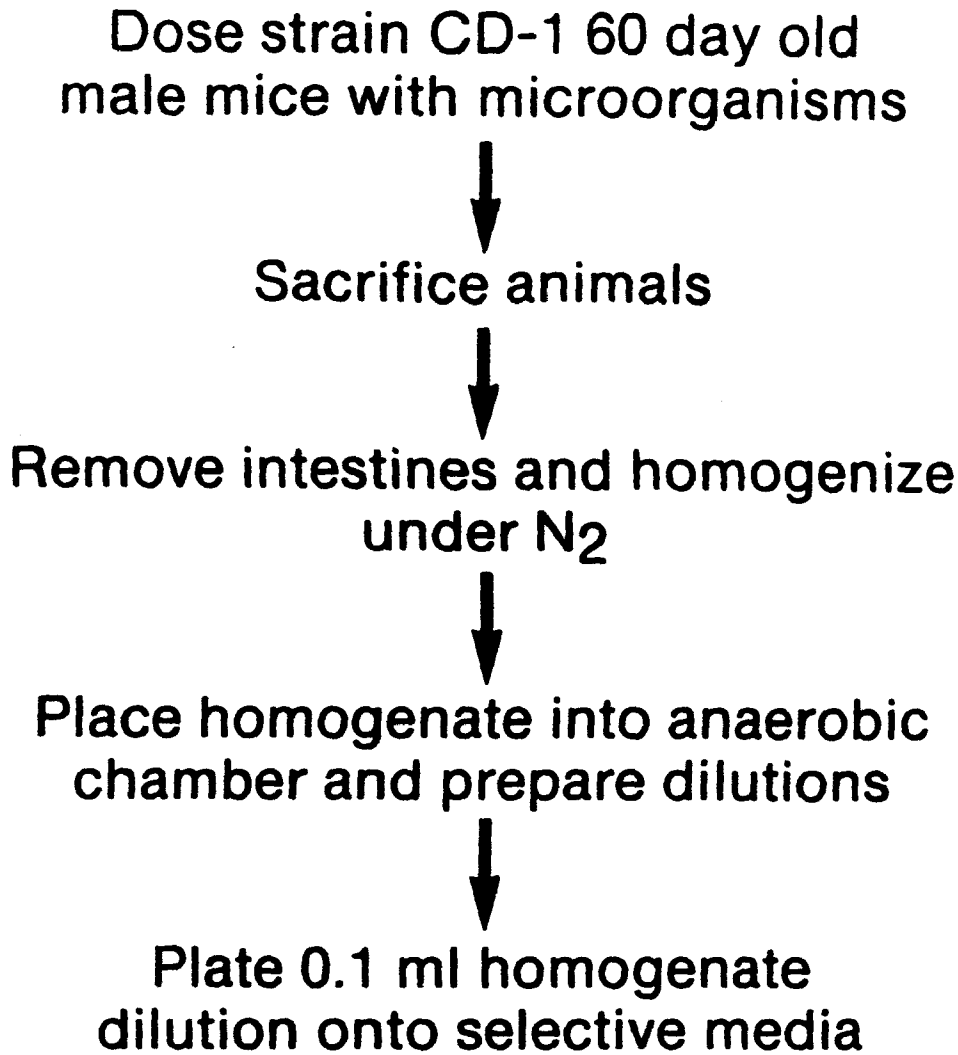
		48 hour study ^a	ampicillin pretreatment 48 hour study ^a	14 day study ^b metabolism cages	14 day regular cages
Lactose positive colonies	Dose	0.012	0.271	0.615	0.338
	Time	0.002	0.451	n.d. ^c	n.d.
	Nonadditivity	0.000	0.757	n.d.	n.d.
Total aerobic colonies	Dose	0.292	0.270	0.234	0.100
	Time	0.044	0.374	n.d.	n.d.
	Nonadditivity	0.866	0.684	n.d.	n.d.
Obligately anaerobic Gram- negative rods	Dose	0.013	n.d.	0.027	0.236
	Time	0.000	n.d.	n.d.	n.d.
	Nonadditivity	0.347	n.d.	n.d.	n.d.
Total anaerobic colonies	Dose	0.292	0.073	0.063	0.338
	Time	0.044	0.079	n.d.	n.d.
	Nonadditivity	0.866	0.513	n.d.	n.d.

^a P-values from a two-way analysis of variance of the data obtained from enumeration of the normal intestinal flora.

^b P-values from a T-test of the data obtained from enumeration of the normal intestinal flora.

^c not determined

Figure 1. Protocol for animal study



Anaerobic media

- Brucella laked blood agar
- Brucella laked blood agar + vancomycin and kanamycin

Aerobic media

- *Pseudomonas* isolation agar
- MacConkey agar
- Brucella laked blood agar

BIOASSAY DETERMINATION OF SOIL ASSIMULATIVE CAPACITY

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ABSTRACT

Bacteria, earthworms and ants were used to define the assimilative capacity of various soil types for heavy metals (Cu, Cd, Zn) wood treating and petrochemical waste products. Mixed soil microbial respiration activity to Copper increased proportionally (less toxic) to the increase in organic content of the soils studied. Zinc toxicity to the mixed microbial respiration activity was only reduced in the high organic Rifle Soil. The earthworm Eisenia fetida was exposed to varying concentrations of Cu, Cd, and Zn added to filter paper and artificial soil substrates. Zinc toxicity was similar in each substrate. The bioavailability of Cd and Cu to E. Fetida decreased approximately two and four-fold respectively in artificial soil relative to their availability in the filter paper aqueous contact test. Harvester ants, Pogonomyrmex owyheei, were exposed to 1.0% (wt/wt) aqueous concentrations of wood treating, drilling fluid and oil slop wastes, applied to filter paper and Ritzville silt-loam-soil. The toxicity rank order was similar for both the filter paper and Ritzville soil substrates. However, Ritzville soil assimilative capacity ranged from a two-fold reduction of toxicity for wood treating waste to a five-fold toxicity reduction for both the drilling fluid and slop oil wastes. These results demonstrate the capability of bioassays to define the assimilative capacity of soils for selected waste products. They also show that the assimilative process is very complex and that the capacity varies with the waste product, and that it is not easily predicted from a soils, silt, sand, clay, or organic content.

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RCRA LAND DISPOSAL RESTRICTION PROGRAM

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ABSTRACT

The Congress decided in its reauthorization of RCRA in 1984 to restrict the disposal of various classes of hazardous wastes e.g., solvents and dioxins in land-based units like landfills. EPA promulgated a complex land disposal restriction rule to implement this new law. Solvents and dioxins, for example, have been restricted from land disposal since November of 1986.

Laboratories play an important role in this matter because wastes and soil must be tested to ascertain whether they meet a specified treatment standard for land disposal. The testing procedure is called the Toxicity Characteristic Leaching Procedure (TCLP). It is an improved Extraction Procedure (EP) although it is questionable whether it is an effective measure of leaching potential where hydrophobic chemicals in natural soils are the concern.

The following is an overview of the Land Disposal Restrictions program.

INTRODUCTION

On November 8, 1984 Congress passed the Hazardous and Solid Waste Amendments (HSWA) to the Resource Conservation and Recovery Act. Among other things, these amendments require EPA to evaluate all hazardous wastes according to a strict schedule to determine which wastes should be restricted from land disposal. For wastes that are restricted, the amendments require EPA to set levels or methods of treatment which substantially diminish a waste's toxicity or reduce the likelihood that a waste's hazardous constituents will migrate from the disposal site. Beyond specified dates, restricted wastes which do not meet treatment standards are prohibited from land disposal.

On November 7, 1986 EPA promulgated the first phase of the Land Disposal Restrictions (LDR) by restricting the land disposal of solvent (F001-F005) and dioxin containing wastes. This paper will discuss development and implementation of this first phase of LDR.

In the final rule, EPA defined land disposal to include, but not be limited to, any placement of hazardous waste in:

- Land fills
- Surface Impoundments
- Waste sites
- Injection wells
- Land treatment facilities
- Salt domes or salt beds
- Underground mines or caves
- Concrete vaults or bunkers

The LDR rule covers hazardous wastes placed in land disposal units after the effective dates of the prohibitions. Wastes disposed of before November 7, 1986 do not have to be removed from land disposal for treatment. There are extensions and variances which extend the effective date of particular wastes under certain circumstances.

HSWA required EPA to set levels or methods of treatment which substantially diminish the toxicity of waste or reduce the potential for migration of hazardous constituents. These levels or methods referred to as treatment standards, must minimize short and long term threats to human health and the environment.

To establish treatment standards, EPA identified wastes with similar characterizations (physical and chemical). EPA then categorized these similar wastes into "waste treatability groups." EPA then evaluated identified technologies used to treat the wastes to determine the best demonstrated available technology (BDAT) for each waste treatability group.

Once BDAT is identified, EPA then established the treatment standards as either a specific technology or as a performance level (i.e., the concentration level of hazardous constituents that is representative of treatment by BDAT). For the November 7, 1986 rule covering solvents and dioxins, this is expressed as a concentration level of hazardous constituents in an extract of the waste using the Toxicity Characteristic Leaching procedure (TCLP).

The TCLP is an analytical method used to determine whether the concentrations of hazardous constituents in a waste extract or an extract of the treatment residual meet the applicable treatment standards. EPA promulgated the TCLP for use only in solvents and dioxins final rule, and only then treatment standards are expressed as concentration levels of hazardous constituents in an extract. The TCLP is an "improved" Extraction Procedure (EP) toxicity test. There is some question of the TCLP effectiveness when testing hydrophobic chemicals in natural soils.

In response to the LDR, the Office of Waste Programs Enforcement embarked on a campaign of implementing and enforcing the new regulations. This included development of a comprehensive LDR inspection guidance manual to educate and prepare enforcement personnel.

The LDR guidance manual is composed of 3 main sections. The first section consists of the statutory and regulatory overview. This covers requirements for each potential handler of restricted solvent waste, as well as a brief discussion on applicable extensions and variances. The second section focuses on major concerns with regard to enforcing these new restrictions. It brings to light areas of potential non-compliance and instructs on proper response and documentation. The third section is an inspection checklist. This checklist is comprehensive to include all possible situations encountered at any type facility handling restricted solvent waste.

The checklist gives the inspector a tool to track and record information during an inspection and to gather necessary information to determine a facility's status of compliance with LDR.

A more detailed discussion on the LDR Guidance Manual as well as other enforcement efforts will be presented.

RCRA LABORATORY AUDIT INSPECTION (LAI) PROGRAM

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ABSTRACT

The Resource Conservation Recovery Act (RCRA) requires the owner or operator of a surface impoundment, landfill or land treatment unit that is used to manage hazardous waste to implement a ground-water monitoring program capable of determining a facility's impact on the uppermost aquifer. The Agency has developed guidance entitled, RCRA Ground-Water Monitoring Technical Enforcement Guidance Document, which details the technical aspects of ground-water monitoring system design and operation deemed essential by the Agency in order for a ground-water monitoring system to meet the goals of the RCRA program.

Once it has been established that owner/operators have adequately designed and constructed their ground-water monitoring systems and that these systems are providing representative ground-water samples, EPA must confirm that these samples are being properly analyzed.

The Laboratory Audit Inspection (LAI) will be conducted to determine the quality of owner/operator's laboratory analyses. At present, the LAI is envisioned to be a two phased inspection. The first phase will determine whether the laboratory is properly staffed and equipped, if there are adequate quality assurance/quality control procedures and whether the samples are properly logged and tracked throughout the laboratory. The second phase of the LAI, if needed, will be a more detailed review of the analytical procedures being performed. This phase of the inspection will require an intensive effort by qualified chemists to evaluate the equipment and the methodologies that are used for particular analyses.

INTRODUCTION

The Resource Conservation Recovery Act (RCRA) requires the owner or operator of a surface impoundment, landfill or land treatment unit that is used to manage hazardous waste to implement a ground-water monitoring program capable of determining a facility's impact on the uppermost aquifer. The Environmental Protection Agency has developed a guidance entitled, RCRA Ground-Water Monitoring Technical Enforcement Guidance Document, which details the technical aspects of ground-water monitoring system design and operation deemed important by the Agency in order for a ground-water monitoring system to meet the goals of the RCRA program. Once it has been established that owner/operators have adequately designed and constructed their ground-water monitoring systems and that these systems are providing representative ground-water samples, EPA must confirm that these samples are being properly analyzed.

The Office of Waste Programs Enforcement, RCRA Enforcement Division, is developing a RCRA Laboratory Audit Inspection (LAI) program. The goal of the inspection is to determine whether the laboratory that the

owner/operator is using for ground-water sample analyses is properly equipped, maintained and staffed, whether there are adequate quality assurance/quality control procedures and whether samples are properly logged and tracked throughout the laboratory.

A Workgroup of Headquarters, Regional, and State laboratory and program personnel was assembled in March 1987 to begin development of the LAI. The purpose of this outline is to summarize the scope of the LAI as developed in the first workgroup meeting. The content and format may change as the inspection is further developed.

OUTLINE

- I) Introduction
 - A. Purpose
 - B. Intended audience
 - C. Relationship to the RCRA ground-water monitoring program
 - D. Resources available for conducting laboratory audits
 - E. Laboratory audits in other EPA programs
 - F. Not a laboratory certification program
 - G. Scope of the LAI
 - H. Relationship with other inspections
 - I. Overview of the inspection
- II) Laboratory Audit Inspection Process
 - A. Schematic of the LAI process
 - B. Targeting of laboratories to receive an LAI
 - 1) potential use of Performance Evaluation (PE) samples
 - 2) results of other inspections
 - C. Review of owner/operator's sampling and analysis plan
 - 1) identification of the laboratory performing the analysis
 - 2) parameters the laboratory is responsible for
 - 3) review of the laboratory's QA/QC plan
 - 4) identification of detection limits
 - 5) highlight activities that need to be confirmed during the laboratory visit
 - 6) use of the sampling and plan review checklist
 - D. Conducting the on-site inspection
 - 1) initial briefing with laboratory management
 - 2) laboratory walk-through
 - 3) use of the laboratory audit checklist
 - 4) debriefing at the conclusion of the inspection
 - E. Inspection report preparation
 - 1) summary of inspection findings
 - 2) recommendations
- III) Sampling and Analysis Plan Review Checklist
- IV) Laboratory Audit Checklist

OPERATION AND MAINTENANCE GUIDANCE FOR RCRA GROUND-WATER MONITORING SYSTEMS

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ABSTRACT

The performance of wells and sampling devices changes over time resulting in the generation of spurious data. An operation and maintenance program is therefore necessary to ensure that conditions are constant, and thus data from one time remain comparable to those of another. The necessity for operation and maintenance is further underscored by the fact that initial design of a monitoring network may change owing to human or natural factors. The continued reliability of data from monitoring wells is an important facet of generating ground-water quality information, and one about which laboratories should be made aware.

The Office of Waste Programs Enforcement is currently involved in the development of guidance to assist the Regions and States in the performance of new types of inspections, one of which is the operation and maintenance inspection. What follows is an outline of the key areas the workgroup will use as a starting point for writing the document. A final document is scheduled for release in late 1987.

OUTLINE

1.0 Introduction

- 1.1 Preface — audience, closure, operating permits
Fundamental: Is company in compliance with regs. and permit?
- 1.2 Importance of O&M Program
- 1.3 Relationship with Other Inspections
- timeframes, LOE, linkages, triggers
- 1.4 O&M Inspection Scope — long term, high use well systems.
Emphasis on monitoring wells, piezometers
- 1.5 Overview of Inspection: (consistent with body)
- flowchart (products)

2.0 Preparation of the Inspection

- 2.1 Objectives — to identify data deficiencies, problems
- 2.2 Review of Data, Documents, and Plans, Identify Data Gaps
- 2.3 Interviews with Involved Parties
- 2.4 Inspection Goals

2.5 Inspection Strategy — develop project plan: outline equipment selection, health and safety plan, notification, sampling plan (split sample)

3.0 Conducting the Inspection

3.1 Discuss Entry — identification

3.2 Objective: fill data gaps, (within O&M context only)

3.3 Project Plan Outline

3.4 Inspections Methods

4.0 Reporting Results

4.1 Report Format

4.2 Checklist

4.3 Recommendations

UNCONVENTIONAL TECHNIQUES AND UNCOMMON ANALYSES IN HAZARDOUS WASTE ANALYSIS

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ABSTRACT

One of the more productive approaches to hazardous waste analysis in our laboratory has been to attempt to identify all major and minor components in a waste sample, an approach called compositional analysis. This approach has also been applied to the polluted groundwater from several Superfund sites, where an attempt was made to identify all the contributors to the total organic carbon. One example which will be presented is the identification of parachlorobenzene sulfonic acid in groundwater. Examples of the use of infrared spectroscopy and X-ray fluorescence spectroscopy in hazardous waste analysis will be presented.

INTRODUCTION

The National Enforcement Investigations Center of the U.S.E.P.A. provides technical support for the enforcement activities of the Agency. Among the chemical analyses performed for this purpose are, of course, many of the routine tests described in SW-846. However, for many of our projects it is necessary to provide additional information for such purposes as assessing hazards, tracing wastes to their source, aiding in cost recovery efforts, and helping with remedial efforts. It has often been necessary and beneficial to employ techniques, such as infrared spectroscopy and X-ray diffraction and fluorescence, which are not commonly encountered in environmental analysis. Since it has been fruitful and necessary to completely analyze samples, analytes which are not listed wastes have been encountered. This paper will describe some of the less standard methods and analytes which have been encountered.

X-RAY FLUORESCENCE

X-ray fluorescence instrumentation has been a valuable addition to the metals capabilities of our laboratory. Energy dispersive XRF has proven very valuable for sample screening by providing a semi-quantitative elemental analysis. Simply by placing a powder, liquid, tar, or sludge in a small plastic cup with a mylar window and performing a 5 minute analysis, it is possible to determine qualitatively the composition of a waste sample. This allows choosing subsequent analyses in a selective manner and greatly increases efficiency. For instance, solid samples can be eliminated from consideration for EP toxicity tests if the total amount of each of the EP metals is less than the extraction limits (corrected for dilution).

Wavelength dispersive X-ray fluorescence has been used to make quantitative measurements on samples, such as waste oils and contaminated soils, which presented preparation difficulties for solution measurements.

As a specific example, a comparison study between XRF and ICP will be described. The samples which were studied were soil samples which had been heavily contaminated with heavy metals from mining and smelting activity. After grinding, the soils were pressed into pellets, with a boric acid binder, for analysis by XRF. For ICP analysis the samples were fused with KOH. This fusion has proven to be very useful in that siliceous minerals and organic matter are rendered soluble, but the temperature is low enough that lead, cadmium, selenium and other volatile metals (except for mercury) are retained.

Twelve metals were determined by both ICP and XRF, with concentrations in the range from 20 mg/kg to one percent. The results from the two methods compared quite well, with an average relative standard deviation of 20 percent. In our experience, the sampling error for soil sampling is at least 30% larger than the analytical error. This study showed that X-ray fluorescence is suitable for the rapid analysis for a large number of soil samples contaminated with metals. Calibration is more difficult with XRF, but sample preparation is faster, so for large numbers of similar samples XRF is superior.

INFRARED ANALYSIS

Infrared spectroscopy is one of the most widely applicable techniques for chemical analysis and compound identification. Among the many advantages of infrared analysis are the often minimal sample preparation and the ability to qualitatively identify a wide variety of both organic and inorganic species. The application in this laboratory of infrared spectroscopy (IRS) to the analysis of several hundred hazardous waste samples collected from dump sites has greatly increased the quality of the analytical results and the number of components identified.

The applications of IRS to hazardous wastes will be divided into two groups. First is the screening of samples; second is the identification of components best determined by infrared.

Screening samples by IRS is an integral and key step in the analysis of hazardous wastes by our laboratory. Rather than look only for a selected set of compounds, the approach is to determine the major components of a waste present at the one percent level or higher. In order to do this, samples were first analyzed by rapid techniques, including IR, with wide applicability. Subsequent

analyses are then directed towards those techniques which will supply the additional information sought.

Usually, the screening and subsequent analyses are done on the separated phases of the sample, after which the results are combined to give a view of the entire sample. After phase separation, each phase is scanned in the as received condition and after drying in an oven. The spectrum of the volatile portion can be obtained by subtraction.

The screening spectra from each of the separated phases are then interpreted. Although some spectra are complex, a surprising number of spectra are readily interpretable. Often there are only one, two or three major components in a given phase and the experienced analyst can readily sort them out. Even if specific compounds can not be identified, the identification of functional groups or compound type, such as phenolic compounds, can be very valuable.

Infrared spectroscopy can be used to complement and extend the commonly used methods of hazardous waste analyses. In several years experience at NEIC much less than half (probably closer to ten percent) the organic content of hazardous waste samples can be gas chromatographed and thus identified by GC/MS. While often the non-chromatographic components are not the most hazardous, it is important to identify all major components if hazard is to be assessed and if futile efforts to identify additional compounds by GC/MS are to be avoided. The application of IRS to the nonvolatile organic portion of hazardous waste is the best way to identify such components as polymers, glycols, etc.

Infrared spectroscopy also can aid with inorganic analyses, especially with compound speciation. IRS can easily identify some species such as carbonate and insoluble cyanides often missed by other methods. Since almost all species with covalent bonds absorb in the mid-infrared region, IRS has much to offer for inorganic analysis.

FACTOR ANALYSIS

Environmental analyses often produce much data, and obtaining the maximum amount of information from this abundance of data is not a trivial task. Factor analysis is a very useful approach for reducing the dimensionality of the data so that it is much easier to display graphically or otherwise interpret. Factor analysis employs an objective mathematical method to examine the correlations between variables. A reduced number of new variables are constructed which contain most of the information of the original variables.

As a simple example, consider some of the data from the analysis of contaminated groundwater near a hazardous waste dump. Over 30

parameters were measured on water from 25 wells. To plot each of 30 parameters versus all of the others using two-dimensional plots would require 375 plots ($30 \times 25 / 2$). As an illustration of the power of factor analysis to reduce the dimensionality of this data set, the data from the determination of ten metals was treated by factor analysis. Only three variables were needed to express 96% of the total variance from these ten metals. Cadmium, magnesium, manganese, nickel and potassium were all highly correlated, and could be combined into one new variable which expressed most of the changes in these five metals. A second new variable could be constructed from aluminum, chromium, copper and iron which accounted for most of the variation in these metals. Calcium was not correlated with any other variables and remained as an independent variable. Thus almost all of the data for ten elements was reduced to three dimensions, and could be viewed in three plots. Incidentally, the reason that calcium was not correlated with any other variable was that large amounts of sulfate were present at this site, and the sulfate controlled the solubility of calcium.

PARA-CHLOROBENZENE SULFONIC ACID

A detailed example will now be presented which illustrates the identification and quantification of a unique pollutant. A waste site in California received a great deal of hazardous material, particularly sulfuric acid. The groundwater leaving the site followed a well defined path down a canyon, toward a small town. Our laboratory undertook a comprehensive study of the polluted groundwater as extracted from a number of on and off site wells. It soon became clear that the amount of organic carbon in the groundwater far exceeded the total carbon accounted for in the GC/MS analyses. The gas chromatographable compounds determined in the purge and trap, semivolatiles and pesticide analyses accounted for less than ten percent of the total organic carbon.

Besides scientific interest, there were at least three other reasons for wanting to identify the major organic components in the groundwater. First, identification of specific compounds would allow assessment of the hazardous potential of the groundwater. Secondly, identification of specific compounds might tie a particular waste generator to the waste site, and perhaps aid in cost recovery efforts. Third, remedial efforts at the site include organic carbon removal, and compound identification could help with treatment selection and cost estimation.

Among the many parameters determined in the groundwater were the standard ones of chloride and sulfate. The latter was very high, of course, since sulfuric acid had been dumped. In addition, X-ray fluorescence was used to measure total sulfur and total chlorine in the groundwater samples. Comparison of these results showed that significant amounts of chlorine and sulfur were present in addition

to the amounts present as chloride and sulfate. It was possible that organic species containing chlorine or sulfur were present.

Several approaches were tried to isolate nonvolatile organic compounds, but only the most successful approach will be described. A portion of a highly contaminated groundwater sample which was drawn from a well was taken to dryness in a vacuum oven. The dried residue was then extracted with boiling ethanol in order to separate polar organics and ionic organics from inorganic salts. This type of extract has been used to isolate surfactants from complex samples. After filtering and drying, the residue was dissolved in ethanol, water and concentrated hydrochloric acid. Methylene chloride was used to extract organic acids. An infrared spectrum of the extract showed that an aromatic sulfonic acid was present which contained very little if any aliphatic character. Comparison of this spectrum with that of an authentic sample of para-chlorobenzene sulfonic acid (PCBSA) showed that this was indeed the sulfonic acid that was present.

The presence of the PCBSA has been confirmed in many ways. Two of the most definitive ways will be mentioned. Diazomethane was used to make the methyl ester of the sulfonic acid. GC/MS was used to detect and identify the methyl ester. Another derivative was also prepared and then confirmed by GC/MS. This was the pentafluorobenzyl derivative, prepared from pentafluorobenzyl bromide. This derivative has been used to detect organic acids and phenols in water (1).

Quantification was important in order to see if the para-chlorobenzene sulfonic acid was a major organic component in the groundwater. Ion-paired reverse-phase liquid chromatography was found to give good separations and allow control of the elution by varying the amount of methanol in the methanol - water eluent. The ion-pairing reagent was tetrapropylammonium ion. The liquid chromatography gave accurate quantification, as shown by spike recoveries, and good precision.

The para-chlorobenzene sulfonic acid proved to be the highest concentration organic compound in the groundwater at this site, with some wells having over 50% of their organic carbon as this component. One well had 0.4% PCBSA.

The situation just described was very favorable for identifying the PCBSA, since it was present at such high concentrations. The method used to identify this compound would not be applicable to all situations and required some skill to execute. However, it is clear that many of the organic compounds at this and similar sites can be separated and eluted by liquid chromatography. All that is needed is a means to identify peaks. Mass spectrometry detection is making great progress in this regard. Also, work is being done to

interface infrared spectroscopy with liquid and thin layer chromatographies, and shows promise of eventually being applicable to problems such as those just described.

COMPOSITIONAL ANALYSIS

Our overall approach to chemical analyses for enforcement purposes is best described as compositional analysis. Even when the standard tests for hazardous characteristics are done, it is often desirable and necessary to obtain further information in order to assist with hazard assessment, identify waste sources, etc. In our laboratory, compositional analysis refers to the determination of all of the components present in a sample at the one percent level or greater. For most samples this provides all the information that is needed.

The first step in compositional analysis is phase separation followed by a number of widely applicable tests which serve to screen the samples; eliminate those that require no further work and direct any further analytical work towards specific goals. For instance, a qualitative XRF analysis can determine if solid or heavy oil samples need EP tests, and can give a good overall indication of what metals and other elements are present in the sample. In many cases, infrared spectra give a good indication of the overall composition of the sample. A Karl Fischer titration is done to determine the water concentration. The initial screening provides sufficient information to characterize many samples, at least in broad terms and often in some detail. Further work, such as purge and trap GC/MS or EP Toxicity tests can be selected for those samples which require them and which have a high probability of yielding violations or additional information from such tests.

An important part of our approach to waste analysis is to report the information from the analytical tests in a form which can be understood by engineers, investigators, or nontechnical colleagues. Rather than report long lists of compounds which were not detected, we produce a summary of our results. The significant findings for each sample are presented clearly, along with a physical description of the sample. Only those determinations which make a waste sample hazardous or which define its overall composition are presented. For example, rather than list a large number of hydrocarbons from a GC/MS analysis, we report saturated hydrocarbons or oil. Along with this summary table, a series of detailed tables present the complete analytical results.

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LEACHING AND PHYSICAL METHODS

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MODIFICATION TO THE TCLP PROCEDURE FOR PROBLEM MATRICES

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ABSTRACT

The Toxicity Characteristic Leaching Procedure (TCLP) was developed as a technique to measure the potential pollutants to leach from unsecured hazardous waste sites. The method was validated in an interlaboratory study last year. As TCLP has been applied to an increasing number of environmental samples, certain difficult matrices have been observed.

Possible modifications to the TCLP are being tested for three specific matrices, oily wastes, wastes which require size reduction, and stabilized wastes. Oily or nonaqueous liquid wastes cause problems in the TCLP because the matrix can clog the glass fiber filters thus reducing the amount of contaminants leached from a sample by the buffer solution. Samples with solids pose two potential difficulties because (1) solids can contain pollutants that will not come in contact with the buffer, or (2) new surfaces exposed during pulverization steps can change the buffering capacity of the sample. Samples that have been stabilized require physical testing to establish that the contaminants are actually immobilized in a stable form. If the waste is truly stabilized, then it should be tested without size reduction. If the waste is poorly stabilized and can be expected to break up under the effects of weathering (freeze/thaw, wetting/drying, or compression), then the waste must be pulverized prior to TCLP.

Analytical data from samples representing each problem matrix will be presented to demonstrate the effect of proposed method modifications on the precision and accuracy of TCLP.

FURTHER DEVELOPMENT OF THE LIQUID RELEASE TEST

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ABSTRACT

In response to requirements under the 1984 Congressional Amendments to RCRA, the Office of Solid Waste, Methods Section, developed the Liquid Release Test (LRT). The test method is intended to determine whether or not liquids can be released from liquid-loaded sorbent materials under simulated landfill pressures. On December 24, 1986 the EPA proposed rules specifying the LRT for use in evaluating whether or not sorbents employed to remove free liquids would release such liquids under land disposal environment conditions.

Previous LRT development work led to the selection of the Zero-Headspace Extractor (ZHE), originally designed for the Toxicity Characteristic Leaching Procedure, for the LRT. This device uses gas pressure to force a piston against the sample, in effect squeezing any releasable liquid from the material.

A single laboratory evaluation of the test's ruggedness and precision demonstrated that the test is adequately precise and rugged. An initial collaborative study indicated a need for several modifications to the procedure. These modifications included changing the method for piston-position set up and clarification of the written procedure to prevent misinterpretation of the procedural steps.

This paper discusses the development of the LRT and further work to be completed on this test in 1987. This additional work includes conducting a second collaborative study of the improved procedure, testing the use of colored filter paper for improved visual detection of a liquid release, investigating shortening the test time by using increased pressures, and summarizing and responding to public comments submitted in response to the EPA's proposed rules banning free liquids in containers from hazardous waste landfills.

VALIDATION OF TOXICITY CHARACTERISTIC LEACHING PROCEDURE (TCLP) AND APPLICATION TO INDUSTRIAL WASTES

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ABSTRACT

This paper describes one laboratory's analytical method validation process as it was applied to Toxicity Characteristic Leaching Procedure (TCLP) leachate samples. This process involves a determination of the analyzability of the individual analytes in simple standards and the extractability of the analytes from the TCLP leachate. Calculation of method detection limits and report limits are also part of the validation process described in this paper.

Data is presented which demonstrates how detection limits and report limits were established by analyzing replicate spiked samples. The process for determining appropriate spiking levels for the replicate samples is also described.

Following completion of the validation process, the tested methods were applied to leachates prepared from actual industrial waste samples. The data obtained from the industrial waste leachates is presented with a discussion of the results. New problems encountered in the analyses of real samples are discussed with recommendations for resolution of those problems in a production environment.

INTRODUCTION

The EPA has been charged with devising the means to be able to assess certain characteristics or properties of wastes in order to classify the waste as hazardous. A waste is deemed hazardous when, if improperly managed, harm to human health or the environment would occur. Hazardous characteristics, once exhibited by a waste would thereby require that waste to be managed in a specified way.

The identification of wastes with hazards resulting from the potential to leach specific toxicants is defined as the Extraction Procedure Toxicity Characteristic. The leaching test, called the EP, results in an extract analyzed for normal drinking water parameters; eight metals, four pesticides, and two herbicides. The regulatory thresholds for those constituents were set at 100 times the drinking water standard levels.

One of the shortcomings of the EP Toxicity test was its lack of optimization for organic compounds.

EPA proposed in the Federal Register of June 13, 1986, to amend the EP Toxicity Characteristic in order to more properly assess the mobility of both organic and inorganic compounds. The proposed leaching procedure was called the Toxicity Characteristic Leaching Procedure or TCLP. The proposal also expanded the list of constituents to be assessed in the leachate. Table 1 presents the expanded list together with the regulatory levels for the contaminants.

Table 1 - Toxicity Characteristic Contaminants and Regulatory Levels

Contaminant	Regulatory Level (mg/l)
Acrylonitrile	5.0
Arsenic	5.0
Barium	100.0
Benzene	0.07
Bis (2-chloroethyl) ether	0.05
Cadmium	1.0
Carbon Disulfide	14.4
Carbon Tetrachloride	0.07
Chlordane	0.03
Chlorobenzene	1.4
Chloroform	0.07
Chromium	5.0
o-Cresol	10.0
m-Cresol	10.0
p-Cresol	10.0
2,4-D	1.4
1,2-Dichlorobenzene	4.3
1,4-Dichlorobenzene	10.8
1,2-Dichloroethane	0.40
1,1-Dichloroethylene	0.1
2,4-Dinitrotoluene	0.13
Endrin	0.0003
Heptachlor (and its hydroxide)	0.001
Hexachlorobenzene	0.13
Hexachlorobutadiene	0.72
Hexachloroethane	4.3
Isobutanol	36.0
Lead	5.0
Lindane	0.06
Mercury	0.2
Methoxychlor	1.4
Methylene Chloride	8.6
Methyl Ethyl Ketone	7.2
Nitrobenzene	0.13
Pentachlorophenol	3.6
Phenol	14.4
Pyridine	5.0

Table 1
Toxicity Characteristic Contaminants and Regulatory Levels
(continued)

Contaminant	Regulatory Level (mg/l)
Selenium	1.0
Silver	5.0
1,1,1,2-Tetrachloroethane	10.0
1,1,2,2-Tetrachloroethane	1.3
Tetrachloroethylene	0.1
2,3,4,6-Tetrachlorophenol	1.5
Toluene	14.4
Toxaphene	0.07
1,1,1-Trichloroethane	30.0
1,1,2-Trichloroethane	1.2
Trichloroethylene	0.07
2,4,5-Trichlorophenol	5.8
2,4,6-Trichlorophenol	0.30
2,4,5-TP (Silvex)	0.14
Vinyl chloride	0.05

This paper presents our laboratory's approach to the validation of this leaching procedure and the associated analytical methods.

Our laboratory's validation efforts were initiated through participation in two EPA-sponsored studies. The first was a collaborative study on the TCLP conducted by S-Cubed. Subsequently, we were involved in another study program, funded by EPA under the direction of the Dynamac Corporation. One phase of the latter study being reported here was to determine if selected SW-846 methods could be used as written, to detect the various hazardous chemicals at or below the regulatory levels in a synthetic, laboratory-generated leachate.

EXPERIMENTAL DESIGN FOR SYNTHETIC LEACHATE TESTING

Preparation of Synthetic Leachate

The synthetic leachate consisted of the TCLP Extraction Fluid #1. Each liter of leachate was prepared by adding 5.7 ml glacial acetic to 500 ml of laboratory reagent water followed by the addition of 64.3 ml of 1.0 N sodium hydroxide and dilution to 1.0 liter. The pH of the solution was verified to be within the required range of 4.93 +/- 0.05.

Table 2 - Concentrations of TCLP Synthetic Leachate Spiking Levels Compared to Regulatory Levels

	Regulatory Level (mg/l)	Spiking Concentration (mg/l)
<u>Volatile Compounds - Analyzed by Direct Injection</u>		
Acrylonitrile	5.0	2.0
Carbon Disulfide	14.4	2.0
Isobutanol	36	4.0

Volatile Compounds - Analyzed by Purge & Trap

Benzene	0.07	0.05
Carbon Tetrachloride	0.07	0.05
Chlorobenzene	1.4	0.05
Chloroform	0.07	0.05
1,2-Dichloroethane	0.40	0.05
1,1-Dichloroethylene	0.1	0.05
Methylene Chloride	8.6	0.05
Methyl Ethyl Ketone	7.2	0.05
1,1,1,2-tetrachloroethane	10.0	0.05
1,1,2,2-Tetrachloroethane	1.3	0.05
Tetrachloroethylene	0.1	0.05
Toluene	14.4	0.05
1,1,1-Trichloroethane	30	0.05
1,1,2-Trichloroethane	1.2	0.05
Trichloroethylene	0.07	0.05
Vinyl Chloride	0.05	0.05

Table 2 - Concentrations of TCLP Synthetic Leachate Spiking Levels Compared to Regulatory Levels (Continued)

	Regulatory Level (mg/l)	Spiking Concentration (mg/l)
<u>Semivolatile Compounds</u>		
o-Cresol	10.0	0.200
m-Cresol	10.0	0.200
p-Cresol	10.0	0.200
1,2-Dichlorobenzene	4.3	0.100
1,4-Dichlorobenzene	10.8	0.100
Bis(2-chloroethyl) Ether	0.05	0.05
Hexachlorobenzene	0.13	0.100
Hexachlorobutadiene	0.72	0.100
Hexachloroethane	4.3	0.100
2,4-Dinitrotoluene	0.13	0.100
Nitrobenzene	0.13	0.100
Phenol	14.4	0.200
Pentachlorophenol	3.6	0.200
Pyridine	5.0	0.100
2,3,4,6-Tetrachlorophenol	1.5	0.200
2,4,5-Trichlorophenol	5.8	0.125
2,4,6-Trichlorophenol	0.3	0.125
<u>Pesticide Compounds</u>		
Chlordane	0.03	0.002
Endrin	0.0003	0.0005
Heptachlor	0.001	0.0003
Lindane	0.06	0.0002
Methoxychlor	1.4	0.001
Toxaphene	0.07	0.005

Preparation of Spiking Standards

The spiking levels selected for each analyte and the corresponding regulatory level are presented in Table 2. A number of the spiking concentrations are significantly lower than the regulatory limits. This is because those regulatory levels were outside the working analytical range of the method. In those cases, the spiking level was set to be within the upper half of the analytical range.

The base-neutral extractable compounds were nominally spiked at 0.100 mg/l with bis(2-chloroethyl)ether being spiked at 0.050 mg/l which is the regulatory level. The acid extractable compounds were nominally spiked at 0.200 mg/l with the trichlorophenols being spiked at 0.125 mg/l.

The trichlorophenols were expected to coelute and the spiking levels for these compounds were set such that the combined concentration would be equal to the regulatory level set for the 2,4,6-isomer.

Throughout the TCLP validation phases, the trichlorophenols did not coelute, but m-cresol and p-cresol did.

All analyte, surrogate and internal standard spiking solutions were prepared within our Standards Laboratory and, with the exception of a few volatile compounds, neat materials were used.

Tests Included

For each method evaluated the tests depicted in Table 3 were performed.

QC Performed

As presented in Table 3, surrogates were spiked into all test samples including blanks. The surrogates and concentration levels used in EPA's Contract Laboratory Program (CLP) were employed for the study. Table 4 presents the surrogates employed, the concentration of the surrogates in the TCLP extract and, for advisory purposes, the CLP surrogate acceptance criteria.

A synthetic leachate blank containing surrogates was processed to evaluate the leachate background. Method blanks containing only surrogates in laboratory reagent water were extracted and analyzed to demonstrate that the processing and processing environment did not introduce contamination.

A blank spike, containing surrogates and analytes of interest was processed to verify that the entire analytical process was in control.

Table 3 - Tests Performed for Each Method Evaluated

- 1 TCLP Extract Method Blank

 Prepared from Synthetic Leachate Fluid
 (Synthetic Leachate Fluid + Surrogates)

- 3 TCLP Extract Spikes

 Prepared from Synthetic Leachate Fluid
 (Synthetic Leachate Fluid + Surrogates + Analytes)

- 1 Blank Water Spike

 Prepared from Laboratory Reagent Water
 (Reagent Water + Surrogates + Analytes)

- 2 Method Blanks

 Prepared from Laboratory Reagent Water
 (Reagent Water + Surrogates)

Table 4 - Surrogate Spike Concentrations and CLP Acceptance Criteria

Surrogate Compound	Concentration (ug/l)	Percent Recovery Acceptance Range
d8-Toluene	50	88 - 110
4-Bromofluorobenzene	50	86 - 115
d4-1,2-Dichloroethane	50	76 - 114
d5-Nitrobenzene	50	35 - 114
2-Fluorobiphenyl	50	43 - 116
d14-Terphenyl	50	33 - 141
d5-Phenol	100	10 - 94
2-Fluorophenol	100	21 - 100
2,4,6-Tribromophenol	100	10 - 123
Dibutylchloredate	0.1	24 - 154

Triplicate spikes of the synthetic leachates, containing surrogates and compounds of interest were processed to obtain a measure of precision and accuracy.

Analytical Method Employed

The primary source for the analytical methods used in the study was the 2nd Edition of SW-846. Methods 8080, 8240 and 8270 were employed as written. Modifications of 8240 and 8150 were also tested as part of the study, but were not considered part of CompuChem's validation process due to their lack of official status.

Instrumentation

8240 Analyses were conducted using a Finnigan OWA equipped with a Tekmar purge and trap device and 6' x 1/4" 1% SP-1000 on 60/80 mesh Carboxpack B column.

8270 analyses were conducted using a Finnigan OWA equipped with a 30 meter wide bore, thick film DB-5 fused silica capillary column.

8080 analyses were conducted using two Varian gas chromatographs equipped with electron capture detectors. Additionally, one instrument was equipped with a 2mm x 6' column packed with 3% OV-101 on 100/120 mesh Supelcoport and the other instrument was equipped with a 4mm x 6' column packed with 1.95% SP-2250/1.5% SP-2401 on 100/120 mesh Supelcoport.

Instrument QC

Prior to analysis of volatile samples by Method 8240, an acceptable BFB tune and a multipoint calibration were obtained. The BFB acceptance criteria presented in CLP methodologies were applied. The nominal concentrations of the three standards which comprised the multipoint calibration were 40, 80 and 160 ug/l.

Prior to analysis of semivolatile samples by Method 8270, an acceptable DFTPP tune and a multipoint calibration were obtained. DFTPP acceptance criteria presented in CLP methodologies were applied. The nominal concentrations of the acid extractable compounds in the five standards comprising the multipoint calibration were 50, 100, 150, 200 and 250 ug/l. The nominal concentrations of the base-neutral extractable compounds in the multipoint standards were 20, 50, 80, 120 and 160 ug/l.

The %RSD for seven compounds (methylene chloride, 2-butanone, trichloroethylene, benzene, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, and tetrachloroethylene) in the volatile multipoint calibration were greater than the 10% required by the method for use of the average response factor in subsequent sample quantitations.

For these compounds, quantitations were based on linear fit. All other compounds were quantitated using the average response factor from the multipoint calibration.

The ZRSD for each analyte in the semivolatile multipoint calibrations was less than 25% as required by the method for use of the average response factor in subsequent sample quantitations.

Analytical Results

Analyte and surrogate recoveries for each spiked synthetic leachate sample and the blank spike processed for semivolatile, purgeable volatile, direct injectable volatile and pesticide analytes are presented in Tables 5 - 8 respectively.

As illustrated, all analytes were recovered well from the synthetic leachate. Semivolatile recoveries ranged from 50% for phenol to 113% for pentachlorophenol. Purgeable volatile recoveries ranged from 72% for 2-butanone to 107% for 1,2-dichloroethane. Pesticide recoveries ranged from 81% for heptachlor to 106% for toxaphene. The direct injected volatiles exhibited the greatest variation in recovery with acrylonitrile at 48% and isobutanol at 122%. The standard deviation for all compounds except isobutanol and 2-butanone were less than 10%. The standard deviations of those compounds were 21.2 and 12.3, respectively.

OTHER METHOD VALIDATION STUDIES

The Federal Register of June 13, 1986 which presented the proposed rule on TCLP indicated that the quantitation limits for the constituents of interest were based on a water matrix. Further, it states "Since TCLP extracts would also be aqueous in nature, EPA is proposing to use the quantitation limit as observed in water."

In our laboratory we have a formalized method validation program based on various EPA guidance documents. Our typical method validation program involves generating precision and accuracy data on quadruplicate replicates processed through the entire analytical process. The spiking level is typically in the middle of the analytical working range. Then, using information contained in Appendix B of October 26, 1984, Federal Register a Method Detection Limit (MDL) study is performed. For this requirement, a minimum of seven (7) replicates are spiked with each analyte being validated at a level of three to five times the estimated detection limit. Each replicate is processed through the entire analytical process. Mean recoveries are determined as well as the standard deviation of the replicate analyses. The MDL is then derived by applying a Student's "T" value to the standard deviation.

This procedure is performed using laboratory reagent water for aqueous matrix procedures and Ottawa sand or verified blank dirt for procedures designed for solid matrices.

The final aspect of our method validation program employs guidance contained in an Analytical Chemistry article entitled "Principles of Environmental Analysis." The article authored by the ACS Committee on Environmental Improvement, included a discussion on the MDL as well as the Limit of Quantitation (LOQ). The latter is the level above which quantitative results may be obtained with a specified degree of confidence. Whereas, the MDL determination for the analysis of a minimum of seven replicates utilizes a Student's "T" value in the 2.5 to 3.1 range times the standard deviation, the LOQ determination recommends a multiplier of ten (10) to eliminate any degree of uncertainty. If analytes are detected below this level and, when employing GC/MS, a resulting mass spectrum meets identification criteria, the quantitative value is characterized as an estimate and is flagged as such. This is the policy we have attempted to follow for all of the SW-846 methods we have validated. As part of the validation program for SW-846, Third Edition, we compared precision and accuracy data of the analytes involved in the TCLP synthetic leachate study with those same compounds in laboratory reagent water. A comparison of those data is presented in Table 9. The data obtained during our validation of SW-846, Second Edition is also included.

APPLICATION TO INDUSTRIAL WASTES

During the past year we have had occasion to perform compositional analyses of some industrial wastes as well as TCLP and ZHE (Zero Head Space) extractions followed by semivolatile and volatile analyses, respectively. Examinations of the data obtained for both analyses shows that compounds which were present in the raw sample at concentrations greater than 1000 ug/kg were present in the TCLP and ZHE leachates at measurable concentrations. Analytes present in the industrial wastes included benzene, toluene, chlorobenzene, chloroform, 1,2-dichlorobenzene, tetrachloroethylene, o-cresol, p-cresol, hexachlorobenzene and 2,4,6-trichlorophenol.

The major problem encountered in the analysis of real industrial waste samples was buffering during the final pH adjustment of several samples during the semivolatile extraction. For three samples, the pH had increased to greater than 2 by the end of the first acid extraction. These samples required readjustment of the pH at that point and three more extractions with methylene chloride. By verifying the pH at the end of the first base and acid extractions, viable extracts in which all surrogate recoveries were within acceptance ranges were obtained for each leached sample.

Table 5 - Synthetic Leachate Spike Percent Recovery Data for Method 8270

	Concentration of Spike Added (ug/l)	Blank Water Spike	Synthetic Leachate Spikes			Synthetic Leachate	
			#1	#2	#3	----- Spike ----- Average	S.D.
Analytical Compounds:							
1,2-Dichlorobenzene	100	84	87	89	75	84	6.2
1,4-Dichlorobenzene	100	80	84	85	69	79	7.3
2,3,4,6-Tetrachlorophenol	200	102	106	108	106	107	0.9
2,4,6-Trichlorophenol	125	68	68	67	69	68	0.8
2,4,5-Trichlorophenol	125	61	65	63	67	65	1.6
2,4-Dinitrotoluene	100	94	99	97	90	95	3.9
Bis(2-chloroethyl)ether	50	77	84	84	77	82	3.3
Hexachlorobenzene	100	97	116	109	106	110	4.2
Hexachlorobutadiene	100	86	86	91	70	82	9.0
Hexachloroethane	100	79	75	80	65	73	6.2
m-Cresol	200	76	69	85	78	77	6.5
Nitrobenzene	100	81	88	92	79	86	5.4
o-Cresol	200	82	89	81	84	85	3.3
Pentachlorophenol	200	101	110	113	116	113	2.4
Phenol	200	48	47	50	52	50	2.1
Pyridine	100	70	72	70	62	68	4.3
p-Cresol	200	76	69	85	78	77	6.5
Surrogate Compounds:							
2,4,6-Tribromophenol	200	105	106	113	110	110	2.9
2-Fluorobiphenyl	100	89	98	92	83	91	6.2
2-Fluorophenol	200	61	62	66	60	63	2.5
d10-Pyrene	100	124	127	117	131	125	5.9
d14-Terphenyl	100	126	134	122	129	128	4.9
d5-Nitrobenzene	100	78	92	84	73	83	7.8
d5-Phenol	200	44	48	50	46	48	1.6

Table 6 - Synthetic Leachate Spike Percent Recovery Data for Purgeable Volatiles by Method 8240

	Concentration of Spike Added (ug/l)	Blank Water Spike	Synthetic Leachate Spikes			Synthetic Leachate ----- Spike -----	
			#1	#2	#3	Average	S.D.
Analytical Compounds:							
Vinyl Chloride	50	112	94	88	92	91	2.5
Methylene Chloride	50	100	94	84	86	88	4.3
1,1-Dichloroethylene	50	116	96	88	86	90	4.3
1,2-Dichloroethane	50	128	116	102	102	107	6.6
2-Butanone	50	96	88	70	58	72	12.3
1,1,1-Trichloroethane	50	124	106	96	94	99	5.2
Carbon Tetrachloride	50	128	106	96	96	99	4.7
Trichloroethylene	50	116	98	88	84	90	5.9
Benzene	50	110	96	86	84	89	5.2
1,1,2-Trichloroethane	50	116	102	86	84	91	8.1
1,1,1,2-Tetrachloroethane	50	124	110	98	96	101	6.2
1,1,2,2-Tetrachloroethane	50	114	98	82	76	85	9.3
Tetrachloroethylene	50	118	98	88	86	91	5.2
Toluene	50	118	104	96	94	98	4.3
Chlorobenzene	50	122	108	98	96	101	5.2
Surrogates:							
d4-1,2-Dichloroethane	50	114	114	100	100	105	6.6
Bromofluorobenzene	50	108	106	96	84	95	9.0
d8-Toluene	50	108	108	98	96	101	5.2

Table 7 - Synthetic Leachate Spike Percent Recovery Data for Direct Inject Volatiles by Method 8240

	Concentration of Spike Added (ug/l)	Blank Water Spike	Synthetic Leachate Spikes			Synthetic Leachate	
			#1	#2	#3	----- Spike -----	
						Average	S.D.
Analytical Compounds:							
Carbon disulfide	2.0	57	93	104	94	97	5.0
Acrylonitrile	2.0	57	50	59	36	48	9.5
Isobutanol	4.0	43	123	96	148	122	21.2

Table 8 - Synthetic Leachate Spike Percent Recovery Data for Method 8080

	Concentration of Spike Added (ug/l)	Blank Water Spike	Synthetic Leachate Spikes			Synthetic Leachate ----- Spike ----- Average S.D.	
			#1	#2	#3		
Pesticide Spike Series #1:							
Toxaphene	5.00	112	106	108	104	106	1.6
Dibutylchloredate (surrogate)	1.00	97	93	89	93	92	1.9
Pesticide Spike Series #2:							
Endrin	0.50	93	98	96	99	98	1.2
Heptachlor	0.30	81	81	82	81	81	0.5
Lindane	0.20	86	87	86	88	87	0.8
Methoxychlor	1.00	98	99	99	99	99	0.0
Dibutylchloredate (surrogate)	1.00	84	91	86	86	88	2.4
Pesticide Spike Series #3:							
Chlordane	1.00	88	73	87	86	82	6.4
Dibutylchloredate (surrogate)	1.00	88	79	89	89	86	4.7

Table 9 - Comparison of Results of Three Validations

	Synthetic Leachate Study		2nd Edition SW-846		3rd Edition SW-846	
	Average	S.D.	Average	S.D.	Average	S.D.
Semivolatile Compounds:						
Phenol	50	2.1	64	12.3	40	7.3
Bis(2-chloroethyl)ether	82	3.3	88	4.4	75	17.7
1,4-Dichlorobenzene	79	7.3	111	12.6	78	8.0
1,2-Dichlorobenzene	84	6.2	98	6.9	71	6.7
o-Cresol	85	3.3	98	9.2	77	10.8
p-Cresol	77	6.5	96	14.9	84	10.5
Hexachloroethane	73	6.2	96	13.4	70	7.8
Nitrobenzene	86	5.4	95	8.8	81	8.1
Hexachlorobutadiene	82	9.0	106	1.7	77	8.2
2,4,6-Trichlorophenol	68	0.8	111	2.6	81	17.4
2,4,5-Trichlorophenol	65	1.6	121	2.5	84	16.5
2,4-Dinitrotoluene	95	3.9	101	3.6	85	13.4
Hexachlorobenzene	110	4.2	102	1.4	83	10.5
Pyridine	68	4.3	58	15.8	28	9.2
2,3,4,6-Tetrachlorophenol	107	0.9	124	2.2	86	20.5
Purgeable Volatile Compounds:						
Vinyl Chloride	91	2.5	76	3.2	89	12.1
Methylene Chloride	88	4.3	89	1.8	129	16.1
1,1-Dichloroethylene	90	4.3	82	3.9	87	5.6
Chloroform	51	2.4	90	2.5	94	5.6
1,2-Dichloroethane	107	6.6	81	12.2	90	2.7
2-Butanone	72	12.3	89	1.6	NA	NA
1,1,1-Trichloroethane	99	5.2	85	4.6	80	3.4
Carbon Tetrachloride	99	5.2	82	4.9	74	4.1
Trichloroethylene	90	5.9	82	3.1	78	3.1
Benzene	89	5.2	86	3.2	87	3.6
1,1,2-Trichloroethane	91	8.1	84	6.4	88	6.6
1,1,2,2-Tetrachloroethane	85	9.3	78	3.4	81	10.7
Tetrachloroethylene	91	5.2	79	8.5	74	4.0
Toluene	98	4.3	84	3.0	86	2.0
Chlorobenzene	101	5.2	85	2.5	87	2.4

NA = Not Available, Validation Not Complete At This Time

CONCLUSIONS

The experimental data indicated that the TCLP methods would work for the analytes of interest. The results of the various validation studies indicate that extraction fluid #1 is similar to water for the parameters tested. Subsequent application of those methods to actual industrial waste sample leachates proved that the methods are viable for the TCLP. As long as the pH of each sample is checked at the end of the initial base and acid extraction, the semivolatile recoveries, as indicated by surrogate recoveries, should be similar to those obtained for water samples.

PERFORMANCE OF THE TOXICITY CHARACTERISTIC LEACHING PROCEDURE

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ABSTRACT

The U.S. EPA has recently promulgated the use of a leaching procedure (TCLP) to be used as a criterion for disposal of hazardous wastes under the Land Disposal Restrictions Rule. The TCLP has also been included in the proposed rule for Identification and Listing of Hazardous Wastes.

Summarized are results of eight different studies designed to evaluate the TCLP method. Study results indicate that the TCLP procedure can be applied consistently by a diverse group of laboratories.

INTRODUCTION

The Resource Conservation and Recovery Act (RCRA) directs the U.S. Environmental Protection Agency (EPA) to identify and regulate wastes which pose a hazard to human health and the environment. In order to meet this mandate, the EPA listed a number of wastes as hazardous and identified four hazardous waste characteristics. One of these, the Extraction Procedure (EP) Toxicity Characteristic, addresses wastes which exhibit leaching potential into ground water supplies if improperly managed.

In 1984, Congress amended RCRA directing the EPA to make changes in the existing Extraction Procedure to predict more accurately the leaching potentials of hazardous wastes and expand its application to a greater number of toxic constituents. In response, the EPA developed and proposed the Toxicity Characteristic Leaching Procedure (TCLP). The second generation leaching procedure, TCLP, was published as a draft protocol on December 20, 1985; it was officially published in the Federal Register (Vol. 51, No. 9, January 14, 1986) as part of the proposed Land Disposal Restrictions Rule, and again in the Federal Register (Vol. 51, No. 114, June 13, 1986) as part of the proposed rule for Identification and Listing of Hazardous Wastes. The final land disposal restrictions rule was published in the Federal Register (Vol. 51, No. 216, November 7, 1986).

The TCLP protocol calls for an 18-hour extraction of a waste sample with either an acetic acid or sodium acetate solution followed by the determination of metals, pesticides, and semi-volatile and volatile organic compounds in the leachate. Metals, pesticides and semi-volatile organic compounds are extracted using a bottle or jar

similar to the EP procedure. For the extraction of volatile organic compounds (VOCs), a new device known as a Zero Headspace Extractor (ZHE) is used.

To date, there have been various single and multi-laboratory studies conducted to evaluate the TCLP procedure. Approximately 23 laboratories representing government, industry, research and commercial organizations participated in various phases of the evaluation studies. At least 15 different types of wastes were used including metal manufacturing, power plant, refinery, electroplating, textile and Publicly Owned Treatment Works (POTW) wastes.

It is the intent of this paper to combine and condense data from these various studies and to discuss the method (TCLP) performance, i.e., comparison to the EP procedure, ruggedness, precision and reproducibility. Data presented herein represent a fairly comprehensive data base from analyses of the various wastes.

In order to condense available data, only results of analytes detected in greater than 90 percent of the samples are reported. Outliers, as determined by the Dixon Test (1) at the 95 percent confidence level, are also excluded. In several of the studies, the Dixon Test had not been applied to the study results. Where raw data were available, the Dixon test was applied by the author.

Most of the original studies conducted under EPA contact or by private industry as referenced in this paper are available for public review in the EPA RCRA Docket (S-212) at the EPA, Washington, DC.

TCLP VERSUS EP

Two studies comparing the TCLP and EP extractions are summarized. The sponsoring industries selected 13 different wastes for extraction and analysis. Only results reported by more than one laboratory and on analytes which were detectable are included here.

Inter-Industry Collaborative Study

The Inter-Industry Collaborative Study (2) was sponsored by six trade associations with the goal of evaluating the TCLP on wastes of interest to the organizations. Only one waste, waste #6, is reported since it was the only waste analyzed by all six participating laboratories. This waste consisted of a composite of baghouse dust from a steelmaking operation and sinter waste from a lead smelting facility. The results of this study are summarized in Table 1. For the five metals presented, there does not appear to be a large difference in the amount of extractable analytes. Although the RSD_{EP} for lead is significantly greater than the lead RSD_{TCLP} ,

the other four metals indicate similar extraction repeatability. Since the raw data for this study were not available, it is not known whether outliers were discarded. The study concluded that although the TCLP and EP are not precise methods, they are similar in precision.

Table 1. Inter-Industry Collaborative Study Summary Results of EP and TCLP Metal Extracts of Waste #6

Parameter	\bar{X}_{EP}	\bar{X}_{TCLP}	S_{EP}	S_{TCLP}	$\%RSD_{EP}$	$\%RSD_{TCLP}$
Arsenic	0.048	0.039	0.024	0.021	50	44
Barium	5.9	14.5	3.77	10.5	64	72
Cadmium	0.19	0.17	0.061	0.067	32	40
Chromium	0.090	0.069	0.065	0.048	72	69
Lead	293	256	471	241	161	94
Average					76	64

Note: Units = mg/L

\bar{X} = Means values for 11-12 extractions

S = Standard Deviation

$\%RSD$ = Relative Standard Deviation

Electric Power Research Institute

A second study comparing TCLP and EP procedures was sponsored by the Electric Power Research Institute (EPRI) (3). Seven different utility wastes (including fly ashes, bottom ashes, flue gas sludges) were extracted in duplicate by three laboratories. The six extracts of each waste were divided equally among the three laboratories and analyzed in quadruplicate. A summary of the results are reported in Table 2.

During the period of public comment, one of the frequently mentioned concerns of the TCLP was the possible aggressive leaching potential of the acetate extraction medium. The average total metal extractables appear to be somewhat higher for the TCLP extraction (0.50 mg/L vs 0.39 mg/L or 0.29 mg/L vs 0.17 mg/L) if zinc, waste W-2, results are not included; the relatively large zinc values tend to buffer significant differences of the smaller values. Eighty percent of the results in Table 2 showed a ration of $\bar{X}_{TCLP}:\bar{X}_{EP}$ of 0.8-2.0 and 15 percent fell within the 2.0 to 3.0 range (disregarding the data pairs containing "ND"). Although these

Table 2. EPRI Results of EP and TCLP Metal Extracts of Seven Utility Wastes

Metals	\bar{X}_{EP}	\bar{X}_{TCLP}	S_{EP}	S_{TCLP}	$ZRSD_{EP}$	$ZRSD_{TCLP}$
Arsenic, W-2	ND	0.317		0.228		72
W-7	0.051	0.149	0.034	0.021	67	14
Barium, W-1	0.406	0.327	0.178	0.147	44	45
W-3	0.430	0.819	0.219	0.336	51	41
W-7	0.177	0.446	0.060	0.138	34	31
Cadmium, W-1	0.013	0.016	0.004	0.004	31	25
W-2	0.224	0.233	0.038	0.100	17	43
W-5	0.033	0.028	0.133	0.005	25	17
W-6	0.005	0.004	0.002	0.002	48	38
W-7	0.006	0.006	0.001	0.002	18	32
Chromium, W-1	0.427	0.470	0.077	0.070	18 ¹	15
W-2	0.016	0.921	0.023	0.405	145 ¹	44
W-3	0.008	0.010	0.006	0.005	75	46
W-4	ND	0.004		0.002		55
W-5	0.030	0.042	0.026	0.036	85	86
W-7	ND	0.059		0.018		30
Lead, W-2	ND	0.181		0.122		67
Selenium, W-7	0.061	0.135	0.024	0.036	39	27
Zinc, W-1	0.171	0.238	0.142	0.171	83	72
W-2	5.36	5.37	0.3	0.38	6 ²	7 ²
W-3	0.092	0.164	0.129	0.174	140 ²	106 ²
W-5	1.48	1.6	0.12	0.22	8	14
W-6	0.174	0.306	0.052	0.278	30	91
W-7	0.151	0.234	0.073	0.140	48	60
Average	0.3	0.50	0.08	0.13	51	45
Without Zinc, W-2						
Average	0.17	0.29				

Note: Units = mg/L

W-1 = Alkaline fly ash

W-2 = Acidic fly ash

W-3 = Alkaline bottom ash

W-4 = Neutral bottom ash

W-5 = Forced oxidized flue gas desulfurization sludge

W-6 = Flue gas desulfurization sludge

W-7 = Neutral fly ash

ND = 50% of the results were not detected

¹ One lab reported results 10X lower than the other two labs

² Analyses of one lab's extracts were approximately 10X greater than those of the other two labs

Approximately 40 analyses were performed on each waste type.

ratios tend to support a conclusion that the TCLP is more aggressive, all extractable results reported are well below hazardous waste threshold levels. If the TCLP is more aggressive, it would appear to be waste and metal specific.

The EPRI study concluded that the TCLP appears to provide better extraction reproducibility but that inter-laboratory variations are a significant factor in TCLP analyses.

For the majority of wastes analyzed in these two studies, the TCLP and EP are similar in extraction efficiency and method precision. Where differences exist, the TCLP tends to provide better precision, is easier to perform and produces a more aggressive leaching medium.

RUGGEDNESS

Two ruggedness studies have been performed to determine the effect of various perturbations on specific elements of the TCLP protocol. Ruggedness testing determines the sensitivity of small procedural variations which might be expected to occur during routine laboratory application. Method variations which are observed to significantly affect analytical results need to be carefully controlled.

Both studies followed the partial factorial design described by Youden. In this design, seven conditions were slightly altered and eight extractions were performed. This design provided sufficient information to identify those areas of the method which were affected by procedural variations.

Metals

A study by ENSECO(4) investigated the following conditions for metals results on two wastes:

- | | | |
|------------------------|---|---|
| 1) Liquid/Solid ratio | - | 19:1 vs 21:1 |
| 2) Extraction time | - | 16 hrs vs 18 hrs |
| 3) Headspace | - | 20% vs 60% |
| 4) Buffer #2 acidity | - | 190 meq vs 210 meq |
| 5) Acid-washed filters | - | yes vs no |
| 6) Filter type | - | 0.7 μ m glass fiber vs. 0.45 μ m vs polycarbonate |
| 7) Bottle type | - | borosilicate vs flint glass |

Of the seven method variations examined, acidity of the extraction fluid had the greatest impact on the results. Four of 13 metals from an API separator sludge/electroplating waste (API/EW) mixture and two of three metals from an ammonia lime still bottom waste were extracted at higher levels by the more acidic buffer. Because of the sensitivity to pH changes, the method requires that the

extraction fluids be prepared so that the final pH is within ± 0.05 units as specified.

Although not directly apparent from this study, other studies referenced have indicated that flint glass, no acid-washed filters or other filter materials may also affect results. Therefore, glass fiber filters (acid washed for metals analyses) are required and borosilicate glass is recommended.

Volatile Organic Compounds

A separate ruggedness study was performed by ERCO/ENSECO (5) to investigate method variations on volatile organic compounds (VOCs) in API/EW and ammonia lime still bottom wastes. The following parameters were tested:

- | | | |
|---------------------------------|---|-----------------------|
| 1) Liquid/Solid ratio | - | 19:1 vs 21:1 |
| 2) Headspace | - | 0% vs 5% |
| 3) Buffer #1 acidity | - | 60 meq vs 80 meq |
| 4) ZHE device | - | ADM vs Millipore |
| 5) Method of storing
extract | - | Syringe vs Tedlar bag |
| 6) Aliquotting | - | yes vs no |
| 7) Pressure behind piston | - | 0 psi vs 20 psi |

The only parameter having a significant effect on the results was the choice of the extraction device. The original Zero Headspace Extractors (ZHEs) manufactured by Millipore had problems with leaking valves and with piston movement which resulted in loss of volatile compounds. Millipore has since corrected the valve and piston problems so that both the Millipore and ADM ZHEs should perform similarly. No problems were reported with the retrofitted Millipore ZHEs used in the S-Cubed Collaborative Study referenced later in this paper.

The ERCO/ENSECO study reported that the ZHE was "adequately rugged with respect to the parameters investigated."

PRECISION

A substantial amount of data has been generated from TCLP precision (reproducibility) studies. Both single- and multi-laboratory studies to assess method precision have been sponsored by private industry and by the EPA. Precision results from recent studies are encouraging. The general consensus from these studies is that the precision of the TCLP is comparable to or exceeds that of the EP procedure and that method precision is adequate. One of the more significant contributions to poor precision appears to be related to sample homogeneity and inter-laboratory variation which is not surprising due to the nature of waste materials.

Metals

The largest source of TCLP precision data comes from the metals analyses of TCLP extracts. Twenty-three laboratories performed extractions and analyses on 15 different wastes. The results from these analyses are presented in the tables below. Tables 3 and 4 contain single and multi-laboratory results, respectively. Results included in tables 1 and 2 also provide precision information. The general range for mean %RSDs in Tables 1, 2, 3 and 4 is 22-74%. Although not necessarily an indication of a precise method, the range is not unreasonable considering the waste types and the relatively low levels of metals determined. Less than five percent of these wastes would be regulated as hazardous wastes under 40 CFR, Part 261, based on the data presented in these Tables. The results indicate that a single analysis of a waste may not be adequate for waste characterization and identification requirements, a comment reinforced by the Inter-Industry Collaborative Study and in the Background Document (6).

Semi-Volatile Organic Compounds

Semi-volatile organic compounds have not been studied as extensively as the metals. There are, however, two studies, a single-laboratory evaluation (4) and a multi-laboratory collaborative study (7), which provide data from several different waste types. Results of these studies are summarized in Tables 5 and 6.

Single-laboratory precision was excellent with greater than 90 percent of the results exhibiting an RSD less than 25 percent. Over 85 percent of all individual compounds in the multi-laboratory study fell in the RSD range of 20-120 percent. The actual ranges are included in Table 6.

The single-laboratory evaluation (4) reported somewhat better reproducibility for semi-volatiles with the more acidic extraction fluid, #2. However, the multi-laboratory study (7) did not confirm the same relationship between extraction fluid acidity and precision.

Both studies concluded that the TCLP provides adequate precision. It was also determined that the high acetate content of the extraction fluid did not present problems (i.e., column degradation of the gas chromatograph) for the analytical conditions used.

Volatile Organic Compounds

Both single and multi-laboratory data are available for VOC precision. Three studies using a combination of three different waste types are summarized.

A single-laboratory study conducted by S-Cubed (8) involved the use

Table 3. Single-Laboratory TCLP Metals, Precision (Williams, et al)

Waste	Extraction Fluid	Metal	\bar{X}	S	%RSD
Ammonia Lime Still Bottoms	#1	Barium	0.283	0.013	4.6
	#2		0.332	0.015	4.6
	#1	Chromium	0.049	0.023	47
	#2		0.062	0.012	19
	#1	Zinc	0.178	0.126	71
	#2		0.257	0.071	28
API/EW Mixture	#1	Barium	0.696	0.020	2.8
	#2		1.01	0.01	0.6
	#1	Chromium	0.125	0.062	49
	#2		14.4	3.6	25
	#1	Zinc	136	7.5	5.5
	#2		347	14.2	4.1

%RSD Range = 0.6-71
Mean %RSD = 22

Note: All extractions were performed in triplicate.
Extraction Fluid #1 = pH 4.9
 #2 = pH 2.9
Units = mg/L

Table 4. Multi-Laboratory TCLP Metals, Precision (Blackburn and Show)

Waste	Extraction Fluid	Metal	\bar{X}	S	%RSD
Ammonia Lime Still Bottoms	#1	Cadmium	0.053	0.031	60
	#2		0.023	0.017	76
	#1	Chromium	0.015	0.0014	93
	#2		0.0032	0.0037	118
	#1	Lead	0.0030	0.0027	90
	#2		0.0032	0.0028	87
API/EW Mixture	#1	Cadmium	0.0046	0.0028	61
	#2		0.0005	0.0004	77
	#1	Chromium	0.0561	0.0227	40
	#2		0.105	0.018	17
	#1	Lead	0.0031	0.0031	100
	#2		0.0124	0.0136	110
Fossil Fuel Fly Ash	#1	Cadmium	0.080	0.069	86
	#2		0.093	0.067	72
	#1	Chromium	0.017	0.014	85
	#2		0.070	0.040	57
	#1	Lead	0.0087	0.0074	85
	#2		0.0457	0.0083	18

%RSD Range = 17-118

Mean %RSD = 74

Note: \bar{X} = Mean results from 6-12 different laboratories

Units = mg/L

Extraction Fluid #1 = pH 4.9

#2 = pH 2.9

Table 5. Single-Laboratory Semi-Volatiles, Precision

Waste	Compound	Extraction Fluid	\bar{X}	S	%RSD
Ammonia Lime Still Bottoms	Phenol	#1	19000	2230	11.6
		#2	19400	929	4.8
	2-Methylphenol	#1	2000	297	14.9
		#2	1860	52.9	2.8
	4-Methylphenol	#1	7940	1380	17.4
		#2	7490	200	2.7
	2,4-Dimethylphenol	#1	321	46.8	14.6
		#2	307	45.8	14.9
	Naphthalene	#1	3920	413	10.5
		#2	3827	176	4.6
	2-Methylnaphthalene	#1	290	44.8	15.5
		#2	273	19.3	7.1
	Dibenzofuran	#1	187	22.7	12.1
		#2	187	7.2	3.9
	Acenaphthylene	#1	703	89.2	12.7
		#2	663	20.1	3.0
	Fluorene	#1	151	17.6	11.7
		#2	156	2.1	1.3
	Phenanthrene	#1	241	22.7	9.4
		#2	243	7.9	3.3
	Anthracene	#1	33.2	6.19	18.6
		#2	34.6	1.55	4.5
	Fluoranthrene	#1	25.3	1.8	7.1
		#2	26.0	1.8	7.1
API/EW Mixture	Phenol	#1	40.7	13.5	33.0
		#2	19.0	1.76	9.3
	2,4-Dimethylphenol	#1	33.0	9.35	28.3
		#2	43.3	8.61	19.9
	Naphthalene	#1	185	29.4	15.8
		#2	165	24.8	15.0
	2-Methylnaphthalene	#1	265	61.2	23.1
		#2	200	18.9	9.5

%RSD Range = 1-33
Mean %RSD = 12

Note: Units = $\mu\text{g/L}$

Extractions were performed in triplicate

All results were at least 2X the detection limit

Extraction Fluid #1 = pH 4.9

#2 = pH 2.9

Table 6. Multi-Laboratory Semi-Volatiles, Precision

Waste	Compound	Extraction Fluid	\bar{X}	S	%RSD
Ammonia Lime	BNAs	#1	10043	7680	76.5
Still Bottoms (A)		#2	10376	6552	63.1
API/EW	BNAs	#1	1624	675	41.6
Mixture (B)		#2	2074	1463	70.5
Fossil Fuel	BNAs	#1	750	175	23.4
Fly Ash (C)		#2	739	342	46.3

Mean %RSD = 54

Note: Units = $\mu\text{g/L}$

\bar{X} = Mean results from 3-10 labs

Extraction Fluid #1 = pH 4.9

#2 = pH 2.9

%RSD Range for Individual Compounds

A, #1 0-113

A, #2 28-108

B, #1 20-156

B, #2 49-128

C, #1 36-143

C, #2 61-164

of two waste types plus a VOC free sand/water mixture blank. In order to assure that volatile compounds were present to extract, each waste was separated into two fractions and spiked with a mixture of VOCs. One fraction was spiked at ppm levels and the other at ppb levels. The spikes were added to the system at two different times and locations in the extraction process. Group 1 compounds (Table 7) were spiked into the sample immediately after placing the waste in the ZHE (both ADM and Millipore extractors were used). The initial waste liquid was removed by the ZHE, as specified in the TCLP protocol, and collected for analysis. Group 2 compounds were added with the extraction fluid as the fluid was being pumped into the ZHE. At the end of the 18-hour extraction procedure, the extraction fluid was collected from the ZHE and combined with the liquid collected from the first solid/liquid separation. Results of the analyses are presented in Table 7. To reduce the amount of data, compound groups were combined for the API/EW and sand/water blank wastes.

Although the data presented in Table 7 are reported in percent recoveries, the reproducibility of percent recoveries is an indicator of precision. The precision results (RSDs) are excellent. Only tetrachloroethane and trichloroethylene showed atypical recoveries. The report suggested that dehydrohalogenation may have occurred converting tetrachloroethane to trichloroethylene and HCl. Since both Groups 1 and 2 RSDs fall in the same range, it is unlikely that the spiking scheme contributed significantly to the excellent precision.

A second study directed by Oak Ridge National Laboratory (9) evaluated the performance of ZHEs with two wastes. Ten extractions were performed on fortified wastes that were spiked with halocarbons and aromatics in a scheme similar to that described in the S-Cubed study. The liquids from the initial liquid/solid separations and the final extracts were analyzed individually. Four of each set of 10 extractions were analyzed by Oak Ridge National Laboratory while the other six of each set were analyzed by another laboratory. The results are summarized in Table 8.

In general, precision results are similar to those reported in the study involving semi-volatile organic compounds (Table 6). Although both wastes were spiked with a mixture of compounds, the oily characteristics of the API/EW waste apparently prevented partitioning of the volatiles into the extraction fluid. Many of the analytes were either not detected or showed a wide range of concentrations. This can be seen from the larger RSDs for several of the compounds, methylene chloride and benzene. Although the variations may be greater for oily wastes which reduce the effectiveness and applicability of the method, the variations are not out of line when compared to other multi-laboratory results. It

Table 7. Single-Laboratory VOCs, Precision

Waste	Compound	Spike Level	\bar{X}	S	%RSD
Ammonia Lime Still Bottoms	<u>Group 1 Compounds¹</u>				
	Acrylonitrile	200 ppb	69	5.3	7.6
		1 ppm	62	3.6	5.8
	Carbon disulfide	200 ppb	47	3.3	7.1
		1 ppm	20	3.2	16
	2-Butanone	200 ppb	75	6.0	8.1
		1 ppm	66	6.6	10
	Benzene	200 ppb	93	3.0	3.3
		1 ppm	46	8.3	18
	Toluene	200 ppb	87	4.0	4.7
		1 ppm	47	7.1	15
	Chlorobenzene	200 ppb	74	2.2	3.0
		1 ppm	52	5.7	11
	<u>Group 2 Compounds²</u>				
	1,1-Dichloroethylene	200 ppb	80	4.3	5.4
		1 ppm	89	4.2	0.7
	Chloroform	200 ppb	90	2.9	3.2
		1 ppm	94	8.0	8.5
	1,2-Dichloroethane	200 ppb	93	3.0	3.2
		1 ppm	95	7.7	8.1
	1,1,1-Trichloroethane	200 ppb	81	3.3	4.1
		1 ppm	88	3.2	3.6
	Carbon tetrachloride	200 ppb	74	7.0	9.5
		1 ppm	86	4.8	5.6
	Trichloroethylene ³	200 ppb	136	6.9	5.1
		1 ppm	151	13	8.6
	1,1,2-Trichloroethane	200 ppb	86	1.6	1.9
		1 ppm	88	4.1	4.7
	Tetrachloroethylene	200 ppb	63	3.7	5.9
		1 ppm	69	5.0	7.3
	1,1,1,2-Tetrachloroethane	200 ppb	88	1	12
		1 ppm	96	0	11
	1,1,2,2-Tetrachloroethane ³	200 ppb	16	4.6	29
		1 ppm	15	8.4	56

Table 7 (Continued)

Waste	Compound	Spike Level	\bar{X}	S	%RSD
API/EW Mixture	<u>Surrogates</u>				
	^d ₄ -1,4-Dichloroethane	200 ppb	96	3.4	3.5
		1 ppm	95	5.6	5.9
	Bromofluorobenzene	200 ppb	97	5.8	6.0
		1 ppm	99	1.2	1.2
	^d ₈ -Toluene	200 ppb	101	3.9	3.9
		1 ppm	101	4.1	4.1
	Group 1 Compounds	1 ppm ¹	51	ND	7.6
		5 ppm ²	56	ND	9.0
	Group 2 Compounds	1 ppm ²	38	ND	8.5
		5 ppm ¹	35	ND	7.6
	Surrogates	1 ppm	101	ND	3.0
		5 ppm	102	ND	2.5
Sand, Spiked Blank	Group 1 Compounds	20 ppb ¹	62	ND	8.2
		200 ppb ¹	54	ND	10
		1 ppm ²	92	ND	7.2
	Group 2 Compounds	20 ppb ²	91	ND	1.5
		200 ppb ²	93	ND	7.3
		1 ppm ¹	34	ND	20
	Surrogates	20 ppb	95	ND	2.0
		200 ppb	102	ND	4.6
		1 ppm	99	ND	3.6

Mean %RSD⁴ = 9
 %RSD Range = 1-56

Note: \bar{X} = Mean % recovery for five replicates

¹ Compounds spiked into the waste

² Compounds spiked into the extraction fluid

³ Recoveries indicate possible transformations due to dehydrohalogenation

⁴ Surrogate and Blank data not included

ND = Not determined

Table 8. Multi-Laboratory (Two Labs) VOCs, Precision

Waste	Compound	Liquid	\bar{X}	S	%RSD
Ammonia Lime Still Bottoms	Benzene	Free liquid	36.5	11.2	30.7
		Leachate	90.3	47.7	52.8
	Bromodichloromethane	Free liquid	24.7	16.3	66.2
		Free liquid	30.1	18.4	61.2
	Carbon tetrachloride	Free liquid	36	47.1	34.6
		Free liquid	33	25.8	19.4
	Chlorobenzene	Free liquid	32	85.2	19.7
		Leachate	17.2	11.2	65.2
	Methylene chloride	Free liquid	235	136	57.7
		Free liquid	59.8	31.1	52.1
	Toluene	Free liquid	35.2	11.6	32.9
		Leachate	65.2	22.3	34.2
API/EW Mixture	Benzene	Free liquid	274	137	50.0
		Leachate	299	262	87.4
	Toluene	Free liquid	208	71.5	34.4
		Leachate	444	210	47.4
	Methylene chloride	Free liquid	514	545	106
		Leachate	452	424	93.7

Mean %RSD = 53

%RSD Range = 19-106

Note: Units = $\mu\text{g/L}$ \bar{X} = Mean results from 9-10 extractions

does, however, indicate that not all wastes may be effectively characterized by the TCLP method.

A third study, coordinated by S-Cubed (Blackburn and Show), was a collaborative study in which 23 laboratories participated. Only 11 laboratories participated in the ZHE testing. Two wastes, API/EW and mine tailings wastes, were fortified with a mixture of VOCs. All participating laboratories had prior experience in using the ZHEs (ADM and Millipore). A summary of the results is included in Table 9.

Precision results for VOCs tend to occur over a considerable range. However, the range and mean RSD compare very closely to the same collaborative study metals results (Table 4). Blackburn and Show concluded that at the 95% level of significance:

- o Recoveries among laboratories were statistically similar.
- o Recoveries did not vary significantly between the two sample types.
- o Each laboratory showed the same pattern of recovery for each of the two samples.

One can, therefore, conclude that the two samples contributed equally to the leachable VOCs and that the RSD range does not preclude acquiring consistent results.

TCLP AND MUNICIPAL POTW SLUDGES

Two studies have been conducted to determine the impact of the TCLP on Publicly Owned Treatment Works (POTW) wastes. S-Cubed (10) and the EPA's Office of Water (11) have coordinated testing of twelve POTW sludges. These wastes were analyzed as split samples by S-Cubed, the EPA contract laboratory, and by the POTW laboratory or by a POTW contracted laboratory. S-Cubed determined EP and TCLP concentrations of the wastes.

Statistically it is difficult to evaluate the data in terms of RSDs. However, some general observations are in order. Table 10 presents a summary of these studies.

Results reported from POTW wastes indicate that analytes of interest, TCLP constituents, are present in relatively low concentrations. Even though the results do vary, indications are that the TCLP method can adequately and reproducibly be used for municipal wastes. The only compound approaching the threshold limit was 2-Butanone found in one waste. An earlier study by the EPA (12) of six municipal sludges, found that benzene and chloroform also approached the threshold limit for two sludges. It appears that of the most critical application of the TCLP to POTW wastes is the ZHE/VOC analysis.

Table 9. Multi-Laboratory (11 Labs) VOCs, Precision

Waste	Compound	\bar{X}	S	%RSD
Mine Tailings	Vinyl chloride	6.36	6.36	100
	Methylene chloride	12.1	11.8	98
	Carbon disulfide	5.57	2.83	51
	1,1-Dichloroethene	21.9	27.7	127
	1,1-Dichloroethane	31.4	25.4	81
	Chloroform	46.6	29.2	63
	1,2-Dichloroethane	47.8	33.6	70
	2-Butanone	43.5	36.9	85
	1,1,1-Trichloroethane	20.9	20.9	100
	Carbon tetrachloride	12.0	8.2	68
	Trichloroethene	24.7	21.2	86
	1,1,2-Trichloroethene	19.6	10.9	56
	Benzene	37.9	28.7	76
	1,1,2,2-Tetrachloroethane	34.9	25.6	73
	Toluene	29.3	11.2	38
	Chlorobenzene	35.6	19.3	54
	Ethylbenzene	4.27	2.80	66
	Trichlorofluoromethane	3.82	4.40	115
	Acrylonitrile	76.7	110.8	144
Ammonia Lime Still Bottoms	Vinyl chloride	5.00	4.71	94
	Methylene chloride	14.3	13.1	92
	Carbon disulfide	3.37	2.07	61
	1,1-Dichloroethene	52.1	38.8	75
	1,1-Dichloroethane	52.8	25.6	49
	Chloroform	64.7	28.4	44
	1,2-Dichloroethane	43.1	31.5	73
	2-Butanone	59.0	39.6	67
	1,1,1-Trichloroethane	53.6	40.9	76
	Carbon tetrachloride	7.10	6.1	86
	Trichloroethene	57.3	34.2	60
	1,1,2-Trichloroethane	6.7	4.7	70
	Benzene	61.3	26.8	44
	1,1,2,2-Tetrachloroethane	3.16	2.1	66
	Toluene	69.0	18.5	27
	Chlorobenzene	71.8	12.0	17
	Ethylbenzene	3.70	2.2	58
	Trichlorofluoromethane	4.05	4.8	119
	Acrylonitrile	29.4	34.8	118

Mean %RSD = 75
%RSD Range = 17-144

Note: Units = $\mu\text{g/L}$

Table 10. TCLP Summary of Twelve POTW Wastes

<u>Parameter</u>	<u>Laboratory</u>	<u>% of Analytes Detected</u>	
		TCLP	EP
Metals (8 EP Metals)	EPA	27	11
	POTW	36	NA
Semi-Volatiles	EPA	2	NA
	POTW	2	NA
Volatiles	EPA	4	NA
	POTW	10	NA
Analytes Found in at Least 50% of the Wastes	Range of Analyte Concentrations (mg/L)	Proposed Regulatory Threshold (mg/L)	
Barium	0.2-3.9	100	
Cadmium	0.02-0.21	5.0	
Toluene	0.0008-0.95	14.4	
2-Butanone	0.0165-2.2	7.2	
Ethylbenzene	0.0006-0.0085	NL	
Methylisobutylketone	0.0048-0.064	NL	
Xylene	0.0045-0.05	NL	
p-Cresol	0.17-1.5	10	
Phenol	0.0008-0.39	14.4	

Note: NA = Not analyzed
 NL = No limits have been proposed

The results from the POTW study are encouraging in light of the previous studies. Some of the POTW tests were performed by POTW laboratories or other commercial laboratories which did not have experience with TCLP equipment. Quality control guidelines, while included in the method, were not otherwise specified. In other words, the results may be more typical of laboratory variations among all types of laboratories. It could, however, be argued that since the POTW sludge had relatively low levels of constituents of concern, that a true test of laboratories' abilities to perform the test had not been measured.

The POTW study results do not appear to differ greatly from the method performance studies.

CONCLUSION

From strictly an analytical application, the TCLP provides a workable method which performs equally well or better than the EP procedure. With practice and good laboratory skills, the extraction procedure can become routine. As stated earlier, difficulties in achieving sample homogeneity and in collecting representative samples from waste sources, may imply that a single TCLP analysis of a waste could provide misleading information.

The TCLP was designed to provide reproducible extraction results, not to determine total constituents of a waste. It is likely that some waste constituents may give highly variable results which are matrix dependent and unpredictable. Distribution coefficients (K_d s) or sorption coefficients of compounds will vary with the sample matrix. In general, TCLP performance appears to satisfy regulatory needs.

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DETERMINATION OF ORGANIC COMPONENTS IN LEACHATES - A SURVEY

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ABSTRACT

The mobility of organic compounds in the environment is dependent on compound solubility, sorption effects, pH behavior, and pH of the mobile phase. The mobility of model organic compounds has been evaluated to determine the effect of each of these factors.

Model compounds were applied to soil columns containing varying amounts of humic matter. The columns were eluted under different pH conditions and the concentration of the model compounds was determined in the resulting leachates by gas chromatography/mass spectrometry. The leachate volume and compound's concentration in the leachate indicate the compound's mobility under the test conditions. The effect of soil composition, pH of the mobile phase, and compound pH behavior were evaluated using this approach. Leaching behavior and mobility of the compounds studied are explained in terms of these factors.

INTRODUCTION

The effect of physical/chemical factors on migration of toxic compounds in the environment has not been widely investigated. It is known that water soluble organics, such as solvents, quickly migrate from waste to groundwater, and that the migration results from the leaching of the organics from the waste. Little is known, however, about the mechanism controlling the migration of chemicals contained in the leachate.

The migration of toxic organic chemicals in the environment was simulated by applying three organic chemicals to sand and soil columns. Migration was evaluated by varying the pH of the leaching media, the amount of humic material contained in the column, and compound solubility behavior.

EXPERIMENTAL

Two sets of three columns (6 cm by 1.5 cm id.) were prepared. Each column set consisted of a column filled with sand, a 1:1 sand/soil mixture, and soil. One set of columns was eluted, first with water, and then with .1N, pH 5, Acetic Acid buffer. The other set was eluted with water and then .1N, pH 8.5, Acetate buffer. After collection of 20 mL of eluate, 10 mg each of Acetophenone, p-Nitroaniline, and p-Chlorobenzoic acid were added to the columns. Eluates from the columns were collected as 50 mL aliquots. A total of 400 mL of eluate was collected from each column. All chemicals were obtained from Aldrich Chemical and were used without purification.

Eluates were analyzed by GC/MS on a Finnegan MAT INCOS 50 GC/MS system. Samples were injected directly onto a J&W DB-5 column using

split injection technique. The GC was programmed from 100 to 200°C at 10°C per minute. Quantitation was performed using Finnegan Target Compound Analysis (TCA) software using a three point calibration.

RESULTS

The column experiments were designed to evaluate the effects of leachate pH, stationary phase organic content, and acid/base compound behavior on compound migration. A low (pH 5) and high (pH 8.5) pH were chosen to represent the range of pHs that might be reasonably encountered in the environment. The amount of soil in each column was varied to determine what effect, if any, organic carbon contained in soil would have on migration. The three compounds used were selected based on their water solubility and acid/base/neutral characteristics.

In all substrate cases, the Acetophenone was the first compound to elute and was essentially completely eluted within the first 150 mL regardless of pH. Each 50 mL passing through the column is roughly equivalent to 11 inches of rain fall. The behavior of the chlorobenzoic acid and nitroaniline were greatly affected by the pH of the leaching media and to a lesser extent column substrate composition. As expected, the acid eluted more quickly at high pH and the aniline at low pH. The make-up of the column had almost no effect on elution of these compounds.

DISCUSSION

Column composition had very little effect on migration behavior of the compound tested. In this case, the concentration of each compound in the eluate was determined only by the compounds solubility and pH behavior. While this result is not entirely surprising, the widely accepted view that soil attenuates compound migration may not be warranted.

EVALUATION OF THE TCLP FOR DETERMINING THE RELEASE
POTENTIAL OF OILY WASTES

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ABSTRACT

Oily wastes are important because they are produced in large quantities and often contain toxic organic and inorganic constituents. Oily waste constituents may be released from landfills by several mechanisms. They may be released as immiscible liquids or as aqueous colloidal suspensions of these liquids. Infiltrating water may leach various constituents from the wastes, resulting in release of contaminants as dissolved compounds. Case histories demonstrate the potential for oily wastes to contaminate ground water by any or all of these mechanisms; the relative importance of each depends on the properties of the waste and the characteristics of the environment in which it has been disposed.

The TCLP was designed to model release of contaminants from a reasonable worst-case mismanagement scenario: codisposal of 5 percent industrial waste with 95 percent municipal refuse in a sanitary landfill. To evaluate how well the TCLP simulates release of hazardous waste constituents under this scenario, the procedure was performed on 11 different wastes. However, oily wastes were not evaluated. The objective of this research effort is to determine the applicability of the TCLP to oily wastes by answering the following questions: 1) Does the TCLP adequately estimate the amount and quality of hazardous contaminants that will be released as organic liquids from a Subtitle D landfill?; 2) Does TCLP adequately estimate the quality of aqueous leachate that will be generated by the dissolution of oily wastes in a Subtitle D landfill?; and 3) Are there any procedural problems that may be encountered when applying the TCLP to oily wastes? This presentation describes current progress of Phase I of this research effort, addressing Questions 1 and 3. Question 2 will be addressed during Phase 2.

Phase I research will determine whether or not the filter in the initial filtration step of the TCLP acts as an exclusion filter for certain constituents of oily wastes for which soil in landfills does not. To make this determination, the fractional amounts of four specific oily wastes that pass through the TCLP filter will be compared with the fractional amounts of the same wastes that pass through packed soil columns designed to represent landfill conditions. In addition, TCLP extractions will be performed on the four wastes in triplicate to evaluate the single-laboratory precision of the procedure and to identify any procedural problems associated with testing oily wastes in the TCLP test devices. Both TCLP test devices (extraction bottles and the zero headspace extractor) will be included in this evaluation.

METALS AND MISCELLANEOUS ANALYTES

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EVALUATION OF MICROWAVE TECHNIQUES TO PREPARE
SOLID AND HAZARDOUS WASTE SAMPLES FOR ELEMENTAL ANALYSIS

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ABSTRACT

The use of microwave energy to facilitate sample decomposition prior to elemental analysis is now receiving considerable attention. Both wet and dry digestions are achievable. When microwave energy is used in combination with acid mixtures in closed vessels, the combined pressure and rapid heating can reduce digestion times to a few minutes from hours or days that may be required for open beaker digestions. This savings in time and labor is significant and has prompted the Office of Solid Waste to evaluate this technology as a preparation tool for solid wastes. Of particular interest are used oils and other fuels slated for incineration, incinerator ash and particulates from these processes.

This study reports on the evaluation of a commercially available microwave oven sample preparation system for this application. The effect of sample preparation conditions, including the acid matrix, heating time, and pressure were evaluated for fifteen toxic or hazardous elements in particulates, ashes, oils, and oil fuels. Analyses were carried out by inductively coupled plasma emission spectroscopy.

INTRODUCTION

The techniques that are typically used to prepare Resource Conservation and Recovery Act (RCRA) wastes for analysis for metals and other elements are relatively time consuming, requiring several hours to several days to complete. They also often involve the use of acid digestions and thermal decomposition steps which may result in analyte losses, incomplete recoveries, or sample contamination. These limitations are well known to the analytical community and to the end users of these data in EPA, states, and industry. The resulting inefficiency of these techniques reduces laboratory sample throughput, drives up the cost of analytical testing and impedes decision-making. Given these concerns, the USEPA Office of Solid Waste is interested in developing cost effective sample preparation techniques for metals and other elements in environmental and process waste samples. Once developed, these techniques can then be written as methods for inclusion in "Test Methods for Evaluating Solid Waste, SW-846" and made available to the user community.

One particularly attractive sample preparation technique that is now receiving considerable attention is microwave assisted sample dissolution. The use of microwave energy to facilitate sample decomposition prior to elemental analysis has received considerable attention in recent years. Both wet and dry digestions are achievable. When microwave energy is used in combination with acid mixtures in closed vessels, the combined pressure and rapid heating can reduce digestion times to a few minutes, from hours or days that may be required for open beaker digestions. This savings in time and labor is significant and has prompted the Office of Solid Waste to evaluate this technology as a preparation tool for solid wastes. Of particular interest are used oils and other fuels slated for incineration, incinerator ash, and particulates from these processes.

Previous evaluative work with those matrices has been carried out by Nadkarni¹. Using a commercial microwave oven and an HF/aqua-regia digest, National Bureau of Standards (NBS) Coal 1632a and NBS SRM 1633a Fly Ash were solubilized. Copeland² (1985) used a HNO₃/H₂O₂ microwave procedure to prepare waste oils for determination of As, Be, Cd, Ni, and Pb.

This study reports on the evaluation of a commercially available microwave oven sample preparation system. The effect of sample preparation conditions, including the acid matrix, heating time, and pressure were evaluated for toxic or hazardous elements in particulates, ashes, oils and oil fuels. Analyses were carried out by inductively coupled plasma emission spectroscopy.

EXPERIMENTAL METHODS

Microwave Oven

The MDS-81D Microwave system (CEM Corporation, Indian Trail, NC) was used for this study. The oven resembles a standard microwave oven, but is equipped with additional features to facilitate sample preparation. For example, the Teflon-coated microwave cavity has a variable speed corrosion resistant exhaust system. The main element of the system couples a precise microwave variable power system with a programmable micro-processor digital computer. Other elements include a rotating turntable, Teflon vessels with caps and a patented pressure relief valve, a capping system, and a cooling tank.

The Teflon sample vessels and caps are designed to withstand pressures up to 100 psi and temperatures up to 200°C.

Inductively Coupled Plasma Emission Spectrometry (ICPES)

All analytical measurements were performed using an Instrumentation Laboratory 200 ICAP.

Reagents

All inorganic acids used were of "Ultrex" quality, from J. T. Baker Chemical Co. Other chemicals were of analytical reagent grade quality. Deionized water of 18 M Ω /cm specific resistivity was used.

Combustion Source Materials

The evaluation of microwave procedures was carried out using the following materials:

- o NBS coal 1632a
- o NBS coal fly ash 1633a
- o Municipal incinerator dust
- o Oil-fired power plant fly ash
- o Used motor oil

Microwave Preparation Procedures

Total Digestion Procedure A 300 mg sample is placed in a 60 mL Teflon digestion vessel equipped with a relief valve and treated with 1 mL concentrated HNO₃, 3 mL HF, and 0.5 mL HClO₄. The vessel is sealed and heated in the microwave oven at 15 percent power for 5 minutes, followed by 30 percent power for 15 minutes. The vessel is cooled, the cap removed, 3 mL HF added, resealed and heated at 20 percent power for one hour. The cap is then removed and the contents evaporated until fuming ceases. Six mL of 20 percent HNO₃ are added and evaporated to dryness; 15 mL 20 percent HCl added, the vessel sealed and heated at 10% power until dissolution of the residue is achieved. The vessel is uncapped, evaporated to dryness, and 15 mL 5 percent HCl is added and heated until the solution clears. The vessel is cooled and diluted with deionized water to 50 mL. Total digestion time is two hours.

HF/Aqua-Regia Procedure A 300 mg sample is placed in a 60 mL Teflon digestion vessel equipped with a relief valve and treated with 6 mL aqua-regia (3HCl:1HNO₃) and 2 mL HF. The vessel is sealed and heated in the microwave oven at 100 percent power for 3 minutes. The vessel is cooled, the cap removed, the digestate filtered and transferred to a 50 mL volumetric flask with deionized water.

Oily Waste HNO₃ Procedure A 300 mg sample is placed into a 120 mL Teflon digestion vessel equipped with a relief valve and 12 mL concentrated HNO₃ is added. The vessel is sealed, capped, and digested according to the six steps below:

<u>Step</u>	<u>100% power</u>	<u>0% power</u>	<u>Vent</u>
1	6 minutes	2 minutes	vent
2	2 minutes	3 minutes	vent
3	6 minutes	3 minutes	vent
4	7 minutes	4 minutes	vent
5	8 minutes	4 minutes	vent
6	10 minutes	Cool to room temp.	vent

Total digestion time is 40 minutes.

RESULTS

Total Digestion Procedure

A total digestion ($\text{HClO}_4/\text{HF}/\text{HCl}$) microwave procedure was used to prepare NBS SRM 1633a Fly Ash. With the exception of Mn and Pb, recoveries were all within 25 percent of the NBS values (Table 1). In addition, results for most elements are comparable to those from a more time-consuming conventional open-beaker digestion of the same material.

A modified total digestion procedure was used to prepare NBS SRM 1632a coal. Due to the potential explosion hazard of the HClO_4 /coal mixture, the elements with the exception of Mn, Pb, and Co (Table 2). The low recovery for Pb is more likely a result of the analytical procedure (ICP) rather than the microwave preparative step.

Tables 3 and 4 provide results for the total digestion of an oil-fired power plant fly ash and municipal incinerator dust. Results for Al, Mn and V for the power plant ash, and Al, As, Mn and V for the incinerator dust were corroborated by Neutron Activation Analysis.

Table 1. Analysis of NBS SRM 1633a Fly Ash
(Total Digestion Procedure)
(ug/g)

Element	Mean \pm S.D. (n = 3)	NBS Values	% Bias	Conventional ³ Digestion
Al	160,000 \pm 28,000	(140,000)	+14	148,000
Be	11.9 \pm 0.6	(12)	-1	15
Ca	9360 \pm 1060	11,000 \pm 100	-15	11,200
Co	49.5 \pm 0.3	(46)	+8	---
Cr	169 \pm 59	196 \pm 6	-14	160
Cu	90.3 \pm 3	118 \pm 3	-24	110
Mn	133 \pm 2	(190)	-30	175
Ni	130 \pm 25	127 \pm 4	+2	130
Pb	45.1 \pm 20	72.4 \pm 0.4	-38	82
V	313 \pm 3	(300)	+4	310
Zn	205 \pm 8	220 \pm 10	-7	210

n = number of replicates

() = uncertified value

% bias = % difference between certified and experimental results

Table 2. Analysis of NBS SRM 1632a Coal
(Total Digestion Procedure)
(ug/g)

Element	Mean \pm S.D. (n= 2) RTI	NBS Values	% Bias
Al	30,200 \pm 5900	(31,000)	-3
Ca	2050 \pm 340	2300 ⁴	-11
Co	9.72 \pm 0.19	(6.8)	+43
Cr	31.2 \pm 2.8	34.4 \pm 1.5	-3
Cu	15.6 \pm 0.6	16.5 \pm 1	-5
Fe	10,400 \pm 600	11,100 \pm 200	-6
Mg	1030 \pm 180	--	
Mn	19.6 \pm 0.6	28 \pm 2	-30
Ni	17.3 \pm 1.8	19.4 \pm 1	-11
Pb	ND	12.4 \pm 0.6	
Sc	5.45 \pm 0.56	(6.3)	-14
V	44.2 \pm 0.7	44 \pm 3	+0.4
Zn	34.9 \pm 0.3	28 \pm 2	+25

n = number of replicates

() = uncertified value

% bias = % difference between certified and experimental results

Table 3. Analysis of Oil-Fired Power Plant Fly Ash
(Total Digest Procedure)
(ug/g)

Element	RTI	NAA
	Mean \pm S.D. (n=3)	Mean \pm S.D. (n=2)
Al	44,700 \pm 7120	43,600 \pm 200
Ca	12,700 \pm 1860	
Co	697 \pm 86	
Cr	834 \pm 196	
Cu	634 \pm 85	
Fe	77,000 \pm 10,800	
Mg	17,500 \pm 2670	<14,300
Mn	438 \pm 67	504 \pm 40
Ni	15,800 \pm 2010	
Pb	1470 \pm 214	
V	24,700 \pm 3380	23,600 \pm 300
Zn	5,050 \pm 681	

NAA = Neutron Activation Analysis
n = number of replicates

Table 4. Analysis of Municipal Incinerator Dust
(Total Digestion Procedure)
(ug/g)

Element	RTI	NAA
	Mean \pm S.D. (n=3)	Mean \pm S.D. (n=2)
Al	12,500 \pm 1700	11,500 \pm 400
As	87.5 \pm 21.3	115 \pm 6
Ca	27.8 \pm 3.0%	
Cd	75.8 \pm 8.3	<154
Co	5.64 \pm 1.4	
Cr	32.4 \pm 4.5	
Cu	134 \pm 15	<847
Fe	7170 \pm 1150	
Mg	3810 \pm 580	<13,000
Mn	209 \pm 27	169 \pm 5
Ni	13.5 \pm 1.2	
Pb	1970 \pm 230	
Sc	1.82 \pm 0.24	
V	33.9 \pm 1.8	35.0 \pm 3.0
Zn	9210 \pm 1610	

NAA = Neutron Activation Analysis
n = number of replicates

Table 5. Analysis of NBS SRM 1632a Coal
(Modified HF/Aqua-Regia Procedures)
Mean \pm S.D. (n = 3)
(ug/g)

Element	A ^a	B ^b	C ^c	NBS Values
Al	24,600	22,700 \pm 1600	19,600 \pm 3500	31,000
Cd	<0.5	<0.5	<0.5	0.17 \pm 0.02
Co	ND	7.37 \pm 0.73	5.77 \pm 0.74	(6.8)
Cr	20.1	24.6 \pm 2.7	23.8 \pm 5.3	34.4 \pm 1.5
Cu	10.3	11.4 \pm 1.0	10.5 \pm 4.0	16.5 \pm 1
Fe	7270	8940 \pm 267	9180	11,100 \pm 200
Mn	19.6	28.2 \pm 1.9	20.7 \pm 1.9	28 \pm 2
Ni	18.3	12.9 \pm 2.2	16.6 \pm 0.4	19.4 \pm 1
Pb	7.2	15.4 \pm 3.2	9.68 \pm 3.00	12.4 \pm 0.6
V	33.0	47.6 \pm 3.1	38.9 \pm 2.2	44 \pm 3
Zn	23.7	15.1 \pm 2.3	13.7 \pm 5.5	28 \pm 2

^aHF/Aqua-Regia, microwave 3 minutes @ 100% power, n = 1

^bHF/Aqua-Regia, microwave 6 minutes @ 75% power

^cAshed at 400°C 4 hours-HF/Aqua-Regia, microwave 3 minutes @ 100% power

n = number of replicates

() = uncertified value

Table 6. Analysis of Used Motor Oil
(Oily Waste HNO₃ Digestion)

Element	Microwave/ICP Mean \pm S.D.(n=2)	Spike Recovery (%)	NAA Mean \pm S.D.(n=2)
Al	17.2 \pm 3.6	83.9	20.6 \pm 4.3
Ca	632 \pm 6		
Cd	2.23 \pm 0.21		6.64 \pm 1.00
Cr	6.00 \pm 0.59	71.9	
Cu	14.8 \pm 0.3	81.0	17.9 \pm 3.0
Fe	148 \pm 1	93.7	
Mg	380 \pm 6	98.5	418 \pm 71
Mn	38.1 \pm 0.3		25.8 \pm 0.5
Ni	ND	74.5	
Pb	679 \pm 4	102	
Zn	1160 \pm 10		

NAA = Neutron Activation Analysis

n = number of replicates

Table 7. Comparison of Digestion Time of
Microwave and Conventional Techniques

Acid Digestion	Microwave	Conventional
Nitric Acid	40 minutes	4 to 6 hours
Hydrofluoric/aqua-regia	3 to 10 minutes	6 to 8 hours
Total digestion	2 hours	16 hours

Keywords

Microwave oven, RCRA wastes, acids, combustion, preparation, digestion, metals, fuels, incineration, ashes

HF/Aqua-Regia Procedure

NBS SRM 1632a coal was digested using three modifications of the HF/Aqua-Regia microwave procedure (Table 5). The modified conditions were 3 minutes at 100% power (column A), 6 minutes at 75% power (column B), and a dry ash of 4 hours, followed by 3 minutes at 100% power (column C).

Microwave digestion for 6 minutes at 75% power yielded higher recoveries for Co, Cr, Mn, Pb, and V than the other variations, whereas digestion for 3 minutes at 100% power gave highest recoveries for Ni and Zn. Dry ashing followed by microwave HF/aqua-regia gave the lowest recoveries, with the exception of Fe, which was higher.

Oily Waste HNO₃ Procedure

The oily waste microwave procedure was used to digest a used motor oil (Table 6). With the exception of Cd, there is good agreement between microwave/ICP-AES results and those by Neutron Activation Analysis. In addition, acceptable recoveries were obtained from spiking the used oil with NBS 1084 -- wear metals in oil.

CONCLUSIONS

Our preliminary work indicates that substantial time/cost savings can be achieved using microwave digestion -- particularly with closed vessel procedures. Table 7 illustrates the considerable time savings for the three procedures used in this study compared to conventional techniques. There is also a potential reduced need for reagents such as HClO₄ and HF.

Future work will involve refining the acid digestion/microwave conditions for combustion source samples and other RCRA wastes.

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RESULTS OF AN INTERLABORATORY STUDY OF ICP METHOD 6010
COMBINED WITH DIGESTION METHOD 3050*

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ABSTRACT

An interlaboratory study of solid wastes using inductively coupled plasma atomic emission spectroscopy (ICP-AES), which is included in the EPA methods publication SW-846, was performed with nine participating laboratories. The study focused on the application of Method 6010 for the analysis of solid materials including dried sludges, sediments, and fly ash. Spiked predigests of seven different solid materials and several quality control (QC) materials were used to characterize and evaluated Method 6010 when sample digestion was excluded as a variable.

In practice, the digestion of solid samples is necessary to apply Method 6010 to the analysis of solid wastes. Therefore, a parallel study of Method 3050 (Acid Digestion of Sediments, Sludges, and Soils) was included in the collaborative study. A statistically-designed homogeneity study on the seven solids was performed by the coordinating laboratory prior to the collaboration study in order to measure the between-sample and within-sample variability of the solid portions that were to be sent to the participating laboratories. Portions of the seven homogeneous solids with appropriate QC samples and spiking solutions were provided to the participating laboratories to be digested by Method 3050 and analyzed according to Method 6010. Because homogeneous solid samples were used in this study, the typically large variation contributed by sampling was minimized. This collaborative study, based on the experimental design involving undigested and predigested solids, provides data on the interlaboratory and intralaboratory variabilities of Method 3050 and Method 6010 together and independent of one another.

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One spiked predigest had a high level (1,000 ppm) of vanadium and molybdenum in order to test the impact on the measurement of the other analytes. The analytical results on this spiked predigest demonstrate that certain elements, in particular antimony, arsenic, selenium and thallium, give analytical results with high uncertainty when the interfering elements vanadium and molybdenum are present at high levels.

The statistical data obtained on the homogeneity study of the solids used in the study will be reported, and the variation in analytical results as a function of the digestion method and the instrumental method will be discussed. Results obtained on both sequential and simultaneous ICP-AES as well as atomic absorption spectrophotometry will be compared statistically. The use of the method of standard additions (MSA), required in Method 6010, will be evaluated critically, because the application of the MSA affects the economics, turnaround time, and the practicality of the method, as well as the data quality. On average the use of the method of standard additions did not provide ICP-AES data that was more accurate than that obtained by normal calibration when atomic-absorption data were used as the reference values.

INTRODUCTION

An interlaboratory study of solid wastes using the EPA analytical Method 6010 entitled "Inductively Coupled Plasma Atomic Emission Spectroscopy" (ICP-AES), which is included in the EPA methods publication SW-846, was performed with nine participating laboratories. This interlaboratory study concentrated on the application of Method 6010 for the determination of 23 elements in seven solid materials including dried sludges, sediments, and fly ash. The 23 target elements are: Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, K, Pb, Mg, Mn, Mo, Ni, Se, Ag, Na, Tl, V, and Zn. This study followed a single-laboratory evaluation that investigated the application of Method 6010 to a variety of aqueous and solid-waste samples. The different waste matrices studied in the single-laboratory evaluation required the utilization of several different digestion procedures. In contrast, this interlaboratory study examined Method 6010 for the analysis of solid wastes that were digested using a single digestion procedure.

Since the digestion of solid samples is necessary to apply Method 6010 for the analysis of wastes, a thorough study of Method 6010 must also include digestion as a variable. Consequently, a parallel study of Method 3050 (Acid Digestion of Sediments, Sludges, and Soils) was included as an integral part of the interlaboratory study. The present study was designed to determine the performance of Method 6010 both independent of and together with the Method 3050 digestion procedure.

Seven solid materials, representative of solid wastes, were selected as the method evaluation materials. Three of the materials (river sediment, coal fly ash, and estuarine sediment) are Standard Reference Materials from the National Bureau of Standards, and one material (the mine tailing) is an EPA reference material. The other three solids (a contaminated soil and two industrial sludges) were obtained from the EPA. A detailed, homogeneity study was performed by the coordinating laboratory before the solids were distributed to the participating laboratories. The results indicated that the solid samples were homogeneous.

Sixteen grams of these homogeneous solids were distributed to the laboratories to be digested by Method 3050, both unspiked and spiked. The spiking solution that was provided to the laboratories contained 19 of the 23 target elements and was designed to be added to the solids prior to digestion to bring the concentrations of the 19 elements in the laboratories' digests to minimum levels of about 100 times the corresponding "Estimated Instrumental Detection Limits" given in Method 6010. It was not necessary to spike Al, Ca, Fe, and Mg into the bulk digests (or spiked solids) because of the high endogenous concentrations of these metals in the 7 solid samples. Having each laboratory spike portions of the solid samples with the spiking solutions prior to digestion assured that each laboratory used equally spiked aliquots of the solid (which eliminated the need to create uniformly-spiked solids for distribution). The resulting unspiked and spiked solids digests were analyzed by Method 6010.

In order to remove sample-preparation variability from measurement variability, bulk digests of the 7 solid samples were prepared by the coordinating laboratory by digesting 40 grams of each of the seven solid materials. These bulk digests were spiked with standard solutions in sufficient quantity to yield two liters of solution with the concentrations of all 23 elements at about 100 times the corresponding "Estimated Instrumental Detection Limits." Thus, the spiked bulk digests of the seven solid samples were very similar in composition to the spiked solids digests that were prepared by the laboratories. Data from the Method 6010 analysis of these spiked bulk digests could be compared to data from the spiked solids in order to estimate the variances due to the digestion and analysis procedures. In order to test the effects of high levels of V and Mo on the determination of the other analytes by Method 6010, the spiked bulk digest from the fly ash solid was also spiked to contain 0.1 percent of these interfering elements.

In addition to the solid samples and the spiked bulk digests, two QC solutions containing the target elements were provided to the participating laboratories for analysis with and without digestion. Because these solutions were carefully prepared and verified by the coordinating laboratory, the results could be used to estimate the

accuracy of the Methods. Other solutions were provided to the participating laboratories to insure high ICP-AES data quality. These were initial calibration verification solutions and an interference check solution.

The results of this collaborative study yielded quantitative information on the precision and accuracy of Method 6010, independently and together with Method 3050. Data obtained on sequential and simultaneous ICP-AES instruments as well as by atomic absorption spectroscopy (AAS) were compared statistically, and the results are reported. The method of standard additions (MSA) is a conditional requirement of Method 6010, so its effect on data quality was investigated.

RESULTS AND DISCUSSION

The wide range of instrumental detection limits (IDL's) reported by the participating laboratories are listed in Table 1 along with the IDL's required for the 6010 study and other lists. The UNLV "Recommended Required Instrumental Detection Limits" (to ensure more uniform levels of detection) given in Table 1 were obtained by inspection of the reported mean IDL's, the "Estimated Instrumental Detection Limits listed in Method 6010," and the minimum reported IDL's.

This multilaboratory evaluation of Method 6010 demonstrates that the method, as described, is capable of achieving excellent precision as estimated in terms of percent relative standard deviation (percent RSD), for the determination of the 23 elements in quality control (QC) solutions (Table 2). These QC solutions contained the 23 elements at concentrations of approximately 100 times the instrumental detection limits, and the solutions were interference-free in that no interfering elements were present at high concentrations. The percent RSD's for the elements range from 3.1 percent to 9.1 percent for the QC solutions that were analyzed by Method 6010 without digestion and from 2.6 percent to 52 percent for the QC solutions that were analyzed after digestion by Method 3050. The median percent RSD's for the 2 sets of QC solutions are 6.5 and 6.7 percent, respectively. This precision is considered excellent for these solutions. Silver with a percent RSD of 52 is the lone outlier in the QC solution set that was digested before analysis.

The interlaboratory precision for Method 6010, with digestion eliminated as a variable, was determined for the 23 elements in the spiked bulk digests of six representative solid complex matrices, including river and estuarine sediments and industrial sludges (Table 3). The analyte concentrations in these spiked bulk digests were about 100 times the instrumental detection limits. The median percent RSD's for the 6 sediments across 23 elements range from 6.8

TABLE 1. INSTRUMENTAL DETECTION LIMIT SUMMARY (ug/L)

Element	Range for Participating Laboratories	Mean for Participating Laboratories	6010 Study Required IDL	CLP# CRDL	UNLV ⁺ Recommended Required IDL
Al	17-192	101	200	200	100
Sb	25-116	70	200	60	70
As	22-400	139	200	10	100
Be	1-4	2.4	5	5	2
Cd	3-18	6.4	20	5	5
Ca	4-4500	660	5000	5000	50
Cr	4-31	11	30	10	10
Co	4-38	16	50	50	20
Cu	3-24	11	30	25	15
Fe	3-80	30	100	100	30
Pb	10-200	89	200	5	70
Mg	1-3600	550	5000	5000	30
Mn	1-12	4.3	20	15	4
Mo	4-60	22	30	**	15
Ni	8-31	20	60	40	20
Se	41-590	200	300	5	150
Ag	3-20	10	30	10	10
Tl	49-400	180	200	10	150
V	3-50	23	50	50	20
Zn	2-18	9	20	20	10
Ba	1-60	13	200	200	15
Na	13-4940	930	5000	5000	150
K	189-4800	1230	5000	5000	500

* Potassium determination is highly dependent upon operating conditions and plasma position so a value was not included.

** Molybdenum is not assayed in the CLP.

CLP is Contract Laboratory Program of the EPA and CRDL is the Contract Required Detection Levels (where atomic absorption is necessary for some elements).

+ UNLV is University of Nevada at Las Vegas.

TABLE 2. PRECISION VALUES FOR THE MEASUREMENT OF THE 23
TARGET ELEMENTS BY METHOD 6010 IN THE QUALITY
CONTROL SOLUTIONS

Method 6010 ^a		Method 6010 with Method 3050 ^b	
Element	%RSD ^c	Element	%RSD ^c
Ag	9.1	Ag	52
Tl	8.5	As	13
Zn	8.3	Cd	11
Cr	8.2	Se	10.1
Sb	7.7	Tl	9.5
Se	7.5	Mo	8.9
Ca	7.4	V	8.4
Cd	7.0	Sb	7.7
Mo	6.9	K	7.2
K	6.6	Zn	6.8
V	6.6	Ba	6.8
Mg	6.5	Ca	6.7
As	6.4	Ni	6.6
Al	6.3	Mg	6.2
Co	5.9	Na	5.8
Fe	5.9	Pb	5.6
Pb	5.9	Fe	5.3
Be	5.8	Cr	5.2
Ni	5.7	Mn	4.5
Cu	5.6	Co	4.3
Mn	4.3	Al	4.0
Na	4.2	Be	2.9
Ba	3.1	Cu	2.6

a. The median percent RSD is 6.5 percent.

b. The median percent RSD is 6.7 percent.

c. The percent RSD data are presented in order of increasing
precision.

TABLE 3. PERCENT RSD's FOR THE DETERMINATION OF THE 23 TARGET ELEMENTS
IN THE SPIKED BULK DIGESTS

ELEMENTS	HAZARDOUS WASTE 1	RIVER SEDIMENT	FLY ASH	ESTUARINE SEDIMENT	INDUSTRIAL SLUDGE	ELECTRO- PLATING SLUDGE	MINE TAILING
Al	11	19	16	1.9	11	13	7.6
Sb	5.6	52	73	8.7	3.2	24	4.4
As	13	11	83	22	25	8.6	5.3
Be	5.8	5.8	57	4.8	6.4	9.9	8.5
Cd	11	6.6	5.7	7.6	3.1	9.8	12
Ca	8.8	9.4	5.6	5.3	8.5	7.0	8.7
Cr	6.2	5.5	36	7.6	5.8	7.8	39
Co	11	14	21	6.8	6.7	11	13
Cu	4.4	4.3	9.7	6.0	11	7.8	12
Fe	6.6	8.3	8.8	6.0	6.9	8.4	8.4
Pb	15	7.2	22	4.7	3.9	5.6	8.0
Mg	8.8	8.1	15	9.4	8.0	20	10
Mn	10	13	14	10	10	9.6	5.5
Mo	20	33	19	31	36	36	21
Ni	9.4	8.9	24	3.4	5.1	9.2	12
Se	7.5	13	24	6.2	13	13	19
Ag	29	25	56	46	47	19	27
Tl	19	12	55	29	30	20	50
V	12	58	7.5	7.3	5.5	32	18
Zn	9.1	6.7	7.6	15	11	2.5	16
Ba	11	10	8.7	6.4	8.0	20	11
Na	17	38	49	4.7	5.8	9.8	7.9
K	8.8	7.4	4.2	4.8	13	5.8	7.9
MEDIAN PERCENT RSD	10	11	19	6.8	8.0	9.8	11

percent to 11 percent. Thus, the precision for the measurement of the target elements in these complex solutions is very good.

The seventh spiked bulk digest, from coal fly ash, was spiked with very high levels of molybdenum and vanadium (0.1 percent). The median percent RSD's for the determination of the 23 elements in this spiked digest range from 4.2 percent to 83 percent with a median of 19 percent (Table 3). The 12 percent median RSD for fly ash digests without added Mo and V (Table 4) suggests that these two elements decreased the measurement precision of many of the target elements.

When Method 6010 and Method 3050 are applied in combination for the determination of the 23 elements in spiked solids, the apparent measurement precision decreases (Table 4) when compared to the corresponding spiked bulk digest. The median percent RSD's for the 7 solids across the 23 elements range from 10-19 percent. The spiked solid samples were spiked prior to digestion to ensure that the concentrations of the analytes were approximately 100 times greater than the instrumental detection limits.

The median percent RSD's for the same 7 solids, unspiked, range from 17-30 percent (Table 5). This poorer precision when compared to the spiked solids results because over 50 percent of the reported concentration values are less than 100 times the average of the instrumental detection limits. In other words, as the concentrations approach the instrumental detection limits the precision decreases as indicated by the higher percent RSD values. Four elements among those with the highest median percent RSD's are antimony, molybdenum, silver and thallium. For those elements that were present in the digests of the unspiked solids at concentrations 100 times greater than the IDL's (due to their occurrence in high concentrations in the unspiked solids), the precision is comparable to the precision for the spiked solid samples.

The Method 6010 variance and the Method 3050 variance can be calculated from the data base resulting from the analyses of the spiked bulk digests and the spiked solid samples (Table 6). A statistical analysis of the data shows that in general, the digestion procedure and the inductively coupled plasma atomic emission spectroscopic analytical procedure contribute about equally to the overall measurement uncertainty or precision (variance) for the determinations of the 23 target elements in the 7 homogeneous solids.

The accuracy of the determination of the 23 target elements by Method 6010, estimated from the analyses of the quality control solutions, is shown to be excellent, within 99 ± 3 percent of the "true" values with the exception of silver. Similarly, the accuracy of Method 6010 in combination with Method 3050 was also found to be

TABLE 4. PERCENT RSD's FOR THE DETERMINATION OF THE 23 TARGET ELEMENTS
IN THE SPIKED SOLIDS

ELEMENTS	HAZARDOUS WASTE 1	RIVER SEDIMENT	FLY ASH	ESTUARINE SEDIMENT	INDUSTRIAL SLUDGE	ELECTRO- PLATING SLUDGE	MINE TAILING
Al	17	24	20	22	14	18	26
Sb	27	56	25	62	28	40	59
As	13	27	16	22	19	20	22
Be	16	13	7.6	8.6	18	7.0	16
Cd	13	8.4	9.5	13	20	18	19
Ca	7.3	9.0	12	10	13	14	12
Cr	7.9	22	9.7	7.1	18	10	26
Co	18	22	11	9.4	17	13	18
Cu	13	14	11	9.7	19	9.1	12
Fe	14	19	44	12	18	15	18
Pb	15	6.4	9.6	11	20	19	5.8
Mg	5.9	8.5	17	9.0	16	10	10
Mn	14	9.1	11	10	17	19	9.4
Mo	19	31	23	18	18	43	20
Ni	13	20	9.8	10	20	16	17
Se	13	15	10	10	15	18	13
Ag	19	31	50	51	46	52	49
Tl	15	30	40	29	28	39	45
V	18	19	12	10	17	41	24
Zn	14	12	11	13	20	8.2	19
Ba	8.4	9.7	7.2	10	16	30	7.2
Na	14	39	25	9.4	22	15	12
K	19	17	17	15	22	5.7	15
MEDIAN PERCENT RSD	14	19	12	10	18	18	18

TABLE 5. PERCENT RSD'S FOR THE DETERMINATION OF THE 23 TARGET ELEMENTS
IN THE UNSPIKED SOLIDS

ELEMENTS	HAZARDOUS WASTE 1	RIVER SEDIMENT	FLY ASH	ESTUARINE SEDIMENT	INDUSTRIAL SLUDGE	ELECTRO- PLATING SLUDGE	MINE TAILING
Al	19	32	19	23	15	23	17
Sb	38	78	0	0	47	68	57
As	53	48	32	74	83	44	28
Be	31	27	27	35	42	70	41
Cd	37	17	57	51	17	22	59
Ca	8.0	13	10	11	10	17	9.0
Cr	10	19	28	23	12	12	90
Co	35	60	23	12	21	47	30
Cu	24	9.0	16	17	17	12	20
Fe	13	24	52	10	15	12	18
Pb	8.0	12	33	37	16	17	17
Mg	6.0	11	20	10	17	14	9.0
Mn	9.0	17	20	10	18	21	11
Mo	30	42	20	58	57	49	26
Ni	14	39	34	21	16	20	40
Se	42	61	0	30	43	74	77
Ag	41	43	49	0	37	54	60
Tl	31	30	0	0	38	45	130
V	21	72	15	17	28	35	47
Zn	14	12	20	9.0	12	9.0	20
Ba	7.0	11	4.1	14	23	38	9.0
Na	52	52	34	9.0	16	17	13
K	23	34	20	17	32	19	24
MEDIAN PERCENT RSD	23	30	20	17	18	22	26

TABLE 6. ESTIMATED PERCENTAGE CONTRIBUTIONS OF METHOD 6010 ICP VARIANCE AND METHOD 3050 DIGESTION VARIANCE TO TOTAL VARIANCE

<u>Elements</u>	<u>6010 ICP</u>	<u>3050 Digestion</u>
Al	42	58
Cd	47	53
Ca	39	61
Co	52	48
Cu	87	13
Fe	13	87
Pb	57	43
Mg	83	17
Mn	57	43
Mo	95	5
Ni	30	70
Se	100	0
Tl	85	15
V	18	82
Zn	64	36
Ba	28	72
K	19	81
As ¹	100	0
Be ¹	25	75
Ag ¹	17	83
Sb ²	2	98
Cr ²	14	86
Na ²	21	79
Median ³	52	48

1. Method 6010 variance estimates pooled over 6 of 7 spiked bulk digests
2. Method 6010 variance is median over 7 spiked bulk digest variances.
3. Medians based on first 17 elements listed above.

excellent, within 97+3 percent of the "true" values with the exception of silver. Silver showed poorer accuracies of 82 percent and 53 percent respectively. The accuracies are for these solutions that contain the analytes in concentrations of approximately 100 times the IDL's.

The accuracy of the ICP Method 6010 can be estimated for complex matrices by comparing the average concentrations of the target elements in the spiked bulk digest (as determined by Method 6010) to the corresponding concentrations which were determined by AAS by one of the participating laboratories. A null hypothesis approach that is based on the mean and on the corresponding standard deviation was used to determine if the ICP-AES and AAS values are significantly different at the 95 percent confidence level. The results indicate that only two out of 184 elemental measurements by the two methods are significantly different. In some cases where the ICP/AAS ratios are very different (less than 0.75 or greater than 1.25), the standard deviations in the ICP measurements are very high, and therefore, the differences in the means are not significant. Overall, the agreement between ICP and AAS is excellent.

A comparison of the results obtained by using Method 3050 with Method 6010 to analyze the EPA-CLP solid reference material, mine-tailing powder, for which the "true" values were obtained previously by multilaboratory ICP-AES and AAS analyses, gives an indication of the combined method accuracy for a solid matrix. The comparison (Table 7) shows that 21 of the 23 elements are within the control limits for the reference material set by the EPA, while the results on the other two elements (selenium and thallium which are near the LOD) are outside of the limits. It should be noted that in the EPA-CLP, these two elements are normally determined by graphite furnace atomic absorption spectrometry, which is more accurate than ICP-AES for the determination of these elements at low levels. These results indicate that Method 6010 can achieve good accuracy in complex matrices for the analytes studied.

The method of standard additions was required for less than 10 percent of the total analyses. Results by ICP-AES using the method of standard additions were compared with non-MSA data and atomic absorption spectrometry results for the spiked bulk digest samples. The comparison of this limited data set (Table 8) indicates that on the average there is no significant improvement in the data quality when the method of standard additions is used with Method 6010 for the analysis of the solid matrices that were used in this study.

A comparison between data obtained on simultaneous and sequential inductively coupled plasma spectrometers indicated that the data were statistically indistinguishable.

TABLE 7. RESULTS FOR THE ANALYSIS OF THE UNSPIKED MINE TAILING SAMPLE
COMPARED TO THE EPA CLP CONTROL LIMITS*

DUPLICATE No.1								
ELEMENT	MEAN	STD DEV	RSD (%)	CLP TRUE	CONTROL LOWER	LIMITS UPPER	MEAN TRUE	WITHIN LIMITS
Al	13900	1580	11	15200	7500	22900	0.91	YES
Sb	12	19	158	<20	0	44		YES
As	618	161	26	680	380	980	0.91	YES
Be	0.5	0.2	49	<1	0	1.6		YES
Cd	1.7	1.4	83	<1	0	7.8		YES
Ca	9850	718	7	10520	7850	13200	0.94	YES
Cr	12	12	104	17	0	46	0.71	YES
Co	7.2	2.1	29	6.9	0	19	1.05	YES
Cu	215	63	30	265	220	310	0.81	NO
Fe	10300	1580	15	11200	5910	16500	0.92	YES
Pb	5660	1050	19	5830	4310	7340	0.97	YES
Mg	14200	1040	7	14730	10910	18560	0.96	YES
Mn	92800	926	1	91735	68600	114900	1.01	YES
Mo ^a	56	16	29					
Ni	21	8.9	43	22	5.0	39	0.93	YES
Se ^b	43	51	117	<1	0	11		NO
Ag	8.0	6.8	85	<2	0	26		NO
Tl ^c	73	107	146	3.8	0	9.1	19.23	NO
V	13	7.2	54	19	0	46	0.72	YES
Zn	362	71	19	425	317	535	0.85	YES
Ba	397	43	11	430	360	510	0.92	YES
Na	3390	374	11					
K	8130	2620	32	8150	4540	11770	1.00	YES

* Concentrations in mg/kg.

(continued)

^a Mo is not a CLP element.

^b The average LOD for Se is 10 mg/kg.

^c The average LOD for Tl is 9 mg/kg.

TABLE 7. (concluded)

DUPLICATE No.2		STD DEV	RSD (%)	CLP TRUE	CONTROL LOWER	LIMITS UPPER	MEAN TRUE	WITHIN LIMITS
ELEMENT	MEAN							
Al	14300	3170	22	15200	7500	22900	0.94	YES
Sb	5.8	4.2	74	<20	0	44		YES
As	636	185	29	680	380	980	0.94	YES
Be	0.5	0.2	39	<1	0	1.6		YES
Cd	1.6	1.4	87	<1	0	7.8		YES
Ca	9670	962	10	10520	7850	13200	0.92	YES
Cr	15	15	100	17	0	46	0.89	YES
Co	7.8	2.6	33	6.9	0	19	1.13	YES
Cu	235	25	11	265	220	310	0.89	YES
Fe	9810	2070	21	11200	5910	16500	0.88	YES
Pb	5550	846	15	5830	4310	7340	0.95	YES
Mg	14300	1540	11	14730	10910	18560	0.97	YES
Mn	92300	11000	12	91735	68600	114900	1.01	YES
Mo ^a	55	14	26					
Ni	21	8	37	22	5.0	39	0.93	YES
Se ^b	43	49	112	<1	0	11		NO
Ag	7.5	6.3	84	<2	0	26		YES
Tl ^c	74	105	141	3.8	0	9.1	19.54	NO
V	13	8.1	62	19	0	46	0.71	YES
Zn	358	71	20	425	317	535	0.84	YES
Ba	414	27	7	430	360	510	0.96	YES
Na	3340	477	14					
K	7350	1112	15	8150	4540	11770	0.90	YES

TABLE 8. COMPARISON OF MSA AND NON-MSA RESULTS^a

SPIKED BULK DIGESTS									
SAMPLE NAME	ELEMENT	N	NON-MSA		N	MSA		%RATIO	SIG. DIF. ^c
			MEAN CONC. ^b	SD		MEAN CONC. ^b	SD		
HAZARDOUS WASTE	Cd	5	894	117	3	940	84	95	NO
HAZARDOUS WASTE	Tl	5	4410	788	3	4510	1130	98	NO
HAZARDOUS WASTE	Zn	5	4310	426	3	4560	250	95	NO
RIVER SEDIMENT	Tl	7	3160	2210	3	5050	675	63	NO
FLY ASH	Cd	5	754	422	3	897	219	84	NO
FLY ASH	Cr	5	1480	885	3	2390	1090	62	NO
FLY ASH	Pb	4	4100	634	4	6770	3300	61	NO
FLY ASH	Mn	4	1910	233	3	1750	304	109	NO
FLY ASH	Ni	3	1530	154	4	1350	500	113	NO
FLY ASH	Tl	4	5530	3730	3	1950	2470	284	NO
ESTUARINE SEDIMENT	Tl	5	3870	1290	3	3340	2850	116	NO
INDUSTRIAL SLUDGE	Tl	5	4470	872	3	4620	2230	97	NO
ELECTROPLATING SLUDGE	Tl	3	4600	740	4	5350	1120	86	NO
MINE TAILING	Cd	5	850	69	3	985	112	86	NO

^a Only those elements that required the application of the MSA by three or more laboratories are included as statistically significant.

^b Concentration for liquids in ug/L; concentration for solids in mg/kg.

^c Result of a null hypothesis approach used to indicate whether MSA and non-MSA results are significantly different.

N - Number of cases.

% Ratio - non-MSA to MSA mean concentrations.

(continued)

TABLE 8. (continued)

UNSPIKED SOLIDS									
SAMPLE NAME	ELEMENT	N	NON-MSA		N	MSA		%RATIO	SIG. DIF. ^c
			MEAN CONC. ^b	SD		MEAN CONC. ^b	SD		
HAZARDOUS WASTE	Be	4	0.8	0.1	3	0.7	0.2	93	NO
HAZARDOUS WASTE	Cr	6	95	8.4	3	111	10	86	YES
HAZARDOUS WASTE	Co	6	8.0	2.4	3	9.1	1.5	88	NO
HAZARDOUS WASTE (DUP.)	Ni	5	17	1.3	4	13	8.9	128	NO
RIVER SEDIMENT	Sb	6	325	266	3	169	246	192	NO
RIVER SEDIMENT	Cd	6	11	2.5	3	11	3.5	103	NO
RIVER SEDIMENT	Co	5	21	16	4	21	19	99	NO
RIVER SEDIMENT	Ni	6	44	20	3	27	7.0	161	NO
RIVER SEDIMENT (DUP.)	Cd	6	10	1.6	3	10	0.7	107	NO
RIVER SEDIMENT (DUP.)	Ni	6	39	13	3	38	19	105	NO
FLY ASH	Be	6	3.0	0.8	3	2.6	1.2	114	NO
MINE TAILING	Cd	4	2.3	1.6	3	1.9	1.1	122	NO
MINE TAILING	Zn	6	372	44	3	340	119	109	NO
MINE TAILING (DUP.)	Cd	4	2.4	1.6	3	1.5	0.8	158	NO
MINE TAILING (DUP.)	Co	6	7.3	2.5	3	8.8	3.1	83	NO
MINE TAILING (DUP.)	Ni	5	21	5.6	4	21	11	100	NO
MINE TAILING (DUP.)	Zn	6	365	43	3	345	122	106	NO
ELECTROPLATING SLUDGE	Cd	6	113	24	3	96	41	118	NO
ELECTROPLATING SLUDGE	Mn	6	226	31	3	254	126	89	NO
ELECTROPLATING SLUDGE (DUP.)	As	6	33	20	3	41	20	80	NO
ELECTROPLATING SLUDGE (DUP.)	Mo	5	14	11	3	21	7.3	68	NO
INDUSTRIAL SLUDGE	As	4	11	6.6	3	26	11	41	YES

(Continued)

TABLE 8. (concluded)

SPIKED SOLIDS									
SAMPLE NAME	ELEMENT	NON-MSA			MSA			%RATIO	SIG. DIF. ^c
		N	MEAN CONC. ^b	SD	N	MEAN CONC. ^b	SD		
HAZARDOUS WASTE	Co	6	45	8.2	3	30	2.2	149	YES
HAZARDOUS WASTE	Pb	6	340	104	3	238	14	143	NO
HAZARDOUS WASTE	Mo	6	39	20	3	29	2.8	134	NO
HAZARDOUS WASTE	Ni	6	57	10	3	37	2.9	152	YES
HAZARDOUS WASTE (DUP.)	Co	6	48	4.8	3	56	11	85	NO
HAZARDOUS WASTE (DUP.)	Pb	6	390	29	3	338	112	115	NO
HAZARDOUS WASTE (DUP.)	Ni	6	61	3.5	3	58	14	106	NO
ESTUARINE SEDIMENT	Cd	6	46	4.7	3	53	2.2	87	NO
ESTUARINE SEDIMENT	Mo	6	37	19	3	47	2.5	79	NO
ESTUARINE SEDIMENT	Ni	6	65	6.7	3	73	1.3	89	NO
ESTUARINE SEDIMENT	Tl	6	180	65	3	239	24	75	NO
ESTUARINE SEDIMENT (DUP.)	Ni	6	63	6.9	3	74	3.3	86	YES
MINE TAILING	Ni	6	64	7.9	3	60	15	108	NO
MINE TAILING (DUP.)	Ni	6	63	6.9	3	64	19	99	NO
ELECTROPLATING SLUDGE (DUP.)	Tl	6	160	46	3	304	104	53	YES

RECOMMENDATIONS

The experimental design used in this multilaboratory study has resulted in several excellent sets of multidimensional analytical data that deserve consideration beyond the intended scope of this report. Further analysis and interpretation of this data base is suggested.

The presence of high concentrations (0.1 percent) of added vanadium and molybdenum in the fly ash spiked bulk digest could account for the apparent decrease in the precision of Method 6010 for the determination of many of the 23 target elements in this matrix compared to the 6 other solid digests. The interfering effects in this matrix should be studied further.

The poor precision, accuracy, and spike recoveries for silver demonstrated in this study, should be noted in both Method 3050 and Method 6010. The possibility of precipitation in the nitric/hydrochloric acid digestion matrix as well as phototransformation should be discussed in Method 3050.

The poor spike recovery of antimony, observed in this study, should be noted in Method 3050. In particular, the possibility of the formation of oxide and oxo-chloride precipitates of antimony in the nitric/hydrochloric acid digestion matrix should be discussed.

The application of the method of standard additions (MSA), a conditional requirement of Method 6010, affects the economics, the turnaround time of analysis, the practicality of the Method, as well as the data quality. Although this report indicates that, on the average, MSA data was not significantly different from non-MSA data, (based on a selected set of statistically significant data), the requirement for the application of the MSA should be investigated further.

When soil-containing matrices are being analyzed by Method 6010, the authors are of the opinion that the method of standard additions should not be required for those elements that are endogenous to soils in high concentrations. The high-concentration endogenous elements in soils include Al, Ca, Fe, Mg, K, and Na.

Required detection limits should be included in Method 6010 to ensure uniform limits of detection. Recommended required detection limits, based on this interlaboratory study, are presented in Table 1 of this report.

Methods 6010 and 3050 should be reviewed carefully for technical completeness and comprehensiveness in view of the findings and experience obtained in this multilaboratory evaluation.

PRELIMINARY STUDIES OF THE SEPARATION AND DETERMINATION OF
CR(VI) AND CR(III) IN WATER BY
SOLID ADSORBENT EXTRACTION AND GFAA ANALYSIS

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INTRODUCTION

The importance of the speciation of Cr(III) and Cr(VI) has been widely recognized. Cr(III) is an essential trace element in mammalian systems. However, due to the toxic effects of Cr(VI) it is considered an environmental and industrial hazard. The Environmental Protection Agency (EPA) has supported the development of three analytical methods that are approved for the determination of Cr(VI).¹ The approved methods for the analysis of Cr(VI) are based on coprecipitation (Method 7195), colorimetric techniques (Method 7196), and chelation/extraction (Method 7197).

Unfortunately, Cr(VI) is a relatively labile species and will convert to Cr(III) under normal preservation procedures before analysis can be accomplished. Cr(VI) lability is emphasized by the EPA SW-846 methods which require a holding time limitation of only 24 hours prior to analysis. This holding time is extremely difficult to meet under normal circumstances of sampling, shipping, and analysis.

An additional analytical approach based on paired ion chromatography has been used to reliably separate^{2,3,4} and quantify Cr(III) and Cr(VI) using element specific detection. These methods are based on separation of the Cr species in a reverse phase HPLC eluent. The counter ion modifies the surface of a reverse phase HPLC column such that the retention and subsequent separation of Cr(III) and Cr(VI) is possible.

The application of the reverse phase adsorbent combined with the paired ion chromatography phenomena may provide an alternative to the collection and separation of chromium species while extending the sample holding time. This paper will present a preliminary procedure of rapidly separating the Cr(VI) from Cr(III) in aqueous samples during sample collection. The isolation procedure for Cr(VI) and Cr(III) requires comparable time to existing methods and may be performed by field sampling personnel without specialized training.

EXPERIMENTAL

A PRP-1 (styrene divinylbenzene co-polymer, Alltech Associates) sample cleanup cartridge is used to retain the chromium compounds. The sample is prepared by the addition of tetrabutylammonium hydroxide (TBAOH) solution to a known volume of⁴ filtered aqueous sample so that the TBAOH concentration is 5×10^{-4} molar. The PRP-1 sorbent is prepared by wetting the sorbent with 2 mL of methanol. The methanol rinse is followed by 1 mL of sodium hydroxide solution (pH 11.5).

The sample/TBAOH mixture is then passed through the PRP-1 reverse

phase sample cleanup cartridge. The Cr(VI) is retained by the paired ion on the PRP-1 column. The TBAOH/sample solution has a pH of 10 which forms the insoluble Cr(III) hydroxide. This precipitate is filtered from the sample by the column. The Cr(VI) is selectively removed from the column by eluting with two milliliters of sodium hydroxide (pH 11.5). This procedure also allows preconcentration since the Cr(III) and Cr(VI) are totally retained on the column.

After eluting the Cr(VI) from the column with sodium hydroxide, the total chromium determined in the column eluent will represent Cr(VI) only. Cr(III) can be subsequently removed with 2 mL of 10% (v/v) nitric acid and determined as total chromium so that a mass balance may be performed. The sodium hydroxide/nitric acid elution sequence must be followed or Cr(VI) will contaminate the Cr(III). The eluents may be preserved and transported as aqueous samples because the chromium species is no longer a consideration, since only total chromium determination is required for the final analysis.

RESULTS

Six ion exchange resins, one C₁₈ silica based, and one styrene divinylbenzene based resins were evaluated in this study. With each adsorbent various rinse, sample retention, and sample elution solutions were used.

The PRP-1 resin provided the best retention and percent recovery of the resins evaluated. When the procedure as described above was followed, 92.1-102% of the Cr(VI) was recovered. In addition, 92.0-114% of the Cr(III) was recovered. Table 1 provides a summary of the retention, elution, and recovery characteristics of Cr(III) and Cr(VI) using the PRP-1 resin.

Instrumental detection limits for chromium is 1.2 ng/mL. The percent relative standard deviation in 7.3%. These results are determined from 14 determinations of 5 ng/mL standard. Method detection limits have not been determined. However, estimated detection limits from the percent recovery should allow similar detection limits. Trace enrichment may also be used to realistically enhance the detection limits by two orders of magnitude.

The results obtained in this paper are from laboratory water standards. As such, the result may not represent what occurs in a field sampling situation. Therefore, to validate this procedure for environmental samples, a wide range of environmental samples should be spiked and analyzed by current EPA approved methods and this procedure. From this spiking data comparison the applicability of this procedure may be determined.

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TABLE 1
RETENTION, ELUTION, AND RECOVERY CHARACTERISTICS
OF Cr(III) AND Cr(VI) USING PRP-1

<u>Rinse</u> <u>Solution</u>	<u>Retention</u> <u>Solution</u>	<u>% Retention</u>		<u>Eluent</u>		<u>% Elution</u>		<u>% Recovery</u>	
		<u>Cr(III)</u>	<u>Cr(VI)</u>	<u>Cr(III)</u>	<u>Cr(VI)</u>	<u>Cr(III)</u>	<u>Cr(VI)</u>	<u>Cr(III)</u>	<u>Cr(VI)</u>
a	b pH=10	92	98	c	a	100	94	92.0	92.1
d	b pH=10	99	99	c	e	93	88	92.1	87.1
f	b pH=10	96	99	c	e	102	85	97.9	84.2
a	b pH=10	99	99	c	a	115	103	114	102

a NaOH TO pH=11.5 (<0.003 M)

b 0.0005 M TBAOH

c 10% HNO₃

d Deionized water

e Methanol

f 0.0001 M NaOH

EVALUATION OF SW-846 COLD-VAPOR MERCURY METHODS 7470 AND 7471

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ABSTRACT

The protocols for Methods 7470 and 7471 in the SW-846 methods manual are designed for the cold-vapor atomic absorption spectrometric (CV-AAS) determination of total mercury in aqueous (extracts, wastewater, ground water, etc.) and solid (soils, sediments, sludges, etc.) materials, respectively. Aqueous samples are digested with a combination of nitric and sulfuric acid plus permanganate and persulfate solutions at elevated temperature; solid samples are heated either in a water bath with aqua regia and permanganate, or in an autoclave with sulfuric acid, nitric acid and permanganate. The mercuric ions in the digests are then reduced with hydroxylamine, and the elemental mercury is aerated into the AAS cell, either in a closed (recirculating) or an open system, where the absorption at 253.7 nm is determined.

The topic of this presentation are the results of a performance evaluation study of these methods conducted without and with changes by the Battelle Columbus Division Laboratory. The methods were evaluated using aqueous and solid environmental samples of homogeneous and known compositions in order to assess the accuracy and precision of the methods without introducing uncertainties due to sample inhomogeneities. The methods, as originally written, were satisfactory for the analyses of samples containing relatively high concentrations of mercury. However, the method quantification limits for the closed and open systems were inadequate for the determination of mercury in environmental samples such as ground-water samples, when low concentrations were encountered. Spectral interferences which are caused by nonspecific absorption of the analytical radiation degraded the accuracy of the recirculating CV-AAS method.

The protocols in Methods 7470 and 7471 were modified to improve mercury detectability and to minimize the additive effect of nonspecific background absorption. The use of an amalgamation CV-AAS system operated in an open configuration resulted in an improvement of one order of magnitude in instrumental detection limit for mercury over the recirculating method. The results of the analyses of four reference sediment materials and a simulated aqueous waste sample by the amalgamation CV-AAS method indicated acceptable accuracy and precision for most of the environmental samples tested. As expected, the amalgamation CV-AAS method revealed no spectral interferences resulting from nonspecific absorption of the analytical radiation by organic vapors.

The modified protocols are at present the subject of a multi-laboratory evaluation with the Battelle Columbus Division laboratory as the coordinating laboratory. Seven laboratories are participating in this study.

INTRODUCTION

SW-846 Method 7470, entitled "Mercury (Manual Cold-Vapor Technique)" is designed for the determination of total mercury in mobility-procedure extracts, aqueous wastes, ground water, and other aqueous samples. The samples are digested on a steam bath with sulfuric acid and nitric acid, followed by the addition of permanganate to oxidize sulfides and of persulfate to oxidize organics. SW-846 Method 7471, entitled "Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)" is designed for the determination of total mercury in soils, sediments, bottom deposits, and sludge-type materials. The samples are digested on a steam bath with aqua regia; any sulfides remaining are oxidized with permanganate. An alternative sample preparation procedure for Method 7471 (which will be referred to as Method 7471A) specifies digestion with a mixture of sulfuric acid, nitric acid, and permanganate under heat and pressure in an autoclave. In all three methods, excess permanganate, persulfate and any free chlorine present in the digests are reduced with hydroxylamine as the hydrochloride or sulfate, and mercuric ions are reduced with stannous sulfate or chloride. The elemental mercury is then aerated from the digests through the absorption cell of an atomic absorption spectrophotometer and is continuously recirculated in a closed system until a steady-state absorption signal is attained for quantification at 253.7 nm. This technique is based largely on the system, where the mercury vapor is passed through the absorption cell only once, may be used instead of the closed system. Potential interferences listed in the methods are certain unspecified volatile organics, copper, excess chloride, and sulfide.

PHASE 1 - SINGLE-LABORATORY EVALUATION

The objective of the Phase 1 effort was to conduct a single-laboratory evaluation of the current EPA protocols for SW-846 Methods 7470 and 7471 and to determine whether or not any revisions of the current protocols would be warranted to improve analytical performance. The methods were evaluated using aqueous and solid environmental samples of homogeneous and known compositions in order to assess accuracies and precisions of the methods without introducing uncertainties such as those which would be due to sample inhomogeneities. The research was conducted in three phases: (1) evaluation of the EPA protocols, as currently written, (2) implementation and evaluation of selected modifications in the current EPA protocols, and (3) testing of the proposed revisions of

the EPA protocols on representative environmental samples. This single-laboratory evaluation was conducted by, and at, the Battelle Columbus Laboratory.

INSTRUMENTATION AND EQUIPMENT

A Model 603 atomic absorption spectrophotometer (The Perkin-Elmer Corporation) was used for all analytical measurements. The cylindrical glass absorption cell with quartz end windows was mounted on a conventional burner head for convenient positioning and for two-dimensional alignment in the optical path of the instrument. A portable lamp was used to heat the absorption cell to slightly above ambient temperature, thus preventing condensation of water vapor on the end windows. The recirculating CV-AAS system (Figure 1) required in Methods 7470 and 7471 was assembled from components and materials as specified in the current protocols. The recirculating system was configured, as necessary, for evaluation of the open ("once-through") CV-AAS system referenced in the current protocols.

All glassware was washed with hot detergent solution, rinsed with deionized water, and filled with a 1:4 (v/v) reagent-grade nitric acid/deionized water mixture. After standing for at least 4 hours at ambient temperature, the acid solution was removed and the

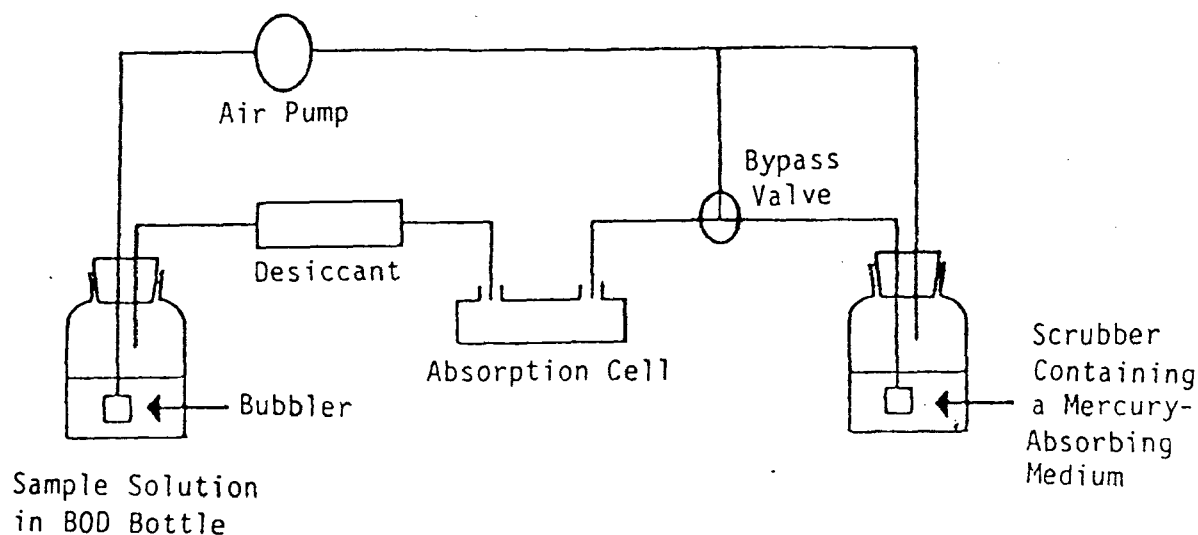


Figure 1. Apparatus for the recirculating CV-AAS method.

glassware rinsed extensively with deionized water. Watch-glass covers and plastic pipet tips were soaked in beakers with 1:4 nitric acid/deionized water, and were then rinsed extensively with deionized water.

REAGENTS AND STANDARDS

All chemicals were of ACS grade or better. Approximately 5 ug of mercury were observed in the digested reagent blanks throughout this study. Inorganic-mercury working standards were prepared fresh daily by serial dilutions with deionized water/nitric acid (100:0.15, v/v) from a commercial 1000-mg/L mercury stock solution (Fisher Scientific Company). Methyl mercuric chloride standards were prepared by diluting, with deionized water, a stock solution previously prepared from the solid material (Pfalz and Bauer), and diphenyl mercury standards were prepared by diluting, with chloroform, a stock solution previously prepared from the solid material (Aldrich Chemical Company).

SAMPLES

One simulated aqueous sample and four solid homogeneous reference samples of known compositions were used to evaluate the methods. The aqueous waste sample was prepared by decanting and combining the aqueous portions from three sludge-type waste samples provided by the EP QA Materials Bank. The pooled aqueous sample was filtered, spiked with 200 ug of mercury as mercuric chloride, acidified with nitric acid to pH 2, and diluted with tap water to 2 liters. The endogenous mercury and the spike added up to a final mercury concentration of approximately 110 ug/L for the simulated aqueous waste.

The solid reference materials were two Standard Reference Materials from the National Bureau of Standards - River Sediment NBS-SRM 1645, 1.1 ug Hg/g, and Estuarine Sediment NBS-SRM 1646, 0.063 ug Hg/g - and two reference materials from the National Research Council of Canada, Ottawa, Ontario, Canada - Marine Sediment NRCC BCSS-1, 0.129 ug Hg/g.

EXPERIMENTAL PROCEDURES

The samples were digested according to the procedures described in the current protocols. However, all sample digestions following Methods 7470 and 7471 were performed in 125-mL Erlenmeyer flasks rather than in the specified BOD bottles. Sample calibration digests could then easily be transferred to volumetric flasks and diluted to calibrated volume for subsequent analysis. The reason for this change was the concern that sample heterogeneity could become a major problem in the analysis of samples with a relatively high mercury content, and that therefore the digestion of small

sample sizes as a means of adjusting the final mercury concentration within the linear dynamic range of the method would be less desirable than the dilution of relatively concentrated sample digests. The NBS Standard Reference Materials, in recognition of the dependence of homogeneity on sample size, are certified only for portions above a specified weight.

To samples subjected to the autoclave digestion in Method 7471A, 5-mL portions of deionized water were added before the addition of the acids. This reduced the possibility of losses from the samples caused by spattering during the addition of the concentrated acids. To all sample digests, hydroxylamine reagent was added while the digests were still in the Erlenmeyer flasks in order to reduce excess permanganate and manganese dioxide particles adhering to the flask walls. Only then were the digests transferred to the 100-mL volumetric flasks or directly to the reduction-aeration vessel, as appropriate, for analysis. The digests or digest aliquots, after transfer to the BOD bottle, were diluted with deionized water to 100 mL, stannous chloride was added to reduce the mercuric ions to the element, and the BOD bottle was connected to the cold-vapor generator apparatus for the mercury determination. The atomic absorption was measured for 120 seconds; the steady-state absorbance peak was reached after approximately 60 seconds.

RESULTS OF THE EVALUATION OF THE CURRENT PROTOCOLS

An evaluation of the current protocols with the minor changes described above gave an instrumental detection limit for the recirculating method of 0.01 ug Hg, based on seven replicate measurements, and of about 0.02 ug Hg for the open method. This corresponds to 0.1 ug/mL and 0.2 ug/mL for aqueous samples, based on 100-mL sample portions. The linear dynamic range for both methods extends from the instrumental detection limit to approximately 1.0 ug Hg. This represents 2 orders of magnitude of linear response.

The precision of the mercury measurements, expressed as percent relative standard deviation, was approximately 1 percent for duplicate analyses of the simulated aqueous waste samples, when Method 7470 was used. When the duplicate set of samples was spiked with mercuric chloride at 1, 2 and 5 times the endogenous mercury levels, the spike recoveries ranged from 101 to 112 percent over the spike range. The precision for triplicate measurements was 4.7 percent for digests of SRM-1645 samples prepared according to Method 7471 and 6.1 percent for Method 7471A, and the recoveries were 0.93 and 1.11 ug/g, respectively, as compared to the certified value of 1.1 ± 0.5 ug/g. When SRM 1646 was digested using Method 7471A, however, the RSD was 26% and the recovery was 0.18 ug/g, as compared to the certified value of 0.063 ug/g. As can be seen from these results, the recirculating CV-AAS method gives good precision and accuracy for samples of high mercury concentrations. However, when

the mercury levels are low as in SRM 1646, high imprecision and poor accuracy result from inadequate detectability and possibly an unidentified background interference.

In order to evaluate potential spectral interferences caused by nonspecific background absorption of primary mercury radiation by volatile organic compounds, benzene and methyl ethyl ketone (MEK) were added to NBS-SRM 1645 digest prior to the reduction and aeration step. The amounts of benzene (3 uL) and MEK (1 mL) were the levels required to produce nonspecific background absorbances equivalent to the mercury absorbance for a clean NRS-SRM 1656 digest. When analyzed, an average mercury concentration of 2.41 ug/g (RSD 6.6%) was found for triplicate measurements in the presence of benzene, and 2.19 ug/g (RSD 2.9%) in the presence of MEK. These data represent an approximate doubling of the apparent mercury concentration when compared to the certified mercury value for SRM 1645 of 1.1 ± 0.5 ug/g. It is anticipated that nonspecific absorption caused by volatile organics may be automatically compensated for by using background correction.

METHOD MODIFICATION AND RUGGEDNESS STUDY

The evaluation of the current protocols revealed that sample preservation and digestion procedures could be used without significant modifications. However, it also showed that the CV-AAS apparatus specified in the protocols - which is based on the Hatch and Ott design - does not represent a state-of-the-art approach when compared to other modern CV-AAS designs described in the literature. The recirculating CV-AAS system in the current protocols was therefore changed to an open CV-AAS system which included an on-line amalgamation/thermal desorption process between the reduction-aeration step and the atomic absorption measurement. This modification is based on an amalgamation method previously described by Long et al.² for the determination of mercury in ambient air. This change offers (1) enhanced detectability for trace mercury determinations and (2) elimination of nonspecific absorption interferences which are due to volatile organics in the sample.

A CV-AAS system using an amalgamation process for on-line mercury preconcentration is not a unique approach but rather represents an established and modern state-of-the-art method. In addition to numerous research groups describing customized variations of mercury preconcentration by amalgamation in the literature²⁻⁶, one major manufacturer of atomic absorption instrumentation incorporates an amalgamation system for mercury preconcentration into a commercial CV-AAS apparatus.

In the modified methods, the ambient air train and the BOD bottle were replaced as a dedicated reduction/aeration vessel. The mercury

aerated from the solution is now concentrated onto silver wool and, after water vapor and other volatile species have been purged from the system, thermally desorbed from the silver wool and swept into the absorption cell of the AAS. A detailed schematic diagram of the amalgamation CV-AAS apparatus is presented in Figure 2. The replacement of the recirculating ambient air train with a flow-through nitrogen purge eliminated the need for the air pump and the mercury scrubber medium. The incorporation of the gas sparging bottle improved the efficiency of the aeration of the mercury vapor from the solution and thus reduced the time required for the amalgamation step. The absorption step allowed purging of the gas train between absorption and thermal desorption. This eliminated any volatile organics and water vapor which could contribute to nonspecific background absorption so that desiccants and automatic instrumental background correction were not required.

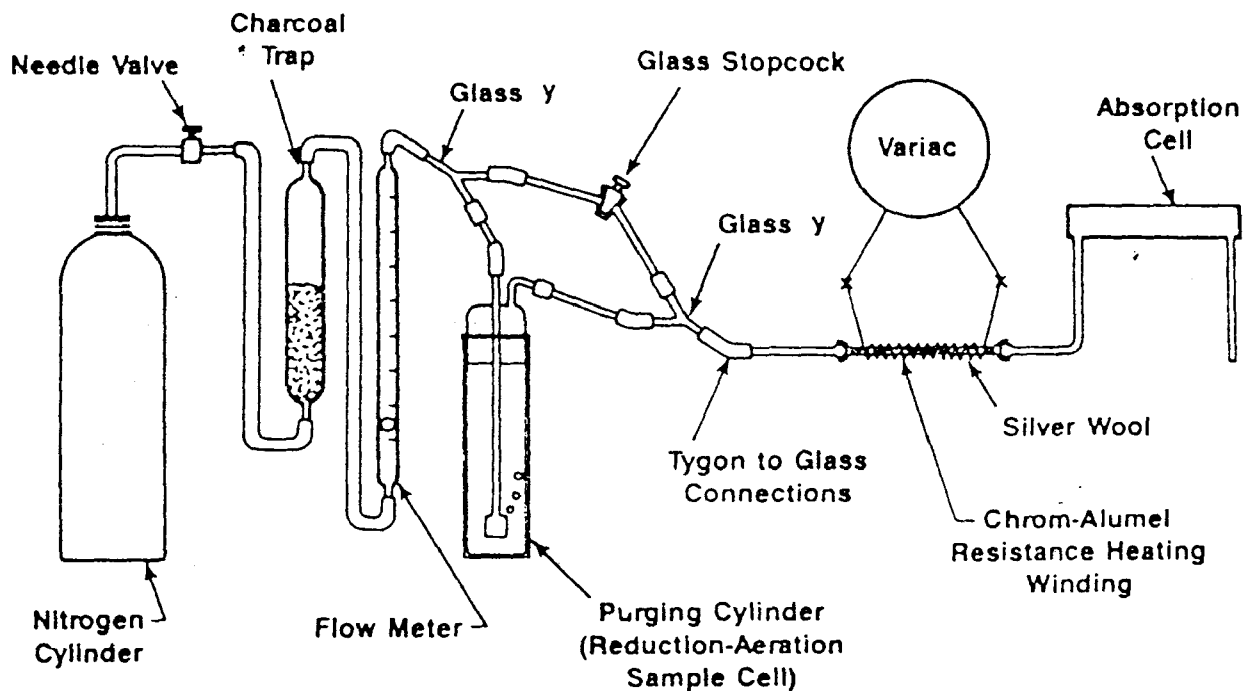


Figure 2. Apparatus for the amalgamation CV-AAS method.

Several operating parameters of the amalgamation CV-AAS system were studied and optimized. The nitrogen gas flow which affects the rate of mercury aeration from the solution in the sparging bottle and, in conjunction with the desorption temperature, determines the rate of mercury desorption, was optimized at 450 mL/min. With this flow rate, more than 90 percent of the mercury vapor of both high and low mercury concentrations was released from the solution within two minutes. A Variac voltage setting of 20 volt output was found to be optimal, rugged and safe at the 540 mL/min nitrogen flow rate, and a 0.7-g portion of silver wool, with a total loading capacity of 3 to 4 ug mercury, was deemed to be adequate for mercury concentrations analyzed within the linear dynamic range of this system.

RESULTS OF THE AMALGAMATION METHOD EVALUATION

An instrumental detection limit of approximately 1 ng mercury was determined for the amalgamation method, using the 3-sigma criterion. This represents an approximately 10-fold improvement over the recirculating CV-AAS system. The linear dynamic range of the amalgamation system extends from the absolute instrumental detection limit to 100 ng. The linear dynamic ranges of both the recirculating CV-AAS method (10 to 1000 ng) and of the amalgamation method span approximately 2 orders of magnitude of mercury concentration. The linear range of the recirculating method is shifted to higher mercury concentrations because of lower sensitivity.

The higher sensitivity of the amalgamation system will require dilution of environmental sample digests containing relatively high mercury concentrations. Multiple dilutions of digests were therefore analyzed to examine whether or not such dilutions would affect the accuracy and precision of the measurements. The results of triplicate measurements on 5-fold and 10-fold dilutions of a NBS-SRM 1645 digest indicate that dilutions of up to 10-fold have no adverse effects on the accuracy and precision of the amalgamation CV-AAS measurements.

The addition of benzene (3 uL) and MEK (1 mL) to NBS-SRM 1645 digests prior to the reduction and aeration step did not increase the apparent mercury recoveries, as was the case for the recirculating method. The average mercury concentrations measured by amalgamation CV-AAS in the sample digests spiked with the organic solvents agreed within the uncertainty limits with the mercury concentrations measured in the sample digests in the absence of these volatile organics. Because background correction with a deuterium arc lamp (or by the Zeeman effect) is not required in the presence of volatile organics when the amalgamation method is used, the spectrophotometer could be operated in the double-beam optical configuration which minimized source-related noise.

The sample digestion procedures described in Methods 7470 and 7471 were tested for mercury recovery from organomercurials. Portions of the simulated aqueous waste and of NBS-SRM 1646 were spiked in triplicate with phenyl mercury and methyl mercuric chloride, respectively, and, for comparison, with mercuric chloride. The spiked samples were digested according to Methods 7470, 7471 and 7471A, and the digests were analyzed by using the amalgamation method. The results listed in Table 1 show that mercury was completely recovered from the inorganic and organic mercury compounds.

To determine potential interferences by sulfide, copper and chloride ions, simulated aqueous waste samples were spiked with these potential interferants, digested according to Method 7470, and analyzed by using the amalgamation method. The results listed in Table 2 show that none of these ions, at the concentrations used, interferes significantly with the mercury determinations.

Table 1.
Mercury Spike Recoveries By Amalgamation CV-AAS Using
Digestion Procedures from Methods 7470, 7471 and 7471A

Average Percent Recovery of Hg Spike			
Sample (Digestion Procedure)	Mercuric Chloride	Diphenyl Mercury	Methyl Mercuric Chloride
Simulated Aqueous Waste ^a (Method 7470)	(109)	125	113
NBS-SRM 1646 ^b (Method 7471 Steam Bath)	86	100	93
NBS-SRM 1646 ^b (Method 7471A Autoclave)	96	107	97

^a 50-mL samples spiked prior to digestion with 4.7 ug Hg as diphenyl mercury and 4.3 ug Hg as methyl mercuric chloride, resp. The endogenous mercury content was 110 ug/L; no HgCl₂ was added.

^b 0.2-g samples spiked prior to digestion with 0.02 ug Hg as mercuric chloride, 0.95 ug Hg as diphenyl mercury, and 0.86 ug Hg as methyl mercuric chloride, resp.

Table 2.
Effects of Selected Inorganic Matrix Ions on Mercury
Determinations in Simulated Aqueous Waste
Digested by Method 7470 and Analyzed by
Amalgamation CV-AAS

Sample ^a Measured, ug/L	Concentration
Simulated Aqueous Waste	111 \pm 3.0
Simulated Aqueous Waste + Sulfide (40mg/L)	108 \pm 1.5
Simulated Aqueous Waste + Copper (20 mg/L)	101 \pm 0.6
Simulated Aqueous Waste + Chloride (68 g/L NaCl)	108 \pm 2.5

^a The endogenous Hg concentration of the simulated aqueous waste is approximately 110 ug/L.

The four solid reference materials listed earlier were digested in triplicate according to the Method 7471 procedure, and were analyzed for mercury by using the amalgamation CV-AAS system with aqueous calibration standard. The digests were also analyzed by the method of multiple standard additions; they were spiked with mercury as mercuric chloride at 1x, 2x, and 4x or 5x the endogenous mercury concentrations in the respective sediment digests. From the results presented in Table 3 it can be seen that when aqueous calibration standards were used the mercury recoveries ranged from 91 percent to 119 percent for three of the materials; the relative precision of the analyses ranged from approximately 1 percent to 7 percent. The recovery for the NRCC BCSS-1 material was marginally high with 125 percent; the same material also showed a similar positive bias when the method of multiple standard additions was used. The results of the analyses using the method of multiple standard additions revealed that multiplicative interferences were not present in the CV-AAS measurement step for the determination of mercury in the reference materials. The relative precisions of the analyses by the method of multiple standard additions ranged from 4 percent to 14 percent.

CONCLUSIONS

The steam bath and autoclave digestion procedures in Methods 7470 and 7471 were generally adequate for the determination of mercury in the spiked and unspiked samples tested, including organomercurial spikes. A potential problem is the reagent blank concentration

which was approximately 5 ng mercury per digest throughout the study. The greater inherent sensitivity of the amalgamation CV-AAS system over the recirculating and the open CV-AAS systems extends the present instrumental detection limit to absolute mercury concentrations of 1 ng, provided that the reagent blank level is sufficiently low. Volatile organic species and water vapor which produce unspecific background absorption interferences with the recirculating CV-AAS system can be eliminated when the amalgamation CV-AAS system is used, and correction with a deuterium arc or Zeeman background corrector is not required.

Table 3.
Mercury Recoveries From Reference Sediment Materials
Using Method 7471 Steam Bath Digestion and
Amalgamation CV-AAS

Concentration (ug/g)			
Sample	Certified Reference Value	Standard Method of Multiple Calibration	Standard Additions
NBS-SRM 1645	1.1 ± 0.5	1.9 ± 0.056	1.07 ± 0.15
NBS-SRM 1646	0.063 ± 0.012	0.075 ± 0.0053	0.063 ± 0.0069
NRCC MESS-1	0.171 ± 0.014	0.185 ± 0.0035	0.193 ± 0.0080
NRCC BCSS-1	0.129 ± 0.012	0.161 ± 0.0020	0.165 ± 0.0089

The amalgamation CV-AAS method requires approximately five minutes per sample for analysis, and longer times may be necessary for samples requiring dilutions because of relatively high mercury concentrations. However, the advantages of the amalgamation CV-AAS methods more than outweigh this disadvantage.

PHASE 2 - MULTI-LABORATORY EVALUATION

The protocols for Methods 7470 and 7471, as modified and tested in Phase 1 of this study, are being evaluated in a multi-laboratory study. The Battelle Columbus Division Laboratory is acting as the coordinating laboratory that is responsible for the design of the study and its execution, including selection of the participating laboratories; sample procurement, analysis and shipping; sample stability studies; standard preparation and shipping; preparation, testing and shipping of the amalgamation component; instruction preparation and data evaluation; and QA/QC.

Battelle Columbus Division staff members have assembled 23 silver amalgamation cells and tested them for uniformity of response. Calibration curves were constructed for each cell using a reagent blank and standards containing 10, 50, and 100 ng of mercury. The slopes of the calibration curves for these cells averaged 0.0055 absorbance/nanogram of mercury with a standard deviation among cells of 0.0006, or a relative standard deviation of 11 percent. These results indicate good consistency of performance among amalgamation cells with respect to amalgamation efficiency and thermal desorption characteristics.

Samples for the study have been collected and characterized, as necessary. These samples have been further evaluated for mercury content, homogeneity, and recovery of predigestion spikes of methyl mercuric chloride and mercuric chloride. The results indicate good overall recoveries. In order to avoid potential problems with low-level spikes added to samples containing chemicals which may prematurely reduce mercury to the elemental state with resultant loss of the spike, the samples have been shipped unspiked. Organic and inorganic mercury standards to be used as predigestion spikes have been sent to the participants with instructions as to how much spike to add.

Form letters were sent to 90 laboratories that were assumed to have the necessary skills, equipment, and interest required to participate in this multi-laboratory evaluation study. Of these laboratories, 22 responded with proposals which included equipment descriptions, qualification statements of personnel, previous relevant experience, and cost bids. Nineteen of these laboratories received Battelle-tested mercury amalgamation cells, a 1000-mg/L inorganic mercury standard, a solid waste sample (NBS-SRM 1633a, Coal Fly Ash), a water sample of low mercury content, standards for spiking the samples, and instructions. The laboratories analyzed the samples according to the instructions and reported the results to the Battelle Columbus Division Laboratory. The final selection of the 7 participants was based on the results of these analyses and, of course, on the available funds.

The selected laboratories received the following samples which will be analyzed in duplicate by three laboratories and in triplicate by four laboratories:

- o Canadian sediment MESS-1
- o Incinerator fly ash
- o Incinerator fly ash spiked with an inorganic mercury compound
- o Columbus municipal sewage sludge
- o Columbus municipal sewage sludge spiked with an organic mercury compound
- o Ground water sample from a farm well
- o Wastewater from an animal facility spiked with an inorganic mercury compound
- o ASTM Type II water spiked with an inorganic mercury compound.

The participating laboratories are at present analyzing these samples. The results obtained from the laboratories will be subjected to statistical evaluation designed to provide estimates of the accuracy and the inter- and intralaboratory precision of Methods 7470 and 7471. In addition, the data will be examined to determine the effects of mercury concentrations and matrix components on the performance of these methods.

The results of the multi-laboratory evaluation study will be used to make any necessary revisions to the Method 7470 and 7471 protocols. It is anticipated that the revised protocols will be available by the end of this fiscal year.

NOTICE

Although the research described in this article has been supported by the United States Environmental Protection Agency under Contract Number 68-03-3226 to the Battelle Memorial Institute, Battelle Columbus Division, through the Acurex Corporation, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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FACTORS AFFECTING EP TOXICITY METALS RESULTS

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ABSTRACT

The EP TOX test, as described in SW-846 method 1310, can produce highly variable results, especially for Pb. This variability is more pronounced in some samples than in others. The magnitude of the variability is greater than would be expected simply as a result of sample inhomogeneity. This phenomenon can be a serious problem since duplicate determinations can differ by orders of magnitude. Thus it may be impossible to determine whether a waste exceeds regulatory action levels.

Using RCRA Performance Sample VI as a model, CRL investigated several possible causes for the problem. The investigation indicated that the problem was not a function of the laboratory water, the acid used for extraction, the type of extractor used, or the sample size. The significant factor seemed to be the technique that was used to adjust the pH. When the pH was adjusted using small aliquots of acid with constant stirring, the results for Pb were relatively low. If larger aliquots of acid were added without constant stirring, the results were significantly higher. The results were most dramatic when the pH of the sample was inadvertently allowed to fall below 4.8. When this occurred, the concentrations of Pb, Cr, Ni, and Zn were significantly higher (and within the "acceptable" range for the PE study).

These observations indicate that the sample pH is a critical factor affecting the EP TOX results. If the pH ever falls below a critical value (presumably 4.8) in any portion of the sample, the results can be significantly elevated.

Based upon these observations, a synthetic sample containing lead nitrate was prepared. It was extracted under varying pH adjustment conditions. The results of these experiments will be reported.

BACKGROUND

Under the Resource Conservation and Recovery Act (RCRA) there are four characteristics attributable to a waste that can classify the waste as hazardous. One of these characteristics is the extraction procedure toxicity (EP TOX). The EP TOX method defines a leaching procedure used to test waste materials. The procedure is intended to simulate the natural leaching that would occur if the waste were

discarded in a leaking landfill. The RCRA regulations set maximum allowable concentration levels for a group of organic chemicals and metals that are extracted from the waste using the EP TOX procedure. This study is concerned with factors which can affect the concentration levels of metals that are extracted using the EP TOX procedure.

In the EP TOX test a waste sample of known weight is separated by filtration into liquid and solid portions. The solid portion is transferred to an extraction vessel. A dilute solution of acetic acid is added to the waste until the pH is lowered to 5.0 ± 0.2 . The sample and acid are mixed for 24 hours using a stirring or tumbling action. During the mixing procedure the pH of the sample/acid slurry is checked frequently and acid is added as necessary to maintain the pH at 5.0 ± 0.2 . After 24 hours of mixing the solid and liquid are separated. The solid residue is discarded. The volume of the liquid is adjusted and the liquid is recombined with the liquid portion of the original sample. This combined liquid is the EP extract. The EP extract is analyzed for the EP TOX metals, As, Se, Pb, Ba, Cd, Cr, Hg, and Ag. Other metals may also be of interest.

As laboratories began large scale testing of wastes for EP TOX metals, CRL Reg. V observed an unexpectedly large degree of variability in the results. This variability seemed more serious for lead than for the other metals. Also, certain samples seemed more sensitive to the variability than others. Table 1 describes lead results for a sample that was analyzed by three laboratories. Laboratory #1, which represented the waste facility produced results which showed the facility to be in compliance with the regulations. Laboratory #2, which represented the regulating agency showed the facility to be in violation. The third laboratory, a referee laboratory, produced results which were just below the action level.

Table 2 describes a similar situation for lead data produced by a single laboratory. For the sample in question results ranged from 1.26 to 49 mg/l (lead). Within this laboratory duplicate analyses for water samples normally agree to within 10%. Results for solid samples normally agree to within 20%. Clearly there are factors unique to the EP TOX procedure which are causing widely scattered results.

EXPERIMENTS AND RESULTS

The Reg. V CRL conducted a series of experiments in order to identify the cause of the variability in the EP TOX metals results. The RCRA Study VII performance evaluation (PE) sample was chosen for study because reference values are available and because CRL had originally reported low results for several metals. Only the affected metals (Cr, Pb, Zn, and Ni) were studied.

Initially CRL hypothesized that the variable results could be caused by using sample weights other than the 100 g. specified in the method. In theory smaller samples would be extracted more thoroughly because of better contact with the acid. However, discussions with other laboratories indicated that the sample size was not a factor: Several labs used small sample weights and produced results near the reference value. Historical data from CRL supports this observation. Also, when CRL reanalyzed the PE sample the results were similar although different sample weights had been used.

CRL investigated the laboratory water as a possible cause for the low results due to CO₂ content of pH effects. CLR extracted the PE sample using distilled, deionized laboratory water as well as distilled water from an outside laboratory. Table 3 shows no significant difference between the results for the two sources of water. Moreover, neither source produced low results.

CRL also investigated the possibility that the nature of the extractor could be causing the difference in results. A stirrer and a tumbler extractor were compared. Table 4 shows slightly higher results for the tumbler. However, the difference is not sufficiently large to explain the variability in results. Again, both mixers produced results which were higher than the originally reported results.

At this point CRL had six sets of data for Pb, Cr, Ni, and Zn. All had been produced by following the specifications of the official method. Three sets of data described low results and three sets described higher results especially for lead. Also, the low results were internally equivalent, as were the higher results. CRL reviewed the analytical technique and observations of the analysts involved in the study. It became evident that lower results were obtained when the technique used to adjust the pH included addition of small aliquots of acid along with constant mixing. When larger aliquots were used and the samples were not mixed continuously the lead results were considerably higher. Also, on one occasion the analyst allowed the pH of the sample extract to fall to 4.4. In this case the results for all four metals were considerably higher (Table 5). It is interesting to note that the results are closest to the reference values in the extract where the pH was allowed to drop below the required level of 5.0 ± 0.2 .

Discussion with other labs supported our observations. In fact some labs intentionally adjust the pH to 4.8 to save time in the pH adjustment step and to assure higher recoveries.

Review of limited historical data indicates that those samples which require frequent pH adjustment are most sensitive to variability in results.

NOTE: Additional experiments could not be conducted because the PE sample had been exhausted. Attempts to synthesize a similar sample were unsuccessful.

DISCUSSION AND CONCLUSIONS

Original sample weight, laboratory water, and type of mixing apparatus did not significantly affect the recovery of EP TOX metals.

Certain "sensitive" sample types are prone to highly variable recoveries for EP TOX metals. These include samples which require frequent pH adjustment in order to maintain the pH at 5.0 ± 0.2 . There is some evidence that samples with high iron contents may contribute to this phenomenon.

For lead, in sensitive samples lower results can be obtained when the pH is adjusted by adding small (1 ml) aliquots of acid with constant stirring. The results are higher when the aliquots of acid are larger and stirring is not constant. This is probably because in the second case the pH is actually falling below 4.8 in some areas of the sample. pH 4.8 is known to be a critical point for the solubility of lead in aqueous solutions.

If the pH of the extract is allowed to fall to 4.4, significantly higher recoveries are observed for Cr, Ni, and Zn in sensitive samples.

RECOMMENDATIONS

If the EP TOX test is to remain in use, EPA should specify the technique for adjusting the pH. We recommend that acid should be added in small aliquots using a buret and that the samples should be stirred constantly. We also recommend that the method include a cautionary statement describing the possible effects of exceeding the allowable pH range of 5.0 ± 0.2 at any time or point in the extraction. We also recommend that the method specify mixing times, rather than chronological time for carrying out the extraction.

TABLE 1. EP Pb Data (3 labs)

Sample matrix - oil

<u>LAB #</u>	<u>Pb (mg/l)</u>	<u>RCRA action level (mg/l)</u>
1	0.2	5.0
2	12.0	5.0
3	4.7	5.0

TABLE 2. EP TOX Pb Data (Single lab)

Sample matrix - industrial solid waste

	<u>Pb (mg/l)</u>	<u>Action Level (mg/l)</u>
	(1.26)	5.0
	11.3	5.0
	49.0	5.0
	40.2	5.0
	19.5	5.0
	34.0	5.0
X	30.9	
S	15.3	

() Outlier - rejected in calculation of statistics

TABLE 3. The effect of the source of water on the EP TOX results for Cr, Pb, Zn, and Ni

Sample matrix - RCRA PE Sample

<u>Metal</u>	<u>Reference value (mg/l)</u>	<u>EP TOX results (mg/l)</u>	
		<u>CRL water (n=2)</u>	<u>Reference Water</u>
CR	1.68	0.2	0.2
Pb	219.0	186.0	184.0
Zn	0.34	0.2	0.14
Ni	65.8	33.0	24.2

TABLE 4. The effect of the extraction vessel on EP TOX results for Cr, Pb, Zn, and Ni

Sample matrix - RCRA PE Sample

<u>Metal</u>	<u>Reference value (mg/l)</u>	<u>EP TOX results (mg/l)</u>	
		<u>Stirrer</u>	<u>Tumbler (n=2)</u>
Cr	1.68	0.2	0.2
Pb	219.0	162.0	198.0
Zn	0.34	0.15	0.2
Ni	65.8	20.3	24.2

TABLE 5. The effect of pH control on EP TOX results for Cr, Pb, Zn, and Ni

Sample matrix - RCRA PE Sample

Metal	Reference value (mg/l)	EP TOX results (mg/l)		
		narrow pH control (n=3)	normal pH control (n=3)	pH below 4.8 (n=1)
Cr	1.68	0.2	0.2	2.7
Pb	219.0	14.0	190.0	250.0
Zn	0.34	1.0	0.2	0.5
Ni	65.8	31.0	23.0	78.0

ACKNOWLEDGEMENTS

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ION CHROMATOGRAPHY FOR THE ANALYSIS OF ANIONS IN HAZARDOUS WASTE MATRICES

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ABSTRACT

Accurate quantitation of inorganic anions is of interest to those involved in the analysis and disposal of hazardous wastes, and those needing information necessary for incineration of the wastes. The information also serves a confirmatory role in cross-checking other parameters such as acid content. Formerly, quantitation of these anions required a unique procedure for each ion necessitating large expenditures of time and materials. Ion Chromatography has the capability of performing single, simultaneous determinations of several anions with a minimal number of interferences and materials. It can also be readily automated.

Ion Chromatography using a carbonate/bicarbonate eluant and chemical suppression is compared to colorimetric and selective ion electrode techniques for the identification and quantification of several anions in hazardous waste matrices. Steps taken to ensure on-line readiness as well as the acceptability of analytical results produced using Ion Chromatography are outlined.

Samples prepared by oxygen bomb digestion were split into equal portions and analyzed using both Ion Chromatography and the previously utilized procedures to quantitate chloride, sulfate and fluoride in typical samples. Evaluation of the results confirmed that under the Ion Chromatographic conditions tested, this method was an acceptable alternative to chlorimetric analysis for sulfate and chloride. However, it was not a suitable substitute for the selective Ion Electrode analysis for fluoride. It was found that several organic anions interfere with the fluoride measurement when using the typical carbonate/bicarbonate eluant. Alternate eluants could possibly eliminate these interferences but were not investigated.

A parallel analysis of hazardous waste samples for incineration, in which two different Ion Chromatographs were used to quantitate chloride, bromide and sulfate gave an overall discrepancy in results of less than 20% in most cases. This was considered acceptable in light of the complex nature of the sample matrices tested.

INTRODUCTION

Knowledge of the type and level of anions present in hazardous waste streams or generated during treatment of these streams is important and necessary for proper hazardous waste management in areas such as solvent-derived fuels programs and incineration units. The anions of interest are Fluoride (F^-), Chloride (Cl^-), Bromide (Br^-), and Sulphate ($SO_4^{=}$), with other anions such as Nitrite (NO_2^-), Nitrate (NO_3^-), and Phosphate (PO_4^{-3}) of interest but lesser importance and frequency in commonly encountered samples.

Prior to the investigation of Ion Chromatography for the measurement of these anions, colorimetric and selective ion electrode methods have been employed. This required separation of the prepared sample into single test portions--one for each anion of interest. Applying calibration standards, blanks, duplications, and fortifications to each test portion, as many as 35 individual determinations were necessary per sample received.

Ion Chromatography has emerged in recent years as an attractive option for the simultaneous separation, identification, and quantification of several anions present in a single sample. The following method of analysis applies specifically to the Dionex 2000i Series, Ion Chromatograph with Autoion 100 Controller, ASM-1 Autosampler and Spectraphysics 4270 Integrator, available from Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94088-3603. Where possible, attempts have been made to generalize the procedures. Information provided may or may not be applicable to other systems.

SCOPE AND APPLICATION

This method addresses the quantification of commonly encountered inorganic anions in liquid and solid, domestic or industrial waste. Detection at the ppm level is achievable in most matrices.

SUMMARY OF METHOD

Theory: The mechanism by which anions are separated in ion chromatography is based on the relative affinities of the various ions for the column resin. The columns are packed with thousands of large sulfonated (negatively charged) resin beads surrounded by a greater number of smaller, aminated (positively charged) resin beads. The positive charges on these aminated beads attract the negatively charged eluant anions--carbonate and bicarbonate. When a sample containing analyte ions is injected into the stream of eluant flowing through the column, they compete with the carbonate and bicarbonate ions for available sites. Monovalent ions, such as chloride, displace the similarly valenced bicarbonate ions; divalently charged ions, such as sulfate, displace carbonate ions. The analyte ions are able to temporarily displace the eluant ions because they have a greater affinity for the resin. The degree of this affinity varies among different species of anions, as evidenced by the chloride peak in a chromatogram emerging before the sulfate peak. Eventually, all of the analyte anions will be displaced by the large volume of eluant ions competing for sites on the resin and pushing analyte ions back into the eluant stream to be flushed onward through the column.

After the eluant, containing the recently separated analyte anions, emerges from the column, it enters the suppressor column. The suppressor enhances the conductivity of the analyte anions and suppresses the conductivity of the eluant ions. In the suppressor, ions are exchanged with dilute acid, which protonates them to create the weakly dissociated, low-conductance carbonic acid from the eluant (to reduce background noise in the chromatogram) and the strongly dissociated, highly conductive analyte acids, such as

hydrochloric and sulfuric acids, which will give strong signals when passed through the conductivity detector and produce peaks in the chromatogram.

Organic compounds may attack the polystyrene-divinylbenzene resin used to pack separator columns. Any sample which contains organic material must be rendered suitable for the ion chromatograph. One method for this involves combustion of the sample in oxygen via a bomb calorimeter. After digestion, dilution, and filtration, samples are injected onto the column for subsequent separation and quantification.

Aqueous samples require only dilution and filtration; solids must first be put into solution. After filtration, samples are injected onto the column for subsequent separation and quantification.

INTERFERENCES

Either one of two species which elute with similar retention times/volumes can interfere with identification and quantification of the other. For example, nitrate can interfere with bromide. It is recommended that the ratio of their concentrations be no greater than 10:1 if they are to be simultaneously quantitated. Similarly, several organic acids of low molecular weight (such as formate and acetate) interfere with the analysis of fluoride since they co-elute. Since detection of ions is based on the specific conductance of the eluting solution, another interference occurs as a negative peak due to the water used in dilution and sample injection. A negative peak ("dip") results from the lower conductance of water relative to that of the carbonate/bicarbonate eluant. This can interfere with the integration of early-emerging peaks which must be sufficiently resolved from the dip to allow successful quantitation. This interference can be eliminated by using the carbonate/bicarbonate eluant to dilute the sample.

In samples containing a component which is present at a high level of concentration, the peak caused by that particular ion can interfere with the analysis of subsequently eluting ions. When the column is overloaded with an extremely high concentration of anions, the eluant ions cannot replace the analyte ions at a high enough rate within the column to achieve the sharp, narrow peaks obtained at lower levels of concentration. The peak broadens, causing not only a shift to longer retention times, but, possibly, a great enough effect to "bury" the next peak by preventing proper resolution. Dilution of the sample to the appropriate degree may minimize the interference.

There are many classes of compounds which can interfere with the accurate quantitation of inorganic anions by occupying available sites on the column resin, thereby making the sites unavailable to analyte ions and reducing column capacity. Some of these contaminants bind irreversibly. They include ClO_4^- , $\text{Fe}(\text{CN})_6^{3-}$, other metal-cyanide complexes, aromatic organics, large aliphatic organics (i.e., surfactants), and humic acids. Also, high concentrations of Fe, Ni, Cu, Zn, hydroxide ion, peroxides,

dissolved gases, and microbial growth have the potential to interfere with analyses. Because these sources of contamination usually cause irreversible damage, prevention may be the best mode of avoiding the interferences they create. Filtration and degasification of eluants can prevent particulate accumulation, as well as the formation of bubbles within lines or columns. Microbial growth, encouraged by the hospitable carbonate/bicarbonate environment provided by the eluant used in this method, can be discouraged by frequent flushing and proper storage of columns. More importantly, fresh eluant should be prepared frequently in small batches to be used immediately. Storage of diluted eluant can allow microbial growth and cause column blockage. Routine column clean-ups with 1N HCl can reduce the concentration of some metals and inorganic species retained by columns.

APPARATUS AND MATERIALS

Ion Chromatograph (see Figure 1) consisting of a pump, an injection valve, a conductivity detector, a suppression device or column, and a set of columns, including a separator suitable for the separation of inorganic anions, a guard column of the same material, and another guard column specifically for trapping out metals that may be found in sample matrices.

Integrator or chart recorder.

Volumetric glassware.

Pipets (5 microliters to 10 milliliters).

Syringes and Luer Lock Adapters.

REAGENTS

Nitrogen gas 99.998% purity (for valve actuation).

18 Megaohm deionized water, filtered through a 0.20 micro membrane filter.

Na_2CO_3 , reagent grade.

NaHCO_3 , reagent grade.

H_2SO_4 , concentrated, reagent grade.

HCl, concentrated, reagent grade.

Sodium Tartrate, reagent grade.

Acetonitrile, HPLC grade.

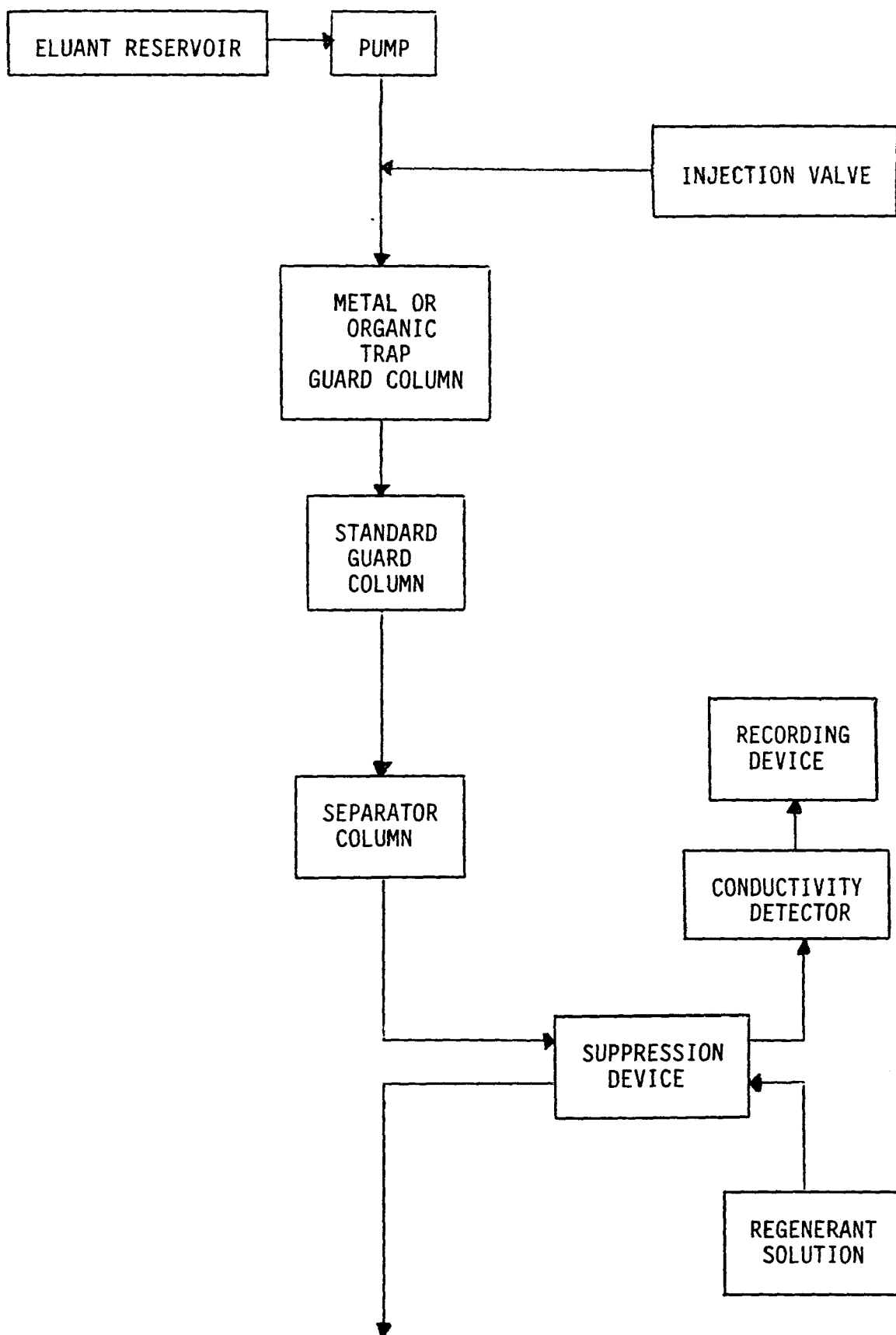


Figure 1
Ion Chromatograph Components

Standards Preparation: 1000 ppm stock solutions should be prepared monthly, as follows, by dissolving oven-dried (at 105°C for 1/2 hour then cooled) reagent chemicals in deionized water. Refrigeration of the resulting solution is recommended:

F^- : 2.2100g NaF/liter

Cl^- : 1.6484g NaCl/liter

NO_2^- : 1.4998g $NaNO_2$ /liter

Br^- : 1.4894g KBr/liter

NO_3^- : 1.3707g $NaNO_3$ /liter

PO_4^{3-} : 1.4330g KH_2PO_4 /liter

SO_4^{2-} : 1.8141g K_2SO_4 /liter

Calibration Standard Preparation: A mixed anionic solution, suitable for use as a calibration standard, is prepared from 1000 ppm stocks in a 1 liter volumetric flask and diluted to volume with deionized water:

5 ppm = 5 mL. each: $F^-/Cl^-/Br^-$

15 ppm = 15 mL. each: $NO_2^-/NO_3^-/SO_4^{2-}/PO_4^{3-}$

A free (inorganic) anion Quality Control Check solution can be prepared from the calibration standard solution by diluting it 1:1. It will have theoretical anion concentrations of 2.5 ppm fluoride, 2.5 ppm chloride, 2.5 ppm bromide, 7.5 ppm nitrite, 7.5 ppm nitrate, 7.5 ppm phosphate, and 7.5 ppm sulfate.

A total anion Quality Control Check solution, which must be oxygen bomb digested to liberate organically bound anions, can be prepared by placing 25 grams each of 2-Chlorobenzothiazole and Bromo-4-fluorobenzene in a 100-ml volumetric flask and making up to volume with Butyl Acetate. This solution will have an approximate heating value of 10,700 BTU/lb., and after digestion, total anionic concentrations of 4.8% Chloride, 12.1% Sulfate, and 7.3% Bromide.

Eluant Preparation: The optimal eluant concentration for separations is dependent on the column set to be used. As a rule, anion separations require a carbonate/bicarbonate eluant which can be prepared in quantity at 100x the recommended strength and diluted with deionized water as needed. Eluant should be filtered and degassed prior to usage. (Bubbling N_2 gas through the eluant or sonication for 20 minutes is sufficient to remove dissolved gases that could cause the pump to lose prime. Allowing the deionized water to reach room temperature before dissolving the salts is also sufficient.) Additionally, an in-line filter for the eluant which includes 35- micron and 5-micron filters enclosed in a Teflon housing, is highly recommended.

Regenerant Preparation: An anion suppression system is used to enhance the signal to noise ratio of analyte relative to eluant anions by converting them to their strongly dissociated, highly-conductive acidic form while also reducing the interfering conductance background of the eluant anions via a similar mechanism. This is achieved by forcing a dilute acid solution to flow through the suppression device in a direction opposite to the direction of eluant flow and providing a membrane interface through which exchange can occur.

SAMPLE COLLECTION, PRESERVATION, AND HANDLING

Free Anion Determinations: Liquid samples should be diluted and filtered. Solids must be put into solution with deionized water before dilution and filtered through a 10 micron filter, followed by 0.20 micron filter. Solutions with organic or metal content should be swirled with an appropriate ion-exchange resin or run through a preparatory extraction column, unless an organic or metal guard column is to be used prior to the separator.

Total Anion Determinations: Samples should be oxygen-bomb digested in a 5% (or greater) (w/w) sodium bicarbonate solution. Bomb washings should be collected in a volumetric flask and diluted to a known volume with subsequent rinsings. The diluted solution obtained from the bomb contents and washings should be filtered in two stages through a 10 micron filter, followed by a 0.20 micron filter. Further dilution may be required to produce on-scale peaks.

PROCEDURE

Instrument Initialization: Ensure eluant and regenerant reservoirs have been filled with the appropriate solutions. Set pump flow rate to 2 mL/minute and begin regenerant flow through the suppression device. Allow system to equilibrate approximately 15 minutes or until pressure and conductivity readings stabilize. The conductivity reading for eluant only should be approximately 15 microsiemens. Flush and load the sample loop by injecting approximately 1 mL of deionized water (from 10 cc luer lock syringe). "Autozero" the conductivity cell. Ensure a flat baseline is observed. If baseline is not flat, check eluant flow-rate, check for leaks, and recheck regenerant regulator pressure. (A high-drifting baseline indicates that pressure is too low, and a low-drifting baseline indicates pressure is too high.) (See Figure 2.) If this does not produce the desired baseline, a longer equilibration period may be required.

Instrument Calibration: If a flat baseline is observed with deionized water, inject the calibration standard to establish retention times for the analyte peaks. Once retention times have been noted, they can be entered, along with the correct concentration values into the integrator and the calibration standard reinjected to serve as a calibration and Instrument Performance Check solution. (See Figure 3.) (If the recording device used does not have this capacity, a series of calibration standards can be used to set up linear calibration curves from which relative sample concentration

values can be calculated using peak heights or areas.)

When using an integrator with the capacity to automatically recalibrate, the calibration standard should be run once for every ten sample runs to ensure accuracy in integrator output. To monitor column degradation, the Instrument Performance Check solution can be used to calculate the theoretical number of plates. (See CALCULATIONS section.)

The Quality Control Check solutions should be injected and concentration values determined to be within acceptable limits prior to sample analyses.

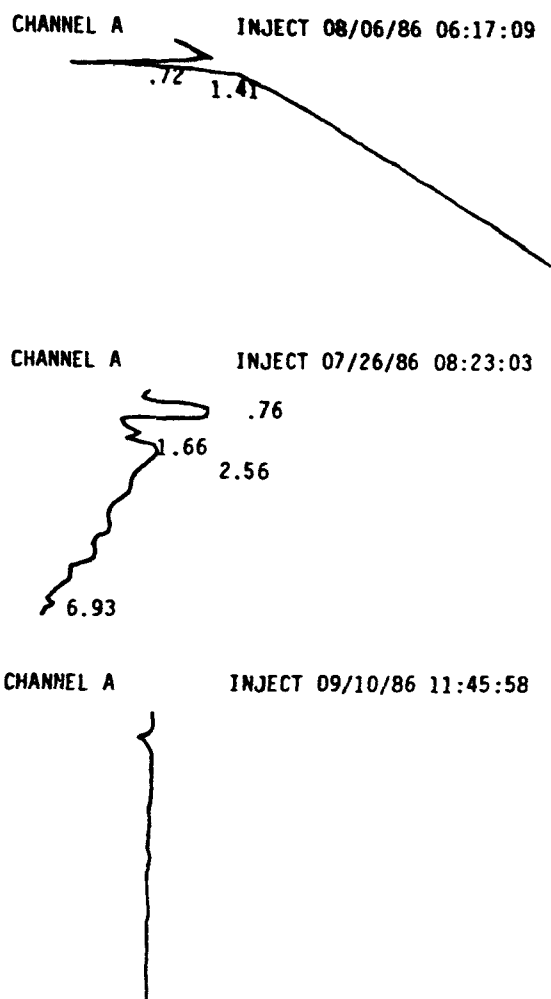
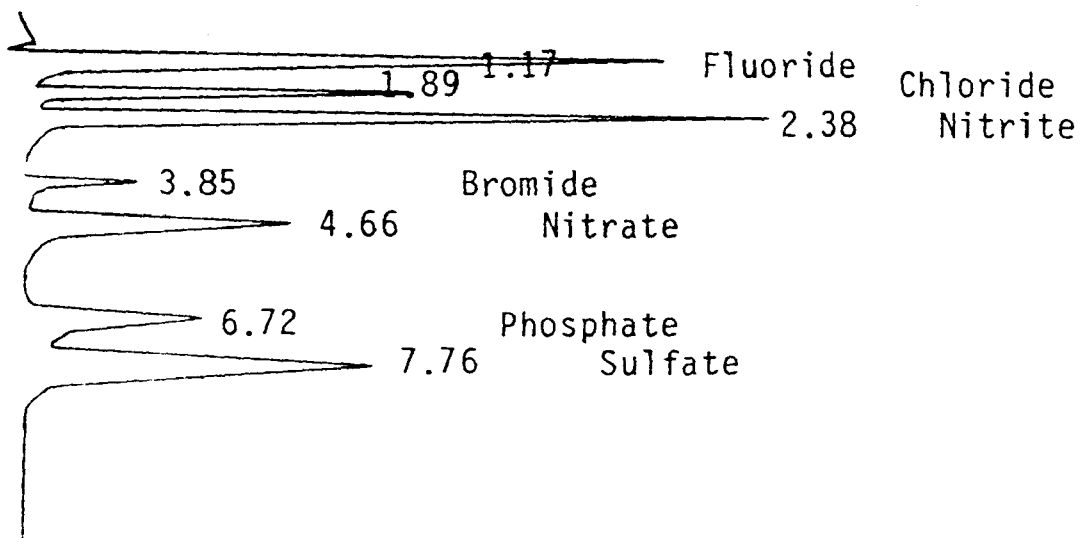


FIGURE 2
BASELINE ADJUSTMENT

a. High-Drifting Baseline b. Low-Drifting Baseline c. Flat Baseline

CHANNEL A

INJECT 07/31/86 13:41:28



Separator Column: Dionex AS4A
Flow Rate: 2 ml/min.
Eluant: Sodium Carbonate/Bicarbonate

FIGURE 3
TYPICAL CALIBRATION SOLUTION RUN

Sample Analyses: When injecting samples manually or using an autosampler, deionized water should be injected frequently (after every 7th sample) to aid in preventing cross-contamination of samples and to provide a continual baseline check.

In the case of a complex chromatogram or insufficient resolution of peaks, a spike solution of known concentration should be added to allow positive identification of ambiguous peak(s). Additional dilution(s) of samples may be necessary to obtain on-scale peaks.

Column Clean-ups: Constant care of columns will help to ensure chromatograms are clear and data reliable. Obvious signs of column degradation include:

Marked increase in system backpressure.

Poor resolution between peaks (i.e., "valleys" less than 50% of peak heights).

Shift in retention times of 10% (i.e., chloride peak shifts from 1.60 to 1.79 or more).

NOTE: Changes in eluant concentration can cause a shift in retention times. When changing eluant, ensure column has been equilibrated and calibration standard analyzed for new retention times.

When column degradation occurs to the extent that the above signs are noted, the guard and/or separator columns should be cleaned.

Separator and Guard Column Clean-up:

Disconnect the suppression device.

Remove organic trap guard column and reverse the order of the separator and guard columns. (Frequent clean-ups on the guard column alone should reduce the necessity for cleaning the separator column.)

Pump deionized water through the column at a flow rate of 2.0 ml/min. for 10 minutes.

Pump 1N HCl through the column at a rate of 2.0 ml/min. for 20 minutes.

Pump deionized water through the column at a flow rate of 2.0 ml/min. for 5 minutes.

Remove the used bed supports and replace with new at column ends.

Organic Trap Guard Column Clean-ups:

Pump a 90% solution of acetonitrile through the column (with all other columns and the suppression device disconnected) at a flow rate of 0.5 ml/min. overnight (or, if necessary, at a rate of 2.0 ml/min. for 1-2 hours).

Pump deionized water through the column at a flow rate of 2.0 ml/min. for 15 minutes.

Replace the bed supports.

After clean-up is complete, replace columns in their proper sequence. Re-equilibrate by pumping eluant through the system.

NOTE: A mild clean-up for guards and separators which is not as harsh but also not as effective as the clean-up described above, consists of pumping 0.10 M Na_2CO_3 over the reverse-ordered (separator followed by guard) columns, with trap and suppressor disconnected, for 20-30 minutes, remove used bed supports and replace with new, resequencing the columns, and, finally, re-equilibrating the system with the proper eluant.

CALCULATIONS

Chromatographic peaks are quantitated on the basis of height or area of peaks. Peak heights are best utilized in instances where peaks are sharp and narrow. Peak areas are more appropriately used when peaks are broad and symmetrical. This depends on several factors, including the type and efficiency of the column used as well as its age and degree of degradation. For integrators which identify and quantify peaks using the method of external standards:

Concentration of Anion (ppm) =

$$\frac{\text{(Peak Height or Area of Sample)}}{\text{(Peak Height or Area of Calibration Standard)}} \times \text{(Dilution Factor)} \times \text{(Concentration of Anion in Calibration Standard)}$$

Column Efficiency/Degradation: As a guide to assist the analyst in determining column efficiency and degradation, calculating the theoretical number of plates and the resolution of two separate peaks can be employed:

Theoretical Number of Plates =

$$N = 5.545 \frac{t^2}{W_1^2} \quad \text{where} \quad t = \text{retention time of peak}$$

$\frac{W_1}{2}$ $\frac{W_1}{2}$ = width at half height of peak

Resolution of Peaks (R)

$$R = \frac{2t}{W_2 + W_1} \text{ where } \begin{array}{l} t = \text{difference in retention times of} \\ \text{the two peaks} \\ W_1 = \text{width of first peak} \\ W_2 = \text{width of second peak} \end{array}$$

Method Detection Limits (MDL): To determine method detection limits, solutions containing a single anionic specie at a concentration near the expected detection limit should be analyzed a minimum of seven times. The mean and standard deviation for these values is then calculated. The method detection limit is equal to three times (3X) the standard deviation. (See Figure 4 for typical values.)

Limits of Quantification (LOQ): In order to ensure a margin of confidence between the method detection limit and reported values, the method detection limit can be multiplied by an additional factor (usually 10) to obtain a lower limit of quantification. (See Figure 4 for typical values.)

In cases where peaks occur below the level of quantification (MDL x 10), use the following calculation to obtain the threshold value for the analysis:

$$(\text{LOQ}) \times (\text{Dilution Factor}) = \text{Conc. of Anion (ppm)}$$

with this result reported as "less than."

<u>ANION</u>	<u>\bar{x} (ppm)</u>	<u>s</u>	<u>MDL (ppm)</u>	<u>LOQ (MDL x 10) (ppm)</u>
Chloride	0.217	0.016	0.048	0.48
Nitrate	0.935	0.040	0.119	1.19
Sulfate	1.155	0.030	0.090	0.90
Phosphate	0.750	0.049	0.146	1.46
Bromide	0.955	0.0095	0.029	0.29

FIGURE 4
TYPICAL VALUES FOR METHOD DETECTION LIMITS AND LIMITS OF
QUANTIFICATION

QUALITY CONTROL

Analyze the Instrument Performance Check solution once per batch of samples. From this, calculate the theoretical number of plates for phosphate, checking to ensure the value is within control limits before proceeding. Analyze the Quality Control Check solution before each batch of samples, checking to ensure values are within control limits for each anion to be analyzed before proceeding. Duplicate and fortify every tenth sample. Duplication error should not exceed 20%. Fortification recovery should be between 80 - 120%. Normally, free anion samples can be fortified with an appropriate amount of the free anion Quality Control Check solution. Total anion (bomb-combusted) samples should be fortified prior to digestion with an appropriate amount of the total anion Quality Control Check solution.

Duplications (10% of Samples)

$$\text{Mean } \bar{X} = \frac{X_2 + X_1}{2} \quad \text{where } \begin{array}{l} X_1 = \text{lower result} \\ X_2 = \text{higher result} \end{array}$$

$$\text{Percent Error} = \frac{(X_2 - \bar{X})}{\bar{X}} \times 100$$

Fortifications (10% of Samples)

$$\text{Theoretical Spike} = \frac{(\text{Conc. of Std.}) \times (\text{Wt. of Std.})}{(\text{Wt. of Sample})}$$

$$\text{Percent Recovery} = \frac{(\text{Spike Result}) - (\text{Avg.} + \text{Dup.} + \text{Orig.})}{\text{Theoretical Spike}} \times 100$$

SAMPLE HANDLING FOR THE ANALYSIS OF CYANIDES IN SOLID AND HAZARDOUS WASTES

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ABSTRACT

Cyanide bearing wastes are among the most common industrial wastes disposed of in this country. Wastes generated from electro-plating, petroleum, metal finishing, pharmaceutical, mining, coking and other manufacturing operations each present unique handling problems due to their complex matrices. EPA has expressed a great deal of concern regarding the disposal of these cyanide wastes and has proposed regulations based on the form of cyanide present in the waste. EPA's intent is to control those cyanide complexes which are toxic to humans and animals and which have potential for formation of hydrogen cyanide gas when mismanaged.

The challenge facing EPA and the regulated community is to develop analytical methodology which will determine those cyanide forms which are a detriment to the environment. The currently approved method for evaluating solid waste (Method 9010 for total and amenable cyanides) is found in SW846. Although this method is sufficient for analyzing these cyanide forms in water and wastewater, it does not deal adequately with complex waste matrices such as tars, oils, sludges or soils.

This paper discusses cyanide forms of environmental significance with regard to decision making criteria. Problems associated with preparing and handling of waste matrices are discussed, and procedures are proposed for solving those problems. Data gathered from the application of proposed methods are presented.

INTRODUCTION

One of the most challenging compounds to analyze, for both industrial and environmental control, is cyanide. Simply understanding definitions and terms associated with the complex forms of cyanides, its analytical chemistry and interferences is a challenge. It is apparent that we need to focus efforts on protocols that not only meet a regulatory need, but also provide users of analytical methods with appropriate information to make decisions.

For environmental decision making, EPA's intent is to control those cyanide complexes which are toxic to humans and animals and which have potential for formation of hydrogen cyanide gas when mismanaged. The challenge, therefore, is to define those cyanide

forms which are toxic under these conditions and provide adequate methodology to accurately test for these forms.

The currently approved method (SW846, Method 9010) is executed wholly from water methods published by EPA. This is an excellent place to begin method development because of the enormous amount of method study conducted by ASTM, Standard Methods Committee, EPA and industry. However, compared to the varied solid, slurried and oily matrices of wastes, analysis of waters is probably a cake walk.

METHOD SELECTION

In order to develop appropriate methodology for handling these varied matrices, we must define and evaluate several variables which influence our ability to predict the impact of cyanide forms on human health and the environment. First, we must determine cyanide forms of interest based on the utility of each form to predict environmental impact. Forms of cyanide identified below were chosen because they are environmentally significant and their analytical methodology is well established, with documented accuracy.

- o Total Cyanide
- o Amenable Cyanide
- o Microdiffusible Cyanide
- o Weak Acid Dissociable Cyanide

The analytical methods for each of these forms have the ability to measure hydrogen cyanide gas which could be evolved when wastes are mismanaged in an acid environment. The total cyanide procedure, however, also measures those cyanides which do not easily dissociate in this management scenario. Method 9010 defines amenable cyanide by the difference between two measurements, total cyanide before and after chlorination.

Experienced analysts are well aware of difficulties which arise from the use of this technique. The microdiffusible cyanide method is a procedure which has recently proven itself as a valuable technique for estimating dissociable cyanides. However, this is a time consuming procedure, and the accuracy and reproducibility of the data are highly dependent on the analyst's experience and skill. The weak acid dissociable cyanide method is a distillation procedure which measures cyanide evolved from a mildly acidic (pH 4-5) solution.

Of the procedures described, total cyanide and weak acid dissociable cyanide procedures remove cyanides from the sample matrix into an easily analyzed matrix of sodium hydroxide in water. These methods are also direct methods, requiring minimal operator manipulation, and both forms can be determined using the same sample handling and preparation procedures.

Total cyanide is typically viewed as a required parameter for the determination of disposal options. This methodology must, therefore, be defined for complex matrices of soils, sludges, oils and tars. Because of the decided advantage of having only one set of sample handling and preparation procedures for both these methods, weak acid dissociable cyanide methodology is the preferred method for evaluating the environmental impact of wastes for the management scenario mentioned earlier. This method provides accurate, reproducible, and rapid results necessary for decision making.

SAMPLE HANDLING

Over the past thirty plus years, water chemists have refined cyanide analyses to provide reliable data of defined accuracy. The basic approach to developing methods for complex solid and hazardous waste matrices is to develop sample preparation procedures that take advantage of these tested methods by removing cyanides into a water matrix. It has been long established that distillation is an appropriate technique to accomplish this transfer.

Directly applying distillation to the matrices of soils, sludges, tars, and oils presents several problems that were not dealt with in previous water method development.

- 1 - How does particle size of a sample effect recovery of cyanides?
- 2 - When solids accumulate in the bottom of the flask, is recovery reduced?
- 3 - When oils or tars are added directly to the flask containing water, is recovery reduced because of lack of miscibility?

These questions and others are being dealt within a joint effort by EPA/OSW and ASTM Committee D-34 on Waste Disposal. The established water methods are being re-evaluated and revised to provide suitable methodology for the wide variety of hazardous and solid wastes.

The first change made was to add magnetic stirring to the classical cyanide distillation apparatus. Data from replicate determinations of solid samples are typically non-reproducible, with high standard deviations, when not agitated. One such set for results is shown below. These poor results are caused by the solids laying undisturbed on the flask bottom, limiting solution contact. Determinations on a similar sample with adequate agitation produced much more acceptable results.

	<u>Replicate</u>	<u>mg/Kg</u>	
<u>Not agitated</u>	1	86	Standard Deviation - 25.3
	2	34	
	3	33	
	4	62	

	<u>Replicate</u>	<u>mg/Kg</u>	
<u>Agitated</u>	1	20	Standard Deviation - 2.6
	2	18	
	3	21	
	4	24	

This approach will not overcome problems with sample non-homogeneity but will significantly improve the chances of obtaining reproducible results.

A second addition to the method is a proposed requirement for particle size reduction to less than 60 mesh for monolithic and granular samples. This proposal is not yet founded in scientific evidence, but is the product of some logic. It is important to handle a sample in a manner that maximizes the method's ability to analyze any cyanide that may be absorbed into a porous sample. After some discussion, 60 mesh was selected as small enough to provide accurate results while still being practical and attainable by almost any laboratory.

Oil and tar present problems of their own because they are immisible in water. An extraction procedure based on the technique for fatty acid removal prior to cyanide analysis is proposed. The sample is quantitatively added to a separatory funnel containing a buffer solution. A suitable solvent (iso-octane, hexane) is added, and the sample is extracted. The buffer solution is then transferred to the distillation flask along with any solids, and analysis is begun. This procedure is still in the development stage, and there are few data available on accuracy and reproducibility.

CASE DATA

Unfortunately, we are just now at the beginning stages of developing new methods, and there are few data available on all the modifications discussed. There are examples of data available, however, that compare results of various methods. One such example is illustrated below.

Cyanide Method	Duplicates		Duplicates	
	(mg/Kg)	(mg/Kg)	(mg/Kg)	(mg/Kg)
Total	4700	4800	1.4	1.6
Weak Acid Dissociable	185	180	0.19	0.22
Amenable	*	*	0.2	0.5
Microdiffusion	99	103	0.100	0.100

***Matrix interference**

Total and weak acid dissociable data were generated using the magnetic agitation modification mentioned. These data represent excellent reproducibility. The asterisk notes that, due to matrix interference, reliable results could not be obtained, pointing out the necessity of applying an alternate technique for this determination.

Cyanide recovery utilizing sample agitation on distilled spike samples is shown in the table below. It is important to note that both the total and weak acid dissociable cyanide procedures demonstrate acceptable recovery. Data for amenable cyanide by difference shows both poor recovery and inadequate detection capability due to interferences.

<u>Cyanide Method</u>	<u>Sample</u> (mg/L)	<u>Percent</u> <u>Recovery</u>
Total	312	93%
Weak Acid Dissociable	1.2	86%
Amenable	<40	70%
Microdiffusion	0.308	83%

SUMMARY

A major effort has been initiated by EPA and ASTM to address the short-comings of published cyanide methods for solid and hazardous wastes. The number and variety of waste matrices requiring testing challenges the analyst's ability to generate reliable data for today's environmental decision making. Progress is being made on developing sample handling procedures and methods should be available in the near future.

DETERMINATION OF TOTAL SULFIDE IN SOLID WASTE

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ABSTRACT

The purpose of this research was to improve, modify and revise Method 9030, in order to make it applicable to a wide variety of waste matrices. As part of this effort, a literature review and experiments were performed to identify potential method interferences and pretreatments to remove them, to identify and appropriate techniques for extracting sulfides from solids and oils, and to develop a procedure to determine acid-insoluble sulfides.

From the literature review, reported data for Method 9030 suggest that recovery is better than 90%. The precision for sulfide determination is relatively good (CV ~6%). The method detection limit was found to be approximately 0.2 mg/L.

Experiments performed in our laboratory demonstrated that sulfite and bisulfite are significant interferences to Method 9030, but they can be removed with formaldehyde. A distillation procedure was developed for this purpose. The recovery obtained with the distillation procedure was approximately 100%, the precision was good (CV ~7.5%), and the method detection limit was approximately 0.2mg/L.

An extraction procedure was developed for solid samples that cannot be distilled. Results of extraction experiments demonstrated that sulfides can be extracted from solids and oils with an alkaline (pH 12) solution. Modifications to the extraction procedure may be required depending upon the physical and/or chemical properties of the matrix.

An acid-insoluble sulfide procedure was also developed. Acid-insoluble sulfide can be determined using a concentrated HCl distillation.

The three developed procedures were tested with industrial wastes to ascertain their range of applicability. A Modified Method 9030 has been prepared incorporating the three developed procedures.

TOX - A SCREENING PARAMETER FOR ENVIRONMENTAL SAMPLES

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ABSTRACT

The EPA procedure for determining Total Organic Halogen in water samples is described in Method 9020, SW-846. In this method, organic halogens present in water samples are adsorbed onto an activated carbon column which is then pyrolyzed in a high temperature oxygen furnace. These organic halogens are converted to hydrogen halide gas, trapped in an acetic acid buffer and microcoulometrically titrated. However, the method as written only applies to TOX determinations in water.

Although not as specific as more complex analytical techniques such as Gas Chromatography or GC/MS, TOX is relatively a rapid and inexpensive technique. Many organic pollutants contain organic halogens. For example, of the 113 organic priority pollutants, 71 are halogenated, with chlorine being the predominant species. TOX, therefore, can be used as a screening tool to determine the extent of pollution in many environmental samples and to assess if more specific chemical analysis is necessary.

In this paper a Mitsubishi TOX-10 Analyzer was used to determine TOX on a variety of environmental samples. These included groundwaters and wastewaters, transformer oils, waste solvents, sludges, soils, and TCLP extracts. The sample preparation and analytical techniques necessary to determine the TOX on each of these sample matrices will be discussed. Brief mention will be made concerning interferences to the TOX determinations. Finally, comparisons will be made between TOX data and data from other analytical methods such as Gas Chromatography and GC/MS.

INTRODUCTION

When using regulatory language, the term TOX (Total Organic Halogen) usually refers to an analytical technique outlined in the EPA Method #9020 found in SW846.¹ The method developed in Germany during the early 1970s from a method to check granular activated carbon filters used in water treatment plants for breakthrough of halogenated organic contaminants. The method was further refined to its present state by the Drinking Water Research Division of the Municipal Environmental Research Laboratory, U.S. EPA, Cincinnati, Ohio.² Instrumentation to semiautomate the method was developed by private industry under contract to the EPA. Presently two commercial

instruments are marketed in the United States. ^{3,4} (A more extensive history of TOX is given in reference 2.)

In Method #9020 a water sample is passed slowly through two activated carbon columns held in series to adsorb the halogenated organic compounds. These carbon columns are then washed with a KNO_3 solution to remove inorganic halides and are combusted in a high temperature furnace. Organic halogens are converted to the hydrogen halide gas and carried by the gas stream to a titration cell where the gas is trapped in a acetic acid/acetate buffer. The hydrogen halide precipitates silver ions from solution and lowers the preset end point potential, which is monitored by a silver/silver/chloride electrode system. A constant pulsed current generates silver ions and coulometrically titrates the solution to the end point potential. By the application of Faraday's law, the concentration of the organic halogen in the sample can be calculated as Cl.

The term TOX requires some qualification. For our purpose, any compound with a C-X bond will be considered an organic halogen. The common halogens, chloride, bromide, and iodide are detected, though not individually, by the method. Fluoride, because of the solubility of AgF in aqueous solution is not detected as TOX.

The term is further qualified by the fact that only those organic halogens which are adsorbed onto the activated carbon and not removed by the washing step will be detected as TOX. It has been shown that some organic halogens, or example chloroacetic acid, weakly adsorb onto the activated carbon and can be removed during the washing step.⁵ In addition, other nonhalogenated organic compounds can compete with halogenated organic halogens for adsorption on the activated carbon column.⁶

One of the main advantages of TOX is that it can serve as a fairly rapid screening technique for pollutants. Many of the contaminants which man has introduced into the environment are halogenated. This halogenation not only makes these compounds more resistant to biodegradation, but also increases their toxicity to aquatic life, animals, and man. Consider the priority pollutant compounds. Seventy-one of the 113 organic compounds on the list are halogenated with chlorine being the predominate halogen. These reasons, combined with the fact that carbon containing compounds are ubiquitous in nature make TOX superior to TOC (Total Organic Carbon), another commonly used analytical tool, as a pollution indicator.

TOX has most often been applied to aqueous samples. In this paper, TOX was determined on a number of different kinds of environmental samples. These included ground waters, TCLP extracts, waste oils, solvents, soils and sludges. Spike recovery data was obtained for a variety of halogenated compounds in these matrices. The precision

of the TOX method was also determined. Comparisons of TOX to other analytical methods were made.

EXPERIMENTAL

Two Mitsubishi TX-10 analyzers were used to determine TOX. These were kept in a positive pressure room free of halogenated solvent vapors.

Aqueous Samples: Samples were loaded through two 40 mg activated carbon columns arranged in series at a rate of 3.3 ml/minute to adsorb organic halogens. Following loading, these columns were washed with 4 ml of 0.8% potassium nitrate solution and transferred to the TOX furnace. The following instrument settings were used for burning the carbon columns:

O ₂ flow	300 cc/min
AR/O ₂ flow	150 cc/min
Furnace temperature (max)	850° C.
Combustion time	2.0 min. before start of titration

End point potential 280-300 mv.

Oils and Solvents: Oil samples were diluted in pesticide grade hexane and injected directly onto a prewashed 40 mg carbon column and immediately placed into the sample entry part of the furnace. An extra delay of one minute was set on the TOX Analyzer. During this period Argon gas flowed over the sample and the sample was slowly inserted into the center of the furnace. This was done to facilitate the evaporation of the hexane solvent and to prevent explosion of the carbon plug.

Solvent samples not miscible with hexane were either injected directly or diluted with methanol or ethyl acetate prior to injection. If these solvent samples were miscible with water, the samples were injected directly onto the top of a carbon column and washed with potassium nitrate solution before the combustion step.

Solid Samples: Two methods were used to extract sludge and soil samples. In the first method the solid sample was mixed with anhydrous sodium sulfate and extracted using 250 ml of a 1:1 (v/v) mixture of acetone/hexane in a soxhlet extractor for four hours. After washing three times with deionized water to remove the acetone, the extract was concentrated to 10 ml on a steam bath using a Kuderna-Danish apparatus and Snyder column⁷. This hexane extract was injected directly onto a washed carbon column and combusted in the instrument.

The second extraction procedure was performed using an Ultrasonics Heat Systems sonic probe fitted with a microtip. The sonicator was set at 50% duty cycle and the output control at 5 to accommodate the microtip. After mixing the solid sample with sodium sulfate, adding 20 ml of hexane, and positioning the microtip in the hexane just above the solid, the sonicator was pulsed for two minutes.

After the sonication, the hexane was decanted and gently suctioned through a Buchner funnel fitted with a glass fiber filter and containing a small amount of sodium sulfate. Solid samples were extracted with three successive 20 ml portions of hexane. After the last sonication, the entire sample was filtered. This residue was then washed with hexane and the extract was made up to a 100 ml using hexane. A portion of this extract was then injected onto a carbon column and introduced into the TOX-10 furnace.

Below are other methods referenced in this paper:

1. PCBs in Oils: Oil samples were dissolved in hexane and eluted through a florisil column with 200 ml of 6% diethylether in hexane. Five ul were injected into a GC under the following conditions:

Injector and detector temperatures - 250°C
Oven temperature - 200°C (isothermal)
Carrier - N₂ (60 ml/minute)
Column - 2 m x 4 mm ID with 1.5% SP-2250/1.95% SP-2401 packing
Detector - electron capture

2. Solvent Identification: Samples were dissolved in carbon disulfide or methanol and injected into a GC under the following conditions:

Injector and detector temperatures - 250°C
Primary column 2 m x 4 mm ID glass column with 1% SP-1000 on 60/80 mesh carbopak B
Oven temperature - initial temperature 80°C with two minute hold, ramp at 8°/min. to 220°C, hold for 15 minutes
Carrier - N₂ (80 ml/min)
Detector - flame ionization detector (FID)

Samples were confirmed on a secondary column.
Secondary column - 60 m x 0.32 mm ID capillary with 1.0 um DB-5 film
Injector and detector temperatures - 250°/320°C
Split injection 16:1
Oven temperature - initial temperature 35°C with 10 minute hold, ramp at 5°/min. to 300°C
Carrier - He
Detector - FID

3. Total Halogen in Oil: Halogens in oil samples were determined gravimetrically as described in ASTM Method D808.¹¹
4. Combustion/Ion Chromatography Method: Oil samples were combusted as above in ASTM D808. The combustion gases were trapped in 10 ml of eluent from the ion chromatograph.

The bomb washings were brought to a final volume of 100 ml. One hundred microliters of these washings were then automatically injected into a Dionex 2020i Ion Chromatograph under the following conditions:

Flow: 2.0 ml/minute
Eluent: 2.8 mM NaHCO₃/2.2mM Na₂CO₃
Column: ASC-3
Suppressor: Anion membrane suppressor (.025 M H₂SO₄)

Chloride was quantitated by peak height using a three level calibration curve.

5. Volatile Organics Compounds were determined as described in EPA Method 624.¹²
6. Acid and Base/Neutral Compounds were determined as described in EPA Method 625.¹³
7. Pesticide/PCBs were determined as described in EPA Method 608.¹⁴

RESULTS AND DISCUSSION

TOX recovery in aqueous matrices. Table 1 shows data on several TOX water standards. The 2,4,6 Trichlorophenol standard was a quality control standard which was determined repeatedly over a period of about six months. This standard was made fresh daily according to Method #9020 in EPA SW-846. An average recovery of 96% and a relative standard deviation (RSD) of 6.7% was obtained. The high recovery indicates that the 2,4,6 TCP was readily adsorbed onto the activated carbon and nearly completely converted to the titratable hydrogen chloride species during the combustion step.

The other standards listed in Table 1 are mixtures of priority pollutant compounds. Table 2 illustrates the composition of the mixture of volatile organic halogens. Twenty ul of this standard were injected into one liter of deionized water to produce the final TOX concentration of 144 ug Cl/liter. Tables 3 and 4 show the composition of the base/neutral and pesticide/PCB standards.

The recovery demonstrated on each one of these standards ranged from 60-70%. Loss of recovery might be attributed to any of the

following: (1) experimental error in making of the standard, (2) weak adsorption onto the activated carbon and breakthrough of organic halogens, (3) loss of volatile organic halogens during the transfer of the carbon column from the loader to the furnace, (4) chemical decomposition of the organic halogen into a form that was not adsorbed or eluted during washing, (5) incomplete combustion of the organic halogen in the furnace to produce a titratable hydrogen halide.

The latter reason seems to be ruled out because of the higher recoveries (nearly 90%) shown on each of these three standards when injected directly into the TOX-10 furnace. (See Table 2 and Table 8.)

A variety of groundwater and waste water spikes are shown in Table 5. In all cases recoveries were very good (85%). None of the sample matrices interfered with the TOX determination.

Application of TOX to TCLP extraction. The Toxicity Characteristic Leaching Procedure (TCLP) has been promulgated and finalized by the EPA.¹⁰ Many of the target compounds which are regulated by the TCLP are halogenated. This is especially true of the volatile organic compounds which are to be extracted using the zero head space extractor (ZHE). Table 6 shows spike data on TCLP extraction fluids spiked with trichloroethene. As seen from the results, the presence of acetic acid in the extracts (0.1 M) did not adversely effect the spike recoveries indicating good carbon adsorption of the TCE.

Even though the TCLP requires that only extraction fluid one be used in the ZHE, extraction fluid two was also spiked. The lower pH of this fluid did not significantly alter recovery of the TOX.

TOX determinations were used to evaluate the extraction efficiency and precision of the TCLP using the ZHE on soil spiked with trichloroethene. The same soil, which had been oven dried and passed through a fine mesh sieve, was used in each of the TCLP extractions shown in Table 7. The TCLP extract of the soil showed a TOX background level of 40 ug Cl/liter. This background was subtracted from the TOX of each TCLP extract. The extraction efficiency was obtained by dividing the total TOX in 500 ml of the TCLP extract (ug Cl) by the total TCE as ug Cl added to the 25 g of soil. Extraction efficiencies of 40-70% were obtained. The soils spiked at the highest concentration showed consistent recovery of about 70%. An RSD at the 95% confidence level of 7.1% was estimated using small number statistics on the TOX of the (replicate) TCLP leachates.

SOLVENTS AND WASTE OILS

Oils and solvents which will be burned as fuel or incinerated pose a potential threat to the environment. Waste oils with a TOX greater than 1000 ppm cannot be sold as a used fuel.¹²

TOX recovery in solvents. Table 8 shows TOX recovery data on a variety of halogenated solvent standards made in methanol and hexane. The volatile, pesticide, and base/neutral compounds are the same as those listed in Tables 2, 3, and 4. The volatile standard, however, was made at a slightly higher concentration (8420 ug Cl/ml). Good to excellent recoveries were observed on each standard. The relative standard deviations, which averaged 3.6%, showed good precision for the technique.

TOX in transformer oils. Table 9 and 10 demonstrate that TOX can be used to screen for the presence of PCBs in transformer oils. It must be realized, however, that other organic halogens, such as chlorinated benzenes often found in transformer oils, will also be detected as TOX. In Table 9 EPA certified standards of three different arochlors are shown. Two levels of standards are shown for the Arochlor 1242 and 1260; three levels are shown for 1254. In all cases except for the two lowest levels of Arochlor 1254, the TOX is within 5% of the certified value (expressed as ug Cl).

The detection limit of this TOX method for solvents was established at 10 ug Cl/ml when 50 ul of hexane were injected directly into the instrument. Lower detection limits are hindered by the volatile and explosive nature of hexane. Fifty ul was found to be the maximum safe injection size.

TOX and PCB determinations of real-world transformer oil samples are compared in Table 10. A "best fit" line was calculated after assigning the total PCB value as ug Cl/g to X and the TOX value to Y. In slope intercept form, the equation of the "best fit" line was: $y = 1.17x + 18.2$. A correlation coefficient of 0.99997 was also obtained. If the results of these methods were identical, a slope of one and intercept of zero would be obtained. However, in this case the slope was greater than one and the intercept was positive, showing that the TOX method has a positive bias. This bias might be explained by the presence of other halogenated organics in the oil or the background from the oil itself. However, a very good linear correlation exists between the TOX and the total PCB content of the samples.

TOX vs GC (FID) solvent analysis. Another comparison between TOX and gas chromatography is shown in Table 11. This table shows data on eight waste solvent and oil samples which were chromatographed to identify and quantitate unknown solvents. The conditions for this chromatography are given above in the experimental section. TOX and

PCBs were also determined on each of the samples. Generally, a good correlation exists between the TOX and GC data. Naturally, the advantage of the GC method is the qualitative identification of the individual halogenated solvents. The GC method, however, is not as sensitive as TOX for most samples.

TOX vs total chlorine in waste oils. A final comparison between TOX and two other combustion methods for determining halogens in fuels and oils is given in Table 12. The ASTM D808 method is a wet chemical method and is very tedious. Because it is a gravimetric method using Ag NO_3 , it is specific to the same halogens (Cl, Br and I) as TOX. The method is incorrectly titled as total chlorine. The ASTM method, theoretically, should also determine inorganic halides present in oil samples. TOX, on the other hand, does not quantitate inorganic halides. In fact, if they are present in waste solvent samples, they will deposit on the walls of the furnace during the combustion step because of their high boiling point. This is often evidenced by poor repeatability, lingering coulometric titrations with slow end points, and erroneously high results. Samples analyzed after the injection of inorganic halides are often affected.

Inorganic halides are usually not a problem with water insoluble oils and solvents because they do not appreciably partition into organic phases. Dilution of oils in hexane also serves to reduce the inorganic halogens in waste oils and solvents. Inorganic halides will strongly interfere with TOX if they are present in water miscible solvents such as some alcohols and acetone and must be removed by washing with KNO_3 prior to combustion.

The combustion/ion chromatography (IC) method listed in Table 12 only quantitates total chlorine. The oxygen bomb combustion was identical to the ASTM D808 method except the eluent from the chromatograph was used to trap the combusted hydrogen chloride.

Of the three methods, the TOX method was the easiest and most rapid to perform. The IC method and TOX method had comparable sensitivities of about 100 ug Cl/g. ASTM D808 was about five times less sensitive.

SOLID SAMPLES

To determine TOX on solid samples such as sludges and soils requires an extraction step. Inorganic halides often tied up in the solid matrix make direct combustion of these samples impossible.

Recovery of TOX in solid samples. Two existing extraction procedures were adapted in this study. Initially, the soxhlet extraction procedure (Method #3540 SW-846) was used. As shown in Table 14, good spike recoveries of nonvolatile halogenated compounds were obtained. The precision of the method was also determined (see

Table 15) and a RSD of 3.7% was found at the 200 ppm level. The sensitivity of this method was 10 ppm and could be pushed as low as one ppm by concentrating the hexane extract to one ml using the KD apparatus.

This extraction procedure, however, precluded volatile halogens because of the concentration step and also the washing step used to remove acetone (see above).

In the second procedure (and the one currently used at Lancaster Laboratories, Inc.) solid samples were extracted with a sonic probe (Method #3550 SW-846). Spike data on a variety of solid samples is given in Table 16. Spike recovery ranged from 56-112% with an average recovery of nearly 84%. Soil I was oven dried and sieved. This soil was stored in our laboratory and used for spiking as part of our quality assurance program. Five different soil samples (sent to our laboratory) were spiked at the same level 2030 ug Cl/g using 1,2,4 trichlorobenzene. An average spike recovery of 88% was obtained and a RSD of 4.7% was found, showing good repeatability. The other samples shown in the table were also real-world samples sent to our laboratory. These were all spiked with 2,4,6 trichlorophenol in methanol at about 2000 ug Cl/g. Only sample #1162542 showed poor recovery. This sample was sent to our laboratory as an unknown solid and resembled charcoal. This material may have retained the 2,4,6 TCP spike preventing recovery. As might be expected, the TCE soil spike showed lower than average recovery. Some of the volatile TCE was most likely lost during the extraction procedure and when the carbon column was injected with TCE prior to being pushed into the furnace.

TOX vs. priority pollutant compounds (GC/MS). To evaluate further the effectiveness of TOX as a screening parameter, a comparison between TOX and organic halogens from the priority pollutant list (expressed as Cl) was made (see Table 17). The volatile, acid and base/neutral compounds were determined by GC/MS. The pesticide/PCBs were determined by gas chromatography.

One must realize that only a limited number of samples are shown on this table and erroneous conclusions can be drawn from a small set of data. More comparison data needs to be accumulated to concretely prove any relation between TOX and priority pollutant data. Nonetheless, a trend is shown in Table 17. TOX in most environmental samples is generally higher than accounted for by GC/MS priority pollutant data. This trend is also supported by the author's experience with TOX. In very few instances have halogenated priority compounds been found when TOX is not detected. When TOX has been found, however, it has not always been accounted for by priority pollutant data. This is especially true of complex solid samples such as the municipal sewage sludges CS1, MB1, and S22 found in Table 17. Note that TOX levels of 230, 63, and 35 ug Cl/g

were found in these samples, respectively. Yet, the GC/MS priority pollutant data could not account for any significant portion of this organic halogen.

SUMMARY AND CONCLUSIONS

Although TOX cannot be used to specifically identify halogenated organic compounds, TOX has been shown to be an effective analytical tool in screening environmental samples. The method is less time consuming and, therefore, less expensive than more complex analytical techniques such as GC and GC/MS. It has been successfully applied to aqueous, organic and solid matrices. Good spike recovery for a variety of halogenated organic compounds was found in all of these sample matrices. Relative standard deviations of about 5% were determined on each matrix. Interferences to TOX were minimal and could be eliminated with proper precautions. In most aqueous samples the detection limit of TOX is in the low ug Cl/liter range when volumes of 100 ml or greater are loaded. This advantage of concentrating the organic halogen in the carbon column, however, is lost with organic and solid samples. In solids and oils, a detection limit of 10 ppm was established. This detection limit was limited by the amount of hexane which could be safely injected into the combustion furnace.

Good correlation was seen between TOX and a variety of gas chromatography methods. These included methods for the determination of PCBs in oils with an electron capture detector and the determination of organic solvents in waste oils and solvents using a flame ionization detector. Comparison was also made to two methods for determining total chlorine in oils using a Parr oxygen bomb combustion. The ASTM D808 method was found to be more laborious and less sensitive than TOX. The combustion/IC method was found to be about as sensitive as TOX and showed equal precision (RSD about 3%). It, however, was more time consuming. The TOX method showed a higher mean than the combustion/IC method on multiple determinations of a waste oil sample.

A general trend showing that TOX was generally higher than accounted for by GC/MS priority pollutant data was also reported. TOX is nonspecific in determining organic halogens and, therefore, would not be limited to detecting only priority pollutant compounds and compounds with chemical and physical properties making them suitable for gas chromatography.

Table 1.

Tox Water Standards

<u>Standard</u>	<u>N</u>	<u>Prepared Conc.*</u>	<u>X*</u>	<u>S*</u>	<u>RSD %</u>	<u>% Recovery</u>
2,4,6 TCP	28	100	96	6.4	6.7	96
Volatiles	5	144	99	10.5	11.0	69
Base/neutrals	4	115	74	4.4	5.9	64
Pesticides	4	104	73	9.4	12.9	70

All standards were prepared in deionized reagent water sample
Volume - 100 ml
Loading rate - 3.3 ml/minute

* ug Cl/liter

Table 2.

Volatile Organic Cocktail

<u>Compound</u>	<u>ug/ml</u>	<u>% Halogen as Cl</u>	<u>as ug Cl/ml</u>
methylene chloride	664	83.5	554.
1,1 dichloroethene	520	73.1	380.
1,1 dichloroethane	588	71.6	421.
trans 1,2 dichloroethene	620	73.1	453.
chloroform	628	89.1	559.
1,2 dichloroethane	744	71.6	533.
1,1,1 trichloroethane	628	79.7	500.
carbon tetrachloride	596	92.2	549.
dichlorobromomethane	672	64.8	436.
1,2 dichloropropane	740	62.7	464.
trichloroethene	652	80.9	528.
dibromochloromethane	776	51.0	395.
2 chlorovinyl ether	668	33.6	224.
bromoform	392	42.0	374.
tetrachloroethane	692	84.5	584.
chlorobenzene	768	31.5	<u>242.</u>
Total			7196.

Standard made in methanol

Direct Injection:

<u>Injection Size (ul)</u>	<u>Conc. of Std. (ug Cl/ul)</u>	<u>Amount as Cl (ug)</u>	<u>N</u>	<u>X (ug)</u>	<u>Average % Recovery</u>
5.	7200.	36.	2.	31.	86.

Table 3.

PESTICIDE/PCB COCKTAIL

Compound	ug/ml	ug Cl/ml
PCB 1248	500.	240.
PCB 1254	500.	270.
Heptachlor	500.	332.
BHC	250.	183.
PCB 1221	500.	105.
4,4' DDD	500.	240.
Heptachlor Epoxide	500.	319.
Toxaphene	500.	340.
Endrin	500.	344.
4,4' DDT	500.	<u>250.</u>
	TOTAL	2620.

Table 4.

BASE/NEUTRAL COCKTAIL

Compound	ug/ml	ug Cl/ml
4 bromophenyl phenyl ether	833.	118.
3,3' dichlorobenzidine	833.	231.
Hexachloropentadiene	833.	648.
1,2,4 trichlorobenzene	833.	488.
Hexachlorobutadiene	833.	<u>144.</u>
	TOTAL	2310.

Table 5.

TOX Aqueous Matrix Spikes

<u>Sample No.</u>	<u>TOX-unspiked Sample*</u>	<u>Spike Added*</u>	<u>TOX-spiked Sample*</u>	<u>Recovery %</u>
1072944	25	100	128	103
1075051	254	100	349	95
1074785	5	100	98	98
1084426	22	50	65	86
1045111	7	100	104	97
1029675	5	100	98	98

Spiked with 2,4,6 TCP

* ug Cl/l

Table 6.

TCLP Extract Matrix Spikes

	<u>pH</u>	<u>N</u>	<u>Spike Conc. (ug/l)</u>	<u>Ave. Spike Recovered</u>	<u>Ave. % Recovery</u>
Extraction Fluid #1	4.98	3	150	139	93
Extraction Fluid #2	2.88	2	150	146	97

Spiked with trichloroethene in methanol.

Table 7.

TCLP of Soil Spiked with TCE

	Conc. of TCE Added to Soil <u>(ug Cl/g)</u>	TOX of TCLP Extract <u>ug Cl/liter</u>	<u>% TCE Extracted</u>
Soil Sample (background)	0.	40	--
Soil Sample 1a	99.	3540	72.
Soil Sample 1b	99.	3620	74.
Soil Sample 1c	99.	3200	65.
Soil Sample 4	1.0	71	62
Soil Sample 5	1.0	62	44

25 g soil spiked and extracted

Final volume of TCLP extract - 500 ml

Table 8.

TOX Solvent Standards by Direct Injection

Compound(s)	N	Injected (ug Cl)	Recovered (ug Cl)	RSD %	Ave. % Rec.
2,4,6-TCP	15	5.00	5.04	3.6	101.
Volatiles	6	25.3	24.2	3.4	96.
Chloroform	6	22.5	22.0	6.1	98.
Bromoform	6	9.80	9.76	2.0	100.
Pesticides	3	13.1	12.2	--	93.
Base/Neutrals	2	23.1	20.6	--	89.
PCB 1242	7	21.1	21.1	3.0	100.

Hexane was the solvent for chloroform and bromoform.

Methanol was the solvent in all other cases.

Table 9.

TOX On EPA Transformer Oil PCB Standards

Aroclor	Calculated	Measured	Percent Recovery
1242	24.0	22.	92.
	239.	249.	104.
1254	6.5	11.	170.
	27.0	30.	111.
	270.	256.	95.
1260	30.0	31.	103.
	341.	331.	99.

TOX values are given in ug Cl/g.

Table 10.

PCBs In Transformer Oils
EC/GC vs. TOX

Sample	G.C.	TOX
373305	128.	145.
373306	125.	143.
373307	122.	153.
377852	4.1	11.
378095	6.0	9.7
379703	527.	637.
381633	8200.	9600.
381778	41.2	99.
381779	36.3	104.

Values are given in ug Cl/g.

Table 11.

**TOX vs GAS CHROMATOGRAPHY
(Waste Solvents and Oils)**

Sample	TOX	GC/FID	Compounds Identified	GC/ECD (PCBs)
Solvent	2.0	2.0	Carbon Tetrachloride	--
Solvent	10.8	11.	Methylene Chloride 1,1,1 Tri-chloroethane	--
Solvent	27.0	30.1	Tetrachloro-ethylene	ND
Solvent	<0.1	ND	--	ND
Oil	0.68	0.8	1,1,1 Tri-chloroethane	ND
Oil	1.3	1.4	1,1,1 Tri-chloroethane	ND
Oil	0.03	ND	--	.019
Oil	<0.01	ND	--	.0019

All values are given as percent halogen by weight.

Table 12.

Comparison of Methods - Halogens in Waste Oils

<u>Sample</u>	<u>TOX</u>	<u>Total Chlorine Comb./IC</u>	<u>ASTM D808</u>
Standard I	1560	1620	1200
1145917	7950 ^a	6440 ^b	6700
1159392	1040	960	600
1159614	570	860	<500
1159615	4970	5250	5200
1160952	240	230	<500
1160953	1150	1120	960

All results as ug Cl/g
Standard I (1680 ug Cl/g) - TCE in mineral oil

a Average 6 trials; s=190

b Average 5 trials; s=220

Table 13.

TOX SPIKE RECOVERY IN SLUDGE BY SOXHLET EXTRACTION

Sample	Spike Material	Spike Added (ug Cl/g)	Spike Recovered (ug Cl/g)	% Recovery
CS1	2,4,6 TCP	241	214	89
CS1	2,4,6 TCP	240	258	108
CS1	PCB 1242	194	190	98
S31	2,4,6 TCP	61	61	100

Table 14.

PRECISION DATA FOR TOX IN SLUDGES

Trial	TOX (ppm)	
CS1	238	
CS2	224	$\bar{x} = 233 \text{ ppm}$
CS3	245	$S = 8.5$
CS4	229	$RSD = 3.7\%$
CS5	228	

Table 15.

TOX Spikes of Solid Samples Sonic Probe Extractions

<u>Sample Matrix</u>	<u>N</u>	<u>Spiking Compound</u>	<u>Spike Added*</u>	<u>Ave. Spike Recovered*</u>	<u>Recovery %</u>
Soil I	3	TCE	510	380	75
Soil I	1	PCB-1260	290	240	83
Soil I	2	1,2 DCB	500	560	112
Natural Soils ^a	5	1,2,4 TCB	2030	1780	88
Carbonaceous Sludge (1162542)	1	2,4,6 TCP	1650	920	56
Paint Sludge (1159794)	1	2,4,6 TCP	2050	1920	94
Industrial Sludge (1139812)	1	2,4,6 TCP	1940	1550	80
Industrial Sludge (1134427)	1	2,4,6 TCP	1940	1620	84

* Units - ug Cl/g

a Five different soil samples - each extracted once

TCE - trichloroethene

DCB - dichlorobenzene

TCB - trichlorobenzene

TCP - trichlorophenol

N - number of determinations

Table 16.

TOX vs. GC/MS Priority Pollutants

<u>Sample Type</u>	<u>TOX</u>	<u>Priority Pollutant Halogen</u>	<u>Compounds Tested</u>	<u>Halogenated Compounds Detected</u>
GW 1	2100 ^a	500 ^a	V,A,B,P	Vinyl chloride trans 1,2 dichloroethene trichloroethene 2,4,6 trichlorophenol
GW 2	810 ^a	767 ^a	V,A,B,P	Chlorobenzene trans 1,2 dichloroethene trichloroethene
WW 3	290 ^a	2 ^a	V,A,B,P	Chloroform
TCLP	3200 ^a	4190 ^a	V	Chloroform 1,2 dichloroethane methylene chloride 1,1,1 trichloroethane 1,1,2 trichloroethane trichloroethylene tetra chloroethene
GW 3	10 ^a	30 ^a	V	methylene chloride 1,1,1 trichloroethane
SW-CS1	230 ^b	ND ^b	V,A,B,P	ND
SW-MB1	63 ^b	ND ^b	V,A,B,P	ND
SW-S22	35 ^b	ND ^b	A,B,P	(some P; Tot <1 ^b)

a Units ug Cl/liter

b Units ug Cl/g

V - Volatiles

A - Acid extractables

B - Base/neutrals

P - Pesticides/PCB

GW - Ground water

WW - Waste water

TCLP - Leachate

SW - Solid waste

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PYROLYSIS/MICROCOULOMETRIC DETERMINATION OF
TOTAL ORGANIC HALIDES IN SOLIDS AND OILY WASTES

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ABSTRACT

Two pyrolysis/microcoulometric methods for the determination of total organic halides in solids and oily waste are described. The first method involves the washing of solid sample in a column packed with granular activated carbon with a dilute nitrate solution to remove interfering inorganic halides. The organic halides are subsequently determined by pyrolysis/microcoulometric measurement of the sample solid-activated carbon mixture. The second method involves the extraction of solid or oily waste samples with a mixture of octanol and 1 M sulfuric acid. The organic halides are extracted into the octanol phase and then analyzed by pyrolysis/microcoulometry. The inorganic halides are removed by extraction into the aqueous phase. The second method has the advantage of extending the technique to the analysis of a broader spectrum of organic halides including polar compounds such as chloroacetic acid.

INTRODUCTION

Organic halides represent one of the most important groups of compounds. Not only these compounds are generally toxic and/or carcinogenic, but they are also the most commonly occurring environmental pollutants. Pesticides and chlorinated organic solvents were once thought to be the primary source of organic halide pollution. However, studies have shown that chlorination of drinking water and wastewater produces significant amount of these compounds(1).

Because many different halides with widely varying characteristics can occur in a sample, it is not practical, if not impossible, to analyze all these compounds individually. It has been estimated that only 10 to 25% of these compounds are amenable to individual analysis(2-3). An effective method for measuring the amount of total organic halides could provide valuable information on the occurrence of organic halide contaminants. Several workers(4-8) have employed pyrolysis/microcoulometry as a means of measuring of organic halides in water. The technique involves isolation, conversion of organic halides into inorganic halides and their subsequent quantitative determination by microcoulometric measurement. The methods used for the isolation of organic halides includes solvent extraction, gas purging and carbon adsorption; the latter is the method presently in use in the U.S. EPA Methods 450.1 and 9020 (9) for determining total organic halides in waters. A semiautomatic system for carbon adsorption, pyrolysis and microcoulometric determination has been developed and is commercially available(10). The primary advantages of this technique are ease of operation, good sensitivity and low background levels. In this study, two pyrolysis/microcoulometric methods for the determination of total organic

halides in solids and oily wastes are described. The first method involves the washing of solid samples in a column packed with granular activated carbon with a dilute nitrate solution to remove interfering inorganic halides. The second method involves the extraction of solid or oily waste samples with a mixture of octanol and 1 M sulfuric acid.

EXPERIMENTAL

Apparatus and Material

The Dohrman DX-20 Total Organic Halide Analyzer was used for the analysis of total organic Halides. Two modes of operation of the analyzer were employed. The boat loading mode was used for analysis of solid samples by Nitrate Wash-Carbon Adsorption Method and the direct injection mode was use for analysis of solid and oily waste samples by Solvent Extraction Method.

A schematic of the Nitrate Wash-Carbon Adsorption apparatus is shown in Figure 1. The apparatus was used for washing of solid samples to remove interfering inorganic halides prior to pyrolysis/coulometric measurement.

All chemical reagents used were reagent grade obtained from Baker Chemical or Aldrich Chemical and were used without further purification. High purity deionized water was obtained form Sparkletts Water Company. The granular activated carbons was Filtrasorb 400(Calgon Corp., Pittsburgh, PA) screened to a 100/200 mesh range. The columns for containing the granular activated carbon and solid sample were 5 cm section of 6 mm O.D. Pyrex tubing with a 2 mm I.D. Since activated carbon adsorbs organic vapor from air quite readily, the carbon was kept in a clean glass bottle with a air

tight cap. Precautions were taken to minimize the time of exposure of the carbon to air during the packing of columns with granular activated carbon.

Standard solutions of different halogenated organic compounds were prepared by dissolving the compound of interest in ethyl acetate. Standard soil samples of the different halogenated organic compounds were prepared. Each soil sample was prepared by adding known volume of the standard solution of interest to an accurately weighed out amount of garden soil which had been dried and passed through a 30-mesh sieve. The soil sample was then shaken vigorously and was allowed to equilibrate at least overnight before use.

Solid Sample Analysis by Nitrate Wash-Carbon Adsorption Method

The glass columns were cleaned with isopropyl alcohol, rinsed with deionized water and dried. A Cerafelt (Johns-Manville, NY) plug was inserted in one end of the glass column. The first column was half filled with granular activated carbon and weighed. The remaining space was filled with sample solid and reweighed to obtain the weight of sample used. A second plug was used to cap the end. The second column was completely filled with granular activated carbon and capped. The two columns were connected in series as depicted in Figure 1. A 500 ppm nitrate wash solution under 65 psig nitrogen was used to wash the columns-in-series. After the desired volume of the nitrate wash was eluted, typically 2 ml, the columns were disconnected for the determination of halogen content in each column. Several samples could be processed simultaneously.

In the pyrolysis-determination step, the solid content from each column was extruded with the use of a plunger and placed directly onto a sampling boat at the inlet of the Dohrman DX-20 analyzer and the analysis cycle was

initiated. The sampling boat first moved into a 250° C vaporization zone, then through a 800°C combustion zone where all organics were oxidized and halides converted to hydrogen halides. The gases then flowed into the microcoulometric titration cell and determined quantitatively by titration with silver ions generated coulometrically.

Solid and Oily Waste Sample Analysis by Solvent Extraction

Accurately weighed out approximately one gram solid sample or delivered from a microsyringe a suitable volume, typically 25 to 100 µl of oily waste into a 14 ml glass vial containing 1 ml of 1 M sulfuric acid and 5 ml of octanol.. The vial was shaken vigorously for one minute to extract the organic halides into the organic phase and then centrifuged for 10 minutes at 1000 G. A 25 to 50 µl aliquot of the octanol phase was withdrawn into a microsyringe and injected directly into the Analyzer set up for direct injection mode for determination of the halide content.

RESULTS AND DISCUSSION

Soild Sample Analysis by Nitrate Wash-Carbon Adsorption Method

The first method developed for the measurement of total organic halides in solids was a modification of the EPA Method 9020 for determining total organic halides in water samples. In the method developed, the solid sample was washed with a 500 ppm nitrate solution to remove any interfering inorganic halides that might be present. Any organic halides eluted from the solid sample were trapped by the granular activated carbon. The use of two columns in series ensured the complete adsorption of the organic halides eluted. The results are given in Table 1. The recoveries for relatively non-

volatile and non-polar compounds ranges from 72.3% to 93.0%. The low recoveries of chloroform and chloroacetone may be ascribed to loss by vaporization during sample preparation. Poor recoveries were also obtained for polar compounds. Study of recovery of organic halides from water using the EPA' method showed similar poor results for polar compounds(10). That study showed that chloroethanol had about 20% recovery while chloroacetic acid showed 0% recovery. The low recoveries may be ascribed to the poor adsorption characteristic of the polar compounds on granular carbon. The result of this study show a 52.1% and 58.3% recoveries for chloroethanol and chloroacetic acid respectively. The higher results were probably due to the incomplete desorption of the organic halides from the soil during the nitrate wash.

Solid Sample Analysis by Solvent Extraction

A method for the determination of organic halides in solid has been developed by Riggins et. al.(7) and involved the use ethyl acetate-water mixture which extracted the organic halides into ethyl acetate phase for pyrolysis/ microcoulometric analysis. Any inorganic halides that may be present were removed into the aqueous phase. To evaluate the applicability of the method, a broad spectrum of organic halides of different known concentrations were analyzed using ethyl acetate-water mixture for extraction. To 1 gram of standard soil sample in a 14 ml glass vial were added 5 ml of ethyl acetate and 1 ml of distilled water. The sample was shaken vigorously for 1 minute and then centrifuged for 10 minutes at 1,000 G. A 25 to 50 μ l aliquot of the ethyl acetate was analyzed by the Analyzer set up for direct injection mode. The results are shown in Table 2. With the exception of bromoform and 1-iodooctane, the generally non-polar

compounds had good recoveries ranging from 86.2 to 129.5%. Bromoform and 1-iodooctane showed slightly lower recoveries and were probably because of the formation of bromine and other undetectable bromine and iodine compounds(8). Polar compounds such as chloroacetic acid shows very poor recoveries. In general, the more polar compounds had a lower overall recoveries. The low recoveries may be ascribed to the loss of the organic halides during the extraction step of the analysis, because the more polar organic compounds were more soluble in water and was partially extracted into the aqueous phase. Equilibrium calculations indicate that the majority of the chloroacetic acid in water dissociated into chloroacetate and hydrogen ions,



The acid dissociation constant for this reaction is 1.38×10^{-3} . This being the case, the chloroacetate ion is more soluble in the aqueous phase than in the organic phase, therefore giving low recoveries for this compound.

One possible means of lowering the solubility of chloroacetic acid and other polar compounds in water is to shift the dissociation equilibrium by adding an excess of hydrogen ions to the aqueous phase. However, ethyl acetate was found to be misible with 1 M sulfuric acid due to an acid catalyzed hydrolysis of the ethyl acetate(11). Instead of having two immisible layers, there was only one layer of solution.

It was decided to try another slightly polar solvent which is immisible with water and do not undergo hydrolysis under acidic conditions. A mixture of 1-octanol and 1 M sulfuric acid was chosen for use in extraction. Unlike the ethyl acetate-sulfuric acid mixture, the octanol and sulfuric acid existed in two separate layers. The results are shown in Table 3. As expected, the non-polar compounds had good recoveries, such as 1-

chloronaphthalene which had 97.9% recovery. The recovery of chloroacetic acid improved substantially from the ethyl acetate-water method from 1.2% to 83.9%. All other compounds less polar than chloroacetic acid showed similar improvements in recoveries.

Oily Waste Analysis

For the purpose of this study, oily waste is qualitatively defined as an organic liquid or an emulsion of organic liquid and water with varying degrees of volatilities and viscosities. Since oily waste sample is in the liquid state, it was thought that a direct analysis of the oil by syringe injection into the pyrolysis tube would be possible. This was found to be impractical because sample with relatively high viscosity such as oils could not be quantitatively injected into the pyrolysis tube. More important, oily waste samples frequently contain water in the form of emulsion which usually contains significant amount of interfering inorganic halides. In view of the difficulties with direct analysis, it was decided to analyze oily waste sample using the octanol-1 M sulfuric acid extraction procedure described earlier for solid. This procedure should eliminate the interference due to inorganic halides by rejecting them into the aqueous phase. Dilution of the sample liquid with octanol during the extraction procedure should lower the viscosity and should result in a solution being relatively easy to inject. Standard oily waste samples containing various organic halide compounds were prepared. Each sample was prepared by pipetting known volume of standard organic halide solution of interest into known volume of toluene. A portion of this oily waste was weighed and was added to a vial which contained 5 ml of octanol and 1 ml 1 M sulfuric acid. The vial was shaken vigorously for one minute and then centrifuged at approximately 1,000 G for

10 minutes. An aliquot of the octanol phase was then analyzed by direct injection with the analyzer set at injection mode. The results obtained are shown in Table 4 and are similar to those from solids by extraction with octanol-1 M sulfuric acid. For non-polar compounds, the average recoveries for 1-chloronaphthalene and 1,2,4-trichlorobenzene are 83.4 and 111.2% respectively. The more polar chloroacetic acid also showed good recovery, giving a value of 81.6%. The recovery for chloroform is low and is probably due to loss by volatilization.

To study the possible interference due to the presence of inorganic halides, a standard oily waste sample containing inorganic chloride was prepared by adding 6.276g of sodium chloride to a mixture made up of 30 ml water, 10 ml methanol, 200 ml toluence and 1.0 ml of triton X-100, an emulsifier. Three layers were obtained from the mixture: octanol, emulsion and water layers. Portions of each layer were weighed and extracted with octanol-1 M sulfuric acid mixture in the same manner as described above. The halide contents of each of the layers were then analyzed by direct injection of the octanol phase. No halide was found in the octanol phase of any of the three layers. The study showed that the presence of inorganic halides in oily waste sample does not interfere with analysis by octanol-1 M sulfuric acid extraction procedure.

CONCLUSIONS

Two methods involving pyrolysis/microcoulometric determination for the measurement of organic halides in solids and oily waste were developed. The first method involving nitrate wash-carbon adsorption is a modification of the U.S. EPA method for the anlysis of total organic halides in water and is

developed for the analysis of solids. This method offers an alternative means for the analysis of organic halides in solids. The second method involving extraction with octanol- 1 M sulfuric acid mixture and is applicable to solid and oily waste samples. For both types of samples, the organic halides are separated from the inorganic halides and are subsequently pyrolyzed and microcoulometrically analyzed. The second method extends the analysis to a broader spectrum of organic halides including polar compounds such as chloroacetic acid.

The pyrolysis/microcoulometric technique using a Dohrman DX-20 analyzer has been shown to provide an effective means for screening and overall determination of organic halides. It is sensitive, relatively fast and easy to use. However, this technique has its limitations. Since a sample can contain a wide variety of compounds or mixture of compounds with different efficiencies of recovery, no suitable standard can be used for calibration. As a result, the accuracy of analysis is expected to be poorer than when a spiked sample for a single known compound is analyzed.

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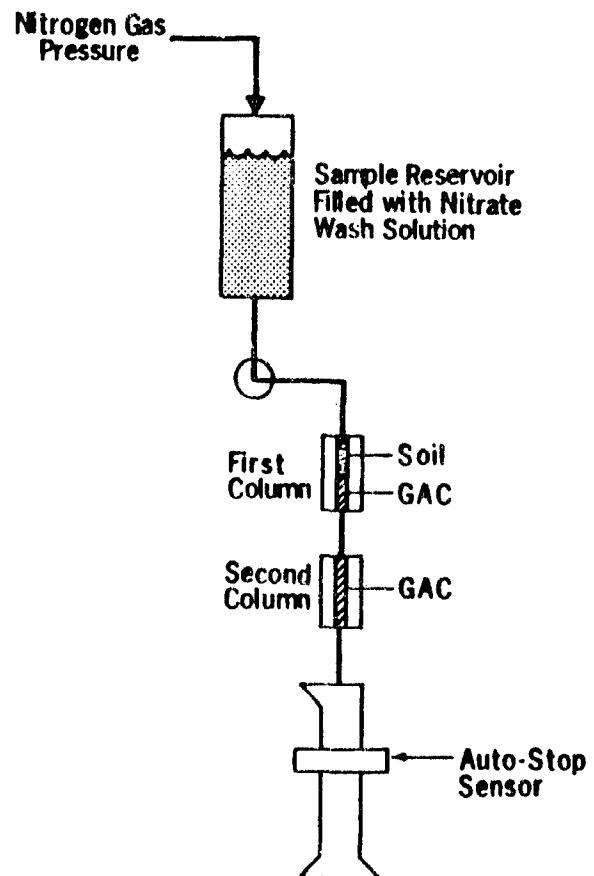


Figure 1. Apparatus for the Recovery of Halogen Compounds in Solid Samples by Nitrate Wash-Carbon Adsorption

Table 1. Recovery of Halogen Compounds in Soils by Nitrate Wash-
Carbon Adsorption Total Organic Halide Determination

<u>COMPOUND</u>	<u>Amount Present (ug/g)</u> <u>as Cl</u>		<u>Number</u> <u>of Runs</u>	<u>Average</u> <u>Recovery</u> <u>(%)</u>	<u>Standard</u> <u>Deviation</u> <u>(%)</u>
	<u>Calculated</u>	<u>Found*</u>			
1-chloronaphthalene	786	731	3	93.0	11.0
1,2,4-trichlorobenzene	810	688	3	84.9	13.7
1,2,4-trichlorobenzene	766	572	5	74.7	6.7
bromocyclohexane	814	589	3	72.3	12.8
chloroform	785	341	1	43.5	---
2,4,6-trichlorophenol	795	575	4	72.3	7.4
2,4,6-trichlorophenol	786	618	4	78.5	5.8
chloroacetone	775	121	3	15.6	9.5
1-chloropropanoic acid	792	17	3	2.1	4.4
1-chloroethanol	689	359	3	52.1	17.7
1-chloroacetic acid	807	470	3	58.3	14.5

*Corrected for the 42 ug Cl/g Soil Originally Present

Table 2. Recovery of Halogen Compounds in Garden Soils by
Extraction with Ethyl Acetate and Water

COMPOUND	Amount Present ($\mu\text{g/g}$) as Cl		Number of Runs	Average Recovery (%)	Standard Deviation (%)
	Calculated	Found*			
chloronaphthalene	10,570	10,464	3	104.0	6.0
chloronaphthalene	815	972	2	129.5	4.8
p-dichlorobenzene	13,600	13,816	2	101.5	0.3
p-dichlorobenzene	833	993	2	119.2	1.0
1,2,4-trichlorobenzene	10,270	8,975	2	87.3	1.3
1,2,4-trichlorobenzene	1,842	1,588	2	86.2	11.4
bromocyclohexane	814	734	2	90.1	5.2
1-iodooctane	812	643	2	79.2	3.1
bromoform	5,976	4,326	2	72.4	2.6
bromoform	863	771	2	89.3	0.3
1-chloropropanoic acid	792	462	2	58.2	6.3
1-chloroacetic acid	13,300	184	6	1.2	0.6
1-chloroacetic acid	807	121	2	15.0	7.4

*Corrected for the $42\mu\text{g Cl/g}$ Soil Originally Present

Table 3. Recovery of Halogen Compounds in Soils by
Extraction with Octanol-1.00M H₂SO₄

<u>COMPOUND</u>	Amount Present (ug/g) as Cl		Number of Runs	Average Recovery (%)	Standard Deviation (%)
	<u>Calculated</u>	<u>Found*</u>			
1-chloronaphthalene	786	770	3	97.9	9.7
1-iodooctane	812	635	3	78.2	4.8
bromocyclohexane	814	707	4	86.8	5.2
2,4,6-trichlorophenol	811	749	3	92.4	6.8
1-chloropropanoic acid	792	574	4	79.5	5.3
1-chloroacetic acid	807	677	5	83.9	2.6

*Corrected for the 42 ug Cl/g Soil Originally Present

Table 4. Recovery of Halogen Compounds in Oily Waste by
Extraction with Octanol-1M H₂SO₄

<u>COMPOUND</u>	<u>Amount Present (ug/ml)</u> <u>as Cl</u>		<u>Number</u> <u>of Runs</u>	<u>Average</u> <u>Recovery</u> <u>(%)</u>	<u>Standard</u> <u>Deviation</u> <u>(%)</u>
	<u>Calculated</u>	<u>Found</u>			
1-chloronaphthalene	31,200	26,021	3	83.4	16.1
1,2,4-trichlorobenzene	614	683	3	111.2	18.9
chloroform	11,700	7,122	2	60.9	6.2
1-chloroacetic acid	36,000	29,376	3	81.6	11.1

DEVELOPMENT AND EVALUATION OF TEST METHODS FOR TOTAL CHLORINE
IN USED OILS AND OIL FUELS

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ABSTRACT

A current EPA regulation prohibits the sale for burning in non-industrial boilers of used oils and oil fuels contaminated above specified levels with certain metals and total chlorine. When burned as fuel in a small boiler, the contaminants may be emitted to the ambient air at hazardous levels. This regulation establishes a rebuttable presumption that used oil containing more than 1,000 ppm total chlorine has been mixed with halogenated solvents and is a hazardous waste. Rebutting the presumption requires the seller of oil to prove that this chlorine is not due to halogenated solvents or other hazardous halogenated organics. If the rebuttal is successful, the oil can be sold as fuel up to a level of 4,000 ppm total chlorine.

Analytical techniques for determination of total chlorine were evaluated or developed to provide regulatory agencies and the regulated community with appropriate chlorine test methods. The techniques evaluated included wet chemical titrations following oxygen bomb combustion, disposable test kits, instrumental microcoulemetry, and x-ray fluorescence spectrometry.

These candidate techniques were subjected to interlaboratory testing to estimate their precision, accuracy, sensitivity, and susceptibility to matrix effects. Information on ease of use and analysis costs was also collected. Based on this study, test methods were prepared for the most promising techniques and subjected to a formal collaborative study to generate precision and accuracy data for each method. These methods are to be proposed in the Federal Register as mandatory for compliance with the existing used oil regulation.

MICROWAVE ACID SAMPLE DECOMPOSITION FOR ELEMENTAL ANALYSIS

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ABSTRACT

Sample preparation is an essential step in achieving both accuracy and precision in analysis of materials. It is also one of the most time consuming portions of many analyses and has become the limiting step for such multi-element techniques as ICP, XRF, and ICP-MS. Acid digestion of biological and botanical samples can take from 4 to 48 hours using classical digestion techniques. Microwave acid digestion requires only 10 to 15 minutes for the decomposition of many biological and botanical samples, dramatically reducing preparation time. Additionally, volatile elements such as selenium, phosphorus, tellurium, vanadium and others can be quantitatively retained using microwave decomposition in a sealed vessel prior to instrumental analysis. To use the technique, however, it is necessary to have an understanding of the fundamental concepts controlling interactions between microwave energy and acid sample molecules.

During the past several years, research has been conducted to determine these fundamental relationships and to develop methods that allow the analyst to predict the conditions that will be generated in the microwave digestion, prior to programming and running the equipment. This has been accomplished by measuring many of the parameters necessary to allow calculation of the microwave power absorption by the mineral acids. The basic theory will be presented that describes the use of fundamental thermodynamic properties to predict the microwave interactions with acids and samples.

The development of real-time monitors for temperature and pressure in the microwave environment permits the investigation of closed vessel digestion using microwave energy as the heat source. The digestion uses Teflon (PFA) vessels. The microwave digestion technique has been tested on all the major sample types including biological, botanical, geological, alloy, and glassy samples and has demonstrated advantages for each of these sample groups.

This work has opened many new applications. Because microwave digestion is a well defined, precisely controlled system, it is suitable for integration into an automated application. Acid digestions have previously been too judgmental and variable for automation to be practical. This microwave technique has direct control of the power, acid temperature, and time of the digestion, and has become structured to the point that it is possible to automate sample decomposition prior to instrumental analysis. New microwave transparent vessels, recently engineered, will permit the use of higher temperatures and pressures, allowing the use of other reagents and making this form of digestion even more efficient.

CHARACTERIZATION OF MUNICIPAL AND HOUSEHOLD HAZARDOUS WASTES

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ABSTRACT

The topics of this paper are the methodologies used to sample and characterize municipal refuse and household hazardous wastes (HHW) that are found in the municipal waste stream. Results of a major characterization project will be used as examples. The paper will provide specific examples of schedules, protocols, procedures and problems. Special emphasis will be placed on HHW. Throughout, the real world of waste characterization will be used to illustrate how textbook guidance and local conditions are accommodated to yield useful results. Statistical techniques used with the data will also be presented.

The paper will be based on a project conducted by SCS Engineers for the Michigan Department of Natural Resources. The project included a year-long waste characterization effort in six counties throughout Michigan. The paper will be divided into four major sub-divisions: Introduction, Quantity, Composition, and Household Hazardous Wastes.

The Introduction will briefly describe the project and its purposes. The general approach taken to coordinate with the host counties, scheduling, logistics, personnel training, and quality control will be presented.

The waste quantity aspects of the project will be presented briefly in a discussion of the refuse weighing program. It will describe the procedures and equipment used including color slides for illustration. Field problems will be highlighted and a comparison made between the program's limited weighing effort and actual weight data. Resulting per capita waste generation rates will also be presented along with the statistical techniques applied to the data.

Waste composition will be a major element of the paper. The overall characterization program will be described including both planning and execution aspects. The waste categories utilized will be presented along with a rationale for their selection and identification of other categories that could be used for other purposes. Actual field procedures for waste sorting will be illustrated through the use of color slides.

Data collection methods will be summarized and the results reported. Illustrations of tabulations will be included.

Statistical procedures applied to the data will be identified and the rationale for their selection will be discussed. The percentage of the sampled waste stream represented by each waste category was of primary interest. Statistical parameters that were calculated include standard deviation, upper and lower confidence limits, and the application of the F-test to assess data variability.

Household hazardous wastes will be another major topic in the paper. Project sponsors were interested to know the types and amounts of HHW in the wastes of each county. The definition of HHW used was one developed by SCS in the preparation of the EPA report, A Survey of Household Hazardous Wastes and Related Collection Programs. The definition relates to environmental concerns rather than to health and safety within the home and parallels the definition of characteristic hazardous wastes. Field procedures used to identify HHW will be described. Additionally, office review of the field data by chemical and environmental engineers was used to edit the field data. The types and quantities of HHW found will be presented.

Two primary issues will be addressed in the paper. They are related to overall waste consumption characterized and to household hazardous wastes. The first issue is, "Can waste composition estimates reasonably be based on national averages?" SCS experience in this project and similar ones will be used to present and discuss this issue. The second issue is, "What kinds and how much household hazardous wastes are found in municipal refuse?". Information collected by SCS during its previous EPA project on this topic plus the field data from the Michigan waste characterization will provide some of the most detailed data to answer that question.

INTRODUCTION

The purpose of this paper is to present methodologies used to sample and characterize municipal refuse and household hazardous wastes (HHW) that are found in the municipal waste stream. Results from a major characterization project provide the basis for specific examples of schedules, protocols, procedures and problems. The real world of waste characterization will be used to illustrate how textbook guidance and local conditions are accommodated to yield useful results.

The overall goal of solid waste characterization is to determine, with a specified degree of accuracy and precision, the quantity, composition and/or other chemical and physical characteristics of solid waste. Specific objectives of waste characterization will vary with individual projects; e.g. total waste quantities, amount of newsprint, or presence of HHW.

Quantity of solid waste generated in a particular locale is important in projecting the life of a planned landfill. For existing landfills this information can be used to reassess the life of the landfill. Knowing the quantity of solid waste generated is also useful in assessing the feasibility of various solid waste management alternatives.

Solid waste composition is a major concern in resource recovery facilities where composition of the waste significantly impacts the practicality of such systems. Through waste characterization, key factors such as problem wastes, and energy and moisture content of wastes can be identified. Waste composition is also critical in planning recycling and composting projects. In addition to identifying desirable waste such as glass or aluminum for recycling or biodegradable material for composting, waste characterization will determine if there are sufficient quantities of the waste to warrant the respective programs.

The goal of waste characterization also determines the degree of accuracy and precision that is necessary. Accuracy requirements depend more on the nature of the study than on the operation in question. For example, waste characterization data from studies performed elsewhere may be adequate for a preliminary feasibility study, whereas a more extensive feasibility study or conceptual design may require more accurate and precise estimates, based instead on local conditions. During the final economic analysis of resource recovery feasibility, accuracy and precision requirements may ultimately be dictated by the buyers of the recovered material, and by those providing financing for the project.

Waste characterization has typically been confined to: (1) use of published data on solid waste quantity and composition, often referred to as "national averages" or "typical characteristics" and (2) limited field characterization involving little or no statistical inference. The questionable results are often compensated by conservative designs or revenue estimates which in turn can impact operating efficiency and economic feasibility. Thus, there is a need for development of statistically sound waste characterization methods.

BACKGROUND

Marquette County's waste stream assessment is one of the programs undertaken by the Michigan Department of Natural Resources (MDNR) to collect data and assess alternatives related to solid waste management in the state. These alternatives include recycling, composting, and energy recovery. Before the feasibility of waste management alternatives can be determined, reliable information on the quantity and composition of wastes generated is needed. In addition, seasonal fluctuations, energy content, and the presence of

household hazardous wastes are important. Although limited waste assessments were conducted in a few areas in Michigan prior to 1985, the methodology used and data generated were found to be deficient and not representative of waste streams. A definite need was found to exist for thorough waste stream assessments in representative areas across the state.

To meet this need, the Resource Recovery Section of the Community Assistance Division within the Department of Natural Resources initiated this project to collect data to determine the quantity and composition of solid waste in six counties within the state. Funded by the Clean Michigan Fund, the project provided specific information about each of the six areas and prepared a guidebook that could be used in other counties to conduct similar waste stream assessment.

OBJECTIVES

The objective of this project was to collect and report current field data regarding solid waste management in Marquette County. The project focused on solid waste quantity and composition for 1986. Additionally, historical data on solid waste and information associated with its management were obtained. The project was to present a concise picture of the amount and composition of waste generated and disposed within Marquette County.

Information obtained from this project may be used to:

- o Assess the feasibility of resource recovery alternatives;
- o Identify materials amenable to recycling, composting, and incineration; and
- o Assess the impact of Michigan's bottle bill on the disposal of returnable containers.

Due to the growing concern of household hazardous wastes in solid waste management, this project also identified types and amounts of HHW in wastes received at designated locations in Marquette County.

PROJECT APPROACH

The major disposal site in Marquette county is the Peninsula Sanitation Transfer Station. Consequently, it was chosen as the location for the weighing and waste composition program. Separate assessments (one week each) were conducted during each of the four seasons to identify seasonal variations. Data were collected Monday through Friday of each week. A second disposal site, the West Marquette Landfill was added to the waste quantity investigation at the request of the county. Limited field data were gathered at the

landfill to better assess total solid waste quantities disposed within the county, and to provide needed information on waste generation from rural areas.

The weighing program involved obtaining a sample of vehicle weights to determine the quantity of waste. Due to the small number of vehicles using the transfer station, the weights of most of the packers and other large vehicles using the site could be obtained several times per week.

The composition and energy content aspects of the project were also conducted at the transfer station. Randomly-selected loads of refuse were sampled using the techniques described in the Waste Stream Assessment Guidebook developed by SCS for MDNR. Trained crews manually separated the refuse into designated categories. In addition to typical waste categories, household hazardous wastes were identified, segregated, and counted. Items considered as HHW were completely described and their quantity estimated. Empty containers were not considered HHW. Returnable beverage containers were also noted. Soft drink and beer containers identified as returnable were counted by type of material: glass, aluminum, or plastic.

One sample from each seasonal sort was collected for laboratory analyses. These analyses included Btu content per unit weight, moisture, ash, and volatiles. These analyses were chosen to provide energy and residue information that will be useful in evaluating the feasibility of recovering energy from the wastes.

WASTE QUANTITIES

Weighing Program

Two sites were chosen as the locations for the weighing/load count portion of the solid waste assessment. The weighing/load count program was conducted for one week, in each of the four seasons at the transfer station. Load count information was collected for three types of vehicles: packers, pickup trucks; and others. Load weights were obtained for packers in all seasons and for others in the summer and fall. Bad weather and operational problems did not allow for weighing the other trucks in winter and spring, and small vehicles were not weighed. Weighing was conducted with a portable truck scale.

The vehicle to be weighed was driven onto a scale where each axle was weighed separately and then added to calculate the gross weight of the vehicle. After weighing, the vehicle unloaded at the compactor. The empty vehicle was reweighed and the tare weight recorded.

All data were then entered into the Marquette County data base for tabulation and calculations. The collection of weight data underwent a two-stage review process. First, one (or both) SCS field personnel checked the transfer of individual weights from facility forms to the SCS forms. Second, the data received an additional review off-site each day. This review included a comparison with previously collected data, so that incorrectly translated weights might be identified and corrected the following day.

Statistical methods were applied to the waste quantity data. Summations were made of the load counts and/or weight of waste delivered by the three vehicle types (packers, pickups, and others). The range of payloads was also identified for each season. Likewise, the arithmetic mean (average) of payloads was calculated for each type of vehicle during each season. Additional statistical values calculated for weight data collected included: standard deviation (SD), lower confidence limit (LCL), and upper confidence limit (UCL).

Results of the load count and weighing program projected to an annual basis are as follows:

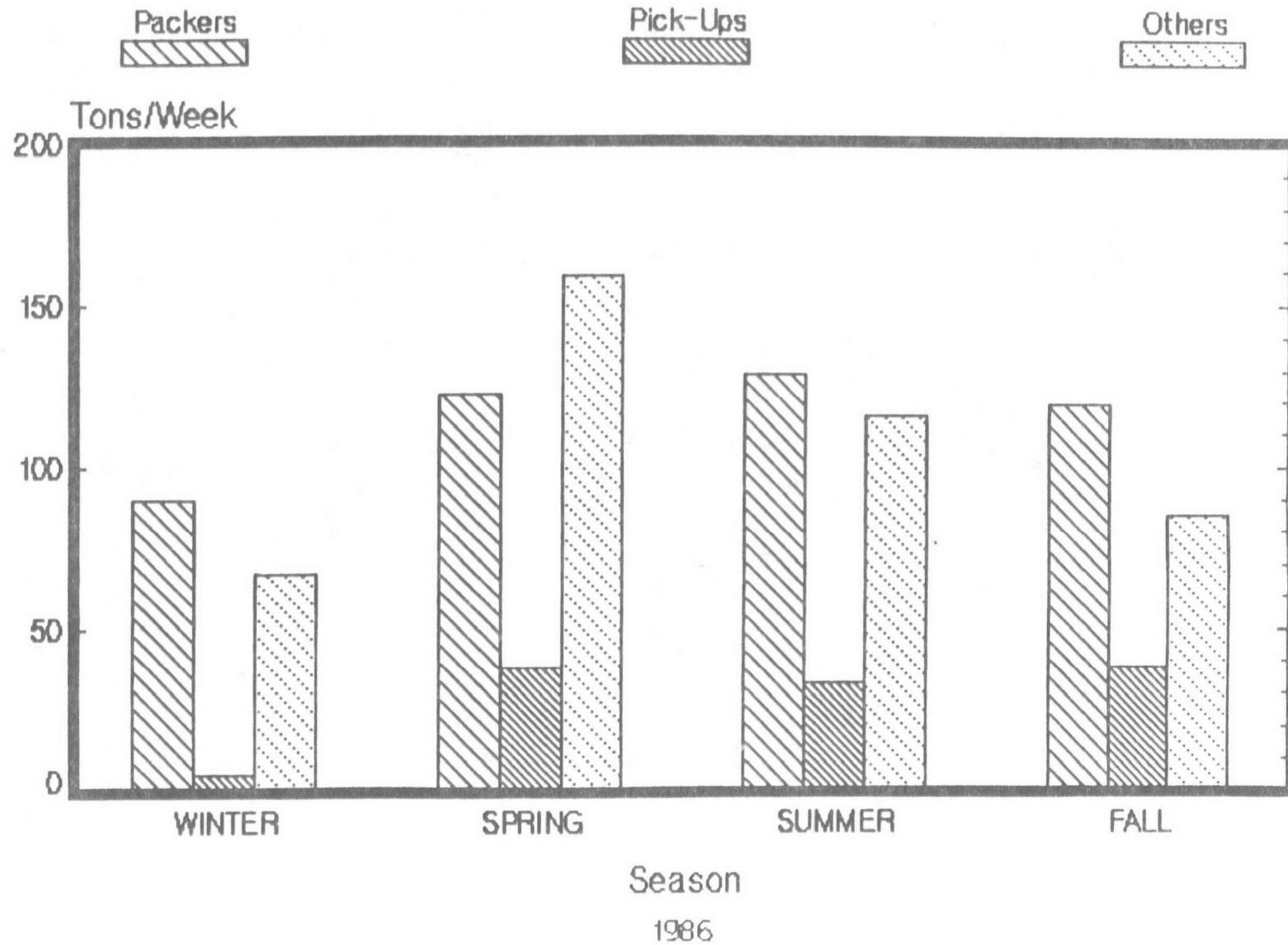
	<u>Annual Loads</u>	<u>Annual Ton</u>
Peninsula Sanitation Transfer Station	8,567	12,984
West Marquette Landfill	15,379	5,070

The dramatic differences between the two sites are the number and types of loads and the quantity of waste. The transfer station serving the City of Marquette receives most of its waste from packers. The ratio of packers to pickup is about 1 to 3. At the landfill, about half of the waste is hauled by pickups, and the packer-to-pickup ratio decreases to about 1 to 23.

SEASONAL VARIATIONS

Waste generation varies throughout the year. In general, less waste than average is generated in winter months and more than average in summer months. Typically, winter is the season of lowest waste generation due to low levels of personal and commercial activity. Summer and fall are often peak waste generation seasons related to high levels of outdoor activities associated with landscaping, travel, recreation, and construction. A graph of waste quantities for the Peninsula Sanitation Transfer Station shown in Exhibit 1 indicates that winter was the season when waste quantities from all types of trucks were at their lowest.

EXHIBIT 1
WASTE GENERATION AT THE PENINSULA SANITATION TRANSFER STATION



The numbers of loads also changed from season to season, except for packers which had a relatively constant frequency of just under 30 loads per week. Again, winter was the season with the fewest loads from pickups and other trucks. Pickups peaked in spring and fall, while spring was the peak season for loads from other trucks.

ESTIMATED GENERATION RATES

Per capita generation rates of solid waste are often estimated and used for planning solid waste facilities such as transfer stations, resource recovery facilities, and landfills. Development of accurate generation rates depends upon reliable population and waste quantity data. Neither are ever known with 100 percent accuracy for communities the size of Marquette County. However, reasonably accurate information is available to support the estimation of an annual per capita solid waste generation rate.

Per capita waste generation rates were calculated for two areas in Marquette County: the City of Marquette and the eight townships served by the West Marquette Landfill. For the purposes of this project, the population of the City of Marquette was estimated at 24,800. Annual waste quantities disposed at the transfer station were estimated at 12,948 tons. Thus, the waste generation is approximately 2.9 pounds per person per day (365 days per year). The eight townships served by the West Marquette Landfill have a combined population of 12,100. The landfill received about 5,070 tons of waste annually, resulting in a generation rate of 2.3 pounds per person per day (365 days per year).

WASTE COMPOSITION

Estimating the composition of waste in Marquette County involved the sampling of refuse delivered to the Peninsula Sanitation Transfer Station and the manual sorting of each sample into numerous components. Because the transfer station receives wastes originating principally from the City of Marquette, the waste composition data collected may be more representative of the urban center than of the entire County. Sampling and characterization were performed during each of the four seasonal assessments. Classification of waste was accomplished Monday through Friday of each week using the same procedures and personnel from day to day and season to season.

Vehicles were selected randomly by the SCS sort team leader for sampling. The drivers of the vehicles were requested to leave approximately one-quarter of each load near the sampling area as a sample. The driver was interviewed as to type of waste (i.e., residential, commercial, or mixture), the area of the County where the waste was collected, any unusual items in the load, and the number of loads expected later that day. The SCS field crew

manually collected 200 to 300 pounds of the load (the sample) and placed it on one of two plastic sheets. SCS field personnel supervised this operation to ensure the random selection of the sample and the safety of the field crew.

Equipment used during the waste composition phase included a sort box, plastic sheets, double-beam scale, 16 plastic trash cans, shovel and push broom and safety equipment.

Refuse was manually separated into the following categories:

- | | |
|---------------------|------------------------------|
| o Newsprint | o Household Hazardous Wastes |
| o Corrugated Paper | o Returnable Aluminum |
| o Office Paper | o Returnable Glass |
| o Yard Waste | o Returnable Plastic |
| o Textiles | o Other Wastes |
| o Plastics | |
| o Other Organics | |
| o Glass | |
| o Ferrous Metal | |
| o Non-Ferrous Metal | |
| o Other Inorganics | |
| o Fines | |

The categories for sorting were identified by the MDNR to best evaluate the composition of solid wastes generated statewide. At the beginning of the first seasonal assessment (the winter sort), each category was explained and examples shown to the sort crew. For consistency, the crew members became specialists in a discreet number of categories. For example, one crew member would identify and remove from the sort box only newsprint, corrugated, office paper, and textiles. Other members would specialize in other categories. If questionable items were found, the leader determined the appropriate category.

The same procedures and crew members were used during the entire waste composition program. This assured consistency from one sample to the next and from season to season.

Actual waste sorting was methodical. Extremely large or heavy waste items were placed directly into the appropriate plastic sorting container. Each item from the sample was placed in the sort box until the box was full. Plastic bags of waste were torn open and each item of waste was manually segregated and placed in the proper plastic container. These steps were repeated until the sample sort was complete.

Upon completion of the sort, the plastic containers were moved to the double-beam scale and weighed. The weights were recorded on a field sort form. After weighing, each container was emptied at the

working face of the landfill. For efficiency, this procedure was performed concurrently with preparation of the area for the next sample. This routine was repeated for up to 10 samples each day for the five-day sort program during each season.

At the end of each day, weight percentages for each sample were calculated and recorded on a daily summary sheet. The daily summary included the mean, standard deviation and coefficient of variation. These statistical parameters served as a basis for checking each days' sorting activities for completeness and accuracy. After review of the data, it was entered into a portable computer and stored for later evaluation.

A summary of the waste composition results is shown in Exhibit 2. Samples were taken only from packers. The average percentages by weight of each category are shown for each seasonal sort. These averages were themselves average to yield an estimated composition of the City of Marquette's residential and commercial waste. A category termed "other wastes" was added to account for bulky, infrequent items arriving at the disposal site, including white goods (i.e., water heaters, stoves, freezers, etc.), tires and furniture. White goods were not identified as a specific category since no major appliances were recorded in the loads sampled and no regular practice of separation occurred at the transfer station.

Seasonal Variations

Data in Exhibit 2 illustrate seasonal variations in waste composition. For example, yard waste increased to 15.3 percent in spring compared to 0.3 percent in winter. The last column in Exhibit 2 numerically compares seasonal variability. An analysis of variance using the F-test was applied to waste composition data. The F-test is a comparison of variances between data points in two groups (e.g., the newsprint data for winter versus the newsprint data for spring). Variances are calculated and checked against values in F-test tables found in most statistics reference books. These tables answered the question, "Do the data in these two groups vary significantly from one another?" As with the LCL and UCL calculations, the 95 percent confidence level was selected for the F-tests.

HOUSEHOLD HAZARDOUS WASTES

Household hazardous wastes (HHW) was a sorting category during each season. These wastes were identified using the definition developed in the U.S. Environmental Protection Agency's (EPA's) first nationwide survey of this topic conducted by SCS Engineers. Wastes identified as originating from residential sources and likely to fail one of the EPA's hazardous waste characteristic tests (ignitability, corrosivity, reactivity, or EP toxicity) were

EXHIBIT 2
PENINSULA SANITATION TRANSFER STATION
WASTE COMPOSITION DATA FOR 1986

Waste Category	Percent by Weight					Varied Significantly?
	Winter	Spring	Summer	Fall	Average	
Newsprint	9.2	4.9	5.3	6.5	6.5	No
Corrugated	2.5	7.0	7.0	10.1	6.7	Yes
Office Paper	3.1	2.7	2.9	3.5	3.0	No
Yard Waste	0.3	15.3	5.7	7.0	7.0	Yes
Textiles	2.1	2.5	1.8	3.8	2.6	Yes
Plastics	3.9	3.6	5.1	3.3	4.0	Yes
Other Organics	57.7	49.7	58.1	52.4	54.5	No
-----	-----	-----	-----	-----	-----	
Subtotal	78.8	85.7	85.9	86.6	84.3	
Glass	7.6	4.4	6.1	5.5	5.9	No
Ferrous Metal	0.6	4.3	4.7	4.3	3.5	No
Non-Ferrous Metal	7.4	0.4	0.5	0.7	2.2	Yes
Other Inorganic	1.2	1.0	0.8	0.2	0.8	Yes
Fines	3.7	2.9	1.7	2.6	2.7	Yes
-----	-----	-----	-----	-----	-----	
Subtotal	20.5	13.0	13.8	13.3	15.1	
Household Hazardous Wastes	Nil	Nil	Nil	Nil	Nil	N/A
Returnable Aluminum	Nil	Nil	Nil	Nil	Nil	N/A
Returnable Glass	Nil	Nil	Nil	Nil	Nil	N/A
Returnable Plastic	Nil	Nil	Nil	Nil	Nil	N/A
Other Waste*	0.7	1.3	0.3	0.1	0.6	Yes
-----	-----	-----	-----	-----	-----	
Total	100.0	100.0	100.0	100.0	**	

* Includes furniture, white goods, tires, and other large or multi-material items.

** Sum of average composition may not be 100 percent due to rounding.

N/A = Not applicable.

considered to be HHW. Through manual sort, items identified as possible HHW were completely described and their quantity estimated. Empty containers of HHW products were not considered as HHW for this study. The MDNR's Hazardous Waste Division generally agrees with this approach and it corresponds to the EPA's approach regarding empty containers.

HHW were categorized as the following:

- Household Cleaners: - drain cleaners, oven cleaners, wood and metal cleaners and polishes
- Automotive: - gasoline and oil additives, grease and rust solvents, carburetor cleaners, air conditioning refrigerant, starting fluids
- Home Maintenance and Improvement - paint thinners, strippers and removers, adhesives and glues
- Lawn and Garden - herbicides, pesticides, fungicides including wood preservatives

Note that not all examples of any generic type included in the list are considered HHW. For example, oven cleaners were included because most would fail the corrosivity test. However, some commercial oven cleaners would not fail this test.

Household hazardous wastes were a small portion of the waste on a weight basis. The largest quantity of HHW identified occurred during the fall sort. During that sampling week, 6.6 pounds of HHW were found in a total weekly sample weight of over 7,491 pounds. Thus HHW amounted to 0.09 percent (by weight) of the wastes sampled that week.

The other seasonal sorts resulted in small percentages of HHW. The winter sort included 3.0 pounds of HHW out of more than 3,784 pounds of waste sampled, representing 0.08 percent of the waste. In the spring, 2.1 pounds of HHW were identified, representing 0.02 percent of the week's sample weight. During the summer sort, no HHW were found. The types and amounts of HHW identified during each seasonal sort are shown in Exhibit 3.

Overall, about 0.06 percent of the waste sampled during the four-week survey was considered to be HHW. This projects to about 7.5 tons per year disposed at the transfer station. The annual percentage and total projected weight of HHW represent the HHW that routinely enter the solid waste stream. Other nationwide studies report that many households tend to stockpile HHW in basements and garages. Thus, HHW may be retained in homes for many years and not

EXHIBIT 3 HOUSEHOLD HAZARDOUS WASTES IN MARQUETTE COUNTY

Household Cleaners	Oz.	Automotive Products	Oz.	Lawn/Garden Products	Oz.	Home Maintenance/ Improvement Products	Oz.	Miscellaneous	Oz.
WINTER 1986									
Oven Cleaner	4							Electrical Contact Cleaner	28
Bathroom Cleaner	16								
Season Totals:	20								28
Total HHW (oz):		48							
Total HHW (lbs):		3							
Total Weekly Sample Wt. (lbs):		3,784							
Percent HHW:		0.08							

SPRING 1986

		Waste Oil	32					30-30 Bullet	2
Season Totals:			32						2
Total HHW (oz):		34							
Total HHW (lbs):		2.1							
Total Weekly Sample Wt. (lbs):		9,544							
Percent HHW:		0.02							

SUMMER 1986

There was no household hazardous waste in the samples for the sorting season.

Season Totals:	0
Total HHW (oz):	0
Total HHW (lbs):	0
Total Weekly Sample Wt. (lbs):	0.0
Percent HHW:	0.0

EXHIBIT 3 (continued)

Household Cleaners	Oz.	Automotive Products	Oz.	Lawn/Garden Products	Oz.	Home Maintenance/ Improvement Products	Oz.	Miscellaneous	Oz.
FALL 1986									
Disinfectant Spray	1	Waste Oil	96			Household Solvent	2	Medicine	1
		Chain Lube	2			Rust Inhibitor	4		
Season Totals:	1		98				6		1
Total HHW (oz):			106						
Total HHW (lbs):			7						
Total Weekly Sample Wt. (lbs):			7,491						
Percent HHW:			0.09						
ANNUAL TOTALS									
Total HHW (oz):			188						
Total HHW (lbs):			12						
Total Sample Wt. (lbs):			20,819						
Percent HHW:			0.06						

disposed of on a regular basis. Periodic collection days, such as those sponsored by grant programs (e.g., the Clean Michigan Fund), civic organizations, or local governments, provide convenient and beneficial outlets for disposal.

The status of waste oil as a hazardous waste is indefinite. Currently, MDNR does not consider waste oil to be hazardous unless specific samples fail one of the characteristic tests. Waste oil was included as a HHW for this study. If it is not hazardous, the quantity of HHW in the waste samples will decrease to 3.8 pounds, but the presence will decrease to 0.01 percent.

Exhibit 4 shows the results of various efforts to quantify HHW. Each characterization study was conducted using a different definition of HHW and each had a different purpose. As a result, the data may not be comparable. However, there appears to be some consistency in HHW representing a very small percentage of solid waste.

FINDINGS AND CONCLUSIONS

The general approach described in this paper can be used in estimating waste quantities, composition, and generation rates in other counties. Although actual results from Marquette County may be used in other counties, considerable caution should be exercised. The basis for comparing Marquette County data to other counties should include (1) population and population density, (2) geographic location, (3) number and size of cities, (4) affluence of the residents, and (5) the bases of the local economy. Other considerations include seasonal population changes in vacation areas or seasonal changes due to agricultural or other activities.

The following findings resulted from this waste characterization effort:

- o Approximately 12,948 tons of solid waste are disposed annually at the Peninsula Sanitation Transfer Station, resulting in a per capita waste generation of 2.9 pounds per person per day (365 days per year).
- o Quantities of waste at the transfer station are highest in the spring and summer and lowest in the winter.
- o Approximately 5,070 tons of waste are delivered annually to the West Marquette Landfill, resulting in a waste generation rate of 2.3 pounds per day per person (365 days per year) for the rural townships served by this site. Little to no commercial waste are disposed at the landfill.

EXHIBIT 4
HHW Characterization Efforts

<u>Date, Location Conducted By</u>	<u>Quantity Sorted</u>	<u>HHW %</u>
1979, Los Angeles County, CA, Los Angeles County Sanitation District	155 tons	< 1% total HW HHW < 20% of HW
1984-85, Los Angeles County, CA, Los Angeles County Sanitation District	15,000 tons scanned for commercial size HW containers	0.00147% total HW
1983, Albuquerque, NM, Albuquerque Environmental Health and Energy Department	Opinion survey of 386 households	0.5%
1986, Six Michigan Counties, SCS Engineers	149 tons	0.08% Range - 0 to 0.3%

- o Waste quantities at the landfill are highest in the summer and fall and lowest in the winter.
- o Household hazardous wastes comprise about 0.06 percent by weight or about 7.5 tons per year projected for the Peninsula Sanitation Transfer Station.
- o Four samples of waste analyzed for energy content yielded an average of 4,318 Btu per pound, essentially equal to the national average of 4,500 Btu per pound.
- o About 84 percent of the waste stream (the organics) is combustible.

Comparison of these findings with national averages result in the following conclusions:

- o Waste generation in the City of Marquette falls within the nationwide range of 2.5 to 3.5 pounds per person per day for residential and commercial wastes.
- o Marquette waste composition is similar to wastes generated in other parts of the nation. Significant quantities of recyclables and compostables are potentially available for recovery, including newsprint, corrugated, glass, and yard wastes. Organized recycling efforts should be focused in the City of Marquette.
- o Household hazardous wastes are present, but in lower concentrations than in other locations nationwide.

ENVIRONMENTAL SCREENING METHODS FOR TOTAL ORGANIC HALOGENS

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ABSTRACT

Approximately two-thirds of the priority pollutants identified by the USEPA are halogenated organic compounds. In response, many analytical techniques have been tested to characterize these compounds in water, waste oils, and soils from spill sites and other waste sites. USEPA Method 9020 documents a very useful and well-established means of monitoring total organic halogens (TOX) in various water samples. (The method is applicable to both identifiable and unidentifiable halogenated organic compounds.) This paper demonstrates the utility of changing analytical parameters of a TOX analyzer for these more challenging sample types.

In cases where chromatographic techniques are requisites for organic halogen identification (EPA Methods 601, 602, 624), time lost due to detector overrange, trap saturation, and/or sample carryover in chromatographic columns becomes critical. For concentrated samples, a fast and easy means of predicting sample and dilution volumes is in high demand. A TOX analyzer is extremely useful in this regard.

For non-liquid samples such as organics, soils and solid wastes, however, GC and GC/MS techniques can not be considered. For these matrices, a fast, accurate and inexpensive method for measuring organic halides is much needed. A TOX analyzer performs accurately and reliably in this capacity.

Finally, when a high proportion of a liquid sample is not chromatographable due primarily to the nature of the sample itself, GC and GC/MS become expensive and unsuitable tools. Although a TOX analyzer does not identify compounds or provide quantitation of individual components, it does provide accurate quantitation of organohalogens including those which cannot be chromatographed.